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TARTARUGAS-VERDES AMEAÇADAS DE EXTINÇÃO NA BACIA POTIGUAR, NORDESTE, BRASIL: PERSPECTIVAS PARA A CONSERVAÇÃO

DANIEL SOLON DIAS DE FARIAS

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Tese apresentada ao Curso de Doutorado em Desenvolvimento e Meio Ambiente, associação ampla em Rede, Universidade Federal do Rio Grande do Norte, como parte dos requisitos necessários à obtenção do título de Doutor.

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APRESENTAÇÃO

A Tese tem como título “TARTARUGAS-VERDES AMEAÇADAS DE EXTINÇÃO NA BACIA POTIGUAR, NORDESTE, BRASIL: PERSPECTIVAS PARA A CONSERVAÇÃO”, e, conforme padronização aprovada pelo colegiado do DDMA local, se encontra composta por uma Introdução geral (embasamento teórico e revisão bibliográfica do conjunto da temática abordada, incluindo a identificação do problema da Tese), uma Caracterização geral da Área de estudo, Metodologia geral empregada para o conjunto da obra e por 03 Capítulos, que correspondem a artigos científicos, intitulados “Population structure of the endangered green turtles (*Chelonia mydas*) in the Potiguar Basin, Northeastern, Brazil: insights for conservation”, “The use of an alimentary index to assess anthropogenic debris on green turtles (*Chelonia mydas*)” e “Bioaccumulation of total mercury, copper, cadmium, silver, and selenium in green turtles (*Chelonia mydas*) stranded along the Potiguar Basin, northeastern Brazil”. Todos os capítulos/artigos estão no formato do periódico ao qual está aceito/submetido; os endereços dos sites onde constam as normas dos periódicos estão destacados em cada capítulo/artigo.

Ao meu pai, que tanto me incentivou a seguir firme nessa jornada, que sempre teve a curiosidade de saber sobre o andamento da minha pesquisa, que se animava ao ver qualquer notícia sobre vida marinha para iniciar uma conversa, que não cansava de me acordar atrasado pela manhã com a desculpa de que soube de um animal encalhado, mas que infelizmente não pôde estar comigo para presenciar essa conquista.

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"*Sei que meu trabalho é uma gota no oceano, mas sem ele o oceano seria menor*" –
Madre Teresa de Calcutá

RESUMO

TARTARUGAS-VERDES AMEAÇADAS DE EXTINÇÃO NA BACIA POTIGUAR, NORDESTE, BRASIL: PERSPECTIVAS PARA A CONSERVAÇÃO

As altas taxas de encalhes anuais de tartarugas-verdes (*Chelonia mydas*) registradas nos últimos anos no Brasil, e mais especificamente na região da Bacia Potiguar, trazem à tona a necessidade de se entender a presença da espécie na região, assim como suas principais ameaças. Nesse sentido, o presente trabalho objetivou analisar o padrão de ocorrência da tartaruga-verde na Bacia Potiguar, região compreendida entre litoral setentrional do Rio Grande do Norte (RN) e leste do Ceará (CE), caracterizando geneticamente os estoques populacionais da espécie, assim como avaliando o impacto dos detritos antropogênicos e a bioacumulação de elementos químicos nos tecidos dos espécimes encontrados encalhados na região. A análise da composição genética demonstrou uma grande diversidade haplotípica das tartarugas-verdes da Bacia Potiguar, com predomínio dos dois haplótipos mais registrados no litoral do Atlântico Sul Ocidental (CM-A8 e CM-A5). Através da adaptação de uma ferramenta utilizada para análise de dieta, o Índice Alimentar (IAi), foi possível acessar o impacto dos detritos antropogênicos, principalmente do plástico flexível transparente em tartarugas juvenis (JUV-I), o que está associado ao hábito alimentar costeiro e de superfície da espécie, onde ocorrem as maiores concentrações de fragmentos de plástico, associados a algas flutuantes. A avaliação da contaminação por elementos químicos (THg, Cu, Cd, Ag e Se) em tecidos (fígado, músculo e rins) de tartarugas-verdes, revelou níveis de contaminação, até então desconhecidos para esses animais na Bacia Potiguar, reforçando o papel das tartarugas marinhas como sentinelas da qualidade do ecossistema marinho. Nossos achados aumentam o entendimento da composição dos estoques populacionais de *C. mydas* na costa brasileira que, associado ao diagnóstico dos impactos causados pelos detritos antropogênicos e contaminantes, principalmente em áreas menos estudadas, como os sítios de alimentação de tartaruga-verde, contribuem para uma maior organização das estratégias de recomposição de áreas de nidificação, com consequente melhoria no status de ameaça da espécie no Brasil e no mundo.

PALAVRAS-CHAVE: *Chelonia mydas*, poluição marinha, ecotoxicologia, detritos antropogênicos, genética populacional.

ABSTRACT

THREATENED GREEN TURTLES IN THE POTIGUAR BASIN, NORTHEAST, BRAZIL: PERSPECTIVES FOR CONSERVATION

The high annual stranding rates of green turtles (*Chelonia mydas*) recorded in recent years in Brazil, and more specifically in the Potiguar Basin region, bring to light the need to understand the presence of the species in the region, as well as its main threats. In this sense, this study aimed to analyse the pattern of occurrence of the green turtle in the Potiguar Basin, a region between the northern coast of Rio Grande do Norte (RN) and east of Ceará (CE), characterizing the population stocks of the species, as well as as evaluating the impact of anthropogenic debris and the bioaccumulation of chemical elements in the tissues of specimens found stranded in the region. The analysis of the genetic composition showed a great haplotypic diversity of the green turtles of the Potiguar Basin, with a predominance of the two most recorded haplotypes on the coast of the Western South Atlantic (CM-A8 and CM-A5). Through the adaptation of a tool used for diet analysis, the Food Index (IAi), it was possible to access the impact of anthropogenic debris, mainly transparent soft plastic on juvenile green turtles (JUV-I), which is associated with coastal eating habits. and on the surface of the species, where the highest concentrations of plastic fragments occur, associated with floating algae. The evaluation of contamination by chemical elements (THg, Cu, Cd, Ag and Se) in tissues (liver, muscle and kidneys) of green turtles, revealed levels of contamination, still unknown for these animals in the Potiguar Basin, reinforcing the role of sea turtles as sentinels of the quality of the marine ecosystem. Our findings increase the understanding of the composition of *C. mydas* population stocks on the Brazilian coast, which, associated with the diagnosis of impacts caused by anthropogenic debris and contaminants, especially in less studied areas, such as green turtle feeding sites, contribute to a greater organization of strategies to recompose nesting areas, with a consequent improvement in the threat status of the species in Brazil and in the world.

KEYWORDS: *Chelonia mydas*, marine pollution, ecotoxicology, anthropogenic debris, population genetics.

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INTRODUÇÃO GERAL

Existem atualmente sete espécies de tartarugas marinhas no mundo, pertencentes a duas famílias distintas: Cheloniidae, que inclui as espécies *Chelonia mydas* (LINNAEUS, 1758), *Caretta caretta* (LINNAEUS, 1758), *Eretmochelys imbricata* (LINNAEUS, 1766), *Lepidochelys olivacea* (ESCHSCHOLTZ, 1829), *Lepidochelys kempii* (GARMAN, 1880) e *Natator depressus* (GARMAN, 1880); e Dermochelyidae, que compreende uma única espécie, a *Dermochelys coriacea* (LINNAEUS, 1766) (MEYLAN; MEYLAN, 1999). De todas as espécies, somente a *L. kempii* e *N. depressus* apresentam distribuição mais restrita: principalmente no Golfo do México e costa oriental dos Estados Unidos, e continente australiano, respectivamente (MÁRQUEZ, 1990; MÁRQUEZ 1994; MEYLAN; MEYLAN, 1999). Apenas essas duas espécies não ocorrem no Brasil (MARCOVALDI; MARCOVALDI, 1999).

As tartarugas marinhas pertencem à linhagem mais antiga de répteis vivos, apresentando ciclos de vida complexos, com migrações transoceânicas e alternância de habitats e recursos alimentares (MÁRQUEZ, 1990; BOLTEN, 2003; LUSCHI et al., 2003). Em razão dessa complexidade, se tornaram ferramentas valiosas para a conservação, uma vez que não reconhecem fronteiras políticas e geográficas entre os países, o que requer esforço coletivo para a preservação das espécies.

Dentre todas as espécies, a tartaruga-verde (*Chelonia mydas*) é uma das mais comuns, apresentando distribuição global, que se estende ao longo dos mares tropicais e subtropicais em todo o mundo (HIRTH, 1997; LEMONS et al., 2011; SEMINOFF et al., 2015), ocupando vários nichos ecológicos ao longo de seu complexo ciclo de vida, com comportamento altamente migratório (MEYLAN; MEYLAN 1999; GODLEY et al., 2002; BOLTEN, 2003; PROIETTI et al., 2012) (Figura 1).

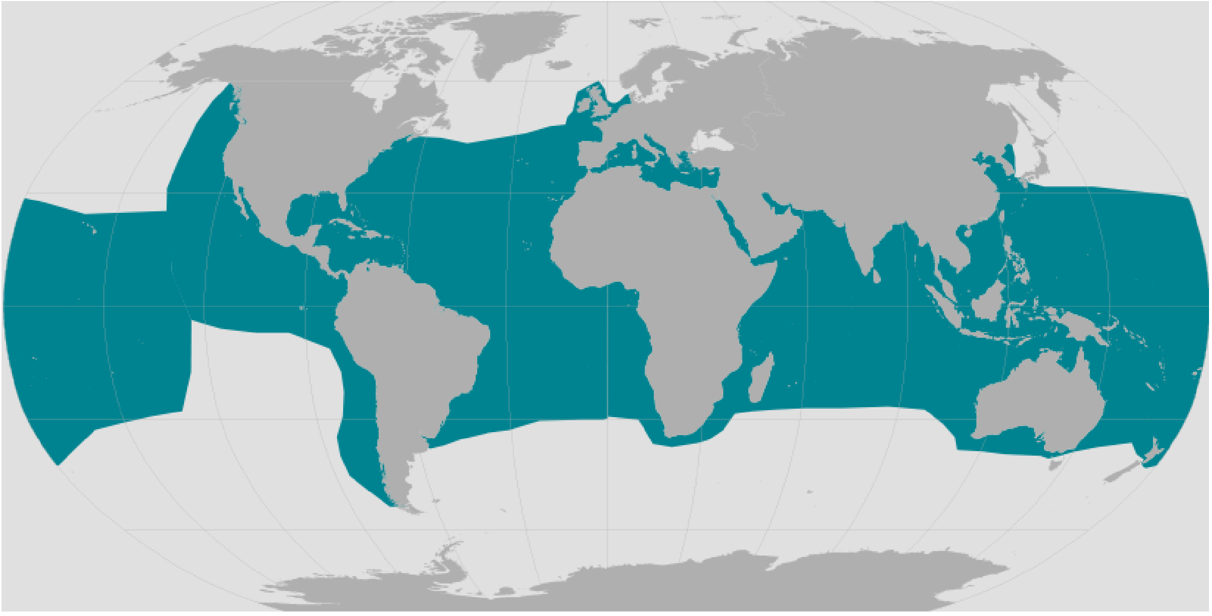


Figura 1. Mapa da distribuição global da tartaruga-verde (*Chelonia mydas*). Fonte: NOAA Pacific Islands Fisheries Science Center.

As características diagnósticas básicas da espécie incluem a presença de um par de placas nasais e quatro pares de placas laterais justapostas na carapaça (Figura 2). A coloração da carapaça pode variar bastante em adultos, com tons de verde-acinzentado a marrom-amarelado e ventre branco-amarelado (MÁRQUEZ, 1990; PRITCHAR; MORTIMER, 1999). A cabeça é arredondada e proporcionalmente pequena em relação ao corpo; a ranfoteca é serrilhada e as nadadeiras geralmente apresentam uma única unha (WYNEKEN, 2001). Os espécimes apresentam maturação sexual tardia, entre 15 e 50 anos (BJORNDAL; ZUG, 1995; SEMINOFF et al., 2002; CHALOUPKA et al., 2004; BELL et al., 2005; WATSON, 2006; GOSHE et al., 2010) e os adultos podem ultrapassar o tamanho de 120 cm de comprimento curvilíneo de carapaça (CCC), chegando a pesar 230 kg (PRITCHARD; MORTIMER, 1999).

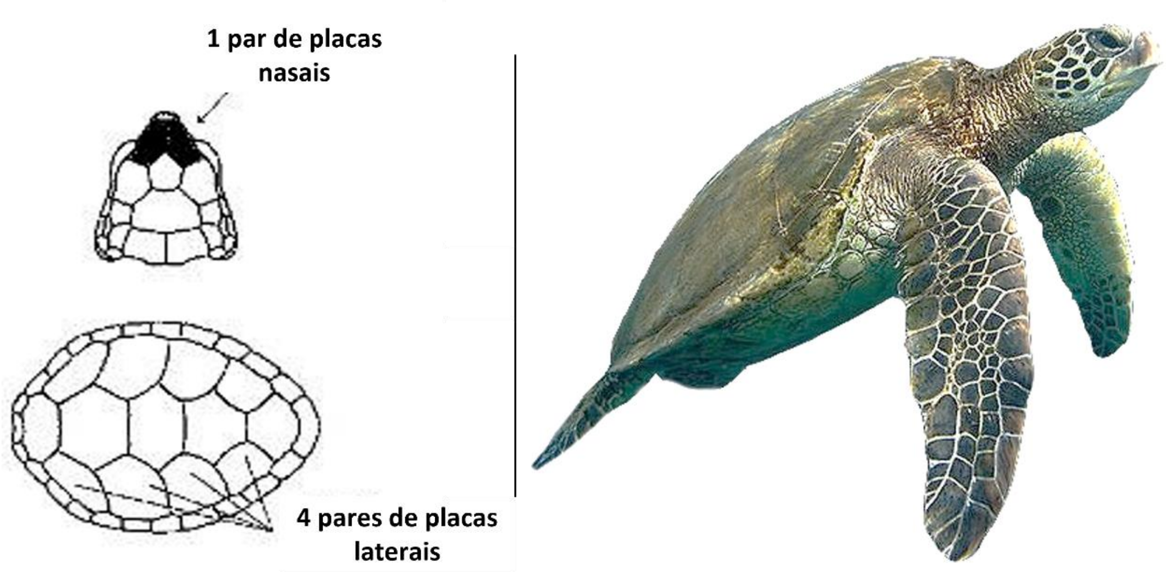


Figura 2. Características diagnósticas básicas da tartaruga-verde (*Chelonia mydas*).

A espécie frequenta toda a costa brasileira, sendo a tartaruga marinha que apresenta hábitos mais costeiros, utilizando inclusive estuários de rios e lagos (HIRTH, 1997; SANCHES, 1999). As desovas ocorrem principalmente nas ilhas oceânicas brasileiras, Ilha da Trindade (ES) (MOREIRA et al., 1995), Atol das Rocas (RN) (BELLINI; SANCHES, 1996; GROSSMAN et al., 2003) e Fernando de Noronha (PE) (BELLINI; SANCHES, 1996). As áreas de alimentação, sobretudo de indivíduos juvenis, se distribuem ao longo de toda a costa, com registros em todos os estados costeiros brasileiros, do Rio Grande do Sul ao Amapá (PRITCHARD, 1976; MASCARENHAS et al., 2003; BRITO et al., 2004; GALLO et al., 2006; NARO-MACIEL et al., 2007; REIS Et al., 2009; FARIAS et al. 2019).

O grande declínio que vem ocorrendo na megafauna marinha tem trazido sérias consequências à viabilidade do ecossistema costeiro numa escala global (JACKSON et al, 2001). Como consequência da intensa exploração ocorrida no passado, provocada principalmente pelo uso direto das fêmeas para alimentação, o consumo dos ovos e a captura intencional na pesca, foi verificada uma depleção de populações de tartarugas marinhas (YALÇIN-OZDILEK; YERL, 2006; FERNANDES et al., 2016). Atualmente, apesar de mais protegidas, as tartarugas marinhas ainda se encontram ameaçadas de extinção, estando a tartaruga-verde classificada como “Em Perigo" (EN), segundo a Lista Vermelha de Espécies Ameaçadas, da União Internacional para a Conservação da Natureza (IUCN, 2021) e como “Vulnerável” (VU), segundo o Ministério do Meio Ambiente do Brasil, no Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (ICMBio, 2018). Os principais impactos estão relacionados a interação negativa com a atividade pesqueira, ingestão de resíduos sólidos, ocupação desordenada e crescente da faixa litorânea e degradação de hábitats (BUGONI et al., 2001; HAMANN et al, 2010, SCHUYLER et al., 2014; FARIAS et al., 2019).

FUNDAMENTAÇÃO TEÓRICA

Durante muito tempo, os esforços conservacionistas foram direcionados para a identificação de ameaças, particularmente em áreas de desova, onde as tartarugas marinhas passam cerca de 1% do seu ciclo de vida (BJORNDAL, 2000). Nesse sentido, vários estudos têm sido realizados, com o objetivo de acessar a composição demográfica de áreas de alimentação, cujo principal interesse é entender os mecanismos responsáveis pelo recrutamento dos animais para estas regiões e as rotas migratórias por eles utilizadas (SEARS ET AL. 1995; BASS; WITZELL, 2000; LUKE et al., 2004; BASS et al., 2006; NARO-MACIEL, 2005, DUTTON et al., 2008; VELEZ-ZUAZO et al., 2008).

Nos primeiros anos de vida (< 25 cm de CCC) os juvenis de tartaruga-verde permanecem em águas oceânicas, com uma alimentação basicamente onívora, com tendência a carnivoria (BJORNDAL, 1997; JONES; SEMINOFF, 2013), alimentando-se de organismos planctônicos, incluindo crustáceos, celenterados e ctenóforos (ARTHUR ET AL., 2008). Após a fase oceânica, já na fase nerítica, passam a ocupar áreas mais costeiras, desenvolvendo uma alimentação tipicamente herbívora, alimentando-se basicamente de macroalgas e fanerógamas, o que se mantém até a fase adulta (Figura 3) (BJORNDAL; BOLTEN, 1988; BJORNDAL, 1997; BRAND-GARDNER et al., 1999; ARTHUR et al., 2008; LENZ et al., 2013). Apesar dessas mudanças de hábitat e de alimentação (BJORNDAL, 1997; REICH et al., 2007; ARTHUR et al., 2008), estudos recentes revelaram diferenças regionais no tempo desse processo, com diferentes níveis de onivoria (CARDONA et al., 2009; LEMONS et al., 2011; MORAIS et al., 2012; REISSER et al, 2013; SANTOS, 2014; CARMAN et al., 2014; MORAIS et al., 2014) e a persistência de uma dieta com matéria animal em adultos (HATASEE et al., 2006; BARROS et al., 2007; PARKER et al., 2011; MORAIS et al., 2012; NAGAOKA et al., 2012; REISSER et al., 2013; MORAIS et al., 2014).

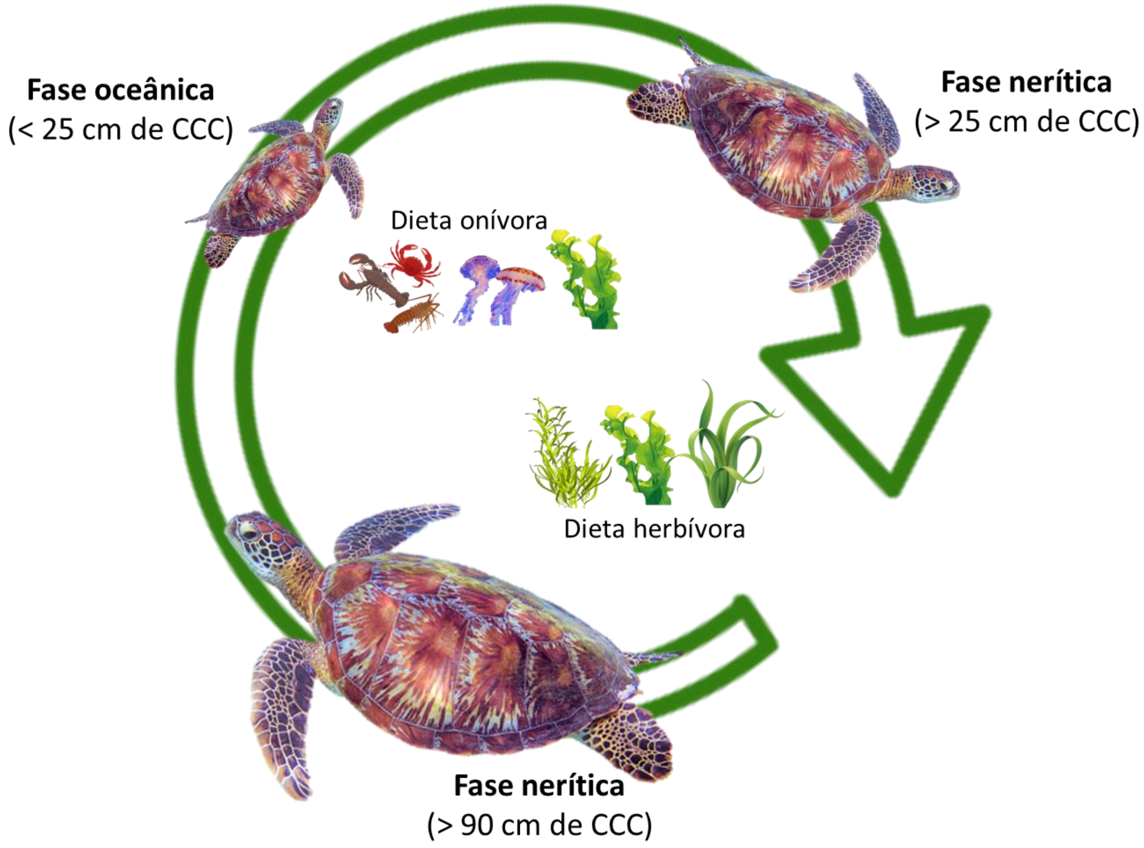


Figura 3. Representação esquemática do ciclo alimentar de tartarugas-verdes (*Chelonia mydas*).

Uma abordagem para estudar os estoques de tartarugas marinhas em áreas de alimentação é através dos dados de encalhe, que fornecem informações consistentes sobre a distribuição de espécies na área marinha adjacente onde elas ocorrem (PYENSON, 2010; PELTIER et al, 2012).

Estudos através de encalhes no litoral setentrional do Rio Grande do Norte e leste do Ceará (Bacia Potiguar) apontam essa região como sendo de grande importância para as tartarugas marinhas, com registros das cinco espécies que ocorrem no Brasil, com predomínio de *Chelonia mydas*, que encontra nessa região melhores condições para o seu desenvolvimento e alimentação (FARIAS, 2019). Entretanto, Attademo (2007) destacou para a Bacia Potiguar alta taxa de alterações ambientais, destruição de manguezais e má exploração da área, caracterizada fortemente pela pesca artesanal dos dois estados, o que reflete direta e indiretamente no grande número de encalhes de tartarugas marinhas nas praias da região.

A Genética como Ferramenta para a Conservação

Dentro desse contexto, a genética da conservação surge como parte integrante da biologia da conservação e é motivada pela necessidade de reduzir as taxas atuais de extinção e preservar a biodiversidade. As tartarugas-verdes demostram um forte “Natal homing”, com as fêmeas retornando à sua região natal para colocar seus ovos, o que ao longo do tempo pode gerar forte divergência genética entre as populações, particularmente ao observar o DNA mitocondrial herdado da mãe (mtDNA) (BOWEN; KARL, 2007; JENSEN et al., 2013). As diferenças nessas frequências de sequências de DNA mitocondrial, denominadas haplótipos, permitem a distinção entre colônias de desova (ENCALADA et al. 1996), podendo elucidar aspectos da história de vida das tartarugas marinhas, incluindo estrutura populacional, filogeografia, sistemática, além da reconstrução de conexões com as áreas de alimentação (MORITZ, 1994; AVISE, 2007; BOWEN; KARL, 2007; PROIETTI et al., 2012).

Estudos prévios em diversas áreas descrevem as até então “populações” de tartarugas marinhas como, na verdade, estoques mistos, reunindo indivíduos provenientes de colônias de desova distintas (LUKE et al. 2004; BASS et al. 2006; DUTTON et al. 2008). As pesquisas com uso dos haplótipos em áreas de alimentação são menos desenvolvidas, quando comparadas aos estudos em colônias reprodutivas, onde as fêmeas são facilmente observadas (BOWEN; KARL, 2007; JENSEN et al., 2013; JORDÃO et al., 2015; ALMEIDA et al., 2021). A limitação de amostragens genéticas em áreas de alimentação para as tartarugas-verdes, impede a compreensão completa da ecologia espacial desses animais (BASS; WITZELL, 2000; BASS et al., 2006; FORMIA et al., 2006; NARO-MACIEL et al., 2012).

Estudos em diferentes áreas de forrageio evidenciaram a presença de haplótipos de tartarugas-verdes de diversas localidades, incluindo Atol das Rocas, Ilha da Trindade, Ilha de Ascension, África, México, Costa Rica e Suriname (NARO-MACIEL et al., 2007; NARO-MACIEL et al. 2012, PROSDOCIMI et al., 2012; PROIETTI et al., 2012; JORDÃO et al. 2015, ALMEIDA et al. 2021). Entretanto, muitas regiões apresentam ainda haplótipos denominados “órfãos” por terem suas áreas de origem ainda desconhecidas, o que indica necessidade de aprofundamento nas investigações da composição genética, principalmente no monitoramento do status nas populações das áreas de alimentação (AVISE, 1995).

A Ameaça do Lixo Marinho

Devido, principalmente ao hábito alimentar costeiro, a tartaruga-verde vem sendo bastante afetada pelas atividades antrópicas (GALLO et al, 2000), principalmente pela pesca artesanal descontrolada (SALES et al, 2008), como a que ocorre na região da Bacia Potiguar (ATTADEMO, 2007). Por outro lado, o ambiente marinho vem sendo cada vez mais afetado pelos detritos antropogênicos, que vem se configurando como uma das maiores ameaças aos ecossistemas marinhos, comprometendo a biodiversidade e os recursos marinhos (MOORE, 2008; BARNES et al, 2009; GALGANI et al., 2010; SUTHERLAND et al., 2010).

A geração de lixo vem crescendo exponencialmente, podendo atingir 6 milhões de toneladas por dia em 2025 (HOORNWEG et al., 2013), sendo grande parte destes resíduos antropogênicos representados pelo plástico (THOMPSON et al., 2009). As tartarugas marinhas são particularmente vulneráveis à poluição por plásticos, afetando todas as espécies em diferentes estágios de vida (WITHERINGTON et al., 2012; SCHUYLER et al., 2014), especialmente enquanto filhotes e juvenis oceânicos, quando se alimentam basicamente na superfície d´água, onde tendem a se associar às agregações de algas flutuantes e resíduos sólidos em áreas de convergência (WITHERINGTON et al., 2012; SCHUYLER et al., 2012; SCHUYLER et al., 2014). Além disso, como a maior parte do lixo possui flutuabilidade positiva (DERRAIK, 2002), as espécies que forrageiam em áreas mais rasas estão mais susceptíveis à ingestão de resíduos sólidos (NELMS et al., 2016), como é o caso da *Chelonia mydas*,

Em 1985, a probabilidade de uma tartaruga-verde ingerir lixo era de 30%. Em 2012 essa porcentagem subiu para 50 % (SCHUYLER et al., 2012), o que provavelmente está relacionado ao aumento da disponibilidade de lixo no oceano e, portanto, ao aumento da chance de encontrar o lixo (SANTOS, 2014; SCHUYLER et al., 2014). Acredita-se que indivíduos de *C, mydas* na fase nerítica, na qual tendem a herbivoria, possam ingerir plástico mais facilmente tanto acidentalmente, quando os resíduos se misturam com as algas e gramas marinhas, como intencionalmente, pela semelhança do lixo com esses alimentos (SCHUYLER et al., 2012; ROBERTSON et al., 2013).

A ingestão de lixo tem efeitos negativos sobre a secreção das enzimas gástricas, prejudicando a absorção de nutrientes e causando a perda de peso corporal do animal; promove falsa sensação de saciedade (SCHULMAN; LUTZ, 1995) e o consequente acúmulo de gases, que interferem na flutuabilidade do animal no ambiente aquático, diminuindo sua capacidade de se alimentar e de escapar de predadores, deixando-o mais suscetível a outros impactos (GEORGE, 1996).

Alguns estudos revelaram detritos antropogênicos em todas as espécies de tartarugas marinhas, o que pode causar a morte direta do animal, através da obstrução do trato gastrointestinal, necrose, laceração e ulceração da mucosa, ou rompimento de vísceras, mesmo quando pequenas quantidades são ingeridas (Figura 4) (BJORNDAL, 1997; TOMÁS et al, 2002). Santos (2014) analisando a causa de óbito em tartarugas marinhas, relacionada à ingestão de resíduo sólido, revelou que a quantidade crítica de lixo necessária para causar a morte de um indivíduo juvenil de *C. mydas* é de 0,5 g. Alguns dos efeitos da ingestão de detritos antropogênicos podem ser considerados como subletais as tartarugas marinhas, como a diluição da dieta ou assimilação de contaminantes derivados de lixo marinho (BJORNDAL et al., 1994; MCCAULEY; BJORNDAL, 1999; ASHTON et al., 2010; YAMASHITA et al., 2011; LAZAR; GRACAN, 2011; FISNER et al., 2013; GALL; THOMPSON, 2015; SCHUYLER et al., 2015 e VÉLEZ-RUBIO et al., 2018).

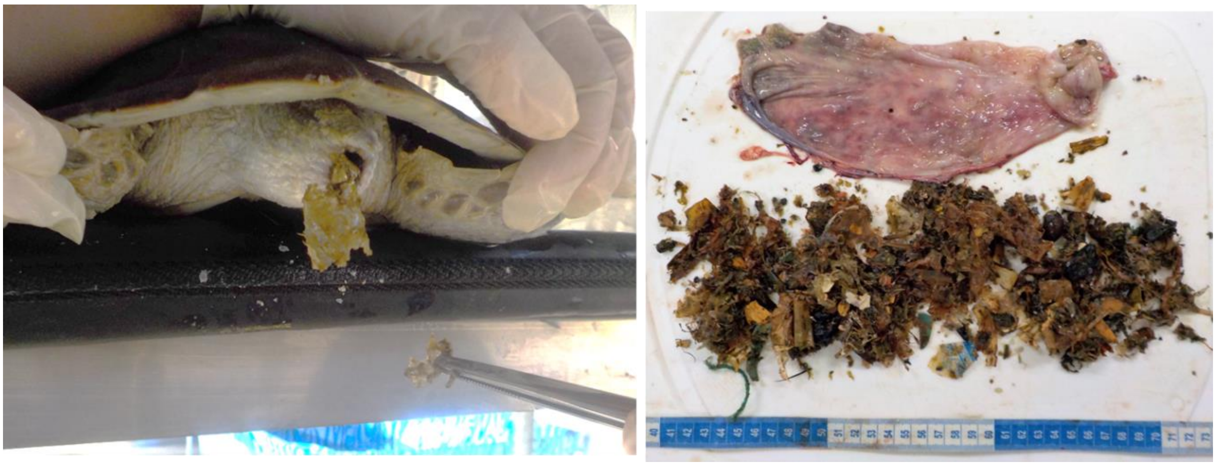


Figura 4. Registro de tartaruga-verde (*Chelonia mydas*) acometida por grande quantidade de lixo, que causou a obstrução do trato gastrointestinal e óbito do animal. Fonte: Projeto Cetáceos da Costa Branca, Universidade do Estado do Rio Grande do Norte (PCCB/UERN).

A Bioacumulação de Elementos Químicos

Outro aspecto relevante para a conservação das espécies de tartarugas marinhas diz respeito à bioacumulação de elementos químicos, como metais pesados e elementos traços. Alguns elementos químicos são essenciais à vida, mas muitos podem bioacumular ou serem metabolizados, tornando-se tóxicos aos organismos. O transporte de longo alcance (rios e ar) desses contaminantes e suas características não biodegradáveis e de biomagnificação aumentam sua toxicidade e potencial ecotoxicológico (D'ILIO et al., 2011; LEY-QUIÑÓNEZ et al., 2011; YARSAN; YIPEL, 2013; BUCCHIA et al., 2015; MATTEI et al., 2015; YIPEL et al., 2016).

A exposição a metais, em geral, tem sido associada à inativação de enzimas e desnaturação proteica, causando efeitos deletérios, como alterações fisiológicas, estresse crônico, problemas neurológicos, função imunológica prejudicada e um aumento na suscetibilidade a problemas intestinais, respiratórios, imunológicos, hepáticos, renais e dermatológicos, seja pela exposição a altas concentrações dos poluentes, como a pequenas quantidades por longos períodos (FOSSI; MARSILI, 2003; DECATALDO et al., 2004; MOFFET et al., 2007; INNIS et al., 2008; DE JESUS; DE CARVALHO, 2008; MARIJIC et al. 2016; MIGUEL; DE DEUS SANTOS 2019).

Animais de vida longa, como as tartarugas marinhas, tendem a acumular níveis mais elevados de metais em seus tecidos (CAURANT et al., 1999; DE LA LANZA-ESPINO et al., 2000; AGUILAR et al., 2002). Esse fato, associado a plasticidade alimentar das diferentes populações e espécies, com mudanças na dieta e de habitat, as torna mais suscetíveis à exposição química (ANDREANI et al., 2008; D'ILIO et al., 2011; MATTEI et al., 2015). Por esses motivos, as tartarugas marinhas são consideradas espécies sentinelas da saúde ambiental das áreas que ocupam (BAPTISOTTE, 2007), funcionando como potenciais bioindicadores de um bom estado ambiental, bem como dos níveis de poluição marinha (GORDON et al., 1998; SAKAI et al., 2000; GARDNER et al., 2006; CAMACHO et al., 2014; YIPEL et al., 2017).

As concentrações de metais tóxicos e elementos químicos diversos têm sido estudadas em tecidos de tartarugas-verdes em diversas regiões do Brasil e do mundo (AGUIRRE et al.,1994; GORDON et al., 1998; GODLEY et al., 1999; SAKAI et al., 2000; ANAN et al., 2002; STORELLI; MARCOTRIGIANO, 2003; LAM et al., 2004; BARBIERI, 2009; KOMOROSKE et al., 2011; BEZERRA et al., 2013; CARNEIRO DA SILVA et al., 2014; PRIOSTE et al., 2015; BRUNO et al., 2021). No entanto, com histórias de vida complexas, com múltiplas mudanças ontogenéticas no habitat, na dieta e grandes migrações entre áreas de forrageamento e nidificação (HAYWOOD et al. 2019), a tartaruga-verde pode apresentar variações no nível trófico e, portanto, também nas concentrações de elementos químicos (como metais e elementos traços) em indivíduos jovens e adultos, nas mais diferentes localidades.

Dessa forma, tem-se como objetivo geral analisar o padrão de ocorrência da tartaruga-verde (*Chelonia mydas*) no litoral setentrional do Rio Grande do Norte (RN) e leste do Ceará (CE), região compreendida como Bacia Potiguar, caracterizando os estoques populacionais da espécie, assim como avaliar a o impacto dos resíduos sólidos e a bioacumulação de elementos químicos nos tecidos dos espécimes que ocorrem na região.

Como objetivos específicos, espera-se:

1) Identificar a composição haplotípica da espécie *Chelonia mydas* encontrada na região da Bacia Potiguar, analisando sua diversidade e variações espaço-temporais;
2) Avaliar o impacto dos resíduos sólidos nas tartarugas-verdes (*Chelonia mydas*) da Bacia Potiguar;
3) Determinar a distribuição e acumulação das concentrações de elementos químicos (metais e elementos-traço) em diferentes órgãos e tecidos das tartarugas-verdes que utilizam a Bacia Potiguar como área de desenvolvimento e alimentação.

CARACTERIZAÇÃO GERAL DA ÁREA DE ESTUDO

O trabalho foi realizado a partir da análise de dados coletados de tartarugas-verdes (*Chelonia mydas*) encalhadas em uma área de abrangência delimitada a noroeste pelo município de Aquiraz (03°49’20.9” S e 38°24’07.8” O), no estado do Ceará, e a leste pelo município de Caiçara do Norte (05°05’28.6” S e 36°17’37.9” O), no estado do Rio Grande do Norte. A extensão total de praias monitoradas corresponde a 332,84 km, 14 municípios costeiros, inteiramente inseridos sobre a Bacia Potiguar, limitada ao sul, leste e oeste por rochas do embasamento cristalino, a noroeste pelo Alto de Fortaleza (limite com a Bacia do Ceará), e ao norte pelo Oceano Atlântico (SOARES et al, 2003).

A pesquisa foi desenvolvida dentro do âmbito do Projeto de Monitoramento de Praias da Bacia Potiguar (PMP-BP), que é conduzido pelo Projeto Cetáceos da Costa Branca, da Universidade do Estado do Rio Grande do Norte (PCCB-UERN) desde 2010, até os dias atuais (2022), como parte de uma condicionante ambiental imposta pelo Instituto Brasileiro do Meio Ambiente e Naturais Renováveis Recursos (IBAMA), decorrente da exploração de Petróleo e Gás (E&P) pela PETROBRAS (Petróleo Brasileiro SA; Contrato nº 2500.005657510.2).

Devido à grande extensão, condições de acesso, infraestrutura local e características ambientais, a área de monitoramento foi dividida em cinco trechos: A: Grossos/RN –

Icapuí/CE, B: Areia Branca/RN - Porto do Mangue/RN, C: Guamaré/RN – Macau/RN; D: Galinhos/RN – Caiçara do Norte/RN e E: Aquiraz/CE – Aracati/CE (Figura 5).

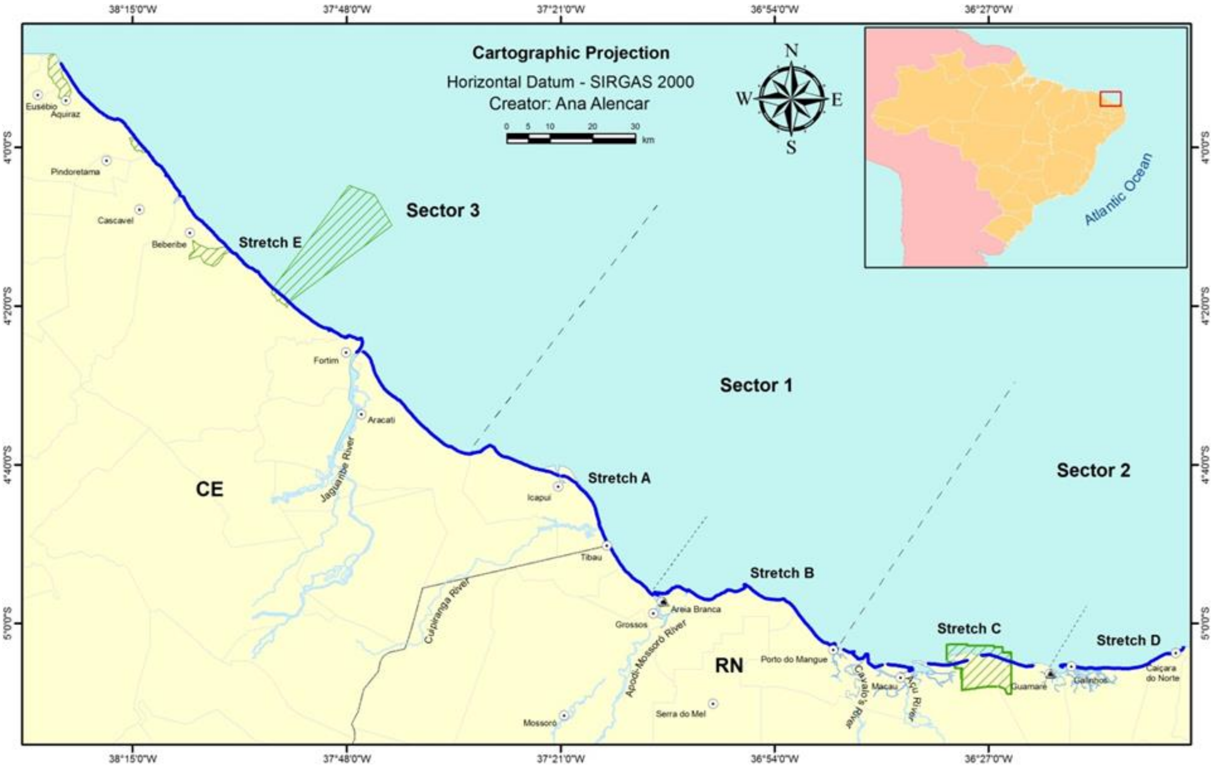


Figura 5. Área de estudo. Fonte: Projeto Cetáceos da Costa Branca, Universidade do Estado do Rio Grande do Norte (PCCB/UERN). Fonte: PCCB/UERN.

METODOLOGIA GERAL

Coleta de dados

Os dados foram obtidos decorrentes do monitoramento diário de toda extensão de praias, conduzido de janeiro de 2010 até dezembro de 2019. Todas as tartarugas-verdes encalhadas foram identificadas quanto à espécie, através da análise de guia de identificação (ECKERT et al., 2000), e local de encalhe, através da coleta de dados geográficos por meio de aparelho de GPS (Global Positioning System). A determinação de “faixa etária” foi realizada a partir da biometria de cada animal (Figura 6), através do comprimento curvilíneo da carapaça - CCC (comprimento da nuca até o entalhe entre as placas supra caudais) (BOLTEN, 1999) utilizando fita métrica flexível, e através de informações coletadas da literatura sobre o menor tamanho de fêmeas das espécies desovando, sendo considerados adultos indivíduos com CCC ≥ 90 cm (ALMEIDA et al., 2011). Para a determinação do sexo, foi analisado o dimorfismo sexual em animais adultos (cauda maior para machos e menor para fêmeas) e em juvenis, quando possível, foram realizadas análises macroscópicas das gônadas.

Figura 6. Registro de biometria e coleta de dados de tartaruga-verde (*Chelonia mydas*). Fonte: Arquivo pessoal.

Durante os encalhes a condição do animal foi avaliada, com o objetivo de identificar algum sinal macroscópico de interação antrópica. As carcaças em bom estado de conservação foram necropsiadas (códigos de carcaça D2 e D3; FLINT et al., 2009; WORK, 2000) e as amostras coletadas (descrição nos tópicos abaixo).

Todos os dados coletados estão previstos na licença SISBIO N. 13694-9 (ANEXO I) e o trabalho foi considerado ISENTO de aprovação pelo Comitê de Ética no Uso de Animais (CEUA), da Universidade Federal do Rio Grande do Norte (UFRN), protocolo n° 060/2019 (ANEXO II).

Determinação dos Haplótipos

De cada tartaruga-verde registrada durante o ano de 2019 foi coletado um pequeno fragmento de pele, de aproximadamente 6 mm de diâmetro, retirados com o auxílio de uma pinça cirúrgica e lâminas estéreis, a fim de evitar contaminação. Os materiais coletados foram armazenados em potes coletores com álcool a 100%. A coleta de fragmentos de tecidos das carcaças encontradas durante o monitoramento de praias, optou pela retirada de fragmentos de regiões menos afetadas pela decomposição. As amostras foram encaminhadas para análise no Laboratório de Patologia Comparada de Animais Silvestres (LAPCOM), da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (USP). Alíquotas de todas as amostras coletadas foram depositadas na coleção de tecidos do Laboratório de Monitoramento de Biota Marinha do Projeto Cetáceos da Costa Branca, da Universidade do Estado do Rio Grande do Norte (PCCB/UERN).

No LAPCOM, o DNA total foi extraído de amostras de pele de 39 tartarugas verdes armazenadas em álcool 100%, usando o kit DNeasy Blood and Tissue (Qiagen, Hilden, Alemanha) de acordo com as instruções do fabricante. Os primers LCM15382 e H950 (ABREU-GROBOIS et al., 2006) foram usados para amplificar aproximadamente 700 pares de bases (pb) da região de controle mitocondrial por reação de PCR de ponto final (25 µL). A reação de PCR de ponto final (25 µL) foi conduzida em microtubos de 0,2 µL contendo 1 µL (10 pmol) de cada iniciador, 200 mM de cada dNTP, 2,5 U de Platinum Taq DNA polimerase (Invitrogen, Life Technologies, Brasil), 1 X Tampão PCR (20 mM Tris-HCl pH 8,4 e 50 mM KCl), 1,5 mM MgCl2, 2,5% DMSO e água ultrapura estéril para um volume final de 25 µL. As amplificações foram realizadas em um termociclador (Bio-Rad, Hercules, Califórnia, EUA) usando o seguinte protocolo de PCR de ponto final: desnaturação por 2 min 94 ° C, seguido por 40 ciclos de 1 min a 94 ° C, 1 min a 57 ° C e 1 min a 72 ° C, e uma extensão final de 10 min a 72 ° C (ABREU-GROBOIS et al., 2006).

Controles negativos (água livre de nuclease) foram incluídos em cada extração de DNA e reação de PCR. Uma amostra de pele com amplificação confirmada e sequenciamento da região de controle mitocondrial foi selecionada como controle positivo. Os produtos de PCR foram visualizados em géis de agarose a 1,5% e as bandas foram excisadas e purificadas usando o kit de extração de gel GFX (GE Healthcare, Illinois, EUA). As amostras positivas foram confirmadas por meio de sequenciamento Sanger direto. Para fazer comparações com outros conjuntos de dados possíveis, encurtamos nossas sequências para 481pb. As sequências foram alinhadas usando BioEdit 5.0.6 (HALL, 1999) e os haplótipos foram identificados de acordo com o Archie Carr Centre for Sea Turtle Research.

Os resultados dos haplótipos estão detalhados no capítulo 01 desse documento, no Manuscrito intitulado “Population structure of the endangered green turtles (*Chelonia mydas*) in the Potiguar Basin, Northeastern, Brazil: insights for conservation”.

Análise de Detritos Antropogênicos

Durante todo o período do estudo (2010 a 2019), conteúdos alimentares presentes no esôfago, estômago e intestino, foram coletados durante as necropsias e fixados em formalina 4% (algas e ervas marinhas) ou álcool 70% (invertebrados). Detritos antropogênicos foram encontrados misturados aos alimentos e separados durante a análise no laboratório. Todo material coletado foi encaminhado para o Laboratório de Morfofisiologia de Vertebrados (LABMORVE), da Universidade Federal do Rio Grande do Norte (UFRN).

No LABMORVE, a análise de conteúdo combinou dois métodos baseados na dieta, os quais foram adaptados para o conteúdo de tartarugas marinhas: (1) frequência de ocorrência (F%): o número de conteúdos analisados contendo um ou mais indivíduos de cada categoria de determinado item foi expresso como uma porcentagem de todos os conteúdos analisados contendo alimentos/detritos antropogênicos (DINEEN, 1951; DUNN, 1954; KENNEDY; FITZMAURICE, 1972) (Figura 7); e (2) método volumétrico por estimativa direta (V%): a medição de cada item alimentar e tipos de detritos antropogênicos ou grupo de itens separados do conteúdo foi realizada em um medidor graduado, segundo Wolfert; Miller (1978), considerando o volume de cada item e expresso em porcentagem de todos os itens alimentares (KAWAKAMI; VAZZOLER, 1980).

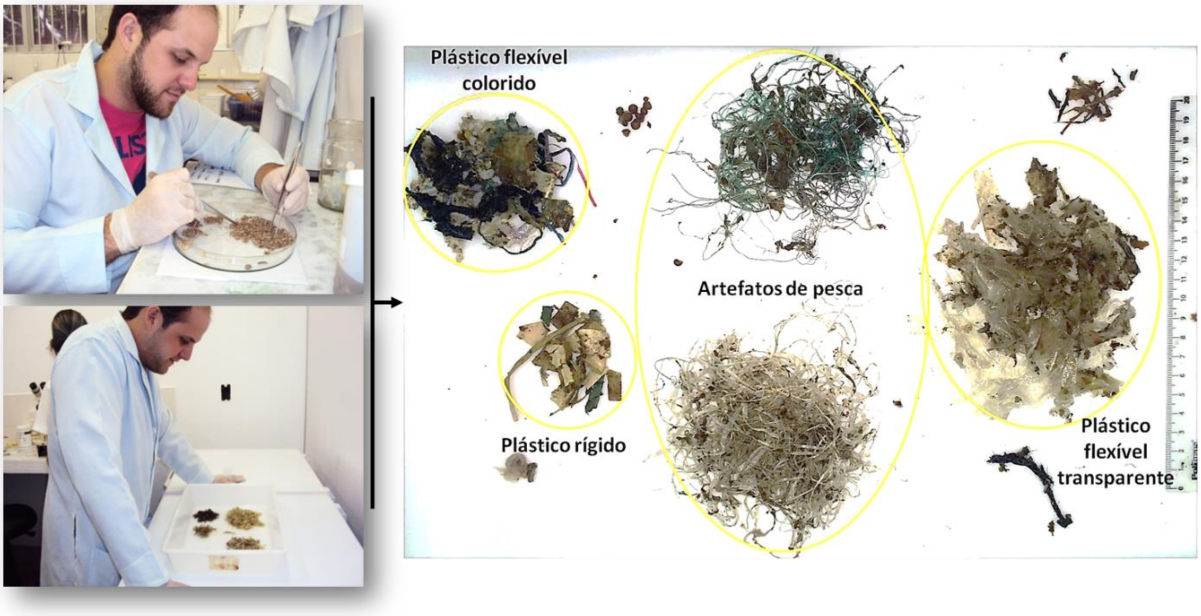


Figura 7. Esquema de análise do método de Frequência de Ocorrência, para determinação dos itens presentes no esôfago, estômago e intestino de tartarugas-verdes (*Chelonia mydas*).

Os detritos antropogênicos foram classificados em cinco categorias: plástico flexível transparente, plástico flexível colorido, plástico rígido, artefatos de pesca e outros tipos. Os alimentos foram classificados em oito categorias: Algas, Porifera, Molusca, material vegetal não identificado, material animal não identificado (estágio avançado de digestão), material orgânica não identificado (estágio avançado de digestão sem distinção entre material animal e vegetal), Sedimento e fezes (matéria digerida). Por fim, o Índice Alimentar (IAi; KAWAKAMI; VAZZOLER, 1980) foi calculado para cada item: $IAi = Fi \times Vi / \sum (Fi \times Vi)$, onde F = Frequência de ocorrência (%) d7 cada item, V = Volume (%) de cada item, e i = 1, 2… n = item determinado. A composição da "dieta" foi analisada considerando o IAi, e os itens alimentares foram classificados nas seguintes categorias de acordo com Rosecchi; Nouaze (1987): IAi <0,25 - item acessório; 0,25 ≤ IAi <0,5 - item secundário; IAi ≥ 0,5 - item principal.

A descrição dos resultados encontra-se detalhada abaixo no capítulo 02, intitulado "The use of an alimentary index to assess anthropogenic debris on green turtles (*Chelonia mydas*)".

Ecotoxicologia de Metais Pesados

Amostras de fígado, músculo e rins coletadas das tartarugas-verdes necropsiadas, no período entre 2014 até o final do ano de 2018, foram encaminhadas para análise no Laboratório de Mamíferos Aquáticos e Bioindicadores, da Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro (MAQUA/UERJ), para a determinação das concentrações de THg, Cu, Cd, Ag e Se.

O mercúrio total (THg) foi determinado de acordo com Bisi et al. (2012). Uma mistura de ácido sulfúrico e ácido nítrico foi adicionada às alíquotas de aproximadamente 0,3 g de amostra. As alíquotas foram então aquecidas em banho-maria a 60 ° C até a solubilização total. As concentrações de THg foram determinadas por Vapor Frio / Absorção Atômica (FIMS-400, PerkinElmer) com boro-hidreto de sódio como agente redutor. A exatidão e precisão dos métodos analíticos foram verificadas usando os materiais certificados padrão (DORM-3 e DOLT-5, National Research Council-NRC, Canadá). Os resultados estão de acordo com os valores de referência e os materiais de referência internos produzidos pelo Laboratório de Mamíferos Aquáticos e Bioindicadores "Profa. Izabel Gurgel" da Faculdade de Oceanografia da Universidade do Estado do Rio de Janeiro (UERJ), Brasil. O controle de qualidade também foi realizado por meio da análise do procedimento de brancos (0,04 ± 0,01 µg. L-1) e repetições de amostras (coeficiente de variação <20%).

Os outros elementos (Cu, Cd, Ag e Se) foram determinados de acordo com os procedimentos de digestão de Dorneles et al. (2007). As duas alíquotas de aproximadamente

0,3 g de amostra foram adicionadas 2 mL de ácido nítrico 65% (HNO3) durante a noite e as soluções foram aquecidas a 60 ° C em banho-maria por 2 horas. Após o resfriamento, as soluções foram colocadas em tubos de 15 mL e água ultrapura foi adicionada para completar o volume de 10 mL. As concentrações dos elementos foram determinadas por espectrometria de absorção atômica eletrotérmica (ZEEnit 60s, Analytic Jena, equipado com correção de fundo Zeeman). Nitrato de paládio - Pd (NO3) 2 e nitrato de magnésio - Mg (NO3) 2 foram usados como modificador de matriz. A exatidão e precisão dos métodos analíticos foram verificadas usando os materiais certificados padrão (TORT-2, DOLT-4 e DOLT-5, National Research Council-NRC, Canadá), com recuperação de elementos (Média ± SD%) em 104 ± 3,61 (THg), 99 ± 7,02 (Cu), 96 ± 7,69 (Cd), 100 ± 6,22 (Ag) e 99 ± 4,95 (Se). O controle de qualidade também foi realizado por meio da análise do procedimento de brancos (<5,00 µg. L-1) e repetições de amostras (coeficiente de variação <25%).

Os limites de detecção (LoD) foram Hg = 0,00002, Cu = 0,00091, Cd = 0,00039, Ag = 0,00025, Se = 0,00402 µg mL-1 e as concentrações são expressas em µg g-1 de peso úmido (w.w.).

A análise dos resultados dessas análises encontra-se detalhada abaixo no capítulo 03 desse documento, intitulado “Bioaccumulation of total mercury, copper, cadmium, silver, and selenium in green turtles (*Chelonia mydas*) stranded along the Potiguar Basin, northeastern Brazil”.

Análises Estatísticas

Para os critérios de definição de uma amostra representativa no capítulo 01, considerou-se a similaridade nos padrões de características macroscópicas entre os indivíduos amostrados e os demais espécimes de tartaruga-verde encalhadas durante 2019. Os padrões analisados foram razão sexual, faixa etária e status do animal (vivo e morto).

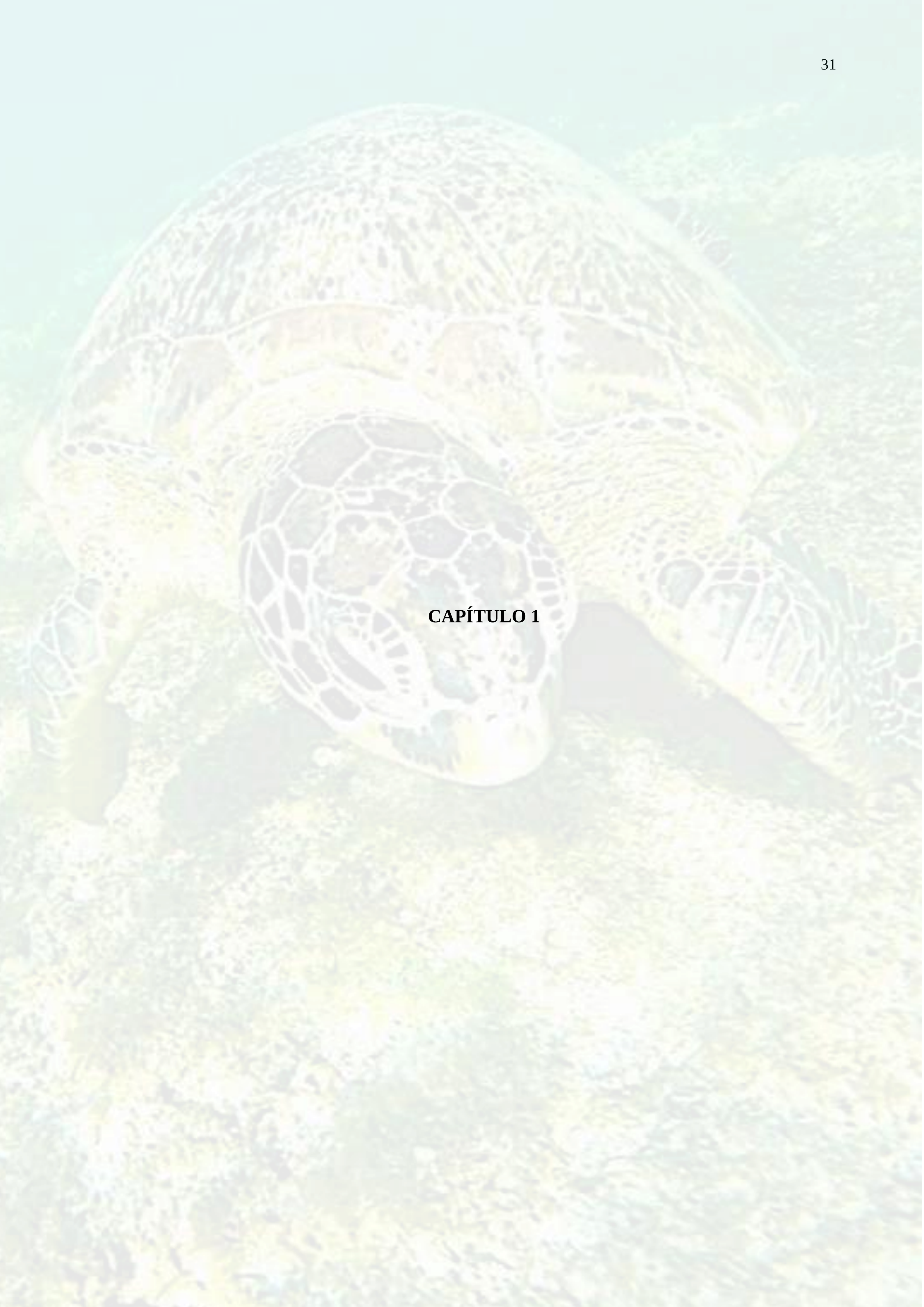
Com relação aos haplótipos, para comparar os dados do nosso estudo com outros realizados no litoral brasileiro, ajustamos uma regressão não paramétrica usando um Generalized Additive Model (GAM) e corrigindo a distribuição dos dados como uma distribuição Beta. A comparação foi feita com estudos realizados no litoral brasileiro em Almofala/CE e Ubatuba/SP (NARO-MACIEL et al., 2007), Bahia, Espírito Santo, Fernando de Noronha e Atol das Rocas (NARO-MACIEL et al., 2012), Ilha Arvoredo/SC e Praia do Cassino/RS (PROIETTI et al., 2012), Complexo Estuarino de Paranaguá (PEC)/PR (JORDAO et al., 2015) e Alagoas (ALMEIDA et al., 2021).

Complementarmente, para analisar os dados de encalhe de tartarugas-verdes juvenis no ano de 2019 foram realizados os testes Kolmogorov-Smirnov e Levene para verificar distribuição e homocedasticidade, respectivamente. Os testes de Kruskal-Wallis e Bonferroni foram aplicados para analisar a frequência mensal de encalhes juvenis de acordo com os grupos de tamanho do CCC (0-29,9 cm, 30-59,9 cm, 60-90 cm). As análises foram realizadas no IBM SPSS Statistics (versão 20) e os resultados foram considerados significativos para valor de $P < 0{,}05$.

Para o capítulo 02, todas as análises foram realizadas após avaliação da normalidade e homocedasticidade (Komolgorov-Smirnov e Levene, respectivamente) e os testes foram realizados no software IBM SPSS (Statistical Package for the Social Sciences, versão 20). Para rastrear a presença de detritos antropogênicos considerando o tamanho da tartaruga, os dados do CCC (26–129 cm) foram convertidos em variáveis qualitativas, por meio da análise de Cluster sob o método do centróide e da Distância Euclidiana Quadrada (CORRAR et al., 2009). Uma vez que classificamos como adultos animais com CCc > 90, segundo Almeida et al. (2011), a análise de cluster foi realizada para indivíduos com CCL de até 89,9 cm, resultando em três grupos: Juvenil I - JUV-I (CCC entre 26 e 54 cm), Juvenil II - JUV-II (54,1-89,9 cm de CCC) e Adulto - ADU (90-129 cm de CCC). Os dois grupos de juvenis foram definidos pela análise de Cluster considerando que o CCC de 53,2 cm foi o maior tamanho no primeiro grupo (JUV-I), e o CCC de 55 cm foi o menor tamanho no grupo JUV-II. O teste de Kruskal-Wallis e a comparação pareada com a correção de Bonferroni mostraram que os três grupos de tamanho eram estatisticamente diferentes em seus valores de CCC ($U = 210{,}156$; $p <0{,}001$).

Adicionalmente, por meio de regressão linear simples, analisamos, se foi possível prever, o volume de determinados tipos de detritos antrópicos, de acordo com os diferentes grupos de CCC definidos pela Análise de Cluster. O R^2, coeficiente de correlação de Pearson ao quadrado, indica a variância explicada. ANOVA unilateral indica a probabilidade de o resultado da regressão ocorrer devido ao erro de amostragem quando p não é significativo.

Com relação a análise dos contaminantes (capítulo 03) os dados foram analisados de acordo com a correlação entre as concentrações de CCC e THg, Cu, Cd, Ag e Se usando o coeficiente de correlação de Spearman (r) e a comparação das concentrações de THg, Cu, Cd, Ag e Se entre o fígado, músculos e os rins foi realizada pela ANOVA de Friedman e teste post-hoc de Nemenyi (apenas indivíduos com presença dos elementos nos três órgãos preencheram os critérios para os testes: $n = 16$). As análises foram realizadas no IBM SPSS Statistics (versão 20) e STATISTICA 7.0 para Windows (StatSoft, Inc. 1984e2004, EUA) e os resultados foram considerados significativos quando o valor de $p <0{,}05$.



CAPÍTULO 1

Population structure of the endangered green turtles (*Chelonia mydas*) in the Potiguar Basin, Northeastern, Brazil: insights for conservation

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ESTE ARTIGO SERÁ SUBMETIDO AO PERIÓDICO Herpetologica E, PORTANTO, ESTÁ FORMATADO DE ACORDO COM AS RECOMENDAÇÕES DESTA REVISTA (https://meridian.allenpress.com/herpetologica)

Abstract

The aim of this study was to analyze the pattern of occurrence of the green turtle (*Chelonia mydas*) in the Potiguar Basin, northeast of Brazil, evaluating the genetic composition, natal origin, and ecological characteristics of the species in this region. A small fragment (6 mm in diameter) of skin was collected from each stranded green turtle, recorded on the coast of the Potiguar basin region during 2019, and sent for haplotypic identification. To define the criteria for a representative sample for genetic analysis, the similarity between three patterns of macroscopic characteristics of the sampled individuals (N=39) and the beached green turtle data during the year 2019 (N=477) was considered: sex ratio, life stage and status of the animal (alive and dead). Considering short sequences (481bp, n=39) of the mtDNA control region, eight haplotypes were identified, with the most common haplotype being CM-A8 (48.7%; 19/39) followed by CM-A5 (30.8 %; 12/39) and all other haplotypes were at frequencies below 6%. According to the data from strandings of green turtles analyzed in 2019, 87.36% corresponded to juveniles, with a predominance of animals from 30 to 59.9 cm CCL. Our findings corroborate other studies on the Brazilian coast, with a predominance of the CM-A8 haplotype, but highlighting the high values of CM-A5 found, compared to other studies in Brazil, which may be related to the geographic position of the Potiguar Basin, in the "corner" of the American continent. The results of this work increase the understanding of the haplotypic composition of *C. mydas* population stocks on the Brazilian coast, filling the information gap on the genetic composition of green turtles in the important feeding site of the Potiguar Basin, reinforcing that the greater the latitude (i.e., further south) the greater the presence of the CM-A8 haplotype. The study also contributes to the organization of strategies to recompose the nesting areas and, consequently, improve the threatened status of the species.

Key words: Sea turtles. Genetic. Endangered species. Ecology.

1. INTRODUCTION

The green turtle (*Chelonia mydas*) is distributed globally and threatened with extinction (IUCN, 2021), occupies several ecological niches throughout its complex life cycle with highly migratory behavior (MEYLAN; MEYLAN 1999; GODLEY et al., 2002; BOLTEN, 2003; PROIETTI et al., 2012). For a long time, conservation efforts have been directed towards identifying threats, particularly in spawning areas, where sea turtles spend about 1% of their life cycle (BJORNDAL, 2000). In this sense, numerous studies have been conducted to access the demographic composition of feeding areas, whose main interest is to understand the mechanisms responsible for the recruitment of animals to these regions and the migratory routes used by them (SEARS et al., 1995; BASS; WITZELL, 2000; LUKE et al., 2004; BASS et al., 2006; NARO-MACIEL, 2007, DUTTON et al., 2008; VELEZ-ZUAZO et al., 2008).

Studies using mitochondrial DNA (mtDNA) have revealed a particularly useful marker for marine species, working to investigate population structuring and adaptation, mainly due to its low effective population size (PIGANEAU; EYRE-WALKER, 2009) and documented functional roles in thermal adaptation and aerobic capacity (MISHMAR et al., 2003; GALTIER et al., 2009; BRADBURY et al., 2010). MtDNA control regions have been increasingly used in sea turtles and the development of genetic markers for these animals has contributed to the acquisition of valuable data which can elucidate many aspects of the sea turtle's life history, including population structure, phylogeographic, systematics, native origins, and natal homing (MORITZ, 1994; AVISE, 2007; BOWEN; KARL, 2007; PROIETTI et al., 2012; NARO-MACIEL et al., 2007; NARO-MACIEL et al., 2012; JENSEN et al., 2013; JORDÃO et al., 2015; ALMEIDA et al., 2021).

Previous studies in several areas describe sea turtle "populations" as actually mixed stocks, composed by individuals from several cohorts and from various nesting beaches (rookeries) which aggregate at feeding grounds (BASS; WITZELL, 2000; LUKE et al., 2004; BASS et al., 2006; AVISE, 2007; BOWEN; KARL, 2007; DUTTON et al., 2008). Understanding their origins, as well as determining possible migratory routes, is crucial for the elaboration of management and conservation plans (AVISE, 2007, BOWEN; KARL 2007; PROIETTI et al., 2012).

One approach to study sea turtle stocks in feeding areas is through stranding data, which provide consistent information on the distribution of species in the adjacent marine area where they occur (PYENSON, 2010; PELTIER et al, 2012). Studies on strandings in the northeast coast of Rio Grande do Norte and eastern Ceará (Potiguar Basin), on the northeast coast of Brazil, point to this region as being of great importance for green turtles, which finds in this region better conditions for its development and feeding (FARIAS et al., 2019). However, several studies (2007) pointed out to the Potiguar Basin a high rate of environmental changes, destruction of mangroves and poor exploitation of the area, which is strongly characterized by artisanal fishing in both states, which directly and indirectly reflects the large number of sea turtle strandings in the beaches in the region (ATTADEMO, 2007; SOARES et al. 2019; FARIAS et al., 2019; BOMFIM et al., 2022).

Based on all this, and to elucidate the missing information gap in this important area, the aim of this study was to analyze the pattern of occurrence of the green turtle (*C. mydas*) in the Potiguar Basin, northeast of Brazil, assessing genetic composition, natal origin, and ecological features of the specie in this region.

2. MATERIALS AND METHODS

2.1 Study site

Green turtles were found stranded along the northeastern Brazilian coast during daily monitoring conducted by field-trained personnel of Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN). This study was carried out along approximately 300 km of the northeastern Brazilian coast, between the municipal district of Caiçara do Norte (58401.1500S, 3684036.4100W) in Rio Grande do Norte State (RN) and the municipal district of Icapuí (4838048.2800S, 37832052.0800W) in Ceará State (CE) within the Potiguar Basin (Figure 1). The Beach Monitoring Program at Potiguar Basin (Programa de Monitoramento de Praias da Bacia Potiguar – PMP-BP) has been conducted by the PCCB-UERN since 2010, as part of an environmental constraint compliance enforced by the Brazilian Institute of the Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA) due to oil and gas exploration by PETROBRAS (Petróleo Brasileiro S.A.; Agreement number 2500.005657510.2).

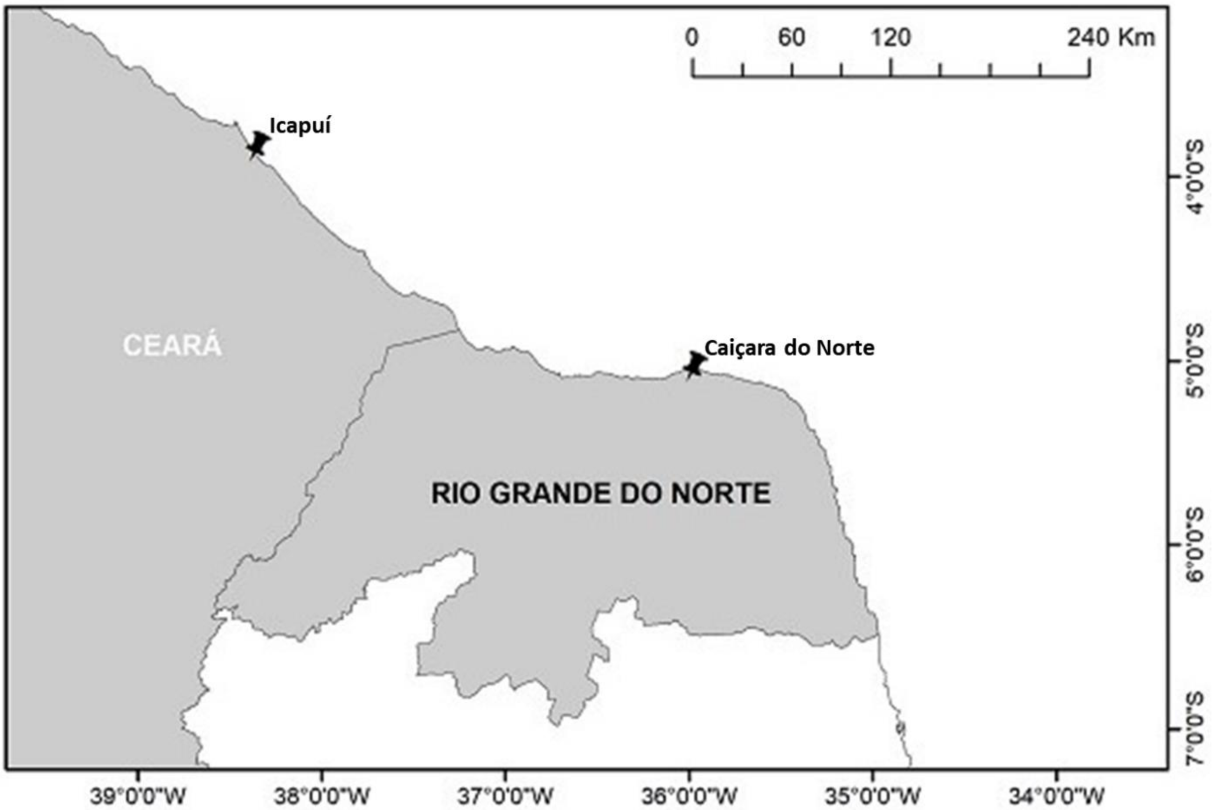

Figure 1. Geographic distribution of the study site, Brazilian Northeastern coast. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN). Fonte: PCCB-UERN.

2.2 Data collection and definition of sampling

A small fragment (6 mm in diameter) of skin was collected from each recorded green turtle, removed with the aid of surgical tweezers and sterile blades, to avoid contamination. The collected materials were stored with absolute alcohol. For the collection of tissue fragments from carcasses during the monitoring of the region, fragments from regions of the body less affected by carcass decomposition were chosen.

As the previous study in the area does not show significant variation in the frequency of *Chelonia mydas* stranding in recent years (FARIAS et al., 2019), we considered data from the most recent year, 2019 (N = 477 animals) and a sample of skin fragments from 39 animals.

For the definition criteria of a representative sample, we considered the similarity in the patterns of macroscopic features between the individuals sampled and the data of green turtle stranded during 2019. The patterns analyzed were sex ratio, life-stage, and animal status (alive and dead) (Table 1).

Table 1 - Relative frequency (%) of macroscopic features patterns of sampled individuals and other specimens from 2019.

	Sex ratio			*Life-stage*			*Animal status*	
	Male	Female	Undetermined	Juvenile	Adult	Undetermined	Alive	Dead
Total stranded animals in 2019	6.37	24.37	69.24	87.36	11.84	0.68	2.5	97.5
Study sampling	7.89	31.57	60.52	97.36	2.63	0	0.9	98.1

To determine de “Life-stage” individual curved carapace length (CCL) was measured in all individuals from the nuchal to notch between supra-caudal scales (BOLTEN, 1999) to distinguish juvenile and adult. The life-stage was determined considering the smallest size recorded for nesting green females in the largest and closest nesting areas the specie in Brazil: ≥ 90 cm (ALMEIDA et al., 2011). For sex determination, we analyzed the sexual dimorphism in adult animals (larger tail for males and smaller for females) and in juveniles, when possible, macroscopic analyses of the gonads were performed.

Once the similarities between the sampled individuals and the rest of the stranded group were verified, the samples were sent for molecular analysis at the Laboratory of Comparative Pathology of Wild Animals (LAPCOM), of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ/USP). Aliquots of all collected samples were deposited in the tissue collection of the Laboratório de Monitoramento de Biota Marinha - PCCB/UERN.

2.3 Molecular analysis

Total DNA was extracted from skin samples of 39 green turtles stored in absolute alcohol, using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The primers LCM15382 and H950 (Abreu-Grobois et al., 2006) were used to amplify approximately 700 base pairs (bp) from the mitochondrial control region by end-point PCR reaction (25 µL). End-point PCR reaction (25 µL) were conducted in 0.2 µL microtubes containing 1 µL (10 pmol) of each primer, 200 mM of each dNTP, 2.5 U of Platinum Taq DNA polymerase (Invitrogen, Life Technologies, Brazil), 1 X PCR buffer (20 mM Tris–HCl pH 8.4 and 50 mM KCl), 1.5 mM $MgCl_2$, 2.5% DMSO, and ultrapure sterile water to a final volume of 25 µL. Amplifications were performed in a thermal cycler (Bio-Rad, Hercules, California, US) using the following End-point PCR protocol: denaturation for 2 min 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 57 °C and 1 min at 72 °C, and a final extension of 10 min at 72 °C (ABREU-GROBOIS et al., 2006). Negative controls (nuclease free water) were included in each DNA extraction and PCR reaction. A skin sample with confirmed amplification and sequencing of mitochondrial control region was selected as positive control. The PCR products were visualized in 1.5 % agarose gels and bands were excised and purified using the GFX gel extraction kit (GE Healthcare, Illinois, US). Positive samples were confirmed through direct Sanger sequencing. To make comparisons with other datasets possible, we shortened our sequences to 481pb. Sequences were aligned using BioEdit 5.0.6 (HALL, 1999) and haplotypes were identified according to the Archie Carr Center for Sea Turtle Research.

2.4 Statistical analyses

To compare the data of our study with others carried out in the Brazilian coast, we adjusted a non-parametric regression using a Generalized Additive Model (GAM) and correcting the data distribution as a Beta distribution. The comparison was made with studies carried out on the Brazilian coast in Almofala/CE and Ubatuba/SP (NARO-MACIEL et al., 2007), Bahia, Espírito Santo, Fernando de Noronha and Atol das Rocas (NARO-MACIEL et al., 2012), Ilha Arvoredo/SC and Praia do Cassino/RS (PROIETTI et al., 2012), Paranaguá Estuarine Complex (PEC)/PR (JORDAO et al., 2015) and Alagoas (ALMEIDA et al., 2021).

To analyze the stranding data of juvenile green turtles Kolmogorov-Smirnov and Levene tests were performed to verify distribution and homocedasticity, respectively. Kruskal-Wallis and Bonferroni tests were applied to analyze the monthly frequency of juvenile strandings according to the CCL size groups (0-29.9 cm, 30-59.9 cm, 60-90 cm). The analyses were performed using IBM SPSS Statistics (version 20) and the results were considered significant at P-value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Ecological features of green turtles

Due to coastal feeding habits, the species *Chelonia mydas* has been greatly affected by human activities (GALLO et al, 2000), mainly by uncontrolled artisanal fishing (SALES et al, 2008), such as that which occurs in the Potiguar Basin region (ATTADEMO, 2007; FARIAS et al., 2019; BOMFIM et al., 2021). This situation directly reflects the high number of stranded animals in the present study, with 97.5% of the fatal cases in 2019.

According to data on strandings of green turtles analyzed in 2019 (n=477), 87.36% corresponded to juveniles, i.e., they have a curved carapace length (CCL) of less than 90 cm (ALMEIDA et al., 2011). The Potiguar Basin region has become an important foraging and feeding area for the green turtle, with a predominance of juveniles (FARIAS et al., 2019). Within the juvenile spectrum, the Kruskal-Walli's test showed that there is an effect of the size of the CCL on the monthly frequency of strandings [X^2 (2) = 31.407; $p < 0.001$]. The pairwise comparison test with the Bonferroni Correction showed that the monthly frequency of strandings of the three groups of CCL sizes are significantly different, being higher for the intermediate size group (30 to 59.9 cm) and frequency smaller for the group with the smallest CCL size (0 to 29.9 cm) (Figure 2).

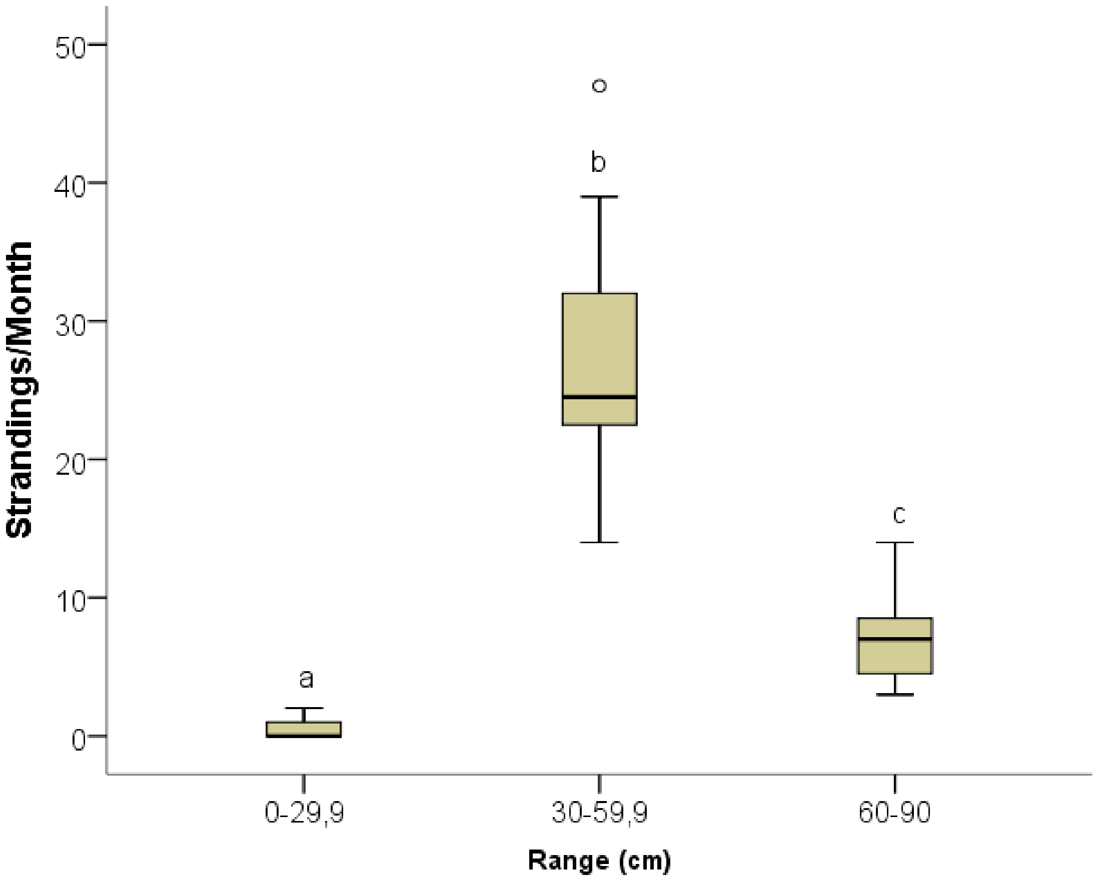

Figure 2: Comparison between the number of strandings of green turtles (*Chelonia mydas*) and the three size groups of CCL (0-29.9, 30-59.9 and 60-90 cm) during the year 2019, in the Potiguar Basin - northeastern Brazil.

In the first years of life (< 25 cm of CCC) juveniles of green turtles remain in oceanic waters, with a basically omnivorous diet, with a tendency to carnivory (BJORNDAL, 1997; ARTHUR ET AL., 2008; JONES; SEMINOFF, 2013). After the oceanic phase, in the neritic phase, they start to occupy more coastal areas, which may be the predominant reality of the green turtles in the Potiguar Basin region (30 to 59.9 cm), developing a typically herbivorous diet, feeding on a diet basically composed of macroalgae and phanerogams, which remains until adulthood (BJORNDAL; BOLTEN, 1988; BJORNDAL, 1997; BRAND-GARDNER et al., 1999; ARTHUR et al., 2008; LENZ et al., 2013).

Regarding the sex-ratio analysis, although the largest records of the present study presented indeterminate sex, comparing the frequency of males and females, it is observed that the green turtles from Portiguar Basin were generally female, which is in line with the most reported sex ratios for green turtles worldwide (HAYS et al., 2014; DE ALMEIDA et al., 2021). Sea turtles have temperature-dependent sex determination, where warmer incubation temperatures generate more females (HAMANN et al., 2013). This ends up being a concern, since climate change could increase temperatures on nesting beaches, which will affect the sex ratios (with a greater number of females), thus accelerating the process of population decline in the coming decades (HARNIK et al., 2012; LALOE et al., 2016).

3.2 Genetic composition of green turtles

Considering short sequences (481pb, n=39) from the mtDNA control region, eight haplotypes were identified: CM-A1, CM-A3, CMA-5, CM-A6, CM-A8, CM-A9, CM-A10, CM-A45 (Figure 2). The most common haplotype was CM-A8 (48.7%; 19/39) followed by CM-A5 (30.8%; 12/39) and all the other haplotypes were at frequencies less than 6% (Figure 3).

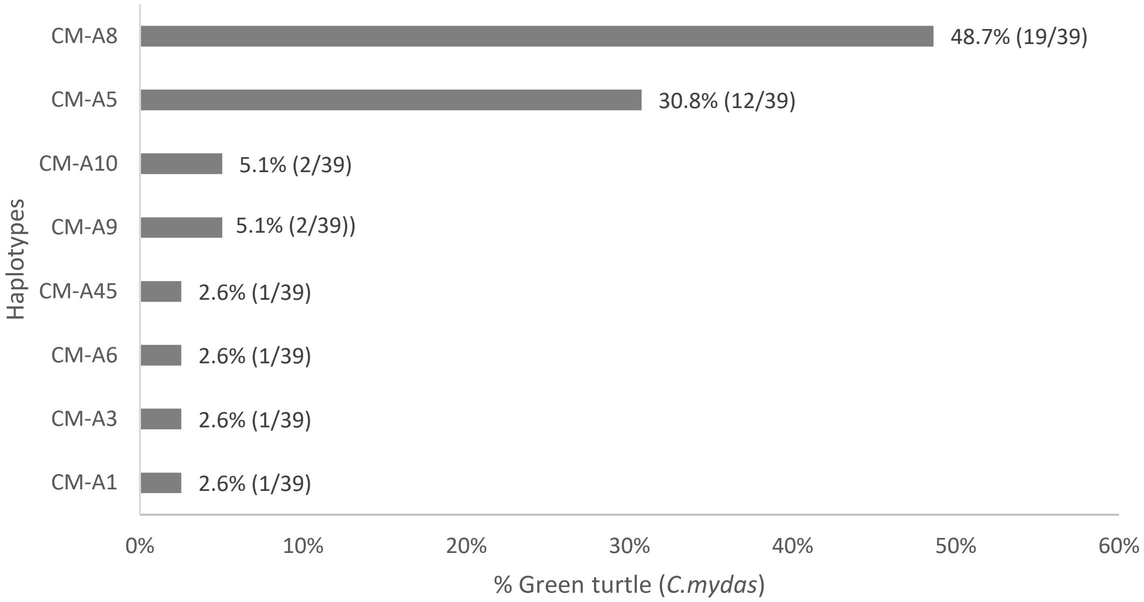

Figure 3. Haplotypes distribution of green turtles (*Chelonia mydas*) (CM-A1, CM-A3, CMA-5, CM-A6, CM-A8, CM-A9, CM-A10 and CM -A45) found in Potiguar Basin foraging grounds.

Studies carried out by Bowen et al. (1992), demonstrated not only the philopatry of *Chelonia mydas* and other species of sea turtles, but also the heterogeneous composition of feeding grounds, where the presence of several haplotypes constituting the mixed stocks of these areas is observed (LAHANAS et al., 1994; ENCALADA et al., 1996; NARO-MACIEL et al., 2007; NARO-MACIEL et al., 2012; JENSEN et al., 2013; JORDÃO et al., 2015; ALMEIDA et al., 2021).

Our results demonstrated a slightly divergent haplotype composition, but the most common haplotypes found were CM-A8 followed by CM-A5, similarly to what is found in other Brazilian feeding grounds in the Southwest Atlantic Ocean (NARO-MACIEL et al., 2007; NARO-MACIEL et al. 2012, PROSDOCIMI et al., 2012; PROIETTI et al., 2012; JORDÃO et al. 2015, ALMEIDA et al. 2021) (Figure 4).

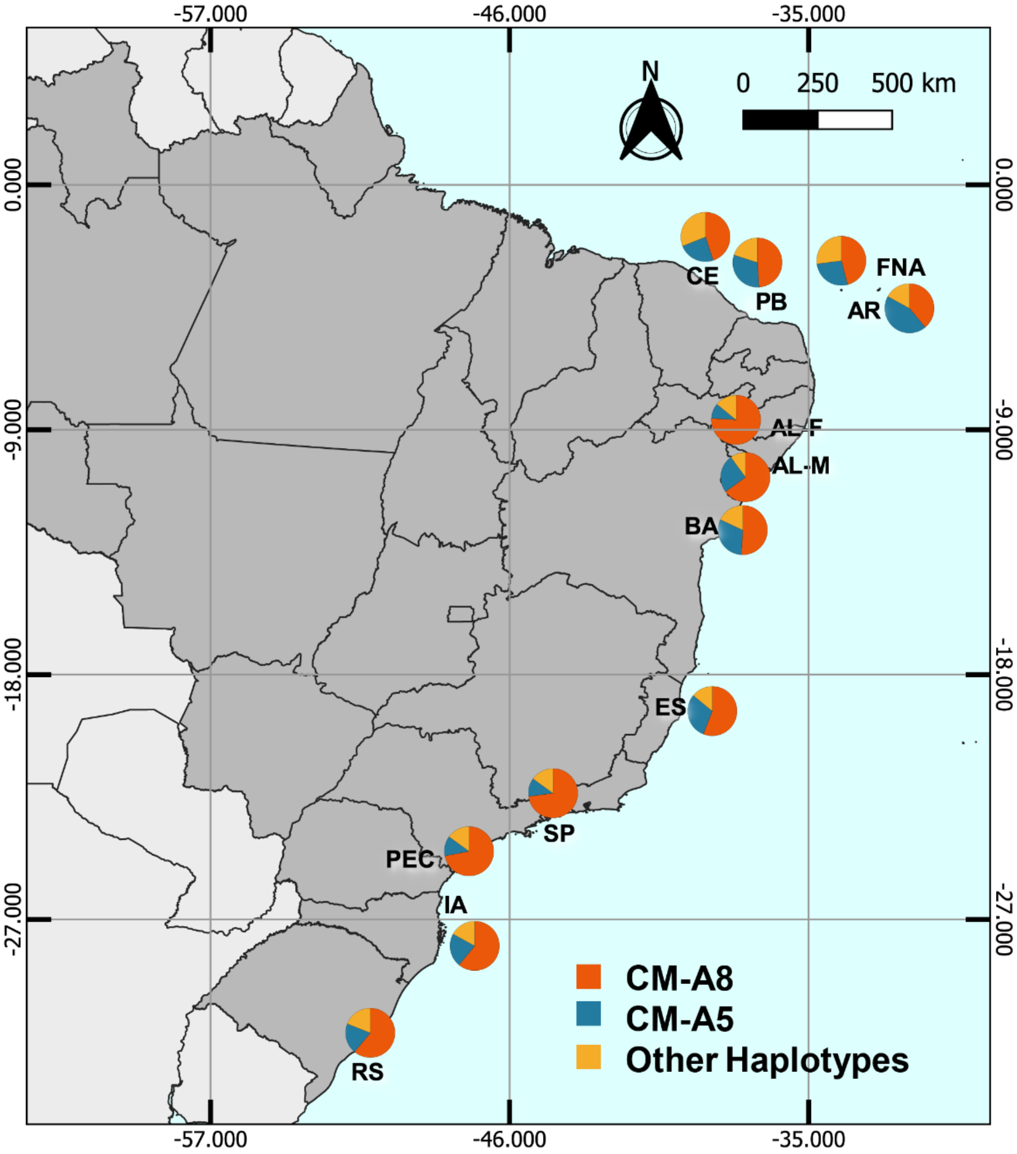

Figure 4: Genetic composition of Brazilian feeding grounds in the Southwest Atlantic Ocean. CE (Almofala) (NARO-MACIEL et al., 2007); PB (Potiguar Basin); FNA (Fernando de Noronha Archipelago) and AR (Atol das Rocas) (NARO-MACIEL et al. 2012); AL-F (Alagoas – Females) and AL-M (Alagoas -Males) (ALMEIDA et al 2021); BA (Bahia) and ES (Espírito Santo) (NARO-MACIEL et al. 2012); SP (Ubatuba) (NARO-MACIEL et al., 2007); PEC (Paranaguá Estuarine Complex) (JORDÃO et al. 2015); IA (Avoredo Island) and RS (Cassino Beach) (PROIETTI et al., 2012).

The best fitted model, considering the haplotype group, explained about 78% of the variance of the entire model, and a R (squared) of 0.76. Also, according to this adjusted model, the data reveal that only for the CM-A8 haplotype, its proportion increases significantly ($p<0.01$). This means that the higher the latitude (ie, further south) the greater the presence of this haplotype in population stocks (Figure 5).

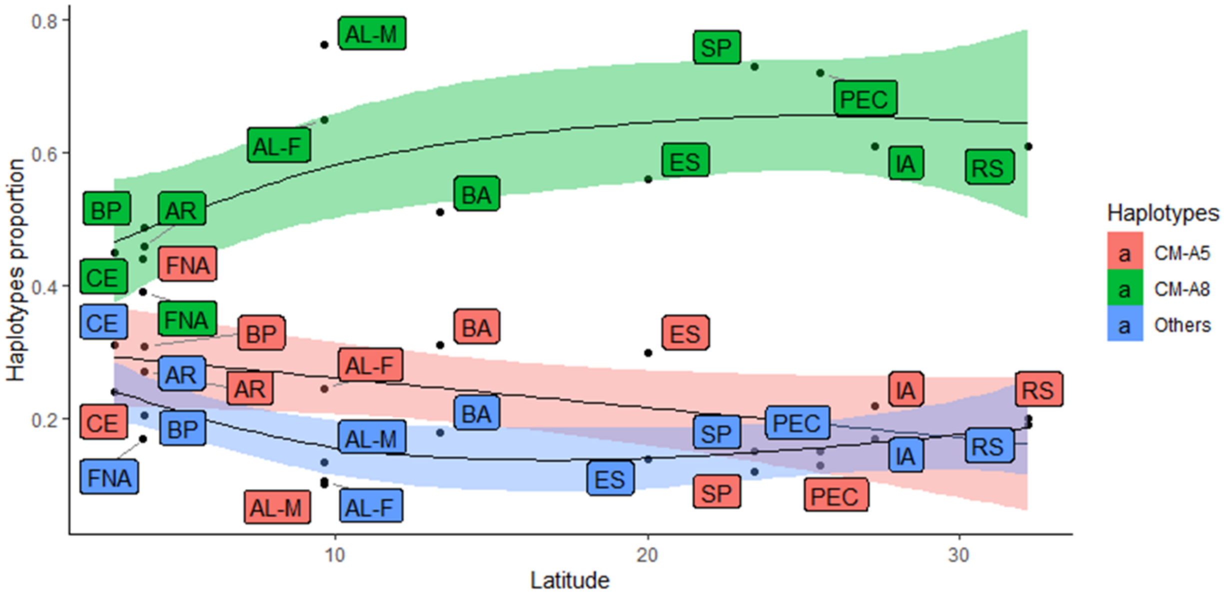

Figure 5: Comparison between genetic haplotype proportion of the Brazilian *Chelonia mydas* feeding grounds in the Southwest Atlantic Ocean according to the latitude. CE (Almofala) (NARO-MACIEL et al., 2007); PB (Potiguar Basin); FNA (Fernando de Noronha Archipelago) and AR (Atol das Rocas) (NARO-MACIEL et al. 2012); AL-F (Alagoas – Females) and AL-M (Alagoas -Males) (ALMEIDA et al 2021); BA (Bahia) and ES (Espírito Santo) (NARO-MACIEL et al. 2012); SP (Ubatuba) (NARO-MACIEL et al., 2007); PEC (Paranaguá Estuarine Complex) (JORDÃO et al. 2015); IA (Avoredo Island) and RS (Cassino Beach) (PROIETTI et al., 2012).

The CM-08 haplotype is predominant in Atlantic colonies and more widespread on equatorial beaches, with frequent records in nesting areas located in Guinea Bissau, Bioko, São Tomé and Príncipe, Ascension Islands, Atol das Rocas, Fernando de Noronha and Trindade (ENCALADA, 1996; FORMIA, 2006; BJORNDAL, 2006; JORDÃO, 2013). For this reason, and due to its central position in the haplotype network, CM-08 was described as the closest to the ancestral haplotype that colonized the Atlantic Ocean (ENCALADA, 1996; FORMIA, 2002).

In the foraging and feeding areas so far elucidated in Brazil, including the Potiguar Basin region of the present study, most of the green turtles (CM-A8) come not only from all known nesting sites in Brazil, which are Rocas Atoll (BELLINI; SANCHES, 1996; GROSSMAN et al., 2003), Fernando de Noronha (BELLINI; SANCHES, 1996) and Trindade Island (MOREIRA et al., 1995), as well as in the vicinity of the African region (Guinea Bissau, Bioko, Sao Tome and Principe) (Figure 6).

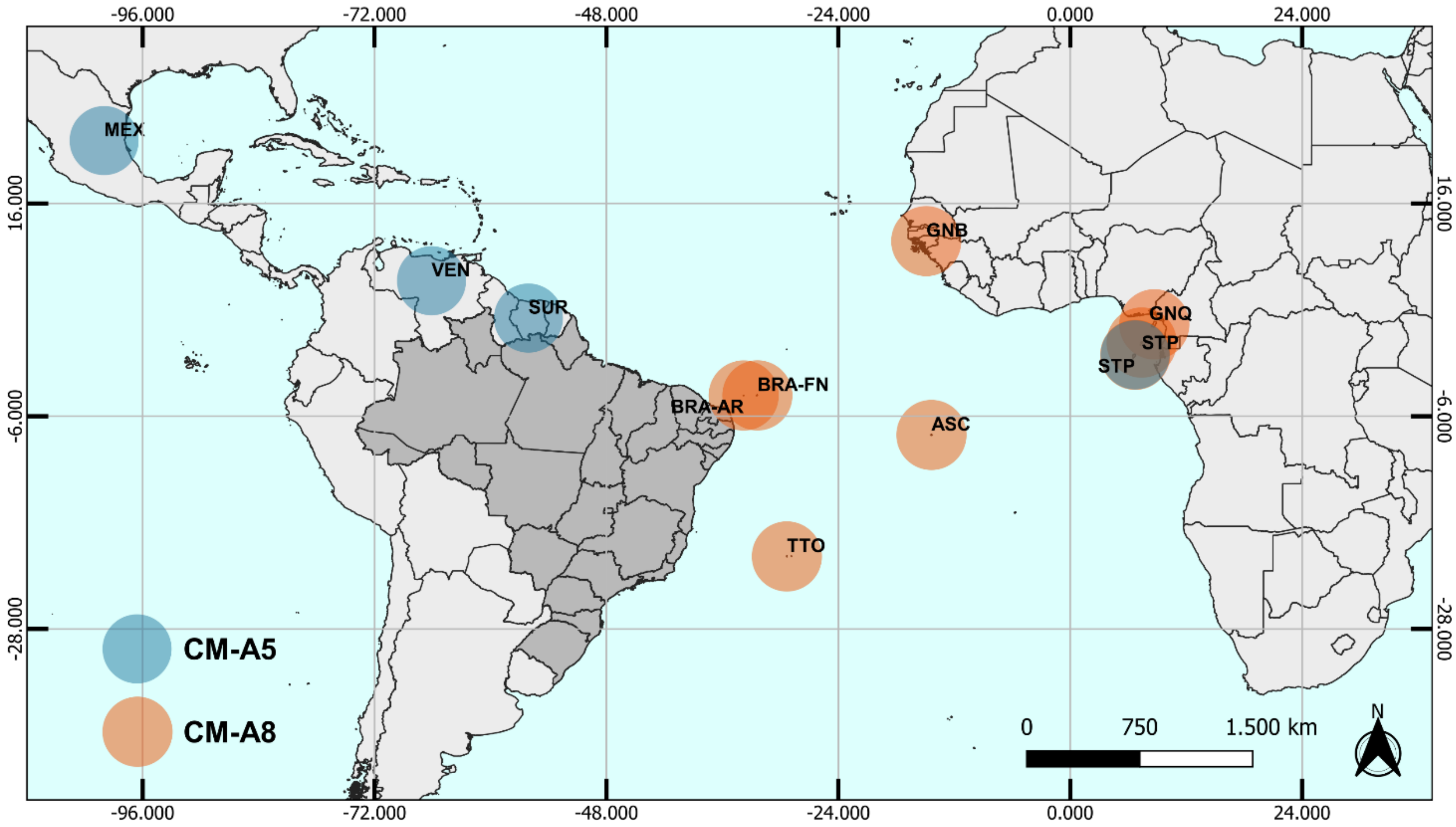

Figure 6: Nesting sites in the Atlantic Ocean of *Chelonia mydas* individuals with the CM-A8 (orange circle) and CM-A5 (blue circle) haplotypes. MEX (México), VEN (Venezuela), SUR (Suriname), BRA-AR (Atol das Rocas, Brazil), BRA-FN (Fernando de Noronha, Brazil), ASC (Ascension Islands), GNB (Guiné-Bissau), STP (São Tomé and Príncipe), GNQ (Bioko) and TTO (Trindade).

The CM-A5 haplotype was the second most recorded being more frequently found in the nesting site closer to the Caribbean and North Atlantic (ENCALADA, 1996; FORMIA, 2006; SHAMBLIN et al., 2012). The CM-A5 values recorded in the Potiguar Basin are considered high (31%) compared to other studies in Brazil (NARO-MACIEL et al., 2007; NARO-MACIEL et al. 2012; PROSDOCIMI et al., 2012; PROIETTI et al., 2012; JORDÃO et al. 2015, ALMEIDA et al. 2021), which may be related to its strategic geographic position, on the “corner” of the American continent, receiving influence from the northern and southern hemisphere, including the two lineages of Atlantic base haplotypes.

Regarding the haplotypes recorded at a lower frequency in the Potiguar Basin (5.1%), the CM-A10 and CM-A9 haplotypes have been recorded at low frequencies in feeding areas in the southern most colonies of Brazil, in Avoredo Island and Cassino Beach (PROIETTI et al., 2012) and in Paranaguá Estuarine Complex (PEC) (JORDÃO et al. 2015), coming from spawning areas located in the South Atlantic (BONDIOLI, 2009).

The CM-A45 and CM-A6 haplotypes were recorded in less than 3% of the cases each in the present study, being the first one described only for the Ascension Islands, with the foraging sites in Brazil in the region of Almofala/CE (NARO-MACIEL et al., 2007), further north of the Potiguar Basin, as well as further south, on Avoredo Island and Cassino Beach (PROIETTI et al., 2012). The CM-A6 haplotype, in addition to the Ascension Islands, was also described for other spawning areas located in Bioko, São Tomé and Suriname. Such records reinforce the contribution of the second largest colony in the Atlantic, the Ascension Islands (BRODERICK, 2006), to the foraging area of the Potiguar Basin.

The CM-A3 and CM-A1 haplotypes, also recorded with low frequency in the Potiguar Basin, have their description limited to the North Atlantic (NARO-MACIEL et al., 2007). CM-A3 records stand out in more than 10% of the cases in the Almofala/CE study (Naro-Maciel et al, 2007) and in Atol das Rocas (NARO-MACIEL et al., 2012), both locations further north. The CM-A1 haplotype, described as coming from Mexico and Florida, is commonly found in feeding areas of the North Atlantic, being recorded in the South Atlantic, on the Brazilian coast, only in Fernando de Noronha and Atol das Rocas (NARO-MACIEL et al., 2012) and now in the present study in the Potiguar Basin.

CONCLUSION

Our results provide the first genetic information about the green turtles (*Chelonia mydas*) from the Potiguar Basin, Brazil, contributing to the understanding of the haplotypes composition of the mixed stock present in this important feeding area, characterized especially by juvenile specimens ranging from 30 to 59.9 cm CCL. Despite the small sample size, our findings corroborate other studies on the Brazilian coast, with a predominance of the CM-A8 haplotype, but highlighting the high values of CM-A5 found, compared to other studies in Brazil, which may be related to the geographic position of the Potiguar Basin, on the “corner” of the American continent, receiving influence from the northern and southern hemispheres, including these two haplotype lineages with an Atlantic base. The results of this work increase the understanding of the haplotype composition of the population stocks of *C. mydas* on the Brazilian coast, filling the gap information in the genetic composition of green turtles in the feeding site of the Potiguar Basin, reinforcing that the greater the latitude (that is, further south) the greater the presence of the CM-A8 haplotype. The study also also contributes to the organization of strategies to recompose the nesting areas and, consequently, improve the threatened status of the species.

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CAPÍTULO 2

The use of an alimentary index to assess anthropogenic debris on green turtles (*Chelonia mydas*)

Running head: Alimentary index to evaluate debris on green turtles

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ESTE ARTIGO FOI SUBMETIDO AO PERIÓDICO Journal of the Marine Biological Association of the United Kingdom E, PORTANTO, ESTÁ FORMATADO DE ACORDO COM AS RECOMENDAÇÕES DESTA REVISTA (https://mc.manuscriptcentral.com/jmba)

Abstract

The ingestion of anthropogenic marine debris (AMD) severely affects green turtles (*Chelonia mydas*), which have been increasingly susceptible to this threat due to their life-stage, location of feeding, and occurrence in the marine ecosystem. In this study, we used the alimentary index (IAi) to quantify and classify AMD ingested by green turtles stranded in the Potiguar Basin, northeastern Brazil. Food items were also examined and classified into categories (e.g., different animal taxa, algae). We classified 295 green turtles as juveniles (JUV) and adults (ADU) and divided them into three size groups: JUV-I (n=190), JUV-II (n=58), and ADU (n=47). We collected samples of the esophagus, stomach, and gut contents during necropsy and analyzed them using the IAi, which is based on frequency of occurrence and volume of each debris and food item. The IAi values were categorized as accessory (IAi<0.25), secondary (0.25≤IAi<0.5), and main (IAi≥0.5). The results revealed that transparent soft plastic was an accessory item for the three size groups and the second most frequent item (IAi=0.23) for JUV-I. Algae was the main food category, with higher IAi values in JUV-II (IAi=0.47), followed by ADU (IAi=0.44) and JUV-I (IAi=0.32). A simple linear regression allowed predicting three types of AMD: colored soft plastic, transparent soft plastic and fishing artifacts. Our study brings insights into the application of the IAi to evaluate the amount and types of AMD ingested by green turtles. In addition, IAi can be used to assess impact of AMD ingestion of sea turtle species or other taxa.

Key words

Sea turtles. Plastic ingestion. Endangered species. Conservation.

1. INTRODUCTION

Anthropogenic marine debris (AMD) is one of the greatest threats to sea ecosystems compromising the biodiversity and marine resources (Moore, 2008; Galgani *et al.*, 2010; Sutherland *et al.*, 2010). The major component of AMD is plastic (Galgani *et al.*, 2015), which has been intensively used by the society (Andrady & Neal, 2009) and, due to its exponential use, plastic waste could reach 6 million tons per day in 2025 (Hoornweg *et al*., 2013). Plastic ingestion has been reported for a large number of species directly affecting over 1,200 marine species from zooplanktons to whales (Stephanis *et al*., 2013; Desforges *et al*., 2015; Santos *et al*., 2021). Studies show that all sea turtle species ingest AMD, which can damage or block the digestive tract, causing lethal or sub-lethal effects, such as dietary dilution or assimilation of contaminants derived from marine litter (Bjorndal e*t al.*, 1994; Bjorndal, 1997; Mccauley & Bjorndal, 1999; Tomás *et al.*, 2002; Ashton *et al.*, 2010; Yamashita *et al.*, 2011; Lazar & Gracan, 2011; Fisner *et al.*, 2013; Gall & Thompson, 2015; Santos *et al.* 2015; Schuyler *et al.*, 2015; Vélez-Rubio *et al.*, 2018; Machovsky-Capuska *et al.*, 2020).

In general, sea turtles are exposed distinctly to types and amounts of AMD, depending on their life-stage and location of feeding in the water column (Cardona *et al.*, 2009; Nagaoka *et al.*, 2012; Carman *et al.*, 2014; Schuyler *et al.*, 2014; Wilcox *et al.*, 2018; Benhardouze *et al.*, 2021). Previous studies have identified drivers for plastic consumption, such as visual cues, as the shape and color plastic items resemble natural preys (Ryan, 1987; Santos *et al*., 2016; Schuyler *et al*., 2012, 2014), and chemical cues (Allen *et al*., 2017; Savoca *et al*., 2016). The plastic ingestion in the ocean is also closely linked to the use of habitats and opportunistic foraging behavior of sea turtles. Neonates have a generalist diet while individuals on the coastal areas have a selective diet (Plotkin, *et al.* 1993; Boyle & Limpus, 2008; Campani *et al.*, 2013; Schuyler *et al.*, 2015; Santos *et al*., 2015; Seney, 2016; Yaghmour *et al.*, 2018; Andrades *et al*., 2019).

The feeding biology of sea turtles is a broad topic. The diet analysis (e.g., stomach content and esophageal lavage) provides insights into the foraging strategies of sea turtles, according to their life stages (Jones & Seminoff, 2013); however, few reliable methods are available to assess plastic ingestion in live green turtle populations (González-Perez *et al.*, 2021). Dietary studies using the lavage (Seminoff *et al.*, 2002; Witherington *et al.*, 2012) or the faecal analyses have reported plastic ingestion by sea turtles (Seminoff *et al.*, 2002; Casale *et* *al.*, 2008); nevertheless, these techniques may underestimate debris ingestion because only a small subset of the gastrointestinal tract is sampled.

Studies on *C. mydas* found stranded during daily monitoring in the Potiguar Basin, northeastern Brazil, showed that this species is severely affected by AMD ingestion and suggested that this region is a feeding area for *C. mydas* (Farias *et al.*, 2019). In this study, we applied an alimentary index to quantify and classify AMD ingested by green turtles at different life stages and stranded in the Potiguar Basin.

2. MATERIALS AND METHODS

2.1 Stranding green turtles

We collected data through the analysis of esophagus, stomach, and gut contents from green turtles stranded between Caiçara do Norte (5° 4'1.15" S; 36° 4'36.41" W) in Rio Grande do Norte State (RN) and Aquiraz (03°49'20.9" S and 38°24'07.8" W) in Ceará State (CE), a region known as Potiguar Basin (Figure 1), in Brazil, over 10 years (from 1 January 2010 to 31 December 2019). The individuals were found during daily beach monitoring conducted by field-trained personnel from Costa Branca Cetacean Project – University of the State of Rio Grande do Norte using a traction vehicle (4-wheel drive) and a portable GPS to record the location of strandings. Since 2010, the PCCB-UERN has conducted the Beach Monitoring Project in the Potiguar Basin (PMP-BP), which is part of an environmental constraint compliance enforced by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) over the oil and gas exploration by PETROBRAS (Petróleo Brasileiro S.A. – Agreement number 2500.005657510.2).

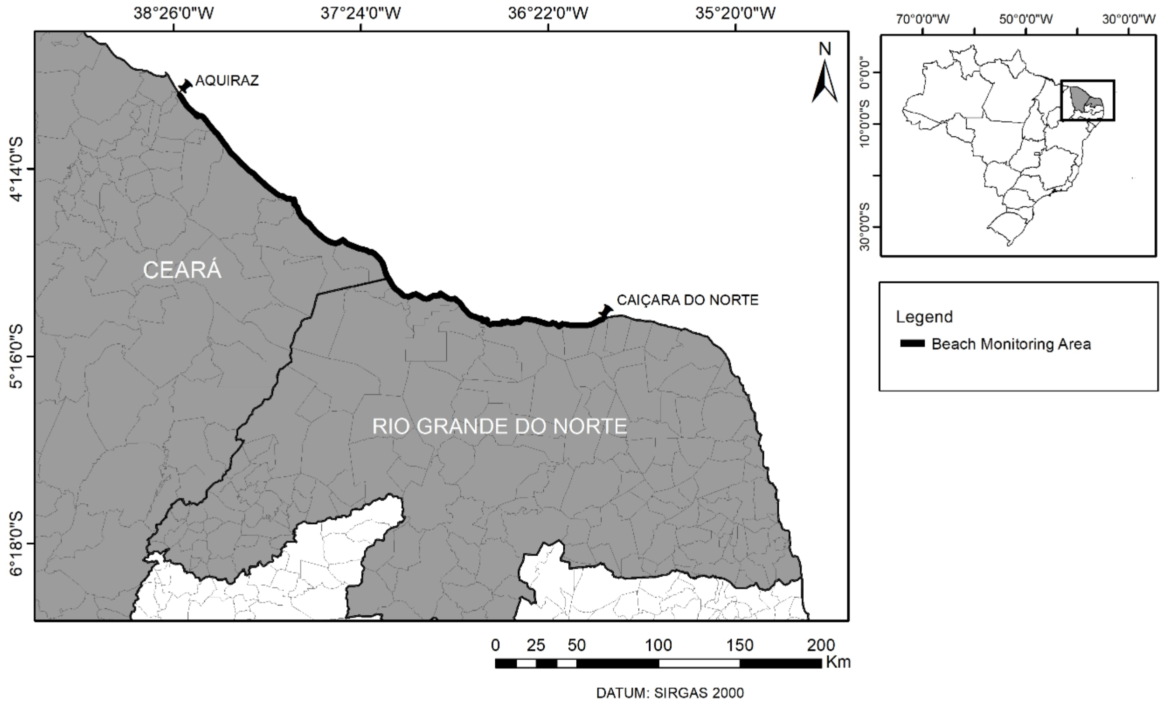

Figure 1 - Geographic distribution of the study site, Brazilian northeastern coast. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN). Fonte: PCCB-UERN.

2.2 Morphometrics, sampling, and content analysis

The curved carapace length (CCL) was recorded using a flexible tape and measured from nuchal notch to the posterior most tip of carapace. Our analysis was based on Almeida *et al*. (2011) who considered the smallest registered size for nesting females in the Brazilian nesting areas = 90 cm CCL. The individuals were taken to the Rehabilitation Center of PCCB-UERN in Areia Branca, Rio Grande do Norte State; however, despite the rehabilitation efforts, the animals died and were thus examined by necropsy. Moderately decomposed individuals (condition codes D2 and D3; Flint *et al.*, 2009) found during beach monitoring were also taken to necropsy examination.

The esophagus, stomach, and gut contents were collected during necropsy and fixed in 4% formalin (algae and seagrass) or 70% alcohol (invertebrates). The AMD was found mixed to the food items and were separated during laboratorial analysis. The analysis of contents combined two methods based on the diet: (1) frequency of occurrence (F%): number of stomachs containing one or more individuals of each item category was expressed as a percentage of all stomachs containing food/anthropogenic debris (Dineen, 1951, Dunn, 1954, Kennedy & Fitzmaurice, 1972); and (2) volumetric method by direct estimation (V%): the measurement of each food category and types of AMD or group of categories sorted from the contents was performed in a graduated measuring device, according to Wolfert & Miller (1978), considering the volume of each category and expressed as a percentage of all food categories (Kawakami & Vazzoler, 1980).

The AMD were classified into five categories: transparent soft plastic, colored soft plastic, rigid plastic, fishing artifacts (e.g., fishing net, fishing raffia, nylon, and rope), and other types (e.g., rubber, elastic, artificial sponges, Styrofoam, and piercing objects), according to Farias *et al.* (2019). In addition, food items were examined and classified into eight categories: Algae, Porifera, Mollusca, non-identified vegetal matters, non-identified animal matters (advanced digestion stage), non-identified organic matters (advanced digestion stage without distinction between animal and vegetable matters), sediment and feces (digested matters). The Alimentary Index (IAi; Kawakami & Vazzoler, 1980) was calculated for each food category: $IAi = Fi \times Vi / \sum (Fi \times Vi)$, where F = Frequency of occurrence (%) of each item, V = Volume (%) of each item, and i = 1, 2… n = determined item. The "diet" composition was analyzed considering the IAi and the items were classified into the following categories, according to Rosecchi & Nouaze (1987): $IAi < 0.25$ - accessory item; $0.25 \leq IAi < 0.5$ - secondary item; $IAi \geq 0.5$ - main item.

2.3 Statistical analyses

All analyses were performed after evaluating data normality and homoscedasticity (Komolgorov-Smirnov and Levene tests, respectively) and tests were performed using the IBM SPSS (Statistical Package for the Social Sciences, version 20) software. The results were considered significant with a P-value < 0.05.

To track the presence of AMD considering the large difference between turtle size, the CCL data (26–129 cm) were converted into qualitative variables. Adult animals were classified according to Almeida et al. (2011) (i.e., CCL >90 cm), resulting in a group ADU (CCL between 90 and 129). The Cluster analysis was performed to individuals with CCL up to 89.9 cm. This analysis uses an exploratory technique of observed data to verify the structure of their relationships and discover groupings of individuals based on the similarity or distances between them (Corrar et al., 2009). To analyze, we considered the Centroid method and the Squared

Euclidean Distance, where the homogeneity of individuals from the same group was maximized as well as the heterogeneity of individuals from different groups (Corrar et al., 2009), resulting in two size groups: Juvenile I – JUV-I (CCL between 26 and 54 cm) and Juvenile II – JUV-II (54.1–89.9 cm CCL). These groups were defined by the Cluster analysis considering that 53.2 cm of CCL was the largest size in the first group (JUV-I), and 55 cm of CCL was the smallest size in the JUV-II group. The three groups, which include all turtles, were analyzed by the Kruskal-Wallis test and pairwise comparison with Bonferroni's correction that showed an effect of CCL values significantly different on the size of the groups ($U = 210{,}156$; $p<0.001$).

We used the simple linear regression to predict the volume of AMD types, according to the different CCL groups defined by the Cluster Analysis. The one-way ANOVA indicates, on the regression model found, whether the CCL value can predict the volume of debris, when p is significant. The R^2, the Pearson's correlation coefficient squared, indicates the explained variance and the model prediction capacity, whose interpretation is the percentage of variation of the AMD volume that is explained by the CCL values (Dancey & Reidy, 2006).

3. RESULTS

We analyzed samples of the esophagus, stomach, and gut contents from 295 green turtles (51.52% with anthropogenic debris; n=152), classified as JUV-I (n=190), JUV-II (n=58), and ADU (n=47) size groups.

The analysis of IAi, according to size groups, revealed that transparent soft plastic had relevance as an accessory item (0.23) for individuals classified into the JUV-I size group, followed by vegetal matters (0.16) and fishing artifacts (0.12). Other types of AMD were also considered accessories, but with lower values than those of transparent soft plastic (Table 1). Regarding food categories, algae was prevalent for all three groups and considered secondary, according to IAi (0.32, 0.44 and 0.47; JUV-I, ADU and JUV-II, respectively). Plant matters were also secondary for JUV-II and ADU size groups (0.44 and 0.43, respectively). Mollusks (cephalopods and gastropods) and porifera were accessory for the JUV-I size group (0.01). Figure 2 shows each category of AMD according to size groups. Table 1 summarizes the data on F%, V%, and IAi of each category (food and AMD) in the digestive tract of the green turtles studied.

Table 1 – Frequency (F%), Volume (V%), and Alimentary Index (IAi) of each category found in the digestive tract of green turtles (*Chelonia mydas*) stranded between January 1, 2010, and December 31, 2019, in the Potiguar Basin - northeastern Brazil, according to size class groups.

Size class groups (cm)	**JUV-I (26-54)**			**JUV-II (54.1-89,9)**			**ADU (90-129)**		
Categories	**%F**	**%V**	**IAi**	**%F**	**%V**	**IAi**	**%F**	**%V**	**IAi**
Anthropogenic debris classified into five categories									
Transparent soft plastic	53.68	21.81	0.23	31.03	7.38	0.04	44.68	10.52	0.09
Colored soft plastic	46.32	7.65	0.07	27.59	2.68	0.01	34.04	2.57	0.02
Rigid plastic	39.47	7.27	0.06	27.59	2.04	0.01	27.66	1.45	0.01
Fishing artifacts	48.95	12.43	0.12	32.76	3.13	0.02	34.04	3.00	0.02
Other type	9.47	0.46	0.00	-	-	-	2.13	0.01	0.00
Food items classified into eight categories									
Algae	61.05	26.61	0.32	63.79	40.67	0.47	72.34	32.82	0.44
Porifera	25.79	2.01	0.01	12.07	0.52	0.00	4.26	0.11	0.00
Mollusca	37.89	1.89	0.01	10.34	0.29	0.00	4.26	0.04	0.00
Non-identified vegetal matters	58.42	14.12	0.16	58.62	40.80	0.44	48.94	48.30	0.43
Non-identified animal matters	22.63	1.08	0.01	6.90	0.64	0.00	2.13	0.17	0.01
Non-identified organic matters	33.16	1.37	0.01	17.24	1.27	0.00	12.77	0.90	0.00
Sediment	26.32	0.96	0.00	3.45	0.41	0.00	-	-	-
Feces	21.58	1.37	0.01	-	-	-	2.13	0.00	0.00

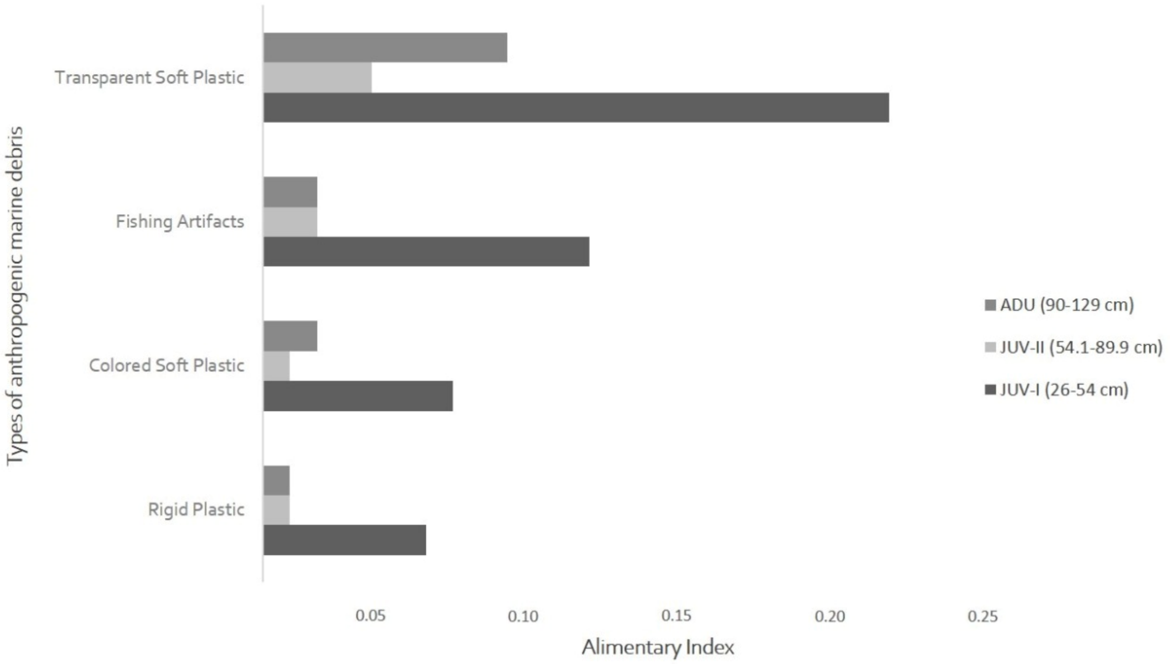

Figure 2 – Comparison between AMD categories of Alimentary Index (IAi) in the digestive tract of green turtles (*Chelonia mydas*) stranded between January 1, 2010, and December 31, 2019, in the Potiguar Basin - northeastern Brazil, according to size groups

The simple linear regression allowed predicting three types of AMD: colored soft plastic, transparent soft plastic, and fishing artifacts. The analyses showed that CCL allowed predicting the volume of colored soft plastic and fishing artifacts in the JUV-I size group [F (1.188) = 11.998; $p = 0.001$; $R^2 = 0.06$] and [F (1.187) = 7.439; $p = 0.007$; $R^2 = 0.038$], respectively, and JUV-II [F (1.56) = 14.817; $p < 0.001$; $R^2 = 0.457$] and [F (1.56) = 4.071; $p = 0.048$; $R^2 = 0.068$], respectively. CCL also allowed predicting the transparent soft plastic in JUV-II [F (1.56) = 5.646; $p < 0.021$; $R^2 = 0.092$], but not in JUV-I [F (1.188) = 3.481; $p = 0.064$; $R^2 = 0.018$]. Thus, for individuals classified into the JUV-I size group, the volume of AMD decreases as their CCL increases. On the other hand, the volume of AMD has a slight increment as CCL of JUV-II increases (Figure 3).

The CCL allowed predicting the volume of colored soft plastic, transparent soft plastic, and fishing artifacts in adult (ADU) individuals (respectively, [F (1.45) = 0.003; $p = 0.954$; $R^2 < 0.001$], [F (1.45) = 0.435; $p = 0.513$; $R^2 = 0.01$] and [F (1.45) = 0.188; $p = 0.666$; $R^2 = 0.004$]) (Figure 3).

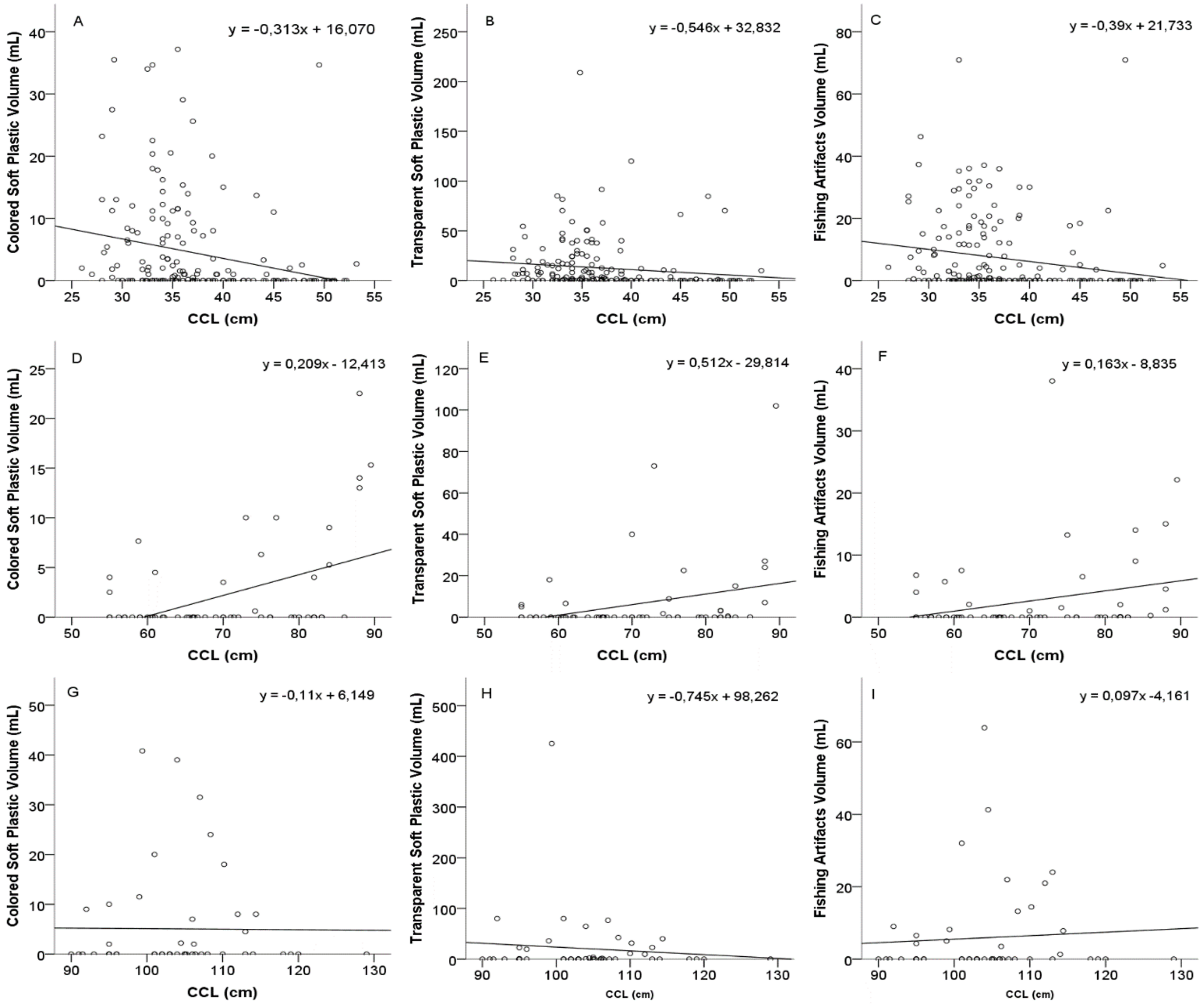

Figure 3 - Relationship between anthropogenic marine debris (colored soft plastic, transparent soft plastic, and fishing artifacts) and CCL size of study *Chelonia mydas*. A, B, C: JUV-I, D, E, F: JUV-II, and G, H, I: ADU.

4. DISCUSSION

Anthropogenic activities pressure ecosystems causing changes to evolutionary traps (Horton *et al*., 2017), such as AMD ingestions, which have been reported for green turtles in many regions worldwide (Plotkin *et al.*, 1993; Boyle & Limpus, 2008; Campani *et al.*, 2013; Schuyler *et al.*, 2015). Our study corroborated this fact, since more than 50% of the green turtles presented some type of AMD in their digestive tract. Waste production has increased exponentially, and it is largely composed by plastic material (Derraik, 2002; Thompson *et al.*, 2009; Hoornweg *et al.*, 2013; Santos *et al.*, 2015). Despite the increasing number of studies reporting plastic ingestion by marine animals, distributed from tropical estuaries to cold deep waters (Santos *et al*., 2021), there are gaps in the knowledge of the impacts and causes of plastic ingestion (Browne *et al*., 2015; Rochman *et al*., 2016; Lynch, 2018), mainly in terms of the quantification of this impact.

The distribution and volume of plastics in the environment combined with the foraging strategies of sea turtles determine the plastic encounter rate, which is an important driving factor for AMD ingestion (Santos *et al*. 2021). Sea turtles are particularly vulnerable to pollution by plastics with records in different life-stages of all species (Bjorndal, 1997; Mccauley & Bjorndal, 1999; Tomás *et al*., 2002; Witherington *et al.*, 2012; Schuyler *et al.*, 2014; Gall & Thompson, 2015; Vélez-Rubio *et al.*, 2018). Neonates and juveniles in pelagic stage can be especially vulnerable to plastic ingestion, once green turtles at this life stage basically feed at the water surface, where plastic material is usually aggregated to floating algae and solid waste in areas of convergence (Plotkin *et al.*, 1993; Boyle & Limpus, 2008; Witherington *et al.*, 2012; Schuyler *et al.*, 2012; Campani *et al.*, 2013; Schuyler *et al.*, 2014; Schuyler *et al.*, 2015). We found similar results, since transparent soft plastic was the main AMD found in the three size groups, mainly JUV-1, in which plastic represented the second item based on the IAi.

Few studies indicate that plastic ingestion by adult *C. mydas*, followed by a largely herbivorous diet in the neritic phase, could lead animals in this life-stage to ingesting plastic material that resembles items of their diet (Schuyler *et al.*, 2012; Robertson *et al.*, 2013). These findings could explain our results, which revealed an increase of transparent soft plastic ingestion by adults in comparison to JUV-II individuals. Regarding food items, algae were the prevalent in the three groups examined corroborating previous studies, which reported algae as the main food type consumed by green turtles (Bjorndal *et al.*, 1994; López-Mendilaharsu *et al.*, 2008; Carrión-Cortez *et al.*, 2010; Santos *et al.*, 2011; Nagaoka *et al.*, 2012).

The great diversity of shapes, colors, and materials of AMD ingested by marine animals may not be explained only by the hypothesis of plastic consumption as a mistaken identify due to similarities with natural food (Derraik, 2002). As the availability of plastic increases, the diversity of feeding signals emitted by plastics also increase (e.g., materials, sizes, shapes, and colors) (Pedrotti *et al*., 2016), and consequently higher encounter rates (Santos *et al*., 2021). Studies show that green turtles ingest large amounts of plastic in the ocean, especially soft plastic, transparent or colored (Schuyler *et al.*, 2012; Santos *et al*., 2015; Schuyler *et al.*, 2014; Wilcox *et al.*, 2018) with a positive relationship between plastic debris abundance and ingestion rates (Wilcox *et al*., 2015; Ferreira *et al*., 2019; Santos *et al.*, 2021); however, this pattern needs to be further explored.

Fishing artifacts represent one of the main causes of mortality for sea turtles, affecting mainly loggerhead turtles (*Caretta caretta* Linnaeus, 1758) (Silva *et al.*, 2010). This category was found in all size-class groups in our study, with a tendency to increase in larger juvenile individuals (JUV-II) and a significant occurrence in smaller young green turtles (JUV-I), in which the fishing artifacts were one of the four most frequent categories. Fishing artifacts and rigid plastic cause different impacts on sea turtles. A single piece of monofilament fishing line or a metal hook can cause damage as they pass through the gut of sea turtles (e.g. necrosis, laceration and ulceration of the mucosa, or rupture of the viscera), resulting in direct death due to the obstruction of the gastrointestinal tract, even when small amounts are ingested (Levitt & Bauer, 1992; Bjorndal, 1997; Tomás *et al.*, 2002; Santos *et al.*, 2015; Jerdy *et al.*, 2017). Santos (2015) analyzed the *causa mortis* in sea turtles due to solid waste ingestion (transparent soft plastic, colored soft plastic, and nylon) and reported that 0.5 g of waste is enough to cause the death of a juvenile individual of *C. mydas*.

A larger number of plastic fragments on water surface are found near coastal zones and in the gyres, presumably due to their proximity to coastal sources or collection points in the gyres (Eriksen *et al.*, 2014; Jambeck *et al.*, 2015). Thus, as green turtles feed at water surface close to coastal shores, they are more likely to ingest larger amounts of AMD and suffer fatal consequences. Our data show the interaction between the feeding area, plastic types, and life-stage of green turtles, since most AMD was recorded in smaller green turtles (JUV-I), when they basically feed at the water surface and when debris are aggregated to floating algae and solid waste.

CONCLUSION

Our results demonstrated the use of a diet analysis tool, the alimentary index (IAi), to assess the impact of AMD on the different life stages of green turtles stranded in the Potiguar Basin. We believed that underestimating the potential death due to debris ingestion might undermine management and conservation actions. The IAi can also be used to study the impacts of anthropogenic debris on other threatened sea turtle species, according to Brazilian and global criteria, and even other taxa.

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CAPÍTULO 3

Bioaccumulation of total mercury, copper, cadmium, silver, and selenium in green turtles (Chelonia mydas) stranded along the Potiguar Basin, northeastern Brazil

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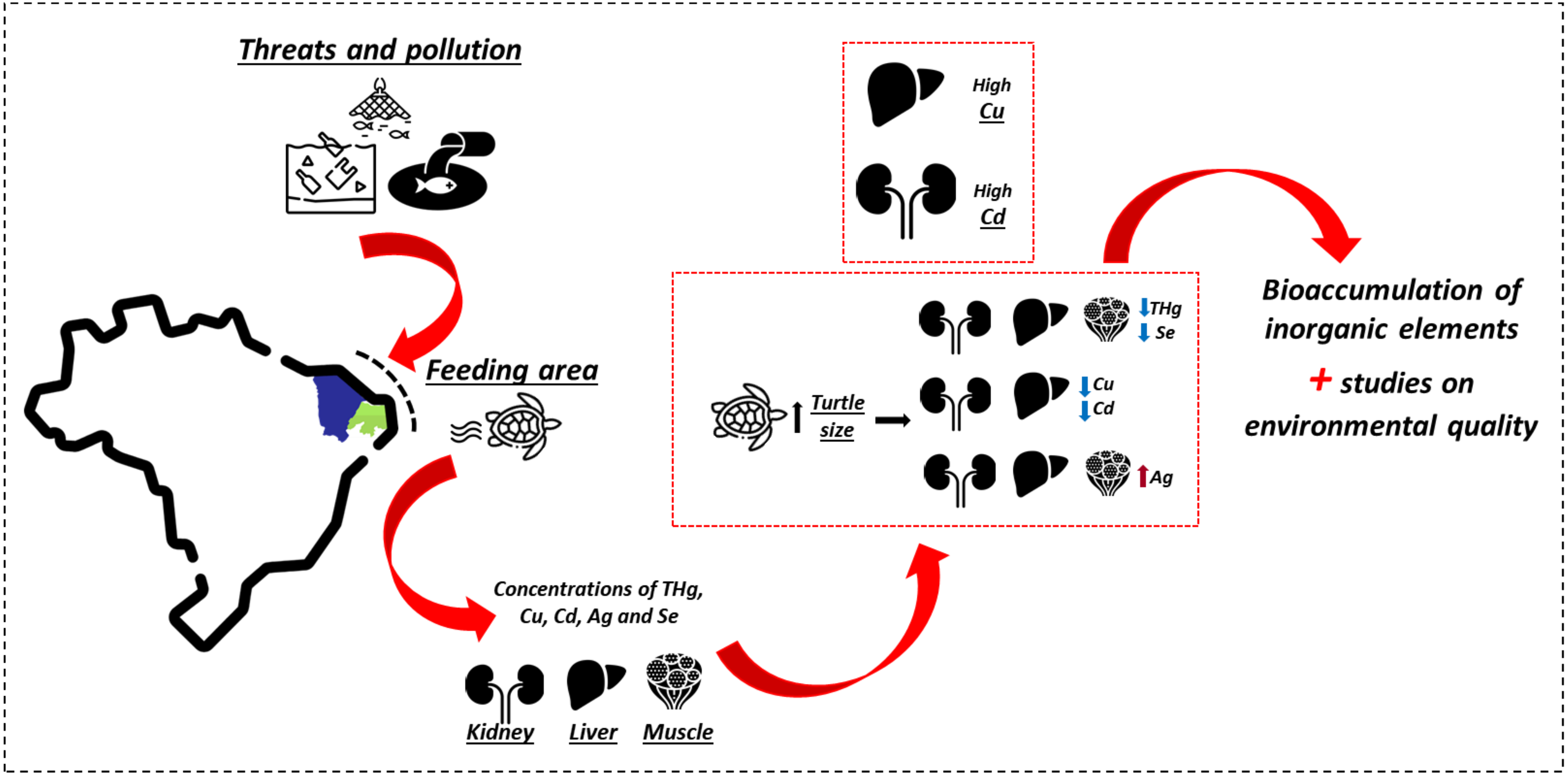
Graphical Abstract



Abstract

Sea turtles face several threats and pollution has become a major concern for their conservation worldwide. We analyzed samples of the liver, muscles, and kidneys of 38 *Chelonia mydas* stranded along the Potiguar Basin, northeastern Brazil, between 2015 and 2018 to determine the total Hg concentration (THg), as well as the concentrations of Cu, Cd, Ag, and Se. The relation between turtle size and element concentrations revealed a negative correlation for THg and Se (liver, muscles, and kidneys), Cu and Cd (liver and kidneys) and a positive correlation for Ag in the three organs analyzed. Concentrations of THg, Cu, Ag, and Se were high in the liver, highlighting the Cu concentration (median = 25.1150 µg g^{-1} w.w.), while the kidneys had the highest Cd levels (median = 12.2200 µg g^{-1} w.w.). There was significant difference between element concentrations and the three organs analyzed, except for Ag and Se concentrations in the muscle and kidney samples. Our study showed that green turtles found in Potiguar Basin, northeastern Brasil, have bioaccumulated inorganic elements which indicate the need of further investigations on the environmental quality of the region.

Keywords: Pollution. Ecotoxicology. Marine turtle. Trace elements. Liver. Kidney. Muscle.

1. INTRODUCTION

Marine pollution caused by chemicals threatens the marine ecosystem and can generate toxic effects to wildlife (Ley-Quiñónez et al., 2011; Yarsan and Yipel, 2013; Bucchia et al., 2015; Yipel et al., 2017). Trace elements are natural components of rocks and soil; however, they are some of the most common pollutants in the marine ecosystem due to accelerated urban growth and increase in industrial activities (Lam et al., 2004). The long-range transport (rivers and air) of trace elements and their non-biodegradable and biomagnification characteristics increases their toxicity and ecotoxicological potential (D'Ilio et al., 2011; Ley-Quiñónez et al., 2011; Yarsan and Yipel, 2013; Bucchia et al., 2015; Mattei et al., 2015; Yipel et al., 2016). Therefore, these pollutants may bioaccumulate and reach toxic concentrations in the tissues of long-lived animals that feed at higher trophic levels, such as species of sea turtles (Järup et al., 1998; Ankley et al., 2006; Agarwal, 2009; Dieter et al., 2014; Mehinto et al., 2014; Cortés-Gómez et al., 2017).

Sea turtles are potential bioindicators of a good environmental status as well as of marine pollution levels (Gordon et al., 1998; Sakai et al., 2000; Gardner et al., 2006; Camacho et al., 2014; Yipel et al., 2017). Their occurrence on coastal regions and proximity to landscapes altered by anthropic actions make sea turtles potentially vulnerable to pollutants (Godley et al., 1999; Maffucci et al., 2005; García-Fernández et al., 2009; Hamann et al., 2010). In addition, sea turtles are long-lived vertebrates that accumulate trace elements through ingestion or directly from the aquatic environment (e.g., inhalation and/or absorption) (Andreani et al., 2008; D'Ilio et al., 2011; Mattei et al., 2015).

Therefore, the effects of trace elements and other chemical pollutants on sea turtles (at individual, population, and ecosystem levels) depend on the levels of trace elements found in organs (Storelli et al., 2005; D'Ilio et al., 2011; Andreani et al., 2008; Bhat, 2013; Camacho et al., 2014; Mattei et al., 2015). Some chemical elements are involved in biological processes and structures and are therefore essential for living beings, such as copper (Cu) which is indispensable for the proper functioning of some metabolic functions (Jakimska, 2011). Studies have reported the capacity of selenium (Se) to reduce toxicity of some heavy metals, such as cadmium (Cd) in deep-sea fish, mercury (Hg) in ringed seals *Phoca hispida* forming the mercuric selenide compound (HgSe), and arsenic (As) in humans (Wagemann et al., 2000; Siscar et al., 2014; Sun et al., 2014). However, little is known about the levels considered thresholds for trace elements in organs of sea turtles and few quantitative data are available linking trace elements to mortality of sea turtles (Anan et al. 2002; Hamann et al., 2010).

On the other hand, several studies have reported on the potential effects of heavy metals in vertebrates (Godley et al., 1999, Caurant et al., 1999; Anan et al., 2002; Storelli and Marcotrigiano, 2003; Maffucci et al., 2005; Moffet et al., 2007; García-Fernández et al., 2009). Mercury is known to be potentially toxic to the nervous and immune systems (Lam et al., 2004, Day et al., 2007); cadmium (Cd) causes negative effects on the metabolic process of essential elements and on the endocrine system and sea turtles tend to concentrate cadmium (Hopkins et al., 1999; Noel et al., 2004; Storelli et al., 2005; Ikonomopoulou et al., 2009; Simoniello et al., 2011); and silver (Ag) in turn can cause ionic and osmotic disturbance in invertebrates (Bianchini et al., 2005; Pedroso et al., 2007).

The Potiguar Basin in northeastern Brazil is an important habitat for sea turtles, mainly green turtles *Chelonia mydas* (Farias et al., 2019). This region presents important economic activities based on exploratory processes of different natural resources (e.g., rocks, soils, salt), agricultural systems and a range of non-renewable (oil and natural gas) and renewable energy elements (biomass, electric energy, wind energy, thermoelectric, and solar bases) (Alves and Amaro, 2019). These activities pose as a potential threat to the quality of estuaries and coastal areas, as well as to endangering marine species (Attademo, 2007; Farias et al., 2019).

Few studies have investigated contamination of sea turtles by trace elements in Brazil (Bezerra et al., 2013; Carneiro da Silva et al., 2014; Prioste et al., 2015; Bruno et al., 2021) and no study has investigated this condition in the Potiguar Basin, to the best of our knowledge. Coastal waters and sediments are impacted by industrial effluents, agricultural residues, and domestic sewage through contamination by metals, such as lead (Pb), zinc (Zn) Ag, Cd, and Cu (Carneiro da Silva et al., 2014). Biomonitoring programs have focused on the bioaccumulation of Hg, Cd, and Pb in the muscle, liver, and kidney (Lam et al., 2004). In Brazil, the Beach Monitoring Project (Projeto de Monitoramento de Praias - PMP) at the Potiguar Basin monitors five elements (total Hg, Cu, Cd, Ag, and Se) in seabirds, marine mammals, and sea turtles.

These elements were previously defined in technical opinions and official guidelines of the Brazilian Institute of the Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA), following the guidelines of the National Council for the Environment (Conselho Nacional do Meio Ambiente - CONAMA) to assess the impacts of oil and gas exploration, production, and transportation activities in Brazil. Therefore, this study aimed to describe the occurrence and concentrations of these five elements in the liver, muscle, and kidney samples of stranded green turtles along the Potiguar Basin in order to provide additional baseline of exposure and/or bioaccumulation in sea turtle organs.

2. MATERIALS AND METHODS

2.1 Study site, green turtles, and sampling

The present study used data collected between January 1, 2015, and December 31, 2018, from records of PMP in the Potiguar Basin. Alive green turtles found during daily monitoring conducted by field-trained personnel of Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN) were rescued and admitted to the rehabilitation center of PCCB-UERN in Areia Branca/Rio Grande do Norte State. The monitored area stretches for roughly 333 km from Caiçara do Norte (5° 4'1.15" S; 36° 4'36.41" W) in Rio Grande do Norte State (RN) to Aquiraz (03°49'20.9" S and 38°24'07.8" W) in Ceará State (CE) (Figure 1). The PMP in the Potiguar Basin has been conducted by the PCCB-UERN since 2010 as part of an environmental constraint compliance enforced by the IBAMA due to oil and gas exploration by PETROBRAS (Petróleo Brasileiro S.A.; Agreement number 2500.005657510.2).

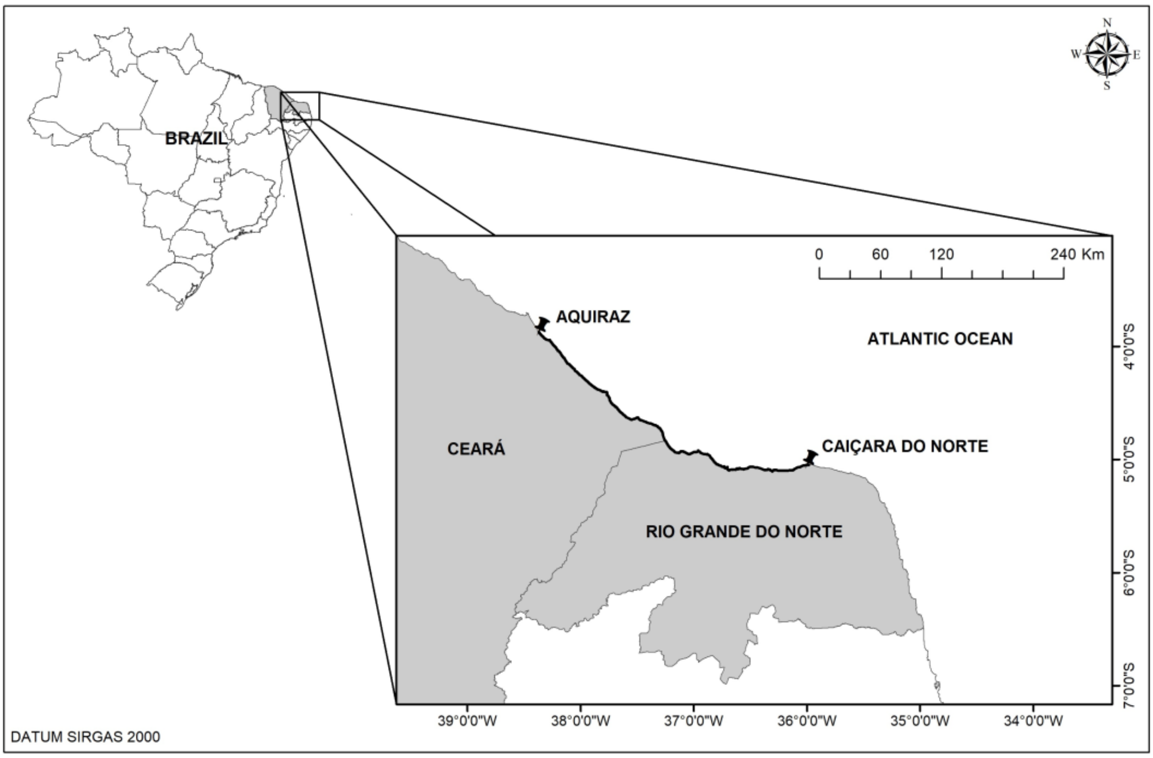


Figure 1 – Area monitored by Beach Monitoring Project at the Potiguar Basin: between Aquiraz/Ceará State and Caiçara do Norte/Rio Grande do Norte State. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN).

Curved carapace length (CCL) was measured in each examined green turtles from the nuchal to notch between supra-caudal scales (Bolten, 1999). Individuals ≥ 90 cm were classified as adults considering the smallest size recorded for nesting females in the largest and closest nesting areas in Brazil (Almeida, et al. 2011). The liver, muscle, and kidney samples were collected from 38 green turtles (27–127 cm CCL): 31 individuals that died at the rehabilitation center of PCCB-UERN and seven individuals were found dead during the beach monitoring. The samples were generally collected immediately post-mortem; however, in some cases, the carcass was frozen and kept at 20 ºC until necropsy (< 12 h). The samples were collected, placed in glass tubes, kept on ice, and stored at -20°C until the laboratory analyses.

2.2 Laboratory analyses

Total mercury (THg) was determined according to Bisi et al. (2012). A mixture of sulfuric and nitric acid was added to the aliquots of approximately 0.3 g of sample. The aliquots were then heated in water bath at 60° C until total solubilization. THg concentrations were determined by Cold Vapor/Atomic Absorption (FIMS-400, PerkinElmer) with sodium borohydride as a reducing agent. Accuracy and precision of the analytical methods were verified using the standard certified materials (DORM-3 and DOLT-5, National Research Council-NRC, Canada). The results agreed with the reference values and the internal reference materials produced by the Laboratório de Mamíferos Aquáticos e Bioindicadores “Profa. Izabel Gurgel” at Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, Brazil. The quality control was also performed through the analysis of procedural blanks (0.04 ± 0.01 µg. L^{-1}) and sample replicates (coefficient of variation < 20%).

The other elements (Cu, Cd, Ag, and Se) were determined according to the digestion procedures of Dorneles et al. (2007). Two aliquots of approximately 0.3 g of sample were added 2 mL of 65% nitric acid (HNO_3) overnight and the solutions were heated at 60°C in water bath for 2 h. After cooling, the solutions were placed in 15 mL tubes and ultrapure water was added to complete a volume of 10 mL. Concentrations of the elements were determined by electrothermal atomic absorption spectrometry (ZEEnit 60s, Analytic Jena, equipped with Zeeman background correction). Palladium nitrate – Pd $(NO_3)_2$ and magnesium nitrate – Mg $(NO_3)_2$ were used as a matrix modifier. Accuracy and precision of the analytical methods were verified using the standard certified materials (TORT-2, DOLT-4, and DOLT-5, National Research Council-NRC, Canada), with elements recovery (Mean ± SD %) at 104 ± 3.61 (THg), 99 ± 7.02 (Cu), 96 ± 7.69 (Cd), 100 ± 6.22 (Ag), and 99 ± 4.95 (Se). Quality control was also performed through the analysis of procedural blanks (<5.00 µg. L-1) and sample replicates (coefficient of variation < 25%). The limits of detection (LoD) were Hg =

0.00002, Cu = 0.00091, Cd = 0.00039, Ag = 0.00025, Se = 0.00402 µg mL^{-1}, and concentrations are expressed in µg g^{-1} wet weight (w.w.).

2.3 Statistical analysis

The Shapiro-Wilk test was performed to verify the distribution of samples and concentrations of the elements were described as median, first quartile, third quartile, minimum and maximum values (range). For comparison, the results were also described as the mean and standard deviation-SD (Mean±SD) and the values presented as dry weight in the literature were converted to wet weight, according to Gordon et al. (1998), whereas w.w.:d.w. ratios for the liver and kidney were 4.9 and 6.8, respectively.

Data were analyzed according to correlation between CCL and THg, Cu, Cd, Ag, and Se concentrations using the Spearman's rank correlation coefficient (r), and comparison of THg, Cu, Cd, Ag, and Se concentrations among liver, muscles, and kidneys were performed by *Friedman* ANOVA and post-hoc *Nemenyi* test (only individuals with the presence of the elements in the three organs met the criteria for the tests: n = 16). The analyses were performed using IBM SPSS Statistics (version 20) and STATISTICA 7.0 for Windows (StatSoft, Inc. 1984e2004, USA) and the results were considered significant when p-value < 0.05.

3. RESULTS

3.1 Concentrations of elements in the liver, kidneys, and muscles

The relationship between concentrations of the elements and CCL showed a weak (r = 0.4–0.7) to strong (r > 0.7) correlation. THg and Se (liver, muscle, and kidney), Cu and Cd (liver and kidney) displayed a negative correlation, showing that concentrations were inversely correlated with the turtle size. THg (kidney; r = -0.744), and Se (liver and kidney; r = -0.728 and -0.722, respectively) showed a strong negative correlation. On the other hand, correlation between Ag levels and turtle size was positive in the liver, muscles, and kidneys (r = 0.727, 0.482 and 0.562, respectively). Cu and CCL (liver), and Cd and CCL (liver and muscle) showed no correlation. Table 1 presents the Spearman coefficient and p-values between CCL and the quantified elements in each organ analyzed.

Copper in the liver presented the highest median and mean values (25.1150 and 32.737±28.866 µg $g^{-}1$ w.w., respectively; n = 32) followed by Cd in kidney (12.2200 and 16.061±21.581 µg g^{-1} w.w., respectively; n = 26), and the lowest median and mean values was quantified for Ag in the muscles (0.0002 and 0.009±0.026 µg g^{-1} w.w., respectively; n = 35). Our results revealed levels below the LoD for all elements in the organs: (1) THg in the liver, muscles, and kidneys (1/32; 3/35; and 2/26, respectively); (2) Cu in the muscles (2/35); (3) Cd in 25.71% of muscles (9/35); (4) Ag in 74.29% of muscle (26/35) and 42.31% of the kidney (11/26) samples; and (5) Se in the liver, muscles, and kidneys (1/17; 3/17; and 3/16, respectively). Table 2 displays the median, the first, and the third quartile values of THg, Cu, Cd, Ag and Se in the organs analyzed. Table 3 summarizes the concentrations of elements compared to previous studies.

Comparison between concentrations of the elements and the organs analyzed revealed significant differences (*Friedman* ANOVA, $p < 0.05$), except for Ag and Se between the muscles and kidneys. The post-hoc *Nemenyi* test showed that THg concentrations in the liver were higher than in the kidneys (p = 0.0020) and muscles ($p = 5.9 \times 10^{-10}$) and levels in the kidneys were higher than in the muscles (p = 0.0084). We observed the same pattern (liver > kidney > muscle) for Cu (liver > kidney: p = 0.0005; liver > muscle: $p = 4.6 \times 10^{-12}$; and kidney > muscle: p = 0.0025). Regarding Cd, the interorgan distribution was kidney > liver (p = 0.0307), kidney > muscle ($p = 5.6 \times 10^{-9}$), and liver > muscle (p = 0.0015). Our results also revealed significant differences between the liver and kidneys for Ag (p = 0.0427) and for Se

($p = 0.0023$) with concentrations in the liver > kidneys and muscles ($p = 0.0038$ and $p = 0.0023$, respectively) (Figure 2).

Table 1 – Spearman coefficient (r) and significance of coefficient (p) between the curved carapace length and quantified elements in each organ (liver, muscle and kidney) of the green sea turtles (*Chelonia mydas*) studied.

Elements	Liver				Muscle				Kidney			
	n	r	p	Correlation	n	r	p	Correlation	n	r	p	Correlation
THg	32	-0.427	0.015	Moderate	35	-0.681	<0.001	Moderate-to-strong	26	-0.744	<0.0001	Strong
Cu	32	-0.170	0.353	No correlation	35	0.433	0.009	Moderate	26	-0.432	0.027	Moderate
Cd	32	-0.114	0.535	No correlation	35	0.102	0.558	No correlation	26	-0.602	0.001	Moderate
Ag	32	0.727	<0.0001	Strong	35	0.482	0.003	Moderate	26	0.562	0.003	Moderate
Se	17	-0.728	<0.001	Strong	17	-0.400	0.111	Weak-to-Moderate	16	-0.722	0.002	Strong

Table 2 – Median, first and third quartile values (µg g^{-1} w.w.) of elements (THg, Cu, Cd, Ag and Se) in samples of green turtles (*Chelonia mydas*).

Elements	Median (first quartile; third quartile)					
	Liver		Muscles		Kidneys	
THg	32	0.2349 (0.1253; 0.3998)	35	0.0220 (0.0030; 0.0487)	26	0.0731 (0.0219; 0.1654)
Cu	32	25.1150 (16.9000; 40.6800)	35	0.2486 (0.1320; 0.3280)	26	1.7542 (1.3166; 1.9925)
Cd	32	4.5048 (3.1084; 7.2510)	35	0.0393 (0.0003; 0.0617)	26	12.2200 (6.3942; 16.9950)
Ag	32	1.3543 (0.2699; 2.9240)	35	0.0002 (0.0002; 0.0100)	26	0.0189 (0.0002; 0.0464)
Se	17	1.8805 (0.7558; 3.6095)	17	0.6955 (0.2880; 1.1528)	16	0.9287 (0.2060; 1.2488)

Table 3 – Concentrations of THg, Cu, Cd, Ag, and Se (μg.g^{-1} w.w.) in the liver, muscles, and kidneys of green turtles (*Chelonia mydas*) compared to previous studies.

Organs	Hg			Cu			Cd			Ag			Se			Study site	Reference
	n	Range	Mean±SD	n	Range	Mean±SD	n	Range	Mean±SD	n	Range	Mean±SD	n	Range	Mean±SD		
Liver	32	<LoD–0.764	0.283±0.202	32	3.836–147.100	32.737±28.866	32	1.105–20.815	5.706±4.349	32	0.023–9.788	2.127±2.315	17	<LoD–7.902	2.440±2.080	Potiguar Basin, northeastern Brazil	Present study
	23	0.00–0.052	0.021 (Mean)	–	–	–	38	2.5–56.9	12.5 (Mean)	–	–	–	23	0.07–2.68	1.18 (Mean)	Southeastern Queensland, Australia	Gordon et al. (1998)
	2	0.0767–0.301	0.159±0.189	2	8.73–13.5	11.115±3.373	2	3.9–12.1	8.0±5.798	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	26	7.429–63.388	28.367±17.551	–	–	–	–	–	–	25	0.408–2.020	1.041±0.469	Yaeyama Islands, Japan	Anan et al. (2001)
	–	–	–	9	18.4–130	–	9	3.21–21.6	–	9	0.67–1.2	–	9	0.87–7.5	–	Yaeyama Islands, Japan	Anan et al. (2002)
	–	–	–	11	1.386–27.143	12.253 (Mean)	11	nd–20.816	0.673 (Mean)	–	–	–	–	–	–	Baja California peninsula, Mexico	Gardner et al. (2006)
	–	–	–	7	18.5–59.0	–	7	2.2–9.2	–	–	–	–	–	–	–	Italian coastal areas	Storelli et al. (2008)
	–	–	–	29	6.408–79.347	20.592 (Mean)	29	0.122–5.184	1.204 (Mean)	29	0.041–0.429	0.63 (Mean)	–	–	–	Southern Atlantic coast of Brazil	Carneiro da Silva et al. (2014)
	4	–	0.04±0.02	4	–	2.13±1.95	4	–	0.54±0.31	–	–	–	4	–	0.46±0.34	Northeast Mediterranean Sea	Yipel et al. (2017)
	–	–	–	42	–	98.7±0.87	42	–	66±0.05	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)
Muscles	35	<LoD–0.090	0.030±0.029	35	<LoD–2.123	0.316±0.354	35	<LoD–0.684	0.072±0.129	35	<LoD–0.147	0.009±0.026	17	<LoD–4.855	0.975±1.141	Potiguar Basin, northeastern Brazil	Present study
	2	0.002–0.007	0.005±0.003	2	0.240–0.270	0.255±0.021	2	0.011–0.034	0.023±0.016	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	42	–	1.65±0.21	42	–	33±0.02	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)
Kidneys	26	<LoD–0.470	0.104±0.107	26	0.426–3.055	1.699±0.613	26	2.134–117.450	16.061±21.581	26	<LoD–1.061	0.087±0.219	16	<LoD–3.697	0.996±1.030	Potiguar Basin, northeastern Brazil	Present study
	23	0.00–0.049	0.020 (Mean)	–	–	–	38	1.7–75.9	15.3 (Mean)	–	–	–	23	0.09–1.85	0.59 (Mean)	Southeastern Queensland, Australia	Gordon et al. (1998)
	2	0-042–0.048	0.045±0.004	2	1.33–1.71	1.520±0.269	2	37.0–45.5	41.250±6.010	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	25	0.354–2.779	1.216±0.597	–	–	–	–	–	–	25	0.309–1.618	0.779±0.353	Yaeyama Islands, Japan	Anan et al. (2001)
	–	–	–	11	0.234–2.994	0.834	11	0.896–96.029	17.794	–	–	–	–	–	–	Baja California peninsula, Mexico	Gardner et al. (2006)
	–	–	–	7	4.8–14.3	–	7	2.2–7.5	–	–	–	–	–	–	–	Italian coastal areas	Storelli et al. (2008)
	–	–	–	29	0.221–5.779	1.794 (Mean)	29	0.309–7.632	4.162 (Mean)	29	0.015–0.176	0.060 (Mean)	–	–	–	Southern Atlantic coast of Brazil	Carneiro da Silva et al. (2014)
	4	–	0.03±0.02	4	–	2.03±1.64	4	–	4.24±1.01	–	–	–	4	–	<LoD	Northeast Mediterranean Sea	Yipel et al. (2017)
	–	–	–	42	–	42.3±0.67	42	–	36±0.02	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)

Values presented as dry weight (d.w.) by Anan et al. (2001) Gardner et al. (2006) and Carneiro da Silva et al. (2014) were converted do wet weight (w.w.) according to Gordon et al. (1998), whereas w.w.:d.w. ratios for the liver and kidneys were 4.9 and 6.8, respectively. nd: Not detected. LoD: Limit of detection. –: Not determined.

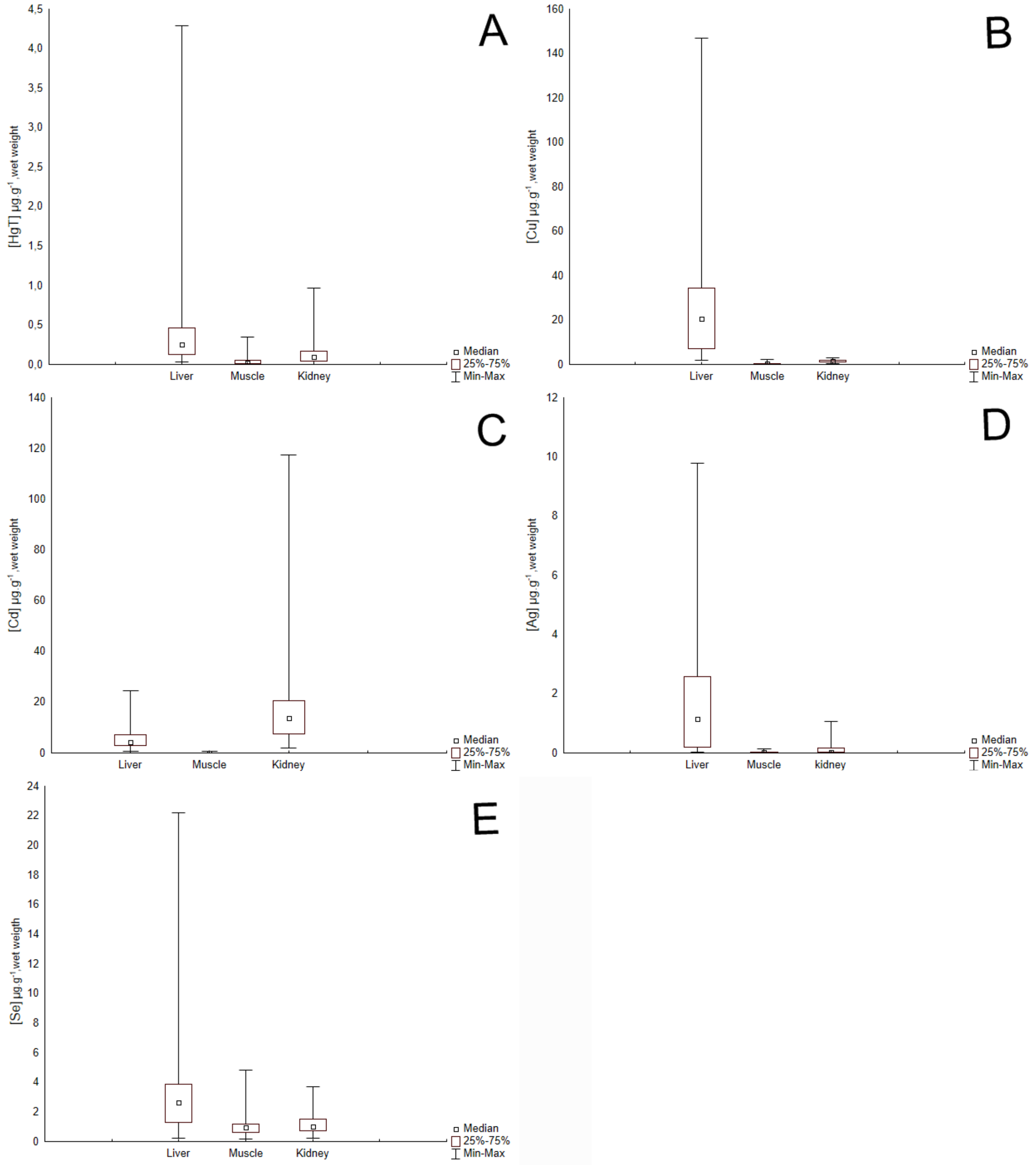


Figure 2 – Concentrations (µg.g^{-1} w.w.) of THg (A), Cu (B), Cd (C), Ag (D) and Se (E) in the liver, muscles, and kidneys of *Chelonia mydas*.

4. DISCUSSION

The diet of sea turtles varies and depends on their age and may be related to the availability of food items in different types of marine ecosystem, which have different ecological and physical characteristics. The diet is the main contamination source of trace elements (Dieter et al., 2014; Mehinto et al., 2014; Sfakianakis et al., 2015; Cortés-Gómez et al., 2017) and green turtles in their pelagic phase are omnivorous, presenting high metal concentrations (Seminoff et al., 2002; Arthur et al., 2008). Our results expressed this trend with a negative correlation (moderate to strong) between CCL and THg concentrations (in the three examined organs), Cu and Cd (in kidney), selenium (in liver and kidney). International environmental conservation policies have advanced recently; nevertheless, the marine environment is still affected by anthropogenic actions, such as contamination by chemical elements and Hg is one of the most studied and worrying toxic metals affecting health parameters of sea turtles even at relatively low levels (Day et al., 2007; Lamborg et al. 2014; Mitchell, 2016; Yipel et al. 2017). In our study, the negative correlation between THg concentration and CCL in all examined organs corroborates previous studies in Brazil and in Japan (Rodriguez et al., 2020; Bruno et al., 2021; Sakai et al., 2000), differing from studies on green turtles from Baja California Sur, Mexico, where no relationship was found between the straight carapace length and Hg concentrations in liver samples (Kampalath et al., 2006). Our findings showed that green turtles had higher THg levels in the liver and kidneys compared to studies conducted in Australia, Japan, and Turkey (Gordon et al., 1998; Sakai et al., 2000; Yipel et al., 2017; See Table 3).

Essential elements, such as Cu, play a crucial role in the metabolism and tissue growth; nevertheless, an excessive Cu amount in the body exerts adverse effects via reactive oxygen species (Halliwell et al., 1990; Andreani et al., 2008). When its concentration is high in the environment, Cu tends to accumulate in the liver associated to metallothionein (Cu–MT) as a non-toxic form of the metal (Andreani et al., 2008; D'Ilio et al., 2011; Yipel et al., 2017). Higher Cu levels in the liver seem to be a general fact in sea turtle species (Caurant et al., 1999; Sakai et al., 2000; Maffucci et al., 2005; Storelli et al., 2005). Our findings revealed the same pattern showed by previous studies: higher mean Cu concentrations in the liver (32.737±28.866 µg g^{-1}) than in the muscles (0.316±0.354 µg g^{-1}) or kidneys (1.699±0.613 µg g^{-1}) (Sakai et al., 2000; Anan et al., 2001; Carneiro da Silva et al., 2014; Sinaei et al., 2021). In our study, high Cu concentration may be related to the consumption of algae (the main food item of *C. mydas*), which accumulate metals at high levels and may be associated to its central role in regulating and detoxing non-essential metals, such as Hg and Cd (Roesijadi, 1996; Gardner et al., 2006).

Our findings revealed that Cd concentrations were higher in the kidneys than in the liver or muscles, corroborating studies in the literature (Gordon et al., 1998; Sakai et al., 2000; Frías-Espericueta et al., 2006; Storelli et al., 2008; Carneiro da Silva et al., 2014; Yipel et al., 2017; Fraga et al., 2018; Bruno et al. 2021; See table 3). Cadmium is one of the most toxic metals in the environment (Dunnick and Fowler, 1988; Barbieri, 2009; Camacho et al., 2013), affecting DNA, RNA, and hormonal attunement (Gerhard et al., 1998; Piasek and Laskey, 1999; Smida et al., 2004; Rana, 2014). Exposure to chronic Cd concentrations can reduce fertility, decreasing the overall reproductive success of marine animals (Singhal et al., 1985). Cadmium does not seem to play a role in biological systems of sea turtles, presenting limited degradation and excretion, defective accumulation, and long-term storage, especially in the kidney, which showed clear bioaccumulation (Storelli and Marcotrigiano, 2003; Frías-Espericueta et al., 2006; Andreani et al., 2008; Storelli et al., 2008; García-Fernández et al., 2009; D'Ilio et al., 2011). Tropism could be explained by the binding between Cd and metallothionein (MT) resulting in the metallothionein complex (Cd-MT) in the liver and intestine, preferably transferred to the kidney, where Cd accumulates (Andreani et al., 2008; Sonne et al., 2009). This association in a metallothionein complex could be a detoxication strategy to mitigate the toxic effects of non-essential elements, such as Cd, Hg, Ag and Pb (Roesijadi, 1996; Das et al., 2000; Anan et al., 2002).

Diet composition and biomagnification in the trophic web could be an important Cd source for sea turtles and previous studies show that cephalopods are important Cd vectors to top marine predators (Bustamante et al., 1998; Turoczy et al., 2001; Maffucci et al., 2005; Nuñez-Nogueira and Rainbow, 2005; Karouna-Renier et al., 2007; Reed et al., 2010; Maulvault et al., 2011). Given that cephalopods can be a complementary food resource during the juvenile life-stage of *C. mydas* (Boyle and Limpus, 2008; Parker et al., 2011), the high mean Cd levels found in our study, especially in the kidney (16.061±21.581 µg g^{-1}), could be related to the ingestion of this type of food by the green turtles. Therefore, our findings suggest that the individuals found stranded in the Potiguar Basin were chronically exposed to Cd.

The mean Ag concentrations in the liver of *C. mydas* (2.127 µg g^{-1}) were higher than in the muscles and kidneys (0.009 and 0.087 µg g^{-1}, respectively), corroborating previous studies conducted in Brazil (0.63 µg g^{-1} in the liver and 0.06 µg g^{-1} in the kidney; Carneiro da Silva et al., 2014). Differently from Cu and Se, Ag is a non-essential element and enters the aquatic environment because of domestic and industrial sewage (Carneiro da Silva et al., 2014). In our study, Ag levels ranged between 0.023–9.788 µg g^{-1} with the maximum value higher than maximum concentration found by Anan et al. (2002) in *C. mydas* from Japan (1.2 µg g^{-1}; See Table 3). Although the effects of Ag on sea turtles are still unknown, the mechanism of Ag toxicity in sea invertebrates and fish induces ionic and osmotic disturbance (Wood et al., 2004; Bianchini et al., 2005; Pedroso et al., 2007, Carneiro da Silva et al., 2014).

The mean Se concentrations in the liver samples (2.440 µg g^{-1}) were higher than previously recorded in Australia (1.18 µg g^{-1}; Gordon et al., 1998), Japan (1.041 µg g^{-1}; Anan et al., 2001) and Turkey (0.46 µg g^{-1}; Yipel et al., 2017). In addition, our findings revealed that the liver had higher mean Se concentrations than the muscles and kidneys, possibly because the concentration in the liver is the formation of the mercury-selenium (Hg-Se) complex in liver tissues, described as a detoxification strategy in sea turtles and marine mammals (Storelli et al., 1998; Jerez et al., 2010; Frouin et al., 2012; Lailson-Brito et al., 2012). Se presence in the samples analyzed can also be related to the capacity of this trace element to reduce Cd toxicity when these toxic metals are stored and relocated, reducing the potential adverse effects of Se (Siscar et al., 2014). Storelli et al. (2005) reported that Se accumulation in some marine mammals is always high and that establishing whether Se concentrations are toxic or at a mere background level in aquatic reptiles is hard.

CONCLUSION

Our results show that green turtles bioaccumulated the inorganic elements monitored, although it is not possible to affirm that the turtles were exposed to the elements in the Potiguar Basin. Our findings bring additional baseline on Hg, Cu, Cd, Ag and Se in green turtles and reinforce the role of these animals as sentinels of the marine ecosystem. This supports the need to continue monitoring inorganic elements and to further the studies on environmental quality of the Potiguar Basin, a feeding site for green turtles and an important economic regional site in Brazil, since these chemical elements could also affect human health.

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Curation, Writing - Review & Editing, Supervision; Aline da Costa Bomfim Ventura: Methodology, Investigation, Writing - Review & Editing; Ana Bernadete Lima Fragoso: Methodology, Project administration; Elitieri Batista Santos-Neto: Methodology, Formal analysis; Flávio José de Lima Silva: Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition; José Lailson-Brito: Methodology, Formal analysis; Julio Alejandro Navoni: Formal analysis, Data Curation; Simone Almeida Gavilan: Resources, Writing - Review & Editing; Viviane Souza do Amaral: Term, Conceptualization, Writing - Review & Editing, Supervision.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests or personal relationships that could influence the work reported in this study.

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CONSIDERAÇÕES FINAIS

Os resultados apresentados neste trabalho trazem novas perspectivas para a conservação das tartarugas marinhas, em especial aquelas da espécie *Chelonia mydas,* que utilizam o litoral da Bacia Potiguar para o seu desenvolvimento e alimentação, e que vem sofrendo com pressões antrópicas nos últimos anos, resultando em elevadas taxas de encalhes anuais. Nossos resultados fornecem as primeiras informações genéticas sobre as tartarugas-verdes (*Chelonia mydas*) da Bacia Potiguar, Nordeste do Brasil, demonstrando uma composição haplotípica variada, com predomínio dos dois haplótipos mais registrados no litoral do Atlântico Sul Ocidental, CM-A8 e CM-A5. Apesar do predomínio da influência haplotípica do CM-A8 no estoque misto de tartarugas-verdes da Bacia Potiguar, os elevados valores de CM-A5 encontrados, em comparação com outros estudos na costa brasileira, podem estar relacionados a posição geográfica da região, na "esquina" do continente americano, recebendo influências mais proporcionais dos dois hemisférios (Norte e Sul). Nossos achados corroboram com outros estudos na costa brasileira, aumentando o entendimento da composição dos estoques populacionais de *C.mydas*, o que contribui para elucidar ainda mais os impactos negativos nas áreas de alimentação e na organização de estratégias de recomposição de áreas de nidificação, com consequente melhoria no status de ameaça da espécie.

Com relação as ameaças antrópicas, através da adaptação de uma ferramenta utilizada para análise de dieta, o Índice Alimentar (IAi), foi possível acessar o impacto dos detritos antropogênicos, principalmente do plástico flexível transparente, nos diferentes estágios de vida das tartarugas-verdes da Bacia Potiguar. Foi revelado um maior impacto dos plásticos em tartarugas juvenis (JUV-I), devido aos seus hábitos alimentares costeiros e na superfície d'água, que coincidem com os locais de maiores concentrações de fragmentos de plástico, associados a algas flutuantes. A região de estudo vem se caracterizando como um importante sítio de alimentação e desenvolvimento para *C. mydas*, porém as pressões antrópicas negativas, como a contaminação crescente dos ambientes marinhos por detritos antropogênicos, precisam cada vez mais mensuradas, para que medidas eficazes de proteção possam ser tomadas. Acredita-se que o método de análise testado e adaptado nesse trabalho possa ser extrapolado para o estudo dos impactos de detritos antropogênicos sobre tartarugas marinhas e outros animais.

Através da avaliação da contaminação por elementos químicos (THg, Cu, Cd, Ag e Se) em tecidos (fígado, músculo e rins) de tartarugas-verdes da região, verificou-se níveis de bioacumulação até então desconhecidos para esses animais na Bacia Potiguar, embora não seja possível afirmar que as tartarugas foram expostas aos elementos na região. Nossos achados reforçam o papel das tartarugas marinhas como sentinelas do ecossistema marinho e destaca a necessidade de uma maior investigação da qualidade ambiental dessa região, uma vez que esses os elementos químicos podem afetar a saúde humana, e que a área estudada se configura como um importante polo econômico regional no Brasil.

Acredita-se que, com o conhecimento da estrutura populacional das populações tartarugas-verdes que ocorrem na região Bacia Potiguar, associado a avaliação adequada de impactos antrópicos, como a ingestão de detritos antropogênicos e a contaminação desses espécimes por elementos químicos, será possível elaborar políticas públicas cada vez mais específicas e eficazes para a conservação, não só das tartarugas marinhas, como também do ambiente marinho que elas ocupam.

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ANEXO I

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 13694-9	**Data da Emissão: 15/07/2019 18:00:48**	**Data da Revalidação*: 01/06/2020**

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: FLÁVIO JOSÉ DE LIMA SILVA	CPF: 485.543.674-72
Título do Projeto: Diversidade, áreas de ocorrência e conservação de mamíferos aquáticos e quelônios marinhos no Rio Grande do Norte (REMANE)	
Nome da Instituição: FUNDACAO UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE - FUERN	CNPJ: 08.258.295/0001-02

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Atendimento aos encalhes de animais marihos	04/2018	04/2023
2	Monitoramento quinzenal das áreas litorâneas	04/2018	04/2023
3	Coleta e armazenamento de material biológico	04/2018	04/2023
4	Análise de material e identificação de espécies	04/2018	04/2023

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Kleberson de Oliveira Porpino	Vice-Coordenador	903.826.094-68	Brasileira
2	ANA BERNADETE LIMA FRAGOSO	Coordenadora de campo e laboratório	013.059.517-94	Brasileira
3	DIOGO MICKAEL ROLIM E SILVA	Coleta de dados e atendimento a encalhes	047.803.734-17	Brasileira
4	ROSEMARY DAYSE SALUSTIANO DE BARROS	Coleta de dados e atendimento a encalhes	025.085.774-05	Brasileira
5	DAMIÃO NASCIMENTO OLIVEIRA	Coleta de dados e atendimento a encalhes	029.635.094-05	Brasileira
6	ALINE DA COSTA BOMFIM	Coleta de dados e atendimento a encalhes	086.298.024-05	Brasileira
7	DANIEL SOLON DIAS DE FARIAS	Coleta de dados e atendimento a encalhes	078.362.394-11	Brasileira
8	EDSON SOARES DA SILVA JÚNIOR	Coordenadora de análises e coleção	080.191.234-23	Brasileira
9	HELOISA CRISTINA DE MORAIS E SÁ LEITÃO	Coleta de dados e atendimento a encalhes	011.473.294-94	Brasileira
10	IARA CECILIA DA COSTA MORAIS	Coleta de dados e atendimento a encalhes	061.329.044-50	Brasileira
11	RADAN ELVIS MATIAS DE OLIVEIRA	Coleta de dados e atendimento a encalhes	076.974.554-71	Brasileira
12	HÉLDER HENRIQUE ALVES DE MEDEIROS	Coleta de dados, atendimento a encalhes, marcação animais	073.865.784-07	Brasileira
13	SIMONE ALMEIDA GAVILAN LEANDRO DA COSTA	Vice-Coordenadora	671.964.034-87	Brasileira
14	Augusto Carlos da Bôaviagem Freire	Coleta de dados, atendimento a encalhes, marcação animais	054.346.194-71	Brasileira
15	JULIANA MAIA DE LORENA PIRES	Coleta de dados e atendimento a encalhes	368.867.028-07	Brasileira
16	Mariana Almeida Lima	Coleta de dados e atendimento a encalhes	117.400.564-50	Brasileira
17	Stella Almeida Lima	Coleta de dados e atendimento a encalhes	117.400.534-35	Brasileira

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Dados do titular

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Nome da Instituição: FUNDACAO UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE - FUERN	CNPJ: 08.258.295/0001-02

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Este documento NÃO exime o pesquisador titular da necessidade de atender ao disposto na Instrução Normativa Ibama nº 27/2002, que regulamenta o Sistema Nacional de Anilhamento de Aves Silvestres.
3	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
4	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio n° 03/2014 ou na Instrução Normativa ICMBio n° 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
6	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
7	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
8	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.
9	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.

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Autorização para atividades com finalidade científica

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Outras ressalvas

1	Está autorizado o transporte de aves resgatadas das praias até o local onde os animais participantes dessa pesquisa serão reabilitados. Esta autorização refere-se apenas às atividades de pesquisa aqui previstas, não autorizando manejo de fauna silvestre não ameaçada, atribuição reservada ao Ibama ou ao órgão estadual de meio ambiente.	CEMAVE Cabedelo-PB

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Área de Atuação da REMANE (Nordeste do Brasil, RN, CE)	RN	Marinho	Não	Fora de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas in situ	Actinopterygii	-
2	Captura de animais silvestres in situ	Aves	-
3	Manutenção temporária (até 24 meses) de vertebrados silvestres em cativeiro	Aves	-
4	Marcação de animais silvestres in situ	Aves	-
5	Coleta/transporte de amostras biológicas in situ	Aves	-
6	Coleta/transporte de amostras biológicas in situ	Elasmobranchii	-
7	Coleta/transporte de amostras biológicas in situ	Cetacea	-
8	Coleta/transporte de amostras biológicas in situ	Sirenia	-
9	Coleta/transporte de amostras biológicas in situ	Mustelidae	-
10	Marcação de animais silvestres in situ	Caretta caretta	-
11	Coleta/transporte de amostras biológicas in situ	Caretta caretta	-
12	Marcação de animais silvestres in situ	Chelonia mydas	-
13	Coleta/transporte de amostras biológicas in situ	Chelonia mydas	-
14	Marcação de animais silvestres in situ	Dermochelys coriacea	-
15	Coleta/transporte de amostras biológicas in situ	Dermochelys coriacea	-
16	Marcação de animais silvestres in situ	Eretmochelys imbricata	-
17	Coleta/transporte de amostras biológicas in situ	Eretmochelys imbricata	-
18	Marcação de animais silvestres in situ	Lepidochelys olivacea	-
19	Coleta/transporte de amostras biológicas in situ	Lepidochelys olivacea	-
20	Marcação de animais silvestres in situ	Cheloniidae	-

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Atividades X Táxons

#	**Atividade**	**Táxon**	**Qtde.**
21	Coleta/transporte de amostras biológicas in situ	Cheloniidae	-
22	Coleta/transporte de amostras biológicas in situ	Dermochelyidae	-
23	Marcação de animais silvestres in situ	Dermochelyidae	-

Materiais e Métodos

#	**Tipo de Método (Grupo taxonômico)**	**Materiais**
1	Amostras biológicas (Aves)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fezes, Fragmento de tecido/órgão, Ovos, Penas, Regurgitação/conteúdo estomacal, Sangue
2	Amostras biológicas (Carnívoros)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fezes, Fragmento de tecido/órgão, Pêlo, Regurgitação/conteúdo estomacal, Sangue, Urina
3	Amostras biológicas (Cetáceos)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Sangue, Secreção
4	Amostras biológicas (Peixes)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Escama, Fezes, Ovos, Sangue, Sêmen, Sêmen, Fragmento de tecido/órgão
5	Amostras biológicas (Sirênios)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fezes, Fragmento de tecido/órgão, Sangue, Secreção
6	Amostras biológicas (Tartarugas marinhas)	Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Fragmento de tecido/órgão, Ovos, Regurgitação/conteúdo estomacal, Sangue, Ovos
7	Método de captura/coleta (Aves)	Outros métodos de captura/coleta(Apenas individuos debilitados nas praias)
8	Método de captura/coleta (Carnívoros)	Outros métodos de captura/coleta(Apenas indivÃ-duos encalhados ou debilitados nas praias)
9	Método de captura/coleta (Cetáceos)	Outros métodos de captura/coleta(Apenas indivÃ-duos encalhados ou debilitados nas praias)
10	Método de captura/coleta (Peixes)	Outros métodos de captura/coleta(Apenas individuos debilitados nas praias)

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Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
11	Método de captura/coleta (Sirênios)	Outros petrechos(Apenas indivÃ-duos encalhados ou debilitados nas praias)
12	Método de captura/coleta (Tartarugas marinhas)	Outros métodos de captura/coleta(Apenas indivíduos encalhados), Outros métodos de captura/coleta(Apenas indivÃ-duos encalhados ou debilitados nas praias), Captura manual
13	Método de marcação (Aves)	Anilha de Alumínio (padrão CEMAVE), Anilha metálica (padrão CEMAVE)
14	Método de marcação (Tartarugas marinhas)	Anilha

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1		Coleção

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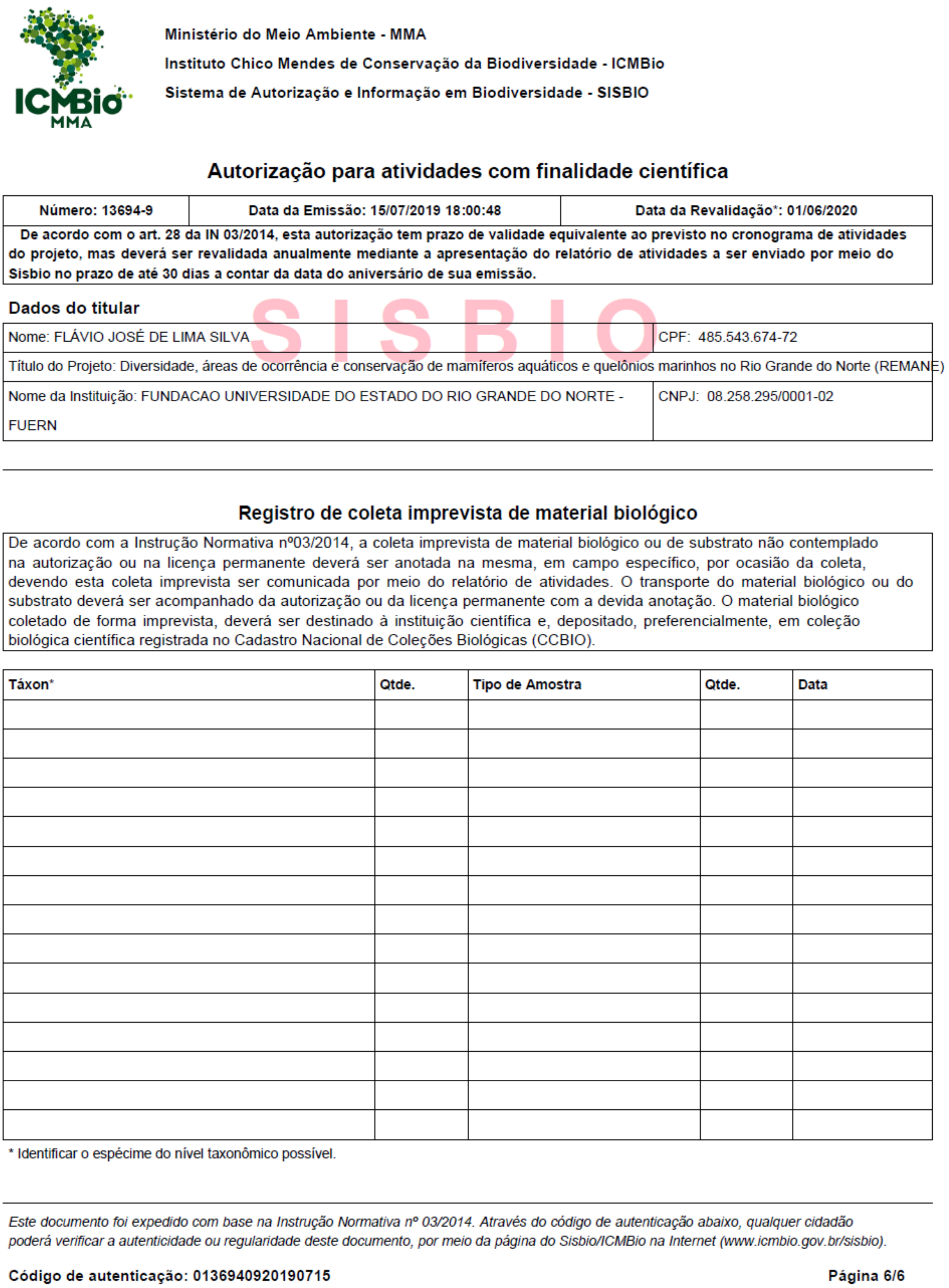

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De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

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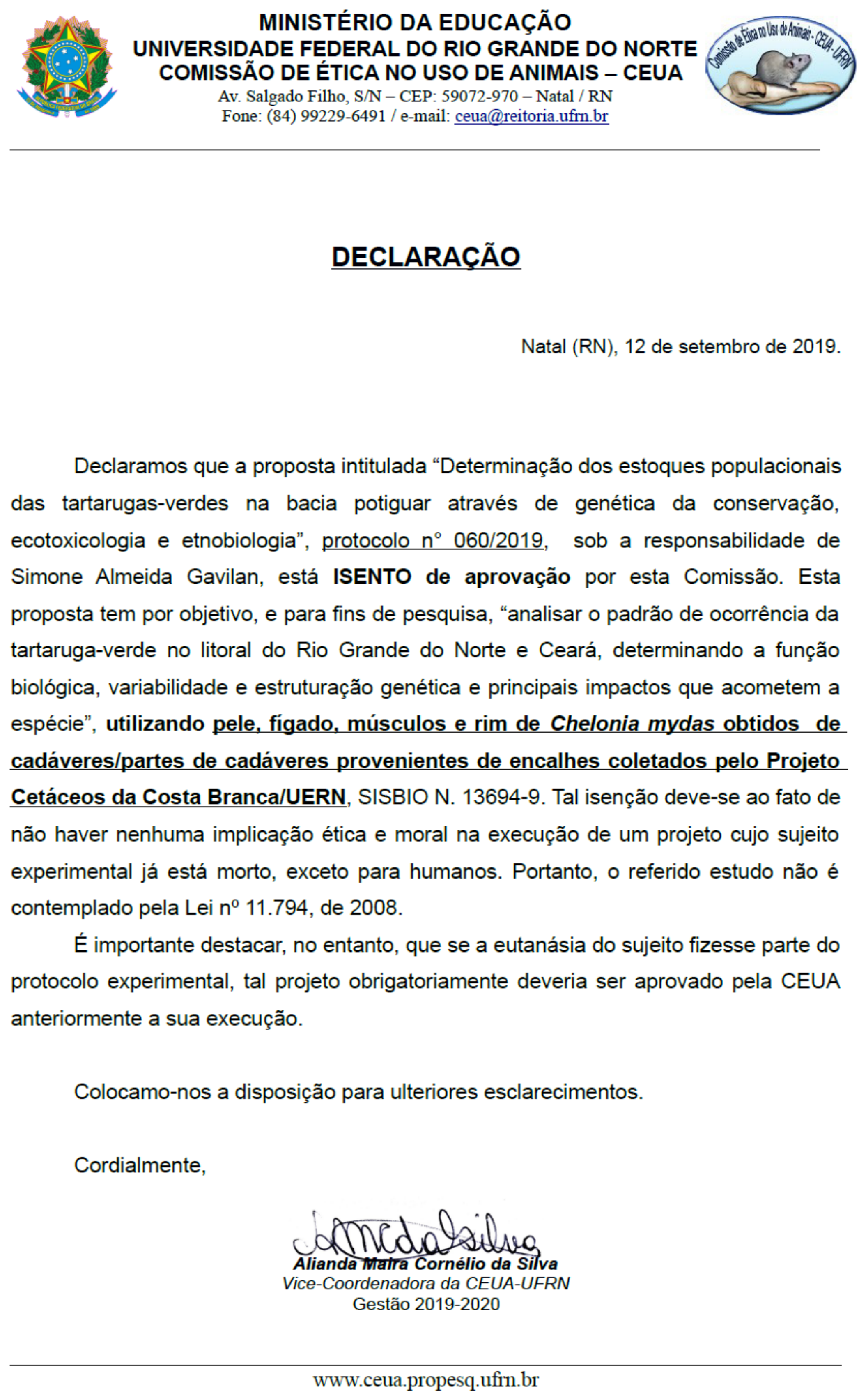
ANEXO II



MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA
Av. Salgado Filho, S/N – CEP: 59072-970 – Natal / RN
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DECLARAÇÃO

Natal (RN), 12 de setembro de 2019.

Declaramos que a proposta intitulada “Determinação dos estoques populacionais das tartarugas-verdes na bacia potiguar através de genética da conservação, ecotoxicologia e etnobiologia”, protocolo n° 060/2019, sob a responsabilidade de Simone Almeida Gavilan, está **ISENTO de aprovação** por esta Comissão. Esta proposta tem por objetivo, e para fins de pesquisa, “analisar o padrão de ocorrência da tartaruga-verde no litoral do Rio Grande do Norte e Ceará, determinando a função biológica, variabilidade e estruturação genética e principais impactos que acometem a espécie”, **utilizando pele, fígado, músculos e rim de *Chelonia mydas* obtidos de cadáveres/partes de cadáveres provenientes de encalhes coletados pelo Projeto Cetáceos da Costa Branca/UERN**, SISBIO N. 13694-9. Tal isenção deve-se ao fato de não haver nenhuma implicação ética e moral na execução de um projeto cujo sujeito experimental já está morto, exceto para humanos. Portanto, o referido estudo não é contemplado pela Lei nº 11.794, de 2008.

É importante destacar, no entanto, que se a eutanásia do sujeito fizesse parte do protocolo experimental, tal projeto obrigatoriamente deveria ser aprovado pela CEUA anteriormente a sua execução.

Colocamo-nos a disposição para ulteriores esclarecimentos.

Cordialmente,

Alianda Maira Cornélio da Silva
Vice-Coordenadora da CEUA-UFRN
Gestão 2019-2020

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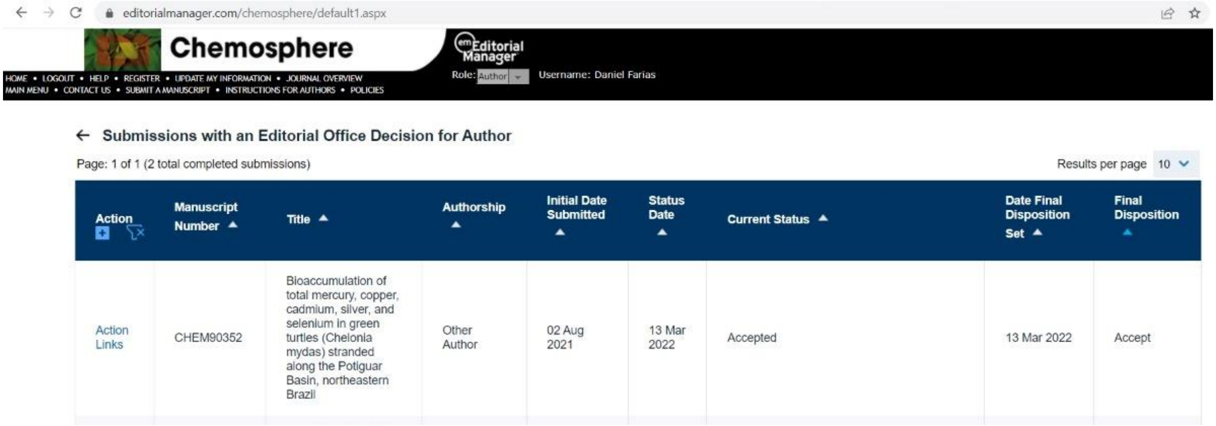
ANEXO III



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Action	Manuscript Number	Title	Authorship	Initial Date Submitted	Status Date	Current Status	Date Final Disposition Set	Final Disposition
Action Links	CHEM90352	Bioaccumulation of total mercury, copper, cadmium, silver, and selenium in green turtles (Chelonia mydas) stranded along the Potiguar Basin, northeastern Brazil	Other Author	02 Aug 2021	13 Mar 2022	Accepted	13 Mar 2022	Accept

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Bioaccumulation of total mercury, copper, cadmium, silver, and selenium in green turtles (*Chelonia mydas*) stranded along the Potiguar Basin, northeastern Brazil

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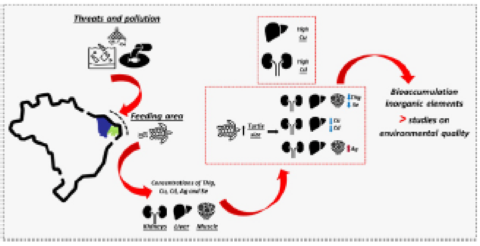
HIGHLIGHTS

- Negative correlation between size and concentrations: THg and Se (liver/muscles/kidneys) and Cu and Cd (muscles/kidneys).
- Ag concentrations in the liver, muscle, and kidney samples showed positive correlation with the curved carapace length.
- Green turtles had high Cd levels in the kidneys and high THg, Cu, Ag, and Se levels in the liver.

GRAPHICAL ABSTRACT



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ABSTRACT

Sea turtles face several threats and pollution has become a major concern for their conservation worldwide. We analyzed samples of the liver, muscles, and kidneys of 38 *Chelonia mydas* stranded along the Potiguar Basin, northeastern Brazil, between 2015 and 2018 to determine the total Hg concentration (THg), as well as the concentrations of Cu, Cd, Ag, and Se. The relation between turtle size and element concentrations revealed a negative correlation for THg and Se (liver, muscles, and kidneys), Cu and Cd (liver and kidneys) and a positive

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Trace elements
Liver
Kidney
Muscle

correlation for Ag in the three organs analyzed. Concentrations of THg, Cu, Ag, and Se were high in the liver, highlighting the Cu concentration (median = 25.1150 μg g^{-1} w.w.), while the kidneys had the highest Cd levels (median = 12.2200 μg g^{-1} w.w.). There was significant difference between element concentrations and the three organs analyzed, except for Ag and Se concentrations in the muscle and kidney samples. Our study showed that green turtles found in Potiguar Basin, northeastern Brasil, have bioaccumulated inorganic elements which indicate the need of further investigations on the environmental quality of the region.

1. Introduction

Marine pollution caused by chemicals threatens the marine ecosystem and can generate toxic effects to wildlife (Ley-Quiñónez et al., 2011; Yarsan and Yipel, 2013; Bucchia et al., 2015; Yipel et al., 2017). Trace elements are natural components of rocks and soil; however, they are some of the most common pollutants in the marine ecosystem due to accelerated urban growth and increase in industrial activities (Lam et al., 2004). The long-range transport (rivers and air) of trace elements and their non-biodegradable and biomagnification characteristics increases their toxicity and ecotoxicological potential (D'ilio et al., 2011; Ley-Quiñónez et al., 2011; Yarsan and Yipel, 2013; Bucchia et al., 2015; Mattei et al., 2015; Yipel et al., 2016). Therefore, these pollutants may bioaccumulate and reach toxic concentrations in the tissues of long-lived animals that feed at higher trophic levels, such as species of sea turtles (Järup et al., 1998; Ankley et al., 2006; Agarwal, 2009; Dieter et al., 2014; Mehinto et al., 2014; Cortés-Gómez et al., 2017).

Sea turtles are potential bioindicators of a good environmental status as well as of marine pollution levels (Gordon et al., 1998; Sakai et al., 2000; Gardner et al., 2006; Camacho et al., 2014; Yipel et al., 2017). Their occurrence on coastal regions and proximity to landscapes altered by anthropic actions make sea turtles potentially vulnerable to pollutants (Godley et al., 1999; Maffucci et al., 2005; García-Fernández et al., 2009; Hamann et al., 2010). In addition, sea turtles are long-lived vertebrates that accumulate trace elements through ingestion or directly from the aquatic environment (e.g., inhalation and/or absorption) (Andreani et al., 2008; D'ilio et al., 2011; Mattei et al., 2015).

Therefore, the effects of trace elements and other chemical pollutants on sea turtles (at individual, population, and ecosystem levels) depend on the levels of trace elements found in organs (Storelli et al., 2005; D'ilio et al., 2011; Andreani et al., 2008; Bhat, 2013; Camacho et al., 2014; Mattei et al., 2015). Some chemical elements are involved in biological processes and structures and are therefore essential for living beings, such as copper (Cu) which is indispensable for the proper functioning of some metabolic functions (Jakimska, 2011). Studies have reported the capacity of selenium (Se) to reduce toxicity of some heavy metals, such as cadmium (Cd) in deep-sea fish, mercury (Hg) in ringed seals *Phoca hispida* forming the mercuric selenide compound (HgSe), and arsenic (As) in humans (Wagemann et al., 2000; Siscar et al., 2014; Sun et al., 2014). However, little is known about the levels considered thresholds for trace elements in organs of sea turtles and few quantitative data are available linking trace elements to mortality of sea turtles (Anan et al., 2002; Hamann et al., 2010).

On the other hand, several studies have reported on the potential effects of heavy metals in vertebrates (Godley et al., 1999; Caurant et al., 1999; Anan et al., 2002; Storelli and Marcotrigiano, 2003; Maffucci et al., 2005; Moffet et al., 2007; García-Fernández et al., 2009). Mercury is known to be potentially toxic to the nervous and immune systems (Lam et al., 2004, Day et al., 2007); cadmium (Cd) causes negative effects on the metabolic process of essential elements and on the endocrine system and sea turtles tend to concentrate cadmium (Hopkins et al., 1999; Noel et al., 2004; Storelli et al., 2005; Ikonomopoulou et al., 2009; Simoniello et al., 2011); and silver (Ag) in turn can cause ionic and osmotic disturbance in invertebrates (Bianchini et al., 2005; Pedroso et al., 2007).

The Potiguar Basin in northeastern Brazil is an important habitat for sea turtles, mainly green turtles *Chelonia mydas* (Farias et al., 2019). This region presents important economic activities based on exploratory processes of different natural resources (e.g., rocks, soils, salt), agricultural systems and a range of non-renewable (oil and natural gas) and renewable energy elements (biomass, electric energy, wind energy, thermoelectric, and solar bases) (Alves and Amaro, 2019). These activities pose as a potential threat to the quality of estuaries and coastal areas, as well as to endangering marine species (Attademo, 2007; Farias et al., 2019).

Few studies have investigated contamination of sea turtles by trace elements in Brazil (Bezerra et al., 2013; Carneiro da Silva et al., 2014; Prioste et al., 2015; Bruno et al., 2021) and no study has investigated this condition in the Potiguar Basin, to the best of our knowledge. Coastal waters and sediments are impacted by industrial effluents, agricultural residues, and domestic sewage through contamination by metals, such as lead (Pb), zinc (Zn) Ag, Cd, and Cu (Carneiro da Silva et al., 2014). Biomonitoring programs have focused on the bioaccumulation of Hg, Cd, and Pb in the muscle, liver, and kidney (Lam et al., 2004). In Brazil, the Beach Monitoring Project (Projeto de Monitoramento de Praias - PMP) at the Potiguar Basin monitors five elements (total Hg, Cu, Cd, Ag, and Se) in seabirds, marine mammals, and sea turtles.

These elements were previously defined in technical opinions and official guidelines of the Brazilian Institute of the Environment and Renewable Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA), following the guidelines of the National Council for the Environment (Conselho Nacional do Meio Ambiente - CONAMA) to assess the impacts of oil and gas exploration, production, and transportation activities in Brazil. Therefore, this study aimed to describe the occurrence and concentrations of these five elements in the liver, muscle, and kidney samples of stranded green turtles along the Potiguar Basin in order to provide additional baseline of exposure and/or bioaccumulation in sea turtle organs.

2. Materials and methods

2.1. Study site, green turtles, and sampling

The present study used data collected between January 1, 2015, and December 31, 2018, from records of PMP in the Potiguar Basin. Alive green turtles found during daily monitoring conducted by field-trained personnel of Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN) were rescued and admitted to the rehabilitation center of PCCB-UERN in Areia Branca/Rio Grande do Norte State. The monitored area stretches for roughly 333 km from Caiçara do Norte (5° 4′1.15″ S; 36° 4′36.41″ W) in Rio Grande do Norte State (RN) to Aquiraz (03°49′20.9″ S and 38°24′07.8″ W) in Ceará State (CE) (Fig. 1). The PMP in the Potiguar Basin has been conducted by the PCCB-UERN since 2010 as part of an environmental constraint compliance enforced by the IBAMA due to oil and gas exploration by PETROBRAS (Petróleo Brasileiro S.A.; Agreement number 2500.005657510.2).

Curved carapace length (CCL) was measured in each examined green turtles from the nuchal to notch between supra-caudal scales (Bolten, 1999). Individuals ≥90 cm were classified as adults considering the smallest size recorded for nesting females in the largest and closest nesting areas in Brazil (Almeida et al., 2011). The liver, muscle, and kidney samples were collected from 38 green turtles (27–127 cm CCL): 31 individuals that died at the rehabilitation center of PCCB-UERN and seven individuals were found dead during the beach monitoring. The samples were generally collected immediately post-mortem; however, in some cases, the carcass was frozen and kept at 20 °C until necropsy (<12 h). The samples were collected, placed in glass tubes, kept on ice, and stored at −20 °C until the laboratory analyses.

2.2. *Laboratory analyses*

Total mercury (THg) was determined according to Bisi et al. (2012). A mixture of sulfuric and nitric acid was added to the aliquots of approximately 0.3 g of sample. The aliquots were then heated in water bath at 60 °C until total solubilization. THg concentrations were determined by Cold Vapor/Atomic Absorption (FIMS-400, PerkinElmer) with sodium borohydride as a reducing agent. Accuracy and precision of the analytical methods were verified using the standard certified materials (DORM-3 and DOLT-5, National Research Council-NRC, Canada). The results agreed with the reference values and the internal reference materials produced by the Laboratório de Mamíferos Aquáticos e Bioindicadores "Profa. Izabel Gurgel" at Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, Brazil. The quality control was also performed through the analysis of procedural blanks (0.04 ± 0.01 μg. L^{-1}) and sample replicates (coefficient of variation <20%).

The other elements (Cu, Cd, Ag, and Se) were determined according to the digestion procedures of Dorneles et al. (2007). Two aliquots of approximately 0.3 g of sample were added 2 mL of 65% nitric acid (HNO_3) overnight and the solutions were heated at 60 °C in water bath for 2 h. After cooling, the solutions were placed in 15 mL tubes and ultrapure water was added to complete a volume of 10 mL. Concentrations of the elements were determined by electrothermal atomic absorption spectrometry (ZEEnit 60s, Analytic Jena, equipped with Zeeman background correction). Palladium nitrate – Pd $(NO_3)_2$ and magnesium nitrate – Mg $(NO_3)_2$ were used as a matrix modifier. Accuracy and precision of the analytical methods were verified using the standard certified materials (TORT-2, DOLT-4, and DOLT-5, National Research Council-NRC, Canada), with elements recovery (Mean ± SD %) at 104 ± 3.61 (THg), 99 ± 7.02 (Cu), 96 ± 7.69 (Cd), 100 ± 6.22 (Ag), and 99 ± 4.95 (Se). Quality control was also performed through the analysis of procedural blanks (<5.00 μg. L-1) and sample replicates (coefficient of variation <25%). The limits of detection (LoD) were Hg = 0.00002, Cu = 0.00091, Cd = 0.00039, Ag = 0.00025, Se = 0.00402 μg mL^{-1}, and concentrations are expressed in μg g^{-1} wet weight (w.w.).

2.3. *Statistical analysis*

The Shapiro-Wilk test was performed to verify the distribution of samples and concentrations of the elements were described as median, first quartile, third quartile, minimum and maximum values (range). For comparison, the results were also described as the mean and standard deviation−SD (Mean ± SD) and the values presented as dry weight in the literature were converted to wet weight, according to Gordon et al. (1998), whereas w.w.:d.w. ratios for the liver and kidney were 4.9 and 6.8, respectively.

Data were analyzed according to correlation between CCL and THg, Cu, Cd, Ag, and Se concentrations using the Spearman's rank correlation coefficient (r), and comparison of THg, Cu, Cd, Ag, and Se concentrations among liver, muscles, and kidneys were performed by *Friedman* ANOVA and post-hoc *Nemenyi* test (only individuals with the presence of the elements in the three organs met the criteria for the tests: n = 16). The analyses were performed using IBM SPSS Statistics (version 20) and STATISTICA 7.0 for Windows (StatSoft, Inc. 1984e2004, USA) and the results were considered significant when p-value < 0.05.

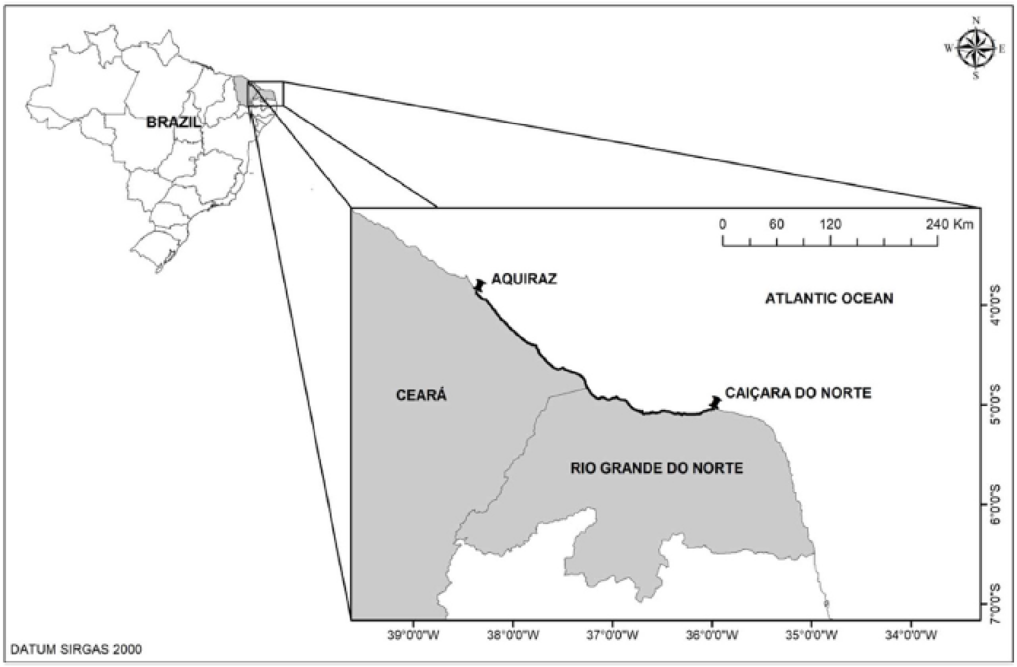

Fig. 1. Area monitored by Beach Monitoring Project at the Potiguar Basin: between Aquiraz/Ceará State and Caiçara do Norte/Rio Grande do Norte State. Source: Projeto Cetáceos da Costa Branca - Universidade do Estado do Rio Grande do Norte (PCCB-UERN).

3. Results

3.1. Concentrations of elements in the liver, kidneys, and muscles

The relationship between concentrations of the elements and CCL showed a weak ($r = 0.4$–0.7) to strong ($r > 0.7$) correlation. THg and Se (liver, muscle, and kidney), Cu and Cd (liver and kidney) displayed a negative correlation, showing that concentrations were inversely correlated with the turtle size. THg (kidney; $r = -0.744$), and Se (liver and kidney; $r = -0.728$ and -0.722, respectively) showed a strong negative correlation. On the other hand, correlation between Ag levels and turtle size was positive in the liver, muscles, and kidneys ($r = 0.727$, 0.482 and 0.562, respectively). Cu and CCL (liver), and Cd and CCL (liver and muscle) showed no correlation. Table 1 presents the Spearman coefficient and p-values between CCL and the quantified elements in each organ analyzed.

Copper in the liver presented the highest median and mean values (25.1150 and 32.737 ± 28.866 μg g^{-1} w.w., respectively; $n = 32$) followed by Cd in kidney (12.2200 and 16.061 ± 21.581 μg g^{-1} w.w., respectively; $n = 26$), and the lowest median and mean values was quantified for Ag in the muscles (0.0002 and 0.009 ± 0.026 μg g^{-1} w.w., respectively; $n = 35$). Our results revealed levels below the LoD for all elements in the organs: (1) THg in the liver, muscles, and kidneys (1/32; 3/35; and 2/26, respectively); (2) Cu in the muscles (2/35); (3) Cd in 25.71% of muscles (9/35); (4) Ag in 74.29% of muscle (26/35) and 42.31% of the kidney (11/26) samples; and (5) Se in the liver, muscles, and kidneys (1/17; 3/17; and 3/16, respectively). Table 2 displays the median, the first, and the third quartile values of THg, Cu, Cd, Ag and Se in the organs analyzed. Table 3 summarizes the concentrations of elements compared to previous studies.

Comparison between concentrations of the elements and the organs analyzed revealed significant differences (*Friedman* ANOVA, $p < 0.05$), except for Ag and Se between the muscles and kidneys. The post-hoc *Nemenyi* test showed that THg concentrations in the liver were higher than in the kidneys ($p = 0.0020$) and muscles ($p = 5.9 \times 10^{-10}$) and levels in the kidneys were higher than in the muscles ($p = 0.0084$). We observed the same pattern (liver > kidney > muscle) for Cu (liver > kidney: $p = 0.0005$; liver > muscle: $p = 4.6 \times 10^{-12}$; and kidney > muscle: $p = 0.0025$). Regarding Cd, the interorgan distribution was kidney > liver ($p = 0.0307$), kidney > muscle ($p = 5.6 \times 10^{-9}$), and liver > muscle ($p = 0.0015$). Our results also revealed significant differences between the liver and kidneys for Ag ($p = 0.0427$) and for Se ($p = 0.0023$) with concentrations in the liver > kidneys and muscles ($p = 0.0038$ and $p = 0.0023$, respectively) (Fig. 2).

Table 2
Median, first and third quartile values (μg g^{-1} w.w.) of elements (THg, Cu, Cd, Ag and Se) in samples of green turtles (*Chelonia mydas*).

Elements	Median (first quartile; third quartile)					
	Liver		Muscles		Kidneys	
THg	32	0.2349 (0.1253; 0.3998)	35	0.0220 (0.0030; 0.0487)	26	0.0731 (0.0219; 0.1654)
Cu	32	25.1150 (16.9000; 40.6800)	35	0.2486 (0.1320; 0.3280)	26	1.7542 (1.3166; 1.9925)
Cd	32	4.5048 (3.1084; 7.2510)	35	0.0393 (0.0003; 0.0617)	26	12.2200 (6.3942; 16.9950)
Ag	32	1.3543 (0.2699; 2.9240)	35	0.0002 (0.0002; 0.0100)	26	0.0189 (0.0002; 0.0464)
Se	17	1.8805 (0.7558; 3.6095)	17	0.6955 (0.2880; 1.1528)	16	0.9287 (0.2060; 1.2488)

4. Discussion

The diet of sea turtles varies and depends on their age and may be related to the availability of food items in different types of marine ecosystem, which have different ecological and physical characteristics. The diet is the main contamination source of trace elements (Dieter et al., 2014; Mehinto et al., 2014; Sfakianakis et al., 2015; Cortés-Gómez et al., 2017) and green turtles in their pelagic phase are omnivorous, presenting high metal concentrations (Seminoff et al., 2002; Arthur et al., 2008). Our results expressed this trend with a negative correlation (moderate to strong) between CCL and THg concentrations (in the three examined organs), Cu and Cd (in kidney), selenium (in liver and kidney). International environmental conservation policies have advanced recently; nevertheless, the marine environment is still affected by anthropogenic actions, such as contamination by chemical elements and Hg is one of the most studied and worrying toxic metals affecting health parameters of sea turtles even at relatively low levels (Day et al., 2005; Lamborg et al., 2014; Mitchell, 2016; Yipel et al., 2017). In our study, the negative correlation between THg concentration and CCL in all examined organs corroborates previous studies in Brazil and in Japan (Rodriguez et al., 2020; Bruno et al., 2021; Sakai et al., 2000), differing from studies on green turtles from Baja California Sur, Mexico, where no relationship was found between the straight carapace length and Hg concentrations in liver samples (Kampalath et al., 2006). Our findings showed that green turtles had higher THg levels in the liver and kidneys compared to studies conducted in Australia, Japan, and Turkey (Gordon et al., 1998; Sakai et al., 2000; Yipel et al., 2017; See Table 3).

Essential elements, such as Cu, play a crucial role in the metabolism and tissue growth; nevertheless, an excessive Cu amount in the body exerts adverse effects via reactive oxygen species (Andreani et al., 2008; Halliwell and Gutteridge, 1990). When its concentration is high in the environment, Cu tends to accumulate in the liver associated to metallothionein (Cu–MT) as a non-toxic form of the metal (Andreani et al., 2008; D'ilio et al., 2011; Yipel et al., 2017). Higher Cu levels in the liver seem to be a general fact in sea turtle species (Caurant et al., 1999; Sakai et al., 2000; Maffucci et al., 2005; Storelli et al., 2005). Our findings revealed the same pattern showed by previous studies: higher mean Cu concentrations in the liver (32.737 ± 28.866 μg g^{-1}) than in the muscles (0.316 ± 0.354 μg g^{-1}) or kidneys (1.699 ± 0.613 μg g^{-1}) (Sakai et al., 2000; Anan et al., 2001; Carneiro da Silva et al., 2014; Sinaei et al., 2021). In our study, high Cu concentration may be related to the consumption of algae (the main food item of *C. mydas*), which accumulate metals at high levels and may be associated to its central role in

Table 1
Spearman coefficient (r) and significance of coefficient (p) between the curved carapace length and quantified elements in each organ (liver, muscle and kidney) of the green sea turtles (*Chelonia mydas*) studied.

Elements	Liver				Muscle				Kidney			
	n	r	p	Correlation	n	r	p	Correlation	n	r	p	Correlation
THg	32	−0.427	0.015	Moderate	35	−0.681	<0.001	Moderate-to-strong	26	−0.744	<0.0001	Strong
Cu	32	−0.170	0.353	No correlation	35	0.433	0.009	Moderate	26	−0.432	0.027	Moderate
Cd	32	−0.114	0.535	No correlation	35	0.102	0.558	No correlation	26	−0.602	0.001	Moderate
Ag	32	0.727	<0.0001	Strong	35	0.482	0.003	Moderate	26	0.562	0.003	Moderate
Se	17	−0.728	<0.001	Strong	17	−0.400	0.111	Weak-to-Moderate	16	−0.722	0.002	Strong

Table 3

Concentrations of THg, Cu, Cd, Ag, and Se (μg.g^{-1} w.w.) in the liver, muscles, and kidneys of green turtles (*Chelonia mydas*) compared to previous studies.

Organs	Hg			Cu			Cd			Ag			Se			Study site	Reference
	n	Range	Mean ± SD	n	Range	Mean ± SD	n	Range	Mean ± SD	n	Range	Mean ± SD	n	Range	Mean ± SD		
Liver	32	<LoD–0.764	0.283 ± 0.202	32	3.836–147.100	32.737 ± 28.866	32	1.105–20.815	5.706 ± 4.349	32	0.023–9.788	2.127 ± 2.315	17	<LoD–7.902	2.440 ± 2.080	Potiguar Basin, northeastern Brazil	Present study
	23	0.00–0.052	0.021 (Mean)	–	–	–	38	2.5–56.9	12.5 (Mean)	–	–	–	23	0.07–2.68	1.18 (Mean)	Southeastern Queensland, Australia	Gordon et al. (1998)
	2	0.0767–0.301	0.159 ± 0.189	2	8.73–13.5	11.115 ± 3.373	2	3.9–12.1	8.0 ± 5.798	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	26	7.429–63.388	28.367 ± 17.551	–	–	–	–	–	–	25	0.408–2.020	1.041 ± 0.469	Yaeyama Islands, Japan	Anan et al. (2001)
	–	–	–	9	18.4–130	–	9	3.21–21.6	–	9	0.67–1.2	–	9	0.87–7.5	–	Yaeyama Islands, Japan	Anan et al. (2002)
	–	–	–	11	1.386–27.143	12.253 (Mean)	11	nd–20.816	0.673 (Mean)	–	–	–	–	–	–	Baja California peninsula, Mexico	Gardner et al. (2006)
	–	–	–	7	18.5–59.0	–	7	2.2–9.2	–	–	–	–	–	–	–	Italian coastal areas	Storelli et al. (2008)
	–	–	–	29	6.408–79.347	20.592 (Mean)	29	0.122–5.184	1.204 (Mean)	29	0.041–0.429	0.63 (Mean)	–	–	–	Southern Atlantic coast of Brazil	Carneiro da Silva et al. (2014)
	4	–	0.04 ± 0.02	4	–	2.13 ± 1.95	4	–	0.54 ± 0.31	–	–	–	4	–	0.46 ± 0.34	Northeast Mediterranean Sea	Yipel et al. (2017)
	–	–	–	42	–	98.7 ± 0.87	42	–	66 ± 0.05	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)
Muscles	35	<LoD–0.090	0.030 ± 0.029	35	<LoD–2.123	0.316 ± 0.354	35	<LoD–0.684	0.072 ± 0.129	35	<LoD–0.147	0.009 ± 0.026	17	<LoD–4.855	0.975 ± 1.141	Potiguar Basin, northeastern Brazil	Present study
	2	0.002–0.007	0.005 ± 0.003	2	0.240–0.270	0.255 ± 0.021	2	0.011–0.034	0.023 ± 0.016	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	42	–	1.65 ± 0.21	42	–	33 ± 0.02	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)
Kidneys	26	<LoD–0.470	0.104 ± 0.107	26	0.426–3.055	1.699 ± 0.613	26	2.134–117.450	16.061 ± 21.581	26	<LoD–1.061	0.087 ± 0.219	16	<LoD–3.697	0.996 ± 1.030	Potiguar Basin, northeastern Brazil	Present study
	23	0.00–0.049	0.020 (Mean)	–	–	–	38	1.7–75.9	15.3 (Mean)	–	–	–	23	0.09–1.85	0.59 (Mean)	Southeastern Queensland, Australia	Gordon et al. (1998)
	2	0-042–0.048	0.045 ± 0.004	2	1.33–1.71	1.520 ± 0.269	2	37.0–45.5	41.250 ± 6.010	–	–	–	–	–	–	Cape Ashizuri, Kochi, Japan	Sakai et al. (2000)
	–	–	–	25	0.354–2.779	1.216 ± 0.597	–	–	–	–	–	–	25	0.309–1.618	0.779 ± 0.353	Yaeyama Islands, Japan	Anan et al. (2001)
	–	–	–	11	0.234–2.994	0.834	11	0.896–96.029	17.794	–	–	–	–	–	–	Baja California peninsula, Mexico	Gardner et al. (2006)
	–	–	–	7	4.8–14.3	–	7	2.2–7.5	–	–	–	–	–	–	–	Italian coastal areas	Storelli et al. (2008)
	–	–	–	29	0.221–5.779	1.794 (Mean)	29	0.309–7.632	4.162 (Mean)	29	0.015–0.176	0.060 (Mean)	–	–	–	Southern Atlantic coast of Brazil	Carneiro da Silva et al. (2014)
	4	–	0.03 ± 0.02	4	–	2.03 ± 1.64	4	–	4.24 ± 1.01	–	–	–	4	–	<LoD	Northeast Mediterranean Sea	Yipel et al. (2017)
	–	–	–	42	–	42.3 ± 0.67	42	–	36 ± 0.02	–	–	–	–	–	–	Northern coast of the Sea of Oman, Median East	Sinaei et al. (2021)

Values presented as dry weight (d.w.) by Anan et al. (2001) Gardner et al. (2006) and Carneiro da Silva et al. (2014) were converted do wet weight (w.w.) according to Gordon et al. (1998), whereas w.w.:d.w. ratios for the liver and kidneys were 4.9 and 6.8, respectively. nd: Not detected. LoD: Limit of detection. –: Not determined.

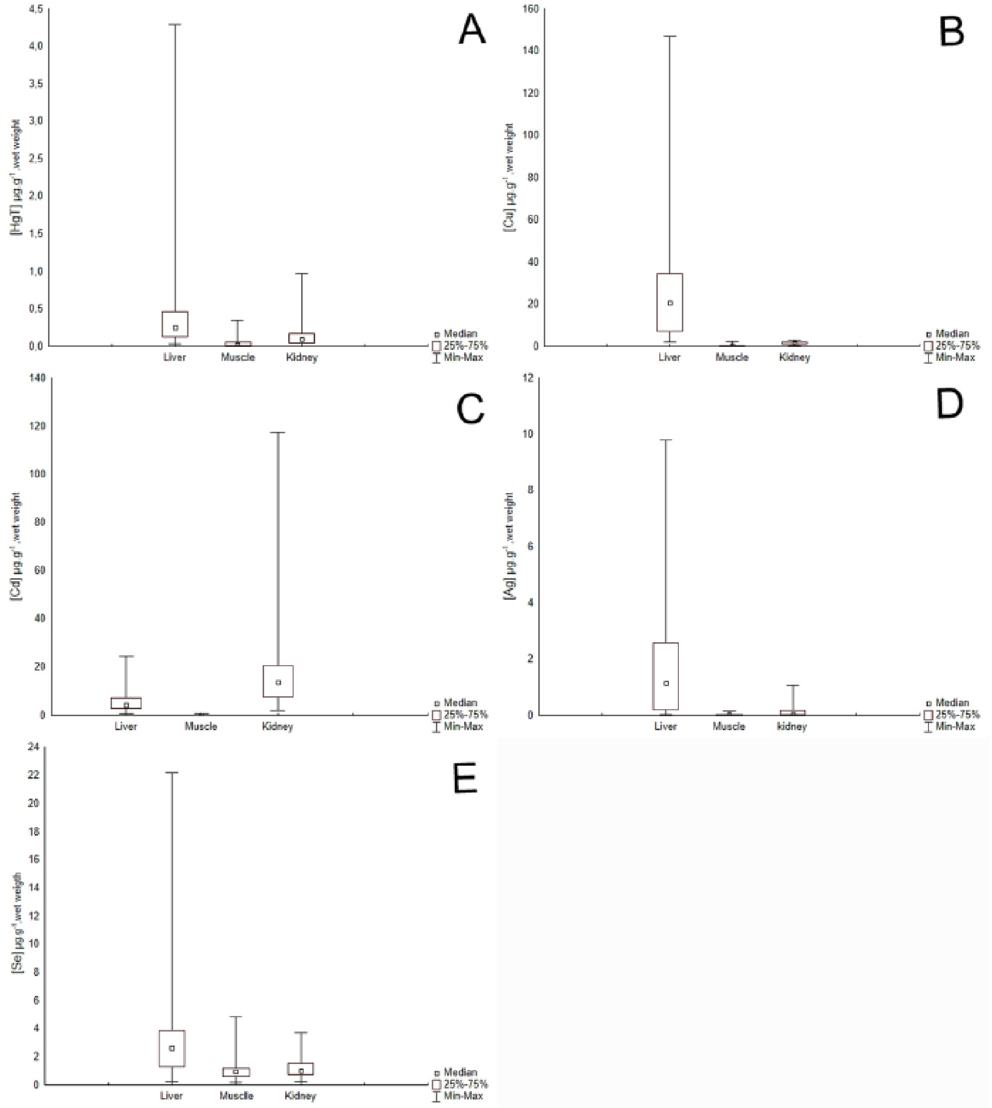

Fig. 2. Concentrations (µg.g^{-1} w.w.) of THg (A), Cu (B), Cd (C), Ag (D) and Se (E) in the liver, muscles, and kidneys of *Chelonia mydas*.

regulating and detoxing non-essential metals, such as Hg and Cd (Roesijadi, 1996; Gardner et al., 2006).

Our findings revealed that Cd concentrations were higher in the kidneys than in the liver or muscles, corroborating studies in the literature (Gordon et al., 1998; Sakai et al., 2000; Frías-Espericueta et al., 2006; Storelli et al., 2008; Carneiro da Silva et al., 2014; Yipel et al., 2017; Fraga et al., 2018; Bruno et al., 2021; See Table 3). Cadmium is one of the most toxic metals in the environment (Dunnick and Fowler, 1988; Barbieri, 2009; Camacho et al., 2013), affecting DNA, RNA, and hormonal attunement (Gerhard et al., 1998; Piasek and Laskey, 1999; Smida et al., 2004; Rana, 2014). Exposure to chronic Cd concentrations can reduce fertility, decreasing the overall reproductive success of marine animals (Singhal et al., 1985). Cadmium does not seem to play a role in biological systems of sea turtles, presenting limited degradation and excretion, defective accumulation, and long-term storage, especially in the kidney, which showed clear bioaccumulation (Storelli and Marcotrigiano, 2003; Frías-Espericueta et al., 2006; Andreani et al., 2008; Storelli et al., 2008; García-Fernández et al., 2009; D'ilio et al., 2011). Tropism could be explained by the binding between Cd and metallothionein (MT) resulting in the metallothionein complex (Cd-MT) in the liver and intestine, preferably transferred to the kidney, where Cd accumulates (Andreani et al., 2008; Sonne et al., 2009). This association in a metallothionein complex could be a detoxication strategy to mitigate the toxic effects of non-essential elements, such as Cd, Hg, Ag and Pb (Roesijadi, 1996; Das et al., 2000; Anan et al., 2002).

Diet composition and biomagnification in the trophic web could be an important Cd source for sea turtles and previous studies show that cephalopods are important Cd vectors to top marine predators (Bustamante et al., 1998; Turoczy et al., 2001; Maffucci et al., 2005; Nuñez-Nogueira and Rainbow, 2005; Karouna-Renier et al., 2007; Reed et al., 2010; Maulvault et al., 2011). Given that cephalopods can be a complementary food resource during the juvenile life-stage of *C. mydas* (Boyle and Limpus, 2008; Parker et al., 2011), the high mean Cd levels found in our study, especially in the kidney (16.061 ± 21.581 $\mu g\ g^{-1}$), could be related to the ingestion of this type of food by the green turtles. Therefore, our findings suggest that the individuals found stranded in the Potiguar Basin were chronically exposed to Cd.

The mean Ag concentrations in the liver of *C. mydas* (2.127 $\mu g\ g^{-1}$) were higher than in the muscles and kidneys (0.009 and 0.087 $\mu g\ g^{-1}$, respectively), corroborating previous studies conducted in Brazil (0.63 $\mu g\ g^{-1}$ in the liver and 0.06 $\mu g\ g^{-1}$ in the kidney; Carneiro da Silva et al., 2014). Differently from Cu and Se, Ag is a non-essential element and enters the aquatic environment because of domestic and industrial sewage (Carneiro da Silva et al., 2014). In our study, Ag levels ranged between 0.023 and 9.788 $\mu g\ g^{-1}$ with the maximum value higher than maximum concentration found by Anan et al. (2002) in *C. mydas* from Japan (1.2 $\mu g\ g^{-1}$; See Table 3). Although the effects of Ag on sea turtles are still unknown, the mechanism of Ag toxicity in sea invertebrates and fish induces ionic and osmotic disturbance (Wood et al., 2004; Bianchini et al., 2005; Pedroso et al., 2007; Carneiro da Silva et al., 2014).

The mean Se concentrations in the liver samples (2.440 $\mu g\ g^{-1}$) were higher than previously recorded in Australia (1.18 $\mu g\ g^{-1}$; Gordon et al., 1998), Japan (1.041 $\mu g\ g^{-1}$; Anan et al., 2001) and Turkey (0.46 $\mu g\ g^{-1}$; Yipel et al., 2017). In addition, our findings revealed that the liver had higher mean Se concentrations than the muscles and kidneys, possibly because the concentration in the liver is the formation of the mercury-selenium (Hg–Se) complex in liver tissues, described as a detoxification strategy in sea turtles and marine mammals (Storelli et al., 1998; Jerez et al., 2010; Frouin et al., 2012; Lailson-Brito et al., 2012). Se presence in the samples analyzed can also be related to the capacity of this trace element to reduce Cd toxicity when these toxic metals are stored and relocated, reducing the potential adverse effects of Se (Siscar et al., 2014). Storelli et al. (2005) reported that Se accumulation in some marine mammals is always high and that establishing whether Se concentrations are toxic or at a mere background level in aquatic reptiles is hard.

5. Conclusion

Our results show that green turtles bioaccumulated the inorganic elements monitored, although it is not possible to affirm that the turtles were exposed to the elements in the Potiguar Basin. Our findings bring additional baseline on Hg, Cu, Cd, Ag and Se in green turtles and reinforce the role of these animals as sentinels of the marine ecosystem. This supports the need to continue monitoring inorganic elements and to further the studies on environmental quality of the Potiguar Basin, a feeding site for green turtles and an important economic regional site in Brazil, since these chemical elements could also affect human health.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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