UNIVERSIDADE FEDERAL DE ALAGOAS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos

JOÃO PAULO FELIX AUGUSTO DE ALMEIDA

DIVERSIDADE GENÉTICA E CONSERVAÇÃO DE TARTARUGAS MARINHAS DO OCEANO ATLÂNTICO SUDOESTE

MACEIÓ - ALAGOAS
Janeiro/2023

JOÃO PAULO FELIX AUGUSTO DE ALMEIDA

DIVERSIDADE GENÉTICA E CONSERVAÇÃO DE TARTARUGAS MARINHAS DO OCEANO ATLÂNTICO SUDOESTE

Tese apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde. Universidade Federal de Alagoas, como requisito para obtenção do título de Doutor em CIÊNCIAS BIOLÓGICAS, área de concentração em Conservação da Biodiversidade Tropical.

Orientador(a): Prof(a). Dr.(a) Tamí Mott
Coorientador: Prof. Dr. Robson G. Santos

MACEIÓ - ALAGOAS
Janeiro/2023

Catalogação na fonte
Universidade Federal de Alagoas
Biblioteca Central
Divisão de Tratamento Técnico
Bibliotecária: Taciana Sousa dos Santos – CRB-4 – 2062

A447d Almeida, João Paulo Felix Augusto de .
Diversidade genética e conservação de tartarugas marinhas do Oceano Atlântico Sudoeste / João Paulo Felix Augusto de Almeida. – 2023.
132 f. : il. color.

Orientadora: Tamí Mott.
Coorientador: Robson G. Santos.
Tese (Doutorado em Ciências Biológicas) – Universidade Federal de Alagoas. Instituto de Ciências Biológicas e da Saúde. Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos. Maceió, 2023.

Bibliografia: f. 105-106.
Anexos: f. 108-132.

1. Tartarugas marinhas. 2. Variabilidade genética. 3. DNA mitocondrial. 4. Hibridização. I. Título.

CDU: 639.248 : 575

AGRADECIMENTOS

A toda minha família, minha mãe, meu pai e todas as pessoas que me apoiaram durante essa jornada. A meus avós, que se foram durante esse período, e que, de perto ou de longe, sempre contribuíram para meu crescimento pessoal desde a infância. A meus amigos, que me ouviram e incentivaram e que muitas vezes tiveram que ouvir que eu não podia sair porque estava ocupado demais com coisas do doutorado.

A todos os amigos do laboratório de biologia integrativa; Grazi, André, Luana, Isaelly, Aline, Letícia e Matheus. Bruna, pelas conversas descontraídas sobre a vida acadêmica, animes e outras coisas aleatórias. Um agradecimento especial para Jessika, pelas conversas, incentivos, conselhos, ajuda no laboratório e com tantas outras coisas! E também por me permitir passar tempo com Jade, essa princesa especial de quatro patas :).

A todos os amigos do laboratório de biologia marinha e conservação; Priscila, Gabi, Nana, Laura, Adriano, Ingredy, Julia, Renata, Larissa, Kallyne, Jimena e especialmente Thaila, pelas conversas e momentos compartilhados em campo e no laboratório. Que venham mais campos como o de Maragogi 2020 haha.

A minha orientadora, Dra Tamí Mott, por ter me recebido em seu laboratório lá em 2011. Por todos esses anos de parceria e aprendizado que me ajudaram a crescer como pessoa e como pesquisador. A meu coorientador, Dr Robson Santos, por ter aberto seu laboratório durante esse período de doutorado e por ter proporcionado essa oportunidade de aprender mais sobre esse grupo fascinante que são as tartarugas marinhas.

A todos os pesquisadores que fizeram parte das bancas avaliativas durante esses mais de quatro anos de doutorado: Dra Maíra Proietti, Dr Kim Barão, Dr Franco de Souza, Dra Tereza Thomé, Dra Camila Domit, Dr Ricardo Miranda, Dra Sibelle Vilaça, Dra Liliana Colman e Dra Taciana Kramer. Da mesma forma, fica aqui meu agradecimento a todos os professores e pesquisadores que fizeram parte da minha formação acadêmica desde os tempos de escola até aqui. Igualmente, a todos os

funcionários que trabalharam para que essas instituições pudessem funcionar e proporcionar educação e formação acadêmica para tantas pessoas.

Ao Dr Paolo Casale, por me receber em seu laboratório na Itália e a todos os membros do departamento, que me receberam muito bem. Em especial Christina, que foi minha guia assim que cheguei por lá e a quem perturbei fazendo as perguntas mais absurdas e aleatórias que uma pessoa pode fazer quando vai morar em um novo país. Em especial também a Giulia, pelas conversas nos intervalos do café e pelas aventuras e conselhos na minha última semana por lá. A Sara e Chiara, por me oferecerem um abrigo em sua casa nas minhas últimas semanas em Pisa. A Chiara, Salvatore e Giulia pelas interações sempre amigáveis mesmo nos poucos momentos que compartilhamos. A todas as pessoas que conheci por lá nos hostels e nas ruas e que proporcionaram ótimas conversas e aprendizados.

A Universidade Federal de Alagoas, que tem sido minha segunda casa desde 2010, por oferecer um ensino público de qualidade e por ter se mantido forte mesmo durante os momentos turbulentos. Que venham ainda muitos anos de formação de profissionais, professores e pesquisadores de qualidade.

Ao Instituto Biota de Conservação, pela parceria na aquisição das amostras utilizadas nessa tese. Aos órgãos de fomento, Fundação Boticário e Fundação PADI, que foram fundamentais para que essa tese pudesse ser desenvolvida. Ao Projeto Ecológico de Longa Duração Costa dos Corais Alagoas, por também ter contribuído direta e indiretamente para o desenvolvimento dessa tese. A Fundação de Amparo à Pesquisa do Estado de Alagoas por ter financiado minha bolsa durante a duração do curso e também minha bolsa de doutorado sanduíche.

Por fim, ao Programa de Pós-graduação em Diversidade Biológica e Conservação nos Trópicos e todos os seus professores, bem como a Julliene que sempre resolve nossas angústias lá na secretaria.

Os sonhos vêm, os sonhos vão e o resto é imperfeito.

Legião Urbana

RESUMO

As tartarugas marinhas são répteis com ciclo de vida complexo, marcado por mudanças ontogenéticas de hábitat. Ameaças às tartarugas marinhas são diversas e podem variar de acordo com seu estágio de vida. Assim, a identificação e caracterização de suas populações é fundamental para elucidar padrões de variabilidade e entender como essas pressões podem estar afetando as tartarugas marinhas. Nesta tese foram avaliadas a razão sexual de tartarugas verdes ao longo de áreas de alimentação do Oceano Atlântico Sudoeste (OAS), a origem natal de fêmeas e machos dessa espécie, a presença de hibridização entre tartarugas marinhas no nordeste do Brasil e se a diversidade genética de tartarugas verdes tem variado ao longo do tempo no OAS e se isso se relaciona com a recuperação de áreas de desova locais. Análises genéticas foram realizadas utilizando-se principalmente a região controle do DNA mitocondrial (mtDNA), porém também utilizamos marcadores nucleares e repetições curtas do DNA mitocondrial (mtSTR) a fim de verificar a presença de estruturações populacionais não reveladas apenas com mtDNA. Foi observada uma razão sexual de tartarugas verdes em favor das fêmeas ao longo do OAS e que fêmeas e machos que se alimentam no nordeste do Brasil possuem origem natal levemente diferente. A variação genética temporal na espécie ao longo do OAS não é perceptível quando se avalia a região como um todo. Porém, foi possível observar que a frequência dos haplótipos variou ao longo do tempo tanto no nordeste quanto no sul do Brasil. Também foi detectada a presença de hibridização entre quatro espécies de tartarugas marinhas ao longo do litoral de Alagoas, nordeste do Brasil, além da presença de um espécime de tartaruga de pente com um haplótipo típico de áreas de desova do Indo-Pacífico. Esses resultados contribuem para a caracterização genética das tartarugas marinhas no OAS, além de discutir questões ecológicas importantes como a presença de hibridização e a diferença na produção de fêmeas e machos por diferentes áreas de desova e como esses processos podem ser acentuados frente às mudanças climáticas e outras pressões atuais.

Palavras-chave: Variabilidade genética. DNA mitocondrial. Aumento de temperatura. Brasil. Hibridização.

ABSTRACT

Sea turtles are reptiles with complex life cycles, marked by ontogenetic habitat shifts. They are under a wide range of threats that vary according to their life stage. Thus, the identification and characterization of sea turtle populations is fundamental to clarify variability patterns and how these anthropic pressures can affect these species. The main goals of this study were to evaluate green turtle sex ratios along feeding grounds in the Southwest Atlantic Ocean (SWA), to investigate natal origins of female and male green turtles, to assess the hybridization process among sea turtles from northeastern Brazil and to evaluate temporal variation in green turtle genetic diversity along the SWA and if this variation is related to the recovery of local nesting sites. The control region of the mitochondrial DNA was employed for most genetic analyses, but nuclear loci and mitochondrial short tandem repeats (mtSTR) were also used to assess population structure. Green turtle sex ratios along the SWA were female-skewed and females and males that feed along the coast of northeastern Brazil have slightly divergent natal origins. Temporal variation on green turtle genetic diversity along the SWA was not noticeable. However, it was possible to observe temporal variation in haplotype frequency and natal origins when analysing data from northeastern and southern Brazil independently. Hybridization was observed among four sea turtle species along the coast of Alagoas, northeastern Brazil. Furthermore, a hawksbill specimen had a haplotype typical from Indo-Pacific nesting sites. This study contributes to sea turtle genetic characterization in the SWA and debates important subjects with ecological implications such as the presence of hybridization in sea turtle populations and varying female and male outputs in local nesting sites. Understanding and monitoring these processes is essential to evaluate how sea turtle populations will respond to ongoing environmental pressures, such as climate change and other anthropic threats.

Keywords: Genetic variability. Mitochondrial DNA. Rising temperatures. Brazil. Hybridization

SUMÁRIO

1 APRESENTAÇÃO

As tartarugas marinhas fazem parte de um grupo de répteis com um complexo ciclo de vida, caracterizado por um crescimento lento e mudanças ontogenéticas de hábitat (BOLTEN, 2003). Cinco das setes espécies de tartarugas marinhas; *Caretta caretta* (tartaruga cabeçuda), *Chelonia mydas* (tartaruga verde), *Dermochelys coriacea* (tartaruga de couro), *Eretmochelys imbricata* (tartaruga de pente) e *Lepidochelys olivacea* (tartaruga oliva); possuem ampla distribuição, podendo ser encontradas em todo o globo, principalmente em regiões tropicais e subtropicais (BOLTEN, 2003). As duas espécies remanescentes, *Lepidochelys kempii* e *Natator depressus*, em geral possuem distribuição mais restrita, sendo encontradas principalmente no golfo do México e no litoral Australiano, respectivamente (LIMPUS, 2007; MÁRQUEZ, 2001).

Atualmente, todas as espécies se encontram sob algum grau de ameaça de acordo com União Internacional para a Conservação da Natureza (IUCN), exceto por *N. depressus*, que se encontra sob a classificação de deficiência de dados (IUCN, 2022). Apesar de serem historicamente exploradas em diversas fases da vida, desde a coleta de ovos até a caça de tartarugas adultas; várias ações de proteção ao redor do mundo têm contribuído para a recuperação e manutenção das populações remanescentes (e.g. MARCOVALDI; MARCOVALDI, 1999; WEBER et al., 2014). Algumas áreas de desova têm permanecido estáveis (ALMEIDA et al., 2011), enquanto outras têm apresentado um crescimento significativo, apesar de ainda representarem apenas uma parte do seu tamanho populacional original (CATRY et al., 2009; WEBER et al., 2014).

Ainda assim, mudanças no ambiente natural decorrentes de atividades humanas têm ameaçado as populações de tartarugas marinhas ao redor do mundo. Mudanças climáticas, pesca incidental, ocupação desordenada do ambiente costeiro, poluição dos ambientes costeiro e marinho, são apenas algumas das ameaças às tartarugas marinhas atualmente (FUENTES et al., 2010; FUENTES; LIMPUS; HAMANN, 2011; SANTOS; MACHOVSKY-CAPUSKA; ANDRADES, 2021). Como consequência desse cenário, a identificação e caracterização das populações remanescentes dessas

espécies se faz cada vez mais necessária, principalmente em áreas que apresentam risco potencial para essas populações, como é o caso do Oceano Atlântico Sudoeste (OAS) (WALLACE et al., 2010). Com exceção de *L. kempii* e *N. depressus*, todas as outras espécies de tartarugas marinhas ocorrem no OAS. É possível encontrar áreas de nidificação e alimentação dessas espécies na região e vários estudos apontam a conexão do OAS com outras regiões do Atlântico (LUSCHI et al., 1998; NARO-MACIEL et al., 2012; PROIETTI et al., 2014). Além disso, a frequência de hibridização entre tartarugas marinhas em algumas áreas do OAS, particularmente no litoral do Brasil, é maior do que em outras regiões do mundo (BRITO et al., 2020; LARA-RUIZ et al., 2006; REIS; SOARES; LÔBO-HAJDU, 2010). Dessa maneira, o contínuo monitoramento dessas áreas e da dinâmica populacional de tartarugas marinhas na região é essencial para guiar futuros estudos e medidas de conservação adequadas para essas espécies.

Esta tese discute um pouco das temáticas expostas acima com um foco nas tartarugas marinhas do OAS. Primeiramente, esses temas são discutidos com mais detalhes na seção de revisão de literatura. Após, seguem-se três capítulos que abordam a diversidade genética e conservação de tartarugas marinhas no OAS. No primeiro capítulo discute-se a razão sexual de tartarugas verdes em áreas de alimentação no OAS bem como a origem natal de fêmeas e machos da espécie em uma área de alimentação no estado de Alagoas, nordeste do Brasil. No segundo capítulo, discute-se a hibridização entre espécies de tartarugas marinhas em áreas de nidificação e alimentação também em Alagoas. No terceiro capítulo é abordada a diferenciação genética entre tartarugas verdes provenientes de áreas de alimentação de Alagoas e do estado do Paraná, sul do Brasil, bem como a variação temporal na diversidade genética da espécie no OAS e como ela se relaciona com a recuperação de áreas de desova da região.

O primeiro capítulo encontra-se publicado no periódico ICES Journal of Marine Science (https://doi.org/10.1093/icesjms/fsab093). O segundo capítulo está publicado no periódico Marine Biology (https://doi.org/10.1007/s00227-022-04168-y) e o terceiro está sendo preparado para submissão no mesmo periódico. Por fim, após os três

capítulos principais da tese, segue-se uma seção de discussão geral e conclusões. Os três capítulos principais estão formatados segundo as normas das revistas citadas acima, enquanto o restante da tese está formatado de acordo com as normas do Programa de Pós-graduação em Diversidade Biológica e Conservação nos Trópicos (PPG-DIBICT) e da Universidade Federal de Alagoas (UFAL).

REFERÊNCIAS

ALMEIDA, A. P. et al. Green turtle nesting on trindade island, Brazil: Abundance, trends, and biometrics. Endangered Species Research, v. 14, n. 3, p. 193–201, 2011.

BOLTEN, A. B. Variation in sea turtle life history patterns: neritic vs oceanic developmental stages. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.), The Biology of Sea Turtles Volume II. CRC Press, Boca Raton, 2003, p. 243–257.

BRITO, C. et al. Combined use of mitochondrial and nuclear genetic markers further reveal immature marine turtle hybrids along the south western Atlantic. Genetics and Molecular Biology, v. 43, n. 2, p. 1–11, 2020.

CATRY, P. et al. Status, Ecology, and Conservation of Sea Turtles in Guinea-Bissau. Chelonian Conservation and Biology, v. 8, n. 2, p. 150–160, 2009.

FUENTES, M. M. P. B. et al. Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic Conservation: Marine and Freshwater Ecosystems, v. 20, n. 2, p. 132–139, 2010.

FUENTES, M. M. P. B.; LIMPUS, C. J.; HAMANN, M. Vulnerability of sea turtle nesting grounds to climate change. Global Change Biology, v. 17, p. 140–153, 2011.

IUCN. The IUCN Red List of Threatened Species. Version 2022-1, 2022. <https://www.iucnredlist.org>

LARA-RUIZ, P. et al. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conservation Genetics, v. 7, n. 5, p. 773–781, 2006.

LIMPUS, C. J. A Biological Review of Australian Marine Turtles. 5. Flatback turtle, *Natator depressus* (Garman). Report for the Environmental Protection Agency Queensland. p. 1–54, 2007.

LUSCHI, P. et al. The navigational feats of green sea turtles migrating from Ascension Island investigated by satellite telemetry. Proceedings of the Royal Society of London B, v. 265, p. 2279–2284, 1998.

MARCOVALDI, M. A.; Marcovaldi, G. G. Marine turtles of Brazil: The history and structure of Projeto TAMAR-IBAMA. Biological Conservation, v. 91, p. 35–41, 1999.

MÁRQUEZ, R. M. Status and Distribution of the Kemp's Ridley Turtle, Lepidochelys kempii, in the Wider Caribbean Region. p. 46–51, 2001.

NARO-MACIEL, E. et al. The interplay of homing and dispersal in green turtles: A focus on the southwestern atlantic. Journal of Heredity, v. 103, n. 6, p. 792–805, 2012.

PROIETTI, M. C. et al. Genetic structure and natal origins of immature hawksbill turtles (*Eretmochelys imbricata*) in Brazilian waters. PLoS ONE, v. 9, n. 2, 2014.

REIS, E. C.; SOARES, L. S.; LÔBO-HAJDU, G. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conservation Genetics, v. 11, n. 4, p. 1587–1591, 2010.

SANTOS, R. G.; MACHOVSKY-CAPUSKA, G. E.; ANDRADES, R. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. Science, v. 373, p. 56–60, 2021.

WALLACE, B. P. et al. Regional Management Units for Marine Turtles : A Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS ONE, v. 5, n. 12, 2010.

WEBER, S. B. et al. Recovery of the South Atlantic's largest green turtle nesting population. Biodiversity and Conservation, v. 23, n. 12, p. 3005–3018, 2014.

2 REVISÃO DA LITERATURA

2.1 Aspectos biológicos básicos das tartarugas marinhas

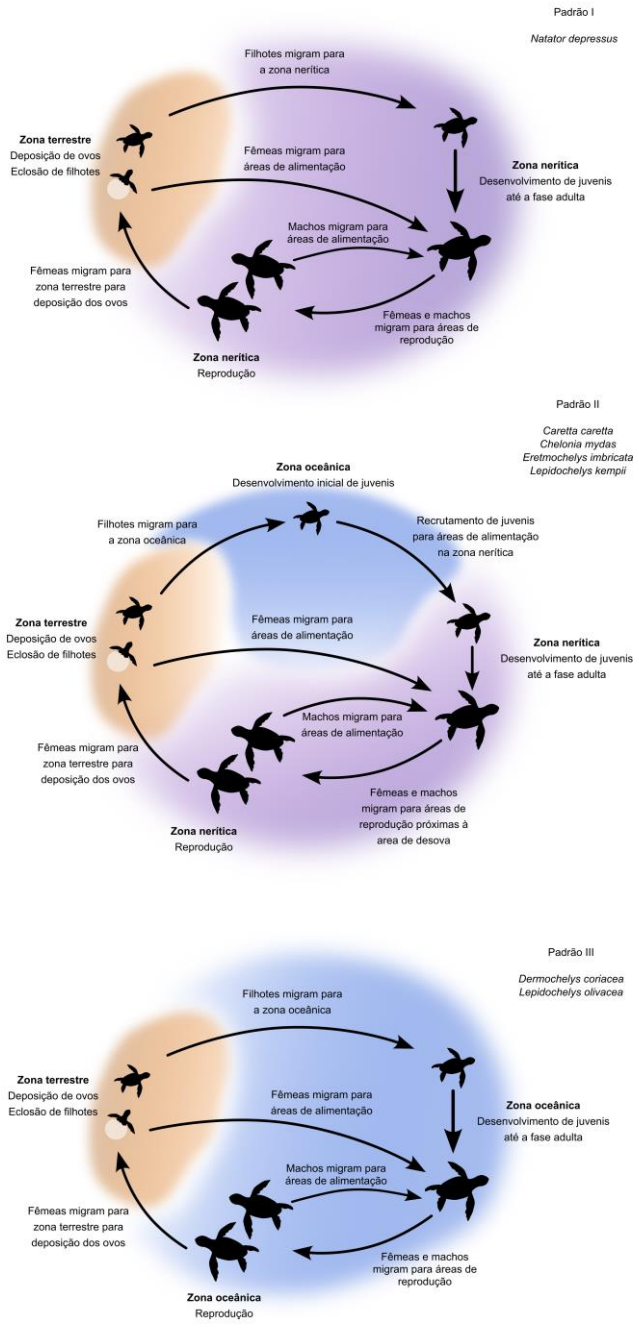
Atualmente, existem sete espécies conhecidas de tartarugas marinhas: *Caretta caretta* (Linnaeus, 1758); *Chelonia mydas* (Linnaeus, 1758), *Dermochelys coriacea* (Vandelli, 1761); *Eretmochelys imbricata* (Linnaeus, 1766), *Lepidochelys kempii* Garman, 1980; *Lepidochelys olivacea* (Eschscholtz, 1829) e *Natator depressus* (Garman, 1980). Duas dessas espécies, *L. kempii* e *N. depressus*, possuem distribuição geralmente mais restrita; sendo a primeira majoritariamente restrita ao golfo do México e a costa leste dos Estados Unidos da América (MÁRQUEZ, 2001), enquanto a segunda é majoritariamente restrita a águas Australianas (LIMPUS, 2007). As outras espécies possuem distribuição cosmopolita, sendo encontradas em águas tropicais e subtropicais de todo o globo (BOLTEN, 2003).

As tartarugas marinhas possuem um ciclo de vida complexo que frequentemente envolve mudanças ontogenéticas de habitat (BOLTEN, 2003). Historicamente, pouco se sabia sobre a fase inicial da vida das tartarugas marinhas, como Carr (1967) escreveu “Where do little sea turtles go, and how do they live after they leave the nesting beach?”. Muito se descobriu depois que Carr fez essas perguntas e o princípio da elucidação do mistério dos “anos perdidos” se deu com o próprio Carr (1967). A hipótese sugerida por ele foi a de que, durante essa fase de vida, as tartarugas marinhas permanecem na superfície de zonas oceânicas, geralmente associadas a campos de sargaço. Vários relatos de pescadores que encontraram pequenas tartarugas nessas áreas foram registrados (e.g. CARR, 1967), o que ajudava a sustentar a hipótese de Carr. Estudos posteriores, principalmente baseados em telemetria por satélite e isótopos estáveis, vieram a confirmar essa hipótese e esclarecer ainda mais a dinâmica migratória dos filhotes recém eclodidos (ARTHUR; BOYLE; LIMPUS, 2008; MANSFIELD et al., 2014; REICH; BJORNDAL; BOLTEN, 2007).

Atualmente, aceita-se a existência de três padrões principais no ciclo de vida das tartarugas marinhas (BOLTEN, 2003). No primeiro deles, a fase juvenil de desenvolvimento e a fase adulta do ciclo de vida dos indivíduos é restrita às zonas neríticas (zonas próximas a costa com profundidade em geral menor que 200m), esse padrão é observado apenas em *N. depressus*. No segundo padrão, logo após a emergência dos ovos, os juvenis migram para zonas oceânicas onde se desenvolvem por alguns anos, posteriormente migrando para zonas neríticas para completar seu desenvolvimento, esse padrão pode ser observado em *C. caretta*, *C. mydas*, *E. imbricata* e *L. kempii*. No último padrão, observado em *D. coriacea* e *L. olivacea*, o desenvolvimento dos indivíduos ocorre inteiramente nas zonas oceânicas (Fig. 1; BOLTEN, 2003).

As áreas de desenvolvimento e alimentação podem se localizar próximas as áreas de desova ou podem estar separadas por até milhares de quilômetros. Quando alcançam a maturidade sexual, fêmeas e machos adultos migram periodicamente das áreas de alimentação para os sítios reprodutivos, que geralmente se localizam em regiões costeiras próximas a seus sítios de nidificação (PLOTKIN, 2003). Tartarugas marinhas apresentam fidelidade tanto aos sítios reprodutivos quanto aos de nidificação (filopatria), ou seja, machos e fêmeas geralmente se reproduzem nas mesmas áreas e as fêmeas da espécie nidificam na mesma região em que nasceram (CARR, 1967; LOHMANN ET AL., 2013). Após o período de acasalamento, os machos retornam aos sítios de alimentação enquanto as fêmeas nadam até a praia para depositar os ovos (HAMANN; LIMPUS; OWEN, 2003; MORTIMER; CARR, 1987). O intervalo entre cada temporada acasalamento para fêmeas gira em torno de dois a quatro anos, enquanto machos podem acasalar mais frequentemente (MORTIMER; CARR, 1987; PLOTKIN, 2003).

Figura 1 – Diferentes padrões de ciclo de vida encontrados em tartarugas marinhas, de acordo com Bolten, 2003.



Fonte: Elaborado pelo autor (2023).

Devido as características filopátricas das tartarugas marinhas, as áreas de desova dessas espécies tendem a ser geneticamente estruturadas, uma vez que elas tendem a se reproduzir na mesma região onde nasceram (ENCALADA et al., 1996; NARO-MACIEL et al., 2014). Entretanto, ao contrário do que acontece nas áreas de desova, as áreas de alimentação podem receber indivíduos de diversas áreas de desova diferentes, compondo o que é conhecido como estoque misto. Nessas áreas há ocorrência não só de indivíduos de diversas origens natais, mas também de classes etárias distintas. Nesse sentido, dados moleculares se tornaram uma ferramenta útil para investigar a conexão entre essas áreas e as possíveis rotas de migração que as tartarugas marinhas utilizam, uma vez que é possível estimar a origem natal de indivíduos em um estoque misto a partir da análise de seu DNA mitocondrial devido às características filopátricas das fêmeas dessas espécies(e.g. BOWEN et al., 1995; NARO-MACIEL et al., 2014; SHAMBLIN et al., 2012).

2.2 Marcadores moleculares no estudo da estrutura populacional de tartarugas marinhas

A estrutura genética das tartarugas marinhas tem sido estudada desde o final da década de 1980 e começo da década de 1990 (BOWEN et al., 1992; BOWEN; MEYLANT; AVISE, 1989; BOWEN; NELSON; AVISE, 1993). Em tartarugas verdes, uma ampla amostragem das áreas de desova da espécie no mundo encontrou vários agrupamentos genéticos utilizando dados de polimorfismos de comprimento de fragmentos de restrição (RFLPs) e forneceu suporte molecular para a hipótese de filopatria em tartarugas marinhas (BOWEN et al., 1992). Porém, por conta da baixa resolução de dados de RFLPs, a estruturação genética não pode ser avaliada em uma escala mais fina. A partir daí estudos subsequentes começaram a empregar dados de sequências de nucleotídeos para estudar a genética de populações das tartarugas marinhas, primeiramente com o gene mitocondrial citocromo b (BOWEN; NELSON; AVISE, 1993), e posteriormente com a região controle do DNA mitocondrial (ALLARD et al., 1994; ENCALADA et al., 1996; LAHANAS et al., 1994).

A região controle do DNA mitocondrial (inicialmente um fragmento de ~400 pares de base) foi utilizada em diversos estudos e se tornou a principal fonte de comparação

para estudos subsequentes (BASS; LAGUEUX; BOWEN, 1998; BJORNDAL et al., 2006; LAHANAS et al., 1998; LUKE et al., 2004; NARO-MACIEL et al., 2007). Com o crescente número de estudos utilizando a região controle, o centro de pesquisa Archie Carr Center for Sea Turtle Research, na Universidade da Flórida, organizou uma lista para tartarugas verdes e tartarugas cabeçudas, as duas espécies mais estudadas, com uma nomenclatura padrão para todos os haplótipos conhecidos para esse fragmento do DNA mitocondrial a fim de evitar possíveis duplicações e confusões nas publicações (https://accstr.ufl.edu/resources/mtdna-sequences/). Novos primers (iniciadores) também foram desenvolvidos visando amplificar uma região maior da região controle (~800 pares de base, ABREU-GROBOIS et al., 2006). À medida que novos estudos passaram a utilizar esse fragmento mais longo, novas estruturações genéticas passaram a ser identificadas (e.g. JORDÃO et al., 2015; NARO-MACIEL et al., 2012; SHAMBLIN et al., 2015).

Outros marcadores moleculares têm ganhado espaço nos últimos anos. Por exemplo, repetições curtas em tandem (do inglês Short Tandem Repeats, STR) e polimorfismos de nucleotídeo único (do inglês Single Nucleotide Polymorphisms, SNP) têm conseguido detectar uma estrutura populacional não observada anteriormente (SHAMBLIN et al., 2015, 2017; TIKOCHINSKI et al., 2012). Da mesma maneira, microssatélites nucleares também em sido desenvolvidos (CARRERAS et al., 2007; FITZSIMMONS; MORITZ; MOORE, 1995; SHAMBLIN et al., 2009). No entanto, alguns estudos têm mostrado que, apesar de exibirem alta estruturação genética (BAGDA; BARDAKCI; TURKOZAN, 2012; NARO-MACIEL et al., 2014), eles têm revelado menos estruturação do que marcadores mitocondriais (e.g. FITZSIMMONS et al., 1997; NARO-MACIEL et al., 2014). Mais recentemente, técnicas de sequenciamento de nova geração, como ddRAD, também têm ajudado a elucidar a estruturação populacional nessas espécies (ARANTES et al., 2020). Ainda assim, a região controle do DNA mitocondrial continua sendo o marcador molecular mais utilizado em genética de populações de tartarugas marinhas.

2.3 Ameaças às tartarugas marinhas

Os desafios para as tartarugas marinhas começam cedo no seu ciclo de vida. Ainda no seu estágio embrionário, estão suscetíveis à predadores naturais que utilizam ovos de tartarugas marinhas como fonte de alimento; aves marinhas, crustáceos, pequenos mamíferos e algumas vezes até plantas fazem parte desse grupo (HEITHAUS, 2013). Seu pequeno tamanho corporal após a emergência dos ovos também as deixa vulneráveis durante todo o seu percurso até o oceano (SANTOS et al., 2016; STEWART; WYNEKEN, 2004). Consequentemente, apenas uma pequena parte dos indivíduos que emergem dos ovos consegue atingir a fase adulta (FRAZER, 1986).

Além dos desafios naturais, elas também têm que lidar com os desafios impostos pela presença humana. O avanço da urbanização em ambientes costeiros promove a diminuição da faixa de areia disponível para desovas das tartarugas marinhas e pisoteamento dos ninhos, além da poluição luminosa que desnorteia os filhotes recém emergidos (COLMAN et al., 2020; SALMON, 2003; TRUSCOTT; BOOTH; LIMPUS, 2017). A eclosão dos filhotes geralmente ocorre no período noturno, e um dos principais guias para os filhotes é a luz da lua que reflete sobre o oceano, que os direciona diretamente para as águas marinhas (SALMON, 2003). Porém, com a iluminação artificial presente nas áreas litorâneas, muitas vezes é possível encontrar filhotes de tartarugas marinhas migrando em direção a essas luzes, em direção oposta ao oceano (TRUSCOTT; BOOTH; LIMPUS, 2017).

Ao chegar ao oceano, as tartarugas marinhas passam a enfrentar uma gama diferente de desafios. Entre eles, a pesca incidental é frequentemente apontada como um dos principais fatores de mortalidade de tartarugas marinhas, e tanto a pesca industrial quanto a artesanal desempenham um papel nesse cenário (LEWISON et al., 2004). Porém, ainda é difícil traçar qual a real amplitude do impacto da pesca incidental nas populações de tartarugas marinhas por conta da dificuldade em realizar medições precisas da quantidade e da causa da mortalidade dos animais (LEWISON et al., 2004).

Devido a isso, embora o número de estudos na área tenha aumentado nas últimas décadas (LEWISON et al., 2014; WALLACE et al., 2013), esse impacto ainda é subestimado (para uma revisão sobre o assunto ver WALLACE et al., 2010b). Uma melhor compreensão sobre a distribuição, hábitos alimentares e rotas migratórias das populações de tartarugas marinhas é essencial para redução da mortalidade desses animais, uma vez que as taxas de pesca incidental geralmente são mais altas em áreas onde há sobreposição de atividades pesqueiras e alta concentração de tartarugas marinhas (WALLACE et al., 2010b)

Apesar da pesca incidental ser considerada como a principal ameaça às tartarugas marinhas (WALLACE et al., 2010b), outras ameaças emergentes começaram a surgir juntamente com o avanço da industrialização. Atualmente, os efeitos das mudanças climáticas, derivadas principalmente de ações antrópicas, têm sido amplamente debatidos e estudados levando ao desenvolvimento de estimativas de como essas mudanças irão afetar o planeta (IPCC, 2021). A constante emissão de gases causadores de um efeito estufa no planeta tem causado grande preocupação e é responsável por um iminente aumento da temperatura global (IPCC, 2021). O aumento da temperatura pode afetar as tartarugas marinhas de diversas maneiras, desde a mudança de hábitats ótimos para alimentação e migração devido a mudanças na temperatura nos oceanos, até alterações na razão sexual de determinadas populações (DAVENPORT, 1997). Todas as espécies de tartarugas marinhas têm o sexo determinado por temperatura, onde cada espécie apresenta uma temperatura pivotal, isto é, uma temperatura ótima onde a geração de fêmeas e machos tem probabilidade igual (DAVENPORT, 1997). Temperaturas de incubação acima dessa temperatura ótima geram mais fêmeas e temperaturas mais baixas, mais machos. Atualmente, várias populações já exibem uma razão sexual em favor de fêmeas (HAYS; MAZARIS; SCHOFIELD, 2014). Esse cenário tende a se intensificar ainda mais com o aumento da temperatura nas praias de nidificação (FUENTES; LIMPUS; HAMANN, 2011).

Apesar de ser o foco de vários estudos recentes, os efeitos desse processo de feminização na dinâmica populacional das espécies ainda não é totalmente claro

(MITCHELL; JANZEN, 2010). Além do aumento da temperatura nas praias, outros fatores abióticos e bióticos também devem ser levados em consideração e podem ajudar a mitigar os efeitos negativos dessa elevação da temperatura. Por exemplo, a presença de vegetação nas praias bem como a coloração da areia podem amenizar a temperatura de incubação dos ovos, afetando assim a razão sexual de determinadas áreas de desova (PATRÍCIO et al., 2019). Da mesma forma, a maior frequência de acasalamento dos machos de tartarugas marinhas, que podem acasalar com várias fêmeas em uma mesma temporada reprodutiva e em temporadas sucessivas, pode compensar a maior abundância de fêmeas na população (HAYS et al., 2017; HAYS; MAZARIS; SCHOFIELD, 2014). Ainda assim, o aumento exacerbado da temperatura em determinadas regiões pode causar um aumento na mortalidade dos filhotes daquelas áreas de desova (DAVENPORT, 1997; HOWARD; BELL; PIKE, 2014). Dessa maneira, áreas de desova que produzem machos são cada vez mais importantes para dinâmica populacional da espécie, uma vez que a tendência atual aponta para um aumento na produção de fêmeas e na mortalidade de filhotes nas áreas de desova.

Outra consequência das mudanças climáticas é o aumento do nível do mar, que juntamente com o avanço da urbanização nas zonas costeiras, ameaça ecossistemas litorâneos nas próximas décadas (IPCC, 2021). Uma consequência direta desse processo para tartarugas marinhas é a inundação cada vez mais frequente de ninhos, além da diminuição da área disponível para nidificação (FUENTES et al., 2010). Isso pode gerar uma maior concentração de fêmeas nas áreas de praia remanescentes, o que pode aumentar as chances de pisoteamento dos ninhos bem como a destruição de ninhos por outras fêmeas durante as temporadas reprodutivas (FUENTES et al., 2010). A diminuição das áreas disponíveis para nidificação também pode gerar maior sobreposição das atividades de reprodução e nidificação entre espécies distintas, o que pode aumentar as chances de eventos de hibridização, que já são comuns em áreas com alta sobreposição em atividades reprodutivas no Brasil (LARA-RUIZ et al., 2006; REIS; SOARES; LÔBO-HAJDU, 2010).

Ainda é incerto, no entanto, como as tartarugas marinhas irão se adaptar a essas alterações ambientais. Mudanças na disponibilidade de hábitats para nidificação irão possivelmente demandar uma adaptação comportamental em um período de tempo relativamente curto (HAWKES et al., 2009). Os efeitos da mudança de temperatura de superfície dos oceanos também podem alterar suas áreas de alimentação devido a ocupação preferencial do hábitat de acordo com a temperatura (WITT et al., 2010). Além disso, a mudança nas temperaturas dos oceanos também pode afetar outras espécies animais e vegetais que fazem parte da sua cadeia alimentar, afetando, por consequência, sua distribuição devido a mudanças na disponibilidade de alimento (HAWKES et al., 2009).

Concomitantemente com as mudanças climáticas, outras ameaças de origem antrópica se fazem cada vez mais presentes com o avanço da urbanização e ocupação dos ambientes costeiros. Duas das principais ameaças decorrentes desses fatores incluem a poluição por plástico e a fibropapilomatose, ambas relacionadas à poluição dos ambientes marinhos (AGUIRRE; LUTZ, 2004; NELMS et al., 2016). A poluição por plástico já afeta milhares de espécies no mundo nos mais variados grupos animais (SANTOS; MACHOVSKY-CAPUSKA; ANDRADES, 2021). O grande aporte de plásticos nos oceanos gera uma grande disponibilidade desse material no ambiente (GEYER; JAMBECK; LAW, 2017), o que se traduz em uma maior probabilidade de interação das tartarugas com itens plásticos. Casos de emaranhamento e ingestão são comuns e representam um grande problema, uma vez que podem interferir no desenvolvimento adequado dos indivíduos (RIZZI et al., 2019; RODRÍGUEZ et al., 2022). Por exemplo, itens plásticos podem apresentar características físico-químicas que se assemelham àquelas de presas das tartarugas, o que as faz consumir esses materiais já que há uma grande abundância deles no ambiente e os mesmos não apresentam resistência (SANTOS; MACHOVSKY-CAPUSKA; ANDRADES, 2021). Esse comportamento pode ser nocivo para saúde das tartarugas uma vez que os itens plásticos não apresentam nenhum valor nutricional e podem gerar complicações como obstrução do trato gastrointestinal, desnutrição e eventualmente a morte (NELMS et al., 2016).

A fibropapilomatose por sua vez, se trata de uma doença viral caracterizada pela presença de tumores externos e/ou internos. A presença e localização desses tumores pode variar de indivíduo para indivíduo e pode ter associação com fatores ecológicos e genéticos (AGUIRRE; LUTZ, 2004; ROSSI et al., 2016). Dependendo da localização desses tumores no corpo dos indivíduos, eles podem interferir na movimentação, visão, alimentação, ou mesmo no funcionamento de órgãos internos, o que pode afetar a habilidade dos indivíduos de interagir com o ambiente (ROSSI et al., 2016). Embora, as causas da fibropapilomatose não sejam totalmente claras, estudos recentes têm ajudado a elucidar os mecanismos de infecção e transmissão da doença (e.g. FARREL et al., 2021; YETSKO et al., 2021), que pode também estar associada à poluição de ambientes marinhos (JONES et al., 2016; SANTOS et al., 2010).

Apesar da grande amplitude das ameaças às tartarugas marinhas, o desenvolvimento de medidas de conservação adequadas é dificultado pelo seu complexo ciclo de vida e recorrentes migrações entre diferentes hábitats, que podem muitas vezes estar separados por milhares de quilômetros (LUSCHI et al., 2003). Zonas de monitoramento mais restritas chamadas Unidades Regionais de Manejo (do inglês Regional Management Units, RMUs) foram então sugeridas a fim de prover uma melhor avaliação do status populacional bem como das ameaças a um nível regional, o que poderia ajudar a melhorar a efetividade das ações de manejo (WALLACE et al., 2010a). Entre as RMUs no Oceano Atlântico, a RMU do Oceano Atlântico Sudoeste (OAS) foi considerada de alto risco para tartarugas marinhas (WALLACE et al., 2011), o que a destaca como uma área prioritária para conservação nessa região.

2.4 Tartarugas marinhas no Oceano Atlântico Sudoeste

Atualmente, cinco espécies de tartarugas marinhas podem ser encontradas no OAS: tartaruga cabeçuda, tartaruga verde, tartaruga de couro, tartaruga de pente e tartaruga oliva. Áreas de desova dessas espécies na região se espalham pelo litoral continental e ilhas oceânicas (MARCOVALDI; MARCOVALDI, 1999). A tartaruga verde possui áreas de desova principalmente nas ilhas oceânicas de Trindade, Fernando de

Noronha e Atol das Rocas, porém as desovas também podem acontecer no litoral continental em menor proporção (ALMEIDA et al., 2011; BJORNDAL et al., 2006). Áreas de desova das outras espécies estão localizadas principalmente no continente. A tartaruga cabeçuda desova entre os estados do Rio de Janeiro e Espírito Santo, no sudeste do Brasil, e entre Bahia e Sergipe no nordeste do Brasil, tendo o litoral norte da Bahia como sua principal área de desova (MARCOVALDI; CHALOUPKA, 2007; REIS; SOARES; LÔBO-HAJDU, 2010). As principais áreas de desova da tartaruga de pente são no litoral norte da Bahia e no litoral sul do Rio Grande do Norte (MARCOVALDI et al., 2007), enquanto áreas de desova da tartaruga oliva se concentram principalmente entre o litoral norte da Bahia e sul de Alagoas (SILVA et al., 2007). Por fim, áreas de desova da tartaruga de couro podem ser encontradas principalmente no litoral norte do estado do Espírito Santo (COLMAN et al., 2019; THOMÉ et al., 2007).

Áreas de desova de tartarugas verdes apresentam uma clara estruturação entre as regiões norte e sul do oceano Atlântico (ENCALADA et al., 1996; NARO-MACIEL et al., 2014), tendo uma composição mista na região da América Central (NARO-MACIEL et al., 2014). No OAS, as áreas de desova também apresentam certo grau de estruturação, embora o haplótipo CM-A8 (considerando o fragmento curto da região controle do DNA mitocondrial), mais comum na região, esteja presente em maior frequência em todas as áreas (BJORNDAL et al., 2006). Considerando o fragmento longo da região controle, o haplótipo CM-A8 pode ser subdividido em cinco haplótipos (subvariantes CM-A8.1–CM-A8.5) distintos. As tartarugas cabeçudas das áreas de desova no litoral norte da Bahia, Sergipe, Rio de Janeiro e Espírito Santo; apresentaram até o momento apenas três haplótipos distintos (CC-A4, CC-A24 e CC-A25), dos quais o haplótipo mais frequente é o CC-A4, representando mais de 86% dos indivíduos analisados até o momento (REIS et al., 2010). Este haplótipo, pode ser subdividido em outros quatro considerando o fragmento longo da região controle (subvariantes CC-A4.1–CC-A4.4). Da mesma forma, apenas três haplótipos (Dc1.1, Dc3.1 e Dc13.1) são encontrados em áreas de desova da tartaruga de couro na região, mesmo considerando o fragmento longo (VARGAS et al., 2019). Três haplótipos já foram

registrados nas áreas de desova da tartaruga oliva, Lo67, LoX3 e LoX4 (BOWEN et al., 1998; Vilaça et al., 2022). Nas áreas de desova da tartaruga de pente, quatro haplótipos podem ser encontrados (Ei-A01, Ei-A32, Ei-A61 e Ei-A62), sendo o haplótipo Ei-A01 o mais frequente (ARANTES; VARGAS; SANTOS, 2020).

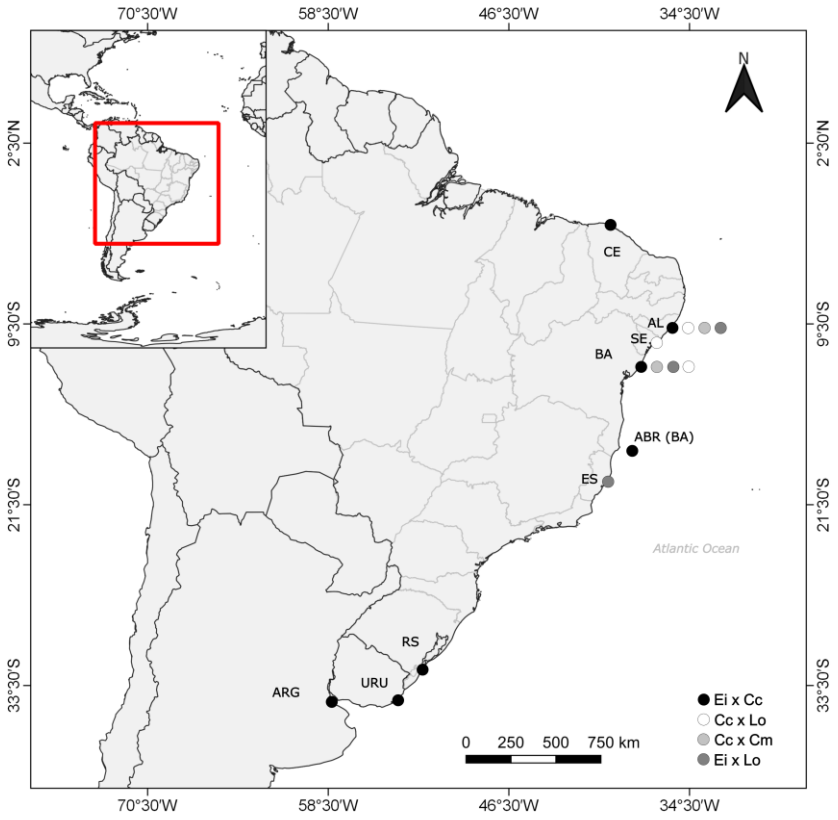
Áreas de alimentação dessas espécies no OAS ocorrem ao longo de todo o litoral da América do Sul e recebem indivíduos de diversas áreas de desova de todo o Atlântico (e.g. NARO-MACIEL et al., 2012; PROIETTI et al., 2014a; PROSDOCIMI et al., 2012). Áreas de alimentação de tartarugas verdes recebem indivíduos principalmente da Ilha de Ascensão, uma das maiores áreas de desova do Atlântico Sul, e também de áreas de desova no caribe (NARO-MACIEL et al., 2012; PROIETTI et al., 2012). A Ilha de Trindade abriga a maior área de desova da espécie no OAS, porém o número de ninhos por temporada chega a ser até quatro vezes menor do que em Ascensão (ALMEIDA et al., 2011; MEDEIROS et al., 2022). Como consequência, sua influência na composição de indivíduos nas áreas de alimentação da região é mais perceptível apenas em regiões geograficamente mais próximas, no sul do Brasil (PROIETTI et al., 2012). Contribuições da área de desova de Guiné Bissau também são notáveis (JORDÃO et al., 2015; NARO-MACIEL et al., 2012), embora em menor proporção (mas veja capítulo 1). Contudo, áreas de desova no litoral Africano do Oceano Atlântico ainda são pouco conhecidas, portanto a contribuição dessas áreas para a composição de indivíduos nas áreas de alimentação do OAS pode vir a mudar a medida em que essas áreas sejam mais bem estudadas.

A diversidade genética de tartarugas cabeçudas em suas áreas de alimentação indica que a maior parte dos indivíduos têm origem em áreas de desova locais, o que é indicado principalmente pela alta frequência do haplótipo CC-A4, que é exclusivo de áreas de desova Brasileiras (REIS et al., 2010). Devidos a seus hábitos oceânicos, estudos em áreas de alimentação das tartarugas de couro e oliva são escassos. As áreas de alimentação da tartaruga de pente no OAS em geral recebem indivíduos principalmente de áreas de desova locais (Bahia e Rio Grande do Norte), embora

haplótipos encontrados em áreas de desova Africanas também já tenham sido observados (PROIETTI et al., 2014a).

O avanço de estudos sobre a diversidade genética das espécies de tartarugas marinhas no OAS também revelou uma alta taxa de hibridização na região (e.g. LARA-RUIZ et al., 2006; REIS; SOARES; LÔBO-HAJDU, 2010), ao contrário do que se encontra em outras regiões do mundo (BRITO et al., 2020). No OAS é possível observar uma alta taxa de hibridização entre a tartaruga cabeçuda e a tartaruga de pente e entre tartaruga cabeçuda e tartaruga oliva (LARA-RUIZ et al., 2006; REIS et al., 2010). Híbridos entre a tartaruga cabeçuda e a tartaruga de pente podem ser encontrados principalmente no litoral norte do estado da Bahia, nordeste do Brasil, região que representa a principal área de desova para as duas espécies e onde as taxas de hibridização chegam até 42% da população já analisada (LARA-RUIZ et al., 2006; SOARES et al., 2018). Onde, a maior parte dos híbridos possui características morfológicas da tartaruga de pente e DNA mitocondrial da tartaruga cabeçuda (LARA-RUIZ et al., 2006). Já híbridos de tartaruga cabeçuda e tartaruga oliva são mais frequentes no litoral do estado de Sergipe, também no nordeste Brasileiro, onde a taxa de hibridização registrada entre as duas espécies chega a 27% da população analisada, onde os indivíduos apresentaram morfologia de tartaruga cabeçuda e DNA mitocondrial de tartaruga oliva (REIS; SOARES; LÔBO-HAJDU, 2010). Essas duas regiões apresentam ampla sobreposição espacial e temporal entre atividades reprodutivas dessas espécies, o que propicia uma maior interação entre elas, favorecendo a hibridização (LARA-RUIZ et al., 2006).

Figura 2 – Áreas de desova e alimentação no Oceano Atlântico Sudoeste com registros de indivíduos híbridos. Cc – *Caretta caretta*, Cm – *Chelonia mydas*, Ei – *Eretmochelys imbricata*, Lo – *Lepidochelys olivacea*. ABR – Arquipélago de Abrolhos, ARG – Argentina, BA – Bahia, CE – Ceará, ES – Espírito Santo, RS – Rio Grande do Sul, SE – Sergipe, URU – Uruguai.



Fonte: Elaborado pelo autor (2023).

Como consequência da alta taxa de hibridização nessas áreas de desova, vários híbridos também já foram registrados em áreas de alimentação dessas espécies, desde o litoral do estado do Ceará até a Argentina (BRITO et al., 2020). Consequências dessas altas taxas de hibridização no OAS ainda não são totalmente claras, porém o perfil genético dos híbridos indica que eles se mantêm férteis e podem se reproduzir também com as espécies parentais (PROIETTI et al., 2014b; VILAÇA et al., 2021). A taxa de emergência de filhotes é levemente menor entre híbridos, mas não difere significativamente das espécies parentais (SOARES et al., 2017). Da mesma maneira, a

viabilidade de híbridos e indivíduos puros parece não diferir significativamente (SOARES et al., 2018). Por outro lado, alguns híbridos morfologicamente identificados como uma espécie parental parecem adotar padrões migratórios da outra espécie (PROIETTI et al., 2014b).

Com o avanço da urbanização em áreas litorâneas e os efeitos das mudanças climáticas na distribuição das tartarugas marinhas (FUENTES et al., 2010), o monitoramento constante das áreas de nidificação e alimentação é fundamental para entender como essas espécies estão sendo afetadas por esses fatores. De fato, no Brasil a identificação e caracterização dessas áreas quanto a sua distribuição espacial e diversidade genética são diretrizes do plano de ação para a conservação das tartarugas marinhas (ICMBIO, 2017). Identificar e caracterizar a diversidade genética dessas áreas é essencial para entender as conexões entre elas, padrões de migração e a dinâmica populacional dessas espécies. Isso é de particular importância no litoral do OAS, especialmente no Brasil, tendo em vista a alta frequência de hibridização na região (BRITO et al., 2020). Assim, estudos nessas temáticas são importantes para elaboração de estratégias de conservação adequadas, especialmente em espécies com hábitos altamente migratórios, como é o caso das tartarugas marinhas, que podem estar sob uma ampla gama de ameaças (HAWKES et al., 2009; NELMS et al., 2016; WALLACE et al., 2013).

REFERÊNCIAS

ABREU-GROBOIS, F. A.; HORROCKS, J.; FORMIA, A.; LEROUX, R.; VELEZ-ZUAZO, X.; DUTTON, P.; SOARES, L.; MEYLAN, P.; BROWNE, D. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Annual Symposium on Sea Turtle Biology and Conservation, 26., 2006, Island of Crete. Book of abstracts of the 26th Annual Symposium on Sea Turtle Biology and Conservation. Athens: International Sea Turtle Society, 2006; p. 179.

AGUIRRE, A. A.; LUTZ, PETERL. Marine Turtles as Sentinels of Ecosystem Health: Is Fibropapillomatosis an Indicator? EcoHealth, v. 1, n. 3, p. 275–283, set. 2004.

ALLARD, M. W. et al. Support for Natal Homing in Green Turtles from Mitochondrial DNA Sequences. Copeia, v. 1994, n. 1, p. 34–41, 1994.

ALMEIDA, A. DE P. et al. Green turtle nesting on trindade island, Brazil: Abundance, trends, and biometrics. Endangered Species Research, v. 14, n. 3, p. 193–201, 2011.

ARANTES, L. S. et al. New genetic insights about hybridization and population structure of hawksbill and loggerhead turtles from Brazil. Journal of Heredity, v. 111, n. 5, p. 444–456, 2020.

ARANTES, L. S.; VARGAS, S. M.; SANTOS, F. R. Global phylogeography of the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Genetics and Molecular Biology, v. 43, n. 2, p. 1–12, 2020.

ARTHUR, K. E.; BOYLE, M. C.; LIMPUS, C. J. Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. Marine Ecology Progress Series, v. 362, p. 303–311, 30 jun. 2008.

BAGDA, E.; BARDAKCI, F.; TURKOZAN, O. Lower genetic structuring in mitochondrial DNA than nuclear DNA among the nesting colonies of green turtles (*Chelonia mydas*) in the Mediterranean. Biochemical Systematics and Ecology, v. 43, p. 192–199, ago. 2012.

BASS, A. L.; LAGUEUX, C. J.; BOWEN, B. W. Origin of Green Turtles, *Chelonia mydas*, at “Sleeping Rocks” off the Northeast Coast of NicaraguaSource: Copeia. [s.l: s.n.]. Disponível em: <http://www.jstor.orgURL:http://www.jstor.org/stable/1447360Accessed:28/08/200811:08>.

BJORNDAL, K. A. et al. Population structure and diversity of brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology, v. 5, n. 2, p. 262–268, dez. 2006.

BOLTEN, A. B. Variation in sea turtle life history patterns: neritic vs oceanic developmental stages. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.), The Biology of Sea Turtles Volume II. CRC Press, Boca Raton, 2003, p. 243–257.

BOWEN, B. W. et al. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution, v. 46, n. 4, p. 865–881, 1992.

BOWEN, B. W. et al. Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. Population Biology, v. 92, p. 3731–3734, 1995.

BOWEN, B. W. et al. Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. Genetica, v. 101, p. 179–189, 1998.

BOWEN, B. W.; MEYLANT, A. B.; AVISE, J. C. An odyssey of the green sea turtle: Ascension Island revisited (mitochondrial DNA/intraspecific phylogeny/gene flow/genetic distance). Proceedings of the Natiotal Academy of Sciences, v. 86, p. 573–576, 1989.

BOWEN, B. W.; NELSON, W. S.; AVISE, J. C. A molecular phylogeny for marine turtles: Trait mapping, rate assessment, and conservation relevance. Proceedings of the National Academy of Sciences, v. 90, p. 5574–5577, 1993.

BRITO, C. et al. Combined use of mitochondrial and nuclear genetic markers further reveal immature marine turtle hybrids along the south western Atlantic. Genetics and Molecular Biology, v. 43, n. 2, p. 1–11, 2020.

CARR, A. F. So Excellent a Fishe: A Natural History of Sea Turtles. New York: Scribner, 1967.

CARRERAS, C. et al. The genetic structure of the loggerhead sea turtle (*Caretta caretta*) in the Mediterranean as revealed by nuclear and mitochondrial DNA and its conservation implications. Conservation Genetics, v. 8, n. 4, p. 761–775, 2007.

COLMAN L. P. et al. Thirty years of leatherback turtle Dermochelys coriacea nesting in Espírito Santo, Brazil, 1988−2017: reproductive biology and conservation. Endangered Species Research, v. 39, p. 147–158, 2019.

COLMAN, L. P. et al. Assessing coastal artificial light and potential exposure of wildlife at a national scale: the case of marine turtles in Brazil. Biodiversity and Conservation, v. 29, p. 1135–1152, 2020.

DAVENPORT, J. Temperature and the life-history strategies of sea turtles. Journal of Thermal Biology, v. 22, n. 6, p. 479–488, 1997.

ENCALADA, S. E. et al. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. Molecular Ecology, v. 5, n. 4, p. 473–483, 1996.

FARREL, J. A. et al. Environmental DNA monitoring of oncogenic viral shedding and genomic profiling of sea turtle fibropapillomatosis reveals unusual viral dynamics. Communications Biology, v. 4, p. 565, 2021.

FITZSIMMONS, N. N. et al. Geographic Structure of Mitochondrial and Nuclear Gene Polymorphisms in Australian Green Turtle Populations and Male-Biased Gene Flow. Genetics, v. 147, p. 1843–1854, 1997.

FITZSIMMONS, N. N.; MORITZ, C.; MOORE, S. S. Conservation and Dynamics of Microsatellite Loci over 300 Million Years of Marine Turtle Evolution. Molecular Biology and Evolution, v. 12, n. 3, p. 432–440, 1995.

FRAZER, N. B. Survival from egg to adulthood in a declining population of loggerhead turtles, *Caretta caretta*. Herpetologica, v. 42, n. 1, p. 47–55, 1986.

FUENTES, M. M. P. B. et al. Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic Conservation: Marine and Freshwater Ecosystems, v. 20, n. 2, p. 132–139, 2010.

FUENTES, M. M. P. B.; LIMPUS, C. J.; HAMANN, M. Vulnerability of sea turtle nesting grounds to climate change. Global Change Biology, v. 17, p. 140–153, 2011.

GEYER, R.; JAMBECK, J. R.; LAW, K. L. Production, use, and fate of all plastics ever made. Science Advances, v. 3, p. e1700782, 2017.

HAMMAN, M.; LIMPUS, C. J.; OWENS, D. W. Reproductive cycles of males and females. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.), The Biology of Sea Turtles Volume II. CRC Press, Boca Raton, 2003, p. 135–161.

HAWKES, L. A. et al. Climate change and marine turtles. Endangered Species Research, v. 7, p. 137–154, 2009.

HAYS, G. C. et al. Population viability at extreme sex-ratio skews produced by temperature dependent sex determination. Proceedings of the Royal Society B: Biological Sciences, v. 284, n. 1848, p. 1–7, 2017.

HAYS, G. C.; MAZARIS, A. D.; SCHOFIELD, G. Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Frontiers in Marine Science, v. 1, p. 1–9, 2014.

HOWARD, R.; BELL, I.; PIKE, D. A. Thermal tolerances of sea turtle embryos: Current understanding and future directions. Endangered Species Research, v. 26, p. 75–86, 2014.

ICMBio (2017) Plano de Ação Nacional para Conservação das Tartarugas Marinhas. https://www.icmbio.gov.br/portal/faunabrasileira/plano-de-acao-nacional-lista/841-plano-de-acao-nacional-para-a-conservacao-das-tartarugas-marinhas.

Intergovernmental Panel on Climate Change (2021). Climate Change 2021: Synthesis Report. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team]. Ed. by Jager-Waldau A and Sapkota T. IPCC, Geneva, Switzerland.

JONES, K. et al. A review of fibropapillomatosis in Green turtles (*Chelonia mydas*). The Veterinary Journal, v. 212, p. 48–57, 2016.

JORDÃO, J. C. et al. Green turtle (*Chelonia mydas*) genetic diversity at Paranaguá Estuarine complex feeding grounds in Brazil. Genetics and Molecular Biology, v. 38, n. 3, p. 346–352, 2015.

LAHANAS, P. N. et al. Molecular evolution and population genetics of Greater Caribbean green turtles (*Chelonia mydas*) as inferred from mitochondrial DNA control region sequences. Genetica, v. 94, p. 57, 1994.

LAHANAS, P. N. et al. Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. Marine Biology, v. 130, p. 345–352, 1998.

LARA-RUIZ, P. et al. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conservation Genetics, v. 7, n. 5, p. 773–781, 2006.

LEWISON, R. L. et al. Understanding impacts of fisheries bycatch on marine megafauna. Trends in Ecology and Evolution, v. 19, n. 11, p. 598–604, 2004.

LEWISON, R. L. et al. Global patterns of marine mammal, seabird, and sea turtle bycatch reveal taxa-specific and cumulative megafauna hotspots. Proceedings of the National Academy of Sciences of the United States of America, v. 111, n. 14, p. 5271–5276, 2014.

LIMPUS, C. J. A Biological Review of Australian Marine Turtles. 5. Flatback turtle, Natator depressus (Garman). Report for the Environmental Protection Agency Queensland. p. 1–54, 2007.

LOHMANN, K. J.; LOHMANN, C. M. F.; BROTHERS, J. R.; PUTMAN, N. F., 2013. Natal homing and imprinting in sea turtles. In: WYNEKEN, J.; LOHMANN, K. J.; MUSIK, J. A. The Biology of Sea Turtles, Volume III. Florida: CRC Press, 2013, p. 59–77.

LUKE, K. et al. Origins of green turtle (*Chelonia mydas*) feeding aggregations around Barbados, West Indies. Marine Biology, v. 144, n. 4, p. 799–805, 2004.

LUSCHI, P. et al. A review of long-distance movements by marine turtles, and the possible role of ocean currents. OIKOS, v. 103, p. 293–302, 2003.

MANSFIELD, K. L. et al. First satellite tracks of neonate sea turtles redefine the “lost years” oceanic niche. Proceedings of the Royal Society B: Biological Sciences, v. 281, n. 1781, 2014.

MARCOVALDI, M. A. et al. Fifteen years of Hawksbill Sea Turtle (*Eretmochelys imbricata*) nesting in Northern Brazil. Chelonian Conservation and Biology, v. 6, n. 2, p. 223–228, 2007.

MARCOVALDI, M. Â.; MARCOVALDI, G. G. Marine turtles of Brazil: The history and structure of Projeto TAMAR-IBAMA. Biological Conservation, v. 91, n. 1, p. 35–41, 1999.

MARCOVALDI, M.; CHALOUPKA, M. Conservation status of the loggerhead sea turtle in Brazil: an encouraging outlook. Endangered Species Research, v. 3, p. 133–143, 2007.

MÁRQUEZ, R. M. Status and Distribution of the Kemp's Ridley Turtle, Lepidochelys kempii, in the Wider Caribbean Region. In: ECKERT, K.L. AND ABREU GROBOIS, F.A. (Eds.). Proceedings, Marine Turtle Conservation in the Wider Caribbean Region: A Dialogue for Effective Regional Management. Santo Domingo: WIDECAST, IUCN-MTSG, WWF, and UNEP-CEP, 2001, p. 46–51.

MEDEIROS, L. et al. Tracking green turtle nesting trends at a remote oceanic rookery. Marine Biology, n. 69, p. 68, 2022.

MITCHELL, N. J.; JANZEN, F. J. Temperature-Dependent sex determination and contemporary climate change. Sexual Development, v. 4, n. 1–2, p. 129–140, 2010.

MORTIMER, J. A.; CARR, A. Reproduction and migration of the Ascension Island green turtle (*Chelonia mydas*). Copeia, v. 1, p. 103-113, 1987.

NARO-MACIEL, E. et al. Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. Journal of Heredity, v. 98, n. 1, p. 29–39, 2007.

NARO-MACIEL, E. et al. The interplay of homing and dispersal in green turtles: A focus on the southwestern atlantic. Journal of Heredity, v. 103, n. 6, p. 792–805, 2012.

NARO-MACIEL, E. et al. From refugia to rookeries: Phylogeography of Atlantic green turtles. Journal of Experimental Marine Biology and Ecology, v. 461, p. 306–316, 2014.

NELMS, S. E. et al. Plastic and marine turtles: A review and call for research. ICES Journal of Marine Science, v. 73, n. 2, p. 165–181, 2016.

PATRÍCIO, A. R., VARELA, M. R., BARBOSA, C., BRODERICK, A. C., CATRY, P., HAWKES, L. A., REGALLA, A., et al. 2019. Climate change resilience of a globally important sea turtle nesting population. Global Change Biology, v. 25, p. 522–535.

PLOTKIN, P. Adult Migrations and Habitat Use. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.). The Biology of Sea Turtles, Volume II. Florida: CRC Press, 2003, p. 225–241.

PROIETTI, M. C. et al. Green turtle *Chelonia mydas* mixed stocks in the western South Atlantic, as revealed by mtDNA haplotypes and drifter trajectories. Marine Ecology Progress Series, v. 447, n. 1, p. 195–209, 2012.

PROIETTI, M. C. et al. Genetic structure and natal origins of immature hawksbill turtles (*Eretmochelys imbricata*) in Brazilian waters. PLoS ONE, v. 9, n. 2, 2014a.

PROIETTI, M. C. et al. Hawksbill × loggerhead sea turtle hybrids at Bahia, Brazil: Where do their offspring go? PeerJ, v. 2014, n. 1, p. 1–14, 2014b.

PROSDOCIMI, L. et al. Genetic composition of green turtle feeding grounds in coastal waters of Argentina based on mitochondrial DNA. Journal of Experimental Marine Biology and Ecology, v. 412, p. 37–45, 2012.

REICH, K. J.; BJORNDAL, K. A.; BOLTEN, A. B. The “lost years” of green turtles: Using stable isotopes to study cryptic lifestages. Biology Letters, v. 3, n. 6, p. 712–714, 2007.

REIS, E. C. et al. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: Colonization hypothesis for the Brazilian rookeries. Conservation Genetics, v. 11, n. 4, p. 1467–1477, 2010.

REIS, E. C.; SOARES, L. S.; LÔBO-HAJDU, G. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conservation Genetics, v. 11, n. 4, p. 1587–1591, 2010.

RIZZI, M. et al. Ingestion of plastic marine litter by sea turtles in southern Brazil: abundance, characteristics and potential selectivity. Marine Pollution Bulletin, v. 140, p. 536–548, 2019.

RODRÍGUEZ, Y. et al. Litter ingestion and entanglement in green turtles: An analysis of two decades of stranding events in the NE Atlantic. Environmental Pollution, v. 298, 2022.

ROSSI, S. et al. Challenges in evaluating the severity of fibropapillomatosis: A proposal for objective index and score system for green sea turtles (*Chelonia mydas*) in Brazil. PLoS ONE, v. 11, n. 12, 2016.

SALMON, M. Artificial night lighting and sea turtles. Biologist, v. 50, n. 4, p. 163–168, 2003.

SANTOS, R. G. et al. Relationship between fibropapillomatosis and environmental quality: A case study with *Chelonia mydas* off Brazil. Diseases of Aquatic Organisms, v. 89, n. 1, p. 87–95, 2010.

SANTOS, R. G. et al. The anti-predator role of within-nest emergence synchrony in sea turtle hatchlings. Proceedings of the Royal Society B: Biological Sciences, v. 283, n. 1834, 2016.

SANTOS, R. G.; MACHOVSKY-CAPUSKA, G. E.; ANDRADES, R. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. Science, v. 373, p. 56–60, 2021.

SHAMBLIN, B. M. et al. Tetranucleotide markers from the loggerhead sea turtle (*Caretta caretta*) and their cross-amplification in other marine turtle species. Conservation Genetics, v. 10, n. 3, p. 577–580, 2009.

SHAMBLIN, B. M. et al. Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Molecular Ecology, v. 21, n. 10, p. 2330–2340, 2012.

SHAMBLIN, B. M. et al. Deeper Mitochondrial Sequencing Reveals Cryptic Diversity and Structure in Brazilian Green Turtle Rookeries. Chelonian Conservation and Biology, v. 14, n. 2, p. 167–172, 2015.

SHAMBLIN, B. M. et al. Mexican origins for the Texas green turtle foraging aggregation: A cautionary tale of incomplete baselines and poor marker resolution. Journal of Experimental Marine Biology and Ecology, v. 488, p. 111–120, 2017.

SILVA, A. C. C. D. et al. Nesting biology and conservation of the olive ridley sea turtle (*Lepidochelys olivacea*) in Brazil, 1991/1992 to 2002/2003. Journal of the Marine Biological Association of the United Kingdom, v. 87, n. 4, p. 1047–1056, 2007.

SOARES, L. S. et al. Comparison of reproductive output of hybrid sea turtles and parental species. Marine Biology, v. 164, n. 1, 2017.

SOARES, L. S. et al. Effects of hybridization on sea turtle fitness. Conservation Genetics, v. 19, n. 6, p. 1311–1322, 2018.

STEWART, K. R.; WYNEKEN, J. PREDATION RISK TO LOGGERHEAD HATCHLINGS AT A HIGH-DENSITY NESTING BEACH IN SOUTHEAST FLORIDA. Bulletin of Marine Science, v. 74, n. 2, p. 325–335, 2004.

THOMÉ, J. C. A. et al. Nesting biology and conservation of the leatherback sea turtle (*Dermochelys coriacea*) in the State of Espírito Santo, Brazil, 1988-1989 to 2003-2004. Chelonian Conservation and Biology, v. 6, n. 1, p. 15–27, 2007.

TIKOCHINSKI, Y. et al. Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: The Mediterranean Sea case study. Marine Genomics, v. 6, p. 17–24, 2012.

TRUSCOTT, Z.; BOOTH, D. T.; LIMPUS, C. J. The effect of on-shore light pollution on sea-turtle hatchlings commencing their off-shore swim. Wildlife Research, v. 44, n. 2, p. 127–134, 2017.

VARGAS, S. M. et al. Revisiting the genetic diversity and population structure of the critically endangered leatherback turtles in the South-west Atlantic Ocean: Insights for species conservation. Journal of the Marine Biological Association of the United Kingdom, v. 99, n. 1, p. 31–41, 2019.

VILAÇA, S. T. et al. Divergence and hybridization in sea turtles: Inferences from genome data show evidence of ancient gene flow between species. Molecular Ecology, n. 30, p. 6178–6192, 2021.

VILAÇA, S. T. et al. Global phylogeography of ridley sea turtles (*Lepidochelys* spp.): evolution, demography, connectivity, and conservation. Conservation Genetics, n. 23, p. 995–1010, 2022.

WALLACE, B. P. et al. Regional Management Units for Marine Turtles: A Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS ONE, v. 5, n. 12, p. 1–11, 2010a.

WALLACE, B. P. et al. Global patterns of marine turtle bycatch. Conservation Letters, n. 3, p. 131–142, 2010b.

WALLACE, B. P. et al. Global conservation priorities for Marine turtles. PLoS ONE, v. 6, n. 9, 2011.

WALLACE, B. P. et al. Impacts of fisheries bycatch on marine turtle populations worldwide: Toward conservation and research priorities. Ecosphere, v. 4, n. 3, p. 1–49, 2013.

WITT, M. J. et al. Predicting the impacts of climate change on a globally distributed species: The case of the loggerhead turtle. Journal of Experimental Biology, v. 213, n. 6, p. 901–911, 2010.

YETSKO, K. et al. Molecular characterization of a marine turtle tumor epizootic, profiling external, internal and postsurgical regrowth tumors, v. 4, p. 152, 2021.

3 CAPÍTULO 1

SEX RATIOS AND NATAL ORIGINS OF GREEN TURTLES FROM FEEDING GROUNDS IN THE SOUTHWEST ATLANTIC OCEAN[1]

João Paulo Felix Augusto de Almeida, Robson Guimarães dos Santos, Tamí Mott

Abstract

Potential effects of climate change on living species are a widely debated topic. Species with temperature-dependent sex determination can be particularly impacted by warmer temperatures because unbalanced sex ratios could threaten population viability. In sea turtles, sex ratio estimates have highlighted the potential feminization of current populations, which tends to increase since warmer temperatures would generate more females. Here, we evaluated temporal variation in sex ratios of green turtles from feeding grounds of the Southwest Atlantic Ocean (SWA) using data from a seven-year time frame, from 2010 to 2016. We also evaluated natal origins of female and male green turtles from SWA based on mitochondrial DNA. Sex ratios of juvenile and adult green turtles were generally female-skewed across collection years. We identified 11 haplotypes in northeast SWA, and haplotype composition of females and males was slightly different. Likewise, estimated natal origins of females and males were divergent. Ascension Island was estimated to be the main source of females while Guinea Bissau was estimated to be the main source of males. Studies evaluating natal origins of females and males independently are rare, this study provides one of the first assessments of the kind for green turtles in the SWA.

Keywords: Brazil, Chelonia mydas, population genetics, sea turtles, warming temperatures

1. Artigo publicado no periódico ICES Journal of Marine Science, qualis A1, percentil 89% pelo Scopus. Almeida, J.P.F.A.A.; Santos, R.G.; Mott, T (2021). Sex ratios and natal origins of green turtles from feeding grounds in the Southwest Atlantic Ocean. ICES Journal of Marine Science, 78(5), 1840–1848. doi: 10.1093/icesjms/fsab093.

3.1 Introduction

Marine ecosystems have been historically transformed by human activities; no area in the world is unaffected and many are strongly impacted by multiple threats (Halpern et al., 2008). As a result, marine wildlife populations have been deeply affected both at local and global scales (McCauley et al., 2015). In addition to a widespread habitat degradation and overexploitation, climate change will likely accelerate population declines in the next decades (Harnick et al., 2012). Although a pattern of historical decline is shared among marine animals (Lotze et al., 2006), species susceptibility to anthropogenic threats may vary.

The impact of this rapidly changing world on migratory species such as sea turtles might be aggravated by aspects of their biology (Robinson et al., 2009). Sea turtles are long-lived animals with a life cycle characterized by multiple habitats shifts, including direct land use during nesting activities, which increases their vulnerability (McCauley et al., 2015). Additionally, sea turtles tend to use the same reproduction sites across mating seasons and females tend to nest on the same beaches they were born (philopatry), which can make them bound to potentially threatened areas (Hamann et al., 2013). They also have temperature-dependent sex determination (TSD), where warmer incubation temperatures generate more females (Hamann et al., 2013). Thus, rising temperatures at nesting beaches can potentially affect sex ratios, which could eventually compromise population viability (Laloë et al., 2016).

Understanding sex ratio variations is key to comprehend sea turtle population dynamics, especially in a world threatened by climate change. However, determining sex of individuals is logistically difficult and cannot always be determined through morphology (Wibbels, 2003). Because of these limitations, most of the information on sex ratio available for sea turtles is based on indirect methods, such as measuring of sand and nest temperatures (e.g. Godfrey et al., 1996; Laloë et al., 2016, 2020). So far, sex ratios at nesting sites (NS) around the globe are usually female-skewed, especially in warmer beaches where females can represent over 90% of new hatchlings (e.g.

Godley et al., 2002; Hays et al., 2014). Only a few NSs are reported to have unbiased or male-biased sex ratios (e.g. Esteban et al., 2016; Patrício et al., 2017b; Laloë et al., 2020).

Sex ratios in feeding grounds (FG) are more difficult to access, but they can also provide important information about population dynamics of sea turtles (e.g. Casale et al., 2006; Maffucci et al., 2013). While assessments of NSs provide sex ratios of hatchlings on specific nesting seasons; FGs harbour individuals of different age classes, which allows the investigation of sex ratios on a wider temporal spectrum (Maffucci et al., 2013). Furthermore, because of the philopatric behaviour of females, a genetic structure can usually be detected in the matrilineal inherited mitochondrial DNA (mtDNA). This genetic structure can be used to trace natal origins of individuals found in FGs. Hence, when the sex of individuals in FGs is known, it is possible to estimate natal origins of females and males independently and thus help to investigate sex ratios in local NSs.

Recently, Jensen et al. (2018) used genetic data of individuals with known sex to investigate sex ratio of green turtles, *Chelonia mydas* (Linnaeus, 1758), using samples from FGs in the Great Barrier Reef (GBR), Australia. They found that sex ratio of green turtles from the northern GBR, one of the largest nesting areas for green turtles in the world, was highly female-skewed (Jensen et al., 2018). This result raises concerns about other green turtle nesting sites, but also demonstrates the usefulness of using data from FGs to investigate local sex ratios.

The Southwest Atlantic Ocean (SWA) is an important region for green turtles, harbouring two of the main NSs in the Atlantic (Ascension Island and Surinam) and it is considered to have high levels of threat to sea turtles (Wallace et al., 2011). Until now, genetic studies of green turtles in the SWA using individuals with known sex are scarce. Studying population genetics of females and males independently can provide important information on population dynamics and help to identify male-producing NSs in the region, which are increasingly important due to the current trend of feminization of

populations (Hays et al., 2014). The identification of NSs that produce mainly or exclusively females is also important to help understand local environmental conditions surrounding extremely skewed sex ratios. This information can be valuable to planning conservation strategies that take into consideration the effects of climate change on green turtle populations (Laloë et al., 2020).

Here, we compile historical data of green turtles from FGs in the SWA and discuss spatiotemporal variations in sex ratios. Additionally, we evaluate genetic data and determine natal origins of sexed specimens of an FG in north-eastern SWA. Our main goals were to evaluate temporal changes in female-male proportions as well as to determine genetic composition of green turtles from north-eastern SWA in order to answer the following questions: i) did sex ratios in the SWA change in the recent years?; ii) is there geographic variation in sex ratios within the SWA? iii) are there differences in natal origins of female and male green turtles feeding in north-eastern SWA? And if so, could that be an indicative of biased sex ratios at source NSs?

3.2 Materials and Methods

3.2.1 Feeding grounds sex ratios

Data from stranded green turtles were obtained from beach monitoring projects performed between 2010 and 2016 in FGs of two geographical regions in north-eastern and one in south-eastern Brazil (Fig. 1). These projects were established by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) as a measure to evaluate environmental impact during the implementation of activities that might alter the surrounding natural environment. Feeding grounds in north-eastern coast encompassed five states and are henceforth denominated Northeast 1 (along the coast of Ceará and Rio Grande do Norte States) and Northeast 2 (along the coast of Alagoas, Sergipe and Bahia States). Feeding grounds in south-eastern, henceforth Southeast, encompassed the coast of Espírito Santo State (Fig. 1, Supplementary Table S1). In

total, we used 3465 records with information on location, date, sex (determined through gonadal inspection) and curved carapace length (CCL) of each stranded specimen.

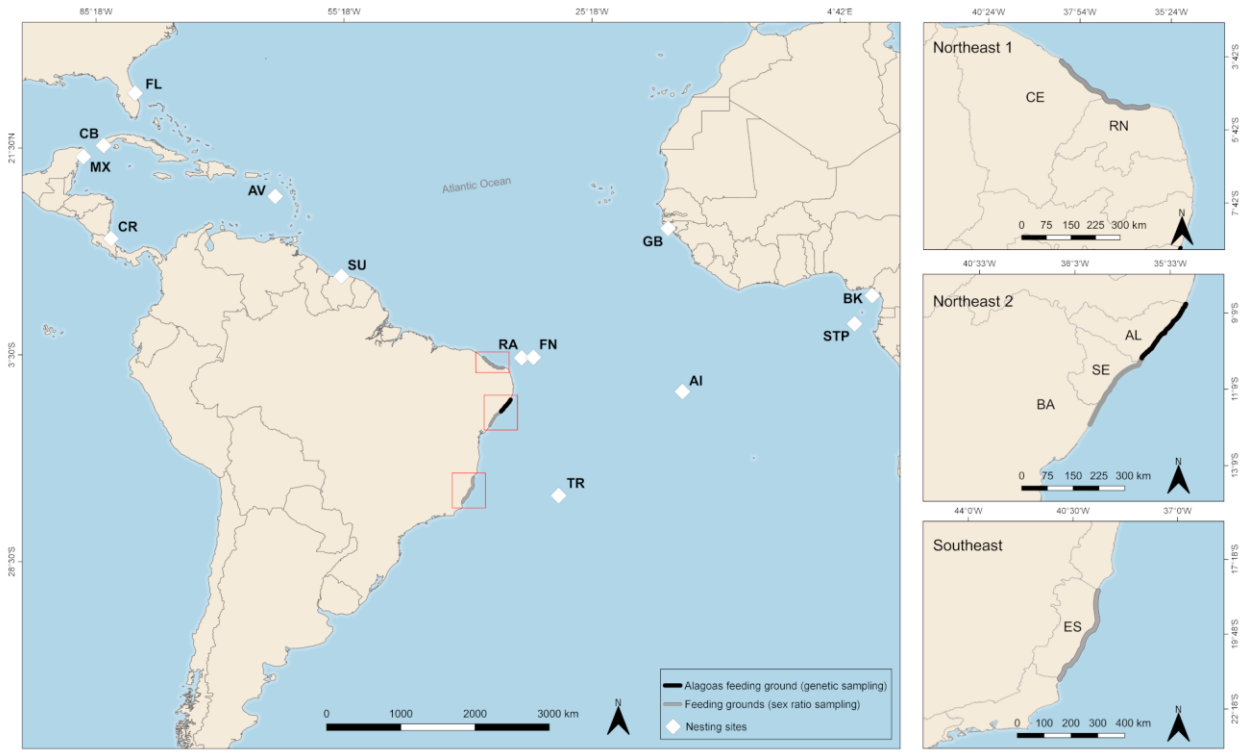


Figure 1. Green turtle sampling included in this study. Areas in gray represent feeding grounds included in sex ratio analyses. Area in black denotes feeding ground included for genetic analyses. White polygons indicate nesting sites used as possible sources in genetic analyses. Feeding grounds: AL – Alagoas, BA – Bahia, CE – Ceará, ES – Espírito Santo, RN – Rio Grande do Norte, SE – Sergipe. Nesting sites: AI – Ascension Island, AV – Aves Island, BK – Bioko, CB – Cuba, CR – Costa Rica, FL – Florida, FN – Fernando de Noronha, GB – Guinea Bissau, MX – Mexico, RA – Rocas Atoll, STP – São Tomé and Principe, SU – Surinam, TR – Trindade Island.

We evaluated sex ratio variations throughout collection years by estimating mean sex ratio among all FGs within each year and applying a Pearson chi-squared test. Subsequently, as we did not detect differences in sex ratios throughout the years (see results), we combined the data from different collection years and grouped females and males into three size classes: juveniles recently recruited to the neritic zone (CCL <40 cm), older juveniles (CCL 40.1–90 cm) and adults (CCL >90 cm). Sizes of recently

recruited juveniles were determined conservatively based on the approximate CCL of the smallest individuals recorded in different FGs in the Atlantic (19–36 cm; Goshe et al., 2010; Lenz et al., 2017; Reich et al., 2007). As estimated growth rates of South Atlantic green turtles with CCL between 30–39.9 cm is around 3.89cm per year (Lenz et al., 2017), we expect that individuals with CCL <40 cm had been recruited to the neritic zone only over the past few years. Size of adults was determined based on information of the smallest nesting females at Trindade NS (around 90 cm; Almeida et al., 2011).

Data from age estimation and growth rates indicate that green turtles in the Atlantic are usually two to seven years old when they recruit to the neritic zone and over 30 years old when they reach sexual maturity (Goshe et al., 2010; Lenz et al., 2017). Thus, by grouping our samples within these three size classes we should have sex ratio estimates for recent years (specimens with CCL <40 cm), for around seven to 30 years (specimens with CCL 40.1–90 cm) and for over 30 years (specimens with CCL >90 cm). It is important to highlight that the size classes we used are not definitive and that the size at which green turtles recruit to neritic zones or reach sexual maturity might vary in different regions (Goshe et al., 2010).

We estimated mean sex ratio in the SWA for each size class using data from all FGs. We then evaluated differences in sex ratios among size classes using a chi-square goodness of fit test. Finally, we tested if sex ratio within each FG differed from the estimated mean sex ratio for the SWA for each size class also using chi-squared goodness of fit tests. All analyses were performed using the software R 3.5.1 and the native package stats (R Core Team, 2018).

3.2.2 Genetic composition and natal origins of female and male green turtles

For molecular analyses, we used muscle samples from 146 specimens (CCL 23–115 cm), of which 89 were from females and 57 were from males. Samples were collected from stranded green turtles found along the coast of Alagoas State between May 2018 and March 2020, covering approximately 230 km of coastline in north-eastern

Brazil (Fig. 1). All specimens were dead, which allowed sex determination through direct inspection of the gonads. Samples were stored in ethanol 92% and kept at -18°C.

Total genomic DNA was extracted using phenol-chloroform method (Sambrook et al., 1989), and a fragment of approximately 800 base pairs (bp) of the mitochondrial control region was amplified through 25 µl polymerase chain reactions using the primers LCM15382 and H950 (Abreu-Grobois et al., 2006). Reactions consisted of 20.8 µl of 1XMaster Mix PCR Buffer with 0.4 mM of each dNTP and 3 mM of MgCl2, 1.0 µl of each primer (10pmol); 2 µl of DNA template (>20ng/µl) and 0.2 µl of Taq DNA polymerase (5U/µl). Amplifications were performed as follows: initial denaturation at 94°C for 7 min followed by 35–40 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, extending at 72°C for 1 min and a final extending at 72°C for 5–7 min. Posteriorly, samples were purified with sodium acetate and isopropanol to remove PCR residuals and sequenced in both directions using Sanger sequencing.

DNA sequences were submitted to the BLAST tool at GenBank database to check for contamination and subsequently edited using BioEdit 7.0.5.3 (Hall, 2011). Sequences were aligned using MAFFT 7.310 (Katoh and Standley, 2013), and trimmed to a 490bp fragment for better comparison with nesting sites. Haplotypes were identified based on the Archie Carr Center for Sea Turtle Research haplotype database (https://accstr.ufl.edu/). Haplotype (h) and nucleotide ($\theta\pi$) diversities were estimated using DnaSP 5.10 (Librado and Rozas, 2009), and haplotypes relationships were evaluated with median-joining haplotype networks (Bandelt et al., 1999) reconstructed with Network 10.1 (https://www.fluxus-engineering.com). Genetic differentiation between females and males was evaluated using an Analysis of Molecular Variance (AMOVA) with 10 000 permutations implemented in Arlequin 3.5.2.2 (Excoffier and Lischer, 2010).

Natal origins of female and male green turtles were estimated independently based on many-to-one mixed stock analyses (MSA), which allows to determine the relative contribution of multiple sources (NSs) to a single mixed population (FG) based on frequency and relative proportions of haplotypes using Bayesian methods (Pella and

Masuda, 2001). Data from the following nesting sites were used: Florida and Mexico (Encalada et al., 1996), Aves Island and Surinam (Bjorndal et al., 2006; Shamblin et al., 2012); Ascension Island (Formia et al., 2007); Costa Rica (Bjorndal et al., 2005); Cuba (Ruiz-Urquiola et al., 2010); Guinea Bissau (Patrício et al., 2017a); Rocas Atoll, Fernando de Noronha and Trindade Island (Bjorndal et al., 2006); São Tomé and Principe and Bioko (Formia et al., 2006). Data from Rocas Atoll and Fernando de Noronha were pooled because of the small sample size and because these two NSs are geographically close and genetically similar (Bjorndal et al., 2006).

We performed MSA analyses using females and males independently. First, we used the size of each NS (MSA1), based on the number of nesting females, as a prior for the analyses (see Supplementary Table S2; also Seminoff et al., 2015). Second, we considered equal weights for all NSs (MSA2), i.e. without the effect of size of NS. Analyses were performed using BAYES (Pella and Masuda, 2001), with 12 chains per run (equal to the number of sources [NSs]) and 50 000 iterations per chain. As default, half of these iterations were discarded as burn-in. Chains convergence was checked using the Gelman-Rubin criterion, considering that convergence has been achieved if values were below 1.2 (Gelman and Rubin, 1992).

3.3 Results

3.3.1 Feeding grounds sex ratios

From the 3465 records of stranded green turtles, 2630 were females and 835 were males (for a detailed description see Supplementary Table S1). Sex ratios in the SWA across collection years were similar, with slightly lower proportions of females in 2015 and 2016 (Fig. 2). Variation observed among years was not significant ($X2 = 10.318$, $p = 0.112$). Comparison of sex ratios among size classes revealed that sex ratios of recently recruited (mean 3.10F:1M) and older juveniles (mean 3.27F:1M) were similar and did not differ significantly ($X2 = 0.976$, $p = 0.323$). Sex ratio of adults was

slightly less female-biased (mean 3.03F:1M), but also did not differ significantly from recently recruited (X2 = 0.097, p = 0.7545) or older juveniles (X2 = 0.658, p = 0.417).

When we compared sex ratios within each FG to mean sex ratios estimated for the SWA for each size class, we found that in recently recruited juveniles sex ratios were not different from the average value for the SWA in Northeast 2 (X2 = 0.891, p = 0.345) and Southeast (X2 = 1.958, p = 0.161), but in Northeast 1 there were fewer females than average (X2 = 8.199, p = 0.004; Fig 3). The number of older juvenile females was significantly higher than the expected in Northeast 1 (X2 = 6.036, p = 0.014), significantly lower in Northeast 2 (X2 = 10.825, p = 0.001) and no different from the average in Southeast (X2 = 1.566, p = 0.210). The number of adult females was no different from the expected in Northeast 1 (X2 = 3.746, p = 0.053), but lower in Northeast 2 (X2 = 13.531, p = 0.0002, Fig 3). We did not compare proportion of females in the Southeast to the SWA because of the low number of adults in this region (five females and five males).

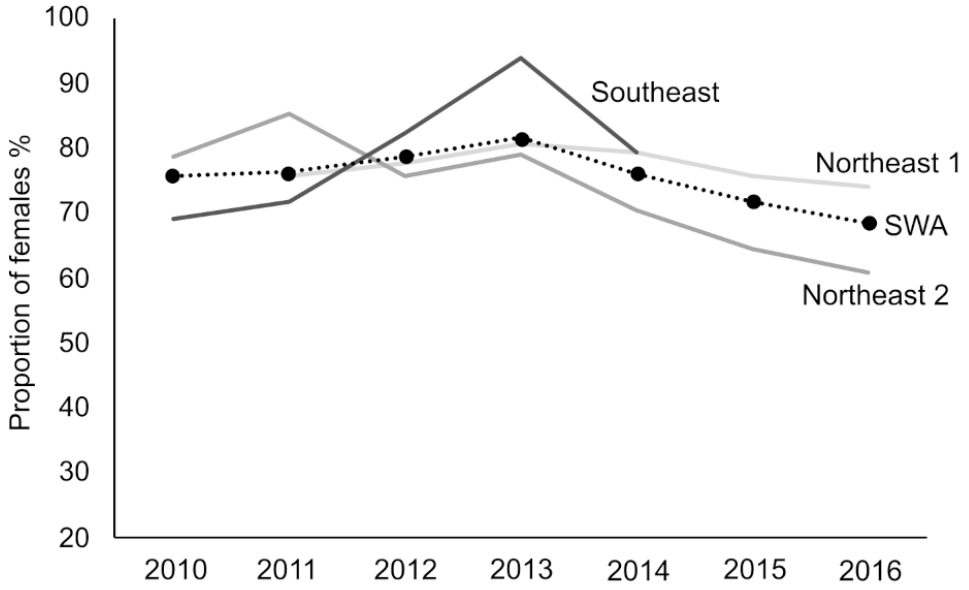


Figure 2. Proportion of female green turtles in Southwest Atlantic Ocean feeding grounds between 2010 and 2016, based on 3,465 records.

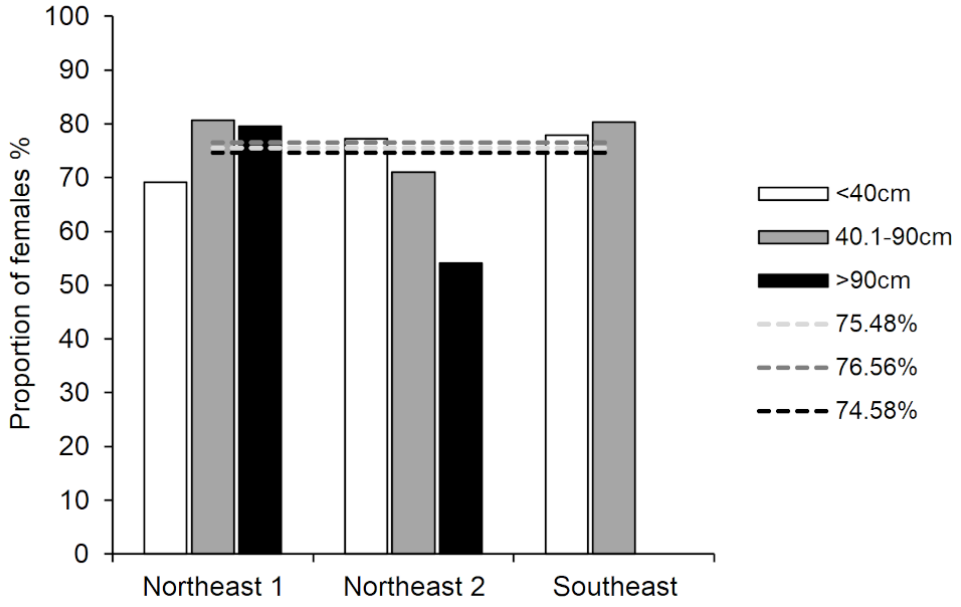


Figure 3. Proportions of female green turtle in Southwestern Atlantic Ocean feeding grounds according to size classes. Dashed light gray, dark gray and black lines indicates mean proportion of females in recently recruited juveniles, older juveniles and adults in the SWA, respectively.

3.3.2 Genetic composition and natal origins of female and male green turtles

We identified eleven haplotypes, of which nine were found in females and seven in males. Five haplotypes were shared between females and males (Fig. 4, Supplementary Table S2). The most common haplotype was CM-A8, 76.4% of females and 64.9% of males, followed by CM-A5, 10.1% of females and 24.5% of males. Nucleotide and haplotype diversities were slightly larger in males ($\theta\pi$ = 0.00234 ±0.00058, h = 0.502 ±0.062) than in females ($\theta\pi$ = 0.00127 ±0.00023, h = 0.389 ±0.063). The AMOVA analysis indicated that most genetic variation was within females and males (95.83%), but variation between them was still significant (FST = 0.0417, p = 0.017). We obtained the longer fragment of the control region (~800 bp) from 106 of 146 specimens and only found more than one variant haplotype among CM-A8 samples: CM-A8.1 (48 females, 28 males), CM-A8.2 (two females) and CM-A8.3 (one male). A full list of long haplotypes is provided in supplementary Table S3.

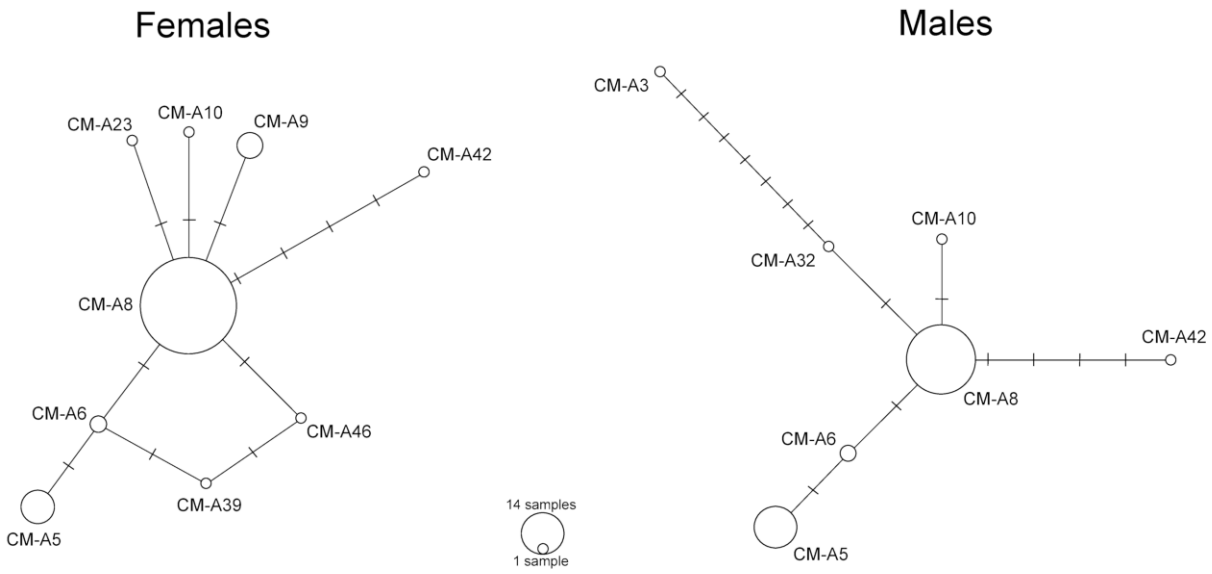


Figure 4. Haplotype networks of female and male green turtles from Alagoas feeding ground, based on 490bp of the control region of the mitochondrial DNA.

Mixed-stock analyses considering the number of nesting females (MSA1) estimated slightly different natal origins for females and males (Table 1). Ascension Island was the main contributor to the composition of females (62.28%), while Guinea Bissau contributed the most to the composition of males (40.28%). Guinea Bissau was the second highest contributor to the composition of females (25.44%), followed by Surinam (8.83%). For males, Ascension Island (30.44%) and Surinam (23.26%) also had large contributions. All other NSs contributed with less than 5% for both females and males (Table 1). When considering equally weighted priors (MSA2), contributions of NSs were similar to MSA1, Ascension Island and Guinea Bissau contributed the most to the composition of females (53.83% and 25.93%, respectively) and males (20.90% and 37.33%, respectively).

Table 1. Mixed-stock analyses of female and male green turtles from Alagoas feeding ground. Analyses were performed using size of nesting sites as prior (MSA1) and equal weights priors (MSA2). Contributions of nesting sites are in % and 2.5% and 97.5% confidence intervals are in parenthesis. Nesting sites with the highest contribution are in bold in each MSA.

Stock	Females		Males	
	MSA1	**MSA2**	**MSA1**	**MSA2**
Florida	0.05 (0–0.58)	0.11 (0–1.16)	0.11 (0–1.29)	0.29 (0–2.94)
Mexico	0.13 (0–1.28)	0.10 (0–1.07)	0.26 (0–2.51)	0.22 (0–2.31)
Costa Rica	0.70 (0–3.27)	0.10 (0–1.06)	2.19 (0.01–7.97)	0.38 (0–3.54)
Aves Island	1.38 (0–12.43)	4.38 (0–15.11)	2.88 (0–28.85)	10.97 (0–34.31)
Surinam	8.83 (0–17.17)	5.88 (0–16.20)	23.26 (0–38.43)	15.57 (0–37.30)
Rocas/Noronha	0.03 (0)	1.13 (0–11.68)	0.17 (0)	4.23 (0–26.04)
Trindade Island	1.06 (0–15.60)	6.22 (0–28.95)	0.19 (0–2.14)	1.36 (0–11.79)
Ascension Island	**62.28 (28.99–88.07)**	**53.83 (17.27–85.80)**	30.44 (1.67–66.65)	20.90 (0–63.33)
Guinea Bissau	25.44 (1.76–55.47)	25.93 (1.27–56.36)	**40.28 (5.94–68.31)**	**37.33 (3.28–67.13)**
Bioko	0.08 (0–0.02)	1.80 (0–18.38)	0.18 (0–0.07)	7.55 (0–77.74)
São Tomé and Principe	0.01 (0)	0.43 (0–4.35)	0.02 (0)	0.95 (0–10)
Cuba	0.01 (0–0.05)	0.10 (0–0.98)	0.03 (0–0.15)	0.27 (0–2.73)

3.4 Discussion

3.4.1 Feeding grounds sex ratios

Sex ratios in SWA feeding grounds were generally female-skewed (Fig. 3), which is in line with most reported sex ratios for green turtles worldwide (Hays et al., 2014). There was no significant change in sex ratios throughout collection years, suggesting that proportion of females and males have been somewhat constant in the SWA in recent years. Sex ratios of green turtles in the SWA have been historically investigated through the evaluation of nesting beach temperatures within or between nesting seasons (e.g. Broderick et al., 2001; Godley et al., 2002). Studies are mainly focused on two major NSs, Ascension and Surinam (Mrosovsky et al., 1984; Godfrey et al., 1996; Godley et al., 2002). Sex ratios in Ascension have been repeatedly reported as female-skewed (Godley et al., 2002, Pintus et al., 2009) and this NS is usually reported as the main contributor to the composition of individuals in FGs in the SWA (e.g. Naro-Maciel et al., 2012; Proietti et al., 2012). Thus, we would expect that this high prevalence of females would be reflected in the composition of individuals in FGs. Our results support this observation, as the proportion of females generally exceeded 70%. Nevertheless, implementation of genetic analyses is still required to corroborate natal origins of these individuals.

Sex ratio of adult green turtles in SWA FGs was slightly less female-biased (2.97F:1M), but not significantly different from juveniles. However, FGs in Northeast 2, exhibited a noticeable lower proportion of adult females (Fig. 3). As green turtles in the Atlantic usually take 30-40 years to reach sexual maturity (Goshe et al., 2010), the similarity between juveniles and adults sex ratios could indicate that female output in local NSs have been constantly high in the recent decades. This result is in agreement with some studies that indicate that female-biased sex ratios were predominant during the last decades in the largest NS in SWA, Ascension Island (Godley et al., 2002; Pintus et al., 2009). However, other extrinsic factors, such as different migration periodicity and differential death rates in female and male adults, could also be playing a role in sex

ratio variation between juveniles and adults (Maffucci et al., 2013). Furthermore, the cause of death in stranded turtles is not always possible to determine accurately, making it difficult to determine if local factors such as fisheries and pollution could bias our results by having distinct impacts in females and males or even juveniles and adults.

3.4.2 Genetic composition and natal origins of female and male green turtles

Haplotype composition of females and males was slightly divergent, but the most common haplotypes were CM-A8 and CM-A5, similarly to what is found in other FGs in the SWA (Naro-Maciel et al., 2012; Proietti et al., 2012; Prosdocimi et al., 2012). Nesting sites in the South Atlantic also exhibit a high prevalence of CM-A8 (e.g. Naro-Maciel et al., 2014; Patrício et al., 2017a), while CM-A5 is more frequently found in NS closer to the Caribbean (Shamblin et al., 2012). The occurrence of two specimens with the haplotype CM-A42 is also noteworthy, since Guinea Bissau is the only NS to which this haplotype was reported so far (Patrício et al., 2017a), and the presence of this haplotype might have influenced MSA results. Considering the longer fragments, the predominant variant of the CM-A8 (N = 79) haplotype was CM-A8.1 (96.2%, N = 76, 48 females and 28 males), similar to what was found in Rocas Atoll, Fernando de Noronha and Trindade Island NSs (Shamblin et al., 2015), all in the SWA, as well as Guinea Bissau (Patrício et al., 2017a). Likewise, the predominant variant of the CM-A5 (N = 14) haplotype was CM-A5.1 (100%, ten females and four males), which is also the primary variant of this haplotype in Suriname, Aves Island and Costa Rica NSs (Shamblin et al., 2012).

Relative contributions of NSs to our study area were slightly divergent between female and male green turtles (Table 1). Considering the size of NSs (MSA1), the major contributors to female composition were Ascension Island and Guinea Bissau. The situation was inverted for males, with Guinea Bissau as the highest contributor followed by Ascension Island (Table 1). While Ascension is usually reported as the major contributor to the composition of individuals in FGs in the SWA, contributions of Guinea Bissau are usually considered unlikely. Data from satellite tracking, tag return and particle dispersion suggest that green turtles from Guinea Bissau most likely feed on

coastal areas of west Africa (Godley et al., 2010). However, contributions of Guinea Bissau can still be substantial in some cases (Naro-Maciel et al., 2007; Proietti et al., 2012). A recent report estimated that foraging aggregations in north-eastern Brazil could receive up to 25% contribution from Guinea Bissau in foraging-centric MSAs (Patrício et al., 2017a). This finding reinforces that individuals from this NS could reach Alagoas FG as well. Additionally, data from hawksbill turtles, *Eretmochelys imbricata* (Linnaeus, 1766), also support transatlantic migration from African NSs to FGs in north-eastern Brazil (Proietti et al., 2014).

Our results using both weighted (MSA1) and equal priors (MSA2) support a high contribution of Guinea Bissau to the composition of individuals, particularly males, in our study area. These results are also in agreement with estimates of balanced sex ratios in this NS (Rebelo et al., 2012; Patrício et al., 2017b), where environmental conditions seem to contribute to cooler nest temperatures and consequently to a higher proportion of male hatchlings when compared to other NSs (see Patrício et al., 2019 for a detailed discussion). In contrast, sex ratios in Ascension Island have been reported to be female-skewed, with estimations varying between 54.2% and 99.6% of females, depending on the specific beach (Godley et al., 2002). This is reflected in the contributions of this NS to the composition of females in our study area, which were high in both MSA1 and MSA2 (Table 1).

Contributions from Surinam to the composition of females and males were also large (9.26% and 23.26%, respectively), which is agreement with reports from other FGs (Naro-Maciel et al., 2012; Proietti et al., 2012). Nevertheless, the noticeably greater incidence of males from this NS is noteworthy and it may be a result of the large relative proportion of CM-A5 haplotype in male samples (24.5%) since this haplotype is predominant in Surinam (Bjorndal et al., 2006; Shamblin et al., 2012). Sex ratios in Surinam have also been reported to be slightly more balanced (68.4%) in relation to some of the largest female-biased beaches in Ascension Island, although it can vary throughout the same nesting season (Godfrey et al., 1996). Our results are concordant with a larger output of males from this NS and seem to reinforce the hypothesis that

Surinam could be an important source of males for green turtle FGs in the SWA, although more studies are still needed.

Male-producing NSs are sparsely scattered around the globe and most NSs of green and other sea turtles usually exhibit a prevalence of females (for a detailed discussion see Hays et al., 2014). In fact, one of the largest green turtle NS in the world, the northern Great Barrier Reef, was recently revealed to be producing over 86.8% females for the past two decades due to increased sand temperatures (Jensen et al., 2018). This raises concerns about population status and sea turtles' response to climate change (Laloë et al., 2020), particularly on beaches where eggs are already incubated above the pivotal temperature, and where effects of a warming climate can be even more accentuated. With the imminent rise of global temperatures which, under different scenarios, can increase over 2.0°C above current temperatures until 2100 according to data from the Intergovernmental Panel on Climate Change (IPCC, 2014), production of females in sea turtle NSs is expected to rise as well, which may promote the feminization of some populations (Hays et al., 2014; Jensen et al., 2018).

The extent to which this feminization process would affect sea turtle populations is still not completely clear (Hays et al., 2017). Undoubtedly, factors like extremely high incubation temperatures and changes in sea surface temperature will likely affect population dynamics of sea turtles (Hamman et al., 2013). However, some studies suggest that the higher reproduction frequency of males and higher availability of nesting females could, to a certain degree, compensate for female-skewed primary sex ratios, at least while complete feminization is not reached (Hays et al., 2017; Tomillo and Spotila, 2020). Additionally, local environmental features such as the presence of vegetation and sand colour seem to play a role on the regulation of nest temperatures (Patrício et al., 2019) and can be valuable tools for management of populations.

Finally, besides the direct effect of rising temperatures on sex determination of sea turtles, a warming climate also poses other challenges, such as: i) the sea level rise, which can compromise nesting activities by reducing the total area available for nests; ii)

increased frequency of lethal incubation temperatures and iii) changes in sea surface temperature and ocean pH that can also affect sea turtles and associated marine communities, such as coral reefs (Fuentes et al., 2010; Hoegh-Guldberg et al., 2019). Moreover, other anthropogenic pressures, such as pollution of marine and coastal environments, must also be taken into account as they can act synergistically as threats to sea turtle populations (Fuentes et al., 2011). Thus, conservation actions that include identification and protection of male-producing rookeries, protection of coastal environments and ultimately direct management of nests could help mitigate some of these harmful effects.

3.5 Concluding remarks

Studies distinguishing female and male sea turtles are rare, because determining the sex of individuals is not always possible. Yet, these studies provide insights on current population dynamics and help developing efficient conservation plans (Jensen et al., 2018). Here, we provided a spatiotemporal evaluation of green turtle sex ratios in FGs in the SWA revealing that a general pattern of female-biased sex ratios has likely been prevalent during the last decades.

We also provided the first independent evaluation of natal origins of female and male green turtles from a feeding ground in the SWA. We were able to detect slightly divergent natal origins in females and males and found a high influence of Guinea Bissau supporting previously proposed transatlantic migrations from this NS in Africa to FGs in the SWA. Furthermore, a prevalence of males from Guinea Bissau and Surinam highlighted the importance of these sites from a conservation standpoint. Nevertheless, more data are still needed to shed more light on the implications of climate change on population dynamics of green turtles.

3.6 Supplementary material

The following supplementary material is available at ICESJMS online: Supplementary file including tables S1–S3 with data regarding composition of female

and male green turtles in feeding grounds sampled in the study, haplotypes of Alagoas feeding ground and nesting sites included in the analyses and long haplotypes from samples of Alagoas feeding ground.

3.7 Acknowledgments

We thank everyone involved in the beach monitoring projects as well as IBAMA for granting access to the database. We thank members of LAMARC and LABI laboratories for help with laboratory work. We also thank the editor and two reviewers for constructive comments on the manuscript. This study was partially funded by PADI Foundation (#47777/2020), Fundação Grupo Boticário de Proteção da Natureza (#1143/20182) and Fundação de Amparo à Pesquisa do Estado de Alagoas (FAPEAL #60030.1564/2016). This study also had the collaboration of Instituto Biota de Conservação. JPFAA thanks FAPEAL for providing PhD scholarship (#23038.023347/2016-74). TM thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (309904/2015-3 and 312291/2018-3).

3.8 Data availability

Raw data used in sex ratio analyses is detailed in supplementary material. All haplotypes used in genetic analysis were previously described by other studies and are available in GenBank.

References

Abreu-Grobois, F. A., Horrocks, J., Formia, A., LeRoux, R., Velez-Zuazo, X., Dutton, P., Soares, L., et al. 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Book of abstracts of the 26th Annual Symposium on Sea Turtle Biology and Conservation, p. 179.

Almeida, A. P., Moreira, L. M. P., Bruno, S. C., Thomé, J. C. A., Martins, A. S., Bolten, A. B., and Bjorndal, K. A. 2011. Green turtle nesting on trindade island, Brazil: Abundance, trends, and biometrics. Endangered Species Research, 14: 193–201.

Bandelt, H. J., Forster, P., and Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16: 37-48.

Bjorndal, K. A., Bolten, A. B., and Troëng, S. 2005. Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Marine Biology, 147: 1449–1457.

Bjorndal, K. A., Bolten, A. B., Moreira, L., Bellini, C., and Marcovaldi, M. A. 2006. Population structure and diversity of brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology, 5: 262–268.

Broderick, A. C., Godley, B. J., and Hays, G. C. 2001. Metabolic Heating and the Prediction of Sex Ratios for Green Turtles (*Chelonia mydas*). Physiological and Biochemical Zoology, 74: 161–170.

Casale, P., Lazar, B., Pont, S., Tomás, J., Zizzo, N., Alegre, F., Badillo, J., et al. 2006. Sex ratios of juvenile loggerhead sea turtles *Caretta caretta* in the Mediterranean Sea. Marine Ecology Progress Series, 324: 281–285.

Encalada, S. E., Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Miyamoto, M. M., and Bowen, B. W. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. Molecular Ecology, 5: 473–483.

Esteban, N., Laloë, J. O., Mortimer, J. A., Guzman, A. N., & Hays, G. C. 2016. Male hatchling production in sea turtles from one of the world's largest marine protected areas, the Chagos Archipelago. Scientific Reports, 6: 1–8.

Excoffier, L., and Lischer, H. E. L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564–567.

Formia, A., Godley, B. J., Dontaine, J. F., and Bruford, M. W. 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conservation Genetics, 7: 353–369.

Formia, A., Broderick, A., Glen, F., Godley, B., Hays, G., and Bruford, M. 2007. Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. Endangered Species Research, 3: 145–158.

Fuentes, M. M. P. B., Limpus, C. J., Hamann, M., and Dawson, J. 2010. Potential impacts of projected sea-level rise on sea turtle rookeries. Aquatic Conservation: Marine and Freshwater Ecosystems, 20: 132–139.

Fuentes, M. M. P. B., Limpus, C. J., and Hamann, M. 2011. Vulnerability of sea turtle nesting grounds to climate change. Global Change Biology, 17: 140–153.

Gelman, A., and Rubin, D. B. 1992. Inference from Iterative Simulation Using Multiple Sequences. Statistical Science, 7: 457–511.

Godfrey, M. H., Barreto, R., and Mrosovsky, N. 1996. Estimating past and present sex ratios of sea turtles in Suriname. Canadian Journal of Zoology, 74: 267–277.

Godley, B. J., Broderick, A. C., Glen, F., and Hays, G. C. 2002. Temperature-dependent sex determination of Ascension Island green turtles. Marine Ecology Progress Series, 226: 115–124.

Godley, B. J., Barbosa, C., Bruford, M., Broderick, A. C., Catry, P., Coyne, M. S., Formia, A., et al. 2010. Unravelling migratory connectivity in marine turtles using multiple methods. Journal of Applied Ecology, 47: 769–778.

Goshe, L. R., Avens, L., Scharf, F. S., and Southwood, A. L. 2010. Estimation of age at maturation and growth of Atlantic green turtles (*Chelonia mydas*) using skeletochronology. Marine Biology, 157: 1725–1740.

Hall, T. 2011. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences, 2: 60–61.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., et al. 2008. A Global Map of Human Impact on Marine Ecosystems. Science, 319: 948–952.

Hamann, M., Fuentes, M. M. P. B., Ban, N. C., & Mocellin, V. J. L. (2013). Climate change and marine turtles. In The Biology of Sea Turtles Volume III, pp. 353–378. Ed. by J. Wyneken, K. J. Lohmann, and J. A. Musick. CRC Press, Boca Raton, FL. 446 pp.

Harnick, P. G., Lotze, H. K., Anderson, S. C., Finkel, Z. V., Finnegan, S., Lindberg, D. R., Liow, L. H., et al. 2012. Extinctions in ancient and modern seas. Trends in Ecology & Evolution, 27: 608–617.

Hays, G. C., Mazaris, A. D., and Schofield, G. 2014. Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Frontiers in Marine Science, 1: 1–9.

Hays, G. C., Mazaris, A. D., Schofield, G., and Laloë, J. O. 2017. Population viability at extreme sex-ratio skews produced by temperaturedependent sex determination. Proceedings of the Royal Society B: Biological Sciences, 284: 1–7.

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Guillén Bolaños, T., Bindi, M., Brown, S., Camilloni, I. A., et al. 2019. The human imperative of stabilizing global climate change at 1.5°C. Science, 365.

Intergovernmental Panel on Climate Change (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.

Jensen, M. P., Allen, C. D., Eguchi, T., Bell, I. P., LaCasella, E. L., Hilton, W. A., Hof, C. A. M., et al. 2018. Environmental Warming and Feminization of One of the Largest Sea Turtle Populations in the World. Current Biology, 28: 154–159.

Katoh, K., and Standley, D. M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30: 772–780.

Laloë, J. O., Esteban, N., Berkel, J., and Hays, G. C. 2016. Sand temperatures for nesting sea turtles in the Caribbean: Implications for hatchling sex ratios in the face of climate change. Journal of Experimental Marine Biology and Ecology, 474: 92–99.

Laloë, J. O., Monsinjon, J., Gaspar, C., Touron, M., Genet, Q., Stubbs, J., Girondot, M., et al. 2020. Production of male hatchlings at a remote South Pacific green sea turtle rookery: conservation implications in a female-dominated world. Marine Biology, 167: 1–13.

Lenz, A. J., Avens, L., and Borges-Martins, M. 2017. Age and growth of juvenile green turtles *Chelonia mydas* in the western South Atlantic Ocean. Marine Ecology Progress Series, 568: 191–201.

Librado, P., and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., et al. 2006. Depletion, degradation and recovery potential of estuaries and coastal seas. Science, 312: 1806–1809.

Maffucci, F., D'Angelo, I., Hochscheid, S., Ciampa, M., de Martino, G., Travaglini, A., Treglia, G., et al. 2013. Sex ratio of juvenile loggerhead turtles in the Mediterranean Sea: Is it really 1:1? Marine Biology, 160: 1097–1107.

McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H., and Warner, R. R. 2015. Marine defaunation: Animal loss in the global ocean. Science, 347.

Mrosovsky, N., Dutton, P. H., & Whitmore, C. P. 1984. Sex-Ratios of 2 Species of Sea Turtle Nesting in Suriname. Canadian Journal of Zoology, 62: 2227–2239.

Naro-Maciel, E., Becker, J. H., Lima, E. H. S. M., Marcovaldi, M. Â., and DeSalle, R. 2007. Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. Journal of Heredity, 98: 29–39.

Naro-Maciel, E., Bondioli, A. C. V., Martin, M., De Pádua Almeida, A., Baptistotte, C., Bellini, C., Marcovaldi, M. Â., et al. 2012. The interplay of homing and dispersal in green turtles: A focus on the southwestern atlantic. Journal of Heredity, 103: 792–805.

Patrício, A. R., Formia, A., Barbosa, C., Broderick, A. C., Bruford, M., Carreras, C., Catry, P., et al. 2017a. Dispersal of green turtles from Africa's largest rookery assessed through genetic markers. Marine Ecology Progress Series, 569: 215–225.

Patrício, A. R., Marques, A., Barbosa, C., Broderick, A. C., Godley, B. J., Hawkes, L. A., Rebelo, R., et al. 2017b. Balanced primary sex ratios and resilience to climate change inmajor marine turtle population. Marine Ecology Progress Series, 577: 189–203.

Patrício, A. R., Varela, M. R., Barbosa, C., Broderick, A. C., Catry, P., Hawkes, L. A., Regalla, A., and Godley, B. J. 2019. Climate change resilience of a globally important sea turtle nesting population. Global Change Biology, 25: 522–535.

Pella, J., and Masuda, M. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin, 99: 151–167.

Pintus, K. J., Godley, B. J., McGowan, A., & Broderick, A. C. 2009. Impact of Clutch Relocation on Green Turtle Offspring. Journal of Wildlife Management, 73: 1151–1157.

Proietti, M. C., Reisser, J. W., Kinas, P. G., Kerr, R., Monteiro, D. S., Marins, L. F., and Secchi, E. R. 2012. Green turtle *Chelonia mydas* mixed stocks in the western South Atlantic, as revealed by mtDNA haplotypes and drifter trajectories. Marine Ecology Progress Series, 447: 195–209.

Proietti, M. C., Reisser, J., Marins, L. F., Rodriguez-Zarate, C., Marcovaldi, M. A., Monteiro, D. S., Pattiaratchi, C., et al. 2014. Genetic structure and natal origins of immature hawksbill turtles (*Eretmochelys imbricata*) in Brazilian waters. PLoS ONE, 9.

Prosdocimi, L., González Carman, V., Albareda, D. A., and Remis, M. I. 2012. Genetic composition of green turtle feeding grounds in coastal waters of Argentina based on mitochondrial DNA. Journal of Experimental Marine Biology and Ecology, 412: 37–45.

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rebelo, R., Barbosa, C., Granadeiro, J. P., Indjai, B., Novais, B., Rosa, G. M., and Catry, P. 2012. Can leftovers from predators be reliably used to monitor marine turtle hatchling sex-ratios? The implications of prey selection by ghost crabs. Marine Biology, 159: 613–620.

Reich, K. J., Bjorndal, K. A., and Bolten, A. B. 2007. The 'lost years' of green turtles: Using stable isotopes to study cryptic lifestages. Biology Letters, 3: 712–714.

Robinson, R. A., Crick, H. Q. P., Learmonth, J. A., Maclean, I. M. D., Thomas, C. D., Bairlein, F., Forchhammer, M. C., et al. 2009. Travelling through a warming world : climate change and migratory species. Endangered Species Research, 7: 87–99.

Ruiz-Urquiola, A., Riverón-Giró, F. B. F., Pérez-Bermúdez, E., Abreu-Grobois, F. A., González-Pumariega, M., James-Petric, B., Díaz-Fernández, R., et al. 2010. Population genetic structure of greater Caribbean green turtles (*Chelonia mydas*) based on mitochondrial DNA sequences, with an emphasis on rookeries from southwestern Cuba. Revista de Investigaciones Marinas, 31: 33–52.

Sambrook, J., Fritsch, E.F. & Maniatis, T. 1989. Molecular cloning: a laboratory manual, 2nd edn, Cold Spring Harbor Laboratory Press, New York.

Seminoff, J. A., Allen, C. D., Balazs, G. H., Dutton, P. H., Eguchi, T., Haas, H. L., Hargrove, S. A. et al. 2015. Status review of the green turtle (*Chelonia mydas*) under the U.S. Endangered Species Act (NOAA Tech Memo NOAA-NMFS-SWFSC-539). La Jolla, CA: Southwest Fisheries Science Center, NOAA.

Shamblin, B. M., Bjorndal, K. A., Bolten, A. B., Hillis-Starr, Z. M., Lundgren, I., Naro-Maciel, E., and Nairn, C. J. 2012. Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Molecular Ecology, 21: 2330–2340.

Shamblin, B. M., Dutton, P. H., Bjorndal, K. A., Bolten, A. B., Naro-Maciel, E., Santos, A. J. B., Bellini, C., et al. 2015. Deeper mithocondrial sequencing reveals cryptic diversity and structure in Brazilian green turtle rookeries. Chelonian Conservation and Biology, 14: 167–172.

Tomillo, P. S., and Spotila, J. R. 2020. Temperature-Dependent Sex Determination in Sea Turtles in the Context of Climate Change: Uncovering the Adaptive Significance. BioEssays, 42.

Wallace, B. P., DiMatteo, A. D., Bolten, A. B., Chaloupka, M. Y., Hutchinson, B. J., Abreu-Grobois, F. A., Mortimer, J. A., et al. 2011. Global conservation priorities for Marine turtles. PLoS ONE, 6.

Weber, S. B., Weber, N., Ellick, J., Avery, A., Frauenstein, R., Godley, B. J., Sim, J., et al. 2014. Recovery of the South Atlantic's largest green turtle nesting population. Biodiversity and Conservation, 23: 3005–3018.

Wibbels, T. (2003). Critical approaches to sex determination in sea turtles. In The Biology of Sea Turtles Volume II, pp. 103-134. Ed. by P. Lutz, J. A. Musick, and J. Wyneken. CRC Press, Boca Raton, FL. 455 pp.

4 CAPÍTULO 2

HYBRIDIZATION AND GENETIC CHARACTERIZATION OF SEA TURTLES IN ALAGOAS, NORTHEASTERN BRAZIL[2]

João P. F. A. Almeida, Oscar K. L. Marques, Tamí Mott, Robson G. Santos

Abstract

Sea turtles are migratory species with wide geographical distributions, usually spanning multiple countries. This characteristic, along with their complex life cycle, makes sea turtle conservation challenging. In Brazil, continued monitoring and recent studies have advanced the knowledge of sea turtle genetic composition and population structure. Some of these studies have shown that hybridization is highly frequent in certain regions along the Brazilian coast, despite being relatively rare globally. Here we investigate the hybridization and genetic diversity of sea turtles in nesting and feeding grounds in the state of Alagoas, northeastern Brazil, using the control region of mitochondrial DNA and three nuclear loci. We were able to identify hybrids between four sea turtle species, but mainly between *Caretta caretta* and *Eretmochelys imbricata* and *C. caretta* and *Lepidochelys olivacea*. Most hybrids were readily identified using morphology and mitochondrial DNA, but some were only detected with nuclear DNA. Apart from hybrids, the genetic profile of each species was congruent with previous studies in Brazil. However, one stranded *E. imbricata* had a haplotype (Ei-IP17) and nuclear allele typically found in the Indo-Pacific, suggesting long distance migration for this species. Our results indicate that hybridization events might be even more geographically spread along the coast of Brazil and provide evidence of the connection between *E. imbricata* from the Atlantic and Indo-Pacific Ocean basins.

Keywords: Population genetics, hawksbills, loggerheads, olive ridleys, green turtles, hybrids

2. Artigo publicado no periódico Marine Biology, qualis A2, percentil 76% pelo Scopus. Almeida, J.P.F.A.A.; Marques, O.K.L.; Mott, T.; Santos, R.G. (2023). Hybridization and genetic characterization of sea turtles in Alagoas, northeastern Brazil. Marine Biology, 170: 14. doi: 10.1007/s00227-022-04168-y

4.1 Introduction

Sea turtles are migratory species with complex life cycles. Five of the seven sea turtle species have wide distributions across different regions of the globe with distinctive habitat changes throughout their lifespan (Bolten, 2003). This migratory behavior can make conservation planning challenging, particularly when sea turtle movement patterns span different countries, thereby requiring collaborative conservation efforts (Wallace et al. 2010). To address this challenge and guide conservation planning at smaller scales, regional management units (RMUs) have been suggested for sea turtles based on distributional and ecological data (Wallace et al. 2010). Among these RMUs, the Southwest Atlantic Ocean (SWA) exhibited considerable threat levels for sea turtle populations (Wallace et al. 2011). Nevertheless, recent studies have reported population recovery at some nesting sites in the region, likely due to continuing conservation efforts in recent decades (Marcovaldi et al. 2007; Colman et al. 2019).

In Brazil, efforts on sea turtle conservation have been historically conducted by TAMAR institute (Marcovaldi and Marcovaldi 1999). Additionally, a National Action Plan for Sea Turtle Conservation (PAN Tartarugas Marinhas) was established by the Brazilian government in 2010 and it is currently in its second phase (ICMBio 2017). Research priorities established by the PAN Tartatrugas Marinhas include the identification and monitoring of nesting and feeding grounds of the five sea turtle species known to occur along the Brazilian coast: *Caretta caretta* (loggerhead turtles), *Chelonia mydas* (green turtles), *Dermochelys coriacea* (leatherback turtles), *Eretmochelys imbricata* (hawksbill turtles) and *Lepidochelys olivacea* (olive ridley turtles) (Marcovaldi et al. 2007), as well as the evaluation of genetic profiles, population dynamics and hybridization between these species (ICMBio 2017). Research on sea turtle genetic diversity in nesting and feeding grounds in Brazil has increased in recent years (Reis et al. 2010b; Naro-Maciel et al. 2012; Proietti et al. 2014a; Jordão et al. 2015; Vargas et al. 2019). Some studies have reported a high hybridization frequency in a few nesting sites in northeastern Brazil (Lara-Ruiz et al. 2006; Reis et al. 2010a) however, this seems to

be rare in sea turtle populations worldwide (Brito et al. 2020). In Brazil, hybridization rates can reach up to 42% between hawksbills and loggerheads on the coast of the state of Bahia (Lara-Ruiz et al. 2006), and 27% between loggerheads and olive ridleys on the coast of the state of Sergipe (Reis et al. 2010a).

A high hybridization rate in wild populations may lead to several evolutionary outcomes, including the enhancement of genetic diversity and adaptative divergence (Abbott et al. 2013). However, it can also compromise small populations by limiting their growth rate through the production of inviable offspring (Todesco et al. 2016). The consequences of these processes in sea turtles are not yet completely understood, but a few studies have observed some differences in behavior and reproductive success between hybrids and parental species (Proietti et al. 2014b; Soares et al. 2017; Arantes et al. 2020a). For instance, while the clutch size of loggerhead and hawksbill hybrids has been reported as intermediate, emergence success was lower in hybrids (Soares et al. 2017; Arantes et al. 2020a). Likewise, post-emergence behavior can also be slightly divergent. Some hybrids, morphologically identified as one parental species, may adopt the migration patterns of the other (Proietti et al. 2014b). Furthermore, these hybrids are not likely to be completely inviable since genetic studies using mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) have detected crosses between F1 hybrids and parental species (e.g., Vilaça et al. 2012; Brito et al. 2020; Arantes et al. 2020c).

Factors promoting this high hybridization frequency in Brazil require further investigation, but the broad spatial and temporal overlapping in sea turtle breeding activities, particularly in northeastern Brazil, certainly favors hybridization (Vilaça et al. 2012). Loggerhead and hawksbill breeding activities overlap along the northern coast of Bahia (Fig. 1), which is the largest nesting site for both species in the SWA (Lara-Ruiz et al. 2006, Marcovaldi et al. 2007). Loggerhead nests extend north along the coast of Sergipe State, where they now coincide with olive ridley nests and several hybrids between the two species have been reported in this area (Reis et al. 2010a). Olive ridley nests extend to the southern coast of Alagoas State, where loggerhead nests become sparse, but still occur.

The coast of Alagoas is an important area for sea turtles, harboring extensive coral reefs that act as feeding and development grounds. Currently, five sea turtle species can be found in this region: loggerheads, green turtles, hawksbills, leatherbacks and olive ridleys; although leatherback sightings are rare (Oliveira et al. 2016; Bonfim et al., 2022). Olive ridley nests are frequent in the southernmost portion of Alagoas, while nests of the other species are present throughout the coast of this state. Green turtle nests are rare, but this species uses the coast of Alagoas as a feeding ground extensively. Furthermore, satellite tracking studies have shown that hawksbills and loggerheads from Bahia, as well as olive ridleys from Sergipe nesting sites, usually feed in Alagoas or pass through the region while migrating to northern feeding grounds (Fig. 1A, Marcovaldi et al. 2012).

These conditions may enable interactions among sea turtle species in Alagoas, facilitating hybridization, but so far only one stranded hybrid (between a hawksbill and loggerhead) has been reported in the region (Brito et al. 2020). Based on the conditions presented above, our hypothesis is that the presence of hybrids in the region is highly possible. Therefore, our main goal was to assess hybridization among sea turtle species occurring along the coast of Alagoas using morphology, mtDNA and nDNA data.

4.2 Methods

We used 53 muscle samples collected along the coast of Alagoas (Fig. 1B) by the Instituto Biota de Conservação between May 2019 and April 2021. Samples were taken from stranded turtles, as well as from hatchlings found dead after emergence events. Our sampling focused mainly on hawksbills and loggerheads as more nest samples were available for these species, but we also included olive ridley and green turtle samples for comparative purposes. Twenty-four samples were taken from turtles that were morphologically identified as hawksbills (15 hatchlings and nine stranded turtles), 23 from loggerheads (14 hatchlings and nine stranded turtles), three from olive ridleys (one hatchling and two stranded turtles) and three from green turtles (all stranded turtles). The morphology of stranded turtles and hatchlings was assessed in the field

upon sample collection by staff of Instituto Biota de Conservação and hatchling morphology was also examined in the laboratory. Species were morphologically identified through the examination of scutes on the carapace, inframarginal scutes on the plastron and prefrontal scales on the head (Pritchard and Mortimer, 1999). Each hatchling sample was collected from a different nest.

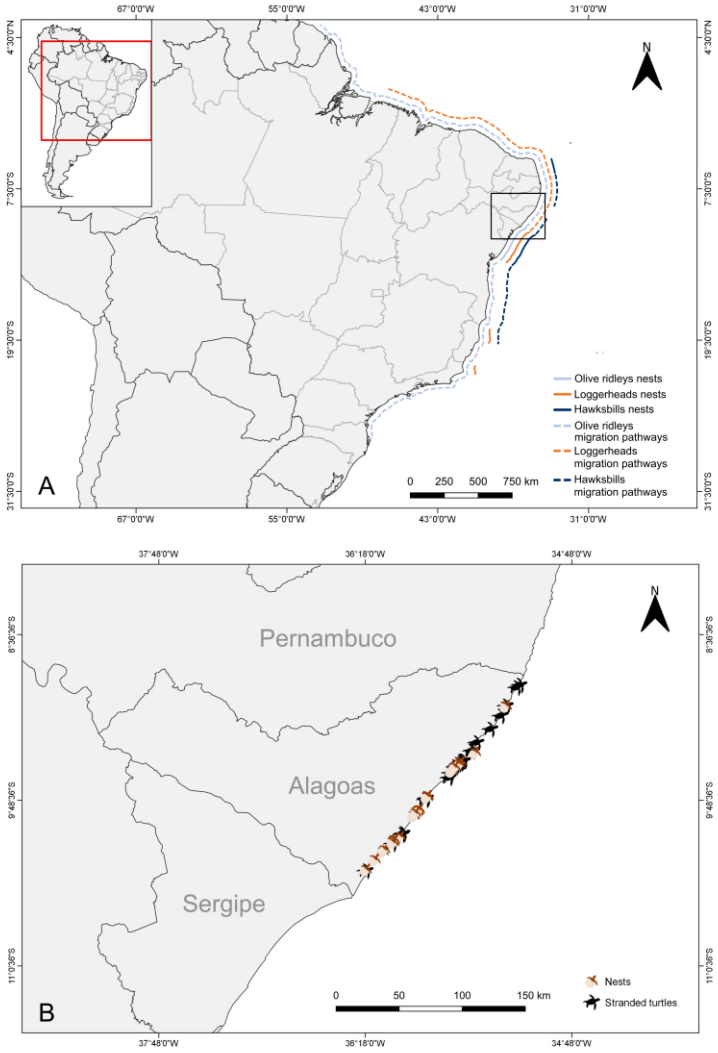


Figure 1. Approximate distribution of the main nesting sites and movement pathways of loggerhead, hawksbill and olive ridley turtles on the coast of the SWA (A). Solid lines indicate nesting sites and dashed lines indicate movement pathways based on satellite tracking studies (Marcovaldi et al. 2010, 2012; Santos et al. 2019; Soares et al. 2021). Sea turtles sampling along the coast of Alagoas State, SWA (B).

Total genomic DNA was extracted using the phenol-chloroform method (Sambrook et al. 1989), and a fragment of 621 base pairs (bp) of the mtDNA control region was recovered through polymerase chain reaction (PCR) using the primers LCM15382 and H950 (Abreu-Gobrois et al. 2006). To better evaluate putative hybrids we also employed three nuclear loci: the oocyte maturation factor mos (CMOS) using the primers LIZ-CMOS and HCMOS-III (Kearney and Stuart 2004) and two anonymous loci, 3061 and 109472 using the primers described by Arantes et al. (2020c). We chose these nuclear loci because they have been shown to present informative variability between loggerhead, hawksbill and olive ridley sea turtles (Vilaça et al 2012; Arantes et al. 2020c). In addition to the primers we used, for the CMOS fragment, we also ran tests with primers developed for sea turtles. We ultimately chose these primers because they provided higher amplification success. We then aligned our CMOS sequences with sequences from GenBank, generated with primers developed for sea turtles, to make sure the fragments overlapped. The CMOS fragment was approximately 550bp while 3061 and 109472 were approximately 320bp each. While mtDNA was sequenced for all samples, nDNA was only amplified for a subset of samples. This subset included all hybrids identified through morphology and mtDNA (see results) and a few representatives of each species (confirmed by morphology and mtDNA) for comparative purposes (Online Resource 1). PCR reactions consisted of 20.8 µl of 1XMaster Mix PCR Buffer with 0.4mM of each dNTP and 3mM of MgCl2, 1.0 µl of each primer (10 pmol); 0.2 µl of Taq DNA polymerase (5 U/µl) and 2 ul of DNA template (10–100 ng/µl). Control region fragments were amplified using the following conditions: initial denaturation at 94°C for 7 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min and a final extension at 72°C for 5 min. Nuclear loci fragments were amplified using the same protocol, except for 109472 annealing temperature which was 58°C. Negative controls were included to check for contamination. Successfully amplified samples were purified with isopropanol and sequenced through Sanger sequencing using the forward primer.

All sequences were checked for contamination using the BLAST tool in GenBank and for some samples we repeated DNA extraction, PCR and sequencing to double check our results. We edited the sequences using Bioedit v7.1.3.0 (Hall 1999) and aligned them with MAFFT v7 using the L-INS-i algorithm (Katoh and Standley 2013). Mitochondrial haplotypes were identified using the Archie Carr Center for Sea Turtle Research database (https://accstr.ufl.edu/resources/mtdna-sequences/) and nuclear sequences were identified using the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Nuclear alleles were reconstructed using the PHASE algorithm implemented in DNAsp v5 (Librado and Rozas 2009). For the purposes of this study, we considered a specimen to be a hybrid when it had the morphology of one species and mtDNA or nDNA of a different species. Additionally, we used nDNA to perform an assignment analysis to determine the most likely association and generation of hybrids. We performed this analysis using three different species pairs: hawksbills and loggerheads, loggerheads and olive ridleys, and hawksbills and olive ridleys. The remaining species were not considered because of their small sample size (see results and Online Resource 1). The analysis was performed using snapclust as implemented in the R package adegenet (Jombart 2008, R Core Team 2021). For each analysis, we set the number of expected clusters to two (k=2), indicated the presence of hybrids between each species pair (hybrids=TRUE) and specified the hybridization coefficient for F1 and first-generation backcross (hybrid.coef = c(.5, .25)). All other parameters were run as default.

4.3 Results

Overall, it was possible to identify all specimens based on the morphological characteristics of each species. However, some specimens were degraded or exhibited morphological characteristics of more than one species (see detailed results below). Mitochondrial haplotypes of all 53 samples were successfully identified according to the Archie Carr Center database, meaning that no new haplotypes were found (Online resource 1). Amplification of nDNA was less effective than mtDNA, particularly for

stranded turtles, likely due to the lower abundance of nDNA coupled with sample degradation caused by long environmental exposure. Consequently, we were only able to recover one locus for most samples (Online Resource 1). In total, we identified nine hybrids out of 53 samples, three (5.6%) with only one source of evidence (weakly supported) and six (11.3%) with more than one source of evidence (strongly supported). Five hybrids were hatchlings from local nests and four were stranded turtles (Table 1). Details on hybridization and overall genetic characterization are given below.

4.3.1 Hybridization

We identified four hybrids from nest samples based solely on morphology and mtDNA. One sample was a hatchling identified as a hawksbill (T6R40), which had the CC-A4 haplotype, typical of loggerheads. The three remaining hybrids (T4R14, T9R1-2019 and MIR1) had loggerhead morphology and the haplotype-F, unique to olive ridleys. Specimen T9R1-2019 was from an olive ridley nest but exhibited malformations and asymmetry of lateral scute counts, five on the right side and seven on the left side (Online resource 2). Regarding the nuclear dataset, we successfully recovered sequences from the three analyzed loci for all four hybrids. The hawksbill x loggerhead hybrid (T6R40) had alleles of both species at the 3061 and 109472 loci, but only hawksbill alleles at CMOS. One of the three loggerhead x olive ridley hybrids (T9R1-2019) only had olive ridley alleles at the three nuclear loci. The second (T4R14) only had loggerhead alleles at 3061, but alleles of both parental species at 109472 and CMOS. The last sample (MIR1) only had loggerhead alleles at 3061 and CMOS, and alleles of both species at 109472.

Using nDNA, we were also able to identify one more hybrid from nests (T9R1-2020), not detected with mtDNA or morphology. The new hybrid had hawksbill morphology and mtDNA (Ei-BR16) but exhibited an olive ridley allele at the 109472 locus while having only hawksbill alleles at 3061 and CMOS (Table 1). This specimen was classified as a hybrid with weak evidence because it had a single olive ridley allele, while morphology and all other genetic evidence indicated that it was a hawksbill.

Nevertheless, this specimen also exhibited dorsal scute malformations (Online resource 2). Membership probabilities of nest hybrids indicated that T6R40 was likely an F1 hybrid (46.9%), T4R14 was likely a loggerhead (41.8%) or a backcross of a F1 hybrid and a loggerhead (40.4%), MIR1 likely a loggerhead (58.1%) and that T9R1-19 was likely an olive ridley (75.6%, Fig. 2). Membership probabilities of T9R1-2020 were higher for hawksbills (55.8%) and for a backcross between an F1 hybrid and a hawksbill (35.7%, Fig. 2).

Among stranded turtles, one of the four hybrids was identified as a hawksbill (T4T363), but had the loggerhead CC-A4 haplotype. The second hybrid had loggerhead morphology, and the CM-A8 haplotype (T5T279), typical of green turtles. The third hybrid had green turtle morphology and the CC-A4 haplotype (T4T8) from loggerheads. The last hybrid (T3T68) was only identified by nDNA. While this specimen had hawksbill morphology and mtDNA, it presented a loggerhead allele at CMOS. Nuclear data from the other hybrids revealed that the hawksbill x loggerhead hybrid (T4T363) only had hawksbill alleles at 3061. One green turtle x loggerhead hybrid (T5T279) only had loggerhead alleles at both 3061 and CMOS loci, while the other sample (T4T8) also only had loggerhead alleles at CMOS (Online Resource 1).

Membership probabilities of T3T68 and T4T363 were higher for loggerheads (42.5%) and hawksbills (52.9%), respectively (Fig. 2). Membership probabilities of green turtle x loggerhead hybrids were not estimated due to the low recovery of green turtle alleles.

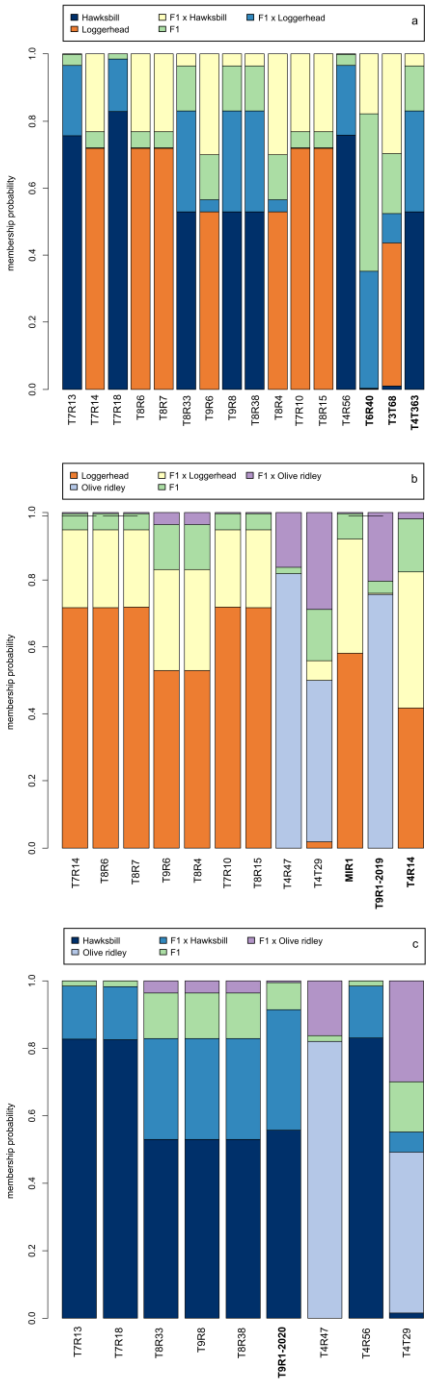


Figure 2. Membership probabilities of hawksbill, loggerhead and olive ridley specimens and their hybrids as recovered using nuclear loci 3061, 109472 and CMOS. Probabilities were estimated for three pairs of species: hawksbills and loggerheads (A), olive ridleys and loggerheads (B) and hawksbills and olive ridleys (C). Hybrids are indicated in bold.

4.3.2 Genetic characterization

Except for the five hybrids, all remaining nest samples (n=25) exhibited haplotypes from their respective species. Eleven hatchlings, identified as hawksbills, exhibited the EiA01 haplotype, one had the Ei-BR16 haplotype and one had the Ei-BR10 haplotype, all typical of the species. All 11 non-hybrid loggerhead hatchlings had the CC-A4 haplotype, typical of the species. The single hatchling identified as olive ridley had the haplotype-F, which is also unique to this species (Online Resource 1). All non-hybrid samples evaluated for 3061 (total N=16), 109472 (total N=11) and CMOS (total N=6) had alleles compatible with their morphological identifications (Online Resource 1).

The eight stranded turtles with hawksbill morphology had haplotypes typical of the species: Ei-A01 (4), Ei-BR10 (2), Ei-BR16 (1) and Ei-IP17 (1). The latter is commonly found in hawksbills from Indo-Pacific nesting sites in the Seychelles Islands and Chagos archipelago. To corroborate the identification of this sample, we performed DNA extraction, PCR and sequencing a second time and the same haplotype was recovered. All eight non-hybrid stranded turtles identified as loggerheads had the CC-A4 haplotype, characteristic of this species. The two turtles identified as olive ridley had the haplotype-F and the two green turtles had the CM-A8 haplotype, both unique to each species (Online Resource 1).

4.4 Discussion

In this study, we contribute to the current knowledge on sea turtle hybridization in the SWA. Here, we found hybrids among four sea turtle species: loggerheads, hawksbills, green turtles and olive ridleys. We also expanded sampling on loggerheads and hawksbills in understudied nesting areas in Alagoas and observed that the genetic profile of these species is very similar to what is found in other nesting sites in the SWA (Lara-Ruiz et al. 2006; Reis et al. 2010b). Remarkably, we observed a hawksbill haplotype typical of the Indo-Pacific in the Alagoas feeding ground. This is not the first time an Indo-Pacific haplotype has been observed in the Atlantic, which reinforces the

connection between these regions (Arantes et al. 2020b). Below we discuss these topics in detail.

4.4.1 Hybridization

Hybrids among sea turtle species have already been reported in the SWA (Fig. 3), where loggerhead x hawksbill and loggerhead x olive ridley hybrids are particularly more frequent (Lara-Ruiz et al. 2006; Reis et al. 2010a; Proietti et al. 2014a; Brito et al. 2020). Although the causes for the high hybridization frequency between these species are still not completely clear, the temporal and spatial overlapping of their breeding activities likely facilitates this process (Reis et al. 2010a). While breeding activities of these species are still understudied in Alagoas, it is possible to find nests of these three species along the coast of this state (Oliveira et al. 2016), which may facilitate hybridization between them. In fact, all hybrids between loggerheads and olive ridleys (3) found here were from nests. All of these samples had loggerhead morphology and olive ridley mtDNA, the same pattern observed in hybrids from Sergipe nesting site (Reis et al. 2010a). The two remaining hybrids from Alagoas nests had hawksbill morphology. One had loggerhead mtDNA (CC-A4, T6R40), the same pattern found in most hybrids from Bahia (Lara-Ruiz et al. 2006), and the other had an olive ridley allele in the 109472 nuclear locus (T9R1-2020). Hybridization between hawksbills and olive ridleys have been reported in the SWA before, but this appears to be much less frequent (Lara-Ruiz et al. 2006; Brito et al. 2020). Nuclear data from nest samples also revealed that these hybrids are likely F1 or backcrosses with parental species (Fig. 2), which indicates that hybridization may be an ongoing process in the region.

Among the stranded turtle hybrids, two had hawksbill morphology. One specimen had loggerhead mtDNA (T4T363) while the other (T3T68) had loggerhead nDNA that matched an Indo-Pacific loggerhead allele (as did its hawksbill mtDNA). The only other hybrid previously reported from Alagoas was a hybrid between these species, a stranded turtle with hawksbill morphology and loggerhead mtDNA (CC-A4) (Brito et al. 2020). However, both species use the Alagoas coastline as both feeding and

reproductive grounds, thus it is difficult to determine how these stranded specimens were using this area. It is plausible that the T4T363 specimen could have originated from the Bahia nesting site, since a particularly high frequency of hawksbill x loggerhead hybrids have been reported there (Lara-Ruiz et al. 2006) and individuals from this nesting site are also reported to migrate through Alagoas (Marcovaldi et al. 2012). On the other hand, the loggerhead Indo-Pacific allele we found in the T3T68 specimen, seems to reinforce that this specimen was indeed from that region (see detailed discussion below).

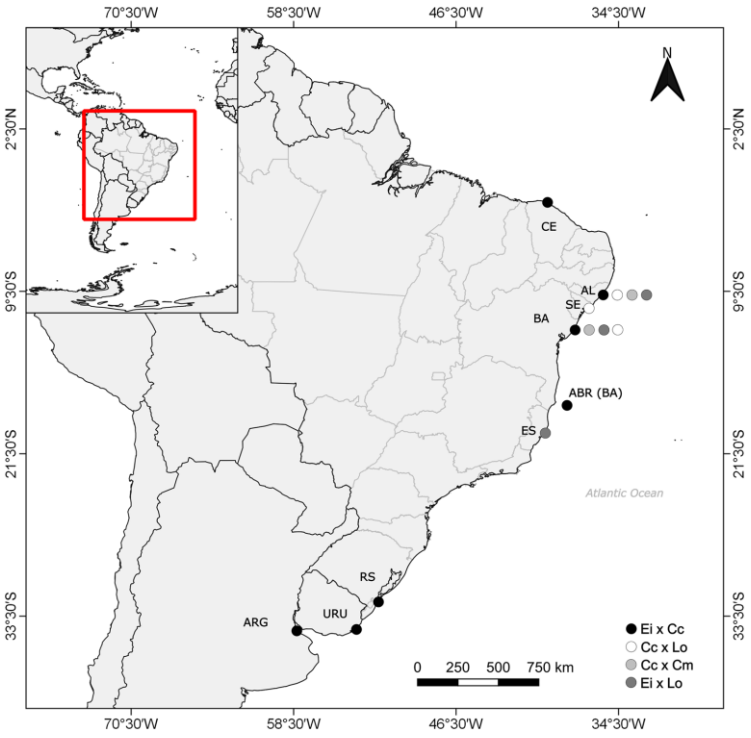


Figure 3. Known reports of sea turtle hybrids in the Southwest Atlantic Ocean. ARG – Argentina, URU – Uruguay, Brazilian States: *AL* Alagoas, *BA* Bahia, *ABR* Abrolhos Archipelago, Bahia, *CE* Ceará, *RS* Rio Grande do Sul, *SE* Sergipe. Source of hybrid records: Brito et al. (2020): AL, BA, CE, ES, RS, URU; Karl et al. (1995): BA; Lara-Ruiz et al. (2006): BA; Proietti et al. (2014b): CE, RS; Prosdocimi et al. (2014): ARG; Reis et al. (2010a): SE; This study: AL.

The remaining stranded hybrids (2) identified through mtDNA were crosses between green and loggerhead turtles. Hybrids between these species are less common in the SWA, probably due to the low overlapping in their nesting activities. While loggerhead nests are mainly found on the Brazilian mainland coast, green turtle nests are mostly concentrated on oceanic islands, such as Rocas Atoll and Trindade, and are sparse on continental areas within the SWA (Marcovaldi and Marcovaldi 1999; Marcovaldi and Chaloupka 2007). Nevertheless, some green turtle nests can be found along the Brazilian coast, mainly in the northern region of Bahia, the main nesting site for loggerheads in Brazil (Lara-Ruiz et al. 2006). So, it is plausible that this region is the probable origin of these hybrids.

Understanding the role hybrids play in sea turtle population structure is particularly important given their status as threatened species (IUCN 2022), especially when we take climate change effects into consideration. Many sea turtle populations are already reported to have strong female bias (Hays et al. 2014; Jensen et al. 2018), which tends to be even more exacerbated with the predicted rise of global temperatures (IPCC 2021). Higher nesting beach temperatures could not only promote higher female output, and consequently higher female proportions in natural populations, but also increase hatchling mortality (Hays et al. 2017). Furthermore, the decrease in beaches available for nesting due to sea level rise and coastal urbanization can potentially cause shifts in habitat use (Fuentes et al. 2010, 2011), which can promote further overlapping of breeding and nesting activities of these species. If these environmental and anthropogenic factors act synergistically, we may likely observe an increase in hybridization frequency over time. Thus, the continuous monitoring of ecological and genetic aspects of these populations is fundamental.

The use of a multilocus approach to investigate hybridization in these populations has been shown to be essential for improving our understanding of the hybridization process (Vilaça et al. 2012; Brito et al. 2020; Arantes et al. 2020c). Here, we were only able to use three nDNA loci, which precludes us from reaching more conclusive results, particularly on hybrid generation. The lower success rate in the amplification of these

loci in stranded specimens, likely due to sample deterioration, also limited our interpretation of these data. The use of nonspecific primers for the CMOS gene also warrants caution in the interpretation of these data. However, all but one allele observed in this locus have been identified before using sea turtle primers. Only one hybrid was defined by CMOS data, all other hybrids can be identified using mtDNA or the other two nDNA loci (Table 1, Online Resource 1). Despite these limitations, the inclusion of nDNA allowed us to identify hybrids that would otherwise not have been observed solely using mtDNA and morphology. Nevertheless, as suggested by previous studies, a better comprehension of hybrid ecology is required to understand how this high hybridization frequency along the Brazilian coast can affect population dynamics (Vilaça et al. 2012; Arantes et al. 2020c).

Studies on the spatial distribution of nesting and feeding grounds, as well as genetic diversity are initial in Alagoas. Consequently, information on breeding periodicity, sex ratios and comprehensive genetic characterizations are still unavailable. Nevertheless, sex ratio studies on loggerhead and hawksbill turtles nesting on the Brazilian coast indicate high female bias (Marcovaldi et al. 1997; Godfrey et al. 1999), thus it is likely that future studies would reveal a similar pattern for Alagoas. Therefore, constant monitoring of this population, regarding shifts in habitat use and population parameters, is extremely important in order to better understand the consequences of hybridization and thereby, improve conservation actions.

4.4.2 Genetic characterization

The genetic diversity of hawksbill and loggerhead nests in the study area was similar to other reproductive areas in the SWA. The CC-A4 haplotype, observed in all non-hybrid loggerhead nests (11), is widely found in loggerhead nesting sites in Brazil (Reis et al. 2010b). Likewise, among the three haplotypes we identified in non-hybrid hawksbill nests (14), the Ei-A01 is widely distributed throughout feeding grounds in the SWA and in the two major hawksbill nesting sites in Brazil: Bahia and Rio Grande do Norte (Proietti et al. 2014a; Simões et al. 2021). The two other haplotypes, Ei-BR16 and

Ei-BR10, are both exclusive to Brazilian nesting sites (Lara-Ruiz et al. 2006). Additionally, the single olive ridley nest sample had the haplotype-F, which is the only haplotype observed for olive ridleys in the SWA to date (Bowen et al. 1997).

We observed that all stranded loggerheads (9) had the same CC-A4 haplotype found in the nests. As mentioned above, this haplotype is the most commonly observed in loggerhead nesting and feeding sites in Brazil and is also exclusive to this region (Reis et al. 2010b). This low haplotype diversity is also in accordance with previous studies and the presence of this exclusive Brazilian haplotype reinforces that the specimens analyzed here likely originated from Brazilian nesting sites (Reis et al. 2010b). We observed a similar genetic profile in stranded hawksbills with haplotypes commonly found in Brazilian nesting sites: Ei-BR10 (2), Ei-BR16 (1) and Ei-A01 (1) (Lara-Ruiz et al. 2006; Proietti et al. 2014a; Simões et al. 2021). Finally, the presence of the Ei-IP17 haplotype was surprising since this haplotype is only found in Indo-Pacific nesting sites (Vargas et al. 2016), implications of which are discussed below.

In general, the genetic profile we observed for both species suggests that feeding grounds are mostly occupied by individuals from local nesting sites. Satellite tracking studies also suggest that the study area is within a migratory corridor for loggerheads and hawksbills migrating from their main nesting area in Bahia to feeding grounds farther north, being also the final destination for some of these individuals (Marcovaldi et al. 2010, 2012).

The occurrence of the Ei-IP17 haplotype among our stranded samples was a surprising and novel result, since this haplotype is typical for Indo-Pacific nesting sites in the Seychelles Islands and Chagos Archipelago (Vargas et al. 2016), suggesting a connection between the Atlantic and Indo-Pacific Oceans. To our knowledge, this is the first time the Ei-IP17 haplotype has been reported in an Atlantic feeding ground. Two other Indo-Pacific haplotypes, Ei-IP16 and EI-IP33, have been previously reported in feeding grounds in Fernando de Noronha and Ascension Island (Fig. 4, Arantes et al. 2020b). Additionally, three orphan haplotypes (Ei-A49, Ei-A70 and Ei-A75) observed in

the Atlantic feeding grounds of Ascension Island, Fernando de Noronha, Cape Verde and Principe Island, group together with haplotypes from the Indo-Pacific (Arantes et al. 2020b). The same occurs with the EATL haplotype observed in the Principe Island nesting site in Africa (Monzón-Argüello et al. 2011; Arantes et al. 2020b).

Haplotype sharing between Atlantic and Indo-Pacific Oceans can also be seen in loggerhead, green and leatherback turtles (Dutton et al. 1999; Bourjea et al. 2007; Shamblin et al. 2014), and migrations in both directions through southern Africa have been suggested. For instance, the CM-A8 haplotype, widely found in green turtle nesting sites in the Atlantic, can also be found in the Mozambique nesting site (Bourjea et al. 2007), a similar pattern to that of the loggerhead CC-A2 haplotype (Shamblin et al. 2014). Loggerhead haplotypes from the Indo-Pacific have also been observed in the Atlantic, suggesting that westward migrations may also occur (Shamblin et al. 2014). Colonization of the Atlantic by olive ridleys is suggested to have occurred through southern Africa (Bowen et al. 1997). Likewise, haplotype sharing between hawksbill nesting sites in Principe Island and in the Indo-Pacific led Monzón-Argüello et al. (2011) to suggest the colonization of this east African nesting site by hawksbill migrants from the Indo-Pacific. Thus, although it seems plausible that the Ei-IP17 haplotype found here could have originated directly from the Indo-Pacific, we cannot disregard putative unsampled nesting sites in east Africa as a possible origin, since hawksbill haplotypes from such sites have already been found in SWA feeding grounds (Proietti et al. 2014a).

The hawksbill sample analyzed here was from a juvenile male (curved carapace length of 42.1cm), which could indicate an occasional incursion. We also observed Indo-Pacific alleles in the CMOS locus of seven additional hawksbill, loggerhead and olive ridley samples (Online Resource 1). Due to the generally slower evolutionary rates of nuclear genes, these alleles may have persisted at low frequencies in Atlantic sea turtle populations after their separation from Indo-Pacific lineages. On the other hand, this may evidence that at least some gene flow between Atlantic and Indo-Pacific sea turtles still exists. Nevertheless, wider sampling of feeding and nesting grounds in the Atlantic is

required to help elucidate hawksbill and other sea turtle population structure and migration pathways.

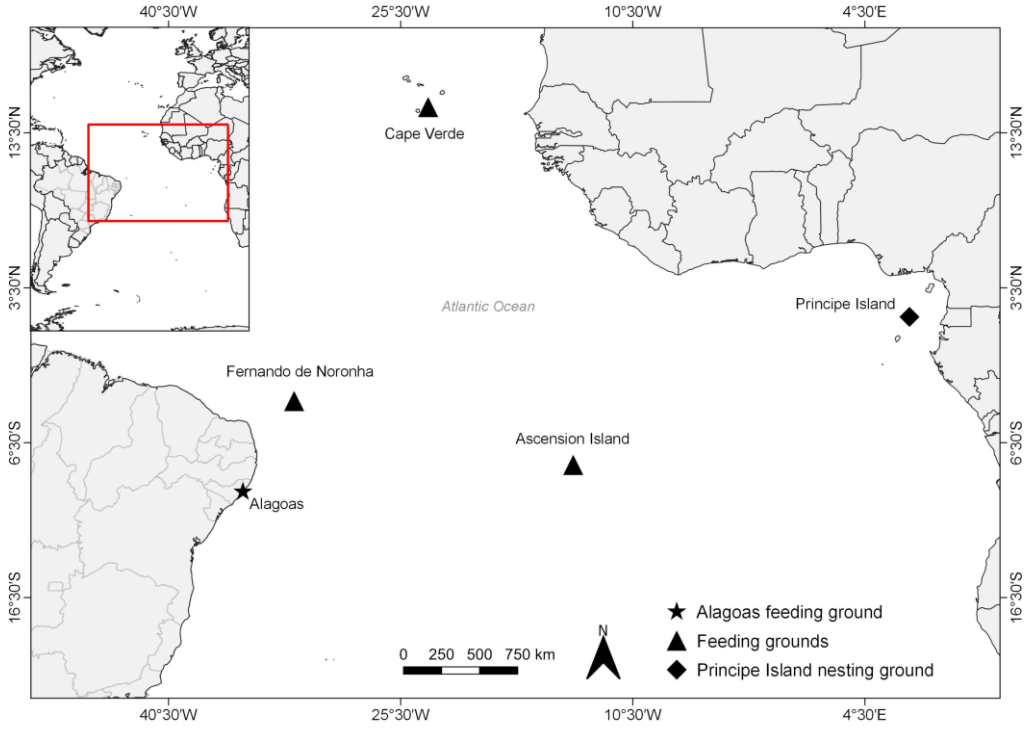


Figure 4. Known occurrences of hawksbill Indo-Pacific haplotypes in south Atlantic feeding and nesting sites. Star denotes the study site in the state of Alagoas, northeastern Brazil.

4.5 Concluding remarks

Although relatively rare in sea turtles, hybridization seem to be very common in Brazilian nesting and feeding grounds (Lara-Ruiz et al. 2006; Brito et al. 2020, our study). Although our total sample size was relatively small, we were still able to detect hybrids in Alagoas nests, as well as in stranded animals (11.3% of our total sampling), including putative crosses between hybrids and parental species. This suggests that hybridization events may be common in the region, as seen in other sites in the SWA, such as Sergipe and Bahia (Lara-Ruiz et al. 2006; Reis et al. 2010a). Most hybrids were readily identified using only morphology and mtDNA, however the use of nuclear data revealed new hybrids that would otherwise remain unidentified, which highlights the

importance of using an integrative approach when studying hybridization (Vilaça et al. 2012; Brito et al. 2020).

The use of mtDNA and nDNA also revealed a possible connection between feeding grounds in the study area and nesting sites in the Indo-Pacific. Understanding these connections and migratory pathways is essential to the development of appropriate conservation strategies and is one of the main priorities in sea turtle research (Hamann et al. 2010). Although a more comprehensive research effort is required to clarify the connections between sea turtles in the Atlantic and Indo-Pacific, our findings represent the fifth hawksbill locality in the South Atlantic with Indo-Pacific haplotypes (Arantes et al. 2020b), reinforcing the connection between these regions.

4.6 Acknowledgments

JPFAA thanks FAPEAL for providing PhD scholarship (#23038.023347/2016-74). TM thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (309904/2015-3 and 312291/2018-3) for financial support. We thank Camila Domit, Maíra Proietti and Kim Barão for kindly read and comment on an earlier version of the manuscript. We also thank the editor and two anonymous reviewers for constructive comments on the manuscript.

4.7 Author contributions

João P. F. A. Almeida, Tamí Mott and Robson G. Santos contributed to the study conception and design. Oscar K. L. Marques participated in sample collection and material preparation. Sample processing and data analysis were performed by João P. F. A. Almeida. The first draft of the manuscript was written by João P. F. A. Almeida and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

4.8 Funding

This study was partially funded by Fundação Grupo Boticário de Proteção da Natureza (#1143/20182) and PADI Foundation (#47777/2020). Beach monitoring and sample collection was partially supported by Long Term Ecological Research – Brazil site PELD-CCAL funded by the Brazilian National Council for Scientific Research and Technological Development (CNPq – #441657/2016-8, #442237/2020-0) and the Research Support Foundation of the State of Alagoas (FAPEAL – #60030.1564/2016, #PLD2021010000001).

4.9 Data availability

All data generated in this study is provided in the article and online resources or was described previously and is already available at the GenBank database.

References

Abbott R, Albach D, Ansell S et al (2013) Hybridization and speciation. J Evol Biol 26: 229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x

Abreu-Grobois FA, Horrocks J, Formia A, LeRoux R, Velez-Zuazo X, Dutton P, Soares L, et al. (2006). New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Book of Abstracts of the 26th Annual Symposium on Sea Turtle Biology and Conservation, pp 179.

Arantes LS, Ferreira LCL, Driller M, Repinaldo Filho FPM, Mazzoni CJ, Santos FR (2020a) Genomic evidence of recent hybridization between sea turtles at Abrolhos Archipelago and its association to low reproductive output. Sci Rep 10:1–13. https://doi.org/10.1038/s41598-020-69613-8

Arantes LS, Vargas SM, Santos FR (2020b) Global phylogeography of the critically endangered hawksbill turtle (Eretmochelys imbricata). Genet Mol Biol 43:1–12. https://doi.org/10.1590/1678-4685-GMB-2019-0264

Arantes LS, Vilaça ST, Mazzoni CJ, Santos FR (2020c) New genetic insights about hybridization and population structure of hawksbill and loggerhead turtles from Brazil. J Hered 111:444–456. https://doi.org/10.1093/jhered/esaa024

Bonfim WAG, Oliveira BSSP, Santos CRM, Medeiros LS, La Fuente MF (2022) First live stranding of a leatherback sea turtle Dermochelys coriacea in Alagoas, north-east Brazil. Herpetol Bull 160: 35–37. https://doi.org/10.33256/hb160.3537

Bolten AB, Lutz PL, Musick JA, Wyneken J (2003) Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. In: Lutz PL, Musick JA, Wyneken J (Eds) The biology of sea turtles, 2nd edn. CRC Press, Boca Raton, pp 243-257

Bourjea J, Lapègue S, Gagnevin L, Broderick D, Mortimer JA, Ciccione S, Roos D, Taquet C, Grizel H (2007) Phylogeography of the green turtle, Chelonia mydas, in the Southwest Indian Ocean. Mol Ecol 16:175–186. https://doi.org/10.1111/j.1365-294X.2006.03122.x

Bowen BW, Clark AM, Chaves A, Reichart HA, Ferl RJ, Rica UDC, Heredia SP, Rica C, Service SF (1997) Global phylogeography of the ridley sea turtles (Lepidochelys spp.) as inferred from mitochondrial DNA sequences. Genetica 101:179–189. https://doi.org/10.1023/a:1018382415005

Brito C, Vilaça ST, Lacerda AL, Maggioni R, Marcovaldi MA, Vélez-Rubio G, Proietti MC (2020) Combined use of mitochondrial and nuclear genetic markers further reveal immature marine turtle hybrids along the south western Atlantic. Genet Mol Biol 43:1–11. https://doi.org/10.1590/1678-4685-GMB-2019-0098

Colman LP, Thomé JCA, Almeida ADP, Baptistotte C, Barata PCR, Broderick AC, Ribeiro FA, Vila-verde L, Godley BJ (2019) Thirty years of leatherback turtle Dermochelys coriacea nesting in Espírito Santo, Brazil, 1988−2017: reproductive biology and conservation. Endang Species Res 39:147–158.

Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK (1999) Global phylogeography of the leatherback turtle (Dermochelys coriacea). J Zool 248:397–409. https://doi.org/10.1017/S0952836999007116

Fuentes MMPB, Limpus CJ, Hamann M, Dawson J (2010) Potential impacts of projected sea-level rise on sea turtle rookeries. Aquat Conserv Mar Freshw Ecosyst 20:132–139. https://doi.org/10.1002/aqc.1088

Fuentes MMPB, Limpus CJ, Hamann M (2011) Vulnerability of sea turtle nesting grounds to climate change. Glob Chang Biol 17:140–153. https://doi.org/10.1111/j.1365-2486.2010.02192.x

Godfrey MH, D'Amato AF, Marcovaldi MA, Mrosovsky N (1997) Pivotal temperature and predicted sex ratios for hatchling hawksbill turtles from Brazil. Can J Zool 77:1465–1473

Hall, TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hamann M, Godfrey MH, Seminoff JA, Arthur K, Barata PCR, Bjorndal KA, Bolten AB, Broderick AC, Campbell LM, Carreras C, Casale P, Chaloupka M, Chan SKF, Coyne MS, Crowder LB (2010) Global research priorities for sea turtles : informing management and conservation in the 21st century. Endang Species Res, 11:245–269. https://doi.org/10.3354/esr00279

Hays GC, Mazaris AD, Schofield G (2014) Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Front Mar Sci 1:1–9. https://doi.org/10.3389/fmars.2014.00043

Hays GC, Mazaris AD, Schofield G, Laloë JO (2017) Population viability at extreme sex-ratio skews produced by temperaturedependent sex determination. Proc R Soc B Biol Sci 284:1–7. https://doi.org/10.1098/rspb.2016.2576

ICMBio (2017) Plano de Ação Nacional para Conservação das Tartarugas Marinhas. https://www.icmbio.gov.br/portal/faunabrasileira/plano-de-acao-nacional-lista/841-plano-de-acao-nacional-para-a-conservacao-das-tartarugas-marinhas. Accessed 26 March 2022

Intergovernmental Panel on Climate Change (2021). Climate Change 2021: Synthesis Report. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team]. Ed. by Jager-Waldau A and Sapkota T. IPCC, Geneva, Switzerland.

Jensen MP, Allen CD, Eguchi T, Bell IP, LaCasella EL, Hilton WA, Hof CAM, Dutton PH (2018) Environmental Warming and Feminization of One of the Largest Sea Turtle Populations in the World. Curr Biol 28:154–159. https://doi.org/10.1016/j.cub.2017.11.057

Jombart T (2008) Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jordão JC, Bondioli ACV, Guebert FM, de Thoisy B, de Toledo LFA (2015) Green turtle (Chelonia mydas) genetic diversity at Paranaguá Estuarine complex feeding grounds in Brazil. Genet Mol Biol 38:346–352. https://doi.org/10.1590/S1415-475738320140353

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kearney M, Stuart BL (2004) Repeated evolution of limblessness and digging heads in worm lizards revealed by DNA from old bones. Proc R Soc London B 271:1677–1683.

Lara-Ruiz P, Lopez GG, Santos FR, Soares LS (2006) Extensive hybridization in hawksbill turtles (Eretmochelys imbricata) nesting in Brazil revealed by mtDNA analyses. Conserv Genet 7:773–781. https://doi.org/10.1007/s10592-005-9102-9

Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Marcovaldi M, Chaloupka M (2007) Conservation status of the loggerhead sea turtle in Brazil: an encouraging outlook. Endanger Species Res 3:133–143. https://doi.org/10.3354/esr003133

Marcovaldi MA, Godfrey MH, Mrosovsky N (1997) Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. Can J Zool 75: 755–770

Marcovaldi M, Lopez G, Soares L, López-Mendilaharsu M (2012) Satellite tracking of hawksbill turtles Eretmochelys imbricata nesting in northern Bahia, Brazil: turtle movements and foraging destinations. Endanger Species Res 17:123–132. https://doi.org/10.3354/esr00421

Marcovaldi MA, Lopez GG, Soares LS, Santos AJB, Bellini C, Barata PCR (2007) Fifteen years of Hawksbill Sea Turtle (Eretmochelys imbricata) nesting in Northern Brazil. Chelonian Conserv Biol 6:223–228. https://doi.org/10.2744/1071-8443(2007)6[223:FYOHST]2.0.CO;2

Marcovaldi MA, Dei Marcovaldi GG (1999) Marine turtles of Brazil: The history and structure of Projeto TAMAR-IBAMA. Biol Conserv 91:35–41. https://doi.org/10.1016/S0006-3207(99)00043-9

Marcovaldi MA, Lopez GG, Soares LS, Lima EHSM, Thomé JCA, Almeida AP (2010) Satellite-tracking of female loggerhead turtles highlights fidelity behavior in northeastern Brazil. Endanger Species Res 12:263–272. https://doi.org/10.3354/esr00308

Monzón-Argüello C, Loureiro NS, Delgado C, Marco A, Lopes JM, Gomes MG, Abreu-Grobois FA (2011) Príncipe island hawksbills: Genetic isolation of an eastern Atlantic stock. J Exp Mar Bio Ecol 407:345–354. https://doi.org/10.1016/j.jembe.2011.07.017

Naro-Maciel E, Bondioli ACV, Martin M, De Pádua Almeida A, Baptistotte C, Bellini C, Marcovaldi MA, Santos AJB, Amato G (2012) The interplay of homing and dispersal in green turtles: A focus on the southwestern atlantic. J Hered 103:792–805. https://doi.org/10.1093/jhered/ess068

Oliveira BSSP, Bonfim WAG, Santos CRM, Santos SM, Silva EA, Medeiros LS, Santiago MC, Marques OKL, Ferreira AA, Izidoro FB, Santos MEF, Melo LL, Silva FMO (2016). Instituto Biota de Conservação: Pesquisa e Conservação de Tartarugas

Marinhas no Estado de Alagoas. In: Correia JMS, Santos EM, Moura GJB (eds) Conservação de Tartarugas Marinhas no Nordeste do Brasil. Editora Universitária da UFRPE, Recife, pp 169–190

Pritchard PCH, Mortimer JA (1999) Taxonomy, external morphology, and species identification. In: Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M (Eds) Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4, Washington, D.C., pp 21–38

Proietti MC, Reisser J, Marins LF, Rodriguez-Zarate C, Marcovaldi MA, Monteiro DS, Pattiaratchi C, Secchi ER (2014a) Genetic structure and natal origins of immature hawksbill turtles (Eretmochelys imbricata) in Brazilian waters. PLoS One 9:e88746. https://doi.org/10.1371/journal.pone.0088746

Proietti MC, Reisser J, Marins LF, Marcovaldi MA, Soares LS, Monteiro DS, Wijeratne S, Pattiaratchi C, Secchi ER (2014b) Hawksbill × loggerhead sea turtle hybrids at Bahia, Brazil: Where do their offspring go? PeerJ 2014:1–14. https://doi.org/10.7717/peerj.255

Prosdocimi L, Bruno I, Diaz L, Carman VG, Albareda DA, Remis MI (2014) Southernmost report of the hawksbill sea turtle, Eretmochelys imbricata, in temperate waters of Argentina and evidence of a hybrid origin supported by mitochondrial DNA analysis. Herpetol Rev 45:1–5.

R Core Team (2021). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing

Reis EC, Soares LS, Lôbo-Hajdu G (2010a) Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conserv Genet 11:1587–1591. https://doi.org/10.1007/s10592-009-9973-2

Reis EC, Soares LS, Vargas SM, Santos FR, Young RJ, Bjorndal KA, Bolten AB, Lôbo-Hajdu G (2010b) Genetic composition, population structure and phylogeography of the loggerhead sea turtle: Colonization hypothesis for the Brazilian rookeries. Conserv Genet 11:1467–1477. https://doi.org/10.1007/s10592-009-9975-0

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Santos EAP, Silva ACCD, Sforza R, Oliveira FLC, Weber MI, Castilhos JC, López-Mendilaharsu M, Marcovaldi MAAG, Ramos RMA, DiMatteo A (2019) Olive ridley inter-nesting and post-nesting movements along the Brazilian coast and Atlantic Ocean. Endanger Species Res 40:149–162. https://doi.org/10.3354/ESR00985

Shamblin BM, Bolten AB, Abreu-Grobois FA, Bjorndal KA, Cardona L, Carreras C, Clusa M, Monzón-Argüello C, Nairn CJ, Nielsen JT, Nel R, Soares LS, Stewart KR, Vilaça ST,

Türkozan O, Yilmaz C, Dutton PH (2014) Geographic patterns of genetic variation in a broadly distributed marine vertebrate: New insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. PLoS One 9:e85956. https://doi.org/10.1371/journal.pone.0085956

Simões TN, Maranhão E, Santos AS, Thaynara E, Menezes C De, Chimendes V (2021) Low Diversity and Strong Genetic Structure Between Feeding and Nesting Areas in Brazil for the Critically Endangered Hawksbill Sea Turtle. Front Ecol Evol 9:704838. https://doi.org/10.3389/fevo.2021.704838

Soares LS, Bolten AB, Wayne ML, Vilaça ST, Santos FR, dei Marcovaldi MAG, Bjorndal KA (2017) Comparison of reproductive output of hybrid sea turtles and parental species. Mar Biol. 164:9. https://doi.org/10.1007/s00227-016-3035-3

Soares LS, Bjorndal KA, Bolten AB, Wayne ML, Castilhos JC, Weber MI, López-Mendilaharsu M, Marcovaldi MA, Vilaça ST, Naro-Maciel E (2021) Reproductive output, foraging destinations, and isotopic niche of olive ridley and loggerhead sea turtles, and their hybrids, in Brazil. Endanger Species Res 44:237–251. https://doi.org/10.3354/ESR01095

Todesco M, Pascual MA, Owens GL, Ostevick KL, Moyers BT, Hubner S, Heredia SM, Hahn MA, Caseys C, Bock DG, Rieseberg LH (2016) Hybridization and extinction. Evol Appl 9: 892–908. https://doi.org/10.1111/eva.12367

Vargas SM, Jensen MP, Ho SYW, Mobaraki A, Broderick D, Mortimer JA, Whiting SD, Miller J, Prince RIT, Bell IP, Hoenner X, Limpus CJ, Santos FR, Fitzsimmons NN (2016) Phylogeography, genetic diversity, and management units of hawksbill turtles in the indo-pacific. J Hered 107:199–213. https://doi.org/10.1093/jhered/esv091

Vargas SM, Lins LSF, Molfetti E, Ho SYW, Monteiro D, Barreto J, Colman L, Vila-Verde L, Baptistotte C, Thomé JCA, Santos FR (2019) Revisiting the genetic diversity and population structure of the critically endangered leatherback turtles in the South-west Atlantic Ocean: insights for species conservation. J. Mar. Biol. Assoc. U. K. 99:31–41. https://doi:10.1017/S002531541700193X

Vilaça ST, Vargas SM, Lara-Ruiz P, Molfetti É, Reis EC, Lôbo-Hajdu G, Soares LS, Santos FR (2012) Nuclear markers reveal a complex introgression pattern among marine turtle species on the Brazilian coast. Mol Ecol 21:4300–4312. https://doi.org/10.1111/j.1365-294X.2012.05685.x

Wallace BP, Dimatteo AD, Hurley BJ, Finkbeiner EM, Alan B, Chaloupka MY, Hutchinson BJ, Abreu-grobois FA, Marcovaldi MA, Mortimer JA, Musick JA, Pilcher NJ, Seminoff JA, Troe S (2010) Regional Management Units for Marine Turtles : A Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS One 5:e15465. https://doi.org/10.1371/journal.pone.0015465

Wallace BP, DiMatteo AD, Bolten AB, Chaloupka MY, Hutchinson BJ, Abreu-Grobois FA, Mortimer JA, Seminoff JA, Amorocho D, Bjorndal KA, Bourjea J, Bowen BW, Dueñas R, Casale P, Choudhury BC, Costa A, Dutton PH, Fallabrino A, Finkbeiner EM, Girard A, Girondot M, Hamann M, Hurley BJ, López-Mendilaharsu M, Marcovaldi MA, Musick JA, Nel R, Pilcher NJ, Troëng S, Witherington B, Mast RB (2011) Global conservation priorities for Marine turtles. PLoS One 6:e24510. https://doi.org/10.1371/journal.pone.0024510

5 CAPÍTULO 3

TEMPORAL VARIATION ON THE GENETIC DIVERSITY OF GREEN TURTLES FROM THE SOUTHWEST ATLANTIC OCEAN

Abstract

Green turtles are migratory animals with a complex life cycle, which makes them vulnerable to a wide range of threats. Despite being historically exploited, continuous conservation efforts are resulting in the recovery of some nesting populations. The recovery, and consequent output increase, of different nesting populations can affect the composition of individuals in feeding grounds since these areas harbour individuals from different nesting sites. In this study we evaluate temporal variations in the genetic composition of feeding grounds in the Southwestern Atlantic Ocean (SWA) to investigate if the recovery of nesting sites in the Atlantic Ocean, particularly Ascension Island, is influencing the composition of individuals in the region. We used the control region of the mitochondrial DNA and mitochondrial short tandem repeats to perform spatial and temporal analyses using samples collected along the SWA in two temporal periods ten years apart. Spatial and temporal genetic variations in the region were not significant. Likewise, estimated natal origins remained similar between the time periods analyzed, with small variations. However, there was significant temporal variation in genetic diversity when considering only the northermost feeding grounds in the SWA, likely related to an increase in the frequency of the CM-A8 haplotype, common in Ascension Island. These results suggest that the genetic diversity in SWA feeding grounds have remained somewhat similar in recent years. However, the constant monitoring of these sites as well as the characterization of new sites are essential to create a strong baseline and understand genetic diversity trends of SWA green turtles.

Keywords: Green turtles, feeding grounds, nesting sites, population recovery

5.1 Introduction

Green turtles (*Chelonia mydas*) are slow growing, long-lived and migratory animals, with a complex life cycle characterized by ontogenetic habitat shifts. Their life history encompasses different ecosystems from terrestrial habitats (nesting sites) to open ocean, as well as a diversity of coastal foraging habitats (Bolten 2003). Adults migrate between foraging grounds and breeding areas, which can be thousands of kilometers apart (Plotkin 2003), and exhibit natal philopatry to nesting beaches (Bowen and Karl 2007). As a result, there is a considerable genetic structure among nesting sites (Bjorndal et al. 2006; Naro-Maciel et al. 2014). In contrast, feeding grounds are commonly mixed stocks, harbouring individuals from several different nesting sites (Naro-Maciel et al. 2007, 2012; Proietti et al. 2012; Prosdocimi et al. 2012).

Because of this complex life cycle, green turtles are usually under multiple threats according to the environments they go through (Wallace 2010). They have been historically exploited, and currently anthropic threats such as climate change, pollution of marine environments and habitat degradation still threaten green turtle populations (Fuentes et al. 2011). Nevertheless, some nesting sites are recovering as a result of continuous conservation efforts (e.g. Catry et al. 2009; Weber et al. 2014). Because of the connection between nesting and feeding grounds, differential recovery of nesting sites can likely alter the composition of individuals at feeding grounds. For instance, a green turtle feeding ground in Lac Bay, Boinare, in the Caribbean have shown an increase in the proportion of individuals from local recovering nesting sites over a ten-year time span as revealed by variations on local genetic diversity based on mitochondrial DNA analyses (van der Zee et al. 2019).

Some nesting sites in the South Atlantic, such as Ascension Island and Guinea Bissau have grown significantly during the last decades (Catry et al. 2009; Weber et al. 2014), while others such as Trindade Island are reported to be stable (Medeiros et al. 2022). Ascension Island has been historically reported as the highest contributor to the composition of green turtles in feeding grounds along the coast of Southwest Atlantic

(SWA) (Naro-Maciel et al. 2012; Prosdocimi et al. 2012). With the rising number of nesting females in this nesting site, contributions of Ascension to local foraging grounds could become even greater. A likely consequence of Ascension Island green turtle nesting growth is that the influence of smaller nesting sites such as Trindade Island could become harder to detect since the number of individuals from this area would be proportionally lower, which could result in variations on the genetic diversity over time. Therefore, our main goal was to evaluate if the recovery of green turtles nesting sites in the Atlantic has influenced the genetic diversity at foraging grounds in the Southwestern Atlantic.

5.2 Methods

We used muscle and skin samples collected from green turtles found stranded along the coast of Alagoas (between May 2018 and January 2021) and Paraná (between January 2018 and November 2021) (Fig. 1). We used samples from these localities because they represent northern and southern distribution of green turtles feeding grounds in the SWA and might different responses to the differential recovery of local nesting sites. Since most green turtles in southern foraging grounds are smaller than 60cm (Barata et al. 2011), we chose this size class as a threshold in sample selection in order to avoid bias caused by migrants from southern to northern foraging grounds. In total, we used 213 samples, of which 140 were from Alagoas (CCL 23-59.2cm) and 73 were from Paraná (CCL 31-57.3cm). Among samples from Alagoas, 100 were from Almeida et al. (2021), and 40 were newly sequenced for this study.

Total genomic DNA was extracted using phenol-chloroform (Sambrook et al. 1989). We amplified the short fragment of the control region of the mitochondrial DNA (493pb) for all samples. For a subset of these samples (49 from Alagoas and 47 from Paraná), we amplified a longer mtDNA fragment (840pb), including the longer fragment of the control region as well as four mitochondrial short tandem repeats (mtSTR) as described in Tikochinski et al. (2012). Control region fragments were amplified using the primers LCM15382 and H950 (Abreu-Grobrois et al. 2006) and mtSTRs were amplified

using the primers CMD1 and CMD5 (Tikochinski et al. 2012). We conducted 25 µl polymerase chain reactions, consisting in 20.8 µl of 1XMaster Mix PCR Buffer with 0.4 mM of each dNTP and 3 mM of MgCl2, 1.0 ml of each primer (10 pmol); 2 µl of DNA template (>20 ng/ml); and 0.2 µl of Taq DNA polymerase (5 U/ml). We amplified the control region fragments using the following protocol: initial denaturation at 94°C for 7 min followed by 35–40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extending at 72°C for 1 min and a final extending at 72°C for 7 min. Mitochondrial STRs were amplified with the same protocol but with a 56°C annealing temperature. We checked for successful amplifications using a 1% agarose gel and samples successfully amplified were purified with isopropanol to remove PCR residuals and sequenced with the forward primer (short control region fragment and mtSTRs) or both primers (long control region fragment) using Sanger sequencing at ACTGene Análises Moleculares.

Sequences were edited with Bioedit 7.2.5 (Hall 1999) and aligned with MAFFT online service (Katoh et al. 2019). We identified haplotypes using the Archie Carr Center for Sea Turtles Research (https://accstr.ufl.edu/resources/mtdna-sequences/) and categorized mtSTRs by counting the “AT” repeats in each of the four loci we analyzed. Thus, each individual sample was represented by a four-digit code as described in Tikochinski et al. (2012). Haplotype and nucleotide diversities were assessed using DNAsp v6.12 (Rozas et al. 2017). We tested for population divergence between Alagoas and Paraná using an analysis of molecular variance (AMOVA) with 10,000 permutations as implemented in Arlequin v3.5.2.2 (Excoffier and Lischer 2010). We performed this analysis in two different ways. Firstly, we used the shorter fragment of the control region, which allowed the inclusion of a larger number of samples. Secondly, we used the longer fragment of the control region plus mtSTRs.

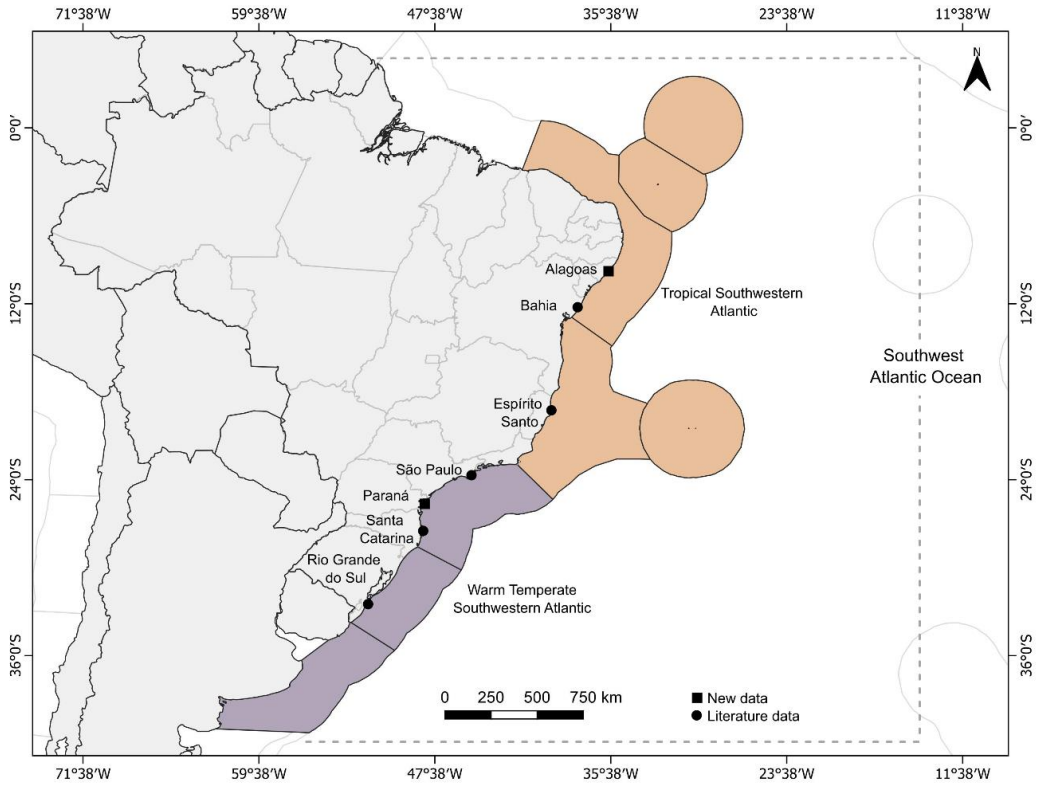


Figure 1. Southwest Atlantic Ocean feeding grounds sampled in this study. Literature data are from Naro-Maciel et al. (2012), Proietti et al. (2012) and Almeida et al. (2021). Colours indicate marine ecoregions by Spaldin et al. 2007, Tropical Southwestern Atlantic in orange and Warm Temperate Southwestern Atlantic in purple.

We assessed temporal variation in genetic composition on the SWA also using two approaches. First, we pooled together our data from Alagoas and Paraná as representative of current genetic composition and compared these data to data collected ten to 15 years ago along the Southwest Atlantic Ocean (total N=743), mainly the Brazilian coast (Naro-Maciel et al. 2012; Proietti et al. 2012). Second, we considered northern and southern feeding grounds independently, according to the marine provinces of Tropical Southwestern Atlantic (TSA, N=341) and Warm Temperate Southwestern Atlantic (WTSA, N=402) (Spalding et al. 2007). To test for genetic differences between time periods, we used AMOVA analyses as mentioned above.

Natal origins of green turtles were estimated using the same groupings described above, first the SWA as a whole and then TSA and WTSA independently. Analyses were carried out through many-to-one mixed stock analysis (MSA; Pella and Masuda 2001). We used 12 nesting sites in the Atlantic Ocean as putative sources of individuals (Table S1). We performed the analyses using BAYES (Pella and Masuda 2001), with 12 chains per run (equal to the number of sources) and 50,000 iterations per chain, discarding half of these iterations as burn-in. Convergence of the runs was checked using the Gelman-Rubin criterion, considering values below 1.2 as adequate, as indicated in the software manual. We used the number of nesting females at each nesting site as a prior in the analyses (Table S1).

5.3 Results

Considering the new samples from Alagoas and Paraná (short fragment), we identified 12 different haplotypes in Alagoas, of which the most frequent were CM-A8 (68%), CM-A5 (20%) and CM-A9 (3%). We also identified a previously undescribed haplotype, H1. Using the CM-A8 haplotype (GenBank accession number Z50130) as reference, the new haplotype has a transition, G to A, at position 89. Among Paraná samples, we identified ten haplotypes, of which CM-A8 (58%), CM-A5 (25%) and CM-A9 (6%) were also the most frequent. Haplotype and nucleotide diversities were higher in Paraná (Hd = 0.611, π = 0.00253) compared to Alagoas (Hd = 0.491, π = 0.00193). Considering the longer fragment of the control region plus mtSTRs, we identified a total of 23 haplotypes in Alagoas and 21 in Paraná. AMOVA results using the shorter fragment of the control region or the longer fragment plus mtSTRs revealed no genetic structure between Alagoas and Paraná (Fst = -0.005, P = 0.645 and Fst = 0.001, P = 0.338; respectively).

Proportion of haplotypes in the SWA as a whole (N=213) was similar between the two sampling periods. We identified 14 different haplotypes currently (2018-2021), of which the most frequent were CM-A8 (64.8%), CM-A5 (21.6%) and CM-A9 (4.2%). The haplotypes CM-A8 (61.5%), CM-A5 (22.6%) and CM-A9 (4.1%) were also the most

frequent in the 2003-2008 sampling period (N=530). Accordingly, AMOVA analysis revealed no genetic differentiation between the two sampling periods (Fst = -0.00275, p = 0.98624). When we considered only samples from the southernmost sampling areas (WTSA), we also observed no genetic differentiation between the two sampling periods (Fst = -0.00014, p = 0.36297). On the other hand, we observed significant genetic differentiation when considering only samples from the northernmost sampling areas (TSA) (Fst = 0.01486, p = 0.03069), even though most genetic variation was within each group (98.51%).

Natal origins of green turtles were similar considering all data (SWA). Ascension Island, Surinam, Guinea Bissau and Trindade Island were the nesting sites with highest contributions to the composition of individuals in SWA foraging grounds both in 2003-2008 (53%, 19%, 12% and 10%, respectively) and 2018-2021 (58%, 16%, 19% and 4%, respectively). Ascension Island was also the highest contributor when considering past and current TSA data (60% and 56%, respectively). Surinam was the second highest contributor using past data (29%), followed by Trindade Island (4%) and Aves Island (3%). Guinea Bissau was the second highest contributor using current data (28%), followed by Surinam (12%) and Aves Island (2%). Considering only data from the WTSA, Ascension Island (62%), Guinea Bissau (16%), Surinam (11%) and Aves Island (5%) were the highest contributors in the past, while Ascension Island (55%), Surinam (21%), Trindad Island (15%) and Guinea Bissau (3%) are highest contributors currently (Fig. 2).

5.4 Discussion

Haplotype frequencies of new samples (Alagoas and Paraná) were similar to what has been reported for the region, with a high frequency of CM-A8 and CM-A5 haplotypes (Naro-Maciel et al. 2012; Proietti et al. 2012; Almeida et al. 2021). We detected no genetic differentiation between the two regions, even though feeding grounds in the Atlantic have been reported to be somewhat structured (Naro-Maciel et al. 2012).

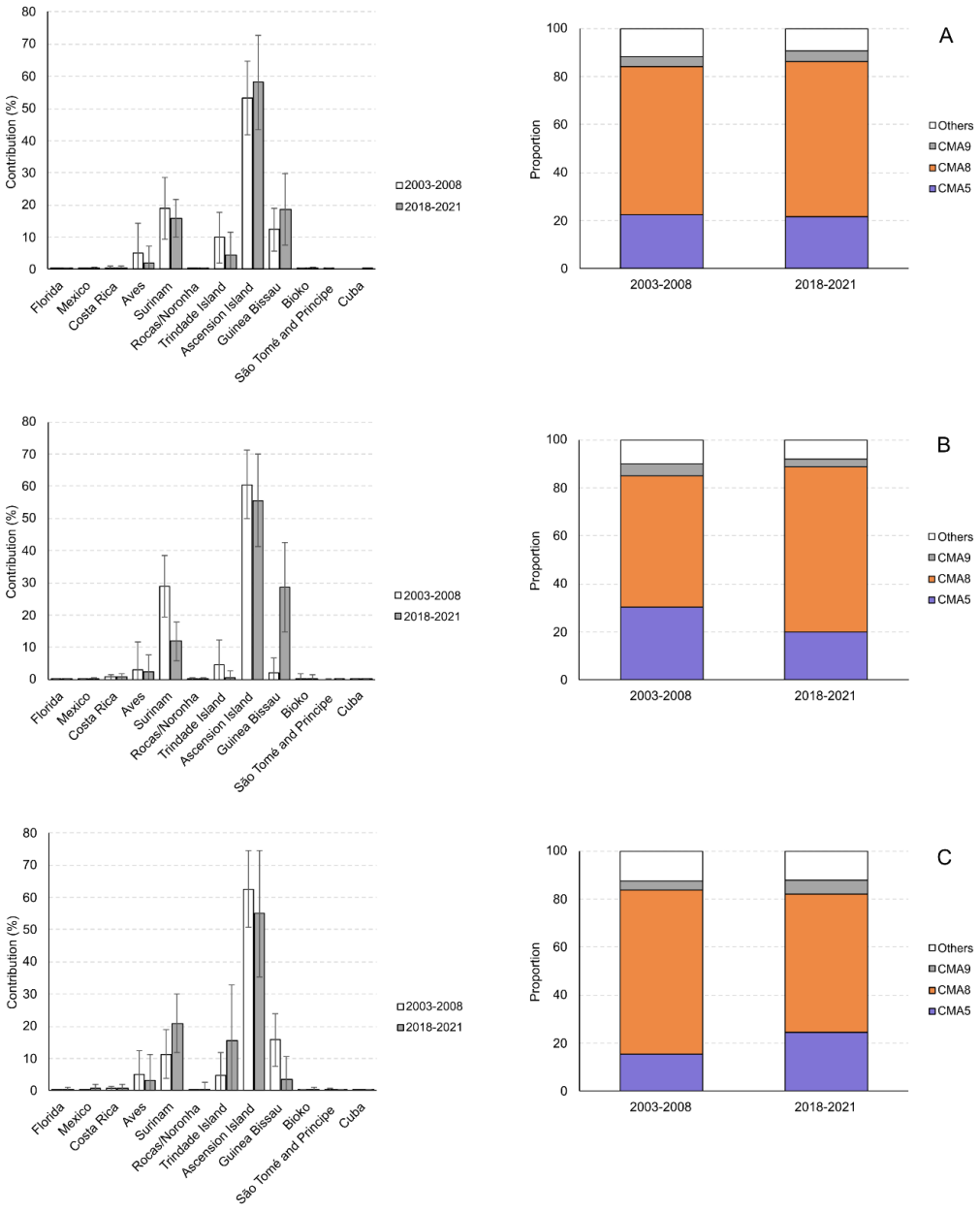


Figure 2. Mixed stock analysis and proportion of haplotypes of green turtles from Southwest Atlantic Ocean (A) and two subsets restricted to the Tropical Southwestern Atlantic (B) and the Warm Temperate Southwestern Atlantic (C), considering past (2003-2008) and current (2018-2021) sampling. Error bars indicate standard deviation.

The use of additional markers, such as mtSTRs has been suggested as a tool to detect deeper genetic variation (Tikochinski et al. 2012; Shamblin et al. 2015; Bradshaw et al. 2018). We sequenced mtSTRs for a subset of our samples and observed a considerable increase in the number of haplotypes, but failed to detect genetic structure, either between Alagoas and Paraná or between subvariants of the most common haplotypes CM-A5 and CM-A8 (Table S2). Although mtSTR data from Trindade Island, Fernando de Noronha and Rocas Atoll nesting sites is available (Shamblin et al. 2015), other major nesting sites such as Ascension Island and Guinea Bissau remain to be sampled. Finer scale analysis of currently known haplotypes could be extremely helpful to reveal deeper genetic structure between nesting sites and help enhance natal origins analyses in the region.

This high frequency of CM-A8 and CM-A5 haplotypes was also observed in data from 2003-2008 and haplotype proportions did not vary greatly between time periods. The CM-A8 haplotype is extremely common across nesting sites in the South Atlantic, including Ascension Island, the second biggest nesting site in the region (Formia et al. 2007). Thus, a high frequency of this haplotype in SWA feeding grounds is expected. The CM-A5 haplotype, on the other hand, is more common in nesting sites near the Caribbean, such as Surinam and Aves Island (Shamblin et al. 2012). A high frequency of this haplotype can be observed on feeding grounds near that region, such as Ceará and Fernando de Noronha (Naro-Maciel et al. 2012). It would make sense that Alagoas also exhibited a similar pattern due to its proximity to the region. However, we observed an even higher frequency of this haplotype in Paraná, which is more than seven thousand kilometers away. Besides the genetic evidence (Naro-Maciel et al. 2012; Jordão et al. 2015), telemetry also indicated that juveniles and subadults green turtles from the Caribbean migrate to foraging grounds along the Brazilian coast, swimming against the Guiana and North Brazil currents (Chambault et al. 2018). Once they reach the northeastern coast of Brazil, they could reach southern foraging grounds with the aid of the Brazilian current as genetic data from our and other studies indicate (Proietti et al. 2012, Jordão et al. 2015).

Natal origin analyses corroborated these observations, as Ascension Island and Surinam had high contribution to the composition of individuals considering all three sample groupings (Fig. 2). When using data from the whole SWA, contributions of Ascension Island, Surinam, Guinea Bissau and Trindade Island were somewhat constant between the two time periods analysed (Fig. 2A). However, there was noticeable variation in TSA and WTSA. Contributions of Surinam to the composition of individuals in the TSA were visibly lower in 2018-2021. Conversely, contributions of Guinea Bissau were visibly higher (Fig. 2B). This might be a result of the decreased proportion of the CM-A5 haplotype, common in the Surinam nesting site (Shamblin et al. 2012). Concomitantly, the increased proportion of the CM-A8 haplotype in the 2018-2021 sampling may have promoted the increased contributions from Guinea Bissau, as the overwhelming majority of individuals from there exhibit the CM-A8 haplotype (Patrício et al. 2017).

Natal origins of samples from the WTSA showed an evident increase in the contributions from Surinam and Trindade Island in 2018-2021, while contributions from Guinea Bissau decreased in the same period (Fig. 2C), likely due to the higher proportion of CM-A5 and CM-A9 haplotypes in the same period. The CM-A9 haplotype have been reported in the nesting sites of Rocas Atoll, Ascension Island and Trindade Island, but with a higher frequency on the latter (Bjorndal et al. 2006). Previous studies also show that contributions from Trindade Island are usually higher to closer feeding grounds in south Brazil (Naro-Maciel et al. 2012; Proietti et al. 2012), which is in agreement with our results.

Overall, our results show that there is no clear influence of the recovery of Ascension Island on the genetic composition of green turtles from SWA feeding grounds, at least not in the time frame analysed here. Contrary to what was expected, contributions of Trindade Island to feeding grounds in the WTSA increased, even though that nesting site have been stable while Ascension have been increasing the number of nesting females (Medeiros et al. 2022). Thus, we would expect an increase in the frequency of haplotypes from Ascension Island in foraging grounds in south Brazil while

haplotypes from Trindade could become less likely to be sampled because their relative frequency would decline. We did not observe such variation in haplotype frequencies. Nevertheless, it is worth noting that the CM-A8 haplotype is the most common haplotype in both nesting sites, which could mask some of those effects since we cannot precisely determine the natal origin of CM-A8 individuals.

Even though our study indicates no significant temporal variation in haplotypes proportion in SWA feeding grounds, continuous monitoring is essential to detect changes in the dynamics of local feeding and nesting grounds. The use of more variable genetic markers can help to detect finer variations on genetic diversity and improve the genetic characterization of known populations, which can be highly useful to improve natal origins estimation. Studies describing the genetic diversity of new feeding grounds or expanding the knowledge on known feeding and nesting grounds also help to create a stronger baseline that allow future studies to detect genetic variability more precisely.

References

Abreu-Grobois FA, Horrocks J, Formia A, Leroux R, Velez-Zuazo X, Dutton P, Soares L, Meylan P, Browne D (2006) New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: Annual Symposium on Sea Turtle Biology and Conservation, Island of Crete. Book of abstracts of the 26th Annual Symposium on Sea Turtle Biology and Conservation. Athens: International Sea Turtle Society, p. 179.

Almeida JPFA, Santos RG, Mott T (2021) Sex ratios and natal origins of green turtles from feeding grounds in the Southwest Atlantic Ocean. ICES Journal of Marine Science 78:1840–1848. doi: 10.1093/icesjms/fsa

Barata PCR, Carman VG, Santos AS, Bondioli ACV, Almeida AP, Santos AJB, Silva ACCD, Gallo BMG, Giffoni BB, Domit C, Baptistotte, Bellini C, Batista CMP, Bezerra DP, Monteiro DS, Albareda D, Lima EHSM, Lima EP, Guebert-Bartholo F, Sales G, Lopez GG, Stahelin GD, Bruno I, Castilhos JC, Thomé JCA, Nunes JAA, Becker JH, Wanderlinde J, Rosa L, Marcovaldi MA, Melo MTD, Mascarenhas R, Estima SC, Naro-Maciel E (2011) Variação latitudinal na distribuição do tamanho de tartarugas-verdes (*Chelonia mydas*) ao longo de parte da costa leste da América do Sul. In: V Jornada sobre Tartarugas Marinhas do Atlântico Sul Ocidental. Florianópolis, Brasil. p. 18–22.

Bjorndal KA, Bolten AB, Moreira L, Bellini C, Marcovaldi MÂ (2006) Population structure and diversity of brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology 5:262–268. doi: 10.2744/1071-8443(2006)5[262:PSADOB]2.0.CO;2

Bolten, AB (2003) Variation in sea turtle life history patterns: neritic vc oceanic developmental stages. In: LUTZ, P. L.; MUSICK, J. A.; WYNEKEN, J. (Eds.), The Biology of Sea Turtles Volume II. CRC Press, Boca Raton, p. 243–257.

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Molecular Ecology, 16: 4886–4907.

Bradshaw PJ, Broderick AC, Carreras C, Fuller W, Snape RTE, Wright LI, Godley BJ (2018) Defining conservation units with enhanced molecular tools to reveal fine scale structuring among Mediterranean green turtle rookeries. Biol Conserv 222:253–260. doi: 10.1016/j.biocon.2017.12.014

Catry P, Barbosa C, Paris B, Indjai B, Almeida A, Limoges B, Silva C, Hono´ H, Pereira H (2009) Status, Ecology, and Conservation of Sea Turtles in Guinea-Bissau. Chelonian Conservation and Biology 8:150–160.

Chambault P, de Thoisy B, Huguin M, Martin J, Bonola M, Etienne D, Gresser J, Hiélard G, Mailles J, Védie F, Barnerias C, Sutter E, Guillemot B, Dumont-Dayot É, Régis S, Lecerf N, Lefebvre F, Frouin C, Aubert N, Guimera C, Bordes R, Thieulle L, Duru M, Bouaziz M, Pinson A, Flora F, Queneherve P, Woignier T, Allenou JP, Cimiterra N, Benhalilou A, Murgale C, Maillet T, Rangon L, Chanteux N, Chanteur B, Béranger C, le Maho Y, Petit O, Chevallier D (2018) Connecting paths between juvenile and adult habitats in the Atlantic green turtle using genetics and satellite tracking. Ecol Evol 8:12790–12802. doi: 10.1002/ece3.4708

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Formia A, Broderick A, Glen F, Godley B, Hays G, Bruford M (2007) Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. Endanger Species Res 3:145–158. doi: 10.3354/esr003145

Fuentes MMPB, Limpus CJ, Hamann M (2011) Vulnerability of sea turtle nesting grounds to climate change. Glob Chang Biol 17:140–153. doi: 10.1111/j.1365-2486.2010.02192.x

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Jordão JC, Bondioli ACV, Guebert FM, de Thoisy B, de Toledo LFA (2015) Green turtle (*Chelonia mydas*) genetic diversity at Paranaguá Estuarine complex feeding grounds in Brazil. Genet Mol Biol 38:346–352. doi: 10.1590/S1415-475738320140353

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20:1160–1166. doi: 10.1093/bib/bbx108

Medeiros L, Chaloupka M, Bolten AB, von Muhlen EM, Santos A, Marcondes ACJ, Thomé JCA, Marcovaldi MÂ, Bjorndal KA (2022) Tracking green turtle nesting trends at a remote oceanic rookery. Mar Biol. doi: 10.1007/s00227-022-04054-7

Naro-Maciel E, Becker JH, Lima EHSM, Marcovaldi MÂ, DeSalle R (2007) Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. Journal of Heredity 98:29–39. doi: 10.1093/jhered/esl050

Naro-Maciel E, Bondioli ACV, Martin M, de Pádua Almeida A, Baptistotte C, Bellini C, Marcovaldi MÂ, Santos AJB, Amato G (2012) The interplay of homing and dispersal in green turtles: A focus on the southwestern atlantic. Journal of Heredity 103:792–805. doi: 10.1093/jhered/ess068

Naro-Maciel E, Reid BN, Alter SE, Amato G, Bjorndal KA, Bolten AB, Martin M, Nairn CJ, Shamblin B, Pineda-Catalan O (2014) From refugia to rookeries: Phylogeography of Atlantic green turtles. J Exp Mar Biol Ecol 461:306–316. doi: 10.1016/j.jembe.2014.08.020

Patrício AR, Formia A, Barbosa C, Broderick AC, Bruford M, Carreras C, Catry P, Ciofi C, Regalla A, Godley BJ (2017) Dispersal of green turtles from Africa's largest rookery assessed through genetic markers. Mar Ecol Prog Ser 569:215–225. doi: 10.3354/meps1207

Pella J, Masuda M (2001) Bayesian methods for analysis of stock mixtures from genetic characters. Fishery Bulletin 99:151–167.

Plotkin P (2003) Adult Migrations and Habitat Use. In: Lutz PL, Musik JA, Wyneken J (Eds.). The Biology of Sea Turtles, Volume II. Florida: CRC Press, p. 225–241.

Proietti MC, Reisser JW, Kinas PG, Kerr R, Monteiro DS, Marins LF, Secchi ER (2012) Green turtle *Chelonia mydas* mixed stocks in the western South Atlantic, as revealed by mtDNA haplotypes and drifter trajectories. Mar Ecol Prog Ser 447:195–209. doi: 10.3354/meps09477

Prosdocimi L, González Carman V, Albareda DA, Remis MI (2012) Genetic composition of green turtle feeding grounds in coastal waters of Argentina based on mitochondrial DNA. J Exp Mar Biol Ecol 412:37–45. doi: 10.1016/j.jembe.2011.10.015

Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302. doi: 10.1093/molbev/msx248

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Shamblin BM, Bjorndal KA, Bolten AB, Hillis-Starr ZM, Lundgren I, Naro-Maciel E, Nairn CJ (2012) Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Mol Ecol 21:2330–2340. doi: 10.1111/j.1365-294X.2012.05530.x

Shamblin BM, Dutton PH, Bjorndal KA, Bolten AB, Naro-Maciel E, Santos AJB, Bellini C, Baptistotte C, Marcovaldi MÂ, Nairn CJ (2015) Deeper Mitochondrial Sequencing Reveals Cryptic Diversity and Structure in Brazilian Green Turtle Rookeries. Chelonian Conservation and Biology 14:167–172. doi: 10.2744/CCB-1152.1

Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA, Lombana A, Lourie SA, Martin KD, McManus E, Molnar J, Recchia CA, Robertson J (2007) Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. Bioscience 57:573–583.

Tikochinski Y, Bendelac R, Barash A, Daya A, Levy Y, Friedmann A (2012) Mitochondrial DNA STR analysis as a tool for studying the green sea turtle (*Chelonia mydas*) populations: The Mediterranean Sea case study. Mar Genomics 6:17–24. doi: 10.1016/j.margen.2012.01.002

van der Zee JP, Christianen MJA, Nava M, Velez-Zuazo X, Hao W, Bérubé M, van Lavieren H, Hiwat M, Berzins R, Chevalier J, Chevallier D, Lankester MC, Bjorndal KA, Bolten AB, Becking LE, Palsbøll PJ (2019) Population recovery changes population composition at a major southern Caribbean juvenile developmental habitat for the green turtle, *Chelonia mydas*. Sci Rep. doi: 10.1038/s41598-019-50753-5

Weber SB, Weber N, Ellick J, Avery A, Frauenstein R, Godley BJ, Sim J, Williams N, Broderick AC (2014) Recovery of the South Atlantic's largest green turtle nesting population. Biodivers Conserv 23:3005–3018. doi: 10.1007/s10531-014-0759-6

6 Discussão geral e conclusões

Cinco das sete espécies de tartarugas marinhas ocorrem no Oceano Atlântico Sudoeste (OAS). Todas essas espécies encontram-se sob algum grau de ameaça, segundo a União Internacional para a Conservação da Natureza (IUCN, 2022). Devido a isso, a identificação e caracterização de áreas de desova e alimentação de tartarugas marinhas na região é fundamental para melhorar cada vez mais a elucidação da dinâmica populacional dessas espécies e entender como elas podem responder a ameaças atuais e futuras (FUENTES; LIMPUS; HAMANN, 2011). Nos estudos desenvolvidos nessa tese, procuramos entender alguns aspectos da diversidade genética e conservação das tartarugas marinhas do OAS.

Observamos que a razão sexual de tartarugas verdes em áreas de alimentação da região é enviesada em favor das fêmeas, onde há cerca de três fêmeas para cada macho. Essa tendência já foi relatada em outras regiões do mundo (HAYS; MAZARIS; SCHOFIELD, 2014), e levanta discussões sobre uma possível feminilização das populações, tendo em vista as mudanças climáticas e um provável aumento na produção de fêmeas em áreas de desova ao redor do mundo (JENSEN et al., 2018). Ainda assim, essa razão sexual é menor do que o observado em várias áreas de desova, onde a produção de fêmeas pode chegar a mais de 90% (HAYS; MAZARIS; SCHOFIELD, 2014), o que sugere que algumas áreas de desova ainda mantém uma produção mais equilibrada de fêmeas e machos (e.g. PATRÍCIO et al. 2017). Assim, a avaliação de áreas de desova e alimentação com relação a sua razão sexual se faz cada vez mais necessária, em todas as espécies de tartarugas marinhas, para o direcionamento de medidas de conservação adequadas a fim de que a viabilidade populacional dessas espécies possa ser mantida. Nesse sentido, a avaliação de dados genéticos de fêmeas e machos em áreas de alimentação pode ajudar a identificar áreas de desova que estejam potencialmente gerando mais fêmeas ou mais machos (JENSEN et al., 2018).

A avaliação desses dados para a área de alimentação de Alagoas, nordeste do Brasil, revelou uma variação entre a origem natal de fêmeas e machos. As análises indicaram que a maior parte das fêmeas que se alimentam na região são provenientes da área de desova da Ilha de Ascensão, enquanto a maior parte dos machos provém da área de desova de Guiné Bissau, no litoral Africano. Esse resultado foi congruente com as análises de razão sexual dessas áreas, que indicam que Ascensão tem uma produção de fêmeas mais acentuada, enquanto Guiné Bissau tem uma produção mais equilibrada entre os dois sexos (GODLEY et al., 2002; PATRÍCIO et al., 2019). A área de desova de Suriname também apresentou alta contribuição para a composição de machos na região. Apesar de ser necessário cautela na interpretação dos resultados devido a limitação das análises, essa avaliação se mostra útil não só para corroborar as análises locais de razão sexual como também para identificar a conectividade da área de alimentação de Alagoas com as áreas de desova da região com relação às fêmeas e machos da espécie.

A análise de outras espécies de tartarugas marinhas no litoral de Alagoas também revelou a presença de hibridização entre quatro espécies: tartaruga de pente e tartaruga cabeçuda, tartaruga de pente e tartaruga oliva, tartaruga cabeçuda e tartaruga oliva e, por fim, tartaruga verde e tartaruga cabeçuda. Foram identificados espécimes híbridos tanto em áreas de desova quanto de alimentação em Alagoas. Isso sugere que esse processo está acontecendo não só entre tartarugas que se reproduzem na região, mas também entre aquelas que se reproduzem em outras áreas e migram para se alimentar no litoral do estado. A alta frequência de hibridização entre tartarugas marinhas no litoral Brasileiro é marcante, principalmente ao longo do litoral dos estados da Bahia e Sergipe (LARA-RUIZ et al., 2006; REIS; SOARES; LÔBO-HAJDU, 2010). Nosso estudo amplia o número de áreas com registro de hibridização e ressalta a importância da ampliação dessa avaliação para outras regiões. O monitoramento dessas áreas com registro de hibridização também é importante para entendermos melhor os efeitos desse processo na dinâmica populacional das espécies e como pressões atuais, como ocupação desordenada do ambiente costeiro e mudanças

climáticas, podem afetar a frequência de hibridização nessas regiões. Isso é de extrema relevância uma vez que estudos recentes têm demonstrado que híbridos e espécies parentais parecem exibir sobreposição ecológica e parecem também se tornar inviáveis a partir da segunda geração (SOARES et al., 2021; VILAÇA et al., 2022). Isso evidencia a necessidade do monitoramento contínuo desse processo no litoral brasileiro, a fim de avaliar com mais clareza a amplitude da ocorrência de híbridos na região e como a frequência de híbridos pode mudar no futuro e se isso pode vir a afetar a viabilidade das populações dessas espécies na região de maneira significativa.

Também foi possível detectar um espécime de tartaruga de pente com um haplótipo típico do Indo-Pacífico na área de alimentação de Alagoas. É precipitado afirmar que esse espécime migrou diretamente daquela região, porém não é a primeira vez que um haplótipo do Indo-Pacífico é registrado em áreas de alimentação no Atlântico (ARANTES; VARGAS; SANTOS, 2020), o que reforça a conexão entre essas duas regiões. Esse padrão de migração e ocupação de habitats que transcendem os limites geográficos de países e continentes é um desafio para a conservação não só da tartaruga de pente, mas das tartarugas marinhas em geral. Isso demanda a coordenação entre diferentes países, uma vez que a proteção do hábitat que essas espécies ocupam em um país, não necessariamente garante a proteção daquela população se os hábitats que os indivíduos daquela população ocupam em outras regiões estiverem sob constante ameaça (WALLACE et al., 2011).

Por fim, foi avaliada a variação temporal na diversidade genética de tartarugas verdes em áreas de alimentação do OAS. Não foi observada uma mudança significativa na proporção de haplótipos quando consideramos o OAS como um todo. Porém, foram encontradas variações genéticas nas áreas de alimentação quando os dados foram analisados de maneira mais regionalizada. A alta frequência do haplótipo CM-A5 no sul do Brasil é digna de nota, uma vez que esse haplótipo é mais comum em áreas de desova do Caribe e os indivíduos têm que nadar ativamente contracorrente para atingirem áreas de alimentação no litoral Brasileiro (CHAMBAULT et al., 2018). A alteração na frequência de haplótipos nas populações de tartarugas marinhas pode ser

um indicador da depleção ou recuperação de áreas de desova locais (VAN DER ZEE et al., 2019). O monitoramento das espécies é essencial para esclarecer esses processos e avaliar possíveis perdas de diversidade genética e o status populacional das espécies. Além disso, a implementação de marcadores moleculares variáveis e de uma abordagem integrativa é crucial para a elucidação de padrões de estruturação populacional, que muitas vezes podem ser mascarados por haplótipos que são altamente difundidos nas populações. A utilização de dados nucleares e de repetições curtas do DNA mitocondrial (mtSTR) ajudou a revelar uma maior variabilidade nos dados analisados nessa tese, que não seria observada apenas com o uso da região controle do DNA mitocondrial, o marcador molecular mais utilizado em estudos com tartarugas marinhas. Assim, estudos futuros que foquem nessa abordagem certamente poderão revelar padrões de estruturação ainda não observados e contribuir para a conservação das espécies de tartarugas marinhas.

REFERÊNCIAS

ARANTES, L. S.; VARGAS, S. M.; SANTOS, F. R. Global phylogeography of the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Genetics and Molecular Biology, v. 43, n. 2, p. 1–12, 2020.

CHAMBAULT, P. et al. Connecting paths between juvenile and adult habitats in the Atlantic green turtle using genetics and satellite tracking. Ecology and Evolution, v. 8, n. 24, p. 12790–12802, 1 dez. 2018.

FUENTES, M. M. P. B.; LIMPUS, C. J.; HAMANN, M. Vulnerability of sea turtle nesting grounds to climate change. Global Change Biology, v. 17, p. 140–153, 2011.

GODLEY, B. J. et al. Temperature-dependent sex determination of Ascension Island green turtles. Marine Ecology Progress Series, v. 226, p. 115–124, 2002.

HAYS, G. C.; MAZARIS, A. D.; SCHOFIELD, G. Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Frontiers in Marine Science, v. 1, p. 1–9, 2014.

IUCN 2022. The IUCN Red List of Threatened Species. Version 2022-1. <https://www.iucnredlist.org>

JENSEN, M. P. et al. Environmental Warming and Feminization of One of the Largest Sea Turtle Populations in the World. Current Biology, v. 28, n. 1, p. 154–159, 2018.

LARA-RUIZ, P. et al. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conservation Genetics, v. 7, n. 5, p. 773–781, 2006.

PATRÍCIO, A. R.; VARELA, M. R.; BARBOSA, C.; BRODERICK, A. C.; CATRY, P.; HAWKES, L. A.; REGALLA, A.; GODLEY, B. J. Climate change resilience of a globally important sea turtle nesting population, v. 25, n. 2, p. 522–535, 2019.

REIS, E. C.; SOARES, L. S.; LÔBO-HAJDU, G. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conservation Genetics, v. 11, n. 4, p. 1587–1591, 2010.

SOARES, L. S. et al. Reproductive output, foraging destinations, and isotopic niche of olive ridley and loggerhead sea turtles, and their hybrids, in Brazil. Endangered Species Research, v. 44, p. 237–251, 2021.

VAN DER ZEE, J. P. et al. Population recovery changes population composition at a major southern Caribbean juvenile developmental habitat for the green turtle, *Chelonia mydas*. Scientific Reports, v. 9, n. 1, 2019.

VILAÇA, S. T. et al. Evidence of backcross inviability and mitochondrial DNA paternal leakage in sea turtle hybrids. Molecular Ecology, v. 32, p. 628–643, 2022.

Wallace, B. P. et al. Global conservation priorities for Marine turtles. PLoS One, v. 6, n. 9, p. e24510, 2011.

ANEXO A – CAPÍTULO 1

Table S1. Female and male green turtles recorded stranded in Brazilian feeding grounds between 2010 and 2016.

Year	Northeast1		Northeast2		Southeast	
	Females	Males	Females	Males	Females	Males
2010	-	-	84	23	31	14
2011	81	26	122	21	204	80
2012	145	42	219	71	260	56
2013	186	45	167	45	59	4
2014	231	61	159	67	92	24
2015	176	57	78	43	-	1
2016	211	74	122	79	4	2

Table S2. Haplotypes of green turtles found in Alagoas foraging ground and Nesting sites used in this study. Number of nesting females are from Seminoff et al., 2015. Full references can be found in the main text reference list. AI – Ascension Island, AV – Aves Island, BK – Bioko, CB – Cuba, CR – Costa Rica, FL – Florida, FN – Fernando de Noronha, GB – Guinea Bissau, MX – Mexico, RA – Rocas Atoll, STP – São Tomé and Principe, SU – Suriname, TR – Trindade Island. I – Encalada *et al*., 1996; II – Bjorndal *et al*., 2005; III – Bjorndal *et al*., 2006; IV – Shamblin *et al*., 2012; V – Formia *et al*., 2006; VI – Formia *et al*., 2007; VIII – Patrício *et al*., 2017.

Haplotype	Alagoas FG		Rookeries											
	Females	Males	FL	MX	CR	AV	SU	RA/FN	AI	TR	GB	BK	STP	CB
CMA1	-	-	11	7	-	-	-	-	-	-	-	-	-	3
CMA2	-	-	1	-	-	-	-	-	-	-	-	-	-	-
CMA3	-	1	12	5	395	5	1	-	-	-	-	-	-	16
CMA4	-	-	-	-	1	-	-	-	-	-	-	-	-	-
CMA5	9	14	-	1	32	62	55	-	-	-	-	-	1	-
CMA6	2	2	-	-	-	-	2	-	11	-	-	5	1	-
CMA7	-	-	-	-	-	-	1	-	-	-	-	-	-	-
CMA8	68	37	-	-	-	-	-	50	204	67	170	45	17	-
CMA9	5	-	-	-	-	-	-	7	9	19	-	-	-	-
CMA10	1	1	-	-	-	-	-	2	5	-	-	-	-	-
CMA11	-	-	-	-	-	-	-	1	-	1	-	-	-	-
CMA12	-	-	-	-	-	-	-	5	-	-	-	-	-	-
CMA15	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CMA16	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CMA17	-	-	-	2	-	-	-	-	-	-	-	-	-	-
CMA18	-	-	-	3	-	-	-	-	-	-	-	-	-	-
CMA20	-	-	-	-	2	-	-	-	-	-	-	-	-	-

CMA21	-	-	-	-	3	-	-	-	-	-	-	-	-	-
CMA23	1	-	-	-	-	-	-	-	1	6	-	-	-	-
CMA24	-	-	-	-	-	-	-	-	7	1	-	-	-	-
CMA25	-	-	-	-	-	-	-	3	1	-	-	-	-	-
CMA27	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA28	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA32	-	1	-	-	-	-	-	1	1	4	-	-	-	-
CMA33	-	-	-	-	-	-	-	-	-	1	-	-	-	-
CMA35	-	-	-	-	-	-	-	-	-	-	-	-	1	-
CMA36	-	-	-	-	-	-	-	-	-	-	-	-	3	-
CMA37	-	-	-	-	-	-	-	-	-	-	-	-	1	-
CMA38	-	-	-	-	-	-	-	-	-	-	-	-	2	-
CMA39	1	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA42	1	1	-	-	-	-	-	-	-	-	1	-	-	-
CMA44	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA45	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA46	1	-	-	-	-	-	-	-	2	-	-	-	-	-
CMA48	-	-	-	-	-	-	-	-	-	-	-	-	-	5
CMA50	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA56	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA57	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total N	89	57	24	20	433	67	59	69	245	99	171	50	26	28
Nesting females	-	-	8,322	24,330	131,751	2,833	13,067	345	13,417	2,016	30,125	850	376	2,226
Source - haplotype data	This study	This study	I	I	II	IV	II, IV	III	VI	III	VIII	V	V	VII

Table S3. Haplotypes of 106 specimens of *Chelonia mydas* from Alagoas feeding ground based on ~800bp of the mtDNA control region.

Haplotype	Females	Males
CMA5.1	4	10
CMA6.1	1	2
CMA8.1	48	28
CMA8.2	2	-
CMA8.3	-	1
CMA9.1	4	-
CMA10.1	1	1
CMA23.1	1	-
CMA32.1	-	1
CMA42.1	1	1
Total	62	44

References

Bjorndal, K. A., Bolten, A. B., and Troëng, S. 2005. Population structure and genetic diversity in green turtles nesting at

Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Marine Biology, 147: 1449–1457.

Bjorndal, K. A., Bolten, A. B., Moreira, L., Bellini, C., and Marcovaldi, M. Â. 2006. Population structure and diversity of brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology, 5: 262–268.

Encalada, S. E., Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Miyamoto, M. M., and Bowen, B. W. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. Molecular Ecology, 5: 473–483.

Formia, A., Godley, B. J., Dontaine, J. F., and Bruford, M. W. 2006. Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conservation Genetics, 7: 353–369.

Formia, A., Broderick, A., Glen, F., Godley, B., Hays, G., and Bruford, M. 2007. Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. Endangered Species Research, 3: 145–158.

Shamblin, B. M., Bjorndal, K. A., Bolten, A. B., Hillis-Starr, Z. M., Lundgren, I., Naro-Maciel, E., and Nairn, C. J. 2012. Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Molecular Ecology, 21: 2330–2340.

Patrício, A. R., Formia, A., Barbosa, C., Broderick, A. C., Bruford, M., Carreras, C., Catry, P., *et al.* 2017. Dispersal of green turtles from Africa's largest rookery assessed through genetic markers. Marine Ecology Progress Series, 569: 215–225.

ANEXO B – CAPÍTULO 2

Table S1. Sea turtles sampled in Alagoas. Samples in bold indicate hybrids. nDNA haplotype names refer to Arantes et al. (2020c).

Field number	Type	Morphology	mtDNA	nDNA		
				3061	109472	CMOS
T7R13	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata* haplotype 1	*Eretmochelys imbricata* haplotype 5 / *Eretmochelys imbricata* haplotype 6	*Eretmochelys imbricata* haplotype 5 JF415101 / *Eretmochelys imbricata* Pacific isolate FJ039966
T7R14	nest	*Caretta caretta*	CC-A4.2	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 1	
T7R18	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata* haplotype 1	*Eretmochelys imbricata* haplotype 4 / *Eretmochelys imbricata* haplotype 5	*Eretmochelys imbricata* haplotype 10 JF415106 / *Eretmochelys imbricata* Pacific isolate FJ039966
T8R6	nest	*Caretta caretta*	CC-A4.1	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 1 / *Caretta caretta* haplotype 2	
T8R7	nest	*Caretta caretta*	CC-A4.2	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 1	
T8R14	nest	*Caretta caretta*	CC-A4.1			
T8R20	nest	*Caretta caretta*	CC-A4.2			
T8R33	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata* haplotype 1		
T8R37	nest	*Eretmochelys imbricata*	Ei-BR16			
T9R6	nest	*Caretta caretta*	CC-A4.1	*Caretta caretta* haplotype 2		
T9R8	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata*		

				haplotype 1		
T8R38	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata* haplotype 1		
T8R4	nest	*Caretta caretta*	CC-A4	*Caretta caretta* haplotype 2		
T9R1/20	nest	*Eretmochelys imbricata*	Ei-BR16	*Eretmochelys imbricata* haplotype 1	*Eretmochelys imbricata* haplotype 5 / *Lepidochelys olivacea* haplotype 3	*Eretmochelys imbricata* haplotype 3 JF415099
T6R86	nest	*Eretmochelys imbricata*	Ei-BR10			
T7R19	nest	*Caretta caretta*	CC-A4.2			
T7R10	nest	*Caretta caretta*	CC-A4.2	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 1	
T8R15	nest	*Caretta caretta*	CC-A4.2	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 2	
T7R5	nest	*Caretta caretta*	CC-A4			
T1R5/21	nest	*Eretmochelys imbricata*	Ei-A01			
T4R47	nest	*Lepidochelys olivacea*	haplotypeF	*Lepidochelys olivacea* haplotype 4	*Lepidochelys olivacea* haplotype 3	*Lepidochelys olivacea*
MIR1	nest	*Caretta caretta*	haplotypeF	*Caretta caretta* haplotype 2	*Caretta caretta* haplotype 1 / *Lepidochelys olivacea* haplotype 3	*Caretta caretta* Pacific isolate FJ009023
T4R56	nest	*Eretmochelys imbricata*	Ei-A01	*Eretmochelys imbricata* haplotype 1	*Eretmochelys imbricata* haplotype 5	*Eretmochelys imbricata* haplotype 5 JF415101 / *Eretmochelys imbricata* haplotype 3 JF415099
T9R1/19	nest	*Caretta*	haplotypeF	*Lepidochelys*	*Lepidochelys olivacea*	*Lepidochelys olivacea* Pacific isolate

		caretta		*olivacea* haplotype 4	haplotype 3	FJ039980
T6R40	nest	*Eretmochelys imbricata*	CC-A4.2	*Eretmochelys imbricata* haplotype 1 / *Caretta caretta* haplotype 2	*Eretmochelys imbricata* haplotype 4 OR 6 / *Caretta caretta* haplotype 1	*Eretmochelys imbricata* haplotype 5 JF415101 / *Eretmochelys imbricata* haplotype 3 JF415099
T6R84	nest	*Eretmochelys imbricata*	Ei-A01			
T1R5/19	nest	*Eretmochelys imbricata*	Ei-A01			
T4R14	nest	*Caretta caretta*	haplotypeF	*Caretta caretta* haplotype 2	*Caretta caretta haplotype 1 / Lepidochelys olivacea haplotype 3*	*Caretta caretta* Atlantic isolate FJ009030 / *Lepidochelys olivacea* Pacific isolate FJ039980
T4R66	nest	*Eretmochelys imbricata*	Ei-A01			
T4R109	nest	*Eretmochelys imbricata*	Ei-A01			
T2T13/20	strand	*Eretmochelys imbricata*	Ei-A01			
T4T166	strand	*Eretmochelys imbricata*	Ei-A01			
T8T348	strand	*Eretmochelys imbricata*	Ei-A01			
T8T350	strand	*Eretmochelys imbricata*	Ei-A01			
T6T155	strand	*Eretmochelys imbricata*	Ei-BR16			
T1T10	strand	*Eretmochelys imbricata*	Ei-BR10			
T6T4	strand	*Eretmochelys imbricata*	Ei-BR10			
T3T68	strand	*Eretmochelys imbricata*	Ei-IP17			*Eretmochelys imbricata* haplotype 5 JF415101 / *Caretta caretta* Pacific

						isolate FJ009023
T1T59	strand	*Lepidochelys olivacea*	haplotypeF			
T4T29	strand	*Lepidochelys olivacea*	haplotypeF		*Lepidochelys olivacea* haplotype 3	
T3T8/20	strand	*Caretta caretta*	CC-A4			
T2T20/20	strand	*Caretta caretta*	CC-A4			
T2T65	strand	*Caretta caretta*	CC-A4			
T4T90	strand	*Caretta caretta*	CC-A4			
T4T182	strand	*Caretta caretta*	CC-A4			
T4T8	strand	*Chelonia mydas*	CC-A4			*Caretta caretta* Atlantic isolate FJ009030 / *Caretta caretta* Pacific isolate FJ009023
T4T100	strand	*Caretta caretta*	CC-A4			
T4T363	strand	*Eretmochelys imbricata*	CC-A4	*Eretmochelys imbricata* haplotype 1		
T4T389	strand	*Caretta caretta*	CC-A4			
T3T74/20	strand	*Caretta caretta*	CC-A4			
T5T279	strand	*Caretta caretta*	CM-A8	*Caretta caretta* haplotype 2		*Caretta caretta* Atlantic isolate FJ009030 / *Caretta caretta* Pacific isolate FJ009023
T9T183/20	strand	*Chelonia mydas*	CM-A8		*Chelonia mydas* haplotype 12	
T8T8/20	strand	*Chelonia mydas*	CM-A8			

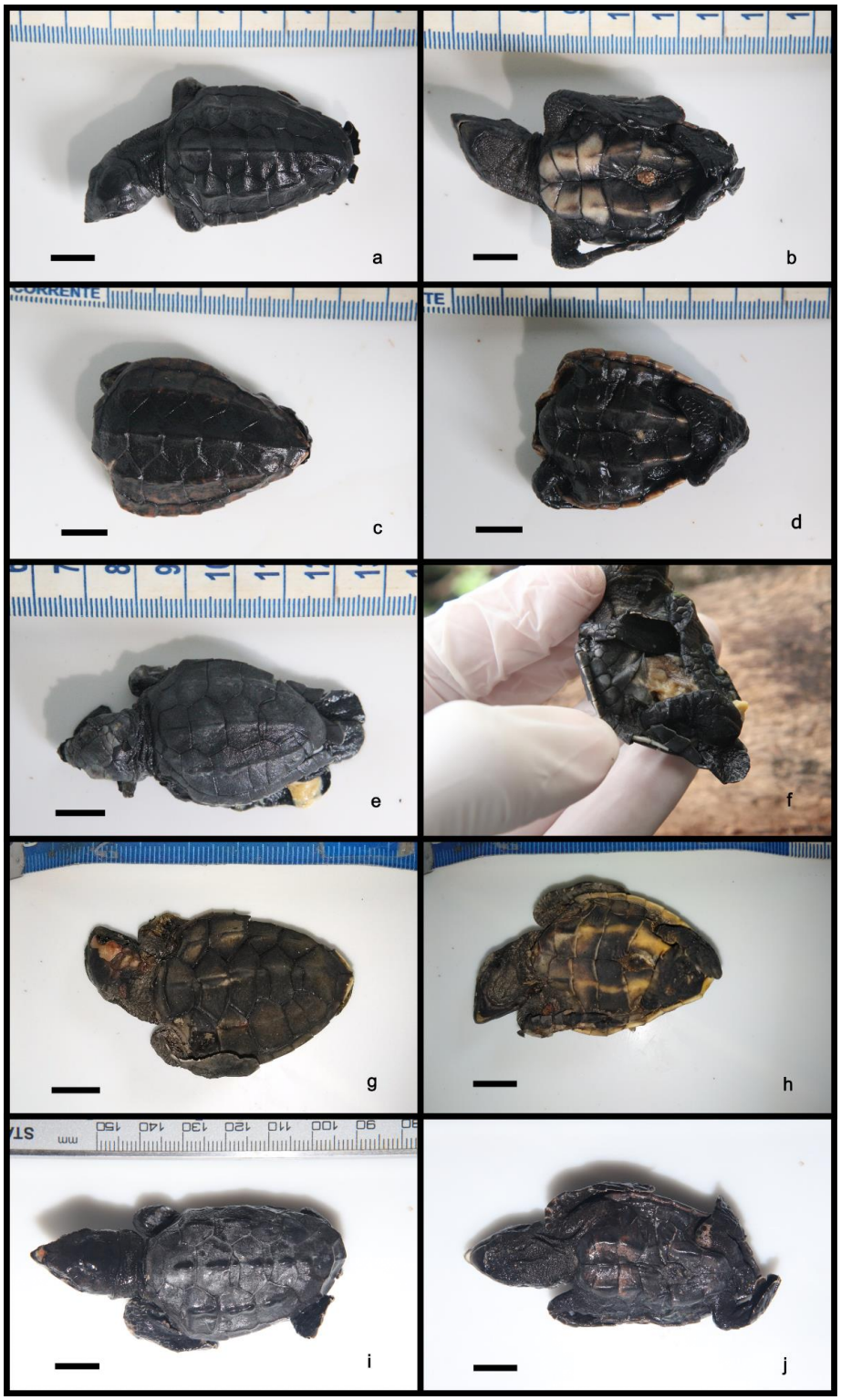


Figure S1. Hatchlings from Alagoas identified as hybrids in this study. T4R14 (a, b), T6R40 (c, d), T9R1-2019 (e, f), T9R1-2020 (g, h), MIR1 (i, j). Black bars indicate 10mm.

Figure S2. Stranded turtles from Alagoas identified as hybrids in this study. T5T279 (a, b), T4T363 (c, d), T4T8 (e, f), T3T68 (g, h). Black bars indicate 100mm. Photos by Instituto Biota de Conservação.

ANEXO C – CAPÍTULO 3

Table S1. Haplotypes of green turtles found in Alagoas foraging ground and Nesting sites used in this study. Number of nesting females are from Seminoff et al., 2015. Full references can be found in the main text reference list. AI – Ascension Island, AV – Aves Island, BK – Bioko, CB – Cuba, CR – Costa Rica, FL – Florida, FN – Fernando de Noronha, GB – Guinea Bissau, MX – Mexico, RA – Rocas Atoll, STP – São Tomé and Principe, SU – Suriname, TR – Trindade Island. I – Encalada *et al*., 1996; II – Bjorndal *et al*., 2005; III – Bjorndal *et al*., 2006; IV – Shamblin *et al*., 2012; V – Formia *et al*., 2006; VI – Formia *et al*., 2007; VIII – Patrício *et al*., 2017.

Haplotype	**Feeding grounds (2018-2021)**		**Rookeries**											
	Alagoas	Paraná	FL	MX	CR	AV	SU	RA/FN	AI	TR	GB	BK	STP	CB
CMA1	-	1	11	7	-	-	-	-	-	-	-	-	-	3
CMA2	-	-	1	-	-	-	-	-	-	-	-	-	-	-
CMA3	1	-	12	5	395	5	1	-	-	-	-	-	-	16
CMA4	-	-	-	-	1	-	-	-	-	-	-	-	-	-
CMA5	28	18	-	1	32	62	55	-	-	-	-	-	1	-
CMA6	4	1	-	-	-	-	2	-	11	-	-	5	1	-
CMA7	-	-	-	-	-	-	1	-	-	-	-	-	-	-
CMA8	96	42	-	-	-	-	-	50	204	67	170	45	17	-
CMA9	5	4	-	-	-	-	-	7	9	19	-	-	-	-
CMA10	2	2	-	-	-	-	-	2	5	-	-	-	-	-
CMA11	-	-	-	-	-	-	-	1	-	1	-	-	-	-
CMA12	-	-	-	-	-	-	-	5	-	-	-	-	-	-
CMA15	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CMA16	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CMA17	-	-	-	2	-	-	-	-	-	-	-	-	-	-
CMA18	-	-	-	3	-	-	-	-	-	-	-	-	-	-
CMA20	-	-	-	-	2	-	-	-	-	-	-	-	-	-

CMA21	-	-	-	-	3	-	-	-	-	-	-	-	-	-
CMA23	1	1	-	-	-	-	-	-	1	6	-	-	-	-
CMA24	-	1	-	-	-	-	-	-	7	1	-	-	-	-
CMA25	-	-	-	-	-	-	-	3	1	-	-	-	-	-
CMA27	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA28	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA32	-	2	-	-	-	-	-	1	1	4	-	-	-	-
CMA33	-	-	-	-	-	-	-	-	-	1	-	-	-	-
CMA35	-	-	-	-	-	-	-	-	-	-	-	-	1	-
CMA36	-	-	-	-	-	-	-	-	-	-	-	-	3	-
CMA37	-	-	-	-	-	-	-	-	-	-	-	-	1	-
CMA38	-	-	-	-	-	-	-	-	-	-	-	-	2	-
CMA39	1	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA42	-	-	-	-	-	-	-	-	-	-	1	-	-	-
CMA44	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA45	-	1	-	-	-	-	-	-	1	-	-	-	-	-
CMA46	1	-	-	-	-	-	-	-	2	-	-	-	-	-
CMA48	-	-	-	-	-	-	-	-	-	-	-	-	-	5
CMA50	-	-	-	-	-	-	-	-	1	-	-	-	-	-
CMA56	-	-	-	-	-	-	-	-	-	-	-	-	-	1
CMA57	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total N	139	73	24	20	433	67	59	69	245	99	171	50	26	28
Nesting females	-	-	8,322	24,330	131,751	2,833	13,067	345	13,417	2,016	30,125	850	376	2,226
Source - haplotype data	This study	This study	I	I	II	IV	II, IV	III	VI	III	VIII	V	V	VII

Table S2. Haplotypes of green turtles from Alagoas and Paraná feeding grounds for the 2018-2021 sampling.

Source	Sample ID	Locality	Size CCL (cm)	mtDNA_short	mtDNA_long	mtSTR_combination
This study	CEM246132	Paraná	37.6	CMA32	CMA32.1	6-12-4-4
This study	CEM179072	Paraná	36.4	CMA8	CMA8.1	7-12-4-4
This study	CEM231761	Paraná	35.4	CMA1	CMA1.1	7-12-4-4
This study	CEM234881	Paraná	33.4	CMA5	CMA5.1	6-12-4-4
This study	CEM237187	Paraná	30.9	CMA8	CMA8.1	7-11-4-4
This study	CEM166390	Paraná	46.6	CMA8	CMA8.1	7-13-4-4
This study	UFP3659	Paraná	36.3	CMA5	CMA5.1	7-12-4-4
This study	CEM178344	Paraná	50.3	CMA8	CMA8.1	7-13-4-4
This study	CEM221919	Paraná	30.9	CMA8	CMA8.1	8-14-4-4
This study	CEM174559	Paraná	38.1	CMA5	CMA5.1	7-15-4-4
This study	CEM111677	Paraná	49.5	CMA8	CMA8.1	7-11-4-4
This study	CEM178008	Paraná	36.7	CMA8	CMA8.1	---
This study	CEM230912	Paraná	35.8	CMA8	CMA8.1	8-14-4-4
This study	CEM234014	Paraná	37.7	CMA8	CMA8.1	7-16-4-4
This study	CEM228272	Paraná	36.1	CMA8	CMA8.1	8-11-4-4
This study	CEM230915	Paraná	35	CMA8	CMA8.1	7-12-4-4
This study	CEM084198	Paraná	31.8	CMA8	CMA8.1	8-13-4-4
This study	CEM216116	Paraná	57.3	CMA8	CMA8.1	---
This study	CEM180600	Paraná	31	CMA8	CMA8.1	7-12-4-4
This study	CEM183851	Paraná	48	CMA8	CMA8.1	5-12-4-4
This study	CEM159204	Paraná	38.4	CMA5	CMA5.1	---
This study	CEM125331	Paraná	39	CMA5	CMA5.1	---
This study	CEM178761	Paraná	39.8	CMA8	CMA8.1	7-11-4-4

This study	CEM115031	Paraná	36	CMA5	CMA5.1	---
This study	CEM185391	Paraná	37	CMA8	CMA8.1	6-16-4-4
This study	CEM253387	Paraná	45.2	CMA8	CMA8.1	7-12-4-4
This study	CEM065774	Paraná	47.8	CMA9	CMA9.1	7-12-4-4
This study	CEM159202	Paraná	49.7	CMA6	-	---
This study	CEM159259	Paraná	37.7	CMA8	CMA8.1	---
This study	CEM114847	Paraná	47.3	CMA8	CMA8.1	7-12-4-4
This study	CEM178778	Paraná	32.8	CMA8	CMA8.1	---
This study	CEM076436	Paraná	35	CMA8	-	---
This study	CEM245371	Paraná	31.2	CMA5	-	---
This study	UFP3432	Paraná	33.5	CMA8	CMA8.1	---
This study	CEM235226	Paraná	35	CMA8	CMA8.1	7-12-4-4
This study	CEM203772	Paraná	32.8	CMA8	CMA8.1	7-12-4-4
This study	CEM186275	Paraná	36.1	CMA5	CMA5.1	7-12-4-4
This study	CEM166112	Paraná	32.2	CMA8	CMA8.1	7-12-4-4
This study	CEM169615	Paraná	31.4	CMA5	CMA5.1	6-12-4-4
This study	CEM170079	Paraná	34.5	CMA9	CMA9.1	7-12-4-4
This study	CEM171526	Paraná	38.5	CMA8	CMA8.1	8-13-4-4
This study	CEM164604	Paraná	38.7	CMA24	CMA24.1	7-12-4-4
This study	CEM159081	Paraná	35.5	CMA10	CMA10.1	7-12-4-4
This study	CEM125337	Paraná	40.2	CMA8	CMA8.1	8-10-4-4
This study	CEM135360	Paraná	35.6	CMA10	CMA10.1	7-12-4-4
This study	CEM101264	Paraná	39.8	CMA32	CMA32.1	7-12-4-4
This study	CEM159121	Paraná	33.2	CMA8	CMA8.1	---
This study	CEM159066	Paraná	36.1	CMA45	CMA45.1	5-13-4-4
This study	CEM157970	Paraná	39	CMA5	CMA5.1	7-11-4-4

This study	CEM172168	Paraná	36.9	CMA5	CMA5.1	7-12-4-4
This study	CEM106437	Paraná	54.5	CMA5	CMA5.1	7-11-4-4
This study	CEM185945	Paraná	45	CMA8	CMA8.1	7-12-4-4
This study	CEM185390	Paraná	45	CMA8	CMA8.1	8-12-4-4
This study	CEM214604	Paraná	45	CMA23	-	---
This study	CEM144799	Paraná	53.2	CMA9	CMA9.1	7-13-4-4
This study	CEM231013	Paraná	36	CMA8	CMA8.1	7-12-4-4
This study	CEM234341	Paraná	35.4	CMA8	CMA8.1	---
This study	CEM76660	Paraná	31.5	CMA5	CMA5.1	7-12-4-4
This study	CEM79414	Paraná	50	CMA8	CMA8.1	7-11-4-4
This study	CEM237331	Paraná	34	CMA8	CMA8.1	6-13-4-4
This study	CEM245254	Paraná	34.5	CMA8	CMA8.1	7-11-4-4
This study	CEM169267	Paraná	45.2	CMA8	CMA8.1	7-12-4-4
This study	CEM159063	Paraná	48	CMA5	CMA5.1	7-12-4-4
This study	CEM172034	Paraná	38.7	CMA5	CMA5.1	6-12-4-4
This study	CEM172061	Paraná	35	CMA8	CMA8.1	7-12-4-4
This study	CEM179075	Paraná	39	CMA8	CMA8.1	7-12-4-4
This study	T9T302	Alagoas	37	CMA8	-	---
This study	T2T48/20	Alagoas	36.5	CMA8	-	---
This study	T2T45/20	Alagoas	38.5	CMA8	-	---
Almeida et al., 2021	T4T99	Alagoas	34.9	CMA8	-	---
This study	T4T173	Alagoas	37.4	CMA8	-	---
This study	CEM125295	Paraná	37.9	CMA8	CMA8.1	7-15-4-4
This study	CEM102273	Paraná	34.3	CMA8	CMA8.1	---
This study	CEM125338	Paraná	37.2	CMA5	-	---
This study	CEM159190	Paraná	34.5	CMA9	CMA9.1	---

This study	CEM166177	Paraná	32	CMA5	-	---
This study	CEM166681	Paraná	39.3	CMA8	-	---
This study	CEM101221	Paraná	33.2	CMA5	-	---
Almeida et al., 2021	T1T110	Alagoas	37.2	CMA8	CMA8.1	7-16-4-4
Almeida et al., 2021	T7T12	Alagoas	45	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T7T124	Alagoas	43	CMA5	-	8-11-4-4
Almeida et al., 2021	T4T223	Alagoas	27.5	CMA8	CMA8.1	7-13-4-4
Almeida et al., 2021	T2T121	Alagoas	52	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T5T78	Alagoas	40.3	CMA8	CMA8.1	7-16-4-4
Almeida et al., 2021	T7T85	Alagoas	37.3	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T4T181	Alagoas	43.5	CMA8	CMA8.1	7-11-4-4
Almeida et al., 2021	T3T37	Alagoas	36.5	CMA5	-	6-12-4-4
Almeida et al., 2021	T7T59	Alagoas	42.3	CMA8	CMA8.1	8-16-4-4
Almeida et al., 2021	T4T228	Alagoas	44.5	CMA8	CMA8.1	7-11-4-4
This study	T4T219	Alagoas	38.1	CMA8	-	3-13-4-4
Almeida et al., 2021	T7T34	Alagoas	47.3	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T4T139	Alagoas	46.4	CMA8	CMA8.1	6-13-4-4
Almeida et al., 2021	T3T5	Alagoas	39	CMA8	-	7-13-4-4
Almeida et al., 2021	T3T81	Alagoas	44	CMA8	CMA8.1	6-16-4-4
Almeida et al., 2021	T7T9	Alagoas	43.4	CMA5	CMA5.1	7-12-4-4
Almeida et al., 2021	T2T8	Alagoas	48.6	CMA8	CMA8.1	7-12-4-4
This study	T9T111	Alagoas	38.5	CMA8	-	7-13-4-4
Almeida et al., 2021	T5T9	Alagoas	48.5	CMA8	CMA8.1	6-12-4-4
Almeida et al., 2021	T5T179	Alagoas	40	CMA8	CMA8.1	---
Almeida et al., 2021	T8T105	Alagoas	47.8	CMA8	-	7-15-4-4
Almeida et al., 2021	T8T195	Alagoas	34.3	CMA8	CMA8.3	7-11-4-4

Almeida et al., 2021	T4T98	Alagoas	51.3	CMA8	CMA8.1	7-11-4-4
Almeida et al., 2021	T5T149	Alagoas	42.3	CMA8	CMA8.1	7-13-4-4
Almeida et al., 2021	T8T84	Alagoas	39.5	CMA5	-	7-12-4-4
Almeida et al., 2021	T8T59	Alagoas	35.4	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T3T124	Alagoas	50	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T5T148	Alagoas	47.2	CMA9	CMA9.1	7-12-4-4
Almeida et al., 2021	T8T70	Alagoas	40	CMA8	CMA8.1	6-12-4-4
Almeida et al., 2021	T3T125	Alagoas	35.5	CMA8	-	7-11-4-4
Almeida et al., 2021	T3T101	Alagoas	38	CMA9	CMA9.1	7-12-4-4
Almeida et al., 2021	T8T53	Alagoas	51.3	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T9T284	Alagoas	47	CMA8	CMA8.1	7-15-4-4
Almeida et al., 2021	T5T123	Alagoas	51.7	CMA8	CMA8.1	8-11-5-5
Almeida et al., 2021	T8T48	Alagoas	38.2	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T4T54	Alagoas	40	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T1T39	Alagoas	45.5	CMA5	CMA5.1	6-12-4-4
Almeida et al., 2021	T1T45	Alagoas	39.8	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T1T15	Alagoas	35.2	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T1T56	Alagoas	40.1	CMA5	CMA5.1	6-12-4-4
Almeida et al., 2021	T1T76	Alagoas	36.3	CMA9	CMA9.1	7-13-4-4
Almeida et al., 2021	T1T85	Alagoas	41.5	CMA8	CMA8.1	6-17-4-4
Almeida et al., 2021	T1T122	Alagoas	49.1	CMA10	CMA10.1	7-13-4-4
Almeida et al., 2021	T1T23	Alagoas	38.1	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T2T123	Alagoas	53.2	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T3T113	Alagoas	46	CMA8	CMA8.1	7-13-4-4
This study	T4T252	Alagoas	45.3	New haplotype	New haplotype	7-12-4-4

Almeida et al., 2021	T4T278	Alagoas	52.2	CMA8	CMA8.1	---
Almeida et al., 2021	T1T81	Alagoas	36.1	CMA8	CMA8.1	---
Almeida et al., 2021	T1T37	Alagoas	31.4	CMA8	CMA8.1	7-13-4-4
Almeida et al., 2021	T1T107	Alagoas	49.8	CMA5	CMA5.1	5-13-4-4
Almeida et al., 2021	T9T315	Alagoas	55	CMA8	CMA8.1	8-12-4-4
Almeida et al., 2021	T2T103	Alagoas	46	CMA8	CMA8.1	8-12-4-4
Almeida et al., 2021	T2T104	Alagoas	53	CMA5	CMA5.1	---
Almeida et al., 2021	T5T158	Alagoas	41	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T7T134	Alagoas	52	CMA8	CMA8.1	5-15-4-4
Almeida et al., 2021	T9T318	Alagoas	36.5	CMA8	CMA8.2	7-11-4-4
Almeida et al., 2021	T4T227	Alagoas	39.8	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T8T126	Alagoas	41	CMA8	CMA8.1	7-12-4-4
Almeida et al., 2021	T9T158	Alagoas	25	CMA8	CMA8.1	8-11-4-4
Almeida et al., 2021	T3T134	Alagoas	50	CMA8	-	-
Almeida et al., 2021	T1T48 /2018	Alagoas	38.1	CMA8	-	-
Almeida et al., 2021	T1T78 /2018	Alagoas	43.5	CMA6	CMA6.1	-
Almeida et al., 2021	T2T11 /2018	Alagoas	47.5	CMA23	CMA23.1	-
Almeida et al., 2021	T2T91 /2018	Alagoas	42.2	CMA5	-	-
Almeida et al., 2021	T1T4 /2018	Alagoas	38.4	CMA8	-	-
Almeida et al., 2021	T1T46 /2018	Alagoas	52.5	CMA5	-	-
Almeida et al., 2021	T1T121 /2018	Alagoas	39.4	CMA8	-	-
Almeida et al., 2021	T2T6 /2018	Alagoas	51.6	CMA8	CMA8.1	-
Almeida et al., 2021	T2T51 /2018	Alagoas	49.1	CMA8	-	-
Almeida et al., 2021	T2T85 /2018	Alagoas	57.5	CMA5	-	-
Almeida et al., 2021	T2T96 /2018	Alagoas	43.2	CMA8	-	-
Almeida et al., 2021	T3T66 /2018	Alagoas	58	CMA8	-	-

Almeida et al., 2021	T3T108 /2018	Alagoas	46.4	CMA8	-	-
Almeida et al., 2021	T3T116 /2018	Alagoas	38.5	CMA8	CMA8.1	-
Almeida et al., 2021	T3T121 /2018	Alagoas	54	CMA3	-	-
Almeida et al., 2021	T4T244 /2018	Alagoas	56.7	CMA8	CMA8.1	-
Almeida et al., 2021	T4T37 /2018	Alagoas	50.8	CMA46	-	-
Almeida et al., 2021	T4T175 /2018	Alagoas	53.7	CMA8	-	-
Almeida et al., 2021	T5T16 /2018	Alagoas	37.2	CMA8	-	-
Almeida et al., 2021	T6T36 /2018	Alagoas	23	CMA6	-	-
Almeida et al., 2021	T7T10 /2018	Alagoas	57.3	CMA5	-	-
Almeida et al., 2021	T7T61 /2018	Alagoas	52.3	CMA8	-	-
Almeida et al., 2021	T5T6 /2018	Alagoas	54	CMA8	-	-
Almeida et al., 2021	T5T67 /2018	Alagoas	44.6	CMA8	-	-
Almeida et al., 2021	T5T114 /2018	Alagoas	59.2	CMA5	-	-
Almeida et al., 2021	T5T115 /2018	Alagoas	59	CMA8	CMA8.1	-
Almeida et al., 2021	T5T157 /2018	Alagoas	59	CMA8	CMA8.1	-
Almeida et al., 2021	T6T26 /2018	Alagoas	42.3	CMA9	-	-
Almeida et al., 2021	T7T166 /2018	Alagoas	44	CMA5	-	-
Almeida et al., 2021	T8T190 /2018	Alagoas	52	CMA39	-	-
Almeida et al., 2021	T9T42 /2018	Alagoas	52.2	CMA8	CMA8.1	-
Almeida et al., 2021	T9T48 /2018	Alagoas	38	CMA8	-	-
Almeida et al., 2021	T9T76 /2018	Alagoas	37	CMA8	-	-
Almeida et al., 2021	T3T47 /2018	Alagoas	55.5	CMA5	CMA5.1	-
Almeida et al., 2021	T4T164 /2018	Alagoas	50.3	CMA6	CMA6.1	-
Almeida et al., 2021	T4T169 /2018	Alagoas	35.7	CMA8	CMA8.1	-
Almeida et al., 2021	T4T235 /2018	Alagoas	58.5	CMA6	CMA6.1	-
Almeida et al., 2021	T3T65 /2018	Alagoas	49.5	CMA8	CMA8.1	-

Almeida et al., 2021	T3T109 /2018	Alagoas	50.7	CMA8	CMA8.1	-
Almeida et al., 2021	T3T123 /2018	Alagoas	33	CMA8	CMA8.1	-
This study	T1T5/2021	Alagoas	36	CMA5	-	-
This study	T1T34/2020	Alagoas	38.5	CMA8	-	-
This study	T4T84	Alagoas	36.5	CMA5	-	-
This study	T4T285	Alagoas	29.8	CMA8	-	-
This study	T7T58	Alagoas	38.4	CMA8	-	-
This study	T8T14	Alagoas	37	CMA8	-	-
This study	T8T179	Alagoas	37	CMA8	-	-
This study	T1T18	Alagoas	43.5	CMA5	-	-
This study	T1T20	Alagoas	41.3	CMA5	-	-
This study	T1T30	Alagoas	55.3	CMA5	-	-
This study	T1T116	Alagoas	40.3	CMA8	-	-
This study	T1T201	Alagoas	47.5	CMA8	-	-
This study	T2T38	Alagoas	42.6	CMA5	-	-
This study	T2T50	Alagoas	40.5	CMA8	-	-
This study	T2T55	Alagoas	41.5	CMA5	-	-
This study	T2T81	Alagoas	59.7	CMA8	-	-
This study	T2T86	Alagoas	57.1	CMA8	-	-
This study	T2T211	Alagoas	41	CMA8	-	-
This study	T2T212	Alagoas	44	CMA5	-	-
This study	T3T71/2020	Alagoas	57.5	CMA5	-	-
This study	T3T72/2020	Alagoas	43	CMA8	-	-
This study	T1T45/2020	Alagoas	33.5	CMA5	-	-
This study	T3T7/2020	Alagoas	33.2	CMA5	-	-
This study	T4T137/2020	Alagoas	54.3	CMA5	-	-

This study	T1T207	Alagoas	44	CMA8	-	-
This study	T2T185	Alagoas	56	CMA5	-	-
This study	T3T49/2020	Alagoas	56	CMA8	-	-
This study	T1T48/2020	Alagoas	56	CMA9	-	-
This study	T2T61/2020	Alagoas	60	CMA8	-	-
This study	T2T68/2020	Alagoas	58.5	CMA8	-	-
This study	T3T13/2020	Alagoas	45	CMA10	-	-
This study	T1T35/2020	Alagoas	45	CMA8	-	-
This study	T3T63/2020	Alagoas	58	CMA8	-	-

References

Almeida JPFA, Santos RG, Mott T (2021) Sex ratios and natal origins of green turtles from feeding grounds in the Southwest Atlantic Ocean. ICES Journal of Marine Science 78:1840–1848. doi: 10.1093/icesjms/fsa.

Bjorndal, K. A., Bolten, A. B., and Troëng, S. 2005. Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Marine Biology, 147: 1449–1457.

Bjorndal, K. A., Bolten, A. B., Moreira, L., Bellini, C., and Marcovaldi, M. Â. 2006. Population structure and diversity of brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology, 5: 262–268.

Encalada, S. E., Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Miyamoto, M. M., and Bowen, B. W. 1996. Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: A mitochondrial DNA control region sequence assessment. Molecular Ecology, 5: 473–483.

Formia, A., Godley, B. J., Dontaine, J. F., and Bruford, M. W. 2006. Mitochondrial DNA diversity and phylogeography of

endangered green turtle (*Chelonia mydas*) populations in Africa. Conservation Genetics, 7: 353–369.

Formia, A., Broderick, A., Glen, F., Godley, B., Hays, G., and Bruford, M. 2007. Genetic composition of the Ascension Island green turtle rookery based on mitochondrial DNA: implications for sampling and diversity. Endangered Species Research, 3: 145–158.

Shamblin, B. M., Bjorndal, K. A., Bolten, A. B., Hillis-Starr, Z. M., Lundgren, I., Naro-Maciel, E., and Nairn, C. J. 2012. Mitogenomic sequences better resolve stock structure of southern Greater Caribbean green turtle rookeries. Molecular Ecology, 21: 2330–2340.

Patrício, A. R., Formia, A., Barbosa, C., Broderick, A. C., Bruford, M., Carreras, C., Catry, P., *et al.* 2017. Dispersal of green turtles from Africa's largest rookery assessed through genetic markers. Marine Ecology Progress Series, 569: 215–225.