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DOUTORADO EM CIÊNCIA ANIMAL

RADAN ELVIS MATIAS DE OLIVEIRA

**MORFOLOGIA E ACHADOS ANATOMOPATOLÓGICOS DO TRATO
DIGESTÓRIO DE TARTARUGAS MARINHAS**

MOSSORÓ

2022

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**MORFOLOGIA E ACHADOS ANATOMOPATOLÓGICOS DO TRATO
DIGESTÓRIO DE TARTARUGAS MARINHAS**

Tese apresentada ao Doutorado em Ciência Animal do Programa de Pós-Graduação em Ciência Animal da Universidade Federal Rural do Semi-Árido como requisito para obtenção do título de Doutor em Ciência Animal.

Linha de Pesquisa: Morfofisiologia e Biotecnologia Animal

Orientador: Prof. Dr. Moacir Franco de Oliveira

Co-orientador: Prof. Dr. Flávio José de Lima Silva

MOSSORÓ

2022

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RESUMO

Embora o conhecimento acerca da biologia e ecologia das tartarugas marinhas esteja sendo ampliado no mundo, ainda são escassas informações acerca da morfofisiologia do trato digestório destes animais, bem como sobre os achados imagiológicos e anatomopatológicos associados principalmente à ingestão de resíduos sólidos. Com isso, o objetivo do presente estudo foi descrever a morfologia do trato digestório de filhotes de uma espécie de tartaruga marinha, *Eretmochelys imbricata*, a fim de compreender os processos evolutivos associados a hábitos alimentares, bem como relatar os principais achados imagiológicos e anatomopatológicos do trato digestório das tartarugas marinhas. Para tanto, a tese foi dividida em nove capítulos. O primeiro capítulo, traz informações sobre as considerações gerais das tartarugas marinhas. O segundo e terceiro capítulos, correspondem a dois artigos experimentais intitulados: Morfologia do assoalho da cavidade orofaríngea de filhotes de tartaruga-de-pente (*E. imbricata*) e suas implicações evolutivas e Caracterização morfológica do tubo digestório de filhotes de tartaruga-de-pente (*E. imbricata*). Já o quarto capítulo, corresponde a um artigo de revisão com o título: Ingestão de resíduos marinhos e o uso do diagnóstico por imagem em tartarugas marinhas: uma revisão. Os últimos cinco capítulos correspondem a artigos de relatos de casos, nominados: Morte de uma tartaruga-cabeçuda (*Caretta caretta*) por ingestão de uma enguia (*Myrichthys ocellatus*); Intussuscepção colocolica associada ao *Octangium* sp. (Digenea: Microscaphidiidae) em uma tartaruga-verde *Chelonia mydas*; Reabilitação bem-sucedida de uma tartaruga marinha oleada (*Lepidochelys olivacea*) afetada pelo maior derramamento de óleo do Brasil; Achados anatomopatológicos de uma tartaruga-verde (*C. mydas*) vítima de colisão com um barco motorizado; e Prolapso peniano em tartaruga-verde (*C. mydas*). Portanto, com base nas características morfológicas observadas no trato digestório de filhotes de *E. imbricata*, existem fortes indícios de uma adaptação a dieta carnívora nessa faixa etária. Os exames de diagnóstico por imagem em tartarugas marinhas têm grande importância na medicina da conservação, pois podem indicar o tipo de material ingerido, bem como sua localização, permitindo a melhor tomada de decisão para o tratamento de um animal. O diagnóstico *ante mortem* das doenças que afetam o trato digestório em tartarugas marinhas é de difícil definição, portanto, os achados anatomopatológicos e os sinais clínicos apresentados aqui fornecerão informações relevantes para definir as melhores terapias para as tartarugas marinhas em centros de reabilitação.

Palavras-chave: Anatomia comparada. Animais silvestres. Cheloniidae. Diagnóstico por imagem. Testudines.

ABSTRACT

Although knowledge of the biology and ecology of sea turtles is expanding in the world, information is still scarce about the morphophysiology of the digestive tract of these animals, as well as imagiological and anatomopathological findings, mainly associated with the ingestion of solid wastes. Thus, the objective of this study was to describe the morphology of the digestive tract of hatchlings of the sea turtle species *Eretmochelys imbricata*, for the purpose of understanding the evolutionary processes associated with the feeding habits and to report the principle imagiological and anatomopathological aspects of the digestive tract of the sea turtles. The thesis is divided into nine chapters. The first contains general information on sea turtles. The second and third chapters examine two experimental articles, entitled Oropharyngeal cavity floor morphology in *Eretmochelys imbricata* (Testudines: Cheloniidae) hatchlings and evolutionary implications; and Morphological characterization of the digestive tube of hawksbill sea turtle (*E. imbricata*) hatchlings. The fourth chapter presents the most relevant information of a review article with the title Marine debris ingestion and the use of diagnostic imaging in sea turtles: A review. The last five chapters involve the following case reports: Death of a loggerhead sea turtle (*Caretta caretta*) from ingestion of an eel (*Myrichthys ocellatus*); Colocolic intussusception associated with *Octangium* sp. (Digenea: Microscaphidiidae) in a green sea turtle *Chelonia mydas*; Successful case of rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil; Pathoanatomical findings of a green turtle (*C. mydas*) victim of collision with a motorboat; and Penile prolapse in green sea turtle (*C. mydas*). Observation of the morphological traits of the digestive tracts of *E. imbricata* hatchlings provides strong indications of adaptation to a carnivorous diet at this life phase. Diagnostic imaging examinations of sea turtles have great importance to conservation medicine, by indicating the types of materials ingested and their location, permitting better decisions for treatment. *Ante mortem* diagnosis of the diseases that affect the digestive tract of sea turtles is difficult, so the anatomopathological findings and clinical signs presented here will provide relevant information to define better therapies for marine turtles in rehabilitation centers.

Keywords: Compared anatomy. Wild animals. Cheloniidae. Imaging diagnosis. Testudines.

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LISTA DE ABREVIATURAS E SIGLAS

AB	Alcian blue
AFA	Alcohol-formalin-acetic acid
CCC	Comprimento curvilíneo da carapaça
CCL	Curved carapace length
CCW	Curved carapace width
CEMAM	Centro de Estudos e Monitoramento Ambiental
DT	Digestive tube
GT	Gomori's trichrome
HCl	Ácido Clorídrico
HE	Hematoxylin and Eosin
IBAMA	Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
ICMBio	Instituto Chico Mendes de Conservação da Biodiversidade
IUCN	International Union for Conservation of Nature
LCC	Largura curvilínea da carapaça
LI	Large intestine
MRI	Magnetic Resonance Imaging
MRT	Magnetic Resonance Tomography
NaCl	Cloreto de Sódio
PAS	Periodic Acid Schiff
PCCB	Projeto Cetáceos da Costa Branca
SEM	Scanning Electron Microscopy
SI	Small intestine
UERN	Universidade do Estado do Rio Grande do Norte
UFERSA	Universidade Federal Rural do Semi-Árido

LISTA DE SIMBOLOS

@	Arroba
®	Marca registrada
%	Porcentagem
+	Soma
<	Menor que
=	Igual
>	Maior que
±	Mais ou menos
×	Multiplicação
μ	Micrômetro
°C	Graus Centígrados

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CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1 INTRODUÇÃO

As tartarugas marinhas são répteis da ordem Testudines e formam um grupo monofilético da subordem Cryptodira (BOWEN; KARL, 1997; MEYLAN; MEYLAN, 1999). Habitam os oceanos desde o Cretáceo, há aproximadamente 100 milhões de anos (HIRAYAMA, 1998). Estes animais são componentes únicos de sistemas ecológicos complexos. Como eles fazem migrações de milhares de quilômetros e levam décadas para atingir a maturidade sexual, as tartarugas servem como indicadores importantes da saúde dos ambientes costeiros e marinhos tanto local quanto globalmente (FRAZIER, 2000).

Atualmente são mundialmente conhecidas sete espécies de tartarugas marinhas, que se encontram distribuídas em duas famílias. A família Dermochelyidae que contém apenas uma espécie vivente, a *Dermochelys coriacea* ou tartaruga-de-couro. As outras seis espécies estão incluídas na família Cheloniidae, *Chelonia mydas* (tartaruga-verde), *Caretta caretta* (tartaruga-cabeçuda), *Eretmochelys imbricata* (tartaruga-de-pente), *Lepidochelys kempii* (tartaruga-lora), *Lepidochelys olivacea* (tartaruga-oliva) e *Natator depressus* (tartaruga-flatback). Das sete espécies existentes de tartarugas marinhas, cinco utilizam a costa brasileira para reprodução e alimentação são elas: a *C. mydas*, *Caretta caretta*, *E. imbricata*, *L. olivacea* e a *D. coriacea* (MARCOVALDI; MARCOVALDI, 1999).

Todas as sete espécies de tartarugas marinhas têm sido mundialmente afetadas por impactos pela presença de resíduos antrópicos, seja por emalhe ou por ingestão destes materiais (LAZAR; GRACAN, 2011; GALL; THOMPSON, 2015; SCHUYLER et al., 2016; CARACAPPA et al., 2018; WILCOX et al., 2018; RIZZI et al., 2019). Destacando ainda, que apesar dos diferentes hábitos alimentares, em geral, as tartarugas marinhas se alimentam de capim agulha, algas, poríferos, cnidários, peixes e crustáceos (DEN HARTOG, 1980; SPOTILA, 2004; COLMAN et al., 2014), itens os quais também são afetados pelos resíduos sólidos ou contaminantes, podendo tanto afetar as tartarugas por meio da ingestão do alimento, como também pela diminuição do estoque alimentar em decorrência desses achados.

A variedade de resíduos ingeridos e encontrados no conteúdo estomacal nas tartarugas marinhas é enorme, desde plásticos, vidro, artefatos de pesca, papel, microplástico e metal, entre outros (ANDRADY, 2011; GALL e THOMPSON, 2015; RIZZI et al., 2019; FARIAS et al., 2019). Dentre os plásticos, a diversidade de materiais encontrados também pode variar

imensamente desde sacolas plásticas à materiais mais densos e de diferentes colorações (LAZAR; GRACAN, 2011; HOARAU et al., 2014; POLI et al., 2015; GALL; THOMPSON, 2015; FUKUOKA et al., 2016; CLUKEY et al., 2017; PHAM et al., 2017; WILCOX et al., 2018; DUNCAN et al., 2019; FARIAS et al., 2019; RIZZI et al., 2019).

O impacto humano sobre os habitats das tartarugas marinhas é reconhecido há décadas, com os esforços para mitigação concentrados no ambiente terrestre (LUTCAVAGE et al., 1997). Apesar de progressos feitos na proteção e recuperação de ecossistemas marinhos em algumas áreas, impactos antropogênicos diretos ou indiretos persistem (HAMANN et al., 2010). Essa condição é um indicativo da importância da avaliação dos efeitos e impactos promovidos pelo lixo em ambiente marinho e torna-se uma prioridade em pesquisas no mundo (NELMS et al., 2016; GALL; THOMPSON, 2015; POLI et al., 2015; CLUKEY et al., 2017; PHAM et al., 2017; DUNCAN et al., 2019; RIZZI et al., 2019).

Portanto, o objetivo deste estudo foi analisar a morfologia do trato digestório de filhotes de uma espécie de tartaruga marinha, *E. imbricata*, fornecendo conhecimentos importantes para a compreensão da fisiologia da digestão desses animais, e ainda, propõe identificar possíveis alterações morfológicas decorrentes das ações antrópicas das outras espécies de tartarugas marinhas que encalharam no litoral do Rio Grande do Norte, e dessa forma, identificando situações que facilitem o diagnóstico das doenças, e consequentemente, a melhoria de informações para ações de conservação desses animais.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Aspectos biológicos das tartarugas marinhas

Tartarugas marinhas possuem diversas adaptações para a vida no mar. Destaca-se para esse grupo taxonômico, o número reduzido de vértebras, que se fusionaram as costelas e formaram uma carapaça de revestimento córneo. A carapaça, por sua vez, tornou-se achatada, proporcionando um corpo hidrodinâmico e facilitando seu deslocamento durante as migrações. Enquanto isso, os membros transformaram-se em nadadeiras, para um movimento mais eficiente debaixo d'água. Além disso, todas as espécies apresentam as glândulas lacrimais grandes e modificadas para remover o excesso de sal do corpo (MEYLAN; MEYLAN 1999; WYNEKEN, 2004).

Estes animais possuem um complexo ciclo de vida e estrutura populacional, caracterizado por migrações de centenas a milhares de quilômetros entre as áreas de alimentação e desova, razão pela qual são difíceis de estudar (BOWEN, 1995), e tanto adultos quanto filhotes e juvenis, parecem utilizar o campo magnético terrestre para guiarem-se durante estas migrações (LOHMAN, 2007).

A identificação das tartarugas marinhas por características externas tem como base o número de placas da cabeça, o formato das mandíbulas, o número de unhas nas nadadeiras e o número e arranjo dos escudos na carapaça. Não apresentam dimorfismo sexual, exceto quando adultas. Machos apresentam a cauda mais longa e uma grande garra curva direcionada para dentro nas nadadeiras anteriores, que o ajudam a segurar a fêmea no momento da cópula (CUBAS; SILVA; CATÃO-DIAS, 2014).

As tartarugas marinhas apresentam respiração do tipo pulmonar, portanto precisam subir à superfície da água para respirar. Os pulmões desses animais estão localizados dorsalmente e aderidos à carapaça e à coluna vertebral. Ventralmente, o pulmão esquerdo se conecta com o estômago pelo ligamento gastropulmonar. O pulmão direito está conectado ao lóbulo direito do fígado pelo ligamento hepatopulmonar. Em sua região posterior, os pulmões são adjacentes às gônadas e se unem ao peritônio, que, por sua vez, recobre os rins e as glândulas suprarrenais. O bordo medial de cada pulmão está firmemente unido, por conexões fibrosas, às superfícies dorsolaterais da coluna vertebral (WYNEKEN, 2004; CUBAS; SILVA; CATÃO-DIAS, 2014).

2.2 Tartaruga-de-pente (*Eretmochelys imbricata*, Linnaeus, 1766)

A *E. imbricata* é uma espécie onívora na fase juvenil e adulta, preferencialmente se alimenta de diversas espécies de poríferos, algumas algas e cnidários. A espécie forrageia nos recifes de corais, rochas, áreas de mangues em baías e áreas estuarinas. Os adultos alimentam-se principalmente de poríferos, desempenhando um importante papel na manutenção dos recifes de coral ao redor do mundo (RINCON-DIAZ et al., 2011; CARRIÓN-CORTEZ et al., 2013; VON BRANDIS et al., 2014) (**Figura 1**).



Figura 1. Tartaruga-de-pente (*Eretmochelys imbricata*) adulta forrageando nos recifes de corais. Foto: (Rainer Von Brandis).

Os indivíduos levam de 20 a 40 anos para atingir a maturidade sexual, dependendo da área geográfica e da disponibilidade de alimento. E no passado, a espécie foi muito caçada devido à beleza de sua carapaça, que era muito utilizado para manufatura de bijuterias, pentes e diversos outros objetos ornamentais e, por causa disso, a espécie foi quase levada à extinção (MORTIMER; DONNELLY, 2008).

A coloração desta espécie pode variar de acordo com a faixa etária, dorsalmente destaca-se um marrom (claro a escuro) em filhotes, muitas vezes mostrando forte pigmentação com estrias de cor âmbar e marrom em juvenis e adultos jovens; e ventralmente observa-se tons de amarelo pálido a branco, às vezes com manchas pretas (particularmente em espécimes do Pacífico). Pode atingir um peso corpóreo de até 80 kg (60 kg em média) (PRITCHARD; MORTIMER, 2000) (**Figura 2**).



Figura 2. Tartaruga-de-pente (*Eretmochelys imbricata*) juvenil. Foto: (Image by U.S. Fish and Wildlife Service Southeast Region [CC BY 2.0 or Public domain], via Wikimedia Commons).

A cabeça é relativamente estreita, com largura de até 12 cm, apresentando dois pares de placas pré-frontais, três pares de placas pós-orbitais e um bico reto, semelhante ao de um pássaro. As nadadeiras anteriores são de comprimento médio comparadas com as de outras espécies, com duas unhas em cada nadadeira. Apresenta uma carapaça oval, com uma margem posterior marcada (serrilhada) e com escudos espessos e sobrepostos (imbricados), exceto em filhotes e em alguns adultos, contém quatro pares de escudos costais, cada um com uma borda posterior levemente rasgada e o comprimento retilíneo da carapaça podendo

atingir até 90 cm. O plastrão é composto de quatro pares de escudos inframarginais (PRITCHARD; MORTIMER, 2000) (**Figura 3**).

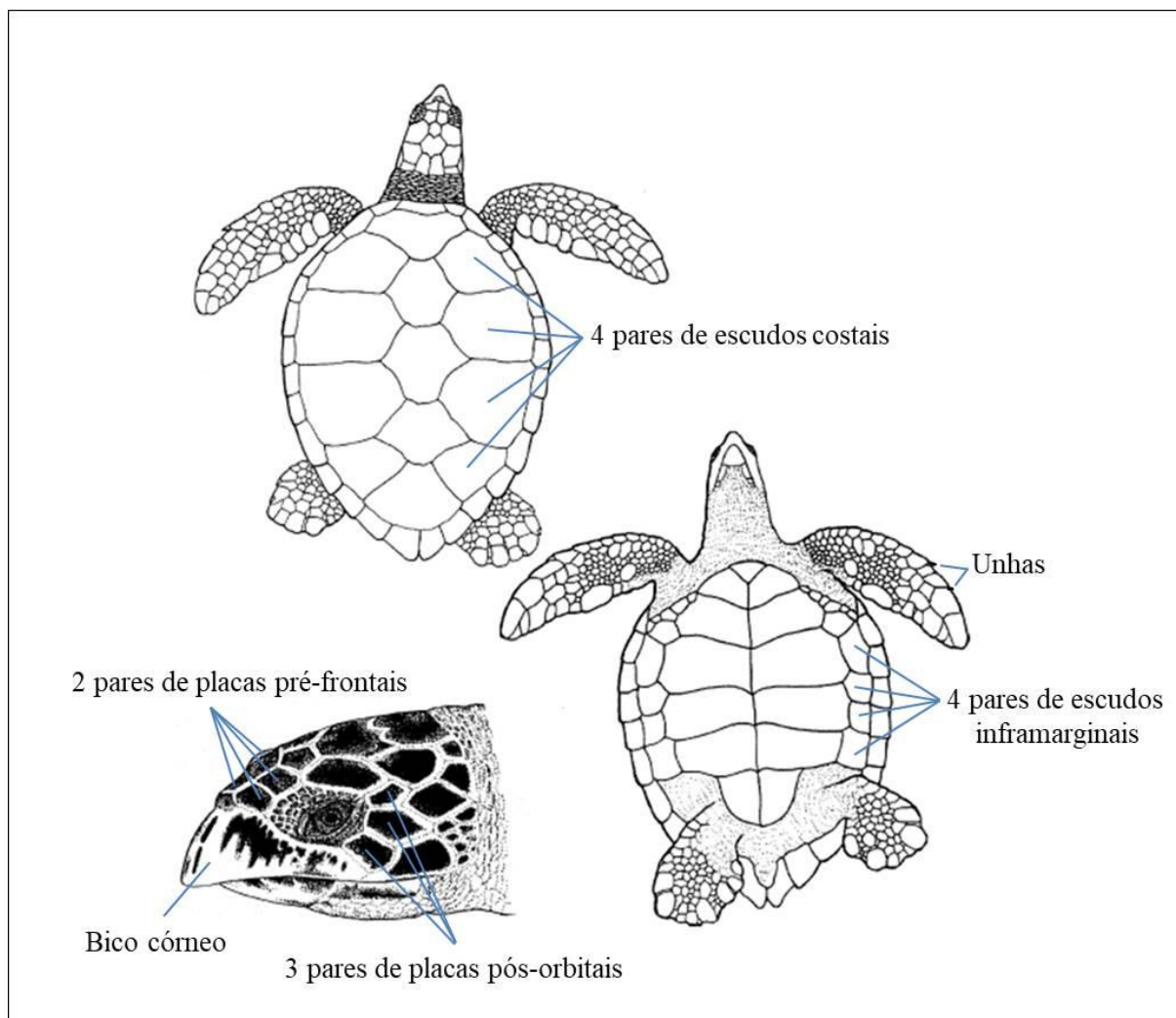


Figura 3. Características morfológicas externas para identificação da tartaruga-de-pente (*Eretmochelys imbricata*). Fonte: (Adaptado de PRITCHARD; MORTIMER, 2000).

2.3 Distribuição

A espécie *E. imbricata* distribui-se globalmente em águas tropicais e, em menor extensão, em águas subtropicais (MORTIMER; DONNELLY, 2008). Esta espécie abrange os oceanos Atlântico, Pacífico e Índico, desde a Califórnia, o Japão, o Mar Vermelho, as Ilhas Britânicas e o sul de Massachusetts até o Peru, e ainda a Austrália, Madagascar, o noroeste da África e o sul do Brasil (ERNST; BARBOUR, 1989).

Juvenis distribuem-se em todo o litoral Norte-Nordeste do Brasil e, com menor frequência, no Sul-Sudeste, sendo as principais áreas de alimentação conhecidas no Brasil o Arquipélago Fernando de Noronha-PE (SANCHES; BELLINI, 1999), o Atol das Rocas-RN (MARCOVALDI et al., 1998), Abrolhos-BA (PEDROSA; VERÍSSIMO, 2006) e a Ilha do Arvoredo-SC (REISSER et al., 2008) (**Figura 4**).

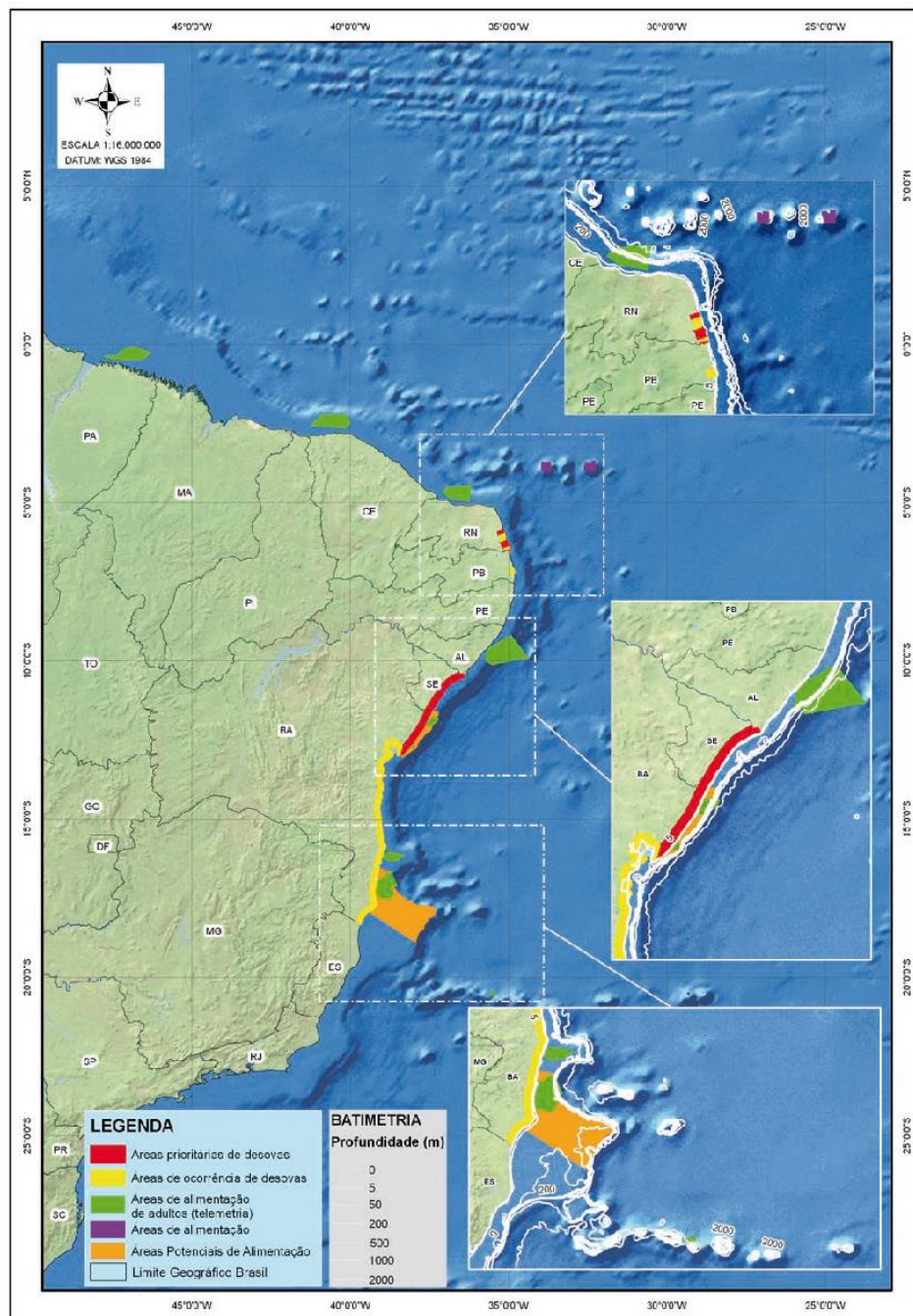


Figura 4. Distribuição geográfica da tartaruga-de-pente (*Eretmochelys imbricata*) no continente brasileiro. Fonte: (MARCOVALDI et al., 2011).

2.4 Reprodução

Todas as espécies de tartarugas marinhas realizam mais de uma postura por temporada, porém, não se reproduzem em anos consecutivos (ROSSI, 2007). O intervalo entre eventos reprodutivos pode variar entre 1 a 9 anos, dependendo da espécie (MILLER, 1997). No caso da *E. imbricata*, as posturas podem ser realizadas aproximadamente a cada 3 anos (GOMES et al., 2006). A temporada de reprodução tem início em novembro e termina em abril, com maior número de desovas nos meses de dezembro, janeiro e fevereiro (MARCOVALDI et al., 2007). O número de ovos em cada desova varia entre 110 a 180 ovos (PRITCHARD; MORTIMER, 2000; BOMFIM et al., 2021) (**Figura 5**).



Figura 5. Desova de tartaruga-de-pente (*Eretmochelys imbricata*) na praia de Pipa, município de Goianinha/RN. A: preparando a cama. B: momento da desova. Foto: (Williane Feitosa de Lima, 2020).

Em aproximadamente 50-70 dias os filhotes nascem e irão emergir da areia, normalmente à noite, quando a temperatura está mais amena, guiando-se até o mar através do reflexo do brilho das ondas, e depois são levados para mar aberto (**Figura 6**). Permanecem em habitat pelágico onde são conduzidos passivamente pelas correntes oceânicas para longe de

suas praias de nascimento, e quando se tornam juvenis, reaparecem na costa nas áreas de alimentação (BOWEN, 1995).



Figura 6. Eclosão de ninho de tartaruga-de-pente (*Eretmochelys imbricata*) na praia de Búzios, município de Nísia Floresta/RN. A: filhotes emergindo da areia. B: a caminho do mar. Foto: (Projeto Cetáceos da Costa Branca – PCCB/UERN, 2019).

As áreas de desova da *E. imbricata* no Brasil distribuem-se desde o Espírito Santo ao Ceará, porém desovas regulares com maior concentração (definindo áreas prioritárias) encontram-se apenas no litoral norte do Estado da Bahia e Sergipe, e no litoral sul do Rio Grande do Norte (MARCOVALDI et al., 2007). Há ainda outras áreas com menor concentração de desovas, mas que devem ser ressaltadas: Paraíba (MASCARENHAS et al., 2004), Ceará (LIMA, 2002), Espírito Santo (MARCOVALDI et al., 2007) e Piauí (SANTANA et al., 2009). Há evidências de desovas regulares, mas também em menor número, no estado de Pernambuco (MOURA et al., 2009) e no norte do Rio Grande do Norte (BOMFIM et al., 2021). No nordeste brasileiro, o período de nidificação ocorre entre os meses de novembro a abril (MARCOVALDI et al., 2011), tendo picos nos meses de

dezembro a fevereiro na Bahia (CAMILLO et al., 2009) e em Sergipe (MARCOVALDI et al., 2011). Na Paraíba, a temporada reprodutiva apresenta picos em fevereiro e março (MASCARENHAS; ZEPPELIN; MOREIRA, 2003) e no Rio Grande do Norte tem seu maior pico no mês de março (BOMFIM et al., 2021).

2.5 Ameaças para as tartarugas marinhas

As ameaças estão associadas a inúmeros fatores: (1) destruição de habitats; (2) ação antrópica nas praias de nidificação (BUGONI; KRAUSE; PETRY; 2001), como iluminação, trânsito de veículos e pessoas, obstáculos (resíduos sólidos, equipamento de trabalho ou veículos estacionados) (WITHERINGTON, 2000); (3) poluentes como organoclorados (KELLER et al., 2004), petróleo (WITHERINGTON, 2000; OLIVEIRA et al., 2021b) e arsênio (SAEKI et al., 2000; FUJIHARA et al., 2003); (4) colisões com embarcações (OLIVEIRA et al., 2021a); (5) atividades pesqueiras; (6) alterações climáticas; (7) desenvolvimento costeiro; e (8) consumo e uso de carne, ovos e carapaça (MAST et al., 2005).

Os poluentes causam efeitos danosos incluindo comprometimento dos aspectos fisiológicos, estresse crônico, imunossupressão e aumento na susceptibilidade às doenças (AGUIRRE et al., 1995). Estudos realizados na Carolina do Norte (EUA) com *C. caretta* demonstraram que compostos organoclorados se acumulam nos tecidos do animal causando hepatotoxicidade, imunotoxicidade, toxicidade reprodutiva, enfraquecimento e efeitos no comportamento neurológico (KELLER et al., 2004). Neste contexto, outros estudos realizados por Saeki et al. (2000) e Fujihara et al. (2003), constataram altas concentrações de arsênio no fígado e no músculo em *E. imbricata*, *Caretta caretta* e *C. mydas*. A intoxicação aguda por arsênio pode causar transtornos gastrointestinais e neurológicos (GONTIJO; BITTENCOURT, 2005).

A captura incidental em atividades de pesca representa uma das principais causas de mortalidade para estas espécies, principalmente redes de emalhe ou arrasto empregadas no entorno das praias de desova (GALLO et al., 2006; MARCOVALDI et al., 2009; SILVA et al., 2010; BOMFIM et al., 2022). Outra grande ameaça, é a ingestão de lixo, causando obstrução intestinal e lesões internas, déficit na digestão e absorção de nutrientes, desnutrição e aumento da flutuabilidade que, por sua vez, pode resultar em problemas de saúde, redução nas taxas de crescimento e na produção reprodutiva, ou morte do animal (NELMS et al., 2016).

De acordo com a lista vermelha de espécies ameaçadas de extinção da União Internacional para a Conservação da Natureza (IUCN) e o Livro Vermelho da Fauna Brasileira Ameaçada de Extinção, a tartaruga-de-pente está classificada como criticamente ameaçada de extinção (MORTIMER; DONNELLY, 2008; ICMBio, 2016).

2.6 Considerações gerais do aparelho digestório dos répteis

O aparelho digestório compreende os órgãos relacionados à recepção, redução mecânica, digestão química e absorção de alimentos e líquidos, bem como a eliminação de resíduos não absorvidos. Este sistema estende-se da cavidade oral ao ânus e é formado pela boca, dentes, língua, faringe, tubo digestório (esôfago, estômago, intestino delgado e intestino grosso), e as glândulas anexas (glândulas salivares, fígado e pâncreas) (COLVILLE, 2010; DYCE; WENSING; SACK, 2010; GETTY; SISSON; GROSSMAN, 2013).

Quelônios em geral têm bico córneo constituído de maxila e pré-maxila queratinizado e mandíbula igualmente queratinizada (CUBAS; SILVA; CATÃO-DIAS, 2014), similares aos bicos das aves, chamados de ranfoteca (DEVOE, 2010).

A cavidade oral da maioria dos répteis apresenta numerosas glândulas salivares lóbulo-alveolares bem desenvolvidas, já em tartarugas marinhas, as glândulas estão localizadas na porção distal do esôfago (CUBAS; SILVA; CATÃO-DIAS, 2014; CALAIS JÚNIOR et al., 2016). As secreções salivares facilitam a ingestão de presas grandes, proporcionando lubrificação (DEVOE, 2010).

Existem muitas diferenças nas características do epitélio dorsal lingual em répteis. Essas diferenças podem ser categorizadas filogeneticamente e, ao mesmo tempo, refletem a adaptação de répteis a seus ambientes até certo ponto (IWASAKI et al., 1996). O epitélio lingual de répteis completamente terrestres tem uma tendência a ser queratinizado (IWASAKI; KUMAKURA, 1994; IWASAKI; YOSHIHARA, 2003; EL-MANSI et al., 2020), enquanto o dos répteis que vivem em ou perto de lagoas, ou pequenos rios tendem a ser não-queratinizado (IWASAKI, 1992; IWASAKI et al., 1992; BEISSER et al., 1998). O epitélio lingual das tartarugas marinhas é predominantemente queratinizado, o que pode ter relação com a dieta desses animais, pois a queratinização fornece proteção contra abrasão (IWASAKI, 2002).

Muitos estudos demonstraram que a superfície dorsal da língua reptiliana é rica em papilas linguais (IWASAKI, 1990; IWASAKI et al., 1992; BEISSER et al., 1998; LINTNER et al., 2012; CIZEK et al., 2019). Em tartarugas marinhas não foi observado a presença de

papilas linguais, em vez disso, observa-se a presença de pregas formadas pelas projeções epiteliais, podendo estar distribuídas por toda a superfície dorsal da língua (IWASAKI et al., 1996)

Em diferentes tipos de répteis, tem sido relatada a ocorrência e distribuição de botões gustativos na língua e assoalho da cavidade orofaríngea (SCHWENK, 1985; IWASAKI; KUMAKURA, 1994, IWASAKI; YOSHIHARA 2003; EL-MANSI et al., 2020). Em quelônios, os botões gustativos foram descritas em tartarugas terrestres da espécie *Manouria emys emys* (HEISS et al., 2011), semi-aquáticas *Heosemys grandis* (LINTNER et al., 2012) e aquáticas *Podocnemis expansa* (SILVEIRA et al., 2015). Já em tartarugas marinhas não há relatos da presença desses botões no epitélio lingual (IWASAKI et al., 1996).

Os quatro órgãos que compõe o tubo digestório (esôfago, estômago, intestino delgado e intestino grosso), possuem características histológicas comuns, representadas pelas quatro camadas ou túnicas concêntricas em suas paredes: mucosa, submucosa, muscular e adventícia ou serosa. Embora esta organização geral seja uniforme ao longo do tubo, os segmentos apresentam variações estruturais diretamente relacionadas com suas atividades funcionais (NASCIUTTI et al., 2014; JUNQUEIRA; CARNEIRO; ABRAHAMSOHN, 2017).

A mucosa é constituída por um epitélio de revestimento, apoiado sobre uma lâmina própria de tecido conjuntivo frouxo ricamente vascularizado e por uma camada fina de tecido muscular liso, denominada muscular da mucosa (NASCIUTTI et al., 2014).

A submucosa é formada por tecido conjuntivo denso irregular com gânglios nervosos, numerosos vasos sanguíneos de médio calibre, vasos linfáticos e nervos (NASCIUTTI et al., 2014; JUNQUEIRA; CARNEIRO; ABRAHAMSOHN, 2017). O plexo nervoso submucoso (ou de Meissner), com gânglios do sistema nervoso autônomo, cujos neurônios são multipolares e motores. Eles controlam o movimento da muscular da mucosa, a secreção das glândulas e o fluxo sanguíneo (GARTNER; HIATT, 2007).

A muscular está organizada em duas camadas de músculo liso. A camada mais interna é formada por células musculares lisas dispostas ao redor do lúmen (camada circular), cuja contração é responsável pela diminuição do diâmetro do lúmen; na camada mais externa, as células musculares se distribuem ao longo do comprimento do tubo (camada longitudinal) e, quando contraídas, reduzem o comprimento do tubo digestório. Na porção superior do esôfago e no esfíncter do ânus, há fibras musculares estriadas esqueléticas (NASCIUTTI et al., 2014). O espessamento do músculo circular em algumas áreas resulta nos esfíncteres, que impedem a passagem do conteúdo luminal com a sua contração (LOWE; ANDERSON, 2016).

A adventícia é formada de tecido conjuntivo em continuidade com os tecidos adjacentes. Nas regiões do tubo digestório suspensas pelo mesentério, a adventícia é substituída por um epitélio pavimentoso simples (mesotélio), formando então a membrana serosa ou o peritônio visceral (NASCIUTTI et al., 2014).

Em geral, o esôfago é um tubo muscular que se estende da faringe ao estômago, sua função é conduzir o alimento ao estômago (COLVILLE, 2010). Nos répteis, é morfologicamente similar ao dos demais vertebrados (CUBAS; SILVA; CATÃO-DIAS, 2014). De acordo com Devoe (2010), o esôfago dos répteis é relativamente fino e distensível na maioria das espécies, especialmente naquelas adaptadas ao consumo de presas grandes. Alguns animais possuem estruturas esofágicas exclusivas que refletem o tipo de presa que consomem. A mucosa esofágica das tartarugas marinhas apresenta papilas pontiagudas ao longo de toda a sua extensão (papilas esofágicas). Essas papilas são orientadas caudalmente e auxiliam na ingestão do alimento (WYNEKEN, 2004; MAGALHÃES et al., 2010; MAGALHÃES et al., 2012; CHEN et al., 2015; CALAIS JÚNIOR et al., 2016) (**Figura 7**).

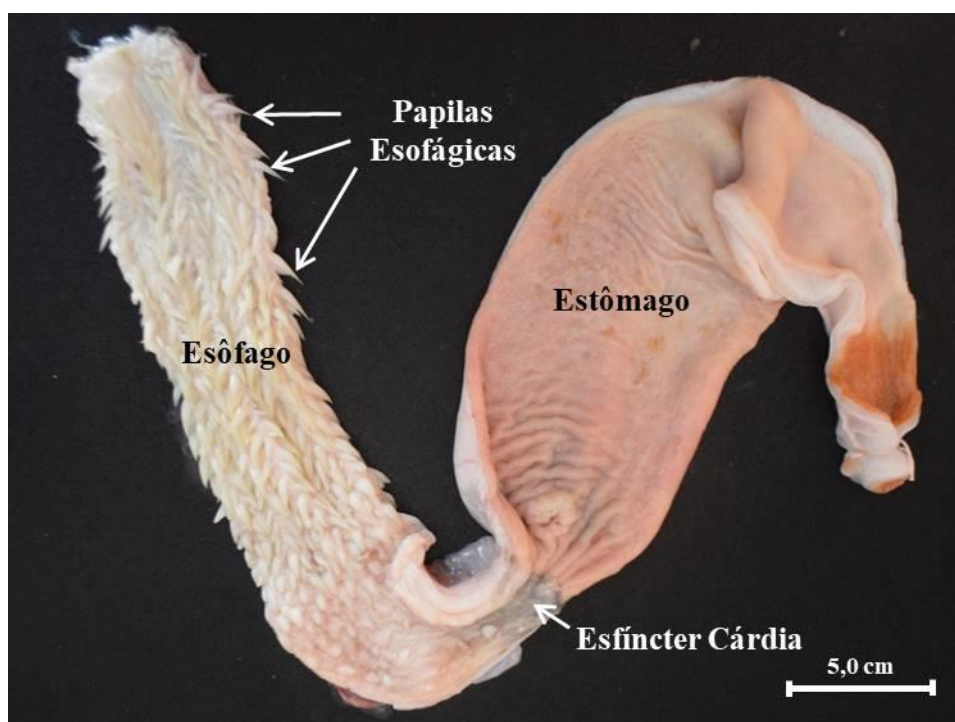


Figura 7. Esôfago e estômago de tartaruga-verde (*Chelonia mydas*) juvenil. Foto: (Projeto Cetáceos da Costa Branca – PCCB/UERN, 2019).

O estômago é a grande dilatação do trato gastrointestinal, delimitado entre o esôfago e o intestino delgado, e está envolvido na digestão mecânica e química dos alimentos. Este órgão varia na forma e tamanho entre as espécies, e esta variação morfológica está

intimamente relacionado com o comportamento alimentar (DEVOE, 2010; GETTY; SISSON; GROSSMAN, 2013).

O estômago das tartarugas marinhas está localizado no antímero esquerdo e forma uma curva ao redor do fígado e do pericárdio. A partir da porção caudal do esôfago, forma-se a região cárdica, seguida de uma grande bolsa, a região fúndica e, por fim, a região pilórica, uma região muscular curta, à direita. Este está ligado ao lobo esquerdo do fígado por um ligamento gastrohepático e ao pulmão esquerdo por um ligamento gastropulmonar (WYNEKEN, 2004; MAGALHÃES et al., 2012; CHEN et al., 2015).

O intestino delgado é o principal local onde a maioria dos nutrientes é absorvido pelo processo de digestão química, através de enzimas secretadas pelo pâncreas exócrino, além das enzimas presentes nas células epiteliais (enterócitos) da própria mucosa do intestino delgado (COLVILLE, 2010; FRANDSON; WILKE; FAILS, 2011). Os eletrólitos (sódio, cloreto, potássio e outros) e as vitaminas podem ser absorvidos completamente pela parede do intestino delgado, enquanto carboidratos, proteínas e gorduras devem ser quimicamente digeridos para serem absorvidos (COLVILLE, 2010).

Em tartarugas marinhas, o intestino delgado é dividido em três regiões, duodeno, jejuno e íleo. A mucosa do duodeno é marcada pela presença de pregas reticulares com disposição semelhantes a “favos-de-mel ou colmeia”, este revestimento texturizado está associado a um aumento da superfície de contato da mucosa. A passagem do duodeno para o jejuno é marcada pela mudança na mucosa, de reticulares para retilíneas. O jejuno e o íleo apresentam pregas retilíneas justapostas (WYNEKEN, 2004; MAGALHÃES et al., 2010; MAGALHÃES et al., 2012). As transições entre as partes do intestino delgado (jejuno e íleo) são difíceis de identificar. As diferenças macroscópicas muitas vezes não são óbvias e são melhores confirmadas por meio de análise histológica. A transição entre o íleo e o cólon é óbvia em animais adultos, onde o íleo termina em um esfíncter muscular, a válvula ileocecal (WYNEKEN, 2004; MAGALHÃES et al., 2012) (**Figura 8**).



Figura 8. Ceco de tartaruga-de-couro (*Dermochelys coriacea*) sub-adulta. Foto: (Projeto Cetáceos da Costa Branca – PCCB/UERN, 2019).

As funções gerais do intestino grosso (ceco, cólon e reto) baseia-se na recuperação de fluidos e eletrólitos e o armazenamento de fezes até a eliminação, este é um órgão do aparelho digestório que pode variar bastante entre as espécies (COLVILLE, 2010). Em tartarugas marinhas, o ceco está localizado na extremidade proximal do cólon e é um saco ligeiramente maior do que o resto do intestino. É mais bem diferenciado em tartarugas marinhas adultas das espécies *C. mydas* e *D. coriacea* e é menos pronunciado em espécies carnívoras e onívoras (WYNEKEN, 2004) (**Figura 8**). O cólon é caracterizado pela alternância de regiões abauladas representadas por saculação e estreitamento. As regiões abauladas apresentam mucosa lisa e o estreitamento é caracterizado por dobras retilíneas. A região caudal, denominada reto, é marcada por dobras retilíneas bem evidentes (MAGALHÃES et al., 2010; MAGALHÃES et al., 2012).

A cloaca da tartaruga marinha é uma estrutura comum aos sistemas digestório e urogenital, caracterizado por uma câmara que coleta as fezes, urina, ovos e fluxo de sêmen, e está conectada ventralmente à bexiga urinária. A cloaca se abre para o exterior e se divide em três compartimentos, o coprodeo, urodeo e proctodeo. O coprodeo recebe as fezes do reto, enquanto o urodeo contém as papilas urinárias e a abertura da bexiga. O proctodeo corresponde à região mais distal da cloaca e contém os ductos genitais do macho ou da fêmea e está associado à cópula (WYNEKEN 2004).

O conteúdo do tubo digestivo é movimentado e misturado por dois tipos principais de contrações de músculo liso, o peristaltismo (contrações peristálticas) e contrações segmentares. As contrações peristálticas movimentam o conteúdo ao longo do trato digestório. Elas são contrações musculares circulares que se movem ao longo do trato como ondas, empurrando o conteúdo do trato digestivo para a frente. As contrações segmentares provocam movimentos de mistura do conteúdo, para a frente e para trás. Elas são contrações musculares concêntricas e periódicas que ocorrem em áreas adjacentes diferentes. As contrações segmentares auxiliam a digestão e absorção ao misturar e diminuir a movimentação do conteúdo do trato digestivo (COLVILLE, 2010).

Nos répteis em geral, o tempo de passagem do alimento no trato gastrointestinal é mais longo e o trato digestório, apresenta-se relativamente mais curto quando comparado aos mamíferos (SILVERMAN; JANSSEN, 1996). A velocidade de digestão está intimamente relacionada com a temperatura, sendo que, em meses mais quentes, a digestão é mais rápida, e em meses mais frios, é mais lenta (CUBAS; SILVA; CATÃO-DIAS, 2014).

O fígado dos répteis desempenha as mesmas funções que o dos mamíferos, onde produz os ácidos biliares (bile) que é secretado no duodeno para emulsificação da gordura e assim facilitar a digestão dos lipídios. Ainda atua na degradação metabólica, desintoxicação do sangue e produz várias substâncias essenciais para o animal. O termo hepático refere-se ao fígado, que é dividido em vários lobos hepáticos, que são subdivididos microscopicamente em lóbulos hepáticos. O fígado é a maior fonte de proteínas sanguíneas importantes, como a albumina (COLVILLE, 2010). Em tartarugas marinhas o fígado é o maior dos órgãos viscerais; localiza-se ventralmente, mas profundamente ao esqueleto peitoral e ao peritônio. A coloração pode variar de marrom escuro a marrom avermelhada e é formado por dois lobos. O lobo direito contém a vesícula biliar na sua superfície ventral e é tipicamente maior que o lobo esquerdo (WYNEKEN, 2004).

O pâncreas é uma glândula composta que possui partes endócrina e exócrina. A parte exócrina produz enzimas como, amilase pancreática, proteases (como a tripsina) e a lipase que participam ativamente do processo digestivo enzimático, ainda, produz o bicarbonato de sódio, que atua na neutralização do conteúdo ácido proveniente do estômago e mantém um pH adequado para as enzimas atuarem no duodeno. Essas substâncias passam pelos ductos pancreáticos e drenam dentro do duodeno próximo à abertura do ducto biliar. A porção endócrina do pâncreas consiste em grupos isolados de células dispersas por toda a glândula. Essas áreas são denominadas de ilhotas pancreáticas, que produzem os hormônios que passam diretamente para a circulação sanguínea, o glucagon e a insulina, que ajudam a regular os

níveis sanguíneos da glicose proveniente do alimento digerido e absorvido (COLVILLE, 2010; FRANDSON; WILKE; FAILS, 2011).

Em tartarugas marinhas, o pâncreas está localizado ao longo do duodeno, logo após o nível do estômago. Estende-se para além do ducto colédoco, com a forma alongada irregular e, muitas vezes, acaba no baço ou logo após. A coloração pode variar entre a cor-de-rosa e amarelo (WYNEKEN, 2004).

2.7 Achados anatomopatológicos do aparelho digestório em tartarugas marinhas

As tartarugas marinhas vêm enfrentando várias ameaças, como poluição, perda de habitat, mudanças climáticas, interação com pesca, e ingestão de resíduos marinhos de origem antropogênica (BUGONI et al., 2001; THOMPSON, 2009; HAMANN et al., 2010). Nesse cenário a presença de lixo no trato digestório que tem sido reportada em diversos estudos como sendo causa de ruptura de órgãos e até mesmo de levar ao óbito os animais afetados (ORÓS et al., 2004; CAPPUA et al., 2007; FICHIA et al. 2016; FARIAS et al., 2019).

As principais alterações gastrointestinais observadas em tartarugas marinhas são: constipações, obstruções, perfurações, torções, íleo paralítico, intussuscepção e presença de corpos estranhos (BUGONI; KRAUSE; PETRY, 2001; DE VICO et al., 2003; ORÓS et al., 2004; CAPPUA et al., 2007; WYNEKEN; LOHMANN; MUSICK, 2013). Isso tudo ocorre devido à ingestão de resíduos provenientes da atividade humana, como: plástico, borracha, metais ou fibras (BUGONI; KRAUSE; PETRY, 2001; WYNEKEN; LOHMANN; MUSICK, 2013).

As principais consequências das lesões da mucosa do trato digestório associado à ingestão de resíduos sólidos, levam à isquemia, necrose e ruptura intestinal (LEVITT; BAUER, 1992; JERDY et al., 2017) (**Figura 9**). Como consequência, pode haver infecção bacteriana secundária e até morte do animal, como relatado em estudos com a presença da bactéria *Enterococcus faecalis* na bexiga, cérebro, intestino, rim, fígado, pulmão e músculos de um exemplar de tartaruga-cabeçuda com perfuração esofágica causada por anzol de pesca e intussuscepção intestinal causada por uma linha de nylon. Nesse caso, as lesões do esôfago ou do intestino, ou ambas, foram as prováveis vias de entrada para essa bactéria, e sua presença em órgãos e vísceras permitiu supor uma septicemia como causa da morte do animal (FICHIA et al., 2016).

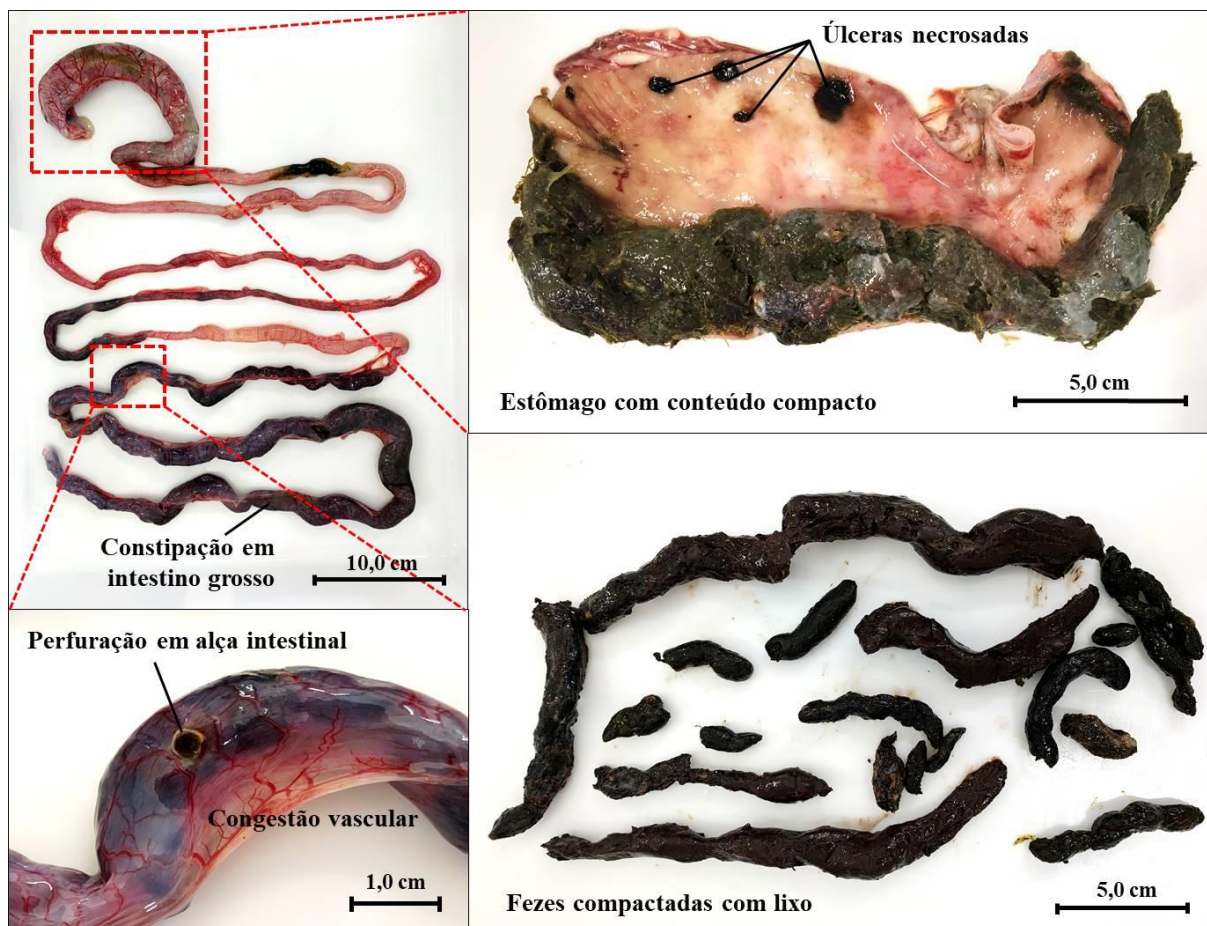


Figura 9. Achados anatomopatológicos no trato digestório em tartaruga-verde (*Chelonia mydas*) decorrente à ingestão de lixo. Foto: (Projeto Cetáceos da Costa Branca – PCCB/UERN, 2020).

Ao ingerir plástico, além dos aspectos físicos e anatômicos, os animais são expostos a uma fonte adicional de toxinas (TEUTEN et al., 2009; ANDRADY, 2011). Os aditivos químicos em plásticos e produtos químicos persistentes, bioacumuláveis e tóxicos, podem ser absorvidos pelo corpo após a ingestão (ENGLER, 2012; FOSSI et al., 2018). Isso amplifica ainda mais a carga de poluentes nos indivíduos por meio da transferência de presas para predadores, nas cadeias alimentares (ERIKSSON; BURTON, 2003).

No exame necroscópico, parasitas e lesões parasitárias são observados comumente em tecidos do sistema digestivo de tartarugas marinhas (INNIS et al., 2009). Muitos trematódeos e nematoides têm sido descritos na literatura mundial, sendo a maioria encontrada no aparelho digestório (GEORGE, 1997). Dentre os helmintos parasitos de tartarugas marinhas na Costa brasileira, apenas nematoides e trematódeos foram relatados. Entre os nematoides, três

espécies foram encontradas em *C. mydas*: *Kathlania leptura*, *Sulcascaris sulcata* e *Tonaudia freitasi* (VICENTE et al., 1993).

Os trematódeos digenéticos têm sido comumente encontrados parasitando o trato gastrointestinal de tartarugas-verdes. No Oceano Atlântico ao norte da Costa Rica já foram registradas várias espécies deste grupo, como *Amphiorchis solus*, *Charaxicephalus robustus*, *Cricocephalus resectus*, *Deuterobaris intestinalis*, *Hapalotrema postorchis*, *Microscaphidium warui*, *Monticellius indicum*, *Pleurogonius sindhii*, *Ritidodoides intestinalis*, *Rhytidodoides similis* e *Octangium hiphalum* (SANTORO et al., 2006).

Os achados necroscópicos associados aos parasitas gastrointestinais resultam da presença de parasitas adultos e granulomas nos vasos sanguíneos, como mais comumente observados, ocasionam oclusão do vaso sanguíneo na serosa do trato gastrointestinal. Assim, ocasionam congestão linfática, estase gastrointestinal e lesão por compressão no cérebro (FLINT et al., 2010; STACY et al., 2010). A análise histopatológica evidencia lesão e inflamação vascular causada pelos êmbolos de ovos dos trematódeos (GORDON; KELLY; CRIBB, 1998; STACY et al., 2010).

Torrent et al. (2002), relataram um caso de um grande divertículo esofágico em uma tartaruga-cabeçuda na junção do esôfago e estômago. O divertículo estava cheio de gás e fluido mucoide verde e uma membrana fibrinonecrótica amarela espessa e difusa cobria a mucosa. O lúmen do divertículo também continha um número moderado de cefalópodes, crustáceos e resíduos antropogênicos, incluindo petróleo bruto, plásticos e linhas de pesca. Histologicamente, verificou-se esofagite fibrinonecrótica difusa grave e o *Aerococcus viridans* foi isolado do divertículo.

Em tartarugas marinhas, casos de intussuscepção intestinal já foram relatados em tartarugas-cabeçudas (DE VICO et al., 2003, FICHIA et al., 2016) e em tartarugas-verde (CAPPUA et al., 2007; OLIVEIRA et al., 2021c). As maiores consequências citadas desta enfermidade são obstrução parcial ou total. A obstrução permanente dos vasos está relacionada com isquemia e necrose, fatores determinantes para o desenvolvimento de perfurações e rupturas intestinais tardias (PATSIKAS et al., 2005). Fatores predisponentes são frequentemente associados como gastroenterites, parasitismo e corpos estranhos (MACPHAIL, 2002; OLIVEIRA et al., 2021c). Todavia, em tartarugas marinhas o principal fator da intussuscepção tem sido associado à presença de resíduos sólidos (DE VICO et al., 2003; ORÓS et al., 2004; CAPPUA et al., 2007). Entretanto, recentemente, um caso de intussuscepção colocolica foi relatado em uma tartaruga-verde associado ao parasita do gênero *Octangium* sp. (OLIVEIRA et al., 2021c).

3 JUSTIFICATIVA

Estudos morfológicos do aparelho digestório das tartarugas marinhas fornecem informações básicas e muito importantes para a biologia das espécies, principalmente àquelas ameaçadas de extinção. Tais estudos podem elucidar os mecanismos de digestão e absorção dos nutrientes, favorecendo, conseqüentemente, o entendimento sobre o comportamento alimentar desses animais em vida livre. Adicionalmente, podendo auxiliar às pesquisas que abordam a composição da dieta em diferentes faixas etárias (filhote, juvenil e adulto). Nesse sentido, existe uma grande lacuna sobre a composição da dieta de filhotes da espécie *E. imbricata*, existindo apenas a hipótese que a dieta nessa faixa etária é totalmente carnívora, portanto, descrever a morfologia do aparelho digestório pode contribuir com a avaliação de processos evolutivos e manejo das espécies para reabilitação e conservação.

O diagnóstico *ante mortem* de doenças do trato digestório em tartarugas marinhas é de difícil definição, e nem sempre diagnosticada prontamente, para isso requer da utilização de tecnologias para a obtenção de um diagnóstico prévio, e uma possibilidade é o uso dos exames de imagens, uma alternativa para identificar as possíveis alterações gastrointestinais, principalmente, decorrentes da ingestão de resíduos antrópicos. Os exames de imagens como um todo, demonstram serem importantes, pois podem indicar o tipo de material ingerido, assim como a localização do mesmo, permitindo, assim, a adequada tomada de decisão para o tratamento do animal.

A ingestão de resíduos antrópicos já foi relatada em todas as espécies de tartarugas marinhas em todo o mundo. Essa condição traz muitos prejuízos a homeostase dos animais, afetando primeiramente o trato gastrointestinal, resultando em alterações teciduais graves, o que compromete a capacidade de alimentação e digestão, causando desnutrição, doenças e redução da taxa de crescimento, podendo inclusive levar o animal ao óbito. Investigar os principais achados anatomopatológicos das tartarugas marinhas por meio do exame necroscópico, em especial, o aparelho digestório, é importante para avaliar os efeitos e impactos promovidos pela ingestão do lixo em ambiente marinho. Portanto, entender como essas alterações teciduais levam ao óbito dos animais, adicionalmente, associando aos sinais clínicos dos animais *ante mortem* em processo de reabilitação, podem fornecer informações importantes sobre o prognóstico dos próximos animais que darão entrada nos centros de reabilitação.

4 HIPÓTESES CIENTÍFICAS

4.1 – As características morfológicas macro e microscópica do trato digestório de filhotes de tartarugas marinhas da espécie *Eretmochelys imbricata* está diretamente relacionado ao hábito alimentar, e, evolutivamente, essas características são primitivas quando comparadas aos quelônios semi-áquaticos e terrestres;

4.2 – O uso do diagnóstico por imagem é uma ferramenta útil para o diagnóstico *ante mortem* das alterações do trato digestório das tartarugas marinhas, decorrentes da ingestão do lixo marinho; e;

4.3 – A ingestão de resíduos antrópicos pelas tartarugas marinhas causam alterações teciduais do trato digestório, trazendo prejuízos aos mecanismos de digestão e absorção dos alimentos, e está relacionado com as causas de encalhes desses animais.

5 OBJETIVOS

5.1 Objetivo Geral

Caracterizar morfologicamente o trato digestório de filhotes de tartarugas marinhas da espécie *Eretmochelys imbricata*, a fim de compreender os processos adaptativos e evolutivos associados a hábitos alimentares, bem como descrever os principais achados imagiológicos e anatomopatológicos do trato digestório dos animais que encalharam nas praias do litoral do Rio Grande do Norte, Brasil.

5.2 Objetivo Específicos

5.2.1 Descrever a morfologia do trato digestório de filhotes de tartarugas marinhas da espécie *Eretmochelys imbricata*, a fim de identificar às variações anatômicas em filhotes e adaptações à dieta;

5.2.2 Realizar uma revisão bibliográfica sobre a ingestão de resíduos marinhos pelas tartarugas marinhas e seus efeitos no trato digestório, bem como os principais achados imagiológicos relacionados à ingestão desses resíduos; e;

5.2.3 Descrever casos clínicos de achados anatomopatológicos em tartarugas marinhas que encalharam no litoral do Rio Grande do Norte, a fim de elucidar as possíveis alterações teciduais no trato digestório, para investigar as causas dos óbitos ou encalhes desses animais, e contribuir com informações para o aprimoramento do manejo das tartarugas marinhas em reabilitação.

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**CAPÍTULO 2 – OROPHARYNGEAL CAVITY FLOOR MORPHOLOGY IN
Eretmochelys imbricata (TESTUDINES: CHELONIIDAE) HATCHLINGS AND
EVOLUTIONARY IMPLICATIONS**

(Artigo experimental)

**SUBMETIDO NA THE ANATOMICAL RECORD: ADVANCES IN INTEGRATIVE
ANATOMY AND EVOLUTIONARY BIOLOGY**

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 2.064

Oropharyngeal cavity floor morphology in *Eretmochelys imbricata* (Testudines: Cheloniidae) hatchlings and evolutionary implications

Running title: Oropharyngeal morphology in Hawksbill Turtle

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Abstract

Studies on chelonian oropharyngeal cavity morphophysiology have become an interesting tool in the understanding of adaptive processes associated with feeding habits in aquatic animals and the transition from aquatic to terrestrial forms. In this context, the aim of the present study was to describe the oropharyngeal cavity floor morphology of hawksbill sea turtle (*E. imbricata*) hatchlings. Ten stillborn hatchlings of undefined sex were obtained from nests hatched on the coast of the state of Rio Grande do Norte, Brazil. The heads of each specimen were fixed, dissected, and analyzed at the macro and microscopic levels. The oropharyngeal cavity floor of the hawksbill sea turtle hatchlings is formed by the tongue, pharynx, floor muscles and the hyolingual skeleton, delimited in the rostral and lateral directions by a keratinized beak, called the rhamphotheca, and in the caudal region at the limit between the pharynx and the esophagus. The tongue muscles and the muscles that support the floor of the oral cavity comprise the following: m. hypoglossohyoideus, m. hypoglossoglossus, m. hyoglossus, m. genioglossus, m. constrictor laryngis, m. geniohyoideus pars lateralis and m. intermandibularis. Histologically, the oropharyngeal cavity floor mucosa is formed by keratinized stratified squamous epithelium and the lamina propria, by loose connective tissue. The floor mucosa is devoid of taste buds. Evolutionarily, we believe that the basic oropharyngeal cavity floor hawksbill sea turtle hatchling characteristics reinforce the idea that these animals are primitive and that semi-aquatic and terrestrial turtles have undergone adaptations to feed out of water.

Keywords: Oral cavity, pharynx, tongue, hawksbill turtle, sea turtle.

Introduction

Hawksbill sea turtles (*Eretmochelys imbricata*) belong to the Testudines order and Cheloniidae family (Meylan & Meylan, 1999). This species is omnivorous, foraging on coral reefs, rocks and in estuarine and mangrove bay areas. Preferential hawksbill sea turtle food items comprise porifera, cnidarians and some algae species, although adults feed mainly on porifera, playing an important role in coral reef maintenance worldwide (Carrión-Cortez et al., 2013; Von Brandis et al., 2014).

Understanding evolutionary mechanisms and patterns can aid not only in reconstructing tetrapod history, but also in improving knowledge concerning major evolutionary transition mechanisms (Stayton, 2011). In this context, one of the most important steps in vertebrate evolution comprises the transition from aquatic to terrestrial forms, which required major

changes in almost all organ systems, as well as in feeding mechanisms (Deban & Marks, 2002).

The oropharyngeal cavity plays an important role in several functions, including feeding, in vertebrates (Druzisky & Brainerd, 2001). The morphological structure of the oropharyngeal cavity of reptiles, especially the dorsal surface of the tongue, has been studied in many chelonians (Iwasaki et al., 1992; Natchev et al., 2010; Pinheiro et al., 2010; Heiss et al., 2011; Natchev et al., 2011; Lintner et al., 2012; Silveira et al., 2015). Scarce data have been published on the morphological structure of the oropharyngeal cavity of sea turtles, however, compared to terrestrial, semi-aquatic and aquatic turtles (Natchev et al., 2010; Pinheiro et al., 2010; Heiss et al., 2011; Silveira et al., 2015).

Most aquatic turtles employ a feeding mode based on the rapid expansion of the oropharyngeal cavity to create inertial or compensatory suction (Aerts et al., 2001). This strategy, however, does not work on land due to the low viscosity and density of air compared to water (Bramble & Wake, 1985). Studies on chelonian oropharyngeal cavity morphophysiology have become an interesting tool in the understanding the evolution of feeding habits in the transition from aquatic to terrestrial forms (Natchev et al., 2010; Heiss et al., 2011; Lintner et al., 2012). In this context, the aim of the present study was to describe the oropharyngeal cavity floor morphology of hawksbill sea turtle hatchlings to establish a standard model for the species concerning this age group, providing information that can further knowledge on the food intake process in these animals from an evolutionary point of view.

Material and Methods

Animals

Ten stillborn hawksbill sea turtle hatchlings of undefined sex (45.93 ± 2.09 mm of curvilinear carapace mean length and 41.89 ± 1.54 mm of curvilinear carapace width) from nests hatched on the coast of the state of Rio Grande do Norte, Brazil, were analyzed. The specimens were stored at the Marine Biota Monitoring Laboratory – Costa Branca Cetacean Project collection, located at the State University of Rio Grande Norte. Sea turtle heads were removed in the field and immediately immersed in aqueous 10% buffered formaldehyde or 2.5% glutaraldehyde solutions. Authorization was obtained from the Federal Rural University of the Semi-Arid Ethics Committee on the Use of Animals (No. 01/2019) and the Biodiversity Authorization and Information System (SISBIO, License No. 13694-8).

Macroscopical analyses

The hawksbill sea turtle hatchling heads were dissected after 72 hours fixing. Initially, an incision was made at the mouth commissures for mandible separation and removal, followed by entire oropharyngeal cavity floor removal, from the limit of the apex of the tongue with the dental bone of the mandible to the limit of the pharynx with the esophagus. The International Committee on Veterinary Gross Anatomical Nomenclature (2017) was employed herein, and the results were compared with reports on other Testudines species.

Light microscopy analyses

The histological techniques applied for the light microscopy analyses followed Tolosa et al. (2003). Briefly, 5 µm thick sections of the tongue and pharynx were obtained using a Leica RM 2125 RT® microtome, adhered to glass slides and left in an oven at 60° C overnight, followed by staining with Hematoxylin and Eosin (HE) and Gomori's trichrome (GT) and histochemistry assessments. The slides were analyzed under a Leica DM 500 HD light microscope with an attached Leica ICC50W camera and images were obtained using the LAS EZ Ink program.

Histochemistry analyses

The 5 µm tongue and pharynx sections were subjected to histochemical reactions using a combination of Periodic Acid Schiff (PAS) associated with Alcian blue (pH 2.5) to label neutral and acidic mucins, respectively (Behmer et al., 1976). Histochemical mucin pattern characterizations (Table 1) were based on the organic secretory unit secretion components, with histochemical reactions classified as negative or positive.

Table 1. Specification of the histochemical stains used on the floor of the oropharyngeal cavity of hawksbill turtle (*Eretmochelys imbricata*) hatchlings.

Method	Stains	Acquired color
Periodic Acid Schiff (PAS)	Stains neutral mucins	Magenta
Alcian blue (AB, pH 2.5)	Stains acid mucins	Blue

Scanning Electron Microscopy (SEM)

For the SEM analyses, samples from the tongue and pharynx were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.4, 4 °C and washed three times in 0.1 M sodium phosphate buffer pH 7.4 for 10 minutes, post-fixed in 2% Osmium Tetroxide buffered with 0.1 M sodium phosphate pH 7.4 for 1 h followed by 20 min in an oven. Subsequently, the samples were treated with 20% HCl at 60 °C for 20 min, followed by three washes in the buffer and two with distilled water to remove any surface mucus and then dehydrated in increasing alcohol series (15%, 25%, 30%, 50%, 70%, 90%), once for 5 min at each concentration, and 100% (three 10 min washes each). The material was dried using a critical point Quorum K 850 apparatus using carbon dioxide mounted on a PELCO TabsTM sample point support stub (Ted Pella, Inc., Redding, CA, USA) for gold sputtering metallization (Q150R ES, Quorum Technologies Ltd., Laughton, East Sussex, UK). Images were captured with a secondary electron SEM detector (TESCAN SEE 3 LMU, Tescan, Czech Republic) operating with a 10kV electron beam.

Results

The floor of hawksbill sea turtle hatchling oropharyngeal cavities is formed by the tongue, pharynx, floor muscles and hyolingual skeleton, delimited in the rostral and lateral directions by a keratinized beak, called the rhamphotheca, and in the caudal region at the limit between the pharynx and the esophagus. The tongue is a muscular and robust organ categorized into apex, body, and root. It has a wide base, which narrows towards the apex, making it triangular with a slightly rounded apex when viewed dorsally, but flattened when viewed laterally. The pharynx is delimited between the root of the tongue and the beginning of the esophagus. The larynx (glottis) entrance is observed in the most rostral portion of the pharynx, covered more rostrally by the glottic cleft, which defines the oropharyngeal junction and is easily identified by the rhyme glottis, formed by two juxtaposed combined laryngeal folds (Figs. 1A, 2A and 2B).

The tongue mucosa is lined by keratinized stratified squamous epithelium. The mucosal epithelium presents four layers (basal, deep intermediate, shallow intermediate and keratinized). Cells are almost spherical in the basal and deep intermediate layers, with spherical and large nuclei. The deep middle layer is composed of only a few layers of cells, flattened and a rapid transition to the keratinized layer in the most superficial intermediate layer (Figs. 1B – 1F). A new finding is reported herein for hawksbill sea turtles, namely the presence of non-keratinized epithelial cells positively stained by AB present on the inner

surface of the glottic cleft (Figs. 1K and 1L). The lamina propria is formed by loose connective tissue and located between the mucosal epithelium and the lingual muscles (Figs. 1C, 1F and 1H). The mucosa is similar to the tongue epithelium observed in a pharyngeal cross-section view. The first tracheal rings are observed in the same region, with the mucosa formed by the pseudostratified cylindrical ciliated epithelium and the submucosa formed by connective tissue and hyaline cartilage, with an extracellular matrix strongly stained by PAS. Parallel to the trachea, a pair of hypoglossal nerves and blood vessels were observed in both antimeres of abductor arytenoid muscles (Figs. 1G, 1H and 1I).

The lingual mucosa of hawksbill sea turtle hatchlings is supported by oral cavity floor musculature and by the hyolingual skeleton. The tongue muscles and the muscles that support the oral cavity floor comprise the following: *m. hypoglossohyoideus*, *m. hypoglossoglossus*, *m. hyoglossus*, *m. genioglossus*, *m. constrictor laryngis*, *m. geniohyoideus pars lateralis* and *m. intermandibularis*. The hyolingual skeleton consists of a central cartilaginous body (*corpus hyoidei*) presenting a thin, triangular-shaped anterior process, the lingual process. The *corpus hyoidei* is articulated laterally to the right and left keratobranchial cornus. The nerve hypoglossum is located anteriorly, immediately ventral to the *corpus hyoidei* in the lingual process region, comprising a cartilaginous element with a thin anterior and posterior process (Fig. 1).

The *m. hypoglossohyoideus* originates laterally in the distal portion of the lingual process of the *corpus hyoidei* and runs caudoventrally to finally insert dorsolaterally into the hypoglossum. The *m. hypoglossoglossus* originates laterally in the anterior portion of the hypoglossum. The muscle fibers of the right and left portion of the *m. hypoglossoglossus* follow straight up and are inserted into the dorsal mucosa of the tongue. More posteriorly, the fibers of the right and left portions curve to the midline, where they meet and formed an arc-shaped myostructure (Figs. 3B and 3D).

The *m. hyoglossus* originates in the proximal part of the gill horn, lateral to the articulation with the *corpus hyoidei* and runs anterodorsally, dorsal to the hypoglossum to then insert into the dorsal lingual mucosa of the anterior tongue, lying between the *m. hypoglossoglossus*, the *m. genioglossus* and the dorsal lingual mucosa (Figs. 1B, 1D, 1E and 1G).

The *m. genioglossus* originates from the dental bone, lateral to the mandibular symphysis, and runs posterolaterally along the entire length of the tongue. Previously, *m. genioglossus* fibers spread vertically and are inserted into the dorsal mucosa of the tip of the

tongue. Later, the fibers begin to orient themselves longitudinally, forming the muscular sides of the tongue (Figs. 1B, 1D and 1E).

The most important muscles that support the oral cavity floor are the *m. geniohyoideus pars lateralis* and *m. intermandibularis*. The *m. geniohyoideus pars lateralis* originates laterally in the mandibular symphysis and runs rostromedially to finally insert into the proximal third of the cornu branchiale to the articulation of the cornu branchiale and corpus hyoidei. The *m. geniohyoideus pars lateralis* is found dorsally to *m. intermandibularis*. The *m. intermandibularis* runs transversely between the halves of the mandible just below the throat integument. It originates medially from the dentary, and both contralateral parts are connected by a medial aponeurosis (*raphe medialis*), which represents the insertion side (Fig. 1G).

Concerning tongue SEM analyses, no lingual papillae were observed on the dorsal tongue surface. Instead, numerous folds are noted, formed by the epithelial projections, distributed over the entire dorsal surface of the tongue, as well as at its root (Figs. 2A and 2B). These lingual folds resemble lingual papillae when viewed under light microscopy in cross-section. Each cell on the surface of the tongue and pharynx exhibits a clearly polygonal profile. Keratin epithelium protrusions are frequently distributed over the dorsal surface of the tongue and pharynx. Thickened cell margins are clearly visible (Figs. 2C and 2D).

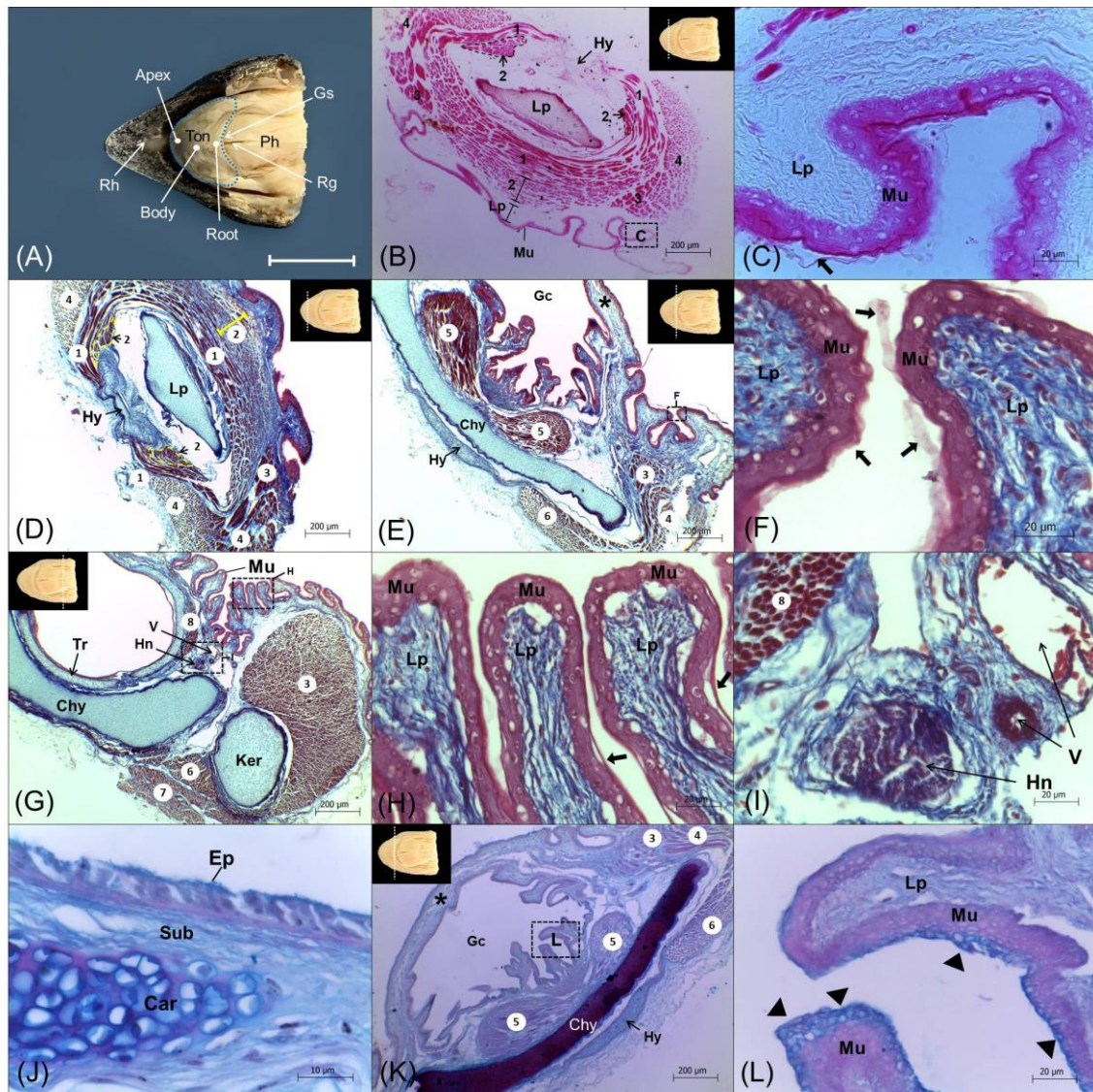


Figure 1. Light microscopy and histochemical characterization of the oropharyngeal cavity floor of hawksbill sea turtle (*Eretmochelys imbricata*) hatchlings. **A:** dorsal view of the floor of the oropharyngeal cavity. Bar: 0.5cm. **B:** cross section of the tongue body. **C:** Detail of Figure B. **D:** cross section of the tongue body. **E:** cross section of the tongue root. **F:** Detail of Figure E. **G:** cross section of the pharyngeal region. **H** and **I:** Detail of Figure G. **J:** tracheal histochemistry - cross section. **K:** tongue root histochemistry- cross section. **L:** Detail of Figure K. Rhamphotheca (Rh); tongue (Ton); pharynx (Ph); glottic cleft (Gc); rhyme glottis (Rg); tongue mucosa (Mu); lamina propria (Lp); lingual process (Lp); corpus hyoidei (Chy); hypoglossum (Hy); right keratobranchial (Ker); m. hypoglossohyoideus (1); m. hypoglossoglossus (2); m. hyoglossus (3); m. genioglossus (4); m. laryngis constrictor (5); m. geniohyoideus pars lateralis (6); m. intermandibularis (7); m. arytenoid abductor (8); keratin detachment (arrow); lingual fold covering the glottic cleft at the base of the tongue (*); cells positively stained with AB (arrowheads); trachea (Tr); blood vessels (V); hypoglossal nerve

(Hn); ciliated cylindrical pseudostratified epithelium (Ep); submucosa (Sub); hyaline cartilage of the trachea (Car). Stains – B and C: Hematoxylin and Eosin. D, E, F, G, H and I: Gomori Trichome. J, K and L: Alcian Blue (pH 2.5) and Periodic Acid Schiff.

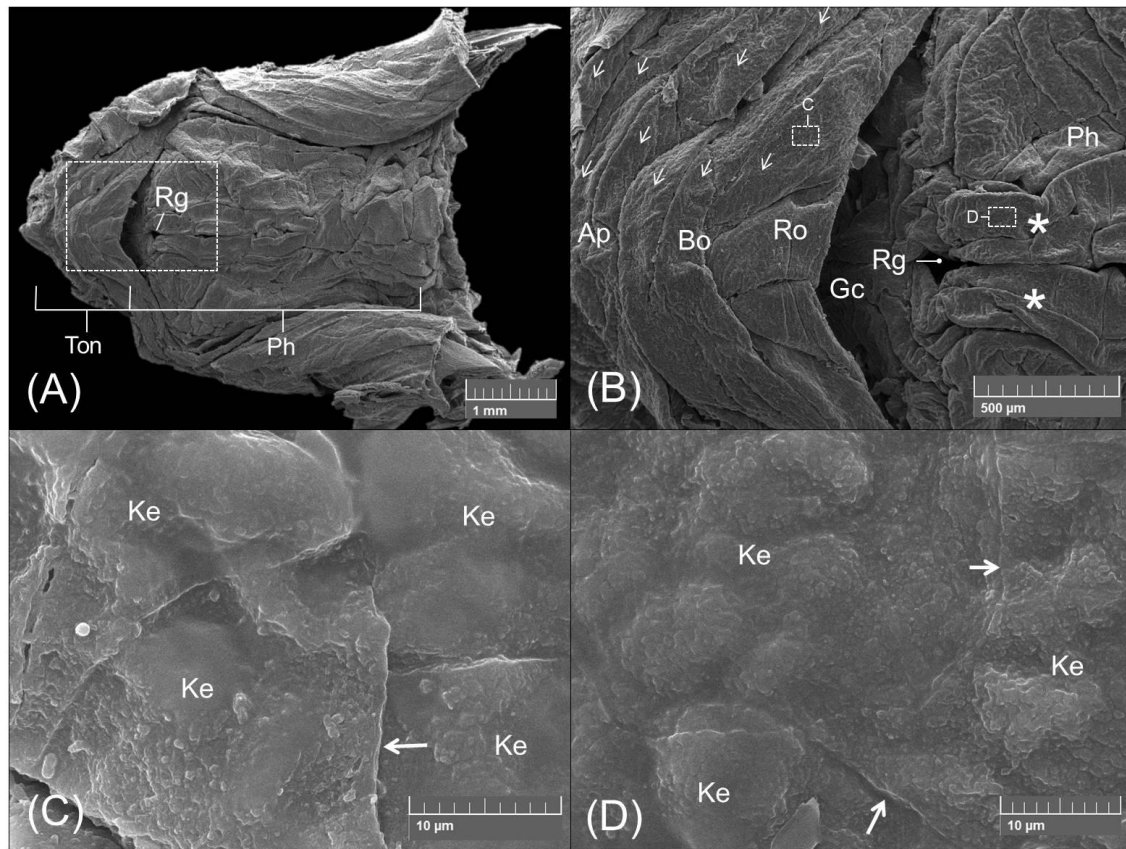


Figure 2. Scanning electron microscopy of the oropharyngeal cavity floor of hawksbill sea turtle (*Eretmochelys imbricata*) hatchlings. **A:** dorsal view of the tongue (Ton), pharynx (Ph) and the rhyme glottis (Rg). **B:** Detail of Figure A, indicating the three regions of the tongue, the apex (Ap), the body (Bo), and the root (Ro), in addition to the pharyngeal region (Ph), glottic cleft (Gc), rhyme glottis (Rg), lingual folds (arrows) and laryngeal folds (*). **C and D:** Details of Figure B, indicating dorsal tongue surface epithelium and pharynx keratinocytes (Ke), cell margin thickening (arrow).

Discussion

The tongue of hawksbill sea turtle hatchlings is a muscular and robust organ, with a triangular shape, a wide base and a slightly rounded apex, similar to the findings described by Iwasaki et al. (1996a) for hawksbill sea turtle and Olive ridley sea turtle (*Lepidochelys olivacea*) hatchlings (Iwasaki et al., 1996b). In contrast, some semi-aquatic, aquatic, and terrestrial turtles have a slightly longer tongue (Natchev et al., 2010; Pinheiro et al., 2010;

Heiss et al., 2011; Silveira et al., 2015). This shape allows for a certain skill in handling food within the oral cavity (Natchev et al., 2011). The evolution of a mobile tongue as part of the food system is one of the key innovations behind aquatic-terrestrial transitions, providing a useful tool for exploiting food resources in the new environment (Lintner et al., 2012).

The findings reported herein for the pharyngeal region are similar to those reported by Sacchi et al. (2004), Silveira et al. (2015) and Fraher et al. (2010) for the semi-aquatic, aquatic and marine turtles *Heosemys grandis*, *Podocnemis expansa* and *Dermochelys coriacea*, respectively, as both authors reported that the entrance of the larynx (glottis) is located at the base of the tongue, which gives continuity caudally to the trachea, whose wall is formed by cartilaginous rings. According to Habova et al. (2022), the rhyme glottis in Testudines is similar to a narrow slit, oriented rostrocaudally. These authors also state that this slit is approximately twice as short and narrow as in terrestrial turtles in aquatic and semi-aquatic turtles, seemingly indicating that a shorter and narrower entry into the larynx of aquatic turtles represents their protective adaptation mechanism against water entering the lower respiratory tract.

Many differences in the characteristics of the dorsal lingual epithelium are noted in reptiles, which can be categorized phylogenetically and, at the same time, reflect reptile adaptations to their environments to some extent (Iwasaki et al., 1996a). For example, the lingual epithelium of completely terrestrial reptiles is mostly keratinized (Iwasaki & Kumakura, 1994; Iwasaki & Yoshihara 2003; El-Mansi et al., 2020), whereas that of reptiles that live in or near ponds, or small rivers tend to be non-keratinized (Iwasaki, 1992; Iwasaki et al., 1992; Beisser et al., 1998). The tongue of reptiles living in amphibious circumstances tends to exhibit both keratinized and non-keratinized epithelium in different regions (Iwasaki, 1990; Iwasaki & Kobayashi, 1992). In our study, hawksbill turtle hatchlings presented a predominantly keratinized tongue and pharynx epithelium, which may be associated to their diet of mainly coral reef sponges (Carrión-Cortez et al., 2013; Von Brandis et al., 2014), as keratinization provides protection against abrasion (Iwasaki, 2002). Because of this, we assume that these animals exhibit greater lingual mucosa protection, as sponges are rigid structures that may cause abrasive oral mucosa lesions. Additionally, the pharyngeal floor of leatherback sea turtles (*D. coriacea*) exhibits prominent longitudinal protrusions and caudally projected pharyngeal spines, a unique feature of this marine species (Fraher et al., 2010).

Morphological and functional tongue characteristics differ significantly among tetrapods. Many studies have demonstrated that the dorsal surface of the reptilian tongue is rich in lingual papillae (Iwasaki, 1990; Iwasaki et al., 1992; Beisser et al., 1998; Lintner et al.,

2012; Cizek et al., 2019), similarly to amphibians (Jasiflski, 1979; Żuwała & Jakubowski, 2001), birds (Jackowiak et al., 2011; Abumandour & El-Bakary, 2017; Skieresz-Szewczyk et al., 2021) and mammals (Okada & Schraufnagel, 2005; Masuko et al., 2007; Cienia et al., 2013; Barbosa et al., 2020; Barboza & Larkin, 2020; Reginato et al., 2020). Only snakes are currently known to lack lingual papillae on the entire tongue surface (Iwasaki & Kumakura, 1994; Iwasaki & Yoshihara, 2003; El-Mansi et al., 2020). In our study, no lingual papillae were observed on the dorsal surface of the tongue of hawksbill turtles. Instead, we detected numerous folds formed by epithelial projections distributed throughout the dorsal surface of the tongue, similar to the findings described by Iwasaki et al. for hawksbill sea, and olive ridley sea turtle hatchlings, respectively (Iwasaki et al., 1996a,b).

The occurrence and distribution of taste buds on the tongue and floor of the oropharyngeal cavity have been reported in different types of reptiles (Schwenk, 1985; Iwasaki & Kumakura, 1994; Iwasaki & Yoshihara, 2003; El-Mansi et al., 2020). According to Schwenk (1985), taste buds in lizards may be present exclusively on the tongue (*Chamaeleo jacksonii*), only on the floor of the oropharyngeal cavity (*Gonatodes antillensis*), or in both (Iguanidae family). In snakes, taste buds are distributed only on the floor of the oral cavity (Iwasaki & Kumakura, 1994; Iwasaki & Yoshihara, 2003; El-Mansi et al., 2020).

In chelonians, taste buds have been described in terrestrial, semi-aquatic and aquatic species, such as *Manouria emys emys* (Heiss et al., 2011), *H. grandis* (Lintner et al., 2012) and *P. expansa* (Silveira et al., 2015), respectively. On the other hand, Iwasaki et al. (1996a,b) did not verify the presence of taste buds in the lingual epithelium when studying the tongue morphology of hawksbill and olive ridley sea turtle hatchlings, similar to the results reported herein. However, the fact that there are no taste buds in the epithelium of the tongue of these animals does not exclude the possibility of the presence of these papillae in another oropharyngeal cavity region, as the presence of taste buds on the floor of the palate has been described in semi-aquatic (Lintner et al., 2012) and terrestrial (Heiss et al., 2011) species.

The mucus produced in the oropharyngeal cavity plays an important role in many functions associated with feeding as a lubricating medium. Likewise, aquatic turtles have only simple oropharyngeal glands, termed goblet cells (Nalavade & Varute, 1976; Winokur, 1988; Iwasaki, 1992; Beisser et al., 1998; Beisser et al., 2001), while land turtles have large glandular entities intricately arranged as tubular, branched or alveolar cells (Kochva, 1978; Wocheslander et al., 1999; Heiss et al., 2011). Regarding sea turtles, no previous studies are available on the existence of any type of mucus-producing cell or gland in the oropharyngeal cavity. In our assessment, however, we observed the presence of small groups of acid mucin-

producing cells in the glottic cleft. We believe that this mucus produced plays a role in lubricating and sealing the glottis, thus preventing water from entering the trachea during diving apnea.

The mobility of the tongue as a unit is largely coupled to hyolingual skeleton movement (Schwenk, 2000). The first step in tongue protraction was suggested by Heiss et al. (2011) when studying a land turtle, *M. emys emys*, where the protraction of the entire hyolingual skeleton involves the contraction of the m. geniohyoideus that is inserted in the m. genioglossus that runs to the dental bone. In a second stage, the contraction of the m. hypoglossohyoideus, which runs between the anterior part of the lingual process and the posterior hypoglossum, compresses these skeletal structures relative to each other. This causes rostral slippage of the hypoglossum in relation to the lingual process of the corpus hyoidei, resulting in further tongue elongation. This movement is probably stabilized and further supported by the contraction of m. medial and posterior hypoglossoglossus. The contraction of the arch-shaped m. hypoglossoglossus serves two functions, stabilizing the slippage of the hypoglossum (caused by m. hypoglossohyoideus) and sustaining this movement by pressurizing the lingual process, causing the hypoglossum to slide anteriorly along the lingual process.

Regarding tongue retraction, Lintner et al. (2012), suggested that the contraction of m. hyoglossus, which runs between the tip of the tongue and the first proximal gill horn, causes retraction of the hypoglossum in relation to the lingual process when studying the semi-aquatic species, *H. grandis*. Further tongue retraction can be caused by retraction of the entire hyolingual skeleton by the coracohyoideus muscle, which runs between the hyolingual skeleton and the shoulder girdle. Furthermore, *H. grandis* can also feed underwater, probably because it can create a certain amount of suction, similar at first to aquatic turtles, which it occurs by turning the hyolingual skeleton posteroventrally (Lauder & Prendergast, 1992; Lemell & Weisgram, 1997; Summers et al., 1998; Lemell et al., 2000; Heiss et al., 2008). We believe that hawksbill sea turtle hatchlings use the same suction mechanisms as aquatic turtles.

Hawksbill sea turtles, like all other sea turtles, lack the epiglottis (Schumacher, 1973). Consequently, closure maintenance depends on the juxtaposition of the laryngeal folds and the glottic cleft. The closing of the latter is produced by m. hyoglossus contraction, which pulls the tongue posteriorly, compressing the two surfaces of the glottic cleft, while the laryngeal folds are compressed against each other, aiding in completely closing the glottis. These effects combine to provide an efficient seal between the respiratory tract and the

oropharyngeal cavity, preventing water from entering the airways during diving and feeding (Fraher et al., 2010). During breathing, glottis opening takes place by the contraction of the two abductor arytenoid muscles (Schumacher, 1973).

Conclusions

Morphologically, hawksbill sea turtle hatchling tongues do not play an important role in feeding, as they have phylogenetically become considerably reduced and devoid of taste buds. The tongue, does, however, play an essential role in glottal closure during diving and feeding periods, under the action of the two hyoglossus muscles. Its function is, therefore, also associated with the breathing system. The pharynx is characterized as a common region for food passage and respiration (the glottis), a feature present in all Testudines which has also evolved into several other groups. The muscles of the floor of the oral cavity and the hyolingual skeleton aid in food ingestion during the suction act. Evolutionarily, we believe that the basic oropharyngeal cavity floor characteristics in hawksbill sea turtle hatchlings reinforce the idea that these animals are primitive and that semi-aquatic and terrestrial turtles have undergone adaptations to feed out of water.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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**CAPÍTULO 3 – MORPHOLOGICAL CHARACTERIZATION OF THE DIGESTIVE
TUBE OF HAWKSBILL SEA TURTLE (*Eretmochelys imbricata*) HATCHLINGS**

(Artigo experimental)

SUBMETIDO NA MICROSCOPY AND MICROANALYSIS

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A2

FATOR DE IMPACTO (2020): 4.127

**Morphological characterization of the digestive tube of hawksbill sea turtle
(*Eretmochelys imbricata*) hatchlings**

Running title: Morphology of the digestive tube of *Eretmochelys imbricata*

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Abstract

Morphological studies concerning the digestive system can further information on animal diets, thus aiding in understanding of feeding behavior. Given the scarcity of information on sea turtle digestive system morphology, the aim of the present study was to describe the digestive tube morphology of *Eretmochelys imbricata* hatchlings to further understand the diet of these individuals in the wild. Digestive tube (DT) samples from 10 stillborn turtles (undefined sex) were analyzed at the macro and microscopic levels. The esophagus, stomach, small intestine (SI) and large intestine (LI) are described. Histologically, the digestive tube is formed by four tunics, the mucosa, submucosa, muscular and adventitia or serosa. The esophagus is lined by keratinized stratified squamous epithelium, while the remainder of the DT is lined by a simple columnar epithelium. The esophagus mucosa is marked by conical, pointed papillae. The stomach comprises three regions, the cardiac, fundic and pyloric, and covered by neutral mucous granular cells. The intestinal mucosa presents absorptive cells with microvilli, neutral and acidic goblet cells and mucosa-associated lymphoid tissue. The SI is significantly longer than the LI (p value = 0.006841). These morphological findings are strong indications of adaptations to a carnivorous diet in this hawksbill turtle age group.

Keywords: Cheloniidae. histochemistry. morphology. morphophysiology. Testudines. Scanning Electron Microscopy.

Conflict of Interest

The authors declare that they have no conflict of interest.

Introduction

The hawksbill turtle (*Eretmochelys imbricata*) displays a complex life cycle and population structure presenting migratory behavior, moving hundreds to thousands of kilometers between feeding and spawning areas (Bowen, 1995). This species is globally distributed in tropical waters and, to a lesser extent, in subtropical waters (Mortimer & Donnelly, 2008), and currently faces several threats, such as pollution, habitat loss, climate change and human interactions (Saeki et al., 2000; Bugoni et al., 2001; Fujihara et al., 2003; Wilcox et al., 2018; Rizzi et al., 2019; Oliveira et al., 2021).

According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, hawksbill turtles are classified as “critically endangered”. Marine debris ingestion represents a major cause of mortality for this species, exposing animals to

gastroenteric disorders and additional toxin exposure (Andrady, 2011; Rizzi et al., 2019). This, in turn, causes nutrient digestion and absorption deficits, resulting in malnutrition and decreased growth and reproductive rates, and even death (Nelms et al., 2016).

Several studies indicate that hawksbill turtles are omnivorous in both the juvenile and adult stages, feeding preferentially on porifera, cnidarians and some algae, although when feeding mainly on porifera when adults, playing an important role in coral reef maintenance worldwide (Rincon-Diaz et al., 2011; Carrión-Cortez et al., 2013; Von Brandis et al., 2014). Some hypotheses indicate that when young, these animals present a completely carnivorous diet in open waters, as described for other young turtle species, such as the green turtle (*Chelonia mydas*) and the loggerhead sea turtle (*Caretta caretta*), which feed on zooplankton, molluscs and crustaceans (Reich et al., 2007).

Morphological digestive tract studies can identify the type of food that animals consume and metabolize, consequently aiding in understanding of feeding behavior (Ku et al., 2001; Tarakçi et al., 2005; Magalhães et al., 2010; Chen et al., 2015). Morphology studies, including with regard to the digestive system, have become increasingly relevant due to global warming, as this phenomenon may directly affect the diet of several species over time. In this context, the aim of the present study was to describe the morphology of the digestive tube of hawksbill turtle hatchlings in order to establish a standard model for this age group in this species. This will further information towards a better understanding of this species diet in the wild, in addition to providing important information for the food management of these animals in captivity during rehabilitation processes, consequently promoting sea turtle conservation actions.

Material and Methods

Animals

Ten stillborn hawksbill turtle hatchlings (undefined sex) (45.93 ± 2.09 mm curvilinear carapace length and 41.89 ± 1.54 mm curvilinear carapace width) from nests hatched on the coast of the state of Rio Grande do Norte, Brazil were assessed. The animals were fixed in aqueous buffered 10% formaldehyde or 2.5% glutaraldehyde by the intracoelomic, oral and cloacal routes and immediately immersed in the corresponding solutions at the same concentrations. All experiments were authorized by the Federal Rural University of the Semi-Arid Ethics Committee on the Use of Animals (No. 01/2019) and the Biodiversity Authorization and Information System (SISBIO, license no. 13694-8).

Macroscopic analysis

After fixing for 72 hours, the specimens were dissected according to Wyneken (2004). The coelomic cavity surrounding the entire plastron was first opened, followed by its removal and *in situ* organ analysis. Then, the entire digestive tube (DT) was removed for *ex situ* assessments from the tongue to the rectum. DT biometry was performed based on Magalhães et al. (2010), where the length in millimeters (mm) was determined with the aid of a cotton cord and a digital caliper. The cord was positioned along the entire DT, cut at the transition between segments and measured with a digital caliper (150mm MTX®). The morphology study was based on the nomenclature adopted by the International Committee on Veterinary Gross Anatomical Nomenclature (2017) and the results compared with the literature concerning other Testudines.

Light microscopy

Histological techniques recommended by Tolosa et al. (2003) were employed for the light microscopy assessments. Sections (5 µm) of the esophagus, stomach, duodenum, jejunum, ileum, colon and rectum were obtained using a Leica RM 2125 RT® microtome, adhered to glass slides and left in an oven at 60 °C overnight, for subsequent Hematoxylin and Eosin (HE) and Gomori's Trichrome (GT) staining and histochemistry analyses. The slides were analyzed under a light microscope (Leica DM 500 HD) with an attached camera (Leica ICC50W) and images were obtained using the LAS EZ Ink software.

Histochemistry analyses

The 5 µm sections were subjected to histochemical reactions using a combination of Periodic Acid Schiff (PAS) staining associated with Alcian blue (pH 2.5) to label neutral and acidic mucins, respectively (Behmer et al., 1976). Histochemical mucin pattern characterizations (Table 1) were based on the organic components of secretory unit secretions, with histochemical reactions classified as negative or positive.

Table 1. Histochemical stains used for digestive tube assessments in hatchling hawksbill turtles (*Eretmochelys imbricata*).

Method	Stains	Acquired color
Periodic Acid Schiff (PAS)	<i>Neutral mucins</i>	Magenta
Alcian blue (AB pH 2.5)	<i>Acid mucins</i>	Blue

Scanning Electron Microscopy (SEM)

Samples were collected from the entire digestive tube and fixed in 2.5% glutaraldehyde buffered with 0.1 molar sodium phosphate at pH 7.4 at 4°C. Three washes in 0.1M sodium phosphate buffer and pH 7.4 for 10 minutes were then performed, followed by post-fixing in 2% Osmium Tetroxide buffered with 0.1M Sodium Phosphate and pH 7.4, for 1h and 20 min in an oven. Subsequently, the samples were treated with 20% HCl at 60 °C for 20 min, followed by three buffer washes and two distilled water washes to remove mucus from the surface, and dehydration in an increasing alcohol series at 15%, 25%, 30%, 50%, 70%, 90% (once for 5 min at each concentration) and 100% (three times for 10 min). The samples were then dried in a critical point apparatus (Quorum K 850) using carbon dioxide (CO₂) and mounted on a “stub” sample point support (PELCO Tabs™, Ted Pella, Inc., Redding, CA, USA) for gold metallization through sputtering (Q150R ES, Quorum Technologies Ltd., Laughton, East Sussex, UK). Images were captured using a secondary electron detector in SEM (TESCAN SEE 3 LMU, Tescan, Czech Republic) employing a 10kV electron beam.

Statistical analyses

Data were analyzed using descriptive statistical procedures, obtaining the means and standard deviations of each analyzed structure. The Wilcoxon test (non-parametric test) was used to detect significant differences between small and large intestine lengths. A p value < 0.05 was considered statistically significant. The R Project for Statistical Computing program (08/2018) was used for all analyses.

Results

The digestive tube (DT) of hawksbill turtle hatchlings is formed by the esophagus, stomach, small intestine (SI - duodenum, jejunum and ileum) and large intestine (LI - colon and rectum) (Figure 1). The mean DT length was 168.22 ± 21.01 mm, 3.66-fold longer than the mean curvilinear carapace length (45.93 ± 2.09 mm). The means and standard deviations of each segment were as follows: esophagus 21.77 ± 3.21 mm, stomach 21.7 ± 1.66 mm, small intestine 69.53 ± 10.09 mm and large intestine 55.21 ± 10.36 mm. Intestine sizes were statistically different (p value = 0.006841), with the highest length values observed for the small intestine (Figure 2).

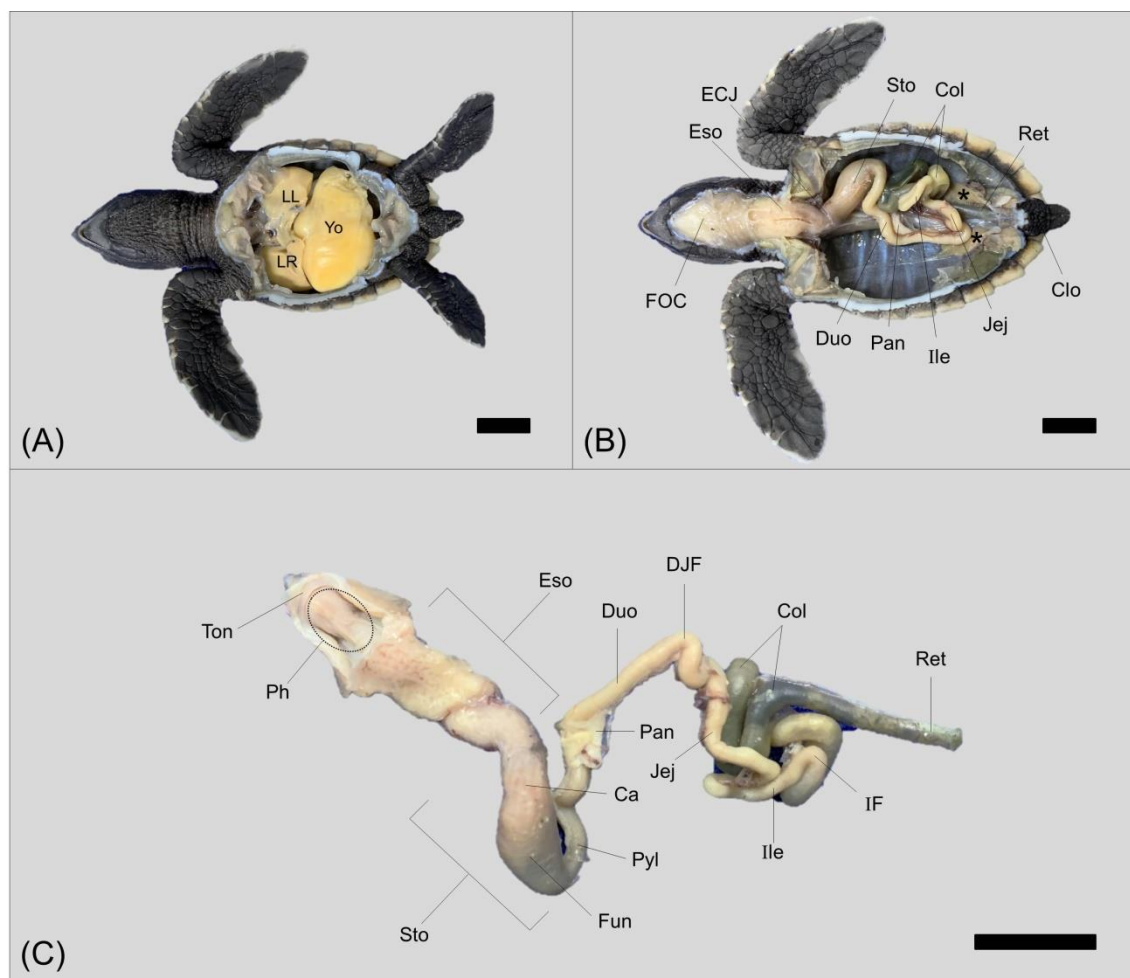


Figure 1. Digestive tube of hawksbill turtle (*Eretmochelys imbricata*) hatchlings. **A:** Ventral view, right hepatic lobe (LR) and left hepatic lobe (LL), yolk (Yo). Bar: 1.0 cm. **B:** *in situ* digestive tract, containing the floor of the oral cavity (FOC), esophagus (Eso), esophagus-cardial junction (ECJ), stomach (Sto), pylorus (Pyl), duodenum (Duo), pancreas (Pan), jejunum (Jej), ileum (Ile), colon (Col), rectum (Ret), Cloaca (Clo) and kidneys (*). Bar: 1.0 cm. **C:** *ex situ* digestive tract, in dorsal view, containing the tongue (Ton), pharynx (Ph),

esophagus (Eso), stomach (Sto), cardiac region (Ca), fundic region (Fun), pylorus (Pyl), duodenum (Duo), pancreas (Pan), duodenojejunal flexure (DJF), jejunum (Jej), ileum (Ile), ileocolic flexure (IF), colon (Co) and rectum (Ret). Bar: 1.0 cm.

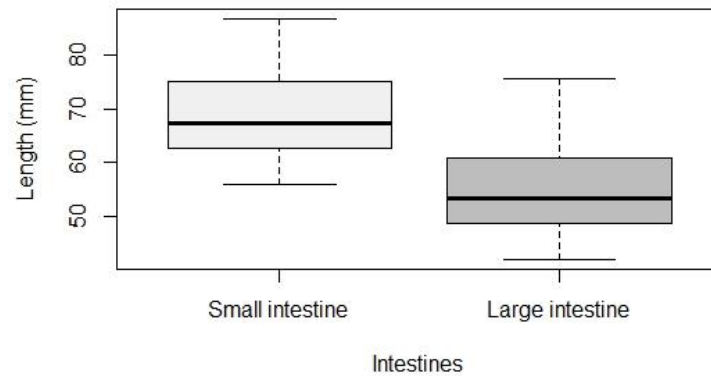


Figure 2. Comparison between small and large intestine lengths (mean and standard deviation) (in millimeters) of hawksbill turtle (*Eretmochelys imbricata*) hatchlings. The small intestine measured 69.53 ± 10.09 mm and the large intestine, 55.21 ± 10.36 mm). Statistical test: p value = 0.006841.

Esophagus

The esophagus of hawksbill turtle hatchlings is an L-shaped tubular muscular organ, considerably wide in the anterior and middle portions, and narrow in the posterior portion, located medially and ventrally to the cervical region. Its posterior section, in the coelomic region, is moved to the left, where the stomach cardia is located. The esophagus is marked by conical, pointed papillae oriented towards the stomach, gradually becoming larger between the anterior and the middle portion and short and blunt in the esophagus-cardia transition (Figure 3A).

The esophageal mucosa is covered by keratinized stratified squamous epithelium (Figures 3C and 3E). The submucosa is thin and thin, and its extension forms the lamina propria of the esophageal papillae, rich in collagen fibers and covered by keratinized stratified squamous epithelium. The epithelium changes from a keratinized stratified squamous to non-keratinized stratified columnar epithelium, and finally to a simple columnar epithelium at the esophagus-cardia transition.

A longitudinal section of the anterior portion of the esophagus verified that the muscular layer is constituted by skeletal muscle tissue, an inner layer with muscle bundles

arranged longitudinally, and an outermost layer arranged in a circular manner, with loose connective tissue between the bundles (Figure 3D and 3F). The muscle layer was visibly thickened in the esophagus-cardia transition compared to the anterior portion, thus forming the cardia sphincter, and the esophageal muscle layer in this portion began exhibiting a two-layer smooth muscle organization, with an innermost circular and external longitudinal wall, in which myenteric plexuses were usually visible (Figures 3H and 3I).

The outermost layer in the anterior and middle portions of the esophagus is formed by the tunica adventitia, consisting of loose connective tissue (Figures 3B and 3F). The serosa is present in the posterior portion of the esophagus when entering the coelomic cavity, also formed by loose connective tissue (Figure 3G). The histochemical analysis indicated negative esophagus staining for AB-PAS.

The SEM analysis indicated numerous pentagon-shaped keratinocytes or hexagonal disks covering the entire surface of the esophagus, with many irregular undulations, in addition to keratin epithelium protrusions distributed at the tip of the esophageal papillae, and no microvilli and goblet cells (Figures 7D, 7E and 7F).

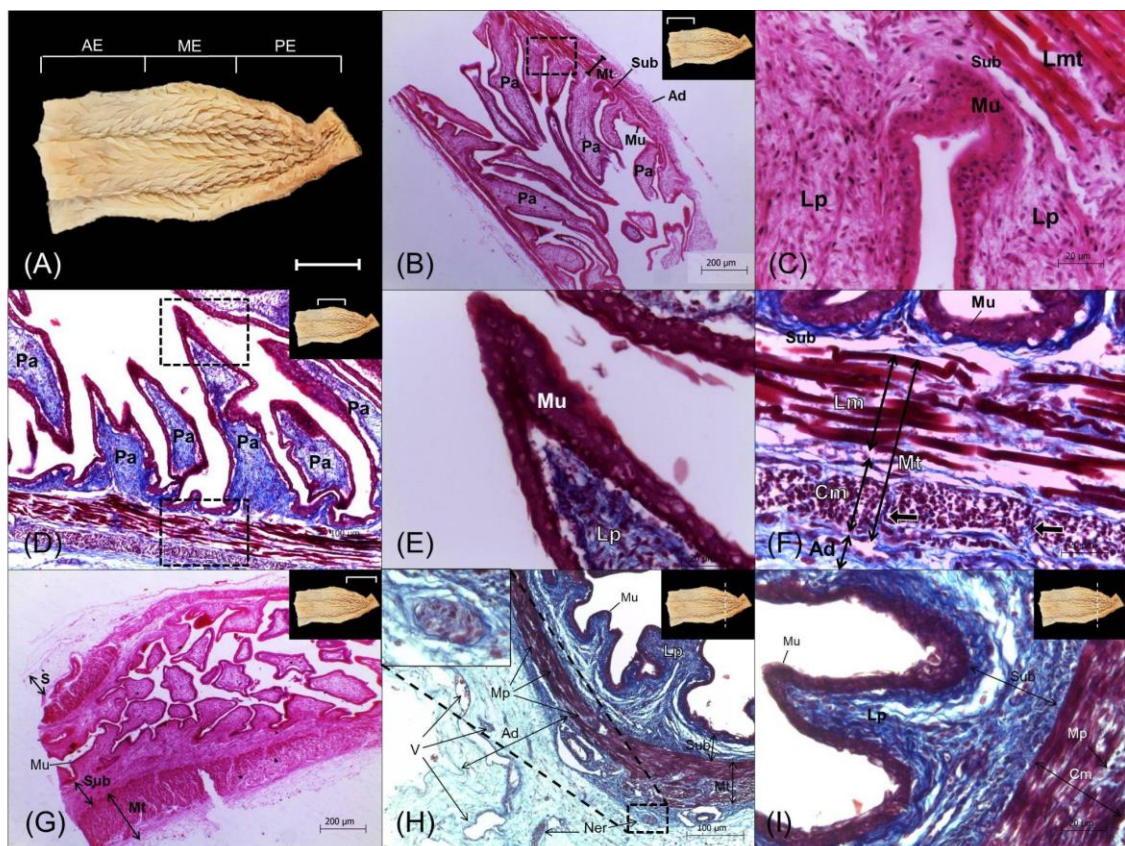


Figure 3. Microscopic characterization of the esophagus of a hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A:** open esophagus observed longitudinally, in ventral view, divided

into three portions, namely the anterior esophagus (LA), middle esophagus (ME) and posterior esophagus (PE), with the entire mucosa lined by conical papillae directed caudally. Bar: 0.5 cm. **B:** longitudinal section of the anterior portion of the esophagus. **C:** enlargement of Figure B, indicating the esophageal mucosa. **D:** longitudinal section of the middle portion of the esophagus. **E:** enlargement of Figure D, indicating the esophageal papilla. **F:** enlargement of Figure D, indicating the four esophageal wall layers. **G:** longitudinal section of the posterior portion of the esophagus. **H** and **I:** cross-section of the posterior portion of the esophagus. Mucous tunic (Mu); submucosal tunic (Sb); muscular coat (Mt); adventitia tunic (Ad); serous tunic (S); esophageal papilla (Pa); lamina propria (Lp); longitudinal muscle tunic (Lm); circular muscle tunic (Cm); perimysium of the muscle bundles of the circular muscle layer (arrows); nerve (Ner); myenteric plexus (Mp); blood vessels (V). Stains - B, C and G: Hematoxylin and Eosin. D, E, F, H and I: Gomori's Trichrome.

Stomach

The stomach is J shaped and located to the left of the midsagittal plane, associating with the left lobe of the liver and the pericardium. The stomach is formed by three regions, where the cardiac region was formed from the posterior portion of the esophagus. The small medium saccular region characterized the fundic region, presents a greater curvature to the left and a lesser curvature to the right, both curving to the left antimerely, and lastly, the pyloric region, a U-shaped tubular region, ascending to the right. At the pyloro-duodenum junction, the stomach is characterized by the pyloric sphincter and connected to the left lobe of the liver by a gastrohepatic ligament and to the left lung by a gastropulmonary ligament. The stomach mucosa presents longitudinal and parallel folds, smaller in animals with a slightly more distended stomach, especially in the fundic region, giving the impression of no folds (Figures 1 and 4A).

Histologically, the gastric wall is formed by four tunics, namely, the mucous tunic, submucosal tunic, muscular tunic and serous tunic. The mucous membrane presents three well-defined stratifications, namely the mucosal epithelium, the lamina propria and the muscularis mucosa. The mucosal epithelium is formed by columnar cells, characterized by an oval nucleus at its base, with mucosal granules stained positively with PAS (Figures 4E - 4I).

The gastric pits exhibit a funnel shape, shallow, usually appearing in epithelial depressions and prevailing in the fundic and pyloric regions (Figure 4E). Gastric glands of the tubuloacinar type are present in all lamina propria regions, more numerous in the cardiac and fundic regions. Gastric glandular cells are cuboidal or low columnar. Their nuclei are globose

or oval, located at the bottom of the cells, and usually contain more than one nucleolus. According to their locations, gastric glands were categorized as cardiac glands, fundic glands and pyloric glands (Figures 4D, 4F and 4G).

The mucosal muscle consists of smooth muscle. The interstitial space of the gastric glands is rich in collagen fibers and contains smooth muscle fibers that extend from the muscularis mucosae accompanied by some fine nerves and capillaries. The gastric submucosal tunic consists of loose connective tissue rich in collagen fibers, containing blood vessels and submucosal plexus (Figures 4C, 4D, 4F and 4G).

The muscle tunic is formed by two layers of smooth muscle tissue, the innermost with a circular arrangement (Cm) and the outermost arranged in the longitudinal direction (Lm). The presence of connective tissue rich in collagen fibers, blood vessels, nerves and myenteric plexuses was observed between these two muscle layers. The Cm layer is noticeably thicker than the Lm (Figure 4C and 4G). The gastric serosa is thin and formed by loose connective tissue, and nerves and blood vessels are also present between the Lm and serosa layers (Figure 4C).

The SEM analyses indicate the arrangement of the four gastric wall layers, in addition to gastric pits visible on top of the folds. Pentagonal/hexagonal epithelial cells are noted in the stomach (Figure 7).

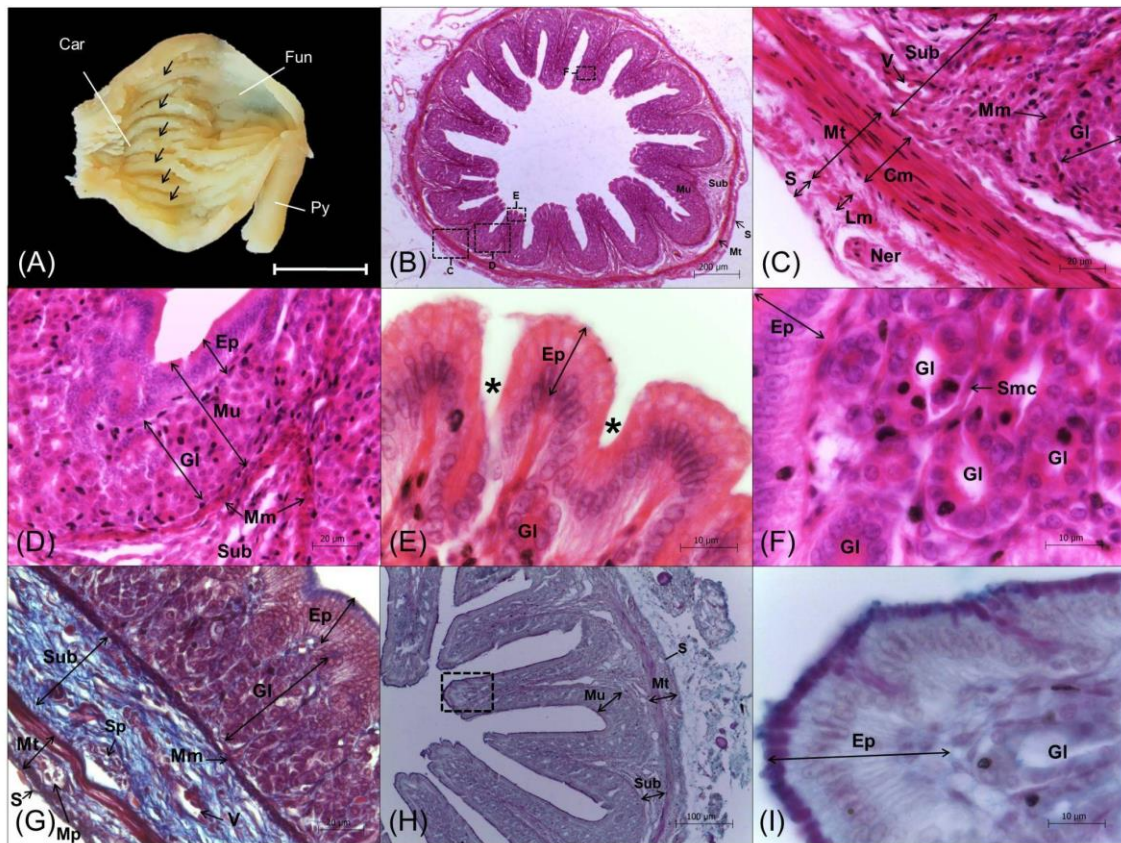


Figure 4. Microscopic characterization of the stomach of hawksbill turtle (*Eretmochelys imbricata*) hatchlings. **A:** stomach opened longitudinally on the lesser curvature. Bar: 0.5 cm. **B:** Cross section of the cardiac region. **C, D, E** and **F:** detail of Figure B. **G:** Cross section of the fundic region. **H:** histochemistry of the cardiac region. **I:** detail of Figure H, showing the gastric mucosa stained positively for PAS. Cardia region (Car); fundic region (Fun); pyloric region (Py); gastric folds (arrows); mucous membrane (Mu); submucosal tunic (Sub); muscular coat (Mt); serous tunic (S); blood vessels (V); Nerve (Ner); myenteric plexus (Mp), submucosal plexus (Sp); circular muscle layer (Cm); longitudinal muscle layer (Lm); mucosal muscle (Mm); mucosal epithelium (Ep); gastric gland (Gl); gastric pit (*); smooth muscle cell (Smc). Stains - B, C, D, E and F: Hematoxylin and Eosin. G: Gomori's trichrome. H and I: Alcian Blue (pH 2.5) and Periodic Acid Schiff.

Small intestine (SI)

The small intestine (SI) extends from the pyloric sphincter to the ileocolic flexure and is formed by three segments, the duodenum, jejunum and ileum. The duodenum, jejunum and ileum mucosa are marked by the presence of longitudinal and rectilinear folds. The jejunum is delimited from the duodenum by the duodenojejunal flexure, while no obvious limits were observed between the jejunum and the ileum (Figure 1).

Histologically, the SI mucosa is characterized by a simple columnar epithelium consisting of brush border absorptive cells and goblet cells. Most epithelial cells are AB-PAS negative absorptive cells. The SI goblet cells are similar in the three segments, positively stained by AB-PAS, although predominantly PAS-stained goblet cells were observed in the proximal and middle portion of the SI, while an increase in goblet cells positively stained by AB was noted in the most distal portion of the SI, in the ileum region (Figure 5).

The lamina propria and submucosa are formed by loose connective tissue, and the muscularis mucosa is formed by a thin smooth muscle layer. The extension of the submucosa into the lumen forms villi, rich in blood vessels and distinct in the duodenum, jejunum and ileum. Duodenal villi are filiform, some elongated and others short, arranged in an intercalated manner (Figure 5A). In contrast, jejunum villi are shorter compared to duodenum villi (Figure 5D), and ileum villi are, in turn, shorter than jejunum villi, each presenting a wide base (Figure 5G).

Nerves and blood vessels, as well as enteroendocrine cells, are located in the SI submucosa. However, the greatest number of enteroendocrine cells is present in the duodenum, presenting a globose or oval nucleus and located at one pole of the cell, while many tiny granules are observed at the other pole, negative for AB-PAS staining (Figures 5A and 5B).

The muscle tunic is formed by two layers of smooth muscle tissue, the innermost with a circular arrangement and the outermost arranged in the longitudinal direction. Connective tissue, blood vessels, plexuses and myenteric nerves are noted between the two muscle layers. The serosa is thin, composed of loose connective tissue (Figures 5A, 5D, 5G and 5H).

The SEM analyses indicate two types of epithelial cells, absorptive cells and goblet cells, both of which emerge in the SI. The absorptive cells contained microvilli, confirming the light microscopy findings, with epithelial cells presenting a brush border. The microvilli in the proximal duodenum are short and fungiform in shape, becoming longer, denser, and fascicular in the distal duodenum and jejunum, and then gradually shortening in the region of the ileum. In contrast, goblet cells appear as small protuberances in the duodenum, jejunum and ileum (Figures 8C, 8F and 8I).

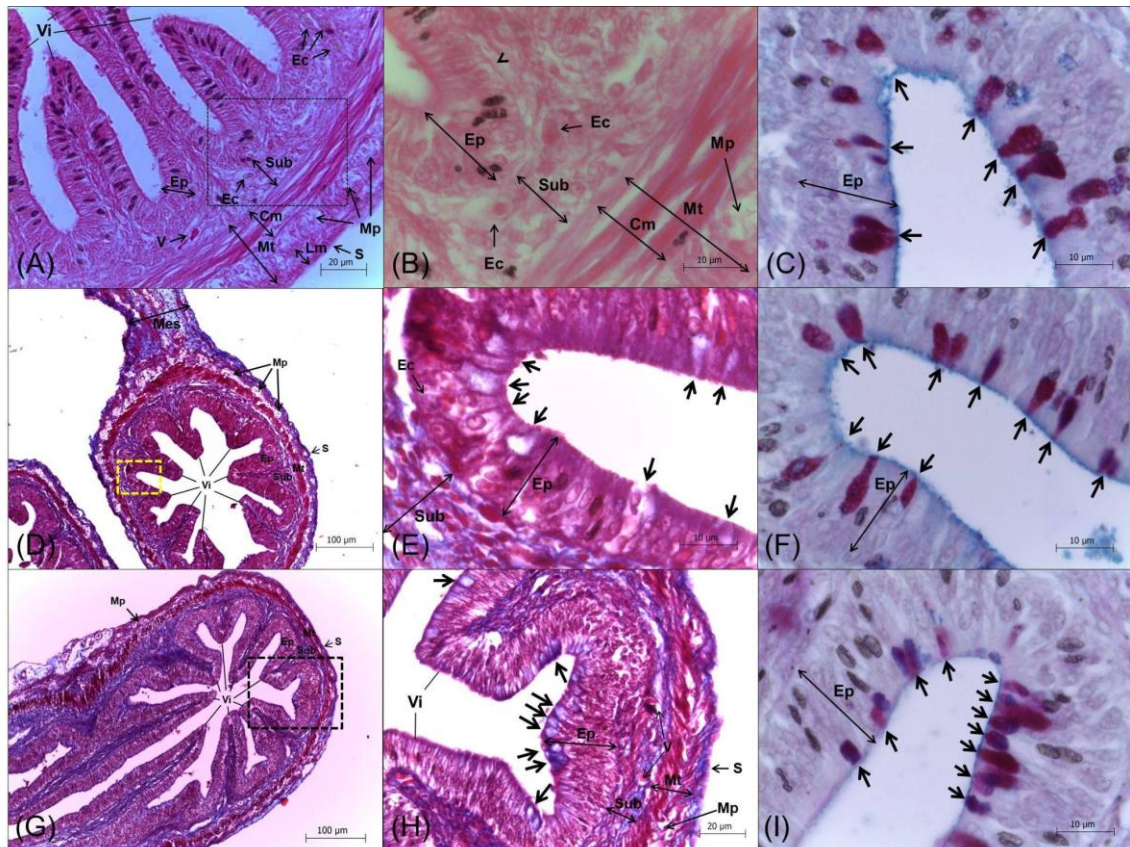


Figure 5. Microscopic characterization of the small intestine of hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A** and **B**: Transverse section of the duodenum. **C**: Duodenum histochemistry - cross section. **D** and **E**: Cross section of the jejunum. **F**: Jejunum histochemistry - cross section. **G** and **H**: Cross section of the ileum. **I**: Ileum histochemistry - cross section. Villi (Vi); mucosal epithelium (Ep); submucosal tunic (Sub); muscular coat (Mt); circular muscle layer (Cm); longitudinal muscle layer (Lm); serous tunic (S); mucosal muscle (arrowhead); myenteric plexus (Mp); blood vessels (V); goblet cells (arrows); enteroendocrine cells (Ec); mesentery (Mon). Stains - A and B: Hematoxylin and Eosin. D, E, G and H: Gomori's trichrome. C, F and I: Alcian Blue (pH 2.5) and Periodic Acid Schiff.

Large intestine (LI)

The LI extends from the ileocolic flexure to the cloaca, formed by two segments, the colon and the rectum. The colon is not clearly delimited from the rectum. No macro or microscopic differences indicate a cecum in this age group. The entire LI mucosa is marked by the presence of longitudinal and rectilinear folds, although with a smooth mucosa in distended colon regions. The diameter of the LI is visibly larger than that of the SI. Most of the LI is located to the left of the midsagittal plane and fixed by the mesentery (Figure 1).

Microscopically, the entire LI is lined with a simple columnar epithelium, consisting of brush border absorptive cells, in addition to abundant goblet cells, which secreted a type of mucin, positively stained by AB-PAS, with a greater predominance of AB positive goblet cells. In addition, mucosa-associated lymphoid tissue was observed in the colon and rectum, especially in the lamina propria (Figure 6).

The lamina propria and submucosa, similar to the SI, are formed by loose connective tissue, while the muscularis mucosa is formed by a thin layer of smooth muscle. The colon villi exhibits a filiform and elongated shape, becoming shorter in the dilated regions. In contrast, rectum villi are longer compared to colon villi. Furthermore, nerves and blood vessels are present. The muscle tunic is formed by two layers of smooth muscle tissue, the innermost with a circular arrangement and the outermost arranged in the longitudinal direction. Connective tissue, blood vessels, nerves and myenteric plexuses are observed between the muscle layers. The serosa is thin and formed by loose connective tissue (Figure 6).

The SEM analyses indicate that the colon and rectum are covered by absorptive cells and goblet cells, with a higher number of absorptive cells. LI microvilli are shorter and denser when compared to the SI. In contrast to the colon, rectal absorptive cells present longer microvilli and are easier to visualize, either in the form of a pentagon or hexagon. On the other hand, LI goblet cells, unlike the SI, are presented in crate form (Figure 9).

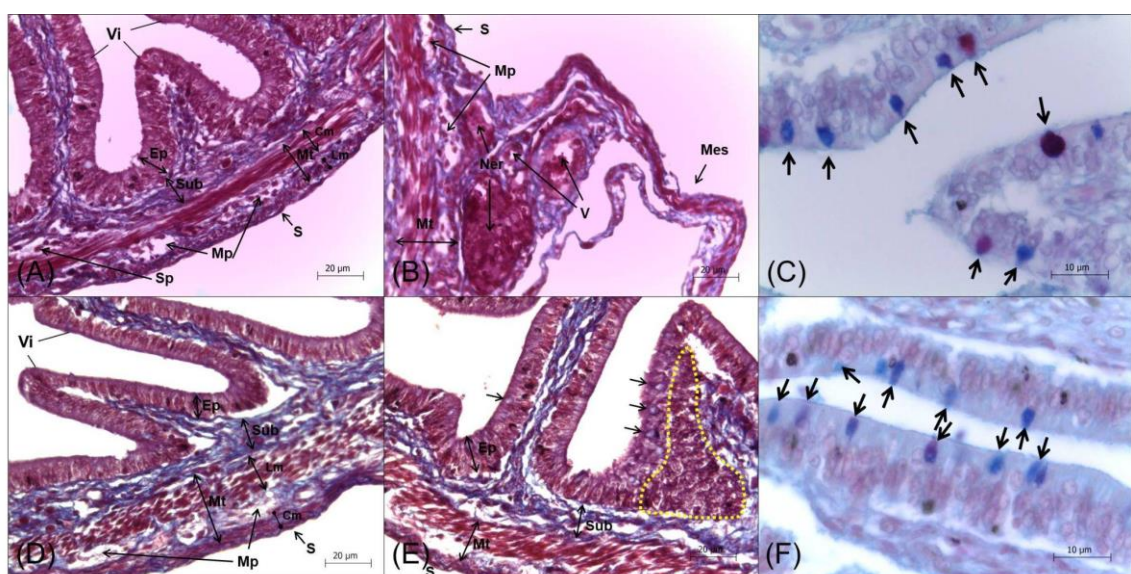


Figure 6. Microscopic characterization of the large intestine of a hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A** and **B**: Cross section of the colon. **C**: Colon

histochemistry - cross section. **D** and **E**: Cross section of the rectum. **F**: Rectum histochemistry - cross section. Villi (Vi); mucosal epithelium (Ep); submucosal tunic (Sub); muscular coat (Mt); circular muscle layer (Cm); longitudinal muscle layer (Lm); serous tunic (S); myenteric plexus (Mp); submucosal plexus (Sp); nerve (Ner); blood vessels (V); mesentery (Mes); goblet cells (arrows); mucosa-associated lymphoid tissue (yellow dashed). Stains - A, B, D and E: Gomori's Trichrome. C and F: Alcian Blue (pH 2.5) and Periodic Acid Schiff.

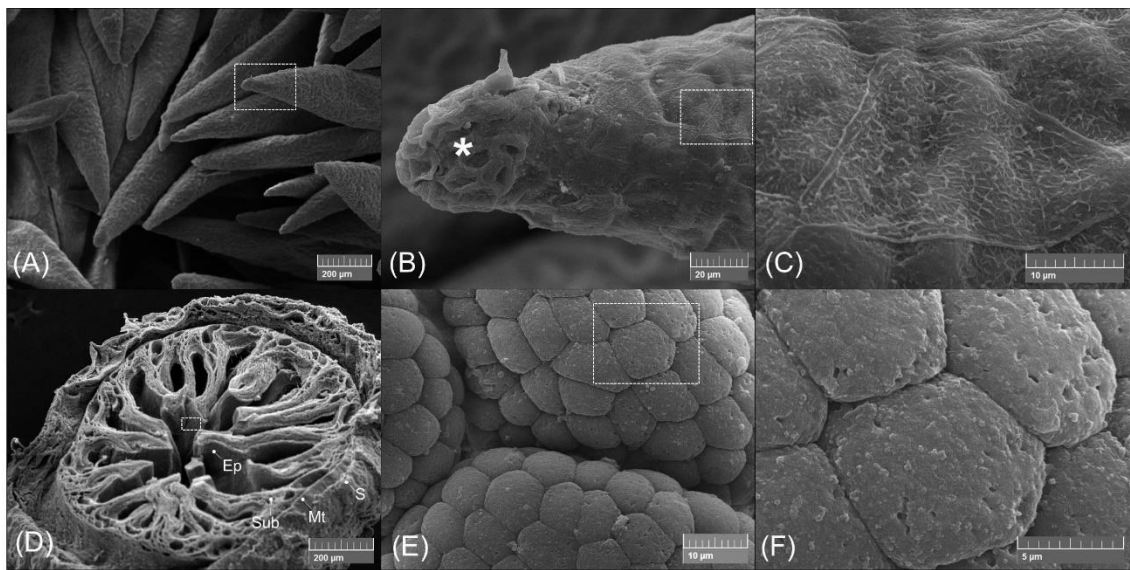


Figure 7. Scanning electron microscopy of the esophagus (A-C) and stomach (D-F) of a hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A**: Esophageal papillae. **B**: detail of figure A, indicating the protrusions of keratin epithelium distributed at the tip of the esophageal papilla (*). **C**: detail of Figure B, where the keratinocytes on the surface of the papillae in the shape of a pentagon can be observed. **D**: cardiac region of the stomach, in cross-section, presenting gastric folds in addition to the mucosal epithelium (Ep), submucosal tunic (Sub), muscular tunic (Mt) and serous tunic (S). **E**: detail of Figure D, indicating the gastric pits, in the shape of a funnel at the top of the gastric fold. **F**: regular pentagonal/hexagonal epithelial cells from the epithelium surface.

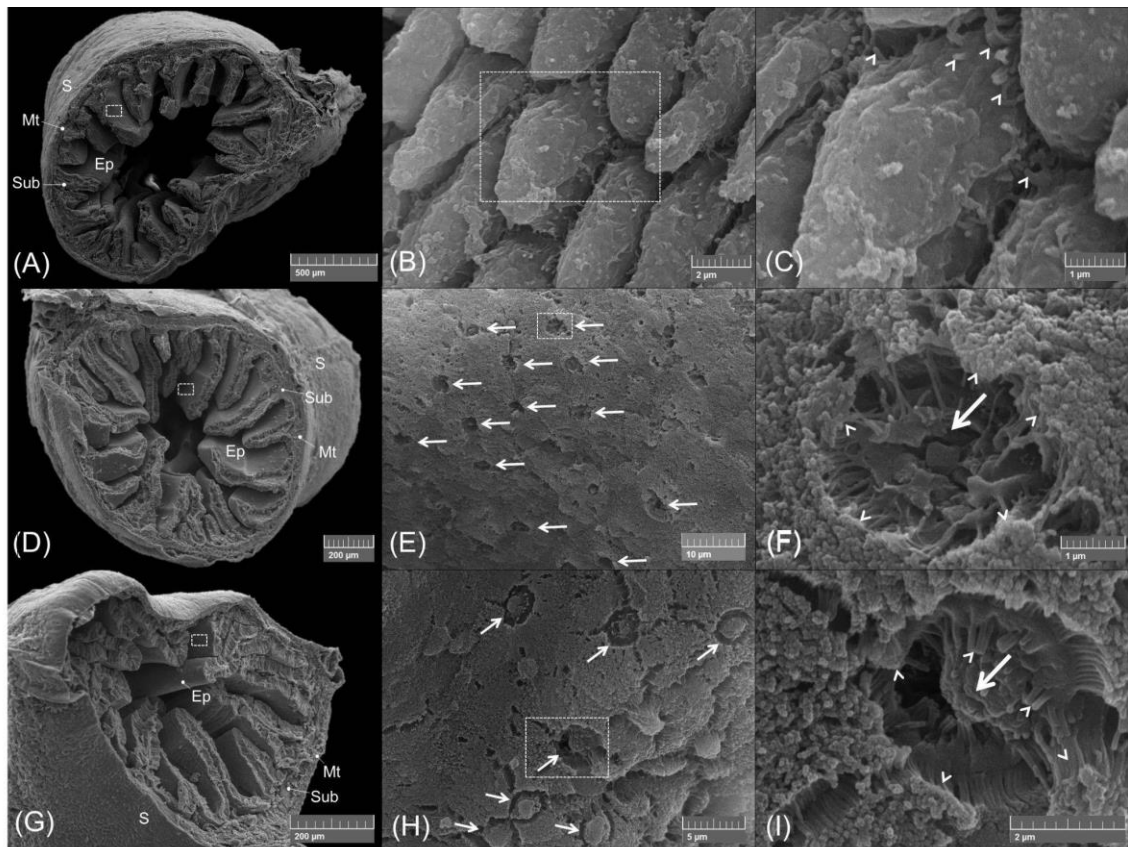


Figure 8. Scanning electron microscopy of the small intestine of a hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A:** Cross section of the proximal duodenum. **B:** detail of Figure A, showing the epithelium of the proximal duodenum. **C:** microvilli of the proximal duodenum. **D:** Cross section of the jejunum. **E:** detail of Figure D, showing the jejunal epithelium. **F:** goblet cells and microvilli of the jejunum. **G:** cross section of the ileum. **H:** detail of Figure G, indicating the ileal epithelium. **I:** goblet cells and microvilli of the ileum. Mucosal epithelium (Ep), submucosal tunic (Sub); muscular coat (Mt); serous tunic (S); microvilli (arrowheads); goblet cells (arrows).

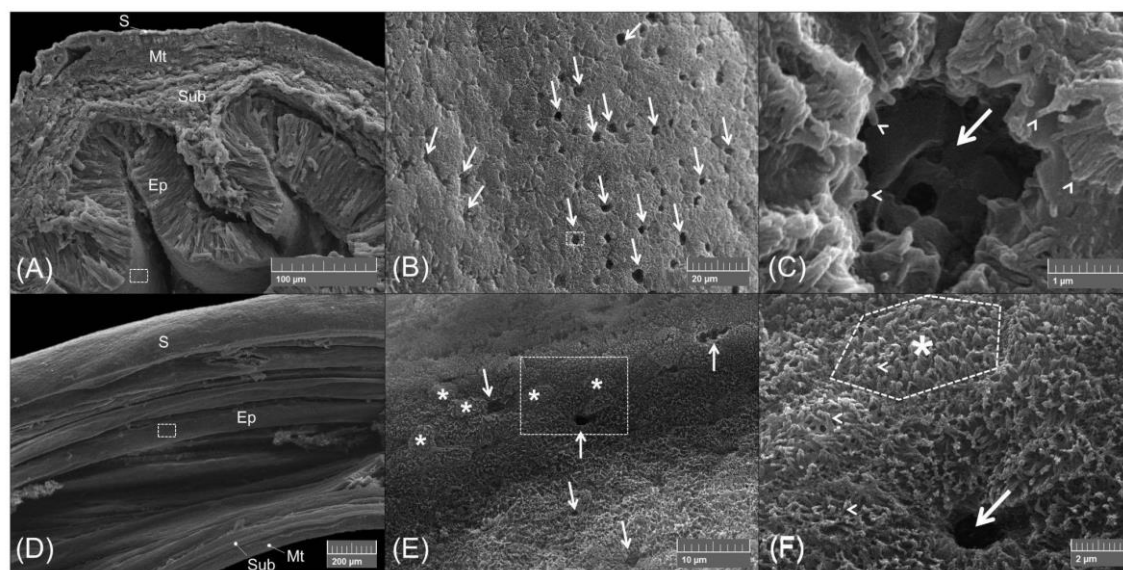


Figure 9. Scanning electron microscopy of the large intestine of a hawksbill turtle (*Eretmochelys imbricata*) hatchling. **A:** Cross section of the colon. **B:** colonic epithelium. **C:** Colonic goblet cells and microvilli. **D:** longitudinal section of the rectum. **E:** detail of Figure D, showing the rectal epithelium. **F:** goblet cells, microvilli and absorptive cells of the rectum. Mucosal epithelium (Ep), tunica submucosa (Sub), tunica muscularis (Mt), tunica serosa (S), microvilli (arrowheads), goblet cells (arrows), absorptive cells in pentagonal/hexagonal shape (*).

Discussion

Esophagus

The esophagus of *E. imbricata* hatchlings presented similar shape and topographical relationships to findings already described for other sea turtle species such as *C. mydas* (Magalhães et al., 2010; Magalhães et al., 2012; Chen et al., 2015; Calais Júnior et al., 2016), *L. olivacea* and *Caretta caretta* (Magalhães et al., 2012). However, Magalhães et al. (2012), report the presence of an exceptionally long esophagus in proportion to the curvilinear length of the carapace in *Dermochelys coriacea*, extending for almost half the length of the body, only then turning to the left and joining the stomach. Our findings also differ from reports for freshwater turtle species such as *Podocnemis expansa*, *Podocnemis unifilis*, *Podocnemis sextuberculata*, *Podocnemis erythrocephala* and *Peltocephalus dumerilianus* (Magalhães et al., 2014), where all present an esophagus in the form of a thin tube and of uniform diameter, while *E. imbricata* hatchlings present an esophagus in the form of a thick tube, wide in the anterior region and narrow in the posterior region.

The *E. imbricata* hatchlings analyzed herein present conical, pointed and cornified papillae covering the entire esophagus, oriented towards the stomach. This characteristic is unique to sea turtles and has been described for both hatchlings and adults of all other sea turtle species (Wyneken, 2004; Magalhães et al., 2010; Magalhães et al., 2012; Chen et al., 2015; Calais Júnior et al., 2016). Another striking feature observed in the esophagus is a developed muscle tunic, mainly in the esophagus-cardia transition. This reinforces the ability of the esophagus to contract to expel excess water through the oral cavity and the nostrils, and the esophageal papillae to retain the ingested solid food, a process during which the gastroesophageal sphincter contracts, preventing the passage of food contents to the stomach (Lutz & Musick, 1997). It is also believed that the strong muscles of the esophagus associated with its pointed papillae can minimally crush food and act in an initial mechanical digestion process.

Calais Júnior et al. (2016), when describing the esophagus morphology of *C. mydas*, observed the presence of PAS-positive mucus-producing glands located in the gastroesophageal region mucosa. Vieira-Lopes et al. (2014) also verified the presence of well-defined glands interspersed in the mucosal epithelium of the esophagus on some freshwater turtles, such as *Phrynops geoffroanus*, formed by clear cells in a semicircular arrangement, while Magalhães et al. (2014), reported the presence of mucus-producing superficial cells throughout the esophagus when studying the morphology of the digestive tube of freshwater turtles belonging to the Podocnemididae family, forming a continuous layer of secretory cells occupying the entire apical region, stained positively with PAS, more intense in the posterior portion. In addition to these mucus-producing cells, the authors also verified the presence of some goblet cells irregularly distributed among the columnar cells in the epithelium but did not observe esophageal glands. In our studies, however, no esophageal glands or mucus-producing cells were observed in the esophagus, suggesting that the esophagus of *E. imbricata* hatchlings exhibits only a mechanical function, mainly because they present a keratinized epithelium, providing protection against abrasion due to feeding on sea sponges, which are composed of rigid structures which may result in abrasive esophageal mucosa lesions.

Stomach

The shape of an animal's stomach is closely related its diet (Work, 2000; Romer & Parsons, 1985). In our study, the stomachs of hawksbill turtle hatchlings are noted as J shaped and located to the left of the midsagittal plane, displaying distinct three regions, the cardiac

region caudal to the esophagus, followed by a small sacculation, corresponding to the fundic region and, finally, the pyloric region, a short and tubular muscular region, on the right, with the mucosa composed of longitudinal folds. These results are similar to those described for other sea turtles, such as *C. mydas* hatchlings (Chen et al., 2015), and juvenile *L. olivacea* and *E. imbricata* (Magalhães et al., 2012), in addition to a freshwater turtle, *P. sextuberculata* (Magalhães et al., 2014). Slight differences are noted compared to two adult sea turtle species, *Caretta caretta* and *D. coriacea* (Magalhães et al., 2012), as the stomachs of these animals are tubular and curvilinear. Juvenile and adult *C. mydas* (Magalhães et al., 2010; Magalhães et al., 2012) also differ in terms of the presence of a large saculation in the fundic region with a blind bottom, while three freshwater turtle species, *P. expansa*, *P. unifilis* and *P. erythrocephala* (Magalhães et al., 2014) present stomachs divided into two compartments separated by a constriction with longitudinal mucosa folds, where the first compartment is formed by the cardiac and fundic regions, both formed by transverse folds throughout the mucosa, while the second curves to the right immediately after constriction, forming a well-developed pyloric region and displays a mucosa organized by longitudinal folds. According to Hildebrand (1995), carnivorous reptiles display a simpler stomach and most herbivorous reptiles exhibit a more complex stomach, which functions as a reservoir or fermentation chamber. In this sense, hawksbill turtle hatchlings exhibit the simplest stomach morphology as carnivores.

According to Chen et al. (2015), the stomach becomes larger in the same sea turtle species during different development stages, providing more space to accommodate food and energy for development. On the other hand, relative animal lengths vary in different species and may be associated to available nutrient content, where sea turtle stomach can adapt to food changes and growth and development needs. In our study, the mean stomach length of a hawksbill turtle hatchling of 2.17 ± 0.166 cm ($n = 10$) is equivalent to 0.47 times the CCC of the animals, while Magalhães et al. (2012) reported a stomach length of 27.7 cm ($n = 1$) in a juvenile hawksbill turtle, about 0.76 times the CCC. Chen et al. (2015) reported the stomach length of *C. mydas* hatchlings of 2.63 ± 0.15 cm ($n = 4$), 0.55 times the CCC. Magalhães et al. (2012), reported a stomach length of 22.37 ± 9.08 cm ($n = 9$) in juveniles of the same species, the equivalent to 0.60 times the CCC. Although both turtle species present different feeding habits, the morphometric data reported for these two age groups (hatchlings and juveniles) are similar, even though both species are only be considered fully omnivorous (*E. imbricata*) and herbivorous (*C. mydas*) in their adult stages. Thus, the relationship between stomach length and the CCC in the adult age group is more notable for *C. mydas*, considering the high

volume of food (seaweed) that these animals ingest during foraging. The entire stomach region of the animals investigated in this study contained gastric glands that resemble those observed in *C. mydas* in all age groups (hatchlings, juveniles and adults) (Magalhães et al., 2010; Chen et al., 2015), and the freshwater species *P. sextuberculata* and *Peltocephalus dumerilianus* (Magalhães et al., 2014), differing slightly from three other freshwater species, *P. expansa*, *P. unifilis* and *P. erythrocephala* (Magalhães et al., 2014), as the glands of these animals are absent in the cardiac and fundic regions and present only in the pyloric region. Thus, gastric gland distribution may reflect an adaptation to feeding habits (Chen et al., 2015). These glands act in chemical food digestion, where they release proteases and hydrochloric acid, the latter playing a role against bacteria and providing the ideal acidic stomach pH for protease activation.

Intestines

According to Magalhães et al. (2012), the duodenum mucosa of juvenile and adult *C. mydas*, *L. olivacea*, *Caretta caretta* and *E. imbricata* sea turtles is characterized by reticular folds, while the jejunum and ileum present longitudinal and rectilinear folds. Chen et al. (2015) characterized the small intestine mucosa of *C. mydas* hatchlings as longitudinal and zigzagging along the entire intestine length. In our study, only longitudinal and rectilinear folds in the three SI segments were observed. The difference observed between the duodenum of juvenile hawksbill turtles (Magalhães et al., 2012) and the offspring of the same species observed herein may be due to the difference investigated age groups and the predilection for a certain type of food.

The LI mucosa of hawksbill turtle hatchlings was marked by the presence of longitudinal and rectilinear folds, with a smooth mucosa in the distended regions of the colon. These findings are similar to those reported by Magalhães et al. (2012), who observed an alternation of saccular regions without folds and narrow with longitudinal folds in the colon juvenile and adult *L. olivacea*, *Caretta caretta*, *E. imbricata* and *C. mydas*, except in the colon of *D. coriacea*, characterized by irregularly distributed folds. Chen et al. (2015), on the other hand, reported the presence of transverse folds in the colon mucosa of *C. mydas* hatchlings, and posterior to the colon, straight folds replacing the transverse folds and were present in the entire rectum. Therefore, fold patterns and distributions in different species may be associated with peristalsis dynamics.

In our study, the brush border observed on the apical surface of the absorptive cells corresponds to the microvilli also observed in the scanning electron microscopy assessments,

suggesting that these cells exhibit an increased absorption surface area (Eichholz and Crane, 1965). These findings corroborate those described for *Caretta caretta* (Ontiveros-Tlachi et al., 2014) and *C. mydas* (Chen et al., 2015) hatchlings. Several mechanisms are involved in nutrient uptake by absorptive cells, including passive diffusion, cytosol, active transfer, and carrier-mediated transport (Sanderson & Mallinson, 2002). Water uptake is driven by the absorption of sodium (Na⁺), potassium (K⁺) and organic compounds and occurs through the formation of osmotic gradients (Blaker & Irving, 2014).

Groups of pale cells observed in the submucosa and between the circular and longitudinal muscle layers of hawksbill turtle hatchlings correspond to the submucosal and myenteric plexuses, which act as important peristalsis modulators and in the compaction of intestinal contents (Holmberg et al., 2002). These findings have also been described for *Caretta caretta* hatchlings by Ontiveros-Tlachi et al. (2014) and in freshwater turtles (*Pseudemys scripta elegans*) by Timmermans et al. (1991). Reflex peristaltic waves mediated by musculomotor neurons drive bowel contents (Worsoe et al., 2011). The intensity of muscle contractions is influenced by the nature of the ingested food, where solid foods induce greater activity than liquid meals, and food items high in glucose result in greater stimulation than those high in fat (Blaker & Irving, 2014).

In sea turtles, intestinal glands have been described only in the duodenum of *C. mydas* hatchlings (Chen et al., 2015) and in juveniles and adults (Magalhães et al., 2010). On the other hand, no intestinal glands were observed herein in the SI and LI, corroborating the report by Magalhães et al. (2014), who did not observe intestinal glands in *P. expansa*, *P. unifilis*, *P. sextuberculata*, *P. erythrocephala* or *Peltocephalus dumerilianus*. Intestinal glands can secrete digestive enzymes to break down proteins and complex carbohydrates in food and hormones to regulate bowel functions. Therefore, the distribution and characteristics of intestinal glands appear different in different species, which may be closely related to their intestinal functions and dietary adaptation (Chen et al., 2015).

Enteroendocrine cells are located in the SI submucosa of hawksbill turtle hatchlings, although most were present in the duodenum. The presence of these endocrine cells was also observed in *Caretta caretta* (Ontiveros-Tlachi et al., 2014) and *C. mydas* (Chen et al., 2015) hatchlings. Gastrointestinal endocrine cells synthesize various types of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract (Bell, 1979). In freshwater turtles, gastrointestinal endocrine cells secrete serotonin, gastrin and insulin in *Mauremys caspica caspica* (Tarakçi et al., 2005), chromogranin A, serotonin, gastrin, somatostatin, cholecystokinin, glucagon and bombesin in *Trachemys scripta elegans*

(Ku et al., 2001). However, no studies that determine which types of hormones are produced by enteroendocrine cells are, available in sea turtles.

The presence of mucosa-associated lymphoid tissue observed in the colon and rectum of hawksbill turtle hatchlings, especially in the lamina propria, is related to the variety and abundance of the bacterial population in the large intestine, and act in the defense system of these microorganisms (Junqueira & Carneiro 2017).

The length of the intestine is directly related to an animal's diet. In general, the SI tends to be longer in carnivores and shorter in herbivores (Stevens & Hume, 1998). Our results confirm these statements, as the SI length in hawksbill turtle hatchlings was greater than the LI, these similar to the carnivorous species *Caretta caretta*, *L. olivacea* and *D. coriacea* (Magalhães et al., 2012) and in *C. mydas* hatchlings (Chen et al., 2015) and in agreement with Reich et al. (2007), who indicate that hawksbill turtles begin their life in the open sea with a generally carnivorous diet similar to that of young *C. mydas* and *Caretta caretta*, which may include zooplankton, molluscs and crustaceans. On the other hand, LI length in herbivorous *C. mydas* in the juvenile and adult stages is greater than the SI length (Magalhães et al., 2010; Magalhães et al., 2012). These length differences comparing the small and large intestine are explained by the fact that foods of animal origin are more easily digested than those of plant origin (Ricklefs, 2003).

Mucins

Neutral mucins, abundant on the luminal surface of the stomach and also secreted by goblet cells, are related to neutralization of the acidic pH of the stomach, thus ensuring stomach and proximal small intestine protection (Lynch et al., 1972; Krause, 1981; Gad, 1982; Allen et al., 1993).

The acidic mucins present in the goblet cells of the hawksbill turtle act in the defense system of the gastrointestinal tract, displaying the ability to associate with protective proteins secreted by the epithelium, either by direct molecular interaction or by offering these proteins an aqueous and viscous-elastic medium to perform their defensive functions (Croix et al., 2011). They also act as lubricants, playing a prominent role in increasing mucus viscosity in the distal bowel region (Sakata & Engelhardt, 1981; Croix et al., 2011).

Conclusions

The esophagus of hawksbill turtle hatchlings presented esophageal papillae as specific structures in these animals, playing a very important role in the act of swallowing, as it

prevents food reflux and, associated with esophageal muscle contractions, aids in the first stage of mechanical digestion. The stomach is characterized as simple, characteristic of carnivorous reptiles. The small intestine is larger than the large intestine, a striking feature of carnivorous animals associated to diet. Hawksbill turtle hatchling morphological digestive tube characteristics are strong indications of an adaptation to a carnivorous diet in this age group. It is important to note that further studies on the morphophysiology of the digestive tube of these animals are required, especially concerning types of intestinal hormones produced by enterodocrine cells.

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**CAPÍTULO 4 – MARINE DEBRIS INGESTION AND THE USE OF DIAGNOSTIC
IMAGING IN SEA TURTLES: A REVIEW**

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Marine debris ingestion and the use of diagnostic imaging in sea turtles: A review

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Abstract: Worldwide, sea turtles are affected by anthropic waste. Animals, unable to differentiate anthropic waste from food, ingest this waste from their surroundings. After ingestion, the waste may cause a digestive tract blockage, thereby compromising the feeding and digestion capacity of the turtles, causing malnutrition, which may lead to death. Radiological imaging can be performed in turtles under rehabilitation to identify alterations of the digestive tract, such as intussusceptions, impactions, obstructions, torsions, neoplasms, and foreign bodies. These alterations are a result of either the ingestion of anthropogenic residues or natural causes. Moreover, diagnostic imaging exams play an important role in interventional medicine as they can indicate the location and type of ingested waste, thereby facilitating decision-making for the treatment of the afflicted animals. The aim of this study was to analyse the published scientific literature surrounding the effects of the ingestion of anthropogenic residue by sea turtles and the main imaging modalities used for their diagnosis. This was undertaken to provide clinicians with a greater amount of information regarding the digestive tract diseases of these animals and consequently, provide better outcomes of the rehabilitation processes. Furthermore, the outcomes of this study provide key information regarding the conservation of these species, among others.

Keywords: Cheloniidae; morphology; plastic; Testudines.

1. Introduction

The impact of anthropic waste intake is one of the greatest problems for marine fauna conservation worldwide (Derraik 2002; Gregory 2009; Hoarau et al. 2014; Gall and Thompson 2015; Poli et al. 2015; Clukey et al. 2017; Pham et al. 2017; Duncan et al. 2019; Rizzi et al. 2019), affecting mammals (Baulch and Perry 2014; Attademo et al. 2015; Poeta et al. 2017; Fossi et al. 2018), birds (Cadee 2002; Provencher et al. 2014; Provencher et al. 2015; Perez et al. 2019), turtles (Lazar and Gracan 2011; Gall and Thompson 2015), fishes, and molluscs (Rochman et al. 2015). This waste, regardless of its origin, remains in the environment and the animals ingest it by confusing it with food (Rizzi et al. 2019). This can cause digestive tract blockages (Laist 1997), which compromises the animal's feeding and digestion capacity, resulting in malnutrition, disease, reduced growth and reproduction rates, and even death (McCauley and Bjorndal 2001).

Sea turtles belong to the order Testudines and form a monophyletic group of the suborder Cryptodira (Bowen and Karl 1997; Meylan and Meylan 1999). The seven known

species of sea turtles are distributed in two families: *Dermochelys coriacea*, or the leatherback sea turtle, belongs to the Dermochelyidae family, while the other six species: *Chelonia mydas* (green sea turtle), *Caretta caretta* (loggerhead sea turtle), *Eretmochelys imbricata* (hawksbill sea turtle), *Lepidochelys kempii* (Kemp's ridley sea turtle), *Lepidochelys olivacea* (olive ridley sea turtle), and *Natator depressus* (flatback sea turtle) (Meylan and Meylan 1999), belong to the Cheloniidae family.

Sea turtles have a complex life cycle and population structure characterised by migrations from hundreds to thousands of kilometres between feeding and spawning areas (Bowen 1995). They face various threats during these migrations, including pollution, loss of habitat, climate change, interactions with fishing practices, and the ingestion of marine waste of anthropogenic origin (Bugoni et al. 2001; Fujihara et al. 2003; Keller et al. 2004; Thompson et al. 2009; Hamann et al. 2010). The presence of waste in the digestive tract is a primary cause of digestive system organ rupture, which leads to death (Orós et al. 2004; Fichia et al. 2016; Farias et al. 2019).

The ingestion of anthropic waste has been reported in all sea turtle species (Macedo et al. 2011; Awabdi et al. 2013; Poli et al. 2015; Schuyler et al. 2016; Wilcox et al. 2018; Rizzi et al. 2019). This indicates the importance of assessing the effects and impacts of marine waste on the development, survival, health, and reproduction of sea turtles, which has become a worldwide research priority (Hamann et al. 2010; Hoarau et al. 2014; Gall and Thompson 2015; Poli et al. 2015; Nelms et al. 2016; Clukey et al. 2017; Pham et al. 2017; Duncan et al. 2019; Rizzi et al. 2019).

The main clinical findings that have been associated with the digestive tract of sea turtles are constipation, obstructions, ulcerations, perforations, necroses, neoplasms, torsions, paralytic ileum, intussusceptions, parasitic infections, and the presence of foreign bodies (Bugoni et al. 2001; Torrent et al. 2002; De Vico et al. 2003; Croft et al. 2004; Orós et al. 2004; Santoro et al. 2006; Werneck et al. 2008; Wyneken et al. 2013; Oliveira et al. 2020a; Oliveira et al. 2020b). In most cases, these findings result from the ingestion of man-made pollutants, such as plastics, nylon strands, rubber, metals, and fishing hooks (Bugoni et al. 2001; Andrady 2011; Wyneken et al. 2013; Clukey et al. 2017; Caracappa et al. 2018; Duncan et al. 2019).

An *ante-mortem* diagnosis of digestive tract diseases is difficult to obtain within a crucial time period. Accurate and detailed knowledge of suitable diagnostic imaging exams for the correct interpretation of alterations to the digestive tract caused by the ingestion of anthropic residue is required (Pressler et al. 2003; Di Bello et al. 2006; Valente et al. 2007a;

Valente et al. 2007b; Erlacher-Reid et al. 2013; De Majo et al. 2016; Spadola et al. 2016b). An early diagnosis, associated with surgical correction, of anthropic waste ingestion is fundamental for the successful treatment of animals in rehabilitation centres. This disorder, when correctly identified and treated, can allow a better prognosis for the animal, depending on the object present and its location in the digestive tract. Most digestive tract disorders in animals are associated with anthropic waste and pollutants. As a result, diagnostic imaging exams are necessary for better outcomes in turtles kept in rehabilitation centres. Therefore, we conducted a bibliographic survey of published studies regarding the effects of the ingestion of anthropic waste by these animals as well as the main imaging procedures used for the diagnosis. This was undertaken to enable greater success in the rehabilitation processes, and consequently, promote the dissemination of important knowledge regarding conservation actions for sea turtles.

2. Morphology of the sea turtle digestive system

Imaging exams conducted on sea turtles require prior knowledge of the animal's morphology. Therefore, the macroscopic and topographic morphologies of the digestive tract of sea turtles are addressed in this section.

The digestive tract of sea turtles comprises the tongue, oesophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (caecum, colon, and rectum), and cloaca (Figure 1) (Wyneken 2004; Magalhães et al. 2010; Magalhães et al. 2012; Chen et al. 2015; Calais Júnior et al. 2016).

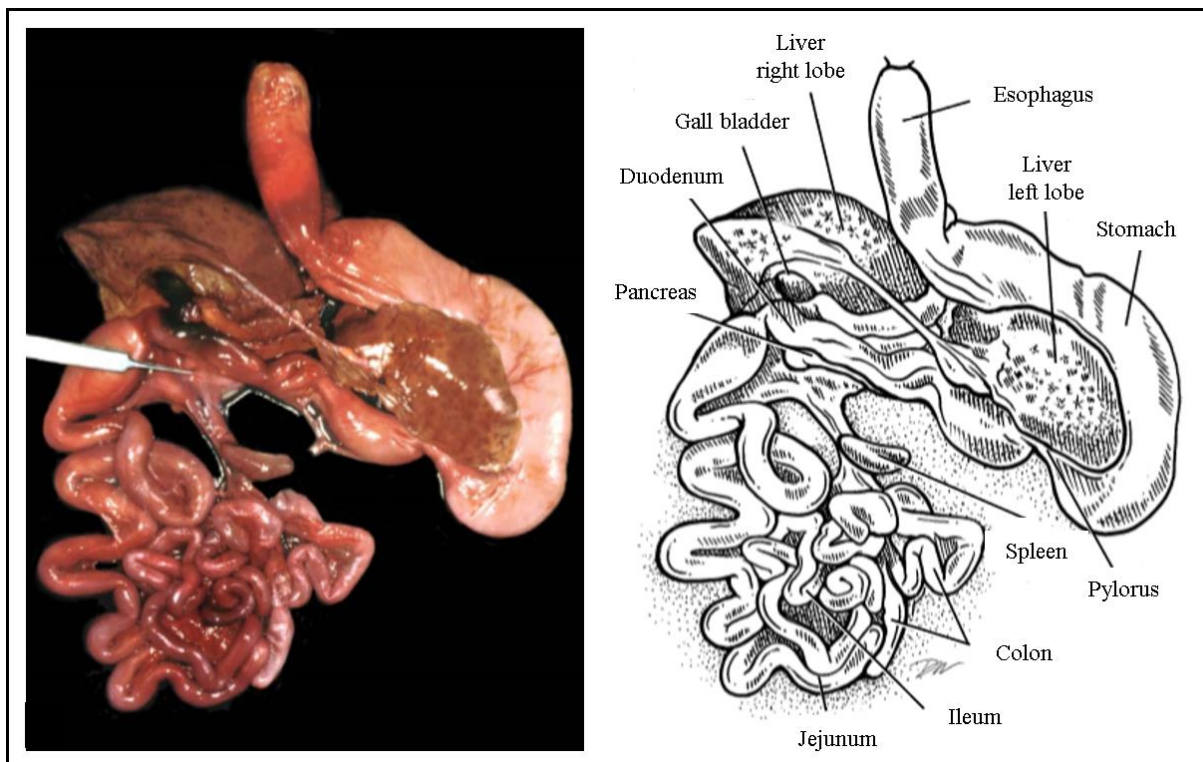


Figure 1. The digestive system of a sea turtle (*Lepidochelys kempii*). Source: Modified after Wyneken (2004).

2.1 TONGUE

Sea turtle tongues are triangular with a slightly rounded apex when viewed from the back. There are no papillae present on the dorsal surface; instead, numerous folds are present there as well as at the root of the tongue. This can be viewed with a scanning electron microscope (Iwasaki et al. 1996).

2.2 OESOPHAGUS

The oesophagus of *C. caretta*, *C. mydas*, *E. imbricata*, and *L. olivacea* consists of a muscular-membranous tubular organ, medially located in the cervical region, which diverts laterally to the left in the celomic region (Magalhães et al. 2010; Magalhães et al. 2012). The oesophagus of *D. coriacea*, on its turn, is exceptionally long (up to 167.00 cm) and extends for almost half of the turtle's body length, and then turns to the left and enters the stomach (Wyneken 2004; Magalhães et al. 2012).

The oesophageal mucosa of all seven species of sea turtles is characterised by pointed papillae along its entire length (oesophageal papillae). These papillae are caudally oriented and become progressively larger in the more caudal portion of this organ, with this being limited to its junction with the stomach (Wyneken 2004; Magalhães et al. 2010; Magalhães et al. 2012; Calais Júnior et al. 2016).

2.3 STOMACH

Sea turtle stomachs are located in the left antimere and form a curve around the liver and pericardium. From the caudal portion of the oesophagus, the cardiac region is formed followed by a large pouch, the fundic region, and finally, the pyloric region, a short muscular region, is formed to the right. This is connected to the left lobe of the liver by a gastrohepatic ligament and to the left lung by a gastropulmonary ligament (Wyneken 2004; Magalhães et al. 2012).

The stomach mucosa is characterised by longitudinal folds. These folds disappear when the stomach is stretched, giving the impression that the stomach is an organ without folds (Magalhães et al. 2010; Magalhães et al. 2012).

2.4 SMALL INTESTINE

The small intestine is divided into three regions; the duodenum, jejunum, and ileum. The duodenum mucosa is marked by the presence of reticular folds with a “honeycomb or beehive-like” arrangement. This textured coating is associated with an increase in the contact surface of the mucosa. The transition from the duodenum to jejunum is characterised by a change in the mucosa, where the folds change from reticular to rectilinear. The jejunum and ileum have juxtaposed rectilinear folds (Wyneken 2004; Magalhães et al. 2010; Magalhães et al. 2012).

The transitions between the components of the small intestine (duodenum, jejunum, and ileum) are difficult to identify. Macroscopic differences are often not obvious and are best confirmed by a histological analysis. The transition between the ileum and colon is obvious in adult animals, where the ileum ends in the ileocaecal valve, which is a muscular sphincter (Wyneken 2004; Magalhães et al. 2012).

2.5 LARGE INTESTINE

The caecum is located at the proximal extremity of the colon and is a slightly larger sac than the rest of the intestine. It is best differentiated in green and leatherback adult sea turtles and is less pronounced in carnivorous and omnivorous species (Wyneken 2004).

The colon is characterised by the alternation of domed regions represented by sacculum and narrowing. The domed regions show smooth mucosa, and the narrowing is characterised by rectilinear folds. The caudal region, referred to as the rectum, is marked by rectilinear folds (Magalhães et al. 2010; Magalhães et al. 2012).

2.6 CLOACA

The sea turtle cloaca is characterised by a chamber that collects the faeces, urine, eggs, and semen flow and is connected ventrally to the urinary bladder.

The cloaca opens to the exterior and is divided into three compartments; the coprodeum, urodeum, and proctodeum. The coprodeum receives the faeces from the rectum, while the urodeum contains the urinary papillae and the opening of the bladder. The proctodeum corresponds to the most distal region of the cloaca and contains the genital ducts of the male or female and is associated with copulation (Wyneken 2004).

3. Ingestion of anthropic waste and its effects

All seven species of sea turtles are globally affected by the impact of anthropic waste, either by getting entangled in or ingesting these materials (Lazar and Gracan 2011; Gall and Thompson 2015; Schuyler et al. 2016; Caracappa et al. 2018; Wilcox et al. 2018; Rizzi et al. 2019). Previous studies have shown that *C. mydas* and *C. caretta* are the most affected species (Lazar and Gracan 2011; Hoarau et al. 2014; Gall and Thompson 2015; Poli et al. 2015; Fukuoka et al. 2016; Schuyler et al. 2016; Pham et al. 2017; Caracappa et al. 2018; Rizzi et al. 2019), followed by *L. olivacea*, *E. imbricata*, *D. coriacea*, *L. kempii*, and *N. depressus* (Gall and Thompson 2015; Schuyler et al. 2016; Wilcox et al. 2018; Rizzi et al. 2019). Different eating habits are observed in sea turtles; their diet ranges from needle grass, algae, sponges, cnidarians, fishes, and crustaceans (Den Hartog 1979; Spotila 2004; Colman et al. 2014). However, man-made solid or contaminating waste affects these turtles through both waste ingestion and a decrease in their food stock as a result of pollution.

Fishing equipment represents one of the main causes of mortality for sea turtles, mainly affecting *C. caretta* (Figure 2) (Silva et al. 2010). Caracappa et al. (2018) reported the presence of fishing hooks in 129 samples of *C. caretta* captured incidentally off the coast of Sicily. These hooks were located in different sections of the digestive tract, but were mostly found in the oesophagus (47.3%) followed by the intestines (24.8%), stomach (14.7%), and mouth (13.2%).

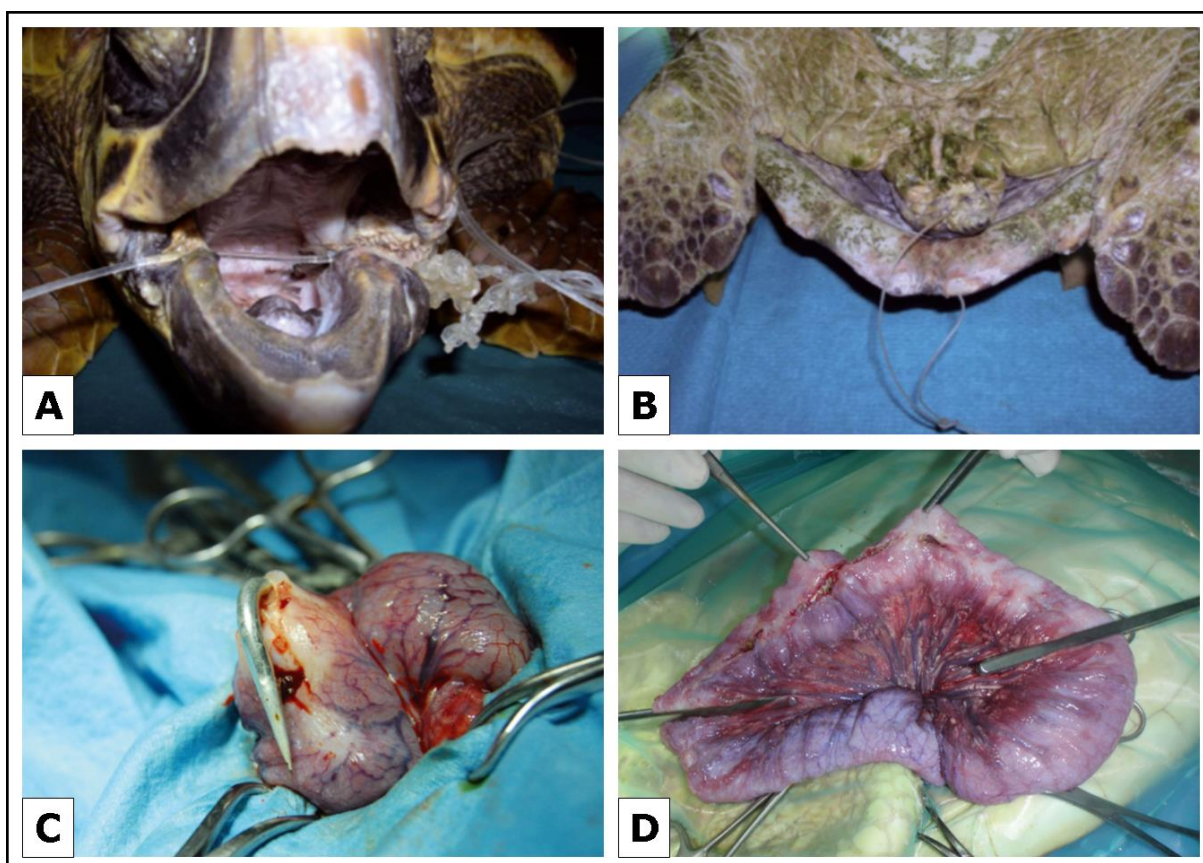


Figure 2. Loggerhead sea turtle with a fishing line protruding from its mouth (A) and cloaca (B). (C) Stomach exteriorisation for the extraction of a large hook perforating the gastric wall. (D) Intraoperative photograph showing the severe laceration of the intestinal wall caused by a fishing strand. Source: Modified Di Bello et al. (2013).

Another major threat, which is also the most worrying threat, is the ingestion of waste (Lazar and Gracan 2011; Gall and Thompson 2015; Caracappa et al. 2018; Rizzi et al. 2019), which, in most cases, causes intestinal obstructions and internal injuries, resulting in deficits in the digestion and nutrient absorption, causing malnutrition and increased buoyancy. This can lead to poor health, reduced growth and reproduction rates, and even death (Nelms et al. 2016).

The waste ingested by sea turtles originates from various sources. Plastics, glass, fishing artifacts, paper, microplastics, and metal, among other types waste material, are most often found in the stomachs of turtles (Andrady 2011; Gall and Thompson 2015; Rizzi et al. 2019). Most waste ingestion incidents occur with plastic fragments of different types and colours (Table 1) (Lazar and Gracan 2011; Hoarau et al. 2014; Gall and Thompson 2015; Poli et al. 2015; Fukuoka et al. 2016; Clukey et al. 2017; Pham et al. 2017; Wilcox et al. 2018; Duncan et al. 2019; Rizzi et al. 2019).

Table 1. Percentage of frequency of occurrence (%F) and wet mass (%mass) of different types and colours of artificial debris in the gut and faeces samples obtained at the Sanriku Coast.

Category	Loggerhead sea turtles				Green sea turtles			
	% F		% mass		% F		% mass	
	Feces (n= 28)	Gut (n= 13)	Feces (n= 28)	Gut (n= 13)	Feces (n= 25)	Gut (n= 10)	Feces (n= 25)	Gut (n= 10)
Type								
Hard plastic	3.6	15.4	2.0	3.1	16.0	30.0	0.3	0.6
Soft plastic	28.6	53.9	67.6	52.7	92.0	100.0	76.7	88.9
Styrofoam	0.0	30.8	0.0	44.1	20.0	30.0	1.3	0.6
Fishing line/Rope	7.1	7.7	19.1	0.1	52.0	80.0	19.9	5.9
Rubber	0.0	0.0	0.0	0.0	4.0	40.0	0.1	0.4
Others	3.6	0.0	11.3	0.0	20.0	40.0	1.7	3.7
Color								
Transparent	28.6	53.9	61.5	51.0	80.0	100.0	46.2	55.6
White	7.1	30.8	20.4	45.8	64.0	100.0	29.3	18.6

Black	3.6	0.0	11.3	0.0	64.0	70.0	10.3	6.5
Colored	10.7	23.1	6.7	3.2	72.0	90.0	14.2	19.4

Source: Fukuoka et al. (2016)

Ischaemia, necrosis, and intestinal rupture are the most frequent digestive tract mucosal injuries associated with the ingestion of solid waste (Levitt and Bauer 1992; Jerdy et al. 2017). Secondary infections and death are consequences of these injuries. For example, the presence of the bacterium *Enterococcus faecalis* in the bladder, brain, intestines, kidneys, liver, lungs, and muscles of a loggerhead turtle with an oesophageal perforation caused by a fishing hook and intestinal intussusception caused by a nylon strand have been found. In this case, the oesophageal and/or intestinal lesions were the likely entry routes for the pathogen, and its presence in the organs and viscera suggested septicaemia as the main cause of the animal's death (Fichia et al. 2016).

Plastic ingestion, in addition to the physical and anatomical aspects, exposes animals to an additional source of toxins (Teuten et al. 2009; Andrady 2011). Following the ingestion of plastics, chemical additives and persistent bioaccumulative and toxic chemicals can be absorbed by the body (Engler 2012; Fossi et al. 2018). This further increases the load of pollutants on the individuals through their transfer from the prey to predators in the food chain (Eriksson and Burton 2003).

Pollutants cause harmful effects, including impaired physiological aspects, chronic stress, immunosuppression, and increased susceptibility to diseases (Aguirre et al. 1995). Studies in North Carolina (USA) involving *C. caretta* have demonstrated that organochlorine compounds accumulate in animal tissues, causing hepatotoxicity, immunotoxicity, reproductive toxicity, and weakening, and affect the neurological behaviour (Keller et al. 2004). Saeki et al. (2000) and Fujihara et al. (2003) identified high concentrations of arsenic in both the liver and muscles in *E. imbricata*, *C. caretta*, and *C. mydas*. Acute arsenic intoxication can lead to gastrointestinal disorders, and ultimately, neurological problems (Gontijo and Bittencourt 2005).

4. Imaging exams on sea turtles

Imaging exams can be used on sea turtles to identify alterations of the digestive tract, such as intussusceptions, impacts, obstructions, torsions, neoplasms, and foreign bodies,

mainly due to the presence of anthropic waste (De Vico et al. 2003; Pressler et al. 2003; Di Bello et al. 2006; Valente et al. 2006; Valente et al. 2007a; Valente et al. 2007b; Erlacher-Reid et al. 2013; De Majo et al. 2016; Spadola et al. 2016b; Pease et al. 2017).

4.1 RADIOGRAPHY

Radiological examinations are used to detect the presence of anthropic residue in the digestive tracts of sea turtles, such as ingested metal hooks. However, it has limited abilities in identifying the internal organs due to the presence of the carapace and lack of visceral fat (Deshaw et al. 1996). Moreover, the low radiopacity of other foreign bodies, such as nylon strands and plastic bags, hampers its effectiveness. However, the use of a barium contrast may enable the identification of the points of obstruction (Figure 3) (Di Bello et al. 2006).

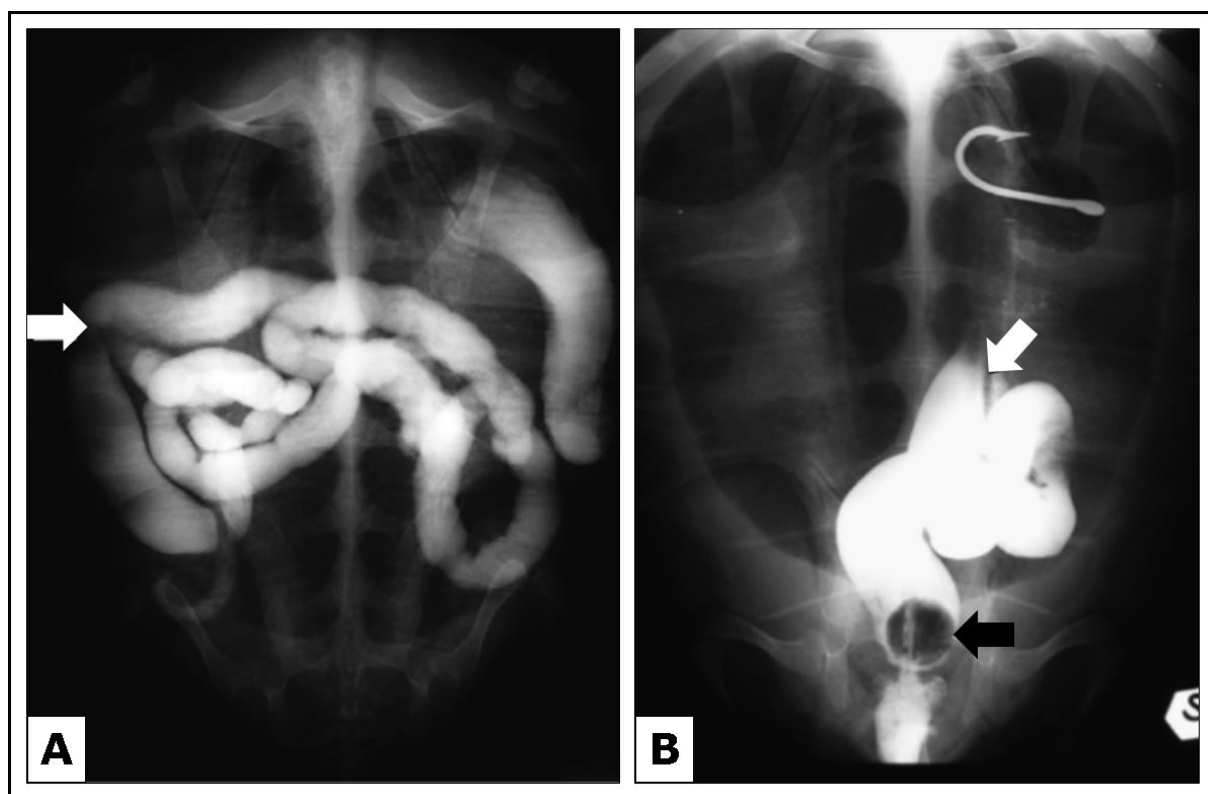


Figure 3. Contrast radiography of a loggerhead sea turtle's (*Caretta caretta*) digestive tract. (A) A dorso-ventral radiograph taken 48 h after the oral administration of a barium contrast. The residual contrast medium in the stomach and completely filled small intestine can be observed as the contrast medium progresses through the ileocolic valve (white arrow). (B) A dorso-ventral radiograph taken after an enema with a barium contrast. No retrograde progression of the contrast medium is shown due to the presence of a fishing strand attached

to the fishing hook in the intestinal tract. A Foley probe is visible in the cloaca region (black arrow). The radiolucent line (white arrow) in the proximal extension of the contrast medium indicates the end of the thick nylon fishing strand. Source: Modified Di Bello et al. (2006).

4.2 ENDOSCOPY, CLOACOSCOPY AND COLONOSCOPY

An endoscopy is a viable diagnostic technique in clinical practice for reptiles (Hernandez-Divers 2004; Spadola et al. 2016b; Franzen-Klein et al. 2020). The endoscopy of the upper digestive tract in sea turtles is relatively straightforward, since it allows the perfect visualisation of the oropharynx and glottis. However, the oesophageal path requires the extension of the neck of the animal and the inflation of the oesophagus with air to facilitate the passage of the endoscope as the oesophageal papillae resist its passage (Figure 4A). However, foreign bodies, such as hooks, which are radiographically identified, are not always visible in endoscopies as they are often lodged between the papillae. However, the gastroesophageal sphincter remains partially open and can be easily utilised by the endoscope, allowing the complete visualisation of the gastric mucosa (Figure 4B) (Pressler et al. 2003).

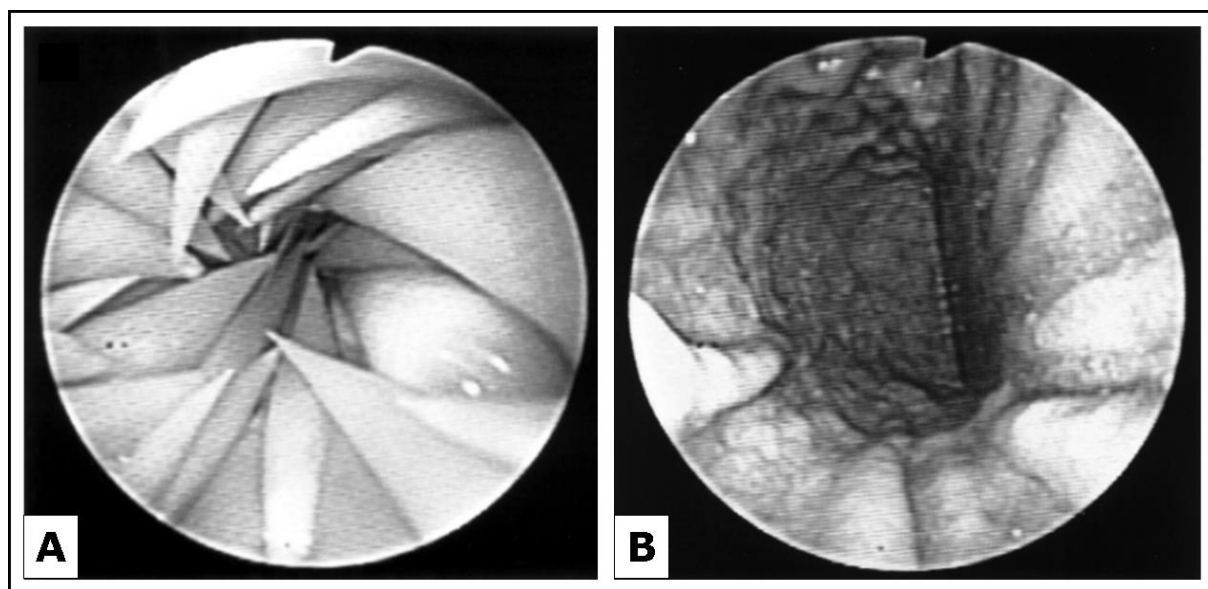


Figure 4. Endoscopy analysis of a loggerhead turtle (*Caretta caretta*). (A) Endoscopic view of the oesophageal papillary region. (B) Endoscopic view of the gastric lumen and pylorus, visible from a distance. Source: Pressler et al. (2003).

A cloacoscopy was first described by Coppoolse and Zwart (1985) and has since been rapidly adopted by reptile specialists. A cloacoscopic analysis of sea turtles allows the

visualisation of different organ systems, such as the gastrointestinal, reproductive, and urinary systems (Figure 5). The term colonoscopy is used when the exam is performed on the colon segment. It is a valuable technique for acquiring clinical information in cases of intestinal stenosis (Erlacher-Reid et al. 2013). Moreover, if any accessible foreign body, such as fishing strands, is present in the cloaca, the colonoscopy enables its removal in a minimally invasive manner (Figure 6) (Pressler et al. 2003; Spadola et al. 2016b).

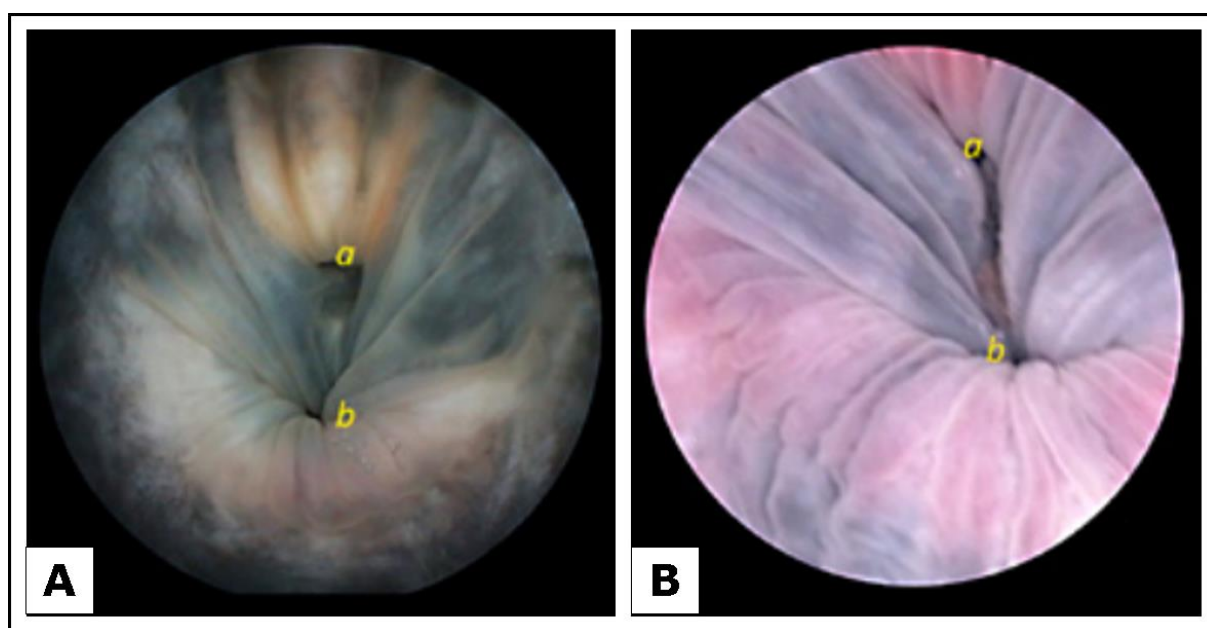


Figure 5. Cloacoscopy of the coprodeum and urodeum after an infusion of sterile saline in a loggerhead sea turtle (*Caretta caretta*). (A and B) The lower part of the image refers to the dorsal part of the animal. Observe the urodeum área (A) and the colon distal sphincter (B) in the adult female. Source: Spadola et al. (2016b).

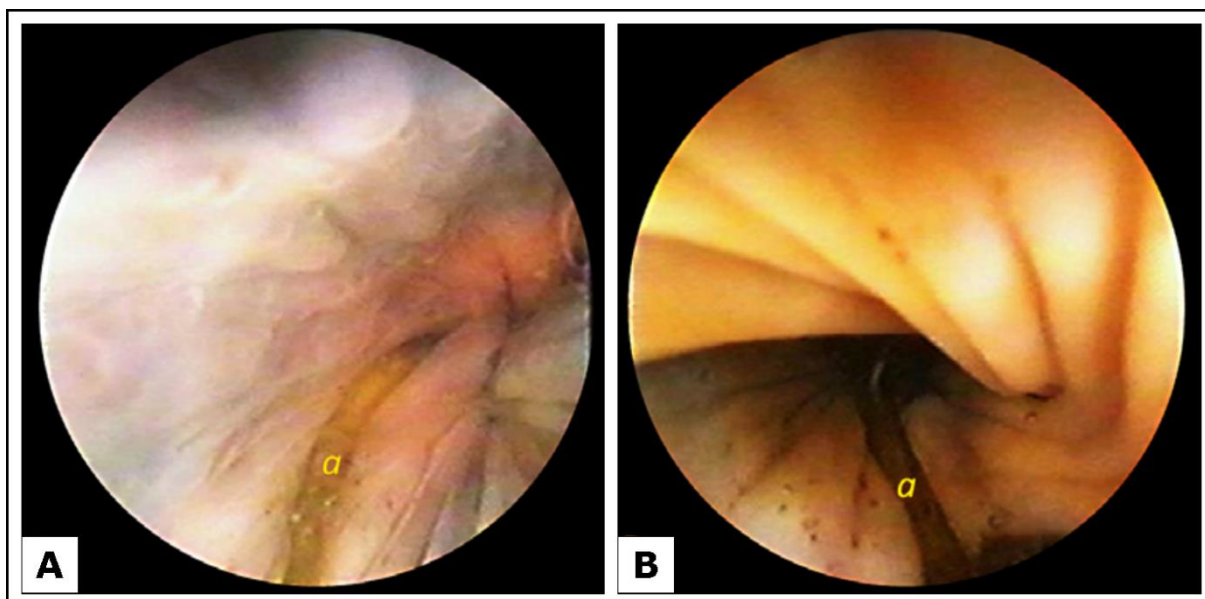


Figure 6. Cloacoscopy analysis of a loggerhead sea turtle (*Caretta caretta*) (A and B) Fishing strands (A) can be seen in the distal colon. Source: Spadola et al. (2016b).

4.3 ULTRASONOGRAPHY

Ultrasonography is a useful tool for the diagnosis of abnormalities of the digestive tract in juvenile and sub-adult sea turtles. It is cost-effective, safe, and straightforward to perform. This method can be used to examine most internal organs (Valente et al. 2007b; De Majo et al. 2016; Franzen- Klein et al. 2020) and for the diagnosis of intussusceptions (De Vico et al. 2003; Leiria et al. 2016; Bercier et al. 2017). The stomach of sea turtles is commonly visualised through the left pre-femoral acoustic window (Figure 7A), while the small and large intestines can be viewed from both the left and right pre-femoral acoustic windows (Figure 7B) (De Majo et al. 2016; Franzen-Klein et al. 2020).

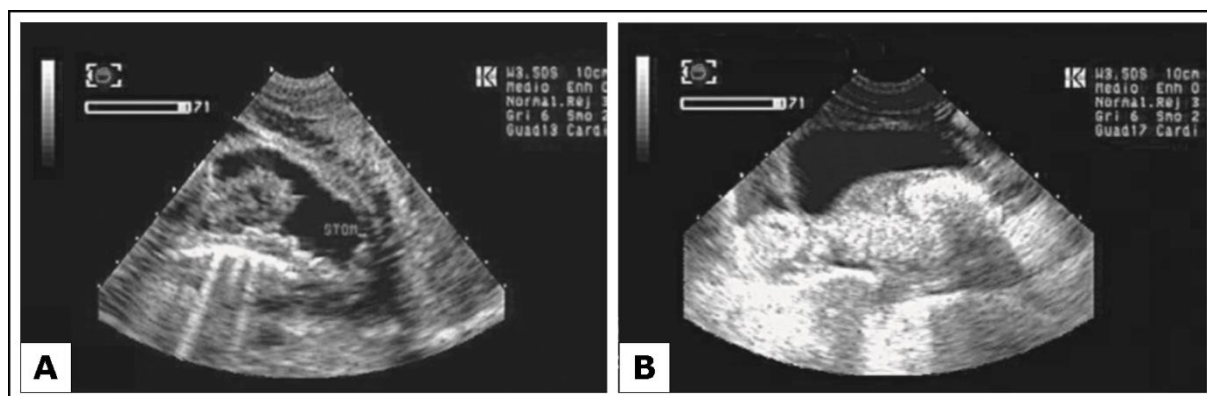


Figure 7. Ultrasonography analysis of a loggerhead sea turtle (*Caretta caretta*) (A) Stomach: Gastric material. (B) Colon: Presence of gas with hyperechoic content, along with acoustic shading. Source: De Majo et al. (2016).

4.4 COMPUTED TOMOGRAPHY (CT)

CT is a rapid and feasible imaging method for the clinical examination of sea turtles (Oliveira et al. 2012; Spadola et al. 2016a), as shown in a study reporting an intestinal mass in a green turtle (Figure 8) (Erlacher-Reid et al. 2013).

In this method, an iodine contrast medium can be used to increase the contrast between the normal structures and pathological tissues and to turn the vascular structures opaque (Caldmeyer and Buckwalter 1999).

Despite its high costs, the use of CT may reduce the treatment time, maximise the chances of successful rehabilitation, and consequently, reduce the cost associated with keeping the animal in captivity (Valente et al. 2007a; Erlacher-Reid et al. 2013; Spadola et al. 2016a). Therefore, CT is a valuable non-invasive method for the clinical examination of sea turtles in the rehabilitation process (Spadola et al. 2016a).

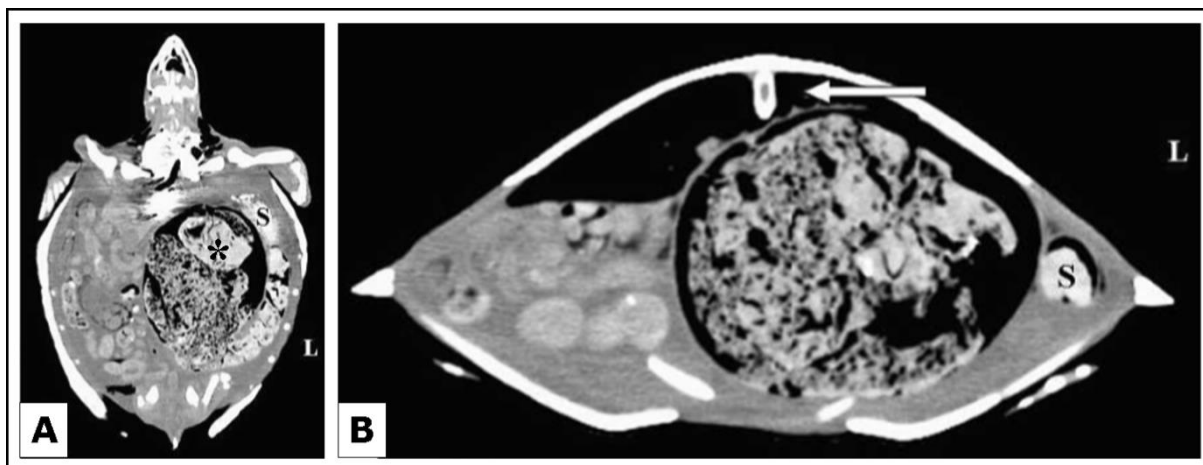


Figure 8. Helical computed tomography of a green sea turtle (*Chelonia mydas*). (A) In the dorsal plane, a large intestinal mass, impaction, or foreign body is predominantly present in the dorsal plane and on the left side of the celomatic (*) cavity, displacing the stomach (S) to the left. (B) An intestinal mass, impaction, or foreign body measuring 8.7 cm in diameter is shown in the cross-sectional plane, displacing the left pulmonary lobe (arrow) dorsally and the stomach (S) to the left. Source: Modified Erlacher-Reid et al. (2013).

4.5 MAGNETIC RESONANCE IMAGING (MRI) OR MAGNETIC RESONANCE TOMOGRAPHY (MRT)

Compared with the other diagnostic imaging tools, MRI offers many advantages. Because no ionising radiation is used in MRI, it is not dangerous for the patients or technical personnel (Schreiber et al. 2001). It is a powerful technique for obtaining soft tissue images in several planes without repositioning the animal (Dennis 1995; Valente et al. 2006) and allows the visualisation of the blood vessels without the need for a contrast medium (Tucker and Gavin 1996). However, the data analysis takes longer compared to other techniques, and the sedation of or administration of anaesthesia to the animal is usually required. MRI is expensive compared to other imaging techniques. However, its use can be justified when treating endangered species, as it collects a significant amount of diagnostic information in a relatively short period of time with little risk to the animal (Walzer et al. 2002; Thornton et al. 2005; Valente et al. 2006).

Because the viscera of chelonians are completely covered by bone structures (carapace and plastron), the use of MRI to visualise and evaluate celomic structures has several advantages over other conventional imaging techniques. These include the representation of detailed cross-sectional anatomical features without distractions from overlapping structures,

variations in grayscale formats, enhanced contrast resolution, and the computer reconstruction of multi-plane images (Figure 9) (Rubel et al. 1994; Valente et al. 2006).

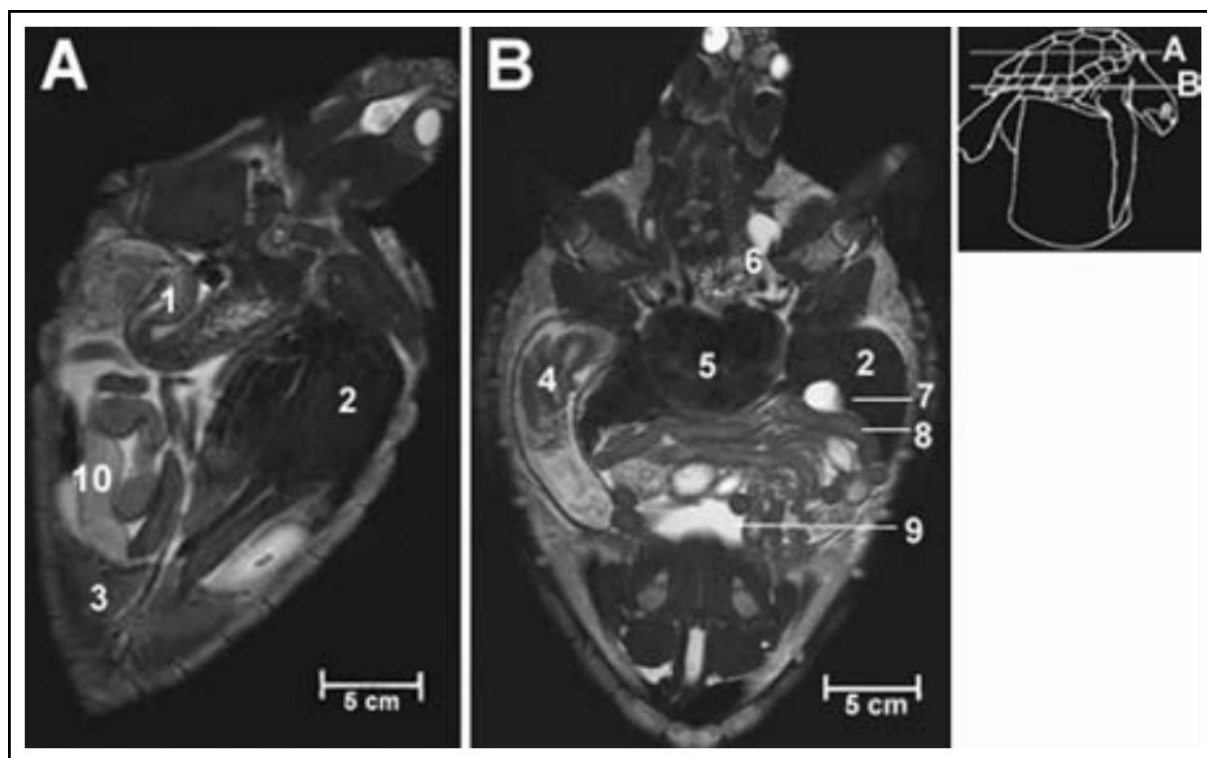


Figure 9. T2-weighted dorsal magnetic resonance images of the celomatic cavity of a live loggerhead turtle at different carapace levels (A and B). Cardiac sphincter (1), liver (2), kidney (3), stomach (4), heart (5), oesophagus (6), gall bladder (7), duodenum (8), urinary bladder or celomic fluid (9), and descending colon (10). Source: Valente et al. (2006).

Generally, digital radiography is the first choice for diagnostic imaging examination because it is widely available and less expensive than other techniques. Moreover, this method provides important information in many cases (Helmick et al. 2000; Di Bello et al. 2013). If a clinical diagnosis is subsequently available, the animal can be treated according to a veterinarian's recommendations, and there is no need for further exams. However, if the diagnosis is doubtful, the use of other tools, such as digital contrast radiography, ultrasonography, endoscopy, or cloacoscopy, should be considered, depending on the results of the clinical evaluation (Pressler et al. 2003; Di Bello et al. 2006; De Majo et al. 2016; Spadola et al. 2016b). Similarly, if a conclusive diagnosis is made by appropriate tests and tools, further exams may not be necessary. Otherwise, more complex and local exams, including CT or MRI, can be conducted based on their availability (Valente et al. 2006;

Williams et al. 2013). A schematic diagram for the decision-making with regard to the choice of diagnostic methodology is provided in Figure 10.

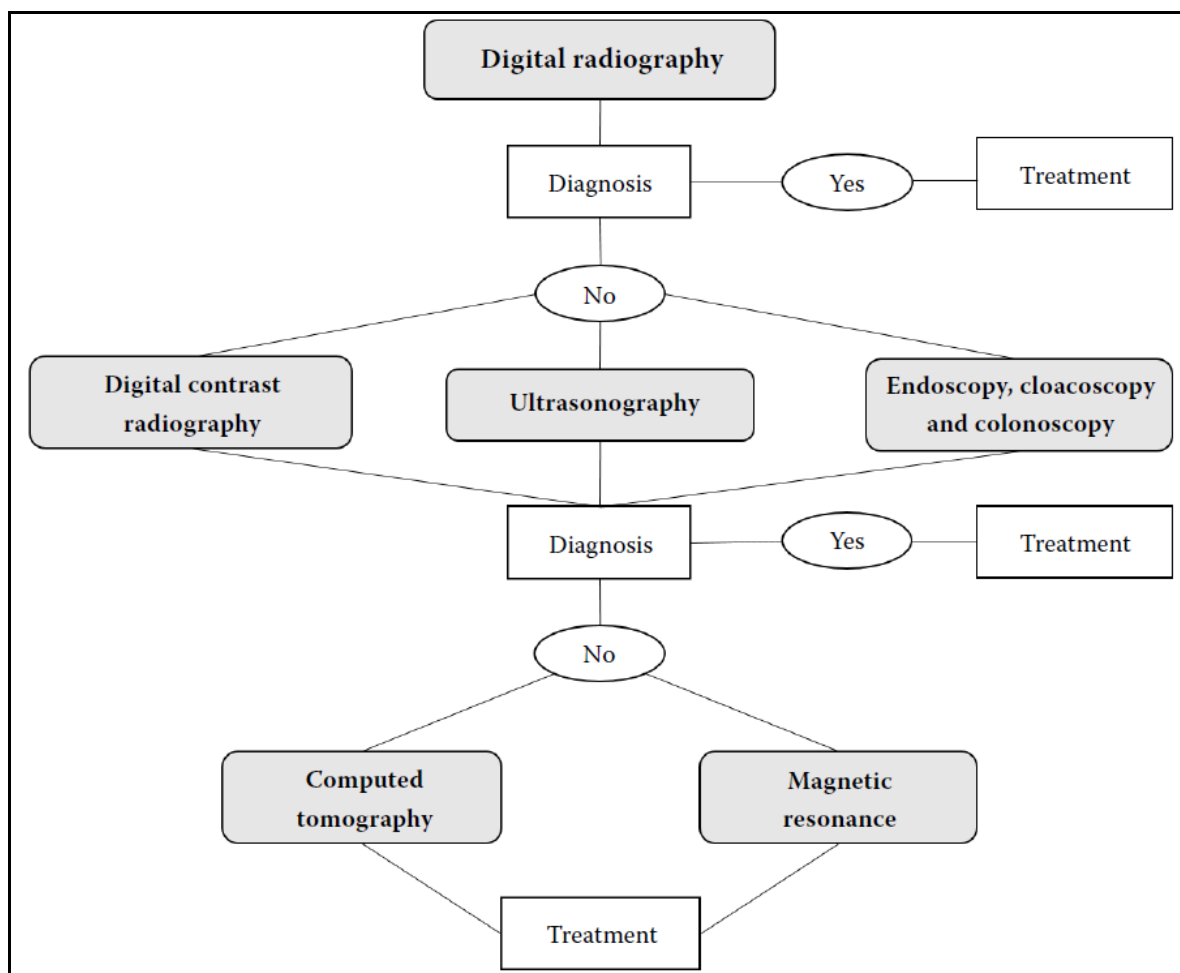


Figure 10. Decision-making flowchart for the digestive tract imaging examinations of sea turtles. Source: Own processing.

5. Pathological findings in the digestive tract through imaging exams

Williams et al. (2013) reported a case where a loggerhead turtle accidentally ingested two rubber stoppers from a water heater in its enclosure. The radiographs initially taken were inconclusive. Subsequently, CT was used, and the ingested items were identified based on their shape and size. Further, a partial obstruction was observed in the region where the objects were located. The animal was treated with a mineral oil-based medical therapy, and a tomographic examination was repeated after two weeks; this identified a persistent partial obstruction. However, the displacement of the rubber stoppers through the intestinal tract was

observed, allowing the authors to conclude that the continued treatment was adequate, since the foreign bodies were expelled in the faeces 26 days after their ingestion.

Franzen-Klein et al. (2020) reported six cases of foreign body obstructions in sea turtles, which were diagnosed using different advanced imaging modalities. Pre-femoral ultrasonography was used to correctly diagnose a monofilament fishing line linear foreign body obstruction in a juvenile female Kemp's ridley sea turtle (*L. kempii*) and a subadult female loggerhead sea turtle (*C. caretta*). The administration of an iodinated contrast confirmed a complete upper gastrointestinal obstruction in a juvenile green sea turtle (*C. mydas*). The traditional radiographs were non-diagnostic in all three animals. Barium-impregnated polyethylene spheres were used to identify a partial gastrointestinal foreign body obstruction in a juvenile green sea turtle. An endoscopy was used in multiple cases as both a diagnostic and therapeutic tool.

A case of an intestinal volvulus in a green sea turtle was reported by Helmick et al. (2000). Based on the radiography, the authors confirmed the gaseous distension of the animal's intestinal loops, suggesting an intestinal obstruction. Subsequently, the animal was admitted for surgery; during the procedure, an intestinal stenosis was observed at the site of the volvulus. After resection of the abnormal intestine, a leiomyoma associated with the focal stenosis was confirmed by the histopathological examination. Six months after surgery, the turtle recovered its normal buoyancy pattern and showed no further clinical signs of a gastrointestinal obstruction, as evidenced by a normal appetite and defecation.

Other cases have reported intestinal or cloacal stenosis resulting in intestinal obstructions in six green sea turtles. The aetiologies of the stenoses were unknown in these cases. The common abnormalities observed following the radiological imaging among most of these animals included constipation, enlarged intestines, and the accumulation of the oral contrast material. A lower gastrointestinal contrast radiography combined with the oral contrast was useful for identifying the suspected area of intestinal obstruction in two cases. A colonoscopy was crucial to visually diagnosing an intestinal stenosis in one case. A diagnosis was achieved in two cases through CT. In both cases, the colon was distended and linear soft tissue attenuation was present, suggesting oedema and inflammation of the intestinal loop. In one case, the MRI enabled the detection of a significant increase of the cloacal and intestinal dilation near the right and left kidneys (Erlacher- Reid et al. 2013).

Cases of intestinal intussusception have previously been reported in loggerhead (De Vico et al. 2003; Fichia et al. 2016) and green sea turtles (Oliveira et al. 2020b), with a partial or total obstruction being the most commonly reported consequences of this disease (Levitt

and Bauer 1992). A permanent blood vessel obstruction resulting from the intussusception is related to ischaemia and necrosis, which are the determining factors for the development of late gastrointestinal perforations and ruptures (Patsikas et al. 2005). Although all intestinal portions can be affected, the incidence of such obstructions occurring in the ileocolic segment is higher than that of those occurring in the other portions in both animals and humans (Levitt and Bauer 1992; Wosar and Lewbart 2006; Batista and Maximiano 2009). Predisposing factors are often associated with gastroenteritis, parasitic infection, and foreign bodies (Macphail 2002). However, the presence of anthropic waste (nylon strands, hooks, plastic bags, and hard plastics) in the digestive tract of sea turtles is commonly reported as the main reason for the intussusception (De Vico et al. 2003; Orós et al. 2004). However, the diagnosis of an intestinal intussusception in sea turtles is relatively limited due to the frequent absence of clinical signs in the afflicted animals. However, ultrasonography and contrast radiography can be used as suitable imaging exams (De Vico et al. 2003; Di Bello et al. 2006).

Croft et al. (2004) investigated the effectiveness of MRI and radiography in the detection of internal tumours in 10 green turtles. Radiography was found to not be efficient in identifying tumours in the digestive tracts of these animals, but could identify tumours of the lungs. However, the MRI was able to detect tumour masses in the stomach of one animal and in the bladder, kidneys, and lungs of other animals.

6. Conclusions

Despite the low radiopacity for most foreign bodies, such as nylon strands and plastic bags, digital radiography remains a very useful tool for identifying solid waste with high radiopacity, such as hooks and rigid waste.

The use of a barium contrast makes this method even more efficient and even allows the obstruction points of the whole digestive tract caused by the presence of low-radiopacity residue to be identified.

Endoscopies and cloacoscopies are less invasive methods, the former being used mainly in the initial segments of the digestive tract, such as the oral cavity, oesophagus, and stomach, while the latter is useful in investigations of the caudal region, rectum, and cloaca.

Ultrasonography is efficient in identifying inflamed areas of the digestive tract and in diagnosing intussusceptions. It can also identify the accumulation of gases and other contents in the intestinal loops, which may indicate possible points of obstruction.

CT and MRI, despite their high costs, are the exams of choice for identifying not only rigid tissues, but also changes in soft tissues in the digestive tract, especially those related to abscesses and neoplasms.

Therefore, the diagnostic imaging exams demonstrate their importance in preservation medicine, as they can indicate the type of material ingested as well as its location, thereby allowing the appropriate decision-making for an animal's treatment.

However, the methodology to be used by a specialist depends on the suspected diagnosis as well as the financial and logistical conditions of a case. It is noteworthy that no imaging exam discards the need for a good anamnesis as well as evaluations using auxiliary exams, such as haemograms and serum biochemistry.

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Conflict of interest

The authors declare no conflict of interest.

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**CAPÍTULO 5 – DEATH OF A LOGGERHEAD SEA TURTLE (*Caretta caretta*) FROM
INGESTION OF AN EEL (*Myrichthys ocellatus*)**

(Artigo de relato de caso)

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Death of a loggerhead sea turtle (*Caretta caretta*) from ingestion of an eel (*Myrichthys ocellatus*)

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Abstract: Gastrointestinal perforation in sea turtles may be associated with the ingestion of solid waste; however, other factors, including the ingestion of marine organisms, may cause intestinal perforation. Herein, we report, presumably, the first case of a death of a loggerhead sea turtle (*Caretta caretta*) from ingesting a live goldspotted eel (*Myrichthys ocellatus*) and describe the necropsy findings. The adult female loggerhead sea turtle was registered alive by a team of the Cetáceos da Costa Branca Project of Rio Grande do Norte State University (PCCB-UERN). The animal died in captivity after 8 days of an attempted rehabilitation process, and the carcass was immediately sent for necropsy. After incision, an encapsulated structure in the cranial region of the left lung was identified as a specimen of the ingested eel. The histopathological examination of the lung showed fibrin and numerous leukocytes, mainly macrophages, in the alveolus and bronchioles. The necropsy revealed that the ingestion of a live goldspotted eel (*M. ocellatus*) caused a gastric perforation in the turtle; this resulted in the displacement of the eel to the celomatic cavity with its cranial portion in the left lung, leading to sepsis and the consequent death of the turtle.

Keywords: Cheloniidae, gastric perforation, septicemia, Testudines.

INTRODUCTION

Loggerhead sea turtles (*C. caretta*) are a globally distributed migratory species and are classified as “vulnerable” to extinction according to the International Union for Conservation of Nature red list of threatened species (Casale and Tucker 2017). Conservation of this species is a complicated task that requires knowledge of its essential biological aspects, such as reproduction, migration, and feeding habits (Burke et al. 1993).

These animals are carnivorous throughout their life cycle, preferentially feeding on fish, followed by pelagic tunicates, crustaceans, mollusks, and other invertebrates (Tomas et al. 2001; Revelles et al. 2007). Dietary data of these specimens are relevant to the understanding of their predator–prey relationships.

Moreover, it is difficult to diagnose gastrointestinal diseases in sea turtles antemortem. Clinical signs found on necropsies provide relevant decision-making information regarding the rehabilitation of stranded animals.

Herein, we report the first case of the death of a loggerhead sea turtle (*C. caretta*) caused by ingestion of a live goldspotted eel (*M. ocellatus*) and have described the necropsy findings.

CASE DESCRIPTION

An adult female loggerhead sea turtle (*C. caretta*), previously registered by the Cetáceos Costa Branca Project of Rio Grande do Norte State University (PCCB-UERN), was rescued alive on March 27, 2018 from the Requenguela beach (4°39'56.4"S, 37°25'41.7"W), Icapuí, Ceará, Brazil. The experiment was conducted with authorization from the Ethics Committee on Animal Use (License No. 01/2019).

The prostrate and debilitated animal was rescued and sent to the Wild Animal Rehabilitation Center of the Cetáceos Costa Branca Project (UERN) in the city of Areia Branca, Rio Grande do Norte (RN), Brazil, where it was kept in a salt water enclosure measuring 200 cm in diameter and 75 cm in depth, with a water temperature of 25–28 °C and natural light from approximately 5:30 a.m. to 5:30 p.m. Biometric data included a curved carapace length of 96.0 cm, curved carapace width of 87.0 cm, and body weight of 91.0 kg.

Clinical examination revealed a regular body score, anorexia, noisy breathing, severe dehydration, and the presence of algae and sessile crustaceans (barnacles) on its carapace and plastron. The turtle received supportive treatment with fluid therapy (KabiPac[®], 0.9% sodium chloride, 20 mL/kg IV, Fresenius Kabi, Brazil), diluted with vitamin (Polivin B12[®], vitamin B12, 0.3 mL/kg IV, Bravet, Brazil), antibiotic therapy (CEF 50[®], ceftiofur hydrochloride, 4 mg/kg IM, Agener União, Embu-Guaçu, São Paulo, Brazil), and analgesia (Tramal[®], tramadol hydrochloride, 5 mg/kg SC, Grünenthal, Germany; Maxicam[®], meloxicam, 0.1 mg/kg IM, Ourofino, Brazil; D-500[®], dipyrrone, 25 mg/kg IM, Zoetis, Campinas, São Paulo, Brazil). The turtle died after 8 days of treatment and was immediately sent for necropsy to the Marine Biota Monitoring Laboratory of UERN in Mossoró, RN.

Complete necropsy was performed following the standard postmortem protocol for sea turtles (Wyneken 2004). The celomatic cavity surrounding the entire plastron was opened for a macroscopic analysis and then removed for an in situ analysis of the organs. Subsequently, all organs were removed outside of the cavity for evaluation, and the most evident macroscopic findings were photographed.

Organ fragments were collected for histopathological examination and fixed in 10% formalin. After fixation for 48 h, the samples were dehydrated using increasing concentrations of alcohol (70%, 80%, 90%, 95%, 100%, 100%, and 100%) for a period of 1 h for each concentration, followed by diaphanization consisting of two xylol baths of 30 min each and subsequent paraffin embedding by immersion in granular histological paraffin with a melting point of 58–62 °C (Synth[®], Labsynth, Brazil) in an oven at 58 °C. After preparation, the

histological blocks were cut into 5- μ m sections using a microtome (Leica RM2125 RT) adhered to glass slides, placed in an oven at 58 °C for deparaffinization, and stained with hematoxylin and eosin (Prophet et al. 1992). Photomicrographs of the most representative images were acquired under a light microscope (Leica ICC50 HD) and obtained using the Leica LAS EZ software (version 2.0.0., Leica Microsystems Ltd., Heerbrugg, Switzerland).

In the initial portion of the digestive tract, the esophagus and stomach showed no food content but contained mucus; the cardiac region of the gastric mucosa showed a small, healed, rounded lesion (Figure 1A). The intestines showed dilated portions containing gas and a greenish-brown liquid. The total length of the small and large intestines was 714 cm. An encapsulated foreign body was found adjacent to the pancreas, and an incision revealed unidentified food content extravasated from the stomach (Figure 1B).

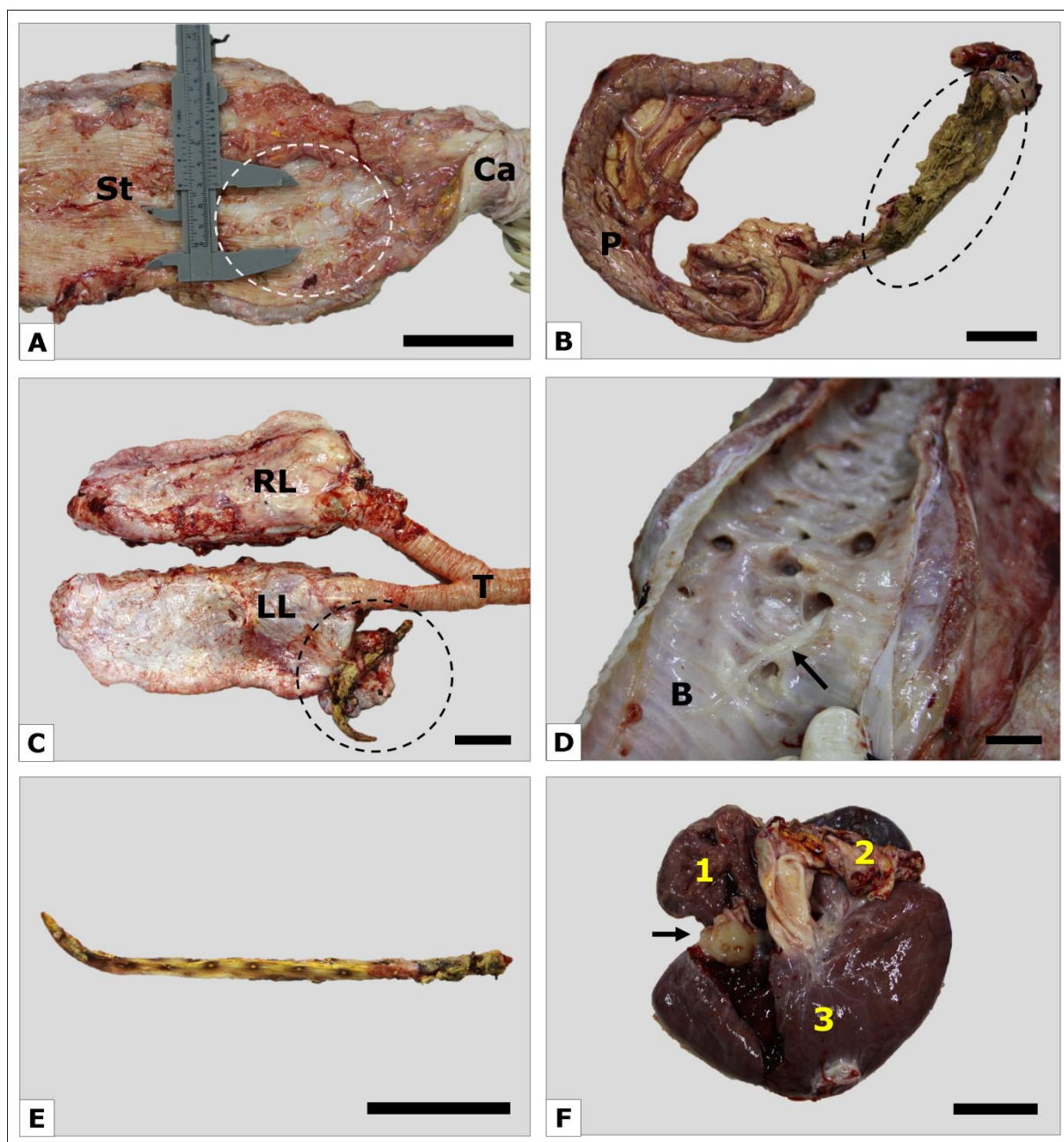


Figure 1. Macroscopic findings of a loggerhead sea turtle (*Caretta caretta*) that died from ingestion of a live goldspotted eel (*M. ocellatus*). (A) Perforation in the stomach (St), with presence of scar tissue (dashed circle) near the cardiac region (Ca) caused by ingestion of the eel. Bar = 5.0 cm. (B) Presence of encapsulated (dashed circle) extravasated stomach content in the pancreas (P). Bar = 5.0 cm. (C) Tracheal region (T) of the respiratory system and the right (RL) and left (LL) lungs with a specimen *M. ocellatus* in the cranial region of the left lung (LL). Bar = 5.0 cm. (D) Presence of yellowish mucoid secretion in the bronchi (arrow). (E) Details of the eel *M. ocellatus*. Bar: 2.0 cm. (F) Dilated flaccid right atrium (1) with a thickened atrioventricular valve (arrow) and left atrium (2) and ventricle (3) without apparent macroscopic changes. Bar = 5.0 cm

The liver was enlarged, with rounded edges, capsular thickening, whitish granules distributed diffusely in the serosa, nutmeg-colored parenchyma, and an abscess (10.0 cm diameter) with caseous content (Figure 2A and 2B). Histopathological examination revealed multifocal areas of hepatocellular necrosis and hepatocyte cord dissociation (Figure 2C and 2D).

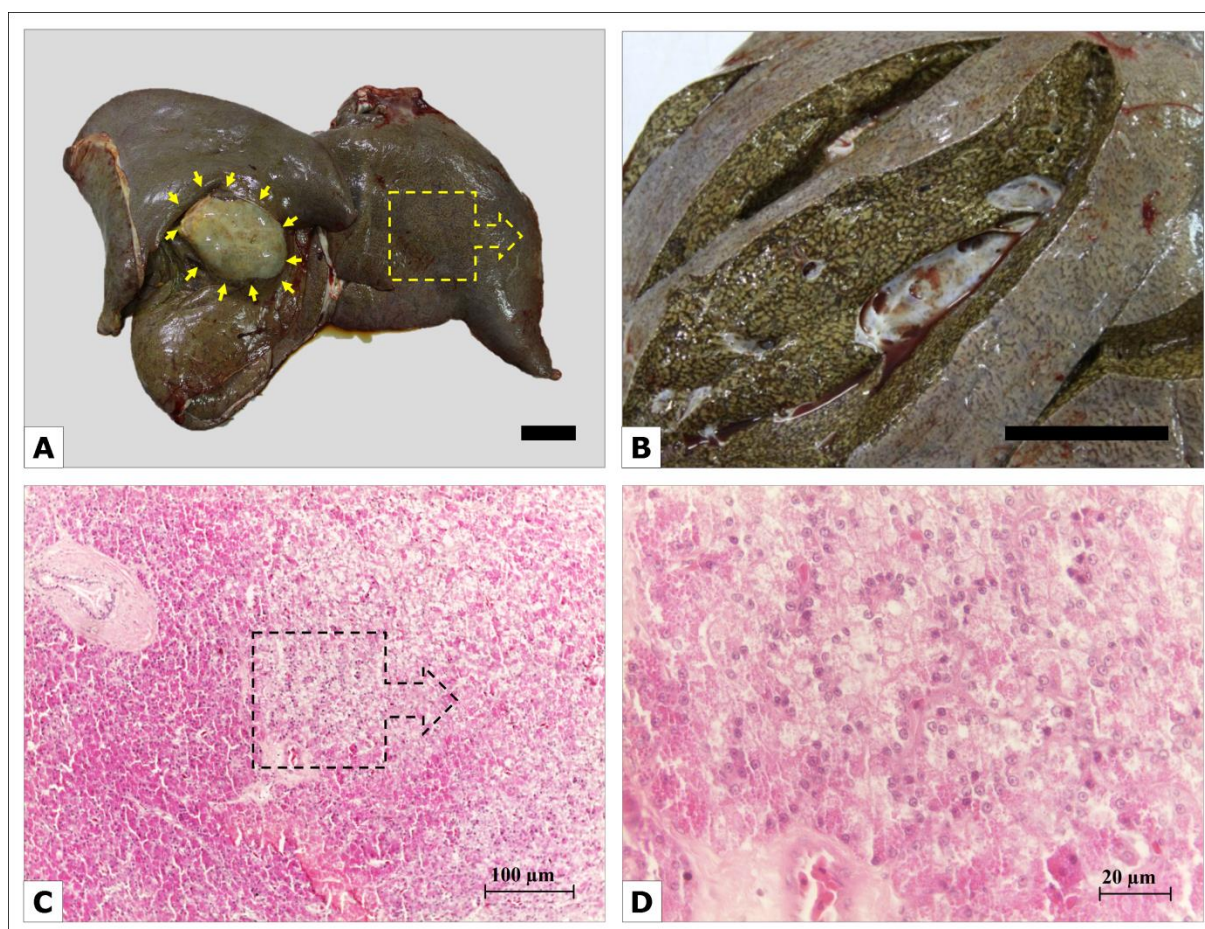


Figure 2. Histopathological findings of the liver of the loggerhead sea turtle (*Caretta caretta*) that died from ingestion of a live eel (*M. ocellatus*). (A) Enlarged liver with rounded, thickened edges and a 10.0-cm diameter abscess with caseous content (arrows). Bar = 5.0 cm. (B) Nutmeg-colored liver parenchyma. Bar = 5.0 cm. (C) Multifocal areas of hepatocellular necrosis. H&E; bar = 100 µm. (D) Zoomed figure 2C showing derangement of cellular architecture resulting from hepatic necrosis. H&E; bar = 20 µm. H&E: hematoxylin and eosin.

In the respiratory system, the trachea and bronchi showed pale-yellow, mucoid secretion (Figure 1D) and solid parenchyma of firm and congestive consistency. An encapsulated structure in the cranial region of the left lung was identified as a specimen of the ingested eel after incision. The eel was removed, and the species was confirmed to be *M. ocellatus* (Figure 1C and 1E).

The histopathological examination of the lung showed fibrin and numerous leukocytes, mainly macrophages (Figure 3A and 3B), in the alveolus and bronchioles. The tissue that surrounded the specimen in the left lung was predominantly vascularised fibrous tissue (Figure 3C and 3D).

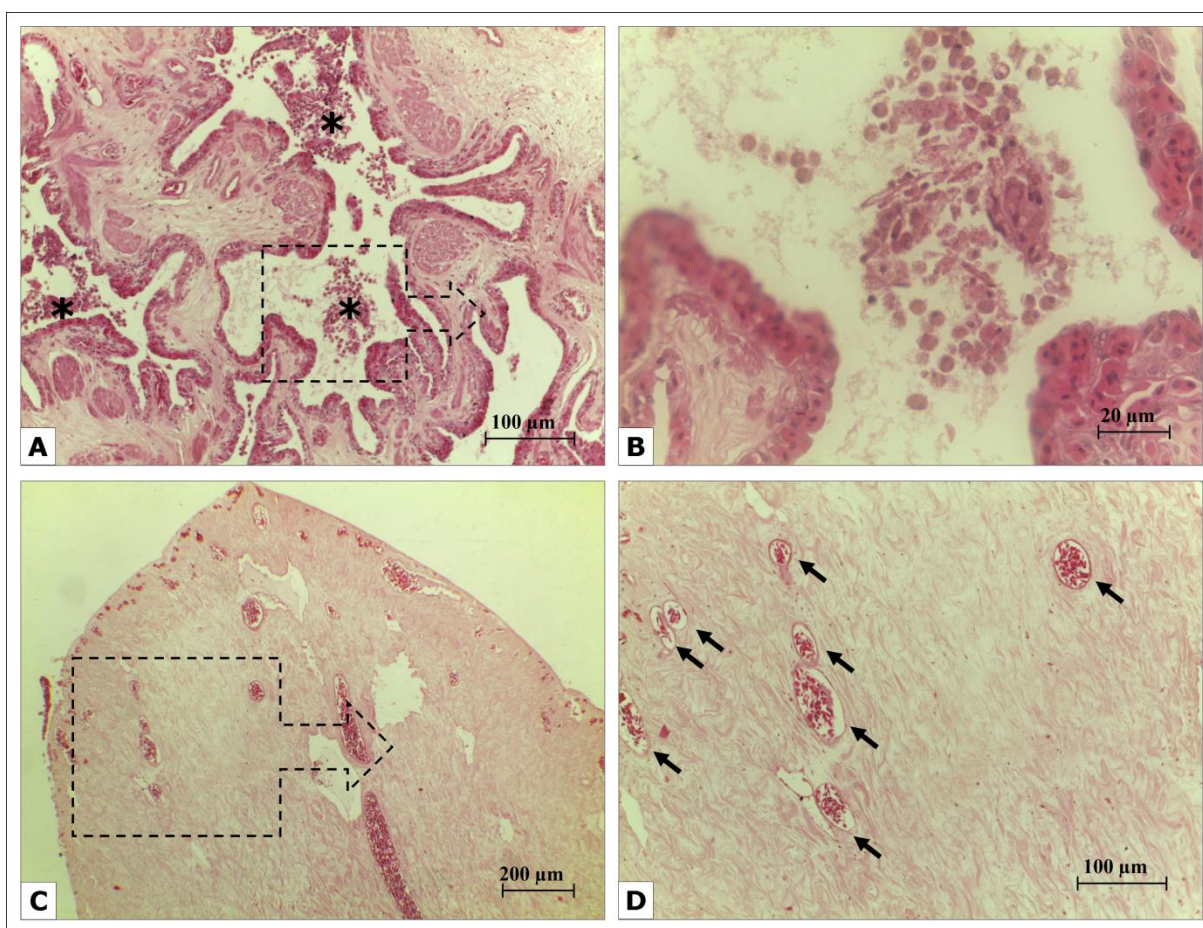


Figure 3. Histopathological findings of the lung of a loggerhead sea turtle lung (*C. caretta*) that died from ingestion of a live eel (*M. ocellatus*). (A) Presence of intra-bronchiolar fibrin-leukocyte exudate (*). H&E; bar = 100 µm. (B) Zoomed figure 3A showing numerous macrophages and dendritic cells and abundant fibrin accumulation. H&E; bar = 20 µm. (C) Fibrovascular tissue that was involved in the eel specimen in the left lung. H&E; bar = 200

µm. (D) Zoomed figure 3C showing fibrous tissue with blood vessels (arrows). H&E; bar = 100 µm. H&E: hematoxylin and eosin.

In the heart, the right atrium was dilated and flaccid with a thickened atrioventricular valve. No apparent macroscopic changes were observed in the left atrium or ventricle (Figure 1F). The other organs showed no noteworthy macro- or microscopic changes.

DISCUSSION AND CONCLUSIONS

Some studies on the feeding ecology of loggerhead sea turtles reported that the diet of these animals consisted mainly of fish, followed by pelagic tunicates, crustaceans, mollusks, and other invertebrates. Loggerhead sea turtles are often opportunistic feeders, eating mainly discarded fish; the prey quantity and diversity increases proportionally with turtle size (Tomas et al. 2001; Revelles et al. 2007).

Goldspotted eels (*M. ocellatus*) are distributed throughout the Atlantic Ocean, from Bermuda to southern Brazil (McCosker et al. 2015). It is commonly found in northeastern Brazil at depths of up to 30 m (Carvalho-Filho 1999). Despite being a prevalent species in the feeding areas of loggerhead sea turtles, the literature regarding eels being the feeding preference of these turtles or causing death is lacking.

In documented cases, gastrointestinal perforation in loggerhead turtles was associated with solid waste ingestion or the presence of hooks due to incidental capture during fishing activities (Horau et al. 2014). A study by Caracappa et al. (2018) on the incidental capture of *C. caretta* along the coast of Sicily reported that 129 of these loggerhead sea turtles had fishing hooks in different parts of the gastrointestinal tract, which were mostly found in the esophagus (47.3%), intestine (24.8%), stomach (14.7%), and oral cavity (13.2%).

Mucosal lesions in the gastroenteric region lead to ischemia, necrosis, and perforation (Jerdy et al. 2017), possibly resulting in secondary bacterial infections and death, as in a case study reporting the bacterium *Enterococcus faecalis* in the urinary bladder, brain, intestine, kidney, liver, lung, and muscle of a loggerhead sea turtle with esophageal perforation caused by a fishing hook and intestinal intussusception caused by a nylon thread. In the present case, lesions in the esophagus, intestine, or both were the probable routes of entry for this bacterium, and their presence in the organs and viscera suggested septicemia as the cause of death (Fichia et al. 2016). The same mechanism could be underlying the present case: The route of entry was the gastric perforation worsened by the presence of the eel in the lung.

Necropsy findings in the lungs, such as intra-bronchiolar fibrin-leukocyte exudate and cellular response, were consistent with a bacterial infection; other findings characteristic of septicemia, such as hepatocellular necrosis and a thickened right atrioventricular valve (endocarditis) were also observed.

These findings revealed that the loggerhead sea turtle had ingested a live eel (*M. ocellatus*) that caused gastric perforation, possibly displacing the eel into the celomatic cavity with its cranial portion in the left lung, and leading to sepsis and consequent death of the turtle.

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Conflict of interest

The authors declare no conflict of interest.

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CAPÍTULO 6 – COLOCOLIC INTUSSUSCEPTION ASSOCIATED WITH *Octangium* sp. (DIGENEA: MICROSCAPHIDIIDAE) IN A GREEN SEA TURTLE *Chelonia mydas*

(Artigo de relato de caso)

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Colocolic intussusception associated with *Octangium* sp. (Digenea: Microscaphidiidae) in a green sea turtle *Chelonia mydas*

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Running page head: Colocolic intussusception in green sea turtle

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Abstract

Reports of intussusception in sea turtles are generally linked to marine debris ingestion; therefore, only a few cases of the disease are associated with parasitic infestations. The objective of this study was to describe the necropsy findings of the first reported case of colocolic intussusception in a green sea turtle *Chelonia mydas*, associated with the helminth *Octangium* sp. A juvenile female green sea turtle, which was registered and rescued by the team from the Cetaceans Project of Costa Branca, State University of Rio Grande do Norte, was examined. The animal died 1 d after being treated and was immediately submitted for necropsy. Our findings indicated that parasitic infestation by *Octangium* sp. in the green sea turtle caused intussusception and consequently led to the animal's death. Early diagnosis and surgical correction are fundamental for a good prognosis and, consequently, for successful rehabilitation of marine species.

Key words: Digestive apparatus, Cheloniidae, helminths, histopathology, parasitism.

INTRODUCTION

Sea turtles face many threats, such as pollution, habitat loss, climate change, interaction with fisheries, and marine debris ingestion (Bugoni et al. 2001; Duncan et al. 2019). The presence of marine debris in the digestive tract has been previously reported to cause organ ruptures and even death of the affected animals (Orós et al. 2004b; Cappua et al. 2007; Fichia et al. 2016).

Green sea turtles (*Chelonia mydas*) present an ontogenetic shift from an omnivorous to a herbivorous diet when they move to the coastal areas after their pelagic phase (Bjorndal 1997). Their diet consists largely of seagrass and/or macroalgae (Spotila 2004).

The parasitic fauna associated with the green turtles is composed of a large number of helminth species belonging to the subclass Digenea and family Microscaphidiidae (Santoro et al. 2006; Werneck and Silva 2016). Sea turtles from the neotropical region of Central and South America present a great diversity of endohelminth, including species of the genus *Octangium* sp. (Werneck and Silva, 2016).

Intussusception is defined as the invagination of a portion of the gastrointestinal tract, called an intussusceptum, on the lumen of the adjacent portion, called the intussusciens (Macphail 2002). The major consequences of this disease are partial or total obstruction of the tract (Levitt and Bauer 1992). Permanent vessel obstruction is related to ischemia and

necrosis, which are the factors that determine the development of perforations and late intestinal ruptures (Patsikas et al. 2005). The diagnosis of intestinal intussusception in sea turtles is quite limited due to the frequent absence of clinical signs in these animals (De Vico et al. 2003).

Gastroenteritis and the presence of parasites and foreign bodies are often considered the predisposing factors of intussusception (Macphail 2002). However, with regard to sea turtles, the presence of marine debris is considered the main factor for causing the condition (De Vico et al. 2003; Orós et al. 2004b; Cappua et al. 2007). However, there are still no reports of this disease being associated with gastrointestinal parasites in the green turtle. Therefore, this is the first published report on the occurrence of colocolic intussusception associated with *Octangium* sp. in green sea turtles.

METHODS

Animal Rescue

The subject examined was a juvenile female turtle found stranded on December 6, 2018 on the rocky reefs of Pirangi beach (05°58'34.6"S, 35°7'18.58"W), Parnamirim, Rio Grande do Norte, Brazil. It was rescued and referred to the Wild Animal Rehabilitation Center of the Cetáceos Costa Branca Project-PCCB-UERN, in the city of Areia Branca, Rio Grande do Norte (RN), Brazil. Biometric data included a curved carapace length and width of 59.5 cm and 53 cm, respectively and body weight 15.8 kg.

Clinical examination revealed a regular body score, anorexia, severe dehydration, and the presence of algae and sessile crustaceans (barnacles) on its carapace and plastron, but there were no evident signs of anthropic interaction (marks of fishing interaction and/or piercing-cutting objects). The animal was kept on a mattress in an enclosure 200 cm in diameter x 75 cm deep, without water. It was apathetic and prostrated, showing no reaction to the stimuli made by the veterinary team. Treatment was started by administering 0.9% NaCl solution (dose: 20 mL/kg/IV) diluted with multivitamin (Hertavita[®]; dose: 1 mL/kg/IV), as well as antibiotic therapy with 0.5% metronidazole (dose: 30 mg/kg/IV), and analgesic therapy with tramadol 50 mg/mL (dose: 5 mg/kg/SC), 2% meloxicam (dose: 0.1 mg/kg/IM), and dipyrone 500 mg/mL (dose: 25 mg/kg/IM). After 24 hours of treatment, the animal died and was immediately submitted for necropsy.

Necropsy

Complete necropsy was performed following a standard *post mortem* protocol for sea turtles (Wyneken 2004). The coelomic cavity was opened for necropsy by removing the plastron and an *in situ* analysis of the organs was done; photographs of the representative findings were taken.

Histopathological Examination and identification of parasites

Tissue fragments from the colon with intussusception were collected and fixed in 10% formalin buffered with sodium phosphate 0.1M and pH 7.4, processed routinely for histology, embedded in paraffin, cut at 5 μ of thickness and stained with haematoxylin-eosin (HE) (Prophet et al. 1992). Photomicrographs of the most representative lesions were acquired under a light microscope (Leica ICC50 HD) and obtained using the Leica LAS EZ software, version 2.0.0.

The parasites were fixed in alcohol-formalin-acetic acid (AFA). After 48 hours, they were transferred and stored in 70% alcohol, and examined using a Stereozoom Microscope model (Labomed Luxeo 4D Digital). We analysed their morphological features following the key to the subfamily Octangiinae proposed by Blair (1987).

RESULTS

The organs in the coelomic cavity, such as the small intestine (duodenum, jejunum and ileum) and large intestine (colon) appeared pale with normal syntopy. Gas was observed in the intestinal loops and the megacolon, and free fluid was found in the coelomic cavity. During the manipulation of intestinal loops, a complete obstruction was observed in the colon portion, and after the obstruction, the loop was firm, similar to compacted faeces. On opening the colon, an intussusceptum was observed in the direction of peristalsis, measuring approximately 20 cm in length, which was inserted into an adjacent segment (intussusciptens) and was firm. There was thickening of the intussusceptum wall and necrotic areas were present in addition to congested areas. The location of the point that initiated intussusception, based on the distance between the cloaca and the point of obstruction, corresponded to approximately 25 cm (Figure 1). No plastic debris was found in the digestive tract, even

though the presence of this type of debris has frequently been mentioned in necropsy findings for the species examined.

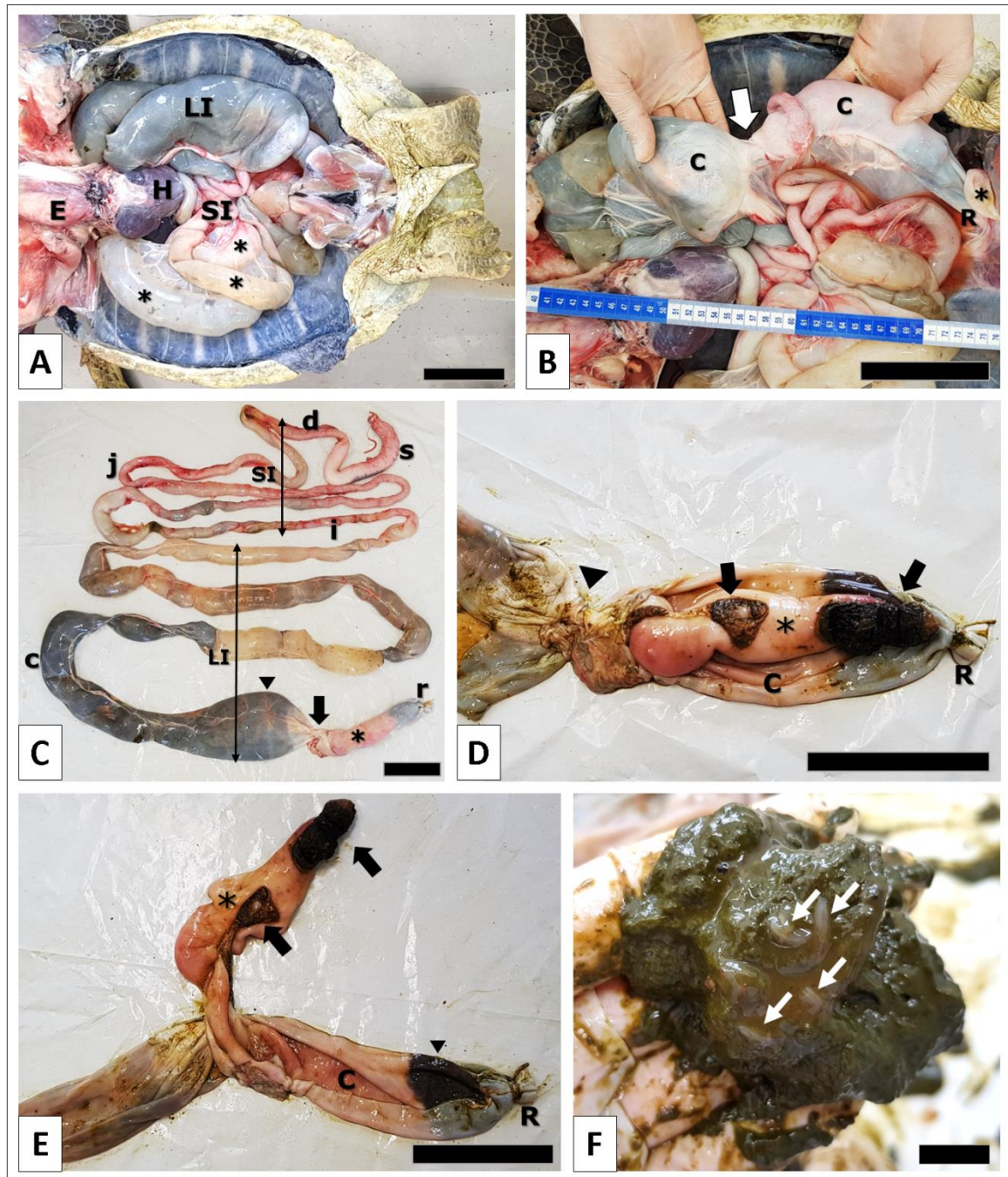


Figure 1. Colocolic intussusception associated with the helminth *Octangium* sp. in *Chelonia mydas*: (A) celomatic cavity in ventral view showing the heart (H), oesophagus (E), small intestine (SI), gases in intestinal loops (*) and a large dilatation in the large intestine (megacolon) (LI) (Scale bar: 10 cm); (B) urinary bladder (*), colon segment (C), and rectum (R) and complete colon obstruction (arrow) (Scale bar: 10 cm); (C) Digestive apparatus divided into small intestine (SI) and large intestine (LI), subdivided into stomach (s),

duodenum (d), jejunum (j), ileum (i), colon (c), and rectum (r); there is still a large dilation of the colon (arrowhead) anterior to the obstruction (arrow) and intussusception (*) (Scale bar: 10 cm); (D) loop obstruction (arrowhead), a portion of the intussusciens open loop, corresponding to the terminal portion of the colon (C) and rectum (R) showing the intussusceptum segment, measuring 20 cm (*), and necrotic region (arrows) (Scale bar: 10 cm); (E) intussusceptum (*) with necrotic region (arrows) and intussusciens (C) with a necrotic region (arrowhead), a place of contact with the necrotic part of the intussusceptum (*); and (F) helminth parasite *Octangium* sp. present in the faeces removed from the dilated region of the colon (Figure 1 C) (Scale bar: 1 cm).

The histopathology of the colon revealed multifocal areas of mucosal epithelial detachment and vascular congestion, in addition to fibrin deposition, forming a fibrinonecrotic membrane over the epithelium. Other lesions such as lymphohistiocytic and eosinophilic colitis, characterized by the infiltration of mononuclear cells (lymphocytes and monocytes) and polymorphonuclear cells (eosinophils and heterophils) in the lamina propria, were observed (Figure 2).

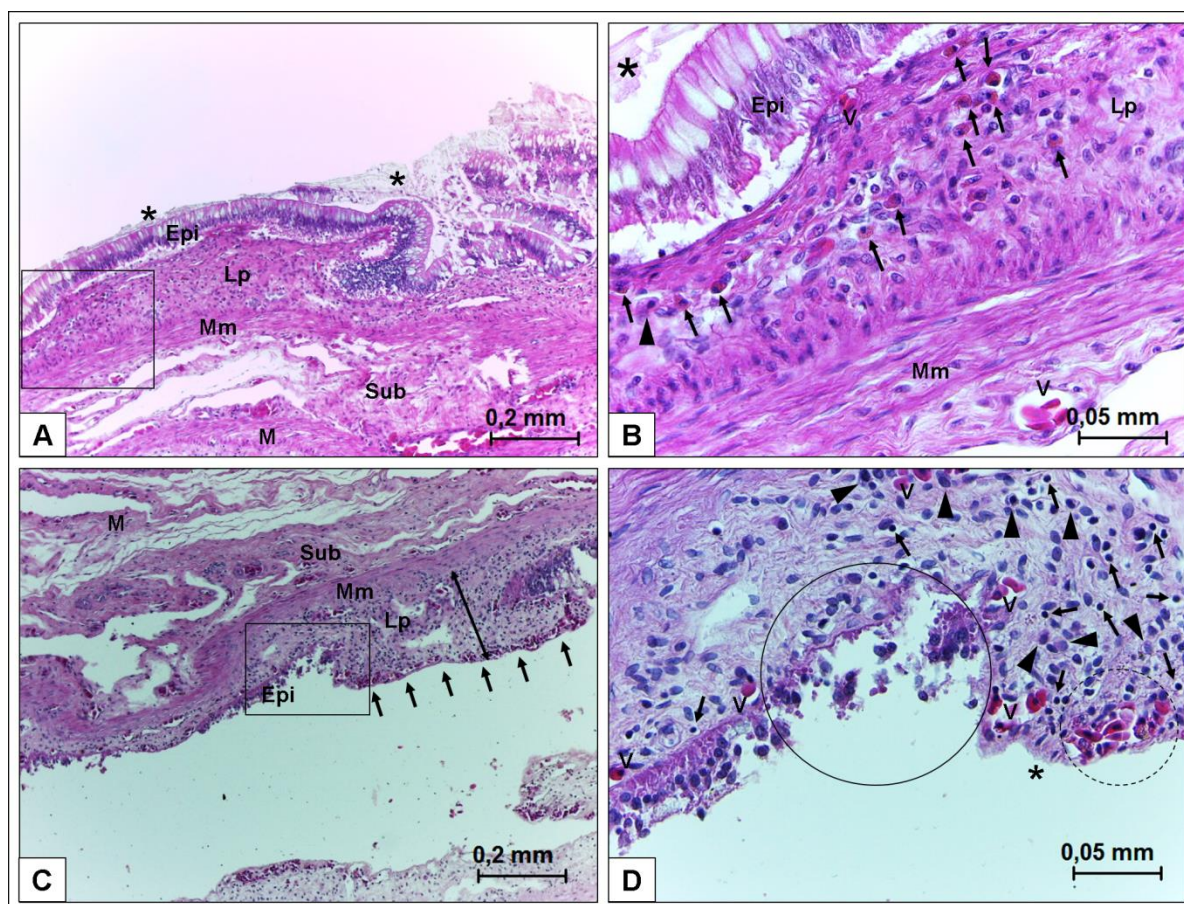


Figure 2. Colitis arising from the colocolic intussusception associated with *Octangium* sp. in *Chelonia mydas*: (A) fibrinonecrotic membrane (*) over the mucosa lining epithelium (Epi), followed by lamina propria (Lp), muscularis mucosa (Mm), submucosa (Sub) and muscularis (M) (Scale bar: 0.2 mm; hematoxylin and eosin staining); (B) enlargement of the area enclosed by the rectangle in panel A, depicting the fibrinonecrotic membrane (*) over the epithelium (Epi), eosinophils (arrows) and monocytes (arrowhead) infiltrate in the lamina propria (Lp), in addition to blood vessels (V) and muscularis mucosa (Mm) (Scale bar: 0.05 mm; hematoxylin and eosin staining) can be observed; (C) The mucosal congestion with detachment of the epithelium (arrows), thickening of the lamina propria (bar) (Scale bar: 0.2 mm; hematoxylin and eosin staining) can be seen; and (D) enlargement of the area enclosed by the rectangle in panel C, showing areas with mucosal epithelium detachment (circle), total absence of the epithelium (*), vascular congestion of the mucosa (dashed circle), inflammatory cells infiltrate: lymphocytes (arrows) and heterophiles (arrowhead), in addition to blood vessels (V). Staining (HE) (Scale bar: 0.05 mm; hematoxylin and eosin staining).

The necroscopic findings revealed the presence of helminth parasites in small intestine (duodenum, jejunum and ileum) and large intestine (colon). We observed the following

features in the examined parasites: body with paired caudolateral projections; simple bladder, receiving excretory ducts on each side; esophagus; esophageal bulb; anterior testis; posterior testis; ovary; uterus; lateral vitelline fields and excretory vesicle. Thus, based on the key for helminths of the subfamily Octangiinae, the examined helminths belong to the genus *Octangium* (Figure 3).

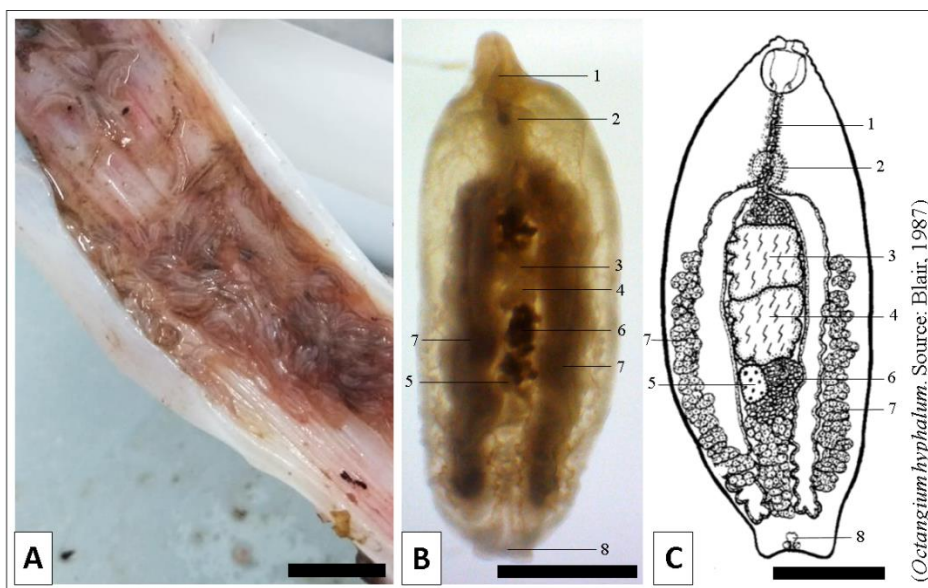


Figure 3. Parasitic infestation by *Octangium* sp. in the gastrointestinal tract of a green sea turtle: (A) several parasites in the final portion of the ileum (scale bar = 1 cm). (B) morphology of the parasite *Octangium* sp. in ventral view (1 = esophagus; 2 = esophageal bulb; 3 = anterior testis; 4 = posterior testis; 5 = ovary; 6 = uterus; 7 = lateral vitelline fields; 8 = excretory vesicle; scale bar = 1 mm). (C) morphology of the parasite *O. hyphalum* (from Blair 1987), with labels corresponding to those in panel B (scale bar = 1 mm).

DISCUSSION

The incidence of intussusception in the gastrointestinal tract has been reported frequently in domestic species, such as dogs and cats, as a result of gastroenteritis caused by bacterial infections (Macphail 2002; Firmino et al. 2017) and, in horses, associated with parasitic infestation with *Anoplocephala perfoliata* (Barclay et al. 1982; Owen et al. 1989) and *Parascaris equorum* (Cribb et al. 2006). It is rarely seen in wild species, as shown in previous studies for the following animals: the Florida manatee (*Trichechus manatus latirostris*), arising from ingestion of fishing artifacts (Forrester et al. 1975), pine snake (*Pituophis melanoleucus*), unknown cause (Wosar and Lewbart 2006), and red corn snake

(*Pantherophis guttatus*), associated with cryptosporidiosis (Bercier et al. 2017). In sea turtles, cases of intestinal intussusception have been described in loggerhead sea turtles (De Vico et al. 2003; Fichia et al. 2016) and in green sea turtles (Cappua et al. 2007), associated with foreign bodies (marine debris), contrary to our findings, where for the first time, we have found an intussusception associated with a parasitic infestation of *Octangium* sp.

The predisposing factors leading to the disease include presence of foreign bodies, granulomas, gastrointestinal masses, infectious or non-infectious gastroenteritis, and intestinal parasitism (Macphail et al. 2002; De Vico et al. 2003; Cappua et al. 2007). The presence of marine debris (nylon lines, hooks, plastic bags, and hard plastics) in the digestive tract of sea turtles is generally described as the main cause of intussusception (De Vico et al. 2003; Cappua et al. 2007). In such circumstances, they cause increased peristalsis, which may lead to intussusception (Valente et al. 2007; Casale et al. 2008).

The intussusception may affect all segments of the digestive tract in animals: esophagogastric and gastroduodenal (Bercier et al. 2017), duodenoduodenal (Cappua et al. 2007), jejunojejunal (Firmino et al. 2017), jejunoileal (Colomé et al. 2006), ileocolic (Wosar and Lewbart 2006), ileoceocolic (Firmino et al. 2017), and colocolic (Hasse et al. 2019), the latter being the type that was observed in our report.

In addition to the number of intussusceptions, there may be a double-compounded intussusception. This was a unique case reported in a specimen of red corn snake (*Pantherophis guttatus*), associated with the protozoan *Cryptosporidium* sp., where the oesophagus was found to be in intussusception with the stomach; this combination had subsequently invaginated into the duodenum, forming a second intussusception (Bercier et al. 2017).

The histopathological findings presented in this report are associated with the reduction or interruption of blood flow, leading to ischemia of the affected area, resulting in tissue damage (Cotran et al. 1994). Cellular changes are directly related to the duration of ischemia and when this is prolonged for a long time, it results in tissue necrosis (Matos et al. 2000). Other findings, such as lymphohistiocytic and eosinophilic colitis presented here, corroborate those described for sea turtles that presented injuries in the digestive tract resulting from marine debris ingestion (Orós et al. 2004b; Fichia et al. 2016; Jerdy et al. 2017), systemic fungal infection by *Candida albicans* (Orós et al. 2004a) and parasitic infestations by larvae of intestinal nematodes belonging to the family Anisakidae (Orós et al. 2005).

The infiltrate of eosinophils observed in the histopathological examination of this report is associated with the inflammatory process, especially in relation to parasitic infestation (Rothenberg 1998). The eosinophils function as antigen-presenting cells and also as cell mediators, releasing degranulatory enzymes, leading to degranulation of mast cells and the release of cytokines, chemokines, and neuronal mediators that are toxic to the intestinal epithelium (Rankin et al. 2000).

The main consequences of digestive tract mucosal lesions associated with intussusception are ischemia, necrosis, and intestinal rupture (Levitt and Bauer 1992; Jerdy et al. 2017). The hypoxia causes the degeneration of mucosal cells, allowing the entry of infectious agents into the gastrointestinal tract. The heterophils and lymphocytes attempt to combat bacterial infections (Jerdy et al. 2017). In sea turtles, necrotizing colitis is characterized by oedema, haemorrhage, and necrosis of the intestinal mucosa, with deposition of fibrin adhered to the surface of the mucosa (Orós et al. 2005).

The parasites of the genus *Octangium* studied in our case report, belongs to the subclass Digenea and family Microscaphidiidae (Santoro et al. 2006; Werneck and Silva 2016). Digenetic trematodes have been commonly found parasitizing the gastrointestinal tract of green sea turtles. In the Atlantic Ocean, to the north of Costa Rica, several species of this group, such as *Amphiorchis solus*, *Charaxicephalus robustus*, *Cricocephalus resectus*, *Deuterobaris intestinalis*, *Hapalotrema postorchis*, *Microscaphidium warui*, *Monticellius indicum*, *Pleurogonius sindhii*, *Rhytidodoides intestinalis*, *Rhytidodoides similis*, and *Octangium hyphalum* can be found (Santoro et al. 2006). According to Werneck and Silva (2016), the neotropical region presents a great diversity of endohelminth parasites in sea turtles, including the only two species of the genus *Octangium* (*O. hyphalum* and *O. sagitta*).

Our findings indicate that parasitic infestation by *Octangium* sp. in sea turtles can cause intussusception and consequently lead to the animal's death. Intussusception is not often reported in marine animals and not always readily diagnosed although it may be related to the death of these animals in the wild or even in captivity. Early diagnosis, along with surgical correction, is fundamental for a good prognosis and, consequently, for successful rehabilitation of marine species.

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**CAPÍTULO 7 – SUCCESSFUL REHABILITATION OF AN OILED SEA TURTLE
(*Lepidochelys olivacea*) AFFECTED BY THE BIGGEST OIL SPILL DISASTER IN
BRAZIL**

(Artigo de relato de caso)

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Successful case of rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil

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Abstract: Standardization of protocols and discussion of therapeutic procedures in the rehabilitation of turtles affected by oil are necessary to optimize the recovery time and increase the chances of survival of these animals. This study aimed to report the processes adopted for stabilization, decontamination, rehabilitation and release of an oiled olive ridley sea turtle (*Lepidochelys olivacea*), rescued alive on September 23, 2019, at Santa Rita beach, Extremoz municipality, Rio Grande do Norte, Brazil. Its entire body was affected by oil. At first, the animal was mechanically dry cleaned using a gauze soaked in mineral oil in the keratinized regions (carapace and plastron) and a gauze soaked in vegetable oil in the oral, nasal, ocular, and cloacal mucous membranes. The second stage of oil removal consisted of washing the animal with heated pressurized water (39 °C) and neutral detergent using a soft foam sponge. The animal received treatment with antitoxins, antibiotics, analgesics, gastrointestinal protectors, and fluid therapy. After 7 days of treatment, the blood count showed that all the parameters were within the normal range. The oil cleaning process and the therapeutic protocol used in the rehabilitation of the olive ridley sea turtle were efficient.

Keywords: Cheloniidae; contamination; crude oil; intoxication; Testudines.

INTRODUCTION

The olive ridley sea turtle is classified as “vulnerable” according to the International Union for Conservation of Nature (IUCN) red list of threatened species (Abreu-Grobois and Plotkin 2008). Interacting with fishing represents one of the main causes of mortality of this species, mainly due to the interaction with gillnets or trawls used on spawning beaches (Silva et al. 2010).

Oil spills affect animals directly through contact with oil or indirectly by reaching all levels food chain thus enduring in the environment on a large scale and for many years (Lauritsen et al. 2017). This occurs because the effects of an oil spill on animals are not always clearly observed and nor immediately after the accident (Soares et al. 2020). Sea turtles are vulnerable to oil at all stages of life, including the embryonic stage, in the nest, in the hatchlings and juveniles stages, in the ocean gyres, in the sub-adult stage, in habitats close to the coast, and the adult stage when they migrate between nests and food areas (Lutcavage et al. 1995).

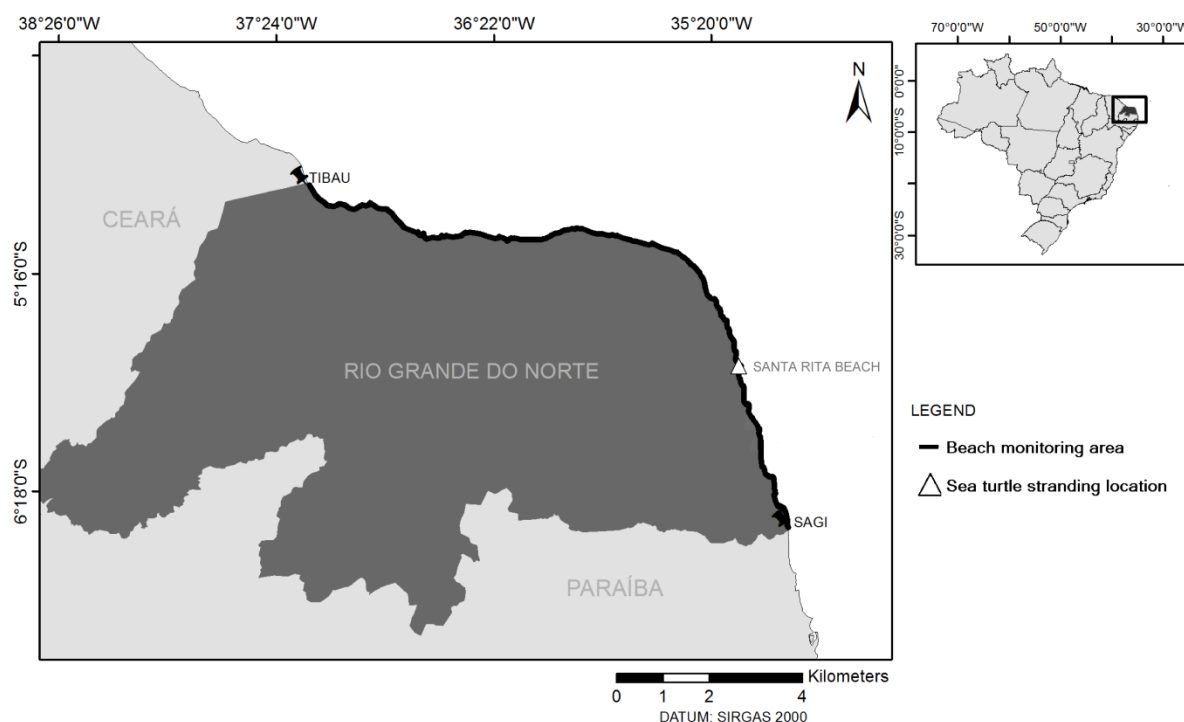
The effects of oil spills on sea turtles are rarely reported, despite the prevalence of this type of accident in areas where these animals inhabit (Wallace et al. 2020). In this sense,

standardization of techniques and discussion of treatments in the rehabilitation of sea turtles affected by oil are necessary to optimize the recovery time and increase the chances of survival of these animals. Thus, this study aimed to report the processes adopted for stabilization, decontamination, rehabilitation and release of an oiled olive ridley sea turtle (*Lepidochelys olivacea*) with 100% of the body affected by oil, in order to guide clinical measures for providing greater success in the rehabilitation processes of oiled turtles and, consequently, to provide relevant knowledge for the conservation planning for these animals.

CASE DESCRIPTION

In September 2019, Northeastern Brazil was affected by an oil spill that reached nine states of the region and that later extended to Southeastern Brazil (Espírito Santo and Rio de Janeiro). The origin of the oil spill remains unknown to date. It caused the death of hundreds of marine animals and put the population living in the country's coastal region at risk. Five months after the first record, oil spills have been recorded on 1,009 beaches, directly affecting 159 marine and estuarine animals, including sea turtles, sea mammals, sea birds, and other aquatic organisms, resulting in the death of 112 animals. Of the animals affected, 105 were sea turtles and, of these, 83 died, making this oil spill the most extensive and severe environmental disaster ever recorded in the history of Brazil. Of the affected sea turtles, 14 were recorded across the Rio Grande do Norte (RN) state, with 13 stranded animals already found dead and one animal found alive (Ibama 2020).

The turtle found alive, which belonged to the species *Lepidochelys olivacea*, was a sub-adult. It was rescued on September 23, 2019, at the Santa Rita beach (5°43'54.37"S, 35°12'15.46"W), Extremoz municipality, Rio Grande do Norte, Brazil (Figure 1). The stranding was notified and the team of the Costa Branca Cetacean Project – State University of Rio Grande do Norte (PCCBUERN) carried out the rescue. The animal was forwarded to the Marine Life Stabilization Center (PCCB-UERN), Natal, Rio Grande do Norte (RN), Brazil. The performance of activities with marine animals is authorized by SISBIO-ICMBio (License No. 13694-9) and the Ethics Committee on the Use of Animals of the Federal Rural University of the Semi-Arid (License No. 01/2019).



Elaboration: Giovanna Almeida Santoro

Figure 1. Beach monitoring area of the Cetáceos da Costa Branca Project and sea turtle stranding location.

The animal had 100% of its body surface affected by crude oil, directly affecting the oral, ocular and cloacal mucous membranes. The animal was active, responsive to stimuli, was tachypneic and with noisy breathing, had a good body score and was moderately hydrated. The specimen had 50.5 cm of curved carapace length (CCL), 54.5 cm of curved carapace width (CCW) and 13.8 kg of body mass. Starting the Stabilization, the animal was first manually dry cleaned using a gauze pad soaked in mineral oil in the keratinized regions (carapace and plastron) and a gauze pad soaked in vegetable oil in the oral, nasal, ocular, and cloacal mucous membranes (Figure 2A, 2B, and 2C). After removing the crude oil from the animal, congestion of the oral, ocular and cloacal mucosa was observed.

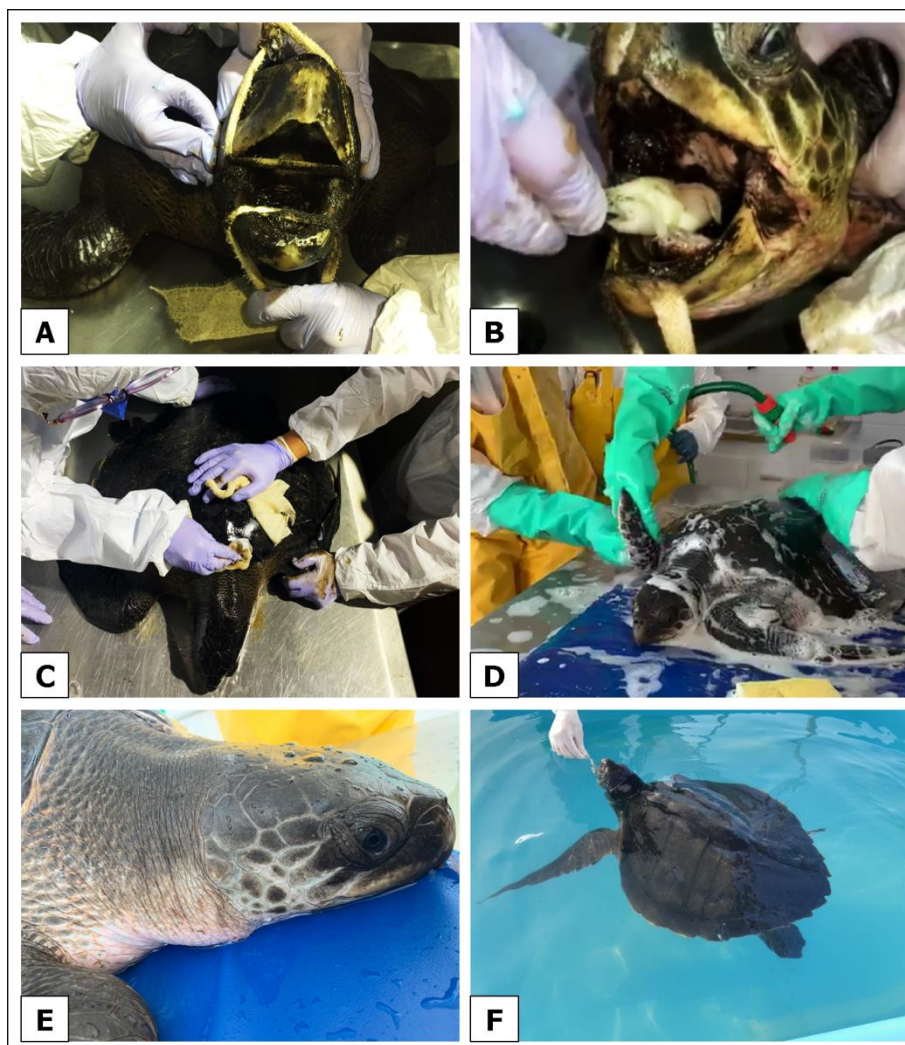


Figure 2. Process of manual removal of oil from the olive ridley sea turtle (*Lepidochelys olivacea*). (A) Presence of oil on the entire body surface and mucous membranes. (B) Manual removal of oil from the oral cavity using a gauze pad soaked with vegetable oil. (C) Removal of oil from the body surface using a gauze pad soaked with mineral oil. (D) Removal of oil with heated water (39 °C) and neutral detergent. (E) Clean animal, without any traces of oil. (F) Animal in a cage feeding at will.

The external jugular veins (right and left) and the dorsal cervical sinus were the routes for blood collection, fluid therapy and drugs administration routes. The animal was maintained out of water on a mattress during the venous assessment either for fluid therapy or drug administration. Containment was performed holding the animal's carapace. This containment was a sufficient to maintain the animal in a satisfactory condition to perform the necessary procedures. After removing oil for the first time, blood samples of the animal were collected sequentially for complementary analyses (blood counts and biochemicals; tables 1

and 2). Treatment administered during the first 24 hours was composed of: fluid therapy with 0.9% NaCl solution (KabiPac[®], dose: 20 ml/kg/i.v., velocity: 30 drops/minute) associated with a multivitamin (Hertavita[®], dose: 1.4 ml/kg/i.v., once a day for 15 days), and antitoxin (Mercepton[®], dose: 0.2 ml/kg/i.v., once a day for 15 days) in addition to activated charcoal adsorbent (Enterex[®], dose: 8g/animal/orally, once a day for 3 days).

Table 1. Blood count tests performed on the oiled turtle (*Lepidochelys olivacea*).

Complete blood count					
Erythrogram					
Parameters	Test 1	Test 2	Reference		
Red Cells	0.37	0.75	0.12-0.40 x 10 ⁶ /μl (Zhang et al., 2011)		
Hemoglobin	102	93	73.7-97.6 g/l (Ramakrisham et al., 2018)		
Hematocrit	35	29	13.6-20.8% (Pinto et al., 2015)		
MCV	945	385	255.5-341.5 fl (Ramakrisham et al., 2018)		
MCHC	290	320	260-359 g/l (Santoro and Meneses, 2007)		
Leukogram					
Parameters	Test 1		Test 2		Reference
					(Zhang et al., 2011)
	Relative (%)	Absolute (μl)	Relative (%)	Absolute (μl)	μl
Leukocytes	100	7,700	100	10,395	3,750-13,000
Heterophiles	79	6,083	75	7,875	2,170-8,840
Eosinophils	0	0	1	105	90-650
Basophils	0	0	0	0	0-70
Lymphocytes	10	770	19	1,995	920-2,540
Monocytes	11	847	4	420	400-2,310
Thrombocyte Count	3,500		30,000		6,750-19,750
Erythrocyte Series	Hemoglobinemia		No visible change		

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration. Test 1 = September 25, 2019; test 2 = October 2, 2019.

Table 2. Biochemical tests performed on the oiled turtle (*Lepidochelys olivacea*).

Biochemical			
Parameters	Test 1	Test 2	Reference (Espinoza-Romo et al., 2018)
ALT	30	31	12-40 g/l x 10 ⁻⁶
AST	275	11	28-402 g/l x 10 ⁻⁶
ALP	46	17	15-94 g/l x 10 ⁻⁶
Albumin	14	15	6-15 g/l
Globulin	46	15	21-37 g/l
Phosphor	0.074	0.075	0.065-0.147 g/l
Calcium	0.087	0.090	0.001-0.098 g/l
Urea	3.82	1.78	0.19-0.78 g/l
Total Proteins	60	30	27-52 g/l

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase. Test 1 = September 25,2019; test 2 = October 2,2019.

On the second day of treatment, other drugs were included in the protocol, antibiotic therapy with ceftiofur hydrochloride (CEF 50[®], dose: 4 mg/kg/i.m., once a day for 30 days), analgesia with tramadol hydrochloride (Tramal[®], dose: 5 mg/kg/i.m., once a day for 5 days), meloxicam (Maxicam[®], dose: 0.1 mg/kg/i.m., once a day for 5 days) and dipyrone (D-500[®], dose: 25 mg/kg/i.m., twice a day for 5 days) in addition to a sucralfate gastric protector (Sucrafilm[®], dose: 0.4 ml/kg/orally, twice a day for 10 days) and a liver protector (Ornitol[®], dose: 0.2 ml/kg/i.v., once a day for 10 days).

After the first 24 hours, the animal was sent to the Oiled Marine Live Decontamination Center of the Cetáceos Costa Branca Project, located at the State University of Rio Grande do Norte (UERN), Mossoró municipality, Rio Grande do Norte, Brazil. The second stage of oil removal consisted of washing the animal with heated pressurized water (39 °C) and neutral detergent using a soft foam sponge (Figure 2D). This procedure was performed within a closed system capable of containing all oil residue and water contaminated by it. Then, they were sent to a specific reservoir prepared to separate the oil from the water and efficiently dispose the by-products generated in the washing process.

After 4 days of washing and complete oil removal (Figure 2E), the animal was transferred to the Wild Animal Rehabilitation Center of the Cetáceos Costa Branca Project - UERN, Areia Branca municipality to continue the rehabilitation. The animal was put in a pool ($1\text{ m}^3 = 200\text{ cm}$ in diameter x 75 cm in depth), water temperatures between $25\text{ }^{\circ}\text{C}$ and $28\text{ }^{\circ}\text{C}$ and with natural lighting from 5:30 am to 5:30 pm approximately. The specimen, which was fed with a small fresh fish right after the washing process, immediately showed interest in the food (Figure 2F). The animal received *ad libitum* feeding corresponding to an average of 500 to 600 grams per meal. One meal was offered in the morning and the other in the afternoon. In addition to fish, the diet included shrimps and crabs.

During treatment, other multivitamins were administered through an esophageal tube (Hemolitan[®], dose: 0.1 ml/kg , once a day for 10 days; Glicopan[®], dose: 0.1 ml/kg , once a day for 10 days; Emulsion Scott[®], dose: 0.4 ml/kg , once a day for 10 days) in addition to 150 g of fish porridge, diluted in 300 ml of 0.9% NaCl solution associated with the sucralfate gastric protector (Sucrafilm[®]) previously mentioned.

On the ninth day of treatment, another gastric protector was included in the protocol, omeprazole (Oprazon[®], 4 mg/kg/i.v. , for 16 days). After 16 days of using this drug, gastroparesis occurred triggering intestinal constipation. Thus, we opt to stop the administration of the drug. Following clinical signs, metoclopramide hydrochloride (Plasil[®], 0.5 mg/kg/i.v. , for 10 days) was included to increase gastrointestinal motility, and the animal spontaneously defecated.

After 67 days of rehabilitation, the animal was reevaluated by the veterinary medical team considering physiological, clinical, and behavioral parameters for the species, and was considered ready for being released. Before releasing it, two metallic washers with individual identification were inserted in both anterior fins (left: BRA17527, right: BRA17528). The animal was transported to the open sea ($4^{\circ}54'20.16''\text{S}$, $37^{\circ}7'51.60''\text{W}$) in a motor vessel and reintroduced there.

DISCUSSION AND CONCLUSIONS

The oil ingested by a sea turtle does not pass quickly through the digestive tract. It can be retained for several days, increasing internal contact and the likelihood of absorption of toxic compounds. The internal effects of oil exposure include significant changes in the blood hematological and biochemical profile (Milton et al. 2010). Prolonged exposure to oil can

worsen the body condition of the animals, interrupting feeding and increasing susceptibility to diseases, and sub sequent mortality (Mitchelmore et al. 2017).

The oil spill disaster that occurred in the Gulf of Mexico in 2010 was one of the largest in the world, affecting not only the environment but the animals living there. All five species of sea turtles that are present in the Gulf of Mexico were affected by this incident. The changes found in the 319 turtles that were analyzed were non-specific, thus it was not possible to observe the toxicological effects (Stacy et al. 2017). However, evidence shows that approximately 30% of all ocean turtles in the region affected by the Gulf of Mexico spill that were not heavily oiled would have died from oil ingestion, while that 100% of heavily oiled turtles would have died from the physical effects of heavy oiling (Mitchelmore et al. 2017). Due to the dispersive, migratory, and surface-dwelling behavior of sea turtles, many populations can be negatively impacted by oil spills. Thus, the spill's impacts may extend far beyond the current focus on the northern Gulf of Mexico (Putman et al. 2015). Although direct toxicological changes could not be observed in our study, supportive and antitoxin prophylaxis treatment was prescribed since the animal arrived at the Rehabilitation Center. On the first day, activated charcoal was used to reduce the absorption of oil toxins according to the recommendations of Walsh (1999), with active function only in the first 24 hours. It was only used in the first three days, in a preventive way, being associated with antitoxins and liver protectors which were both administered intravenously. Considering that the liver is the main site of chemical detoxification, it is expected that the toxic effects cause changes in the serum levels of several liver enzymes (Milton et al. 2010).

Regulation of osmolarity in the blood of sea turtles is highly dependent on the availability of water for consumption, reabsorption of water in the urinary bladder/cloaca, and loss of salts by the salt glands, rather than the reabsorption of water by the kidneys. When a negative water balance occurs, all body systems are affected, and fluid therapy becomes an integral part of the restoration and maintenance of cellular homeostasis, where adequate hydration provides support for cells and a means of transport for molecules (Rudloff 2005).

In an experiment conducted by Lutcavage et al. (1995), the physiological and clinical-pathological effects of exposure to crude oil on loggerhead turtles (*Caretta caretta*) in the southern state of Louisiana were evaluated. Sea turtles ingested oil incidentally, and contamination was observed in the nostrils, eyes, upper esophagus and feces. White blood cell counts of oiled turtles had a fourfold increase, a 50% reduction in red blood cell counts, and changes in the color of red cells. Histological changes were observed on the skin, and mucosa surface of oiled turtles, including acute inflammatory cell infiltrates, dysplasia of the

epidermal epithelium and loss of cellular architecture of the skin layers. Cellular changes in the epidermis are of particular concern, as they may increase susceptibility to secondary infectious conditions (Milton et al. 2010). Thus, the administration of antibiotics in this report is justified as a preventive approach. Vitamin B12 (cyanocobalamin) and B9 (folic acid) are essential nutrients for normal hematopoiesis. Since macrocytic anemia is related to the deficiency of these vitamins in cases of intoxication (Aslinia et al. 2006), supplementing Vitamin B12 and B9 is necessary to reverse this condition observed in the first blood count test (haemoglobinaemia and mean corpuscular volume). After 7 days of treatment, the second blood count showed that all parameters were within the normal range.

Proton pump inhibitors (PPIs) are substances that selectively and completely inhibit the H^+/K^+ -ATPase proton pump in the parietal cell membrane. Thus, gastric acid secretion is suppressed, increasing gastric pH (Strand et al. 2017). Typical doses of PPIs are incredibly useful in inducing remission of esophagitis and gastritis symptoms (Khan et al. 2007; Fass et al. 2012). For this reason, using omeprazole was essential to reduce the damage caused by the ingested oil on the gastroesophageal mucosa and the occasional gastrointestinal adverse effects of meloxicam. Adverse effects related to omeprazole are rare and can occur with prolonged use. Adverse effects in humans include headache, diarrhea, abdominal pain, nausea, skin rashes, constipation, and vitamin B12 deficiency (Marchetti et al. 2003). After 16 days of using omeprazole, the turtle had constipation. Thus, we opted for stopping the administration of the drug.

In conclusion, we found that the oil cleaning process and the therapeutic protocol used in the rehabilitation of the olive ridley sea turtle were efficient. Moreover, the short time between the stranding of the animal and the first visit was essential to avoid greater absorption of oil toxins. Although PPIs are considered safe drugs, caution should be exercised in their use in sea turtles for an extended period. It is worth mentioning that the therapeutic protocol can be tailored to suit individual cases.

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Conflict of interest

The authors declare no conflict of interest.

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**CAPÍTULO 8 – PATHOANATOMICAL FINDINGS OF A GREEN TURTLE
(*Chelonia mydas*) VICTIM OF COLLISION WITH A MOTORBOAT**

(Artigo de relato de caso)

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**PATHOANATOMICAL FINDINGS OF A GREEN TURTLE (*Chelonia mydas*)
VICTIM OF COLLISION WITH A MOTORBOAT**

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Abstract: Here, we report the trauma suffered by a green turtle (*Chelonia mydas*), caused by a collision with a motorboat, and describe the case ante and post-mortem. An adult female green turtle was rescued alive on December 2, 2016 at Ponta Negra beach, municipality of Natal, Rio Grande do Norte, Brazil. The rescued specimen was sent to the Marine Fauna Rehabilitation Center (PCCB-UERN), municipality of Areia Branca (RN), in Brazil. The physical examination revealed an excellent body score, noisy breathing, moderate dehydration, absence of hind limb and cloacal reflexes when stimulated by pressure, and two traumatic injuries characteristic of a collision with a motorboat. After three days of supportive treatment, the animal died and was immediately sent for necropsy. The animal had a complete fracture of the vertebral bodies (dorsal elements D9 and D10) and spinal cord section. The entire gastrointestinal tract had food content, in addition to sharp injuries in the colon caused by the bone fragments of the fractured carapace. The lungs were congested, hemorrhagic with frothy and bloody secretion, and interstitial bronchiole fibrosis. There was also the presence of fibrin and a large number of leukocyte cells, consisting mainly of macrophages. The liver was enlarged, with rounded edges and thickening of the capsule, multifocal areas of hepatocellular necrosis, and dissociation of the hepatocyte cords. The collision resulted in the exposure of the coelomic cavity and spinal cord, causing the animal intense pain, paralysis of the hind limbs and cloaca, septicemia, and consequently, death.

Keywords: Cheloniidae, propeller, Testudines, trauma.

INTRODUCTION

The green turtle (*Chelonia mydas*) is in the “endangered” category of extinction, according to the Red List of Endangered Species of the International Union for Conservation of Nature [1]. Human activities have been the leading cause of threat to the species through the effects of fishing activities [2] and motorboat collisions, which has led to increased mortality of these animals over the years [3].

Among the seven species of sea turtles worldwide, green turtles, followed by loggerhead turtles (*Caretta caretta*), comprise the majority of death records from collisions with motorboats [3]. The record of this cause of death, often underreported, is important for developing public policy measures for conservation, as the descriptions of these cases make it possible to identify which types of ships may be affecting these animals and what the impact on populations is [4].

The state of Rio Grande do Norte has been identified as an important area for the occurrence of the species *C. mydas* [5,6]. Thus, it is important to be aware of the effects of mechanized animal fishing activities in the state to assist researchers and public agencies in the adoption of mitigation measures. Herein, we report the trauma suffered by a green turtle (*C. mydas*) in Rio Grande do Norte, Brazil, due to a collision with a motorboat, describing the case ante and post-mortem.

CASE PRESENTATION

An adult female green turtle (*C. mydas*) was rescued alive on December 2, 2016 at Ponta Negra beach (5°52'50.6 "S 35°10'18.2 "W), municipality of Natal, Rio Grande do Norte, Brazil (Figure 1). The stranding was registered by team members of the Cetaceans Project of Costa Branca, of the State University of Rio Grande do Norte (Projeto Cetáceos da Costa Branca, da Universidade do Estado do Rio Grande do Norte - PCCB-UERN). The execution of activities with marine animals is authorized by SISBIO-ICMBio (License No. 13694-9) and the Ethics Committee on the Use of Animals of the Federal Rural University of the Semi-Arid (License No. 01/2019).

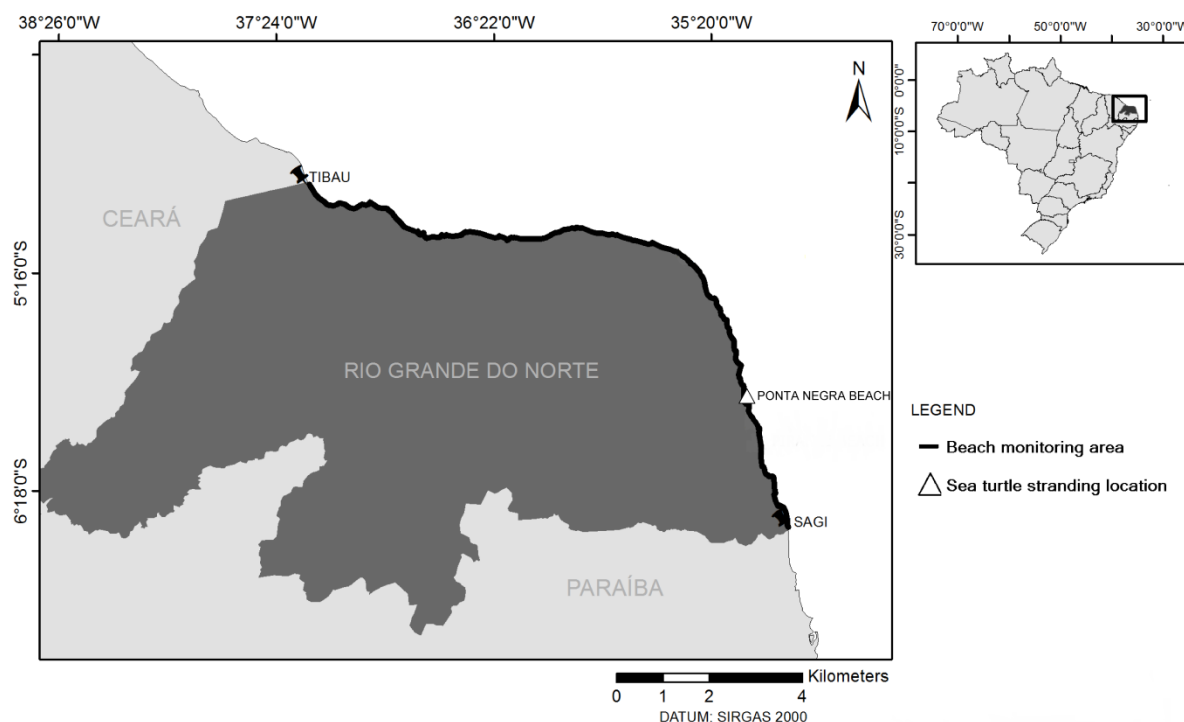


Figure 1. Area for monitoring the beaches for the Cetaceans Project in Costa Branca and site of the animal's stranding.

The animal was found stranded on the sand, prostrate, and having a complete fracture in the horny plates that make up the carapace, with exposure of the coelomic cavity and a fracture in the nasal bone. The turtle was rescued and sent to the Wildlife Rehabilitation Center of the PCCB, in the municipality of Areia Branca, Rio Grande do Norte (RN), Brazil. The animal's biometric data were: 121.0 cm of curvilinear carapace length (CCC), 108.5 cm of curvilinear carapace width (LCC), and 177.5 kg of body mass.

In a detailed clinical examination, some alterations were observed. Upon inspection, there was an excellent body score, noisy breathing, moderate dehydration, and two traumatic injuries, characteristic of a collision with a motorboat. The first injury corresponded to a fracture that extended transversally from the fifth corneal plate on the left side of the carapace to the middle of the fifth contralateral plate (4.4 cm from the largest opening) with exposure of the coelomic cavity (Figures 2A and 2B). The second injury was characterized by a comminuted fracture in the nasal bone (5.1 cm wide) with exposure of the left nasal sinus (Figure 2C), in addition to a sharp injury with lacerated edges in the left anterior fin (4.1 cm wide) (Figure 2D). The neurological examination was based on the methodology by Chrisman

et al. [7] for sea turtles, where the absence of hind limb and cloaca reflexes was found when stimulated by pressure.

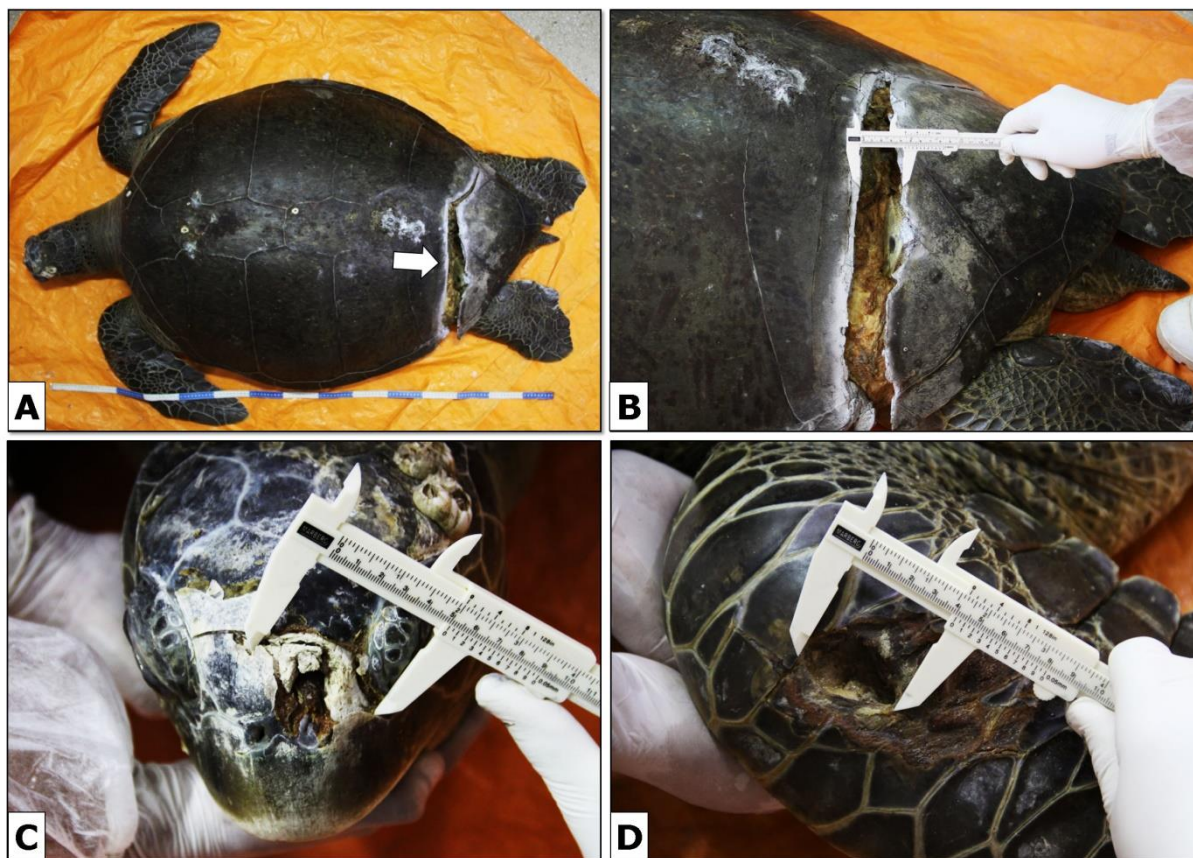


Figure 2. Traumatic injuries of a green turtle (*Chelonia mydas*) due to a collision with a motorboat. (A and B) a fracture is observed transversely from the fifth left corneal plate of the carapace to the middle of the fifth contralateral plate (4.4 cm from the largest opening) with exposure of the coelomic cavity. (C) comminuted fracture in the nasal bone (5.1 cm wide) with exposure of the left nasal sinus. (D) piercing-cutting injury on the left anterior fin with lacerated edges.

Emergency support treatment consisted of electrolyte replacement with 0.9% sodium chloride-based fluid therapy (KabiPac[®], dose: 20 ml/kg/EV/speed: 30 drops/min), diluted with vitamin B12 (Polivin B12[®], dose: 0.3 ml/kg/EV), antitoxic (Mercepton[®], dose: 0.2 ml/kg/EV/SID), and calcium gluconate (Calcifos[®], dose: 0.06 ml/kg/EV, SID), as well as antibiotic therapy with gentamicin sulfate (Gentamax[®], dose: 3 mg/kg/IM, SID), analgesia with tramadol hydrochloride (Tramal[®], dose: 4 mg/kg/SC, SID), meloxicam (Maxicam[®], dose: 0.2 mg/kg/IM, SID), dipyrrone (D-500[®], dose: 25 mg/kg/IM, TID) and dexamethasone

(Dexaflan[®], dose: 0.04 mg/kg/ EV, BID). After three days of treatment, the animal died and was immediately sent for necropsy at the Marine Biota Monitoring Laboratory, at the State University of Rio Grande do Norte, in Mossoró (RN).

For the macroscopic analysis, the Wyneken [8] methodology for sea turtles was used. Initially, the opening of the coelomic cavity was performed, surrounding the entire plastron, followed by its removal and analysis of the organs in situ. Then, all organs were removed for ex situ evaluation, and photographs of the evident macroscopic findings were taken. Tissue fragments were also collected and fixed in 10% paraformaldehyde.

Liver and lung fragments, were collected and fixed in 10% buffered formalin for histopathological examination. For the histopathological analysis, the histological techniques recommended by Tolosa et al. [9] were used. Moreover, 5µ thick sections of paraffin blocks were made in a microtome (LEICA RM 2125 RT[®]), adhered to glass slides, and left in an oven at 60 °C overnight for subsequent staining with hematoxylin and eosin (HE). The histological slides were analyzed under a light microscope (LEICA DM 500 HD) with a camera attached (LEICA ICC50W), and images were obtained using the LAS EZ Ink program.

The animal presented a complete fracture of the vertebral bodies (dorsal elements D9 and D10) and spinal cord section (Figure 3A). The entire gastrointestinal tract had food content, in addition to sharp lesions in the colon, caused by the bone fragments of the fractured carapace (Figure 3B). The lungs were congested and hemorrhagic with frothy and bloody secretion (Figure 3C). Histopathological examination of the lung showed interstitial fibrosis of the bronchioles, in addition to the presence of fibrin and a large number of leukocyte cells, consisting mainly of macrophages (Figure 3D). The liver was enlarged in size, with rounded edges and capsule thickening (Figure 3E). Histopathological examination revealed multifocal areas of hepatocellular necrosis and dissociation of the hepatocyte cords (Figure 3F).

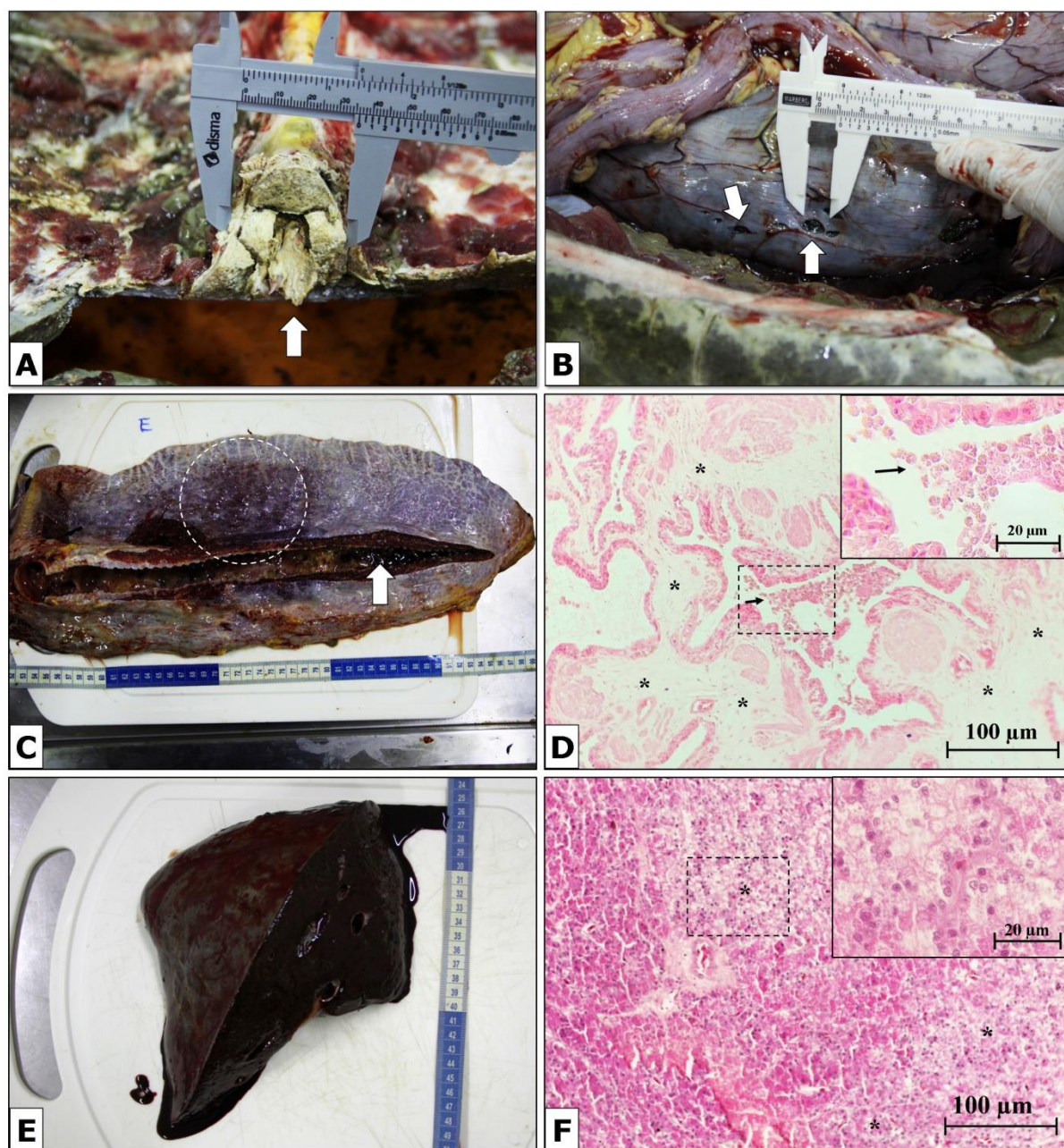


Figure 3. Anatomopathological and histopathological findings of a green turtle (*Chelonia mydas*) killed by collision with a motorboat. (A) complete fracture of the vertebral bodies (dorsal elements D9 and D10) and spinal cord section (arrow). (B) piercing-cutting lesions in the colon (arrows). (C) left lung congested, hemorrhagic (dashed circle), and with frothy and bloody secretion in the bronchus (arrow). (D) interstitial fibrosis of the bronchioles (asterisks), in addition to the presence of fibrin and a large number of leukocyte cells, consisting mainly of macrophages (arrow). (E) an enlarged liver with rounded edges and capsule thickening. (F) multifocal areas of hepatocellular necrosis (asterisks) and dissociation of the hepatocyte strands (highlighted figure of F). D and F = staining (HE).

DISCUSSION

The increased number of motorboats worldwide explains the rise in mortality rates of marine animals due to being run over [10]. As sea turtles are lung-breathing, they often need to surface to breathe, leaving them vulnerable to collisions with motorboats. Another behavior that increases the chances of the green turtle colliding with boats is that these animals feed in more coastal environments [11].

The cause of extensive and deep lesions on the shell of sea turtles may be due to them contacting the propellers, hulls, or keels of boats [3,4]. The type and severity of the injury in some situations may be related to the part of the boat that collided with the animal and be related to the boat speed at the time of the collision [4,12]. In this sense, the propellers produce multiple, linear, parallel, penetrating, and regularly spaced injuries at the same angle [4,13], while the hull or keel of the boat causes blunt wounds [14]. According to the evidence and severity of the injuries shown in this case report and considering the large size of the animal, we believe that the collision is related to the hull or keel of a boat, which was most likely going at high speed.

Hazel et al. [12], when carrying out an experimental study with green turtles to assess the escape behavior of these animals, revealed that the speed of boats directly influences the animals' escape. That is the proportion of turtles that fled to avoid collisions significantly decreased as the speed of boats increased. In this sense, there is the possibility that the animal reported here has been run over by a boat at high speed, for example, a speedboat, considering that this type of boat is authorized on the beach at the place of stranding [15].

According to Hazel and Gyuris [3], from 1990 to 2002, mortality records related to collisions with ships on the east coast of Queensland, in the United States, accounted for between 12% and 16% of the annual stranding records of sea turtles. Of the total records related to collisions with boats, the majority corresponded to green turtles (76%), followed by loggerhead turtles (*Caretta caretta*; 14%). Regarding age group, 72% of cases corresponded to adult turtles or sub-adults. Adult turtles have a higher reproductive value than juveniles, so the increased mortality of individuals in this age group should be given higher priority [16].

Sea turtles have an axial skeleton composed of a carapace, plastron bones, vertebrae, ribs, and rib derivatives [8]. In this case, the injury, probably due to a collision with a boat, produced fractures in the vertebral bodies (dorsal elements D9 and D10). Barten [17] states that fractures in this region can cause spinal cord injuries and severe and irreversible neurological signs. Fichi et al. [5] reinforce that the exposure of the coelomic cavity and

injuries to the internal organs trigger serious bacterial infections, leading to the animal's death. Affected animals are usually found dead or in a very critical condition [18]. In this report, the bacterial entry point were the multiple fractures in the animal, particularly in the spinal cord section, which may have evolved into bacterial meningitis. The histopathological findings of the lungs, such as intrabronchiolar fibrin-leukocyte exudate and the cellular response, are consistent with a possible bacterial infection [19]. Consequently, another finding such as hepatocellular necrosis was evidenced and characteristic of septicemia, which is considered to be due to the trauma of the boat collision.

Aleksić-Kovačević et al. [20], in a study based on histopathological and microbiological examinations through biopsies of the pathoanatomical findings of the skin, plastron, carapace and skeletal system of 150 European pond turtles (*Emys orbicularis*), all taken from their natural habitats in lakes in the Serbia, found that in the plastron, inflammatory and degenerative processes were frequently found, while osteopathies and mechanical injuries were the dominant findings. Microbiological examinations showed the presence of a variety of bacterial and fungal agents, primary pathogens or potential pollutants, which invaded the skin and carapace. These agents were present in cloacal swab samples. Bacterial infections were diagnosed in 76.66% of the turtles, mainly those with skin and carapace necrosis, while mycoses were diagnosed in 33.33% of the animals. Therefore, these findings reinforce that lesions at the integument level will lead to possible infections by bacterial and fungal agents present in the natural environment of these animals. In our study, although we did not perform microbiological tests, we hypothesized that agents present in the marine environment were responsible for the infections and alterations observed in necroscopic and histopathological examinations in the sea turtle.

According to the findings presented here, we assume that the green turtle suffered a collision with a motorboat. This resulted in the exposure of the coelomic cavity and spinal cord and caused intense pain, hind limb paralysis, and cloaca to the animal, in addition to septicemia and the animal's death. This report highlights the need to implement mitigation measures to reduce this type of impact on sea turtles, such as promoting conservation through environmental education tools, regulations, and inspections.

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Authors' contributions

REMO, FLNA, DSDF, ACB, ACBF, SAG, FJLS, and MFO participated in interpretation of data and drafting of the manuscript. REMO, ACBF, JMLP, and RMO performed laboratory analyses (necropsy and histopathology). REMO, FLNA, FJLS, and MFO involved in conceptualization, methodology, interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ANATOMOPATOLOŠKA NALAZI U ZELENOJ KORNJAČI (*Chelonia mydas*) ŽRTVI SUDARA S PLOVILOM

Apstrakt: Ovdje izvještavamo o traumi koju je pretrpjela zelena kornjača (*Chelonia mydas*), uzrokovanu sudarom s motornim čamcem, te opisujemo slučaj prije i nakon obdukcije. Odrasla ženka zelene kornjače spasena je živa 2. decembra 2016. godine na plaži Ponta Negra, opština Natal, Rio Grande do Norte, Brazil. Spašeni primjerak poslan je u Centar za rehabilitaciju morske faune (PCCB-UERN), općina Areia Branca (RN), Brazil. Fizikalnim pregledom utvrđeno je odlično ocjenjivanje tijela, bučno disanje, umjerena dehidracija, odsutnost zadnjih ekstremiteta i kloakalnih refleksa pri stimulisanju pritiskom te dvije traumatske ozljede karakteristične za sudar s motornim čamcem. Nakon tri dana suportivnog tretmana, životinja je uginula i odmah je poslana na obdukciju. Životinja je imala potpuni prijelom tijela kralježaka (leđni elementi D9 i D10) i presjeka leđne moždine. Cijeli gastrointestinalni trakt imao je sadržaj hrane, osim oštih ozljeda u debelom crijevu uzrokovanih fragmentima kostiju slomljene ljuske. Pluća su bila zakrčena, hemoragična sa pjenastim i krvavim izlučevinama i intersticijskom fibrozom bronhiola. Također je bilo prisutno fibrin i veliki broj leukocitnih stanica, koje se sastoje uglavnom od makrofaga. Jetra je povećana, sa zaobljenim rubovima i zadebljanjem kapsule, multifokalnim područjima hepatocelularne nekroze i disocijacijom žica hepatocita. Sudar je rezultirao izlaganjem celomske šupljine i leđne moždine, uzrokujući životinji intenzivan bol, paralizu stražnjih udova i kloake, septikemiju, a posljedično i smrt.

CAPÍTULO 9 – PROLAPSO PENIANO EM TARTARUGA-VERDE (*Chelonia mydas*)

(Artigo de relato de caso)

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Prolapso peniano em tartaruga-verde (*Chelonia mydas*)*Penile prolapse in green sea turtle (*Chelonia mydas*)*

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Resumo

A ocorrência de prolapso peniano em reptéis é observada tanto em quelônios terrestres, quanto em squamatas, porém há a escassez de relatos em tartarugas marinhas documentados até o momento. Desta forma, objetivou-se relatar o processo de reabilitação de uma tartaruga-verde (*Chelonia mydas*) com prolapso peniano, de modo a nortear procedimentos clínicos e terapêuticos que possam propiciar maior sucesso na reabilitação destes animais. Uma tartaruga-verde (*C. mydas*), adulto, macho, foi resgatada no dia 02 de maio de 2020 na praia de Muriú, Ceará Mirim, Rio Grande do Norte, Brasil. O espécime encontrava-se ativo, responsivo, com bom escore corpóreo, hidratação moderada, presença de prolapso peniano com mucosa associada hiperêmica e parte necrosada, além disso, encontrava-se com a nadadeira anterior esquerda amputada. O animal recebeu suporte terapêutico à base de analgésicos, antibióticos, anti-inflamatórios, polivitamínicos e fluidoterapia. Foram realizados procedimentos cirúrgicos para remoção total do úmero e redução mecânica do prolapso peniano. Após 50 dias de reabilitação o animal teve alta médica e foi reintroduzido de volta ao mar. Conclui-se que o protocolo utilizado, tanto na reabilitação, quanto no tratamento conservativo do prolapso peniano mostrou-se eficaz na redução do edema, evitando assim a recidiva do prolapso.

Palavras-chave: Chelonidae, falo, reabilitação, tartaruga marinha, Testudines.

Abstract

*The occurrence of penile prolapse in reptiles has been observed both in terrestrial turtles and squamates. However, there are few reports of penile prolapse in sea turtles published to date. This study aimed at reporting the rehabilitation process of a green sea turtle (*Chelonia mydas*) with penile prolapse, in order to guide clinical and therapeutic measures that can provide greater success in the rehabilitation of these animals. An adult male green sea turtle (*C. mydas*) was rescued on May 2, 2020 at Muriú beach, Ceará Mirim, Rio Grande do Norte, Brazil. The specimen was active and responsive, with a good body score and moderate hydration, and showed the presence of penile prolapse with associated hyperemic mucosa and necrosis. It also had the left anterior fin amputated. The animal received therapeutic support based on antibiotics, analgesics, anti-inflammatories, multivitamins, and fluid therapy. The surgical procedure for the complete removal of the*

humerus and mechanical reduction of penile prolapse was performed. After 50 days of rehabilitation, the animal was discharged and was reintroduced back to the sea. It is concluded that the protocol used was effective in reducing edema, thus preventing the recurrence of prolapse, both in rehabilitation and in the conservative treatment of penile prolapse.

Keywords: *Chelonidae, phallus, rehabilitation, sea turtle, Testudines.*

Introdução

A tartaruga-verde (*Chelonia mydas*) encontra-se globalmente na categoria “em perigo” de extinção de acordo com a União Internacional para a Conservação da Natureza (IUCN, 2020). A ocorrência de prolapso peniano em reptéis é observada tanto em quelônios, quanto em squamatas (Ramos et al., 2009; Hedley e Eatwell, 2014), porém há a escassez de relatos em tartarugas marinhas documentados até o momento. O prolapso peniano já foi diagnosticado como possível causa morte de uma tartaruga-verde na Austrália (Flint et al., 2010).

Nas espécies de quelônios terrestres, como causas do prolapso peniano, são observados: traumas em geral, tração durante o acasalamento, infecção, inflamação, déficits traumáticos abrangendo os músculos retratores do pênis ou esfíncter cloacal, distensão causada por parasitas intestinais e impacto da cloaca com corpos estranhos gastrointestinais, como areia, lascas de casca de árvore ou cascalho (Barten, 2006).

O tratamento e o prognóstico não são totalmente compreendidos na prática da medicina veterinária, principalmente quando acometem tartarugas marinhas de vida livre. Em uma tartaruga terrestre na Índia da espécie *Pangshura tentoriacircumdata*, o tratamento conservativo mostrou sucesso na redução do prolapso peniano (Talukdar et al., 2019). Neste sentido, padronização de técnicas e discussão de tratamentos e manobras terapêuticas na reabilitação de tartarugas marinhas com prolapso peniano fazem-se necessário, a fim de aumentar as chances de recuperação de animais que por ventura possam vir a encalhar, a exemplo deste achado, assim como aqueles mantidos sob condição de cativeiro. Desta forma, objetivou-se relatar o processo de reabilitação de uma tartaruga-verde (*C. mydas*) com prolapso peniano, de modo a nortear procedimentos clínicos e terapêuticos que possam propiciar maior sucesso na reabilitação de tartarugas marinhas e, consequentemente, promover conhecimentos e ações para conservação destes animais.

Material e Métodos

Origem do animal

O registro da tartaruga viva, refere-se a um indivíduo macho da espécie (*C. mydas*), adulto, resgatado no dia 02 de maio de 2020 na praia de Muriú (05°33'59.3"S 35°14'15.2"W), município de Ceará-Mirim, Rio Grande do Norte, Brasil. O animal foi resgatado e encaminhado para o Centro de Reabilitação de Fauna Marinha do Projeto Cetáceos da Costa Branca, Universidade do Estado do Rio Grande do Norte (PCCB – UERN), localizado no município de Areia Branca, Rio Grande do Norte (RN), Brasil. A execução das atividades com os animais marinhos está autorizada pelo SISBIO-ICMBio (Licença nº 13694-9) e Comissão de Ética no Uso de Animais da Universidade Federal Rural do Semi-Árido (Licença nº 01/2019).

Anamnese

O animal encontrava-se ativo, responsivo, com bom escore corpóreo, hidratação moderada, glicemia de 94 mg/dL, presença de prolapso peniano com mucosa associada hiperêmica e parte necrosada (Figura 1A). Além disso, encontrava-se com a nadadeira anterior esquerda amputada, com exposição dos côndilos do úmero e tecidos moles adjacentes (Figura 2), provavelmente decorrente de emalhe por rede de pesca, que foi removida do animal ainda no local do encalhe. O espécime apresentava 99 cm de comprimento curvilíneo da carapaça (CCC), 94 cm de largura curvilínea da carapaça (LCC) e 130 kg de massa corporal. No exame hematológico verificou-se um quadro de leucocitose, com valores aumentados de heterófilos, linfócitos, basófilos e monócitos.

Tratamento

A estabilização clínica foi preconizada antes de proceder com a correção cirúrgica da nadadeira amputada e redução do prolapso peniano. Para isso, o animal foi mantido em recinto com água salgada, a fim de evitar maiores lesões sobre o pênis. O tratamento de suporte emergencial consistiu na reposição eletrolítica com fluidoterapia a base de cloreto de sódio 0,9% (*KabiPac*®) no volume de 20 mL/kg/IV por 15 dias, diluído com vitamina B12 (*Polivin B12*®) na dose de 0,3 mL/kg/IV por 15 dias, antitóxico (*Mercepton*®) na dose de 0,2

mL/kg/IV/SID durante 15dias. Também foi iniciada a antibioticoterapia com ceftriaxona (*Blau Farmacêutica*[®]) na dose de 15 mg/kg/IV/BID durante 20dias e fosfato de clindamicina (*Dalacin*[®]) na dose de 10 mg/kg/IM/SID durante 15dias. A analgesia foi realizada com cloridrato de tramadol (*Tramal*[®]) na dose de 4,0 mg/kg/SC/BID por 10 dias, meloxicam (*Maxicam*[®]) na dose de 0,2 mg/kg/IM/SID por 5dias e dipirona (*D-500*[®]) na dose de 25 mg/kg/IM/TID por 10dias.

O tratamento conservativo do prolapso peniano para redução do edema consistiu da realização de compressas geladas com solução de manitol a 20% e solução hipersaturada de sacarose granulado e água filtrada 1:1, ambas a cada 8 horas, durante 2 dias.

Assim, após estabilização clínica por um período de 48 horas, o animal foi submetido ao procedimento cirúrgico para remoção total do úmero (Figura 3) e a redução mecânica do prolapso peniano (Figura 1). Para obtenção do plano anestésico foi utilizado como medicação pré-anestésica cetamina (*Quetamina*[®]) na dose de 10 mg/kg/IM e midazolam (*Hipolabor*[®]) na dose de 0,2 mg/kg/IM, com período de latência em torno de 30 minutos, para a indução o propofol (*Provive*[®]) na dose de 5,0 mg/kg/IV. A manutenção anestésica foi realizada com propofol (0,05mcg/min) em bomba de infusão.

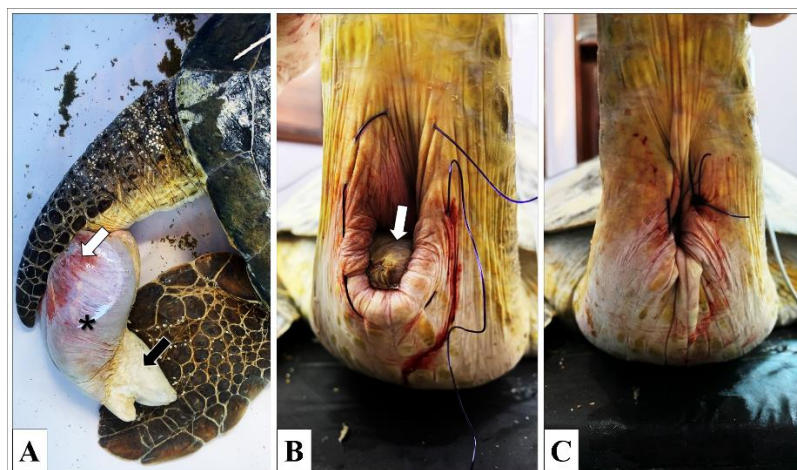


Figura 1. Prolapso peniano em tartaruga-verde (*Chelonia mydas*). (A) Pênis edemaciado (*) com mucosa hiperêmica (seta branca) e áreas de necrose (seta preta). (B e C) Pênis (seta branca) reduzido manualmente para o canal da cloaca, sendo a luz submetida a estreitamento com sutura tipo bolsa de tabaco.

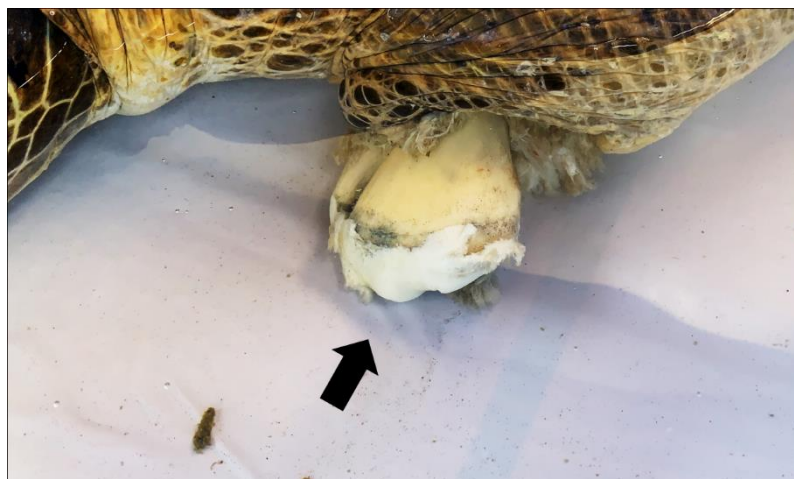


Figura 2. Amputação de nadadeira de tartaruga-verde (*Chelonia mydas*), decorrente de emalhe por rede de pesca, com exposição dos côndilos do úmero da nadadeira anterior esquerda (seta).

Para a amputação total do úmero esquerdo, inicialmente foi realizada a incisão da pele, circundando todo o membro com uma distância média de 2,0 cm dos bordos da lesão, logo em seguida, realizou-se a incisão e dissecação dos músculos: bíceps braquial (superficial e profundo), tríceps braquial (parte umeral e parte escapular), deltóide e o supracoracóideo (parte anterior e parte posterior), por fim, realizou-se a desarticulação do ombro e remoção do úmero. A hemostasia dos vasos sanguíneos foi realizada com o auxílio de pinças hemostáticas traumáticas do tipo Kelly curva. Na sutura da musculatura e dos grandes vasos do plexo braquial foi utilizado o fio absorvível sintético poliglecaprone número 0 (*Bioline*[®]), enquanto na sutura da pele, foi utilizado o fio não absorvível de nylon número 0 (*Technofio*[®]). A sutura contínua simples foi realizada para aproximação dos bordos musculares, e na pele foi realizada a sutura contínua festonada (Figura 3B).

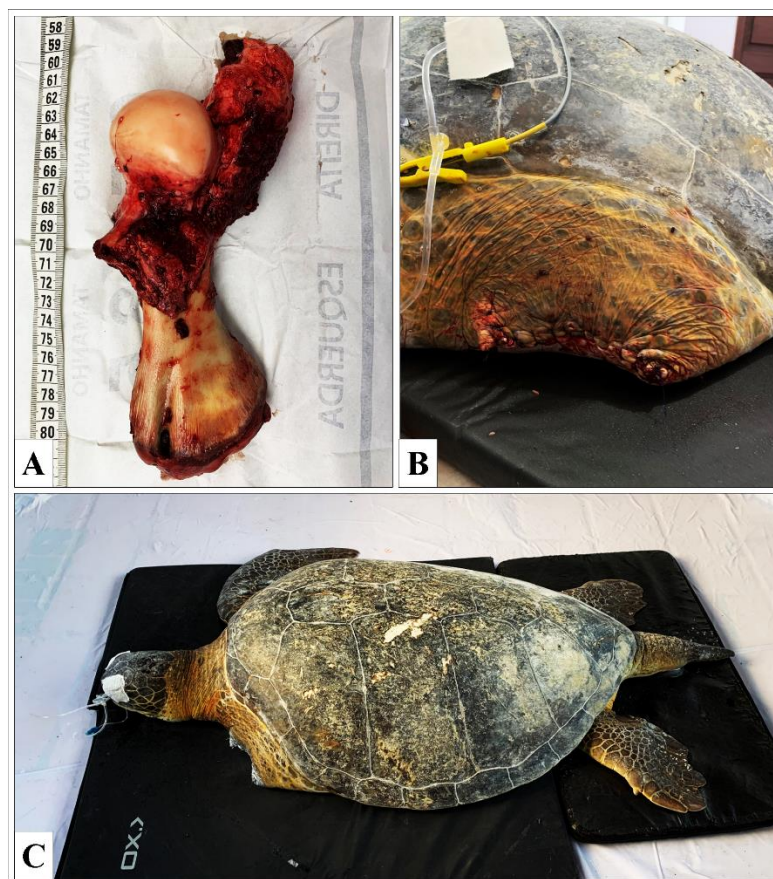


Figura 3. Amputação completa do úmero de uma tartaruga-verde (*Chelonia mydas*) com prolapso peniano. (A) úmero amputado, (B) sutura simples contínua da pele, (C) pós-operatório imediato do animal ainda sob efeitos anestésicos.

Na redução mecânica do prolapso peniano, a antisepsia do pênis e da cloaca foram realizadas previamente com solução de clorexidine degermante a 2% e solução de cloreto de sódio a 0,9%, além do desbridamento mecânico da mucosa necrosada, com auxílio de uma lâmina de bisturi número 24, logo em seguida, realizou-se a manobra para realocar o pênis no canal da cloaca, e, concomitante, foi realizado compressas geladas com solução de manitol a 20% para diminuir o edema. Após a manobra de redução mecânica do prolapso, foi realizado o estreitamento do óstio da cloaca com a sutura do tipo bolsa de tabaco, utilizando-se o fio absorvível sintético poliglecaprone número 0 (*Bioline*[®]) com espaçamento aproximado de 1,0 cm entre cada ponto de sutura. Finalizada a sutura, o óstio da cloaca mediu em torno de 2,0 cm de diâmetro para evitar uma nova protrusão do pênis e permitir a passagem dos excrementos (Figuras 1B e 1C). Por se tratar de um fio absorvível, não foi necessária remoção.

Após o procedimento cirúrgico foi dada continuidade ao tratamento medicamentoso. O tratamento tópico da ferida cirúrgica consistiu-se de limpeza com solução de cloreto de sódio a 0,9% seguido com a aplicação de antibiótico tópico à base de cloridrato de oxitetraciclina associado a hidrocortisona (*Terra-Cortril Spray*[®]) duas vezes ao dia, durante 30 dias. Além disso, realizou-se compressas geladas na região da cloaca a cada 12 horas para involução do edema, seguido de infusão de antibiótico, mucolítico e antisséptico à base de gentamicina (*Gentrin*[®]) uma vez ao dia, por 30 dias.

Durante a reabilitação do animal, foi fornecida uma dieta restritiva quanto ao volume e composição de fibras, a fim de diminuir a frequência de defecações e reduzir o tamanho do bolo fecal, visando preservar a região afetada pela lesão.

Resultados e Discussão

Após 50 dias de reabilitação, o animal foi reavaliado pela equipe médica veterinária e considerado apto para soltura, sendo avaliados parâmetros fisiológicos, clínicos e comportamentais para a espécie. Antes de proceder com a soltura, duas anilhas metálicas com identificação individual foram inseridas nas nadadeiras do animal (BRA7572 e BRA7573).

O prolapso peniano em quelônios terrestre está relacionado ao esforço ou tenesmo e disquezia causados por infecção, inflamação ou trauma. O prolapso também pode originar-se em decorrência de constipação ou impactação das fezes provocada pela ingestão de um corpo estranho. Também é verificado casos decorrentes de disfunção neurológica (Martinez-Jimenez e Hernandez-Divers, 2007). Neste relato, sugere-se que a causa evidente relacionada ao prolapso diagnosticado, foi o acentuado estímulo doloroso decorrente da amputação da nadadeira esquerda. Entretanto, outras causas já foram relatadas em quelônios terrestres como quadro infeccioso agudo, cálculo vesical ou cloacal, parasitas intestinais e separação forçada do pênis durante a cópula, déficits traumáticos envolvendo os músculos retratores do pênis ou esfíncter cloacal (Barten, 2006; Ramos et al., 2009).

Nos casos de prolapso peniano em que o tecido é avaliado como viável clinicamente, é realizado o tratamento conservativo do órgão, pois a penectomia impediria que o animal pudesse voltar a reproduzir e contribuir com a conservação da espécie. Nesses casos é recomendado a utilização de técnicas para redução do edema, evitando, deste modo, a necessidade de submeter o paciente a um procedimento cirúrgico e anestésico (Bennett e Mader, 2006). Previamente é necessário que seja realizada a limpeza e reparação das lacerações do pênis prolapsado, assim como reduzir o edema existente utilizando-se

compressas frias (Barten, 2006) e soluções de dextrose de alta concentração para reduzir os tecidos edematosos (Martinez-Jimenez e Hernandez-Divers, 2007; Serafini et al., 2012). Da mesma forma, neste relato, preconizamos o uso de compressas geladas de solução hipersaturada de sacarose granulada, além de compressas com solução de manitol à 20%.

O procedimento de redução do prolapso peniano deve ser planejado para que o órgão não volte a prolapsar, sendo importante inserir as suturas em uma distância que evite recidiva, e limitação das necessidades fisiológicas de excrementos (Nisbet et al., 2011). Neste sentido, foram levadas em consideração todas essas precauções, quando foi realizado o estreitamento do óstio da cloaca preservando uma abertura suficiente para passagem dos excrementos. Após o procedimento, não foi observado recidiva de prolapso durante todo o processo de reabilitação em cativeiro.

O procedimento pós-cirúrgico deve ser criterioso e com uso de antibióticos, analgésicos e anti-inflamatórios, evitando que uma evolução infecciosa ou inflamatória ou até mesmo em decorrência de forte dor, leve ao insucesso do procedimento e o órgão novamente apresente prolapso. O tratamento local e sistêmico preconizado para o animal apresentou boa evolução do quadro, com resposta satisfatória no caso clínico relatado. A limpeza da ferida e do ambiente em que o animal estava mantido foi fundamental para o sucesso do procedimento, pois evitou que infecções secundárias ocorressem.

Depois da lesão inicial, o tecido lesado pode rapidamente se tornar edemaciado impedindo seu retorno à cloaca. Com esta exposição constante, o órgão torna-se sujeito a novos traumas e infecções secundárias. A evolução do quadro pode levar à necrose do pênis e consequentemente da cloaca, agravando mais ainda o prognóstico do paciente (Ramos et al., 2009). Se a evolução do quadro, diferentemente do relatado neste trabalho, ocorrer desta forma, para preservar a vida do animal, deve ser realizada a penectomia após uma análise criteriosa da viabilidade dos tecidos envolvidos (Ramos et al., 2009).

Considerações Finais

A estabilização clínica e tratamento pré e pós-cirúrgico preconizado neste relato demonstraram-se eficientes no controle dos estímulos dolorosos e processo infeccioso já instalado. O tratamento conservativo com o uso de compressas geladas com soluções supersaturada de sacarose e manitol a 20% mostraram-se eficazes na redução do edema. A técnica de sutura em bolsa de tabaco com o uso do fio absorvível sintético poliglecaprone, garantiu boa resistência, evitando assim a recidiva do prolapso.

A *C. mydas* é uma espécie ameaçada de extinção e por isso, todos os indivíduos são fundamentais para o processo reprodutivo, na tentativa de minimizar o grau de ameaça da espécie, a tentativa de redução do prolapso e redução da dor, além de um aspecto ético da medicina veterinária, torna-se fundamental para a conservação da espécie, pois permite que o animal retorne ao ambiente natural e possa exercer a atividade reprodutiva com fêmeas em vida livre.

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6 CONSIDERAÇÕES FINAIS

A presente tese apresentou resultados significativos no que diz respeito a morfofisiologia do sistema digestório da tartaruga marinha da espécie *E. imbricata*. Com base nas características morfológicas observadas no trato digestório de filhotes de *E. imbricata*, são fortes indícios de uma adaptação a dieta carnívora nessa faixa etária. Evolutivamente, acreditamos que algumas características morfológicas evidenciadas no trato digestório desses animais, reforçam a ideia que as tartarugas marinhas são realmente animais primitivos quando comparadas aos quelônios semiaquáticos e terrestres. Esses resultados serão importantes para o manejo alimentar desses animais em cativeiro quando estiverem em reabilitação.

Os exames de diagnóstico por imagem em tartarugas marinhas têm grande importância na medicina da conservação, pois podem indicar o tipo de material ingerido, bem como sua localização, permitindo a melhor tomada de decisão para o tratamento de um animal, seja de forma conservativa ou cirurgicamente. Ressalta-se que nenhum exame de imagem dispensa a necessidade de uma boa anamnese, bem como avaliações por meio de outros exames complementares, como hemograma e bioquímica sérica.

O diagnóstico *ante mortem* das doenças que afetam o trato digestório em tartarugas marinhas é de difícil definição, portanto, os achados anatomopatológicos e os sinais clínicos apresentados aqui fornecerão informações relevantes para a tomada de decisão quanto à reabilitação de tartarugas marinhas em centros de reabilitação. O conhecimento dos principais achados anatomopatológicos no trato digestório são necessárias para otimizar o tempo de recuperação e aumentar as chances de sobrevivência desses animais em cativeiro.

ANEXO A – COMPROVANTE DE SUBMISSÃO DO ARTIGO NA REVISTA THE ANATOMICAL RECORD

**OROPHARYNGEAL CAVITY FLOOR MORPHOLOGY IN *Eretmochelys imbricata*
(TESTUDINES: CHELONIIDAE) HATCHLINGS AND EVOLUTIONARY
IMPLICATIONS**

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 2.064

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Dear Dr. Radan Elvis Matias de Oliveira:

Thank you for submitting your manuscript, titled "Oropharyngeal cavity floor morphology in *Eretmochelys imbricata* (Testudines: Cheloniidae) hatchlings and evolutionary implications", to be considered for publication in The Anatomical Record.

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CRediT Taxonomy

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Oropharyngeal cavity floor morphology in *Eretmochelys imbricata* (Testudines: Cheloniidae) hatchlings and evolutionary implications

By Radan Elvis Matias de Oliveira

**ANEXO B – COMPROVANTE DE SUBMISSÃO DO ARTIGO NA REVISTA
MICROSCOPY AND MICROANALYSIS**

**MORPHOLOGICAL CHARACTERIZATION OF THE DIGESTIVE TUBE OF
HAWKSBILL SEA TURTLE (*Eretmochelys imbricata*) HATCHLINGS**

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A2

FATOR DE IMPACTO (2020): 4.127

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**ANEXO C – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA VETERINÁRNÍ
MEDICÍNA**

**MARINE DEBRIS INGESTION AND THE USE OF DIAGNOSTIC IMAGING IN
SEA TURTLES: A REVIEW**

QUALIS: Quadriênio 2013-2016: B2 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 0,565

DOI: <https://doi.org/10.17221/50/2020-VETMED>

ANO DE PUBLICAÇÃO: 2020

Marine debris ingestion and the use of diagnostic imaging in sea turtles: A review

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Citation: Oliveira REM, Attadamo FLN, Moura CEB, Araujo Junior HN, Costa HS, Reboucas CEV, Silva FJL, Oliveira MF (2020): Marine debris ingestion and the use of diagnostic imaging in sea turtles: A review. *Vet Med-Czech* 65, 511–527.

Abstract: Worldwide, sea turtles are affected by anthropic waste. Animals, unable to differentiate anthropic waste from food, ingest this waste from their surroundings. After ingestion, the waste may cause a digestive tract blockage, thereby compromising the feeding and digestion capacity of the turtles, causing malnutrition, which may lead to death. Radiological imaging can be performed in turtles under rehabilitation to identify alterations of the digestive tract, such as intussusceptions, impactions, obstructions, torsions, neoplasms, and foreign bodies. These alterations are a result of either the ingestion of anthropogenic residues or natural causes. Moreover, diagnostic imaging exams play an important role in interventional medicine as they can indicate the location and type of ingested waste, thereby facilitating decision-making for the treatment of the afflicted animals. The aim of this study was to analyse the published scientific literature surrounding the effects of the ingestion of anthropogenic residue by sea turtles and the main imaging modalities used for their diagnosis. This was undertaken to provide clinicians with a greater amount of information regarding the digestive tract diseases of these animals and consequently, provide better outcomes of the rehabilitation processes. Furthermore, the outcomes of this study provide key information regarding the conservation of these species, among others.

Keywords: Cheloniidae; morphology; plastic; Testudines

**ANEXO D – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA VETERINÁRNÍ
MEDICÍNA**

**DEATH OF A LOGGERHEAD SEA TURTLE (*Caretta caretta*) FROM INGESTION
OF AN EEL (*Myrichthys ocellatus*)**

QUALIS: Quadriênio 2013-2016: B2 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 0,565

DOI: <https://doi.org/10.17221/83/2020-VETMED>

ANO DE PUBLICAÇÃO: 2020

Death of a loggerhead sea turtle (*Caretta caretta*) from ingestion of an eel (*Myrichthys ocellatus*)

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Citation: Oliveira REM, Pires JML, Batista JS, Attadamo FLN, Farias DSD, Freire ACB, Bomfim AC, Lima LRP, Oliveira RM, Gavilan SA, Silva FJL, Oliveira MF (2020): Death of a loggerhead sea turtle (*Caretta caretta*) from ingestion of an eel (*Myrichthys ocellatus*). Vet Med-Czech 65, 415–420.

Abstract: Gastrointestinal perforation in sea turtles may be associated with the ingestion of solid waste; however, other factors, including the ingestion of marine organisms, may cause intestinal perforation. Herein, we report, presumably, the first case of a death of a loggerhead sea turtle (*Caretta caretta*) from ingesting a live goldspotted eel (*Myrichthys ocellatus*) and describe the necropsy findings. The adult female loggerhead sea turtle was registered alive by a team of the Cetáceos da Costa Branca Project of Rio Grande do Norte State University (PCCB-UERN). The animal died in captivity after 8 days of an attempted rehabilitation process, and the carcass was immediately sent for necropsy. After incision, an encapsulated structure in the cranial region of the left lung was identified as a specimen of the ingested eel. The histopathological examination of the lung showed fibrin and numerous leukocytes, mainly macrophages, in the alveolus and bronchioles. The necropsy revealed that the ingestion of a live goldspotted eel (*M. ocellatus*) caused a gastric perforation in the turtle; this resulted in the displacement of the eel to the celomatic cavity with its cranial portion in the left lung, leading to sepsis and the consequent death of the turtle.

Keywords: Cheloniidae; gastric perforation; septicaemia; Testudines

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**ANEXO E – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA JOURNAL OF
AQUATIC ANIMAL HEALTH**

**COLOCOLIC INTUSSUSCEPTION ASSOCIATED WITH *Octangium* sp. (DIGENEA:
MICROSCAPHIDIIDAE) IN A GREEN SEA TURTLE *Chelonia mydas***

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 1,625

DOI: <https://doi.org/10.1002/aah.10115>

ANO DE PUBLICAÇÃO: 2021

COMMUNICATION

Colocolic Intussusception Associated with *Octangium* sp. (Digenea: Microscaphidiidae) in a Green Sea Turtle *Chelonia mydas*

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 Received May 29, 2020; accepted September 30, 2020

**ANEXO F – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA VETERINÁRNÍ
MEDICÍNA**

**SUCCESSFUL REHABILITATION OF AN OILED SEA TURTLE (*Lepidochelys
olivacea*) AFFECTED BY THE BIGGEST OIL SPILL DISASTER IN BRAZIL**

QUALIS: Quadriênio 2013-2016: B2 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 0,565

DOI: <https://doi.org/10.17221/81/2020-VETMED>

ANO DE PUBLICAÇÃO: 2021

Successful rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil

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Citation: Oliveira REM, Attademo FLN, Galvinctio JS, Freire ACB, Silva AS, Pires JML, Lima LRP, Aguiar JMF, Moreira AB, Melo LIS, Gavilan SA, Lima SA, Lima MA, Silva FJL, Oliveira MF (2021): Successful rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil. Vet Med-Czech 66, 313–319.

Abstract: The standardisation of protocols and discussion of therapeutic procedures in the rehabilitation of turtles affected by oil spills are necessary to optimise the recovery time and increase the chances of survival of these animals. This study aimed at reporting the processes adopted for the stabilisation, decontamination, rehabilitation and release of an oiled olive ridley sea turtle (*Lepidochelys olivacea*), rescued alive on September 23, 2019, at Santa Rita Beach, Extremoz municipality, Rio Grande do Norte, Brazil. Its entire body was covered by oil. At first, the animal was mechanically dry cleaned using a gauze soaked in mineral oil in the keratinised regions (carapace and plastron) and a gauze soaked in vegetable oil was used on the oral, nasal, ocular, and cloacal mucous membranes. The second stage of the oil removal consisted of washing the animal with heated pressurised water (39 °C) and a neutral detergent using a soft foam sponge. The animal received treatment with antitoxins, antibiotics, analgesics, gastrointestinal protectors, and fluid therapy. After 7 days of treatment, the blood count showed that all the parameters were within the normal range. The oil cleaning process and the therapeutic protocol used in the rehabilitation of the olive ridley sea turtle were efficient.

Keywords: Cheloniidae; contamination; crude oil; intoxication; Testudines

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ANEXO G – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA ACTA VETERINARIA (BEOGRAD)

**PATHOANATOMICAL FINDINGS OF A GREEN TURTLE (*Chelonia mydas*)
VICTIM OF COLLISION WITH A MOTORBOAT**

QUALIS: Quadriênio 2013-2016: B1 / Novo Qualis: A4

FATOR DE IMPACTO (2020): 0,800

DOI: <https://doi.org/10.2478/acve-2021-0040>

ANO DE PUBLICAÇÃO: 2021

Acta Veterinaria-Beograd 2021, 71 (4), 490-499

UDK: 598.122:[591.2:616-001.3

656.629.084

DOI: 10.2478/acve-2021-0040

*Case report***PATHOANATOMICAL FINDINGS OF A GREEN TURTLE
(*Chelonia mydas*) VICTIM OF COLLISION WITH A MOTORBOAT**

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Augusto Carlos da Bôaviagem FREIRE^{2,3}, Juliana Maia de Lorena PIRES^{2,3}, Daniel
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(Received 18 August, Accepted 18 November 2021)

In this paper we report the trauma suffered by a green turtle (*Chelonia mydas*), caused by a collision with a motorboat, and describe the case *ante* and *post-mortem*. An adult female green turtle was rescued alive on December 2, 2016 at Ponta Negra beach, municipality of Natal, Rio Grande do Norte, Brazil. The rescued animal was sent to the Marine Fauna Rehabilitation Center (PCCB-UERN), municipality of Areia Branca (RN), in Brazil. Physical examination revealed an excellent body score, noisy breathing, moderate dehydration, absence of hind limb and cloacal reflexes when stimulated by pressure, and two traumatic injuries characteristic of a collision with a motorboat. After three days of supportive treatment, the animal died and was immediately sent for necropsy. The animal had a complete fracture of the vertebral bodies (dorsal elements D9 and D10) and spinal cord section. The entire gastrointestinal tract had food content, in addition to sharp injuries in the colon caused by the bone fragments of the fractured carapace. The lungs were congested, hemorrhagic with frothy and bloody secretion, and interstitial bronchiole fibrosis. There was also present some fibrin and a large number of leukocyte cells, consisting mainly of macrophages. The liver was enlarged, with rounded edges and thickening of the capsule, multifocal areas of hepatocellular necrosis, and dissociation of the hepatocyte cords. The collision resulted in the exposure of the coelomic cavity and spinal cord, causing the animal intense pain, paralysis of the hind limbs and cloaca, septicemia, and consequently, death.

Keywords: Cheloniidae, propeller, Testudines, trauma

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**ANEXO H – PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NA REVISTA
BRASILEIRA DE REPRODUÇÃO ANIMAL**

PROLAPSO PENIANO EM TARTARUGA-VERDE (*Chelonia mydas*)

(Artigo de relato de caso)

QUALIS: Quadriênio 2013-2016: B4 / Novo Qualis: B4

FATOR DE IMPACTO (2020): Não possui

DOI: <https://doi.org/10.21451/1809-3000.RBRA2021.012>

ANO DE PUBLICAÇÃO: 2021



Prolapso peniano em tartaruga-verde (*Chelonia mydas*)

Penile prolapse in green sea turtle (Chelonia mydas)

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Resumo

A ocorrência de prolapso peniano em répteis é observada tanto em quelônios terrestres, quanto em squamatas, porém há a escassez de relatos em tartarugas marinhas documentados até o momento. Desta forma, objetivou-se relatar o processo de reabilitação de uma tartaruga-verde (*Chelonia mydas*) com prolapso peniano, de modo a nortear procedimentos clínicos e terapêuticos que possam propiciar maior sucesso na reabilitação destes animais. Uma tartaruga-verde (*C. mydas*), adulto, macho, foi resgatada no dia 02 de maio de 2020 na praia de Muriú, Ceará Mirim, Rio Grande do Norte, Brasil. O espécime encontrava-se ativo, responsivo, com bom escore corpóreo, hidratação moderada, presença de prolapso peniano com mucosa associada hiperêmica e parte necrosada, além disso, encontrava-se com a nadadeira anterior esquerda amputada. O animal recebeu suporte terapêutico à base de analgésicos, antibióticos, anti-inflamatórios, polivitamínicos e fluidoterapia. Foram realizados procedimentos cirúrgicos para remoção total do úmero e redução mecânica do prolapso peniano. Após 50 dias de reabilitação o animal teve alta médica e foi reintroduzido de volta ao mar. Conclui-se que o protocolo utilizado, tanto na reabilitação, quanto no tratamento conservativo do prolapso peniano mostrou-se eficaz na redução do edema, evitando assim a recidiva do prolapso.

Palavras-chave: Chelonidae, falo, reabilitação, tartaruga marinha, Testudines.

Abstract

The occurrence of penile prolapse in reptiles has been observed both in terrestrial turtles and squamates. However, there are few reports of penile prolapse in sea turtles published to date. This study aimed at reporting the rehabilitation process of a green sea turtle (Chelonia mydas) with penile prolapse, in order to guide clinical and therapeutic measures that can provide greater success in the rehabilitation of these animals. An adult male green sea turtle (C. mydas) was rescued on May 2, 2020 at Muriú beach, Ceará Mirim, Rio Grande do Norte, Brazil. The specimen was active and responsive, with a good body score and moderate hydration, and showed the presence of penile prolapse with associated hyperemic mucosa and necrosis. It also had the left anterior fin amputated. The animal received therapeutic support based on antibiotics, analgesics, anti-inflammatories, multivitamins, and fluid therapy. The surgical procedure for the complete removal of the humerus and mechanical reduction of penile prolapse was performed. After 50 days of rehabilitation, the animal was discharged and was reintroduced back to the sea. It is concluded that the protocol used was effective in reducing edema, thus preventing the recurrence of prolapse, both in rehabilitation and in the conservative treatment of penile prolapse.

Keywords: Chelonidae, phallus, rehabilitation, sea turtle, Testudines.

ANEXO I – AUTORIZAÇÃO PARA ATIVIDADES COM FINALIDADE CIENTÍFICA SISBIO



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: 13694-8	Data da Emissão: 15/05/2018 10:58	Data para Revalidação*: 14/06/2019
* 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

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Título do Projeto: Diversidade, áreas de ocorrência e conservação de mamíferos aquáticos e quelônios marinhos no Rio Grande do Norte (REMANE)	
Nome da Instituição: UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE	CNPJ: 08.258.295/0001-02

Cronograma de atividades

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

Observações e ressalvas

1	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.
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Outras ressalvas

1	As áreas de atuação deste projeto deverão ser previamente bem definidas, a fim de não sobrepor áreas já licenciadas no litoral do RN e CE. As atividades de marcação de tartarugas marinhas autorizadas nesta licença só poderão ser realizadas mediante formalização de protocolo de cessão de marcas com o Centro Tamar-ICMBIO, utilizando marcas com a sequência alfanumérica fornecidas por este Centro. Este protocolo deverá ser apresentado em conjunto com a licença. Todos os dados de marcação deverão ser regularmente inseridos no Banco de Dados para Conservação das Tartarugas Marinhas – BDCTAMAR. Para viabilizar este procedimento, solicitamos que o pesquisador entre em contato com o TAMAR pelo e-mail: cecilia.baptistotte@icmbio.gov.br .
2	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.

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Autorização para atividades com finalidade científica

Número: 13694-8	Data da Emissão: 15/05/2018 10:58	Data para Revalidação*: 14/06/2019
* 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 : UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE	CNPJ: 08.258.295/0001-02

Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
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4	DIOGO MICKAEL ROLIM E SILVA	Coleta de dados e atendimento a encalhes	047.803.734-17	1907955 ITEP-RN	Brasileira
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7	ALINE DA COSTA BOMFIM	Coleta de dados e atendimento a encalhes	086.298.024-05	002356291 SSP-RN	Brasileira
8	RADAN ELVIS MATIAS DE OLIVEIRA	Coleta de dados e atendimento a encalhes	076.974.554-71	2411987 SSP-RN	Brasileira
9	SIMONE ALMEIDA GAVILAN LEANDRO DA COSTA	Vice-Coordenadora	671.964.034-87	001096305 SSP-RN	Brasileira
10	EDSON SOARES DA SILVA JÚNIOR	Coordenadora de análises e coleção	080.191.234-23	002459529 ITEP-RN	Brasileira
11	HÉLDER HENRIQUE ALVES DE MEDEIROS	Coleta de dados, atendimento a encalhes, marcação animais	073.865.784-07	2490805 SSP-RN	Brasileira
12	JULIANA MAIA DE LORENA PIRES	Coleta de dados e atendimento a encalhes	368.867.028-07	329580747 SP-SP	Brasileira
13	Stella Almeida Lima	Coleta de dados e atendimento a encalhes	117.400.534-35	2863939 ssp-RN	Brasileira
14	Mariana Almeida Lima	Coleta de dados e atendimento a encalhes	117.400.564-50	002863915 SSP-RN	Brasileira
15	HELOISA CRISTINA DE MORAIS E SÁ LEITÃO	Coleta de dados e atendimento a encalhes	011.473.294-94	002280825 SSP-RN	Brasileira
16	AUGUSTO CARLOS DA BOAVIAGEM FREIRE	Coleta de dados, atendimento a encalhes, marcação animais	054.346.194-71	6398168 SDS-PE	Brasileira
17	IARA CECILIA DA COSTA MORAIS	Coleta de dados e atendimento a encalhes	061.329.044-50	002501899 ITEP-RN	Brasileira

Locais onde as atividades de campo serão executadas

#	Município	UF	Descrição do local	Tipo
1		RN	Área de Atuação da REMANE (Nordeste do Brasil, RN, CE)	Fora de UC Federal
1	Amostras biológicas	Sangue, Sêmen, Ectoparasita, Urina, Regurgitação/conteúdo estomacal, Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Fragmento de tecido/órgão, Pêlo		
2	Método de captura/coleta	Captura manual, Outros métodos de captura/coleta		
3	Método de marcação	Foto-identificação, Transponder, Outros métodos de marcação		

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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: 13694-8	Data da Emissão: 15/05/2018 10:58	Data para Revalidação*: 14/06/2019
* 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 : UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE	CNPJ: 08.258.295/0001-02

Atividades X Táxons

#	Atividade	Táxons
1	Captura de animais silvestres in situ	Aves
2	Coleta/transporte de amostras biológicas in situ	Chelonia mydas, Lepidochelys olivacea, Actinopterygii, Elasmobranchii, Aves, Cetacea, Sirenia, Mustelidae, Dermochelys coriacea, Cheloniidae, Caretta caretta, Eretmochelys imbricata, Dermochelyidae
3	Manutenção temporária (até 24 meses) de vertebrados silvestres em cativeiro	Aves
4	Marcação de animais silvestres in situ	Caretta caretta, Dermochelys coriacea, Aves, Eretmochelys imbricata, Lepidochelys olivacea, Chelonia mydas, Dermochelyidae, Cheloniidae

Material e métodos

1	Amostras biológicas (Aves)	Penas, Fragmento de tecido/órgão, Fezes, Ectoparasita, Animal encontrado morto ou partes (carcaça)/osso/pele, Regurgitação/conteúdo estomacal, Sangue
2	Amostras biológicas (Carnívoros)	Urina, Sangue, Regurgitação/conteúdo estomacal, Pêlo, Fragmento de tecido/órgão, Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Ectoparasita
3	Amostras biológicas (Cetáceos)	Secreção, Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Sangue
4	Amostras biológicas (Peixes)	Fezes, Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Escama, Sêmen, Ovos, Sangue, Fragmento de tecido/órgão
5	Amostras biológicas (Sirênios)	Fezes, Ectoparasita, Sangue, Secreção, Animal encontrado morto ou partes (carcaça)/osso/pele, Fragmento de tecido/órgão
6	Amostras biológicas (Tartarugas marinhas)	Sangue, Regurgitação/conteúdo estomacal, Fragmento de tecido/órgão, Animal encontrado morto ou partes (carcaça)/osso/pele, Fezes, Ovos
7	Método de captura/coleta (Aves)	Outros métodos de captura/coleta(Apenas indivíduos debilitados nas praias)
8	Método de captura/coleta (Carnívoros)	Outros métodos de captura/coleta(Apenas indivÁ-duos encahados ou debilitados nas praias)
9	Método de captura/coleta (Cetáceos)	Outros métodos de captura/coleta(Apenas indivÁ-duos encahados ou debilitados nas praias)
10	Método de captura/coleta (Peixes)	Outros métodos de captura/coleta(Apenas indivíduos debilitados nas praias)
11	Método de captura/coleta (Sirênios)	Outros petrechos(Apenas indivÁ-duos encahados ou debilitados nas praias)
12	Método de captura/coleta (Tartarugas marinhas)	Captura manual, Outros métodos de captura/coleta(Apenas indivÁ-duos encahados ou debilitados nas praias)
13	Método de marcação (Aves)	Anilha metálica (padrão CEMAVE), Anilha de Alumínio (padrão CEMAVE)
14	Método de marcação (Tartarugas marinhas)	Anilha

Destino do material biológico coletado

#	Nome local destino	Tipo Destino
1	UNIVERSIDADE DO ESTADO DO RIO GRANDE DO NORTE	coleção

Este documento (Autorização para atividades com finalidade científica) foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº 03/2014, 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).

Táxon*	Qtde.	Tipo de amostra	Qtde.	Data

* Identificar o espécime no nível taxonômico possível.

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ANEXO J – PARECER CEUA-UFERSA


UNIVERSIDADE FEDERAL RURAL DO SEMI-ÁRIDO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Mossoró, 29 de janeiro de 2019.

PARECER 01/2019

O projeto intitulado “Morfologia e achados anatomopatológicos do aparelho digestório de *Eretmochelys imbricata* e *Lepidochelys olivacea* (Testudines: Cheloniidae) encalhadas na bacia potiguar, Brasil” processo numero 23091.013527/2018-75, sob responsabilidade de Moacir Franco de Oliveira o qual envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da lei 11.794 de 8 de outubro de 2009 e com as normas editadas pelo Conselho Nacional de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal Rural do Semi-Árido –UFERSA em reunião no dia 22 de janeiro de 2019.

Vigência do projeto	Fevereiro de 2019 a julho de 2020
Espécie/linhagem	<i>Eretmochelys imbricata</i> e <i>Lepidochelys olivacea</i>
N. de Animais	40
Peso/idade	Filhotes, juvenis e adultos
Sexo	Machos e Fêmeas
Origem	Animais provenientes de encalhes na costa do RN.

Emanuelle Fontenele Rabelo
 Emanuelle Fontenele Rabelo

Presidente CEUA-UFERSA