Universidade
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FIBROPAPILOMATOSE EM TARTARUGAS-VERDE (*Chelonia mydas*, Linnaeus, 1758) NO SUDOESTE DO OCEANO ATLÂNTICO:

EPIDEMIOLOGIA E PARÂMETROS CLÍNICOS LABORATORIAIS

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito para a obtenção do título de Doutor.

Orientador: Profa. Dra. Ana Paula Frederico Rodrigues Loureiro Bracarense.

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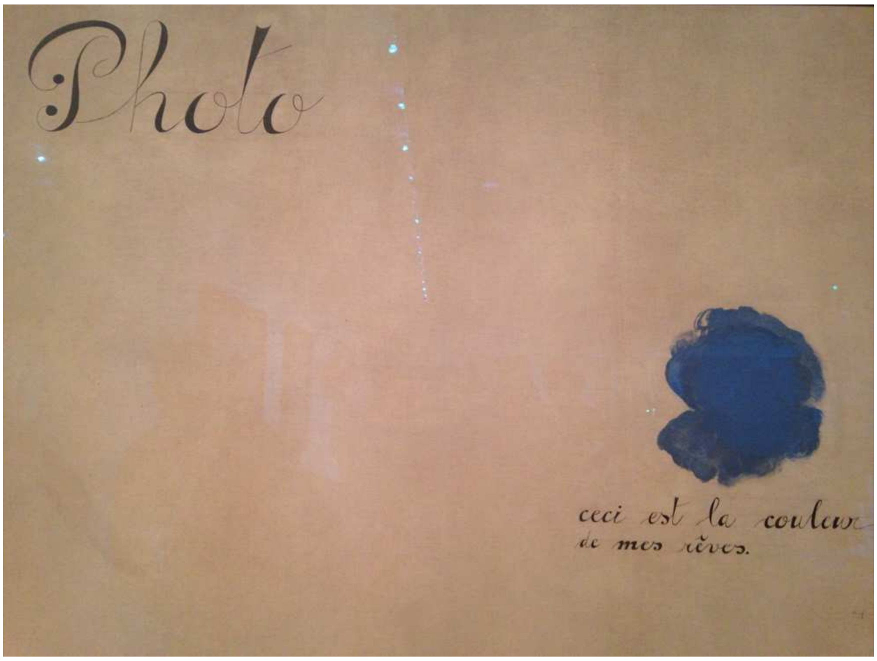

Joan Miró (1893-1983)

‘This is the color of my dreams’ (1925)

‘Esta é a cor dos meus sonhos’

DOMICIANO, Isabela Guarnier. **Fibropapilomatose em tartarugas-verde (*Chelonia mydas*, Linnaeus, 1758) no sudoeste do oceano Atlântico:** epidemiologia e parâmetros clínicos laboratoriais. 2016. 93f. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2016.

RESUMO

A tartaruga-verde, *Chelonia mydas*, é a espécie de maior ocorrência no Brasil e habita áreas mais próximas à costa, onde estão suscetíveis a ameaças naturais, como doenças, ou decorrentes de atividades antropogênicas. A fibropapilomatose é uma doença crônica caracterizada por tumores externos e internos, principalmente em tartarugas-verde juvenis, que podem dificultar atividades como alimentação e natação ou levar a morte. O artigo A teve como objetivo revisar a epidemiologia da fibropapilomatose no Brasil e avaliar informações inéditas sobre a doença em três estados (Rio de Janeiro-RJ, Paraná-PR e Santa Catarina-SC). Foram levantadas informações na literatura sobre a prevalência, comprimento curvilineo da carapaça (CCC), metodologia utilizada (encalhe, captura incidental ou intencional em rede de pesca ou desova) e gravidade da doença em tartarugas-verde ao longo da costa (Latitudes 02°52`S a 26°02`S), de 2000 a 2014. Além desses parâmetros, foram avaliadas nos três estados, a condição corpórea do animal, a histologia e a presença de Chelonid Herpesvirus-5 (ChHV5) em tumores de animais amostrados de 2007/2009 a 2014. Os dados disponíveis em 12 dos 17 (70,6%) estados da costa brasileira foram comparados entre dois períodos (2000 a 2006 e 2007 a 2014) e prevalências demonstraram diferenças significativas de acordo com (i) a metodologia utilizada, (ii) o período e (iii) a latitude. Houve diminuição da doença no Ceará, Rio Grande do Norte, Espírito Santo e São Paulo; manutenção na Paraíba e RJ; aumento na Bahia e SC e ausência nas ilhas oceânicas. As prevalências da doença foram 13,6% (12/88), 14,5% (17/117) e 9,6% (38/395) no RJ, SC e PR, respectivamente, em animais encalhados e 23% (10/43) em animais intencionalmente capturados no PR. A análise total dos casos em toda a costa indicou a manutenção da doença de 2000 a 2014 [14% (1090/7804) de 2000 a 2006 e 13,8% (1106/8035) de 2007 a 2014]. A patogenia e prevalência da doença podem estar associados ao CCC e poluição ambiental. Achados histopatológicos dos tumores sugerem infecção viral, porém, apenas uma lesão apresentou corpúsculos de inclusão (1/121). A análise molecular das amostras detectou cinco variantes de ChHV5, semelhantes ao grupo de variantes do Atlântico e Atlântico oeste/leste do Caribe. No artigo B, o objetivo foi avaliar parâmetros hematológicos e bioquímicos de tartarugas-verde capturadas intencionalmente em duas ilhas na zona estuarina do litoral do PR. Os animais foram capturados por sete dias em 2014 e amostras de sangue foram colhidas para análise de 36 parâmetros. No total, 43 juvenis foram capturados e 10 apresentavam fibropapilomatose (23,2%), sendo que a maioria apresentava bom estado nutricional (42/43; 97,7%). O CCC e massa corpórea (MC) de animais com tumores (CCC=43,51±4,42cm; MC=10,42±2,81kg) foram semelhantes aos animais sem tumores (CCC=41,06±4,66cm; MC=8,58±2,97kg). Níveis menores de colesterol (57,10±9,24mg/dl) e ureia (7,80±0,99mg/dl) e maiores concentrações de eosinófilos (3,10±1,09 103/µl) foram observados em animais com tumores em relação aos sem tumores (colesterol=85,66±6,74mg/dl; ureia=37,85±8,59mg/dl e eosinófilo=0,88±0,26 103/µl). A despeito da doença, o CCC foi relacionado positivamente aos níveis de proteína total, albumina/globulina e hematócrito e negativamente ao cloreto. O estado de saúde das tartarugas-verde pode refletir a saúde e impactos no ambiente marinho, que devem ser continuamente monitorados para efetiva conservação dessa espécie ameaçada de extinção.

Palavras-chave: Tartaruga marinha. Doença. Bioquímica. Hematologia. Conservação. Brasil.

DOMICIANO, Isabela Guarnier. **Green turtle (*Chelonia mydas,* Linnaeus 1758) fibropapillomatosis off Southwestern Atlantic Ocean:** epidemiology and blood parameters. 2016. 93p. Thesis (Doctor´s Degree in Animal Science) – Universidade Estadual de Londrina, Londrina, 2016.

ABSTRACT

The green tutle, *Chelonia mydas*, is the most commom species that inhabit coastal areas off Brazil, and are susceptible to natural hazards (including diseases) and anthropogenic activities. Fibropapillomatosis is a chronic disease characterized by skin and internal tumours that can affect vital activities as feeding and swimming or can lead to death. The objective of article A was to review the epidemiology of fibropapillomatosis in Brazil and also evaluate new data from three states (Rio de Janeiro–RJ, Santa Catarina–SC and Paraná–PR). Local and international peer-reviewed studies summarizing prevalence, curved carapace length (CCL) sampling method (stranded, bycaught, intentionally captured or nesting), and severity of disease were searched for green turtles throughout the Brazilian coast (Latitude 02°52`S to 26°02`S) from 2000 to 2014. The body condition, histology and detection of Chelonid Herpesvirus-5 (ChHV5) were also evaluated of afflicted animals sampled from 2007/2009 to 2014 in RJ, PR and SC. Data from 12 of 17 (70.6%) states of Brazilian coast were compared between two periods (from 2000 to 2006 and from 2007 to 2014). Prevalences varied according to (i) sampling methods, (ii) period evaluated and (iii) latitude degrees. Prevalence decrease was observed in Ceará, Rio Grande do Norte, Espírito Santo and São Paulo, maintenance in Paraíba and RJ; increase in Bahia and SC and were absent in oceanic islands. The prevalences were 13.6% (12/88), 14.5% (17/117) and 9.6% (38/395), in stranded animals off RJ, SC and PR; and 23% (10/43) in animals intentionally captured off PR. Considering all data from Brazilian coast, prevalence of fibropapillomatosis was similar from 2000 to 2014 [14% (1090/7804) from 2000 to 2006 and 13.8% (1106/8035) from 2007 to 2014]. The pathogenisis and consequent prevalence values can be associated with CCL and environmental pollution levels. Microscopical findings suggested viral infection, but eosinophilic inclusion bodies were observed in only one tumor (1/121). Molecular methods detected five variants of ChHV5 that were clustered with Atlantic groups, excepting one that clustered with western Atlantic/ eastern Caribean group. In article B, the objetive was to evaluate hematological and biochemistry samples of green turtles intentionally captured in two islands off Paraná coast. The animals were caught in gillnets for seven days in 2014 and 36 parameters of blood samples were evaluated. A total of 43 juvenile green turtles were caught, of which 10 had fibropapillomatosis (23.2%). The majority was classified in good nutritional status (42/43, 97.7%). The CCC and body mass (BM) of fibropapillomatosis afflicted animals (CCL = 43.51 ± 4.42 cm; BM = 10.42 ± 2.81 kg) were similar to green turtles without tumours (CCL = 41.06 ± 4.66 cm; BM = 8.58 ± 2.97 kg). Significant lower levels of cholesterol (57.10 ± 9.24 mg dl–1) and urea (7.80 ± 0.99 mg dl–1), but greater absolute numbers of eosinophils (3.1 ± 1.1 103 µl–1) were observed in turtles with tumours compared to turtles without tumours (cholesterol = 85.66±6.74 mg dl–1, urea = 37.85 ± 8.59 mg dl–1, eosinophils=0.9 ± 0.3 103 µl–1). Irrespective of disease, CCL was positively associated with total protein, albumin, albumin-to-globulin and hematocrit and negatively associated with chloride. The green turtles health may reflect hazards and the health status of marine environment. Therefore, monitoring is important to green turtle conservation.

Keywords: Sea turtles. Diseases. Biochemistry, Hematology. Conservation. Brazil.

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1 INTRODUÇÃO

As tartarugas marinhas apresentam complexo ciclo de vida, característico de animais que realizam deslocamentos, muitas vezes, longos entre as áreas de nascimento, alimentação e reprodução (BOWEN e KARL, 2007). Dessa forma, são consideradas sentinelas ambientais e indicadores da saúde do ambiente costeiro e marinho, tanto em escala local como mundial, refletindo impactos durante o longo período de vida (AGUIRRE e LUTZ, 2004).

Existem sete espécies no mundo e, entre elas, cinco são encontradas no Brasil: *Chelonia mydas* (tartaruga-verde), *Caretta caretta* (tartaruga-cabeçuda), *Lepidochelys olivacea* (tartaruga-oliva), *Eretmochelys imbricata* (tartaruga-de-pente) e *Dermochelys coriacea* (tartaruga-de-couro) (SANTOS *et al*., 2011). *Lepidochelys kempii* (Kemp's Ridley) ocorre apenas no Atlântico Norte e Golfo do México e *Natator depressus* (Flatback turtle), no oceano Índico e Pacífico (IUCN, 1996a e 1996b). Todas estão inclusas nas listas de espécies ameaçadas de extinção, principalmente devido a impactos causados por atividades humanas (IUCN, 2016; ICMBio, 2014).

A tartaruga-verde possui distribuição cosmopolita, desde os trópicos até zonas temperadas e apresenta hábitos mais costeiros em relação às outras espécies de tartarugas marinhas, utilizando inclusive estuários e lagos (ALMEIDA *et al*., 2011). No Brasil, as áreas de desova da espécie localizam-se nas ilhas oceânicas Trindade (Espírito Santo), Atol das Rocas (Rio Grande do Norte) e Fernando de Noronha (Pernambuco), mas há casos de desovas no norte do litoral da Bahia e nos Estados do Espírito Santo, Sergipe e Rio Grande do Norte (ALMEIDA *et al*., 2011). As tartarugas-verde juvenis são as mais comuns na região costeira do mar continental do Brasil, onde se alimentam, e representam o maior número de ocorrências de encalhes, avistagens e capturas em rede de pesca (SANTOS *et al*., 2011).

A fase juvenil das tartarugas-verde é um período em que ocorrem mudanças de habitat, morfológicas e de dieta (NISHIZAWA *et al*., 2010, GAMA *et al*., 2016). Os animais migram de zonas oceânicas para áreas de alimentação neríticas (próximas à costa), etapa conhecida como recrutamento, quando passam por mudanças morfológicas graduais no crânio e de uma dieta onívora para predominantemente herbívora (CARDONA *et al*., 2010, NISHIZAWA *et al*., 2010, GAMA *et al*., 2016). Mesmo após o recrutamento para zonas neríticas, espécimes ainda se deslocam entre zonas pelágicas oceânicas e neríticas em busca de alimento, em um período de transição de ingestão de itens de origem animal e vegetal (GONZÁLEZ-CARMAN *et al*., 2012).

Essas alterações são peculiares à espécie e estágio de desenvolvimento e, em conjunto com outros fatores (ex. sazonalidade e disponibilidade de alimentos), podem refletir em mudanças na homeostase do animal e consequente desempenho corpóreo para atividades vitais (AMOROCHO e REINA, 2008; WILLIARD, 2013). O entendimento sobre os parâmetros fisiológicos e sobre os limites aceitáveis para uma condição saudável dos indivíduos/populações são importantes e servem como base também na avaliação de potenciais impactos naturais ou causados por atividades humanas (WILLIARD, 2013).

Alguns dos impactos naturais incluem ataques de predadores, doenças como a fibropapilomatose, mudança abrupta de temperaturas da água (“cold stunning”) e deficiências nutricionais, porém os mais impactantes envolvem atividades humanas (GEORGE, 1997; FLINT *et al*., 2009; ANDERSON *et al*., 2011). A captura incidental em pescarias, tais como emalhe e arrasto, ingestão de resíduos sólidos, principalmente o plástico, e a exposição aguda ou crônica a poluentes químicos também interferem no estado de saúde desses animais (SCHUYLER *et al*., 2014; KELLER, 2013; LEWISON *et al*., 2014). Na natureza, esses fatores atuam de forma simultânea e, mesmo que não levem diretamente à morte, influenciam no nível de estresse que está associado à queda da imunidade e aumento da suscetibilidade das tartarugas marinhas a patógenos e desenvolvimento de doenças (MILTON e LUTZ, 2003).

A fibropapilomatose, por exemplo, destaca-se pela distribuição mundial e por afetar principalmente tartarugas-verde juvenis (JONES *et al*., 2016). Existem, porém, diferentes lacunas no entendimento sobre a gravidade, a progressão da doença à morte, a patogenia e sobre as diferentes prevalências da doença entre regiões, mesmo em pequena escala geográfica (WORK *et al*., 2004; VAN HOUTAN *et al*., 2010; GUIMARÃES *et al*., 2013). Dessa forma, a avaliação da enfermidade e também da saúde dos animais afetados pela fibropapilomatose, considerando a interação entre os fatores ambientais (ex. impactos, características físicas e químicas), o agente etiológico e o hospedeiro (ex. mudanças na fase juvenil), trazem uma resposta mais holística e servem como base para monitoramento e conservação desses animas considerados sentinelas do ecossistema marinho.

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2 REVISÃO DE LITERATURA*

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Tartarugas-verde, *Chelonia mydas*, como sentinelas do ambiente marinho e costeiro: atividades antrópicas e enfermidades

The green turtle *Chelonia mydas* as marine and coastal environmental sentinels: anthropogenic activities and diseases

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Resumo

A tartaruga-verde, *Chelonia mydas*, possui ampla distribuição geográfica, reprodução tardia e complexo ciclo de vida que englobam habitats nos ambientes costeiros e marinhos. Devido a essas características, os animais ficam expostos a diferentes tipos de ameaças para a conservação e podem sinalizar sobre variações e gravidade desses impactos ao longo do tempo, sendo considerada sentinela ambiental. Este trabalho teve por objetivo discorrer sobre relatos de atividades antrópicas impactantes e doenças infecciosas e parasitárias em *C. mydas*, com ênfase nas ocorrências no litoral brasileiro. A captura acidental em petrechos de pesca é uma das principais ameaças à conservação desses animais, assim como a ingestão de resíduos sólidos, colisão com embarcações e dragas e bioacumulação de contaminantes químicos, os quais podem atuar diretamente ou indiretamente na morte dos animais. Dessa forma, quando não matam o animal, podem servir como porta de entrada para agentes patológicos e agirem como imunossupressores que influenciam no desenvolvimento de doenças secundárias. Os agentes patológicos associados a lesões incluem vírus, bactérias, fungos, protozoários e helmintos. A fibropapilomatose, por exemplo, é caracterizada por tumores cutâneos e viscerais que afetam *C. mydas* juvenis no mundo todo, e o provável agente é o Herpesvirus Chelonid-5. As bactérias do gênero *Vibrio*, *Aeromonas*, *Pseudomonas* são frequentemente encontradas no ambiente aquático, como também em animais com lesões em diferentes órgãos. Os adultos e ovos de trematódeos da família Spirochiidae também são frequentemente relatados em afecções cardiovasculares sistêmicas em *C. mydas*. O impacto causado por atividades antrópicas e o desenvolvimento de doenças é sinérgico e afetam a saúde e conservação desses animais. A identificação de doenças e a causa da morte em *C. mydas* ainda são esporádicas em diversas regiões do mundo, assim como no Brasil. A avaliação contínua, sistemática, por meio de exames macroscópicos, histopatológicos e auxílio de análises moleculares para identificação de agentes patogênicos é necessária para avaliar o estado de saúde e subsidiar decisões de gestão costeira, de pesquisa e para desenvolver medidas de mitigação apropriadas para a conservação de *C. mydas*.

Palavras-chave: captura acidental, ingestão de resíduos, agentes patogênicos, morbidade, conservação, tartaruga marinha

Abstract

The green turtle, *Chelonia mydas,* has a wide distribution, slow maturing and a complex life cycle that encompass oceanic and coastal environments. The species are exposed to different threats and are considered environmental sentinels that sinalize variations and severity of hazards to marine ecosystem. This study aimed to describe anthropogenic impacts and infectious and parasitic diseases in *C. mydas* – including cases off Brazilian coast – and implications to conservation. The bycatch is one of the main threats to species conservation, following by debris ingestion, boat and dredge collision and enrivormental contamination. All those impacts may cause direct death or indirect implications to death, as facilitating pathological agents contact and imunossupression to secondary diseases. The pathological agents associated to lesions in *C. mydas* inclued virus, bacteria, fungi, protozoa and helmints. The fibropapillomatosis is an example of a disease characterized by cutaneous and visceral tumours that affect mostly juveniles *C. mydas* worldwide and is associated with Herpesvirus Chelonid-5. The bacterias *Vibrio*, *Aeromonas*, *Pseudomonas* are found in aquatic environment, as in animals with lesions in differents organs. Trematode adults and eggs of family Spirorchiidae are also frequent observed in sistemic cardiovascular diseases in *C. mydas*. The impact of anthropogenic activities and diseases are synergic and may affect the health and species conservation. Therefore, the monitoring and systematic diagnosis of cause of death – including necropsy, histopathology and molecular exams – are necessary to assess populations health, to make appropriate decisions of coastal management and to target research topics to reach *C. mydas* conservation.

Key-words: byctach, debris ingestion, pathological agents, morbidity, conservation, sea turtle.

2.1 BIOLOGIA E ECOLOGIA DE *CHELONIA MYDAS*

As tartarugas marinhas compreendem apenas sete espécies e se distribuem mundialmente em quase todos os oceanos (WALLACE et al., 2010). As características das populações desses animais, como reprodução, morfologia, tamanho e tendências de crescimento populacional variam geograficamente, assim como o grau de ameaças e impactos associados às atividades humanas (WALLACE et al., 2011).

A tartaruga-verde, *Chelonia mydas*, possui distribuição cosmopolita, desde os trópicos até zonas temperadas e apresenta hábitos costeiros em relação às outras espécies de tartarugas marinhas, utilizando inclusive estuários e lagos (ALMEIDA et al., 2011) (Figura 1).

Figura 1. Tartaruga-verde, *Chelonia mydas*, encalhada no litoral do estado do Paraná.

Fonte: Laboratório de Ecologia e Conservação/UFPR.

O estágio de desenvolvimento, assim como taxas de crescimento desses animais são estimadas pelo comprimento curvilíneo da carapaça (CCC) ou comprimento linear da carapaça e a idade, pelas linhas de crescimento do úmero (BJORNDAL et al., 2013; ANDRADE et al., 2016). No Brasil, por exemplo, o valor mínimo e máximo de CCC de *C. mydas* fêmeas desovando em Atol das Rocas variou entre 96 e 132 cm (BELLINI et al., 2013), e a idade estimada para maturação sexual, entre 26 e 40 anos (ALMEIDA et al., 2011).

O dimorfismo sexual é verificado apenas no estágio adulto, quando a cauda e garras do macho apresentam tamanhos maiores em relação à fêmea (WYNEKEN, 2001). No estágio juvenil, as características externas são semelhantes, portanto, a diferenciação sexual pode ser observada após análise de níveis hormonais, laparoscopia ou histologia das gônadas (DUARTE et al., 2011, FORATTINI, 2011).

O ciclo de vida de *C. mydas*, em geral, envolve diferentes habitats: (i) o desenvolvimento de embriões e nascimento dos filhotes em regiões costeiras ou ilhas oceânicas, (ii) seguido pelo desenvolvimento dos filhotes até a fase juvenil em áreas oceânicas e (iii) posterior recrutamento às áreas costeiras, onde permanecem e compartilham o hábitat com tartarugas de outras regiões (estoques populacionais) nas áreas de alimentação (BOWEN; KARL, 2007). Animais adultos retornam novamente às áreas oceânicas durante a migração para áreas de reprodução (BOWEN; KARL, 2007; PUTMAN; NARO-MACIEL, 2013). O CCC de *C. mydas* juvenis recrutadas para zonas costeiras varia entre 19 e 40 cm e entre um e 10 anos, de acordo com a região (SEMINOFF et al., 2015).

No Brasil, as áreas de desova incluem as ilhas oceânicas em Ilha da Trindade (Espírito Santo), Atol das Rocas (Rio Grande do Norte) e Fernando de Noronha (Pernambuco), mas há casos de desovas no norte do litoral da Bahia e nos Estados do Espírito Santo, Sergipe e Rio Grande do Norte (ALMEIDA et al., 2011). Os sítios de alimentação, porém, estão distribuídos ao longo da costa brasileira, e são habitados principalmente por indivíduos no estágio juvenil, que apresentam estrutura genética e padrões de residência variados nesses locais (BOWEN; KARL, 2007; ALMEIDA et al., 2011).

Os juvenis que chegam às zonas costeiras apresentam hábitos alimentares onívoros e, em geral, mudam gradativamente para herbivoria (JONES; SEMINOFF, 2013), sendo os itens de origem animal amostrados principalmente em espécimes oceânicas ou recém recrutados, menores que 45 cm, em diferentes regiões geográficas (JONES; SEMINOFF, 2013, GAMA et al., 2016; VÉLEZ-RUBIO et al., 2016). A frequência dos itens consumidos pode variar também de acordo com a disponibilidade dos mesmos em diferentes habitats ao longo do tempo (AMAROCHO; REINA, 2007; SANTOS et al., 2015a). No estado do Paraná, por exemplo, as *C. mydas* encalhadas apresentaram maior consumo de grama marinha na estação chuvosa e algas na estação seca (GAMA et al., 2016).

Alterações na morfometria geométrica corpórea também ocorrem nessa fase juvenil inicial até o estágio adulto (NISHIZAWA et al., 2010). *Chelonia mydas* entre dois a cinco anos possuem tamanhos de crânio semelhantes, e por volta dos seis anos, CCC médio de 46 cm, ocorre o aumento na estrutura corpórea, inclusive do crânio, possivelmente relacionada a adaptação de disponibilidade de alimento, mudança na dieta e uso de habitat (COELHO, 2015). Além da mudança associada ao tamanho, a morfologia craniana de *C. mydas* também varia de acordo com a genética, diferindo, por exemplo, entre os estoques populacionais de

Ascenção (Atlântico Sul) e Suriname (América central) amostrados no litoral sul do Brasil, ressaltando as diferenças que podem ser observadas em animais provenientes de diversas regiões e que utilizam a mesma área de alimentação (COELHO, 2015).

As tartarugas-verde que habitam as áreas de alimentação na costa brasileira são procedentes principalmente da Ilha de Ascensão, localizada no Oceano Atlântico Sul (LUSCHI et al., 1998), mas também por animais da América Central, Ilha da Trindade e Atol das Rocas e possivelmente e do continente africano (PRITCHARD, 1976; LIMA; TROËNG, 2001; NARO-MACIEL et al., 2012; COELHO, 2015). A distância da Ilha de Ascensão até a costa brasileira é cerca de 2300 km, mas os animais chegam a percorrer 5000 km a partir da Costa Rica e 5300 km da Nicarágua (LUSCHI et al., 1998; LIMA et al., 1999; LIMA; TROËNG, 2001).

2.2 MORBIDADE E MORTALIDADE DE *CHELONIA MYDAS* ASSOCIADA À IMPACTOS ANTRÓPICOS

Devido ao grande deslocamento e diversidade de habitats utilizados ao longo do ciclo de vida das tartarugas marinhas, esses animais ficam expostos a diferentes tipos de ameaças, decorrentes de atividades antropogênicas ou naturais, de consequências agudas ou crônicas (GEORGE, 1997; FLINT, 2013).

Entre as atividades humanas que afetam direta ou indiretamente a morbidade e mortalidade de *C. mydas*, destaca-se a interação com petrechos utilizados durante a pesca ou aqueles descartados no ambiente; colisão com embarcações; contaminação química e transporte de agentes patogênicos derivados de efluentes agrícola, industrial e doméstico; ingestão de resíduos sólidos e destruição de habitat, com consequente alteração de áreas de desova e diminuição de itens alimentares (HARVELL et al., 1999; FLINT, 2013; GAMA et al., 2016; SCHUYLER et al., 2014a, 2014b).

A captura incidental de tartarugas marinhas em petrechos de pesca é um dos principais impactos causados por atividades humanas, a qual aumenta em locais de maior sobreposição de uso de habitat pelos animais e esforço pesqueiro, como em áreas de alimentação, corredores migratórios e próximos a áreas de reprodução, envolvendo, portanto, a captura de diferentes níveis de ramificação ou estágios de desenvolvimento da população (WALLACE et al., 2011; LEWISON et al., 2013). Estimativas em diferentes áreas indicam que o número de animais retirados é maior do que a população suporta (WALLACE et al., 2013; CASALE; HEPPELL, 2016), e isso pode se intensificar uma vez que os animais possuem grande distribuição geográfica, sem limites geográficos e oceânicos, onde ficam sujeitos a diferentes escalas de esforço pesqueiro (WALLACE et al., 2013).

As consequências da captura podem ser diretas, levando a morte, ou causar traumas e infecções cutâneas, redução da natação, dificuldade de alimentação e inanição quando petrechos permanecem presos aos animais ou quando ingeridos, pois podem provocar perfuração das estruturas bucais e do esôfago (Figura 2A), além de alterações ao longo do trato gastrointestinal, como intussuscepção e infecção bacteriana secundária (ORÓS et al., 2005; WORK et al., 2015a; NELMS et al., 2016). Em casos de afogamento associados à rede de pesca as alterações descritas foram edema pulmonar (Figura 2B), atelectasia e enfisema pulmonar, marcas profundas na pele (Figura 2C), necrose muscular e hemorragia na cavidade celomática (WORK; BALAZS, 2010; PHILLIPIS et al., 2015). No Havaí, uma das principais causas de morte de *C. mydas* foi associada à captura incidental em redes de pesca, sendo que a análise externa nem sempre revelou sinais de interação com redes ou linhas de pesca, ressaltando a importância do exame interno do animal quanto à presença de outros indícios da interação (WORK et al., 2015a).

Ainda, existem registros de doença descompressiva em tartaruga-cabeçuda (*Caretta caretta*) capturadas incidentalmente, as quais quando retiradas rapidamente vivas de redes a profundidades entre 10 a 75 m, apresentavam sinais neurológicos progressivos, coma ou morte (GARCÍA-PÁRRAGA et al., 2014). Os achados macroscópicos e microscópicos incluíam congestão, hemorragia e edema pulmonares. No entanto, o principal achado foi a presença de bolhas gasosas intravasculares no pulmão, fígado, rim, baço, mesentério, além dos grandes vasos e no coração (Figura 2D) (GARCÍA-PÁRRAGA et al., 2014). Esse tipo de enfermidade ainda é pouco estudado em *C. mydas*.

No Brasil, a captura incidental é considerada a principal ameaça para *C. mydas*, com registros em toda a costa e também nas zonas oceânicas (GALLO et al., 2006; PUPO; SOTO; HANAZAKI, 2006; SALES et al., 2008; SANTOS et al., 2011; FIEDLER et al., 2012; LÓPEZ-BARRERA; LONGO; MONTEIRO-FILHO, 2012; MONTEIRO et al., 2016). Em Santa Catarina, por exemplo, análises etnobiológicas revelaram captura nos sítios de alimentação de *C. mydas*, sendo o local, a profundidade e o tamanho da malha da rede fatores que influenciam quantitativamente a captura (PUPO; SOTO; HANAZAKI, 2006). No Paraná, a maior incidência de captura incidental de *C. mydas* em pescarias artesanais ocorre no período de estação seca (outubro a dezembro) e está associada à localização (áreas de alimentação do animal), espessura do fio, tamanho da malha (entre nós 12-16 cm) e tempo de permanência da rede na água (24h) (LÓPEZ-BARRERA; LONGO; MONTEIRO-FILHO, 2012).

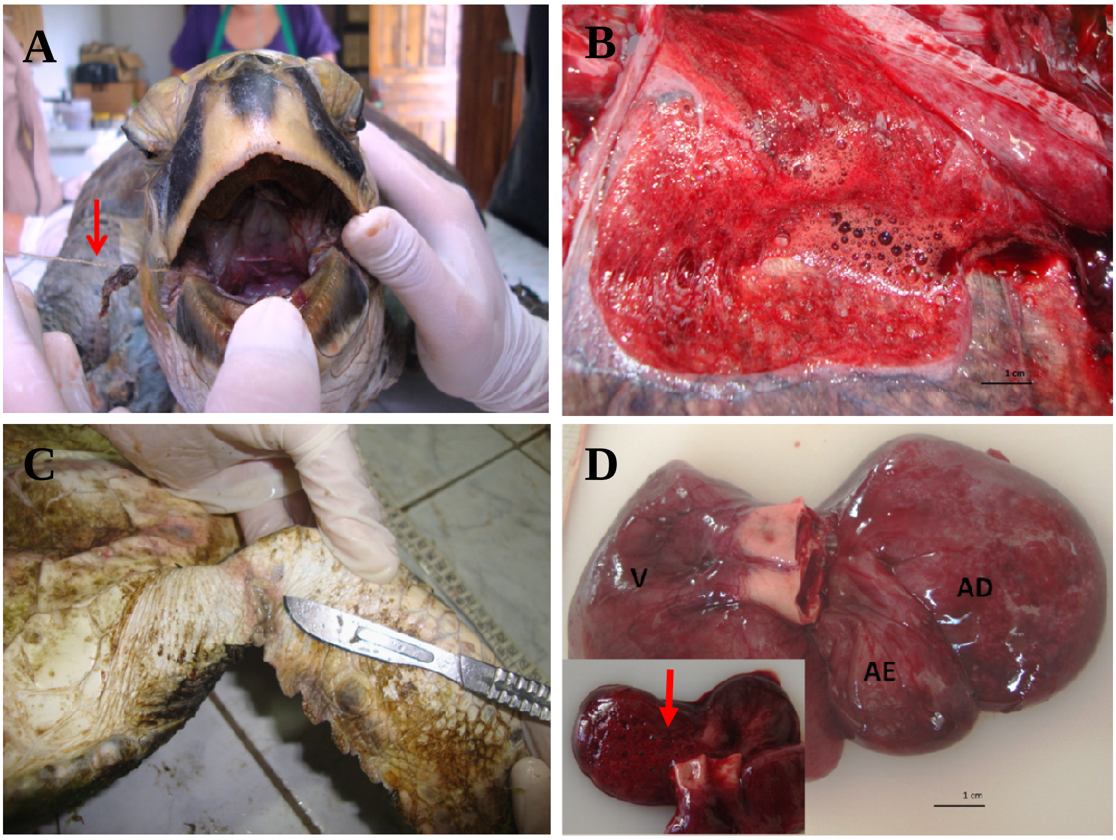

Figura 2. Achados macroscópicos em *Chelonia mydas* indicativos de interação com pesca. A. Ingestão de fio de nylon. B. Edema pulmonar. C. Trauma linear e exsudato caseoso em região axilar esquerda pelo contato com petrecho de pesca. D. Presença de pequenas bolhas em átrio direito (AD), e saindo de grandes vasos (seta, detalhe), indicativas de embolia gasosa. Fonte: Laboratório de Ecologia e Conservação/UFPR.

Na colisão com embarcações ou impactos com hélices, pode-se observar trauma e hemorragia na carapaça, plastrão e cabeça, como também amputação de membros (ORÓS et al., 2005). Quando não matam imediatamente o espécime, colisões com embarcação podem levar à desorientação e debilidade, além de causar lesões que servem como porta de entrada para infecções secundárias, principalmente no pulmão e rim, anatomicamente aderidos à carapaça e à coluna vertebral, locais onde os traumas são frequentes (ORÓS et al., 2005). Lesões semelhantes à colisão com embarcação ocorrem nos casos de impacto de tartarugas marinhas com dragas em áreas de alimentação e reprodução (GOLDBERG et al., 2015). As dragagens ocorrem para retirada de sedimento e aprofundamento dos canais, adequando à passagem do calado de navios que se deslocam em áreas portuárias (TORRES et al., 2009). Nesses casos, as tartarugas colidem ou são sugadas junto ao sedimento e podem ser dilaceradas, como nos casos observados principalmente com *C. mydas* no Rio de Janeiro, sudeste do Brasil, próximas a áreas de dragagens (GOLDBERG et al., 2015).

Outro impacto causado também pelas atividades de dragagem é a disponibilização de contaminantes ambientais, como os inorgânicos arsênio (As), chumbo (Pb), zinco (Zn) e os orgânicos hidrocarbonetos policíclicos aromados (HPAs), que estão presentes no sedimento e são novamente biodisponibilizados para a cadeia alimentar (TORRES et al., 2009). Adicionado à contaminação ambiental por efluentes agrícolas, industriais e domésticos no ambiente costeiro, os contaminantes podem causar efeitos agudos ou crônicos na saúde da fauna marinha (ISLAM; TANAKA, 2004). Os contaminantes podem ser bioacumulados em *C. mydas*, sendo que as concentrações variam de acordo com as características do princípio ativo, a região geográfica do espécime amostrado, dieta, comprimento corpóreo e tecidos analisados (GAUS et al., 2012; KELLER, 2013). Animais juvenis que se alimentam de itens de origem animal, por exemplo, tendem a apresentar maiores níveis de elementos traço comparados aos adultos que se alimentam principalmente de itens de origem vegetal (SILVA et al., 2016), assim como animais residentes em áreas com maior aporte de contaminantes orgânicos apresentam maiores níveis em relação a áreas menos impactadas (LABRADA-MARTAGÓN et al., 2011).

As concentrações de contaminantes nos tecidos de *C. mydas* juvenis amostradas em áreas de alimentação próximas a zonas industriais e urbanas na Bahia, e também em áreas menos impactadas no Ceará, estados no nordeste do Brasil, indicam altos níveis de elementos como o mercúrio total, cádmio, níquel, chumbo e zinco, semelhantes a outras regiões impactadas na China, Austrália e Japão (BEZERRA et al., 2015, MACÊDO et al., 2015).

A ação e as doses associadas a efeitos adversos dos contaminantes químicos ainda são pouco conhecidas em tartarugas marinhas (GAUS et al., 2012). Geralmente, os elementos traço ou contaminantes orgânicos são detectados em pequenas concentrações em *C. mydas* (GAUS et al., 2012), porém admite-se que sejam suficientes para causar alterações nos parâmetros clínicos sanguíneos e induzir estresse oxidativo, contribuindo possivelmente com a formação de tumores nesses animais (KOMOROSKE et al., 2011; LABRADA-MARTAGÓN et al., 2011; SILVA et al., 2016).

A contaminação por ingestão de resíduos sólidos (ex. plásticos rígidos ou moles, linhas de nylon, isopor) ou líquido (ex. petróleo) também afeta as tartarugas marinhas (SCHUYLER et al., 2014a). A ingestão pode ocorrer de forma passiva, quando os itens são consumidos junto à dieta, ou ativamente, devido à semelhança visual com os itens normais da dieta e, possivelmente, devido à percepção olfativa e gustativa, pela formação de biofilmes microbianos nos resíduos (SCHUYLER et al., 2014b; NELMS et al., 2016). Os itens ingeridos podem permanecer desde alguns dias até quatro meses no trato digestório (LUTZ, 1990) e os efeitos podem ser letais ou subletais, incluindo abrasões na mucosa, obstrução e ruptura do trato digestório, absorção de contaminantes como bisfenol A e ftalato presentes nos plásticos, redução da alimentação e da absorção dos nutrientes, inanição, ocasionando menores taxas de crescimento e vulnerabilidade à predação (BJORNDAL, 1997; NELMS et al., 2016)

A frequência de animais que apresentam resíduo no trato gastrointestinal varia de acordo com a região amostrada e o estágio de desenvolvimento do animal (SCHUYLER et al., 2014b), variando em *C. mydas* juvenis entre 73% (Uruguai e no estuário do Rio da Prata) a 90% (Argentina e Uruguai) (GONZÁLEZ-CARMAN et al., 2014; TERYDA, 2015), área considerada junto ao Brasil como corredor migratório dos animais no Atlântico Sul Ocidental. No Brasil, as frequências de resíduos no trato gastrointestinal em *C. mydas* juvenis relatadas foram de ~60% a 100% no Rio Grande do Sul (BUGONI et al., 2001; TOURINHO et al., 2010), 70% no Paraná (GUEBERT-BARTHOLO et al., 2011, GAMA et al., 2016), 45% a 70% em São Paulo (BEZERRA, 2014; MENDES et al., 2015), ~60% entre Rio de Janeiro e Espírito Santo (DiBENEDITTO; AWABDI, 2014; SANTOS et al., 2015b) e 70% no Sergipe (SANTOS et al., 2015b). A frequência de relatos sobre a gravidade e os tipos de lesões observadas como obstrução, ruptura do trato gastrintestinal e consequente morte, entretanto, é baixa. Nos casos em que essas análises foram realizadas, a frequência de morte dos animais variou entre 0 a 29%, nos estados do sul e sudeste do Brasil, sendo que 0,5g de resíduo no trato são considerados suficientes para obstrução e consequente inanição (BUGONI et al., 2001; TOURINHO et al., 2010; SANTOS et al., 2015b).

As interações negativas com resíduos sólidos no ambiente também envolvem dificuldade de desova em praias com alta concentração de lixo, como também no desenvolvimento de filhotes devido à modificação térmica do sedimento e obstáculos durante o deslocamento dos filhotes em direção ao mar, tornando-os mais suscetíveis a predadores e soterramento na areia (NELMS et al., 2016).

2.3 MORBIDADE E MORTALIDADE DE *CHELONIA MYDAS* ASSOCIADA A DOENÇAS INFECCIOSAS E PARASITÁRIAS

Os registros de doenças infecciosas e parasitárias em tartarugas marinhas de vida livre e em cativeiro envolvem ampla variedade de agentes patogênicos, muitas vezes, oportunistas, presentes na dieta e no meio abiótico marinho (LAUCKNER, 1985; GLAZEBROOK; CAMPBELL, 1990a; ORÓS et al., 2005; FLINT et al., 2010, WORK et al., 2015a). A avaliação da gravidade da doença e sua relação com a causa da morte, entretanto, é complexa em animais silvestres, uma vez que a simples presença de agentes infecciosos e principalmente parasitários pode ser considerada um achado compatível com a saúde do animal e não causar alterações detectáveis (WOBESER, 2007). Dessa forma, foram levantados na literatura relatos de agentes e doenças associadas assim como achados secundários em *C. mydas*, de acordo com os sistemas afetados.

A fibropapilomatose, por exemplo, é uma doença caracterizada por tumores cutâneos ou viscerais observada em tartarugas marinhas, principalmente em *C. mydas* juvenis em zonas costeiras tropicais de todo o mundo (AGUIRRE; LUTZ, 2004; GREENBLATT et al., 2005; BAPTISTOTTE, 2007). Esta neoplasia é benigna, entretanto, de acordo com a quantidade e tamanho dos tumores, podem dificultar a visão, natação e alimentação, quando ocorrem nas pálpebras, regiões axilar e inguinal, nadadeiras e aparelho bucal (HERBST, 1994) (Figura 3A).

Existem registros de regressão espontânea dos tumores cutâneos, possivelmente associados com a resposta imunológica dos animais frente à doença (FOLEY et al., 2005; SANTOS et al., 2010; GUIMARÃES et al., 2013) ou, também, com a resposta do agente patogênico a fatores ambientais como temperatura e exposição a raios ultravioleta que podem agir na replicação do vírus (HERBST, 1994). As taxas de regressão, entretanto, ainda são subestimadas uma vez que existem poucos estudos com esse tipo de análise.

Os tumores viscerais são observados em diferentes órgãos como pulmão, rim, coração, músculo esquelético, trato gastrointestinal e baço, medindo entre 0,1 a 20 cm de diâmetro, e são classificados como fibromas, mixofibromas ou fibrosarcomas de baixo grau de malignidade (HERBST, 1994; WORK et al., 2004). Podem prejudicar as funções dos órgãos e predispor os animais a infecções secundárias, deixando-os debilitados ou levar a óbito (WORK et al., 2004; FLINT et al., 2009). No Brasil, os registros de tumores viscerais em *C. mydas* incluem fibromas no rim, pulmão e coração (BAPTISTOTTE, 2007; DUTRA et al., 2012). A frequência desses tumores no Brasil é, possivelmente, menor em relação a outras regiões, como no Havaí em que ocorreu em 39% dos casos (WORK et al., 2004; BAPTISTOTTE, 2007; DUTRA et al., 2012).

O agente etiológico e a forma de transmissão da doença ainda não foram determinados, mas a etiologia viral é a mais aceita, principalmente devido à detecção do herpesvírus Chelonid Herpesvirus 5 (ChHV5) e à presença de lesões histopatológicas sugestivas de infecção viral (DOMICIANO et al., 2013; WORK et al., 2015b, JONES et al., 2016). O desenvolvimento da doença está associado ao pós-recrutamento de *C. mydas* jovens para o ambiente costeiro, uma vez que animais no ambiente oceânico são livres da doença (ENE et al., 2005, JONES et al., 2016). Alguns fatores ambientais estariam associados à patogenia, como a exposição a níveis elevados de contaminação química e da temperatura da água, assim como a diferentes concentrações e estirpes virais (HERBST et al., 1994; SANTOS et al., 2010; RODENBUSCH et al., 2014).

Além da fibropapilomatose, relatos de doenças cutâneas em *C. mydas* incluem lesões claras e pequenas, nas quais foram observadas hiperplasia de epiderme e inclusões virais no exame microscópico, associadas ao *Chelonia mydas* papilomavirus (CmPV) (MANIRE et al., 2008). Dermatites lacerativas e papulares causadas por herpesvirus seguidas por infecção bacteriana secundária foram relatadas em animais de cativeiro (GLAZEBROOK; CAMPBELL, 1990a). Além de lesões associadas a vírus foram descritas em animais de vida livre e de cativeiro dermatites ulcerativas associadas à fibrose em locais parasitados por sanguessugas ou cracas (SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007; FLINT et al., 2009) e dermatites ulcerativas decorrentes de trauma (LAUCKNER, 1985).

As infecções no sistema respiratório incluem relatos nos quais os animais apresentaram sinais clínicos como secreção nasal, inapetência, torpor, crepitação pulmonar e dificuldade de manter o controle da flutuabilidade (LAUCKNER, 1985; ORÓS et al., 2005). Em *C. mydas* de cativeiro foram observadas no trato superior rinite obstrutiva e traqueíte e, no trato inferior, broncopneumonia, frequentemente associada a casos de estomatite ulcerativa, de onde foram isoladas bactérias dos gêneros *Vibrio, Aeromonas*, *Pseudomonas* e *Cytophaga-Flavobacterium*. Pneumonia granulomatosa associada à infecção por *Mycobacterium* spp. e fúngica (Figura 3B), como o *Paecilomyces* spp. também foi relatada em animais de cativeiro (BROCK et al., 1976; GLAZEBROOK e CAMPBELL, 1990a). Outros relatos em cativeiro incluem detecção de herpesvirus também relacionados à traqueíte, pneumonia acentuada e conjuntivite (JACOBSON et al., 1986). Em *C. mydas* de vida livre foram observados casos de traqueíte causada pelos fungos oportunistas *Veronaea botryosa*, *Cladosporium* spp., *Ochroconis* spp. e *Cochliobolus* spp. (DONNELLY et al., 2015) e pneumonia causada por *Mycobacterium* spp. (FLINT et al., 2010).

Os relatos no Brasil incluem um espécime de *C. mydas* oriundo de Santa Catarina que apresentava pneumonia granulomatosa crônica acentuada associada à infestação por ovos de Spirochiidae induzindo à debilidade e óbito (GOLDBERG et al., 2013). O pulmão e o baço foram os órgãos mais afetados por ovos desses parasitas em *C. mydas* encalhadas no litoral do Espírito Santo. Nesse caso as lesões não foram associadas à morte dos animais (BINOTI et al., 2016), sugerindo que há diferentes níveis de suscetibilidade e cargas parasitárias nessas infecções.

No sistema cardiovascular as lesões são associadas principalmente à presença de trematódeos adultos da família Spirorchiidae no coração e grandes vasos (STACY et al., 2010). Na macroscopia apresentam-se aderidos à superfície ou livres nas câmaras cardíacas e nos grandes vasos sanguíneos causando endocardite necrotizante, infarto, aneurisma, arterite, trombose e hemorragia (GORDON et al., 1998; SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007; FLINT et al., 2009). Esses trematódeos se reproduzem e disseminam os ovos pela corrente sanguínea para diferentes órgãos, como baço, tireoide, trato gastrointestinal, pulmão, rim e cérebro, onde também causam lesões de diferentes intensidades, principalmente formação de êmbolos parasitários, reações granulomatosas e proliferativas, envolvendo espessamento de tecido conjuntivo e de vasos (GORDON et al., 1998; WORK et al., 2005; SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007; STACY et al., 2010). Os ovos são identificados como tipo 1 – fusiformes, com processos afilados nas bordas – característicos, por exemplo, dos gêneros *Learedius*, *Monticellius* e *Hapalotrema*, tipo 2, ovais com uma das bordas afiladas – *Carettacola* e *Haemoxenicon*, e do tipo 3 – redondos a ovais – de espécies do gênero *Neospirorchis* (WOLKE et al., 1982).

A condição corpórea boa ou ótima bem como os achados de necropsia indicaram que *C. mydas* parasitadas e resgatadas na Flórida, Costa Rica e Taiwan tiveram a morte associada a outras causas, sendo as lesões associadas aos trematódeos da família Spirorchiidae consideradas incidentais (SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007; STACY et al., 2010; CHEN et al., 2012). O mesmo parece ocorrer na maioria dos casos observados no Brasil (BINOTI et al., 2016), porém, casos descritos no Havaí e Austrália, indicaram que os parasitas causam lesões acentuadas que levaram animais à morte e que a carga parasitária pode ser maior quando os animais apresentam piores condições corpóreas (GORDON et al., 1998; WORK et al., 2005). Dessa forma, um maior número amostral abrangendo diferentes regiões geográficas e identificação das espécies de parasitas pode melhorar a análise de patologias associadas em *C. mydas*, de acordo com diferentes classes etárias e estados nutricionais (GORDON et al., 1998; STACY et al., 2010). Além de lesões associadas a trematódeos, fibromas cardíacos também foram reportados em *C. mydas* no sudeste do Brasil (DUTRA et al., 2012).

Os relatos de alterações no trato gastrintestinal incluem esofagite causada por *Rameshwarotrema uterocrescens* (Digenea: Pronocephalidae) em *C. mydas* (SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007), e casos de ingestão de anzóis com consequente estomatite caseosa e esofagite fibrinonecrótica devido à perfuração esofágica, associadas à presença de bactérias como *Vibrio alginolyticus*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Escherichia coli*, *Staphylococcus* spp. (ORÓS et al., 2004). Lesões caseosas associadas a bactérias gram positivas e trematódeos (família Pronocephalidae, Microscaphididae, Cladorchiidae e Rhytidodidae) também foram observadas no esôfago de *C. mydas* no litoral do Espírito Santo, levando à obstrução total do lúmen esofágico em 24,3% (9/37) dos casos (CALAIS JR, 2015).

No estômago, trematódeos *Charaxicephaloides* spp. e *Charaxicephalus robustus* (Digenea: Pronocephalidae) foram relacionados a hiperplasia da mucosa, petéquias e necrose da mucosa gástrica em *C. mydas* (SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007). Nematódeos *Sulcascaris sulcata* foram relatados ocasionalmente em *C. mydas*, inclusive em espécime no Brasil (FREITAS; LENT, 1946), ocorrendo em áreas em que moluscos apresentavam-se parasitados (LICHTENFELS; BIER; MADDEN, 1978). Podem provocar úlceras gástricas, gastrite fibrino-purulenta ou crônica (LAUCKNER, 1985). Úlceras hemorrágicas, gastrite e cistos em serosa também foram relacionados a nematódeos da família Anisakidae, *Anisakis simplex* ou *A. typica*, em animais de cativeiro (BURKE; RODGERS, 1982; GLAZEBROOK; CAMPBELL, 1990a) e um espécime de vida livre no Brasil (XAVIER, 2011). A transmissão nesses casos ocorreu, possivelmente, por dieta de peixe contaminado e como as tartarugas marinhas são hospedeiros paratênicos, as larvas não completam o ciclo reprodutivo e permanecem nos animais, podendo migrar para a cavidade celomática provocando granulomas e fibrose na lâmina própria e submucosa (GLAZEBROOK; CAMPBELL, 1990a).

No intestino são descritas intussuscepção, ruptura e obstrução pela presença de corpo estranho, enterite catarral, fibrinosa e necrotizante, abscessos e granulomas, assim como serosite fibrinosa associada à presença de bactérias como *Bacillus* spp., *Escherichia coli*, *Pasteurella* spp., *Proteus* spp., *Serratia marcescens*, *Staphylococcus* spp., *Streptococcus* spp. e *Vibrio alginolyticus* (ORÓS et al., 2005; FLINT et al., 2009, WORK et al., 2015a). Diversos parasitas foram descritos no intestino, porém, os poucos relatos de lesões descrevem enterite exsudativa difusa acentuada associada ao protozoário *Charyospora cheloniae* em surtos envolvendo *C. mydas* de cativeiro e vida livre (GORDON; KELLY; LESTER, 1993; FLINT et al., 2009; CHAPMAN et al., 2016). Ainda, há relatos de espessamento da parede intestinal com áreas enegrecidas ou esbranquiçadas multifocais ao exame macroscópico (Figura 3C) acompanhado de edema, enterite e vasculite granulomatosa associados a ovos de trematódeos da família Spirorchiidae e bactérias, assim como serosite fibrino-hemorrágica associada a bacilos gram negativos (RAIDAL et al., 1998; SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007; FLINT et al., 2010; WORK et al., 2015a).

Em estudos realizados no estado do Rio de Janeiro, sudeste do Brasil, *swabs* colhidos das regiões oral, cloacal e de ferimentos de *C. mydas* indicaram a presença de *Vibrio* spp. em aproximadamente 88,2% dos casos e *Aeromonas* spp. em 52,9%, demonstrando elevada incidência nesses espécimes e possível ameaça à saúde das tartarugas e do ecossistema marinho, como também para o consumo humano desses animais (REIS et al., 2010; ZAVALA-NORZAGARAY et al., 2015).

As lesões hepáticas podem ser de origem nutricional, como a lipidose, observada em animais caquéticos ou por disfunção metabólica e endócrina (FLINT et al., 2009). Lesões de origem infecciosa englobam processos granulomatosos e necrotizantes, como os causados por ovos de trematódeos da família Spirorchiidae, fungos ou bactérias (RAIDAL et al., 1998; FLINT et al., 2009, STACY et al., 2010, WERNECK et al., 2015, WORK et al., 2015a). Os melanomacrófagos são encontrados normalmente no fígado de tartarugas marinhas, assim como de outros répteis, anfíbios e peixes e possuem a função de fagocitar hemácias velhas e agentes infecciosos e parasitários (JOHNSON et al., 1999). Em estudo *in vitro*, melanomacrófagos de tartarugas apresentaram maior atividade fagocitária frente ao alvo *E. coli* em baixas temperaturas (2-7ºC) quando comparados a macrófagos de mamíferos, resultado esperado para animais pecilotérmicos (JOHNSON et al., 1999). A avaliação da hipertrofia e hiperplasia de melanomacrófagos pode ser associada à gravidade de lesões inespecíficas, por exemplo, em resposta a estresse e inflamações crônicas (FLINT et al., 2009).

Na vesícula biliar observou-se hiperplasia e espessamento da mucosa, infiltrado linfocitário, dilatação de vasos capilares e linfáticos em *C. mydas*, associados à presença do trematódeo *Rhytidodoides similis* (SMITH et al., 1941; SANTORO; MORALES; RODRÍGUEZ-ORTÍZ, 2007). A bile apresenta normalmente coloração verde escura, no entanto pode estar alterada com aspecto cristalino ou espesso na presença de parasitas (FLINT et al., 2009). Hepatite granulomatosa discreta e presença do trematódeo *R. similis* na vesícula biliar foram relatadas em *C. mydas* encontrada no estado do Espírito Santo, Brasil (WERNECK et al., 2015).

As alterações de origem infecciosa no sistema genitourinário envolvem degeneração e necrose tubular, nefrite granulomatosa (Figura 3D) e nefrite intersticial crônica associadas a protozoários *C. cheloniae* e ovos de trematódeos (LAUCKNER, 1985; STACY et al., 2008; FLINT et al., 2010). Fibromas renais em animais afetados pela fibropapilomatose e alterações como degeneração e necrose tubular, granulomas e fibrose renal decorrentes de deposição discreta, multifocal, de cristais de oxalato de cálcio também foram observados em *C. mydas*, porém, no último caso, a origem e metabolismo dessa substância são desconhecidos (WORK et al., 2004; STACY et al., 2008). Outros achados no sistema genitourinário incluem edema e hiperplasia da mucosa, serosite e granulomas na bexiga e gônada causados por trematódeos (LAUCKNER, 1985; FLINT et al., 2009 e 2010).

No sistema nervoso central de *C. mydas* foram observados meningoencefalites granulomatosas e abscessos associadas a bactérias gram-negativas, *Corynebacterium* spp., formas adultas e ovos de trematódeos e ao protozoário *C. cheloniae* (GEORGE, 1997; RAIDAL et al., 1998; FLINT et al., 2009). No globo ocular foram observados enoftalmia (associada à desidratação), ceratoconjuntivite e blefarite associadas à infecção bacteriana pós-trauma em animais de cativeiro. Outras alterações no globo ocular incluem ceratite fibrinosa, fibropapilomas e infestação de sanguessugas na pálpebra (FLINT et al., 2009).

Traumas na pele e mucosas, assim como as vias aéreas (aspiração de água do mar) são as portas de entrada para bactérias e fungos patogênicos e oportunistas que infectam as tartarugas marinhas, podendo levar à septicemia (GEORGE, 1997; WORK et al., 2003). Casos de septicemia foram observados em *C. mydas* com múltiplos abscessos em vísceras e presença de bactérias gram negativas em centros necróticos (WORK et al., 2015a). Em animais com bacteremia foram identificadas *Citrobacter freundii, Burkholderia capacia, Shewanella putrefasciens, Staphylococcus sciuri, Salmonella* spp., *Moraxella* spp., *Bacillus* spp., *Escherichia coli*, *Aeromonas* spp., *Pseudomonas* spp. e *Vibrio* spp. Essas espécies de bactérias foram identificadas tanto em animais vivos como mortos de vida livre e de cativeiro (GLAZEBROOK e CAMPBELL, 1990a,b; RAIDAL et al., 1998; WORK et al., 2003).

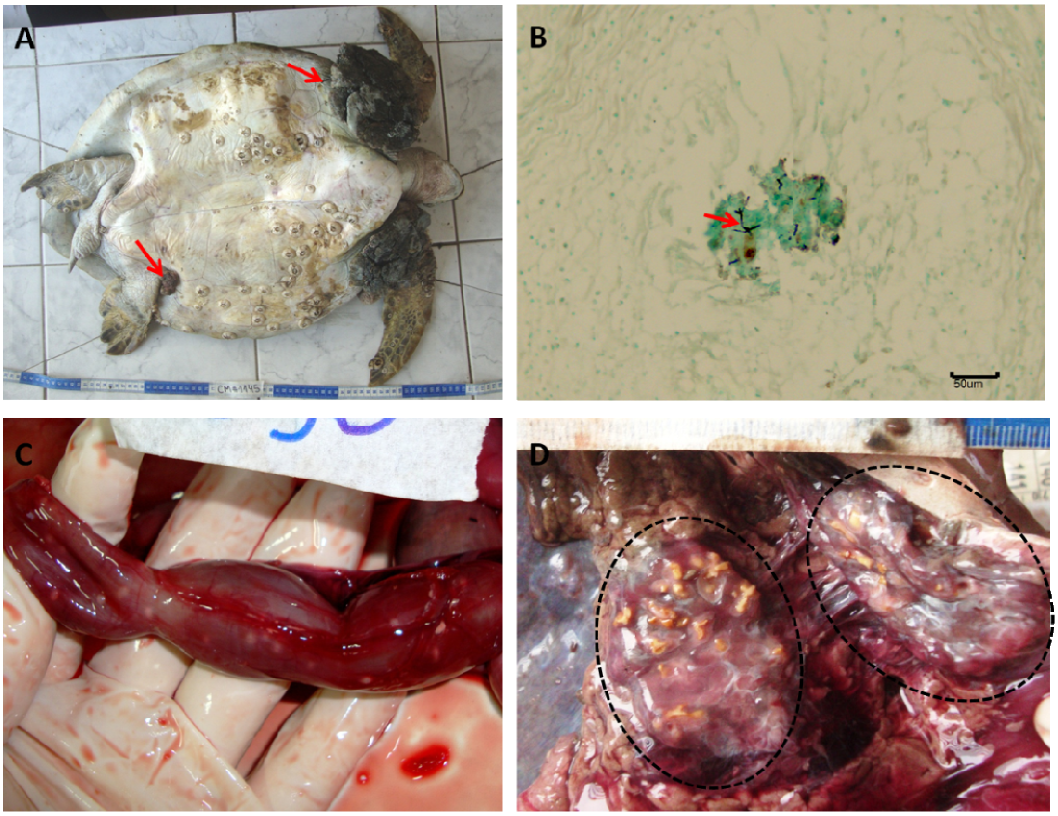

Figura 3. Doenças de origem infecciosa e parasitária em *Chelonia mydas*. A. Fibropapilomatose, caracterizada por tumores cutâneos em região de axilar e inguinal (setas). B. Pneumonia granulomatosa com hifas intralesionais (seta; Grocott) C. Granulomas parasitários, arredondados e esbranquiçados no intestino delgado. D. Nefrite granulomatosa parasitária, com áreas de necrose amareladas (rins delimitados por linha tracejada). Fonte: Laboratório de Ecologia e Conservação/UFPR, Laboratório de Patologia Animal/UEL.

A septicemia também pode ocorrer secundariamente a outras doenças em *C. mydas*, como a fibropapilomatose moderada e acentuada e parasitose (RAIDAL et al., 1998; WORK et al., 2003). A presença de úlceras nos fibropapilomas e as lesões vasculares causadas durante o deslocamento de ovos de parasitas nos órgãos seriam possíveis vias para disseminação de bactérias na circulação sanguínea, que encontrariam um ambiente favorável devido à doença primária, com possível imunossupressão ou colapso do sistema imune em estabelecer resposta a agentes infecciosos (RAIDAL et al., 1998; WORK et al., 2003).

2.4 CONCLUSÃO

A suscetibilidade a diferentes impactos antrópicos e doenças e as consequências para as populações de *C. mydas* resultaram na classificação de espécie ameaçada de extinção pela

"International Union for Conservation of Nature" (IUCN). Essas ameaças são sinérgicas, portanto, podem atuar simultaneamente no mesmo espécime e população, sendo necessárias ações em diferentes frentes para reverter o quadro atual, como melhoria no controle e fiscalização dessas atividades antrópicas e na qualidade sanitária do ecossistema marinho.

Nas últimas décadas tem crescido o número de registros de interação negativa com atividades humanas e doenças em animais marinhos, inclusive o surgimento de enfermidades em tartarugas marinhas, ainda sem identificação dos agentes patogênicos. No Brasil, pouco se sabe sobre as doenças que acometem as *C. mydas*, mesmo as mais relatadas como a fibropapilomatose e as parasitoses, e os impactos gerados nas populações desses animais. Portanto, a avaliação e monitoramento do estado de saúde, doenças e causas de morte devem ser realizados continuamente, de forma sistematizada, com exames macroscópicos, histopatológicos e auxílio de análises moleculares para identificação de agentes patogênicos. Essas informações podem subsidiar decisões de gestão costeira, de pesquisa e para desenvolver medidas de mitigação apropriadas para a conservação de *C. mydas*.

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3 OBJETIVOS

3.1 OBJETIVO GERAL

- Avaliar a epidemiologia da fibropapilomatose na costa brasileira e parâmetros clínicos laboratoriais associados à doença em tartarugas-verde registradas no litoral Paraná, sul do Brasil.

3.2 OBJETIVOS ESPECÍFICOS

- Comparar as prevalências da fibropapilomatose em diferentes períodos e estados (latitude) na costa do Brasil;
- Avaliar dados inéditos de prevalência da fibropapilomatose em tartarugas-verde nos estados do Paraná (PR), Santa Catarina (SC) e Rio de Janeiro (RJ);
- Identificar possíveis co-fatores associados a diferenças de prevalências nos estados (latitude) e períodos avaliados;
- Avaliar alterações histológicas dos tumores de tartarugas-verde resgatadas no PR, SC e RJ;
- Investigar a ocorrência de herpesvírus presentes em lesões de fibropapilomatose de tartarugas-verde resgatadas no PR, SC e RJ;
- Avaliar parâmetros bioquímicos e hematológicos de tartarugas-verde capturadas intencionalmente no litoral do Paraná.

4 ARTIGO A

Fibropapillomatosis in green turtles (*Chelonia mydas*) off Brazilian coast

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Abstract

The green turtle (*Chelonia mydas*) is an endangered migratory species and different impacts during all life stages lead to species vulnerability. Fibropapillomatosis (FP) is a pandemic chronic disease that afflicts mainly juvenile green turtles and a marked difference in prevalence has been noticed in small to large spatio-temporal scale. The aim of this study was to review the epidemiology of fibropapillomatosis in Brazil and also evaluate new data from three states (Santa Catarina-SC, Paraná-PR and Rio de Janeiro-RJ). Local and international peer-reviewed studies summarizing prevalence, curved carapace length (CCL), sampling method (stranded, bycaught, intentionally captured or nesting), and severity of disease were searched for green turtles throughout the Brazilian coast (Latitudes 02° 52`S to 26° 02`S) from 2000 to 2014. The body condition, histology and detection of Chelonid Herpesvirus-5 (ChHV5) were also evaluated of afflicted animals sampled from 2007/2009 to 2014 in SC, PR and RJ. Data from 12 of 17 (70.6%) states of Brazilian coast were compared between two periods (from 2000 to 2006 and from 2007 to 2014). Prevalences varied according to (i) sampling methods, (ii) latitude degrees, and (iii) chronologically. Prevalence decrease between periods in Ceará, Rio Grande do Norte, Espírito Santo and São Paulo, maintenaice in RJ and Paraíba, increase in SC and Bahia and were absent in oceanic islands. Prevalences were 14.5% (17/117), 9.6% (38/395) and 13.6% (12/88) off SC, PR and RJ; and 23% (10/43) in animals intentionally captured off PR. Considering all data, fibropapillomatosis mainteined similar from 2000 to 2014 [14% (1090/7804) from 2000 to 2006 and 13.8% (1106/8035) from 2007 to 2014]. Afficted animals were larger (>CCL) than turtles without FP, and similar findings were observed in different areas worldwide. The results suggest that green turtles get infected after recruitment, when they shift slowly from omnivorous into an herbivorous diet and are exposed to different environmental factors, including virus infection that culminate in severity/death or regression of disease. The severity of disease was mainly mild in SC and PR, and moderate and severe in RJ. The severe cases often lead to difficult in feeding, diving and affect vision. Microscopical findings suggested viral infection, but eosinophilic inclusion bodies were observed in only one tumor (1/121). Five variants were detected and clustered with Chelonid Herpesvirus 5 and no papillomavirus DNA was amplified. Therefore, continuous monitoring of disease and other features that affect host and infectious agent in global scale are also necessary for a better understanding of FP and green turtle conservation.

Keywords: Sea turtles, Tumorous disease, Epidemiology, Herpesvirus, South Atlantic

4.1 INTRODUCTION

The green turtles (*Chelonia mydas*) are highly migratory species and use a variety of habitats during their life cycle, encompassing nest beaches where eggs are incubated, ocean areas where hatchlings grow for few years, and neritic (nearshore) developmental areas where early juveniles recruit and continue maturing at different foraging grounds (Bolten, 2003). Despite their wide distribution, the vulnerability to threats during all life stages, including egg and nesting females overexploitation, fisheries bycatch, coastal development, pollution and pathogens concentration, associated with their slow maturing and population recovery, qualify green turtles for endangered status (IUCN, 2016).

In an effort to guide future researches and effective sea turtle conservation in the 21st century, Hamann *et al.* (2010), including 13 nations working with biology and conservation of sea turtles, assembled a list of priority of global research questions about reproductive biology, biogeography, population ecology, threats and conservation strategies. One of these important questions request information of the etiology and epidemiology of fibropapillomatosis (FP) and how this disease can be managed (Hamann *et al.*, 2010).

Fibropapillomatosis is a pandemic chronic disease characterized by single to multiple tumours ranging from 0.1 cm to greater than 30 cm in skin, plastron and carapace, although visceral tumours diagnosed as fibromas, myxofibromas and fibrosarcomas occur frequently in some populations (Work *et al.*, 2004). According to size, location and number of tumours, the disease contributes to progressive debilitation; impair swimming, feeding and breathing and can correlate to physiological condition and death (Ene *et al.*, 2005). This disease was reported in loggerhead turtle (*Caretta caretta*), olive ridley (*Lepidochelys olivacea*), hawksbill turtle (*Lepidochelys imbricata*), leatherback turtle (*Dermochelys coriacea*) and flatback turtle (*Natator depressus*) (Herbst, 1994, Huerta *et al.*, 2000), but occurs primarily in juveniles and, to a lesser extent, in adult green turtles (Herbst, 1994).

Investigations relative to the etiological agent of FP have demonstrated that the disease is transmissible experimentally, because tumours are induced by inoculation of cell-free tumour extracts; and strong evidence exists of its association with a Chelonid herpesvirus 5 (ChHV5) (Herbst *et al.*, 1995; Quackenbush *et al.*, 2001, Patrício *et al.*, 2012, Rodenbush *et al.*, 2014). Although the efforts to cultivate the virus using *in vitro* methods remained ineffective until now, ChHV5 was detected in fibropapillomas by Polymerase Chain

Reaction (PCR), histological and immunohistochemical assays (Herbst *et al*., 1999, Work *et al*., 2009, Work *et al*., 2015).

Furthermore, environmental factors must play a role in disease expression because posthatchling green turtles that live in oceanic habitat are free of FP and juveniles express the disease only after recruiting to neritic habitats (Ene *et al*., 2005). Cofactors such as seasonal elevation on temperature, presence of possible mechanical vectors (e.g. marine leeches) and geographic localization are suspected to affect host susceptibility, transmission and the relative frequency of the virus that may vary considerably across locations (Haines and Kleese, 1977, Greenblatt *et al*., 2004, Foley *et al*., 2005, Rodenbusch *et al*., 2014).

In Brazil, FP is considered one of the most important diseases threatening sea turtles (Baptistotte, 2007, Santos *et al*., 2015) but information are sparse. Therefore, the aim of the present study was to highlight the epidemiological data of FP in green turtles throughout the Brazilian coast, including reviewed and new data, comparisons to other worldwide regions and analysis of factors involving green turtle biology, ecology and ChHV5 detection.

4.2 MATERIAL AND METHODS

The study was separated into two components. The first involved a review of the available literature from 2000 to 2014, detailing the percentage occurrence (prevalence) of FP among green turtles along the Brazilian coast (Latitudes 02° 52’S to 26° 38’S). The second component comprised more detailed analyses of the disease among individual specimens sampled from 2007/2009 to 2014 in three states (encompassing 22° 24' to 26° 02’S).

4.2.1. Fibropapillomatosis occurance in green turtles off Brazil

Local and international peer-reviewed studies summarizing epidemiological data describing FP in green turtles off Brazil were searched using major online databases. Attention was also directed towards the historical proceedings of the International Symposium of Sea Turtles (http://www.seaturtlesociety.com/proceedings.html) and the Symposium of Research and Conservation of Sea Turtles in the South West Atlantic (http://cicmar.org/archives/2532). From these papers, general information was collected describing when, where and how (e.g. nesting, stranded on beaches, or incidentally or intentionally caught for research) the specimens were obtained, the number of animals sampled, their morphological data (minimum and maximum or mean ± SD curved carapace length–CCL), the prevalence and severity of FP. It is important to note that ‘stranded’ green turtles can actually have died as an ‘incidentally caught’, disentangled and washed ashore.

The prevalence of FP was compared chronologically between two periods, according to effort of disease monitoring off Brazilian coast: from 2000 to 2006 and from 2007 to 2014. The same areas were considered to periods evaluation, except Santa Catarina (192 km of distance between areas). Spatially evaluation – comparisons between states – was conducted using only data from 2007 to 2014. FP information were available in multiple studies in some regions (e.g. Serra in Espírito Santo, Itaipu in Rio de Janeiro, Cananéia in São Paulo and Arvoredo in Santa Catarina), then studies including systematic monitoring, longer effort and most complete data were preferred. Controversially, prevalence analyzes of afflicted green turtles from regions in Rio Grande do Sul (RS) were precluded owing to small sample size (e.x. 4/19) (Rodrigues *et al.*, 2012). All information is available in Figure 1 and detailed in Supplementary Material (Table S1).

4.2.2 Fibropapillomatosis occurrence and epidemiology among green turtles off southeastern and southern Brazil (new data)

Between 2007/2009 and 2014, stranded or bycaught green turtles were collected during beach monitoring, or provided by fishers from three states: Santa Catarina (26° 02' S and 48° 50' W; n=117 animals), Paraná (25° 29' S and 48° 20' W; n=395 animals) and Rio de Janeiro (22° 54' S and 43°10' W; n=88 animals). An additional 43 green turtles were intentionally caught in Cobras (25°28' S and 48° 25' W) and Mel (25° 29' S and 48° 20' W) islands off Paraná during seven consecutive days in April/May 2014 as part of an ancillary study.

All specimens were measured for curved carapace length (CCL to the nearest 0.1 cm), with those ≥ 96 cm CCL classified as adults (following Bellini *et al.*, 2013). General body condition was estimated by visual inspection and classified based on Torezani *et al.*, (2010) adapted from Walsh (1999). Briefly, a turtle with FP was classified as ‘good’ if the plastron was convex, eyes were normal, the neck areas had fat tissues and axillary and inguinal areas were protuberant; ‘fair’ if the plastron was less convex and fat was not protuberant as in ‘good’ condition; and ‘emaciated’ if the plastron was concave, eyes were sunken, muscles of the neck area were obvious with little or no surrounding fatty tissues, and inguinal and axillary areas were very thin.

Skin tumours were evaluated for size (± 0.1 cm) and grouped into four categories (1: <1.0 cm; 2: 1.0–4.0 cm; 3: 4.0–10 cm; and 4: >10 cm). Where present, the number of FP was counted to establish a severity score, ranging from non-afflicted (FPS-0), mild (FPS-1), moderate (FPS-2) to heavily tumoured (FPS-3), following Work and Balazs (1999).

4.2.3 Microscopic and molecular evalution of green turtle tumours off southeastern and southern Brazil

Tumour samples were excised from afflicted turtles, fixed in 10% buffered formalin solution, routinely processed, embedded in paraffin, and stained with Hematoxylin and Eosin for histopathological analysis. Histological criteria were evaluated according to Herbst *et al*., (1999) and included: epithelial hyperplasia (acanthosis and ortokerathosis), epithelial integrity (ulceration, spindle and basal cell degeneration and dermal-epidermal cleft), inflammatory infiltrate (perivascular inflammatory cell infiltrate and foreign body granulomas), conjunctive tissue proliferation and pathogens (intranuclear inclusion bodies, bacteria in superficial skin and Spirorchiid ova). Tissue-egg burdens were microscopically examined and the average of eggs was obtained by counting three fields at 200× magnification.

Tumour were also sampled and stored at -80°C for subsequent molecular analyses. DNA was extracted using the commercially available ‘Qiagen DNeasy blood and tissue kit’ (Qiagen Sample and Assay Technologies, Hilden, Germany) in accordance with the manufacturer’s instructions. A set of primers were designed to amplify ~450 bp of polymerase gene of herpesvirus, ChMyHV1 (FW: 5’- GGACAAGGGTTACCAAGGG-3’) and ChMyHV2 (RV: 5’- CGGTGTCCCCGTAAATGACA-3’) and another two set of primers FAP59 (5’– TAACWGTIGGICAYCCWTATT-3’) and FAP64 (5’-CCWATATCWVHCATITCICCATC-3’) to amplify ~480 bp L1 ORF and AR-E1F2 (5′-ATGGTNCAGTGGGCNTATGA-3′) and AR-E1R9 (5′-CATTWGTDGTDAYMAGSAKRGGVGGGCA-3′) that target a fragment of E1 ORF of papillomavirus (Forslund *et al*., 1999; Robles-Sikisaka *et al*., 2012). Polymerase Chain Reaction assay was performed using 5 µl of the extracted DNA and 45 µl of PCR-mix consisting of 1 µl (20 pmol) from each primer; 200 mM of each dNTP (Invitrogen, Life

Technologies, Carlsbad, USA); 2.5 units of Platinum Taq DNA polymerase (Invitrogen, Life Technologies, BR); 1 × PCR buffer (20 mMTris–HCl pH 8.4 and 50 mM KCl); 1.5 mM of $MgCl_2$; 2.5% DMSO and ultrapure sterile water to a final volume of 50 µl. Amplification was performed in a thermal cycler (Swift MaxPro, Esco Healthcare, USA) with the following cycling profile: an initial step of 5 min at 94^{o}C, followed by 35 cycles of 1 min/94^{o}C, 1 min/52^{o}C, 1 min/72^{o}C, and a final extension step of 7 min/72^{o}C for herpesvirus amplification. The cycling profile was similar to papillomavirus, except fot the annealing temperature at 50°C (FAP59/FAP64) and 47°C (AR-E1F2/E1R9). Aliquots of 5 µl from the PCR products were analyzed by electrophoresis in 2% agarose gel in TBE buffer pH 8.4 (89 mM Tris; 89 mM boric acid; 2 mM EDTA) at constant voltage (90 V) for approximately 45 min, stained with ethidium bromide (0.5 mg/ml), and visualized under UV light.

In order to ensure the primers specificity, the products of PCR were submitted to sequence analyses. The PCR products were purified using a PCR DNA and Gel Band Purification kit (Invitrogen, Life Technologies, BR), and quantified using Quant-iT ^{TM} dsDNA BR Assay kit (Invitrogen, Molecular Probes, Eugene, OR, USA) in the Qubit ^{TM} Fluorometer (Invitrogen, Molecular Probes, Eugene, OR, USA). The direct sequencing was performed by using the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, USA) with the forward and reverse corresponding primers, in the 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, USA), according to the manufacturer's instructions. The obtained sequences were examined with the PHRED software (http://asparagin.cenargen.embrapa.br/phph) for quality analysis of chromatogram readings. The sequences were accepted if base quality was equal to or higher than 20. Consensus sequences were determined by CAP3 software (http://asparagin.cenargen.embrapa.br/cgi-bin/phph/cap3.pl) and the sequence identity was verified with all sequences deposited in the GenBank using the BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

A multiple alignment was performed in CLUSTAL W (version 1.4) using MEGA package version 5.1 software and sequence identity matrix using BioEdit software version 7.0.8.0. Phylogenetic trees were obtained by the Maximum-likelihood method with two parameter distance estimate (Takamura *et al*., 2011), using MEGA package version 5.1 software. Statistical analyses of phylogenetic trees were determined by bootstrap method on 1000 replicates. The similarity among sequences, were obtained using BIOEDIT version 7.0.9 software (Hall, 1999).

4.2.4 Statistical analyses

Statistical analyses included reviewed and new data of Paraná, Santa Catarina and Rio de Janeiro states for spatio-temporal comparisons of FP prevalence. Other parameters (e.g. CCL, tumour score, histological features) were evaluated using new data only.

Parametric and non-parametric statistical tests were used depending on whether or not the data adhered to assumptions of normality and equal variance. Fisher's exact test and Chi-Square test were used to compare FP prevalence according to sampling method (stranded/bycatch *versus* intentionally captured), between periods (from 2000 to 2006 and from 2007 to 2014) and also used to compare FP prevalence between states along Brazilian coast in the second period (from 2007 to 2014). The Mann-Whitney test was used to compare CCL of turtles with and without FP. The ANOVA and Tukey´s test were used to compare CCL of turtles and tumour-score categories (FPS-1 to FPS-3). The Kruskal-Wallis test was used to compare CCL and trematode egg burdens observed microscopically according to states, followed by parwise *post-hoc* multiple comparisons. Fisher's exact and Chi-Square tests were also used to compare histological features according to states and association within degenerative lesions (spindle cell degeneration, basal degeneration and cleft). A standard alpha level of $p<0.05$ was used for all statistical tests using Assistat 7.7 beta (pt).

All sampling procedures were in accordance with protocols for wildlife conservation and authorized by the Brazilian Institute for Biodiversity and Conservation – Instituto Chico Mendes (ICMBio) through SISBIO permits (SISBIO/ICMBIO n° 43.4431).

4.3 RESULTS AND DISCUSSION

4.3.1 Fibropapillomatosis occurance in green turtles off Brazil

Green turtles inhabit foraging grounds along Brazilian coast and islands, from Amapá (AP) to Rio Grande do Sul (RS), while nesting areas are concentrated mainly offshore, in Rocas Athol (RA/RN), Fernando de Noronha Archipelago (FN/PE) and Trindade Island (TI/ES) (Santos *et al.*, 2011). Data of FP prevalence were available in most of the green turtle distribution areas including 12 of 17 (70.6%) states of Brazilian coast, and monitoring effort of stranding and bycatch or intentional captures varied from one week to eight years in these studies (see Supplementary material for more details).

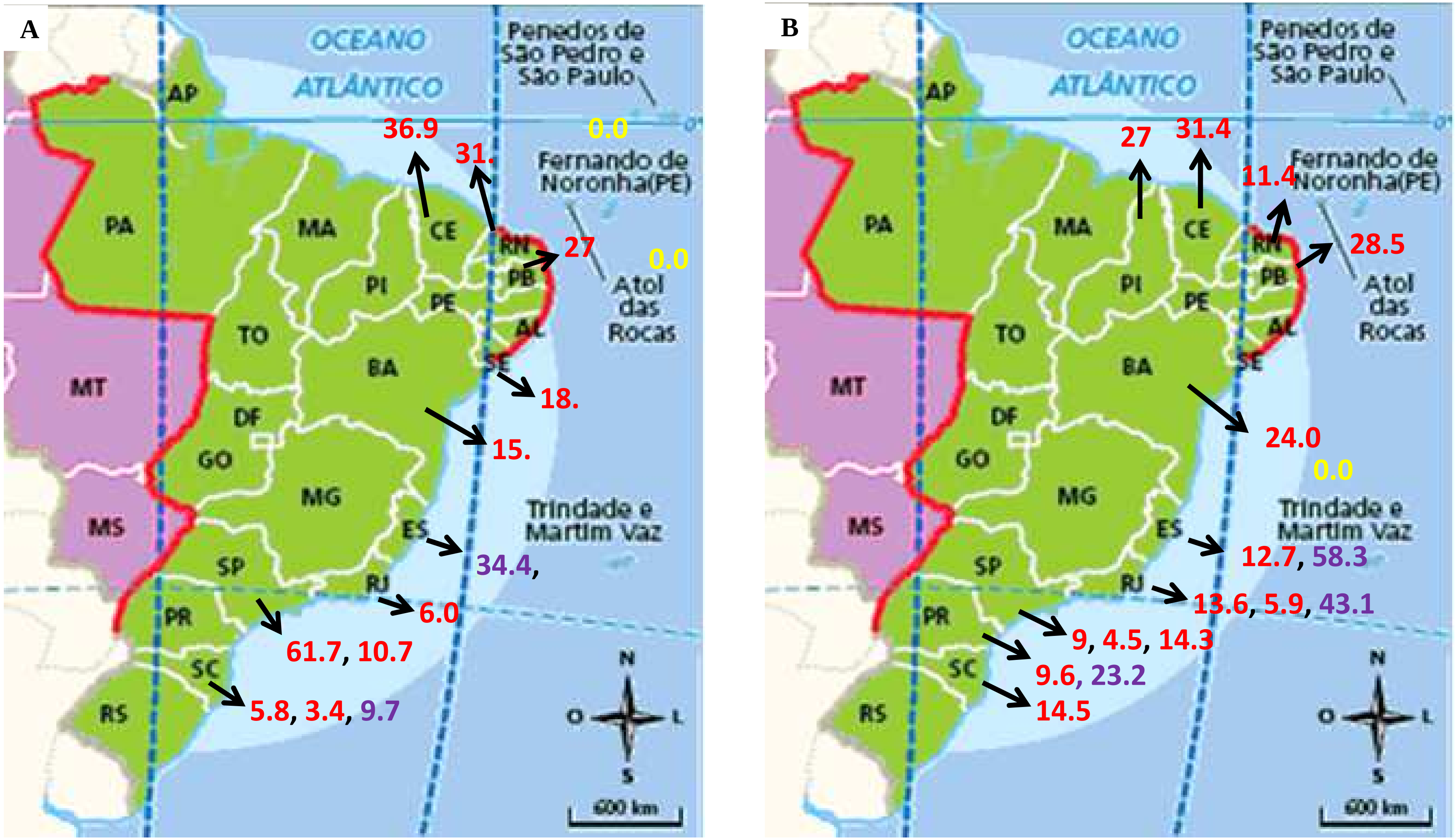
The first record of FP in a green turtle on the Brazilian coast was in 1986 and initial monitoring of the disease was incidentally documented in stranded or bycaught turtles by Projeto TAMAR-IBAMA (1986-1999) in specific nesting and feeding areas (Baptistotte *et al*., 2001). Positive cases were observed in northeastern and southeastern along Brazilian coast. However, in nesting areas, only few cases of FP were recorded in Trindade Island and the disease was absent in the Fernando de Noronha Archipelago and Rocas Athol (Baptistotte *et al*., 2001). After this period (1986-1999), systematic monitoring was gradually performed and it was possible to evaluate the spatio-temporal epidemiological data, based on literature and complemented with new data of the three states analyzed here: Santa Catarina (SC), Paraná (PR) and Rio de Janeiro (RJ).

Overall, prevalence along Brazilian coast from 2000 to 2014 ranged from 0% in offshore nesting islands to 61.7% in a foraging ground area in SP coast. The same range (0-61.7%) was observed from 2000 to 2006 and 0% in offshore nesting islands to 58.3% in a foraging ground area in ES coast from 2007 to 2014 (Figure 1, Supplementary Table 1). The FP prevalence of the three states evaluated here was 14.5% (17/117) in SC and 9.6% (38/395) in PR from 2007 to 2014 and 13.6% (12/88) in RJ, from 2009 to 2014, of stranded/bycaught green turtles. Furthermore, 23.2% (10/43) of green turtles intentionally captured in 2014 were FP-afflicted in PR.

According to sampling methods used in reviewed studies, live and dead stranded animals and also few incidentally bycaught cases reported by fishers were sampled, but no distinction of FP prevalence was made for each sampling method (stranded *x* bycaught). Therefore, these cases were considered as the methodology ‘stranded/bycaught animals’ for further comparisons (Figure 1).

Fibropapillomatosis prevalence of stranded/bycaught animals (SIcC) *versus* intentionally captured animals (InC) in ES, PR, RJ and SC were analyzed (Figure 1). Prevalence was different in ES in the first period (SIcC: 21.2% *x* InC: 34.4%, p=0.000) and in the second period (SIcC: 12.7% *x* InC: 58.3%, p=0.000) (Torezani *et al*., 2010, Santos *et al*., 2010, Rodenbusch *et al*., 2014); in PR (SIcC: 9.6% *x* InC: 23.2%, p=0.017) (This study) and RJ (SIcC: 13.6% *x* InC: 43.1%, p=0.000) (This study, Tagliolatto, 2015), but not in SC (SIcC: 3.4% *x* InC: 9.7%, p=0.195) (Proietti *et al*., 2007, Baptistotte, 2007). These results suggests a greater FP prevalence in intentionally captured animals than stranded animals in some areas. It is possible that population density of green turtles, including afflicted animals, was higher

Figure 1. Prevalence of fibropapillomatosis in green turtles off Brazilian coast, (A) from 2000 to 2006 and (B) from 2007 to 2014, and sampling methods utilized in studies.



Sampling methods are differentiated by colors: red – stranded/bycaugth; purple - intentionally captured, and yellow - nesting areas/no cases of FP.

in areas where intentional capture was performed or that afflicted turtles could spend more time feeding in these areas than non-afflicted animals. Although these are tempting hypotheses, further studies are necessary to assess this issue.

Given the situation stated in previous sentence, spatio-temporal comparisons between prevalence rates were adjusted according to sampling methods. In a large scale, FP prevalence of stranded/bycaught green turtles along Brazilian coast was 14% (1090/7804) in the first period (based mostly in Baptistotte, 2007), and 13.8% (1106/8035) in the second period based on reviewed and new data. These values were statistically similar (p=0.712), suggesting that disease is maintained despite time along Brazilian coast. The prevalence observed in Brazil are lower than FP prevalence on stranded turtles from Hawaiian Archipelago (28%), and bycatch turtles from Indonesia (21.5%), but higher than prevalence on turtles from Australia (0.5–2.1%) (Adnyana, Ladds and Blair, 1997, Limpus *et al*., 2005, Chaloupka *et al*., 2008, Flint *et al*., 2010a). Overall, these values gives a spatial broad view of the disease but details about epidemiology in a small spatial scale are also necessary because substantial differences trough time may be found over relatively short distances linked to environmental features (Foley *et al*., 2005, Van Houtan *et al*., 2010).

The comparisons of FP prevalence between periods along Brazilian coast indicated increase during time in Santa Catarina (Florianópolis *x* Babitonga bay–SC) (p=0.036) and Bahia (Northern–BA) (p=0.000), maintenance in Rio de Janeiro (Northern–RJ) (p=1.000), and Paraíba (João Pessoa–PB) (p=0.751), decrease in Ceará (Almofala–CE) (p=0.046), Rio Grande do Norte (Southern–RN) (p=0.000), ES (Northern and Southern) (p=0.000), São Paulo (Ubatuba–SP) (p=0.000) and was absent in nesting islands Rocas Athol and Fernando de Noronha Archipelago (Figure 1). These results suggests significant decrease in the highests prevalence along Brazilian states evaluated, CE (36.9% to 31.4%) and SP (61.7% to 4.5%) and a warning in BA (15.8% to 24%) and SC (3.4% to 14.5%). It is important to note, however, that comparisons were precluded in nine states (52.9%), owing to lack of available information.

Spatially, comparison between states along Brazilian coast during the second period, demonstrated a significant trend of greater prevalence from PI to BA (northeastern Brazil) compared to ES to SC (southeastern and southern Brazil). Exceptions are RN (northeastern Brazil) that presented similar prevalence to that observed from ES to SC and for Cananéia and Ubatuba (SP, southeastern Brazil) that demonstrated lower prevalence compared to all states evaluated (Supplementary Table 2).

In main Hawaiian Islands, FP prevalence varied from absence to almost 100% in regional scale, and elevated disease rates clustered in watersheds with highst nitrogen input and land use (Van Houton *et al*., 2010). In Brazil, spatial difference of the disease has also been related to poor environmental quality in a FP-endemic area in Espírito Santo compared to a FP-free area classified as good quality habitat in the Fernando de Noronha Archipelago, located 344Km from the coastline (Santos *et al*., 2010).

Pollution affects the biochemical quality of water, pathogen input and toxicity to biota that can lead to physiological and immunological effects (Sindermann, 2006). Chemical contaminants have been reported in green turtles along Brazilian coast (Barbieri, 2009, Silva *et al*., 2014, Bezerra *et al*., 2015, Macêdo *et al*., 2015), including high levels of total mercury (T-Hg), cadmium (Cd), nickel (Ni), lead (Pb) and zinc (Zn) in turtles from industrialized foraging ground in BA, with levels among the highest ever reported in juvenile green turtles (Bezerra *et al*., 2015, Macêdo *et al*., 2015). Lower, but representative levels of T-Hg were reported in a pristine feeding area in CE (Bezerra *et al*., 2015). These states are among the highest FP prevalence on Brazilian coast (CE=31.4% and BA=23.9%), and when compared to lower FP prevalence rates in Cananéia (SP=9%) (Barbieri *et al*., 2009), levels of some trace elements in the former states are higher.

The contamination by trace elements (e.g. copper and iron) has been associated with oxidative stress and enzyme antioxidant action that can result in toxic products, cell damage and tumor induction, exemplified in green turtles fibropapillomatosis-affected (Silva *et al*., 2016). Nevertheless, organic contaminants have not been linked as a direct factor for the onset of FP in green turtles, but they could contribute to the progression of disease in severely emaciated cases owing to mobilization of contaminants from adipose tissue into the blood leading to immunosuppression or toxic effects (Keller *et al*., 2014).

4.3.2 Biological characteristics of green turtles and fibropapillomatosis

The host, pathogen features and environment interplay (‘the disease triangle’) are also important to understand infectious diseases and how epidemics might be predicted, limited and controlled (Scholthof, 2007). Green turtle CCL has been considered a risk factor of FP (Van Houtan *et al*., 2010; López-Mendilaharsu *et al*., 2016), because the disease is not randomly represented in populations but is found primarily among turtles in the 40-85 cm size range (Limpus *at al*., 2005). The mean±SD CCL of turtles with FP were 47.2±7.0 cm (stranded/bycaught) and 43.5±4.4 cm (intentionally captured) in PR, 46±7.8 cm in SC (stranded/bycaught) and 52.9±8.3 cm in RJ (stranded/bycaught) (Supplementary table 1). These values were similar, for example, in Florida (46.3 cm), Australia (peak at 45-50 and 80 cm), Hawaii (Straight Carapace Length-SCL=35-65 cm), Uruguay (50.3±6.7 cm), in previous study along Brazilian coast (47.9 cm), and turtles intentionally captured from SP (44.5 cm), RJ (44.8 cm) and ES (peak at 45-50 cm) (Murakawa *et al*., 2000, Folley *et al*., 2005, Limpus *et al*., 2005, Baptistotte, 2007, Santos *et al*., 2010, Guimarães *et al*., 2013, Zwarg *et al*., 2014, López-Mendilaharsu *et al*., 2016)

Furthermore, comparisons of CCL demonstrated that afflicted turtles are generally larger than turtles without FP (Aguirre and Balazs, 2000, Folley *et al*., 2005, Baptistotte, 2007, Torezani *et al*., 2010, Zwarg *et al*., 2014, López-Mendilaharsu *et al*., 2016) and it was also observed with turtles stranded in PR (CCL of non-afflicted animals=38.6±5.1 cm; Mann-Whitney, $Z_{1,96}$=6.81, n=357), SC (CCL of non-afflicted animals=40.4±7.4 cm; Mann-Whitney, $Z_{1,96}$=2.98, n=76) and RJ (CCL of non-afflicted animals=43.5±9.2 cm; Mann-Whitney, $Z_{1,96}$=3.42, n=80), but not with turtles intentionally captured in PR (CCL of non-afflicted animals= 41.1±4.7cm; p=0.149, n=33). It is interesting to note that size distribution of green turtles is not uniform along Brazilian coast and amplitude, increasing from southern to northeastern regions (Barata *et al*., 2011). It was also observed here, once turtles in RJ are larger than turtles from PR and SC (Kruskal-Wallis, $H_{5,99}$=32.17, n=601), including afflicted turtles (Tukey´s test, $F_{3,16}$=3.30, p=0.041, n=61).

Green turtles settle in neritic areas (recruitment) between 19 cm and 40 cm of CCL worldwide (Seminoff *et al*., 2015), and spend the majority of their lives in coastal foraging grounds, where they might get infected by specific ChHV variants (Ene *et al*., 2005). Therefore, CCL range of afflicted turtles along Brazilian coast indicates that most of green turtles get infected after recruitment, when they slowly shift from omnivorous into an herbivorous diet (Cardona *et al*., 2010) and are exposed to different environmental factors, including anthropogenic impacts, that increase likelihood of small immature turtles to become unhealthy (Flint *et al*., 2010b). Controversially, FP are rare in larger green turtles (>80 cm CCL) (Foley *et al*., 2005), as observed in a previous study performed along Brazilian coast (Baptistotte, 2007). The rarity of disease in the largest size is associated to mortality of animals before they reach a larger size, increased resistance and consequent tumour regression or a combination of both (Foley *et al*., 2005).

The severity of disease vary according to the number and size of tumours and is scored as mild (FPS-1), moderate (FPS-2) and severe (FPS-3) (Work and Balazs, 1999). The score is used to indicate the prognosis of the disease, diagnose FP as the cause of death and indicate trends of mortality in a population (Work and Balazs, 1999, Work *et al*., 2004, Chaloupka *et al*., 2008). In PR (FPS-1=14, FPS-2=5 and FPS-3=5) and SC (FPS-1=9, FPS-2=3 and FPS-3=3), turtles presented mostly a FPS-1 score, indicating a mild severity disease (58.3% and 60%, respectively), whereas moderate and severe cases (87.5%) were observed in RJ (FPS-1=1, FPS-2=2 and FPS-3=5). Although sample size of tumour score was small here, it represents 64.9% (24/37) of afflicted animals in PR, 88.2% (15/17) in SC and 66.7% (8/12) in RJ.

The tumour scores FPS-2 and FPS-3 were observed in 88.5% (23/26) stranded afflicted turtles in BA, 77.8% (28/36) in SP and in 94.2% (82/87) of intentionally captured in ES (Supplementary table 1), suggesting that despite higher (e.g. BA and ES) or lower (e.g. SP) prevalence, the severity is also an important feature of the disease. In Hawaiian Islands, FP severity was associated with immunossuppression and poor body condition (Work *et al*., 2001 and 2004, Chaloupka and Balazs, 2005). Apparently, severe cases can cause reduction in ability to dive and affect vision that can lead to difficulty in feeding and negatively affect health in those cases (Work *et al*., 2001, Torezani *et al*., 2010).

The mean CCL of the individuals in the three states analyzed was similar despite the score (FPS-1=48.3±6.8 cm, FPS-2=45.7±10.7 cm and FPS-3=49.7±6.9 cm) ($p=0.511$). Therefore, an expected increase of disease severity in turtles with greater CCL, presumably older and with greater chances to get exposed to the possible causative agent or co-factors was not observed. However, this association was observed in ES, Brazil, where individuals with FPS-2 and FPS-3 demonstrated greater sizes and smaller growth rates, compared to FPS-1 and non tumoured turtles (Torezani *et al*., 2010).

4.3.3 Histology and molecular characterizarion

The microscopical analyses of skin tumours were performed in 23 green turtles stranded and intentionally captured off PR (mean=2.9 tumours per turtle; 1 to 13 tumours), six green turtles off RJ (mean=6.5 tumours per turtle; 1 to 15 tumours) and six green turtles off SC (mean=3.7 tumours per turtle; 2 to 7 tumours), despite tumour size or body localization. One hundred and twenty-one lesions were classified as fibropapillomas, with both epidermal and dermal hyperplasia; three fibromas, with relatively normal epidermis and dermal hyperplasia, and four were not classified, due to lack of epidermis. The main histological findings are shown in Table 1.

Table 1. Relative frequency of main histological features in skin tumours of green turtles sampled from 2007 to 2014 off Paraná (PR), Santa Catarina (SC) and from 2009 to 2014 off Rio de Janeiro (RJ) states, south and southeastern Brazil.

Characteristic Histological Features	**Relative Frequency (%)**		
	PR *(67 sections)*	*SC* *(22 sections)*	*RJ* *(39 sections)*
Conjunctive tissue proliferation	100^{a}	100^{a}	100^{a}
Acanthosis	90.8^{a}	100^{a}	92.1^{a}
Orthokeratosis	98.4^{a}	91^{a}	100^{a}
Basal cell degeneration	68.5^{a}	100^{b}	82^{a}
Dermal-epidermal cleft	72.3^{a}	91^{a}	84.6^{a}
Spindle cell degeneration	79.4^{a}	100^{b}	89.5^{ab}
Erosion or ulceration	26.5^{a}	22.7^{a}	25.6^{a}
Inflamatory infiltrate	52.2^{ab}	76.2^{a}	46.1^{b}
Foreign body granuloma	48.6^{a}	54.5^{a}	7.7^{a}
Pathogens			
Virus (intranuclear inclusions)	1.7	0	0
Spirorchiid ova	55.2^{a}	77.3^{b}	23.1^{a}
Bacteria in superficial skin	17.9^{a}	9.1^{ab}	2.6^{b}

Different letters in the same line indicate frequences of lesion were statistically different (chi-square test, $p<0.05$)

Proliferation of fibroblasts was observed in all tumours. In fibropapillomas, it was also observed varying amounts of epidermal proliferation, including acanthosis and/or orthokeratosis, from five to 15 layers along epidermal tissue. In several cases, orthokeratosis lead to production and retention of cornified inclusion cysts (18.2-60.5% of tumours). Spindle cell degeneration, vacuolation and necrosis in basal cell cytoplasm were also common features in the epidermis of fibropapillomas.

Similar to tumours analyzed in Floridian and Hawaiian green turtles (Herbst *et al*., 1999), basal cell vacuolation was associated with cleft formation between dermis and epidermis (p=0.000), but, controversially, we also observed association between basal cell vacuolation and spindle cell degeneration (p=0.0001), and between spindle cell degeneration and cleft formation (p=0.006) in tumours of green turtles from the three states evaluated here. These results suggest that tumour development include various degrees of epithelial cell degeneration, one of the hallmarks of herpesvirus dermatitis, that could lead to secondary focal to locally extensive cutaneous erosions or ulcerations (Herbst *et al*., 1999).

Foreign body granulomas and a few scattered lymphocytes were observed with Spirorchiid egg emboli or embedded in conjunctive tissue, but most eggs demonstrated to be innocuous. The median of eggs in tissues was one in PR and RJ (min=1, max=9 eggs), statistically different of three eggs in SC (min=1, max=23 eggs) (Kruskall-Wallis, $H_{5.99}$=18.74). Bacteria were observed opportunistically in superficial epidermal layer of tumours, in FPS-1 and FPS-2 (3/6, 50% each), however, few scattered cases were associated with inflammatory reaction. Spirorchiid eggs (p=0.139) and bacteria (p=0.328) presence were not associated with tumour score in this study. Similarly to Hawaii, splenic egg burden was not associated with FP severity, but with increasing size and poor body condition (Work *et al*., 2005), while bacteria in tissue and bacteremia were associated with score FPS-2 and FPS-3 of captured and stranded green turtles (Work *et al*., 2003). Therefore, low infestation and infection of these agents in tumours, and the good body condition observed in PR, SC and RJ suggests incidental findings without potential health implications, but further analyses of different organs and parameters are needed for a better understanding.

Fibropapillomatosis is associated with a non-cultivable alphaherpesvirus, Chelonid herpesvirus 5 (ChHV5) (Work *et al*., 2009). Extensively studies evidenced intranuclear inclusions with viral particles, mostly associated with ballooning degeneration in epidermal layers (Herbst *et al*., 1999, Rodenbusch *et al*., 2014, Work *et al*., 2015), but foci of ballooning degeneration were not common in the evaluated samples. This change was found only in tumours of turtles from PR (21%) and eosinophilic intranuclear inclusion was rare, observed in one tumour of an animal from PR (Figure 2).

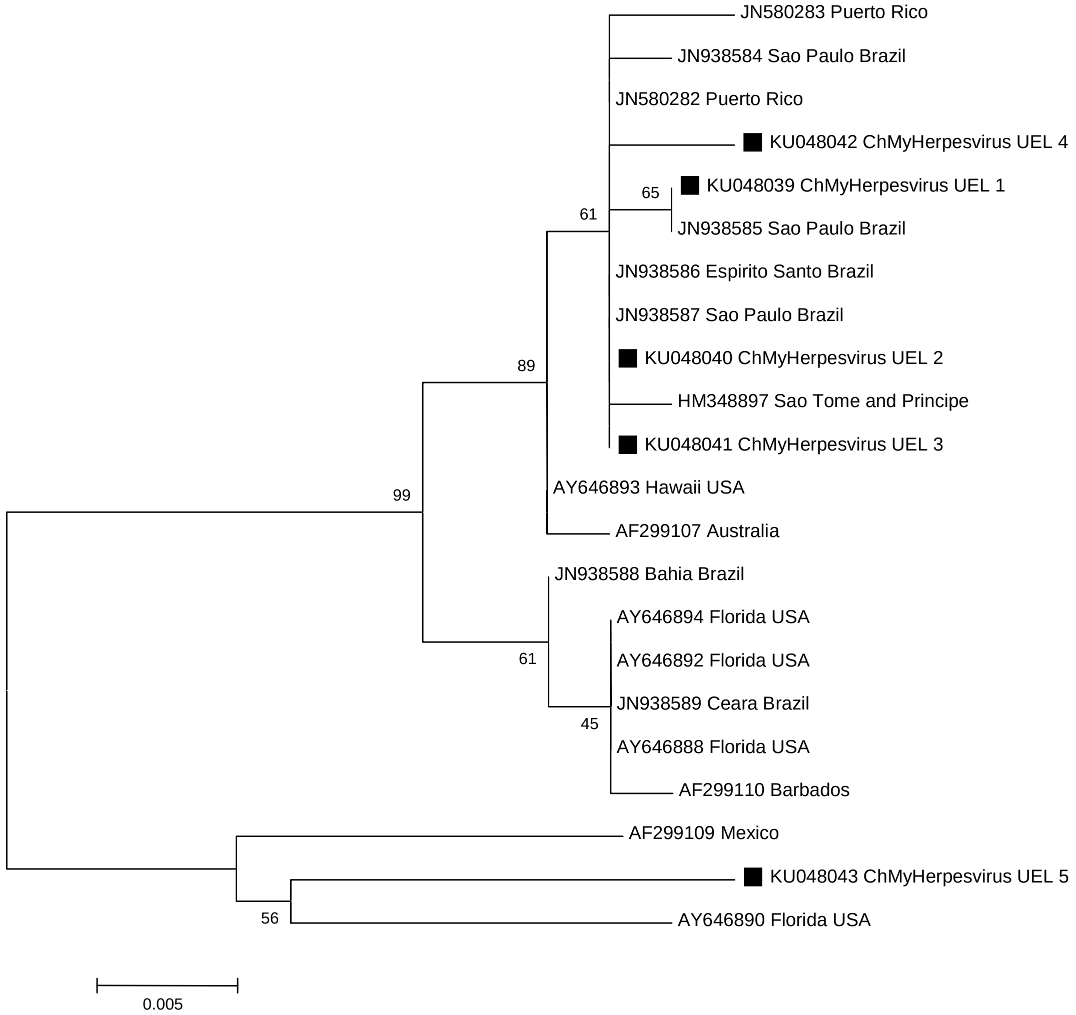
The distribution frequency of eosinophilic intranuclear inclusion here (1/121, 1%) was similar to Floridian green turtles tumours (2/119, 1.7%), but lower compared to afflicted Hawaiian green turtles tumours (27/381, 7.1%) and green turtle tumours from other four states on Brazilian coast (5/43, 11.6%) (Herbst *et al*., 1999, Rodenbusch *et al*., 2014, Work *et al*., 2015). Low frequency distribution of eosinophilic intranuclear inclusion and sporadic virus shedding is likely a trend of the disease (Work *et al*., 2015). Few tumours and infectious individuals in a population contribute disproportionately to secondary infections, suggesting that transmission of ChHV5 may depend on superspreaders (Work *et al*., 2015).

Figure 2. Eosinophilic inclusion bodies in ballooning epithelial cells (arrows) of skin fibropapilloma of green turtle stranded in Paraná state.

The ChHV has been associated with sea turtles for millions of years (Herbst *et al.*, 2004) and four phylogeographic ChHV5 groups have been described from tumours found in green turtles: Atlantic (samples from the Gulf of Guinea and Porto Rico), western Atlantic/eastern Caribbean (samples from Florida and Barbados), mid-western Pacific (samples from Australian and Hawaiian) and eastern Pacific (samples from San Diego, Costa Rica and Mexico) (Patricio *et al.*, 2012). In Brazil, variants in green turtle tumours sampled in southeastern coast were closest to Atlantic group while those from northeastern coast were more similar to the western/Atlantic eastern Caribbean (Rodenbusch *et al.*, 2014). In this study, 19/28 (67.8%) afflicted animals contained DNA of ChHV5 and nine cases had no DNA of ChHV5 amplified (32.2%). Five genetic variants of ChHV5 were found, and the sequence analyzes demonstrated that herpesvirus strains are closest to Atlantic cluster, excepting one that is closest to western Atlantic/eastern Caribbean cluster (Figure 3). The strains sequences were deposited in GenBank as ChMyHerpesvirus_UEL-1 until 5, with the respectively number KU048039 to KU048043. The similarity among sequences are demonstrated on Table 2. To the best of author’s knowledge it is the first time that ChHV5 was detected in turtles from Paraná, Santa Catarina and Rio de Janeiro.

Additionally, the primers AR-E1F2 and AR-E1R9 allowed the papillomavirus DNA amplification of approximately 580 bp in one sample, which was negative in herpesvirus identification. However, the low quality of the sequencing product failed to identify the PV type. The degenerate primers FAP59/FAP64 did not allowed amplification of the samples. The results reinforce the herpesvirus association with fibropapillomatosis in green turtles, and the participation of papillomavirus infection was not excluded. The ChHV-5 variant is possibly widespread on southern Brazil, but more studies are necessary to understand the dissemination of the viral strain between population stocks on the Brazilian coast, and the role of PV infection in fibropapilloma pathogenesis.

Figure 3. Phylogenetic tree obtained by the maximum-likelihood method based on the nucleotide sequences of a 453 bp fragment within polymerase gene of *Chelonia mydas* Herpesvirus



Bootstrap values are indicated when 40% as a percentage was obtained from 1,000 replications. The herpesvirus strains ChMyHerpesvirus_UEL 1 to 5 are marked as square. The sequence names are labelled as follows: GenBank accession number and geographic location. The scale bar represents a genetic distance of 0.005 substitutions per site.

Table 2. The highest similarity percentage of herpesvirus types with ChMyHerpesvirus_UEL-1 to 5

Sample	HV strain	Closest related type	Similarity
1	ChMyHerpesvirus_UEL 1	JN938585_Sao_Paulo_Brazil	100%
2	ChMyHerpesvirus_UEL 2	JN580282_Puerto_Rico, JN938586_Espirito_Santo_Brazil, JN938587_Sao_Paulo_Brazil	100%
3	ChMyHerpesvirus_UEL 3	ChMyHerpesvirus_UEL 2	100%
4	ChMyHerpesvirus_UEL 4	JN580282_Puerto_Rico and ChMyHerpesvirus_UEL 2 and 3	99.5%
5	ChMyHerpesvirus_UEL 5	JN938589_Ceara_Brazil	95.8%

4.4 CONCLUSIONS

This is the first study that analyzed spatio-temporal FP prevalence and associated factors along Brazilian coast. Data suggests (i) maintenance of disease from 2000 to 2014 with variation according to state (latitude); (ii) prevalence data can vary according to sampling methods; (iii) CCL range of afflicted turtles was similar in different regions, but differs of non-afflicted animals and (iv) histological findings and molecular evalution reinforces viral infection. These findings exemplify the role of environmental and host features in disease, and the understanding is a challenge specially considering a hazard and complex migratory host as green turtles. Therefore, continuous monitoring is necessary for a better understanding of FP trends and consequent conservation of these animals.

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Supplementary Table 1. Biological parameters and prevalence of fibropapillomatosis in green turtles (*Chelonia mydas*) along and within Brazilian states.

Region	**State**	**Local**	**Prevalence (%) (2000-2006)**	**Prevalence (%) (2007-2014)**	**CCL (cm) affected**	**CCL (cm) affected and non affected**	**Stage of development**	**Score**	**Sampling method**	**Effort**	**Reference**
North	Amapá	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
	Pará	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
Northeast	Maranhão	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
	Piauí	Delta do Parnaíba		27		49 (25-114)	Adults and juveniles	NA	S, IcC	jan-ag 2011 (7 months)	7
	Ceará	Almofala		31.38	33-76*		Juveniles	1 (30.35%), 2 (39.3%) 3 (30.35%)	S, IcC	2009-2010	12
		Almofala	36.9		$47.9^{\#}$		Aduls and juveniles	NA	S, IcC	2000-2005	1
	Rio Grande do Norte	Tibau do Sul	31.4		$47.9^{\#}$		Aduls and juveniles	NA	S, IcC	2000-2005	1
		Areia Branca to Caiçara do Norte		11.4	NA	NA	NA	NA	S, IcC	2011-2012	14
		Rocas Athol	0		$47.9^{\#}$		Aduls and juveniles	NR	S, IcC, Nesting	2000-2005	1
	Paraíba	Joao Pessoa and Cabedelo	27			34.9-106	Adults and juveniles	NA	S, IsC	2002-2006	3
		Joao Pessoa and Cabedelo		28.5	54.0 (37.9-76.4)		mostly juveniles (90.3%)	NA	S, IcC	Ag 2009-Julho 2010 (11 month)	4
	Pernambuco	FN	0		NR	52.2 (15.5-88)	Aduls and juveniles	NR	S, IcC, Nesting	2000-2005	1
	Alagoas	NA	NA	NA	NA	NA	NA	NA	NA	NA	-
	Sergipe	North and South	18.5		$47.9^{\#}$		Aduls and juveniles	NA	S, IcC	2000-2005	1
	Bahia	North	15.8		$47.9^{\#}$		Aduls and juveniles	NA	S, IcC	2000-2005	1
		North		23.98	33-76*		Juveniles	1 (11.5%), 2 (30.8%), 3 (57.7%)	S, IcC	2009-2010	12
Southeast	Espírito Santo	Serra	34.4			40.3 (25.2-	Juveniles	1 (35.2%), 2	InC	2001-2006	15

						77.5)		(52.5%), 3 (12.3%)			
		North to South	21.2		42.4		mostly juveniles	NA	S, IcC	2001-2006	15
		Vitória		58.3	peak 45-50 (30-60)		immature	1 (5.7%), 2 (35.6%), 3 (58.6%)	InC	March 2007-April 2008	2
		North to South		12.69	33-76*		Juveniles	1 (51.4%), 2 (17.1%), 3 (31.4%)	S, InC, IcC	2009-2010	12
		Trindade Island		0	NR	97-130	Adults	NR	Nesting	2011	12
	Rio de Janeiro	North	6		47.9#		Aduls and juveniles	NA	S	2000-2005	1
		Center and North		5.9	NA	NA	NA	NA	S	Jan a Dez 2009 (11 meses)	5
		Niterói		43.09	60.2		Juveniles	NA	InC	2008-2014	13
		Center		13.6	52.9 (37-66)		Juveniles	1 (12.5%), 2 (25%), 3 (62.5%)	S, IcC	2009-2014	This study
	São Paulo	Ubatuba	10.7		47.9#		Aduls and juveniles	NA	S, IcC	2000-2005	1
		Mostly Ubatuba (Northen SP)	61.7		44.5		Juveniles	NA	S, IcC	2006-2007	6
		Ubatuba		4.54	33-76*		Juveniles	1 (22.2%), 2 (25%), 3 (52.8%)	S, IcC	2009-2010	12
		Baixada santista and South		14.3	NA	NA	mostly juveniles (95.3%)	NA	S, IcC	2004-2011	8
		Cananéia		9		38.4 (31-49)	Juveniles	NA	S, IcC	2005-2008	10
South	Paraná	CEP		8.5	47.8 (33-62)		Juveniles	1 (58.3%), 2 (20.8%), 3 (20.8%)	S, IcC	2007-2014	This study
		Cobras Island in CEP		23	42.8 (35.4-51.5)		Juveniles	NA	InC	2014 (one weak)	This study
	Santa Catarina	Babitonga and SFS		14.5	46.0 (31.9-63.2)		juveniles	1 (60%), 2 (20%), 3 (20%)	S, IcC	2007-2014	This study
		Arvoredo	9.7		54.5-67		juveniles and adults	NA	InC	2004-2006	9
		Florianópolis	3.4		47.9#		Juveniles	NA	S, IcC	2003-2005	1
		Araranguá a Passo de Torres, Penha a Itapema	5.8		NA	NA	Juveniles	NA	S, IcC	2000-2004	11

NA: not analysed

Score: 1 (mild), 2 (moderate) and 3 (severe)
S: stranded, IcC: incidentally caught in fishing gears, InC: intentionally captured for research
$^{\#}$ Mean CCL of green turtles from different states evaluated (Baptistotte, 2007)
*CCL range of green turtles evaluated in three different states (Rodenbusch et al., 2014)

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Supplementary Table 2. Spatial differences of FP prevalence in green turtles stranded/incidentalty caught among and within states on Brazilian coast (2007-2014).

	SC	PR	SP (Baix)	SP (Can)	SP (Ubat)	RJ (Guan)	RJ (Quis)	ES	BA	PB	RN	CE	PI
SC		No	No	Yes	Yes	No	No	No	**Yes**	**Yes**	No	**Yes**	**Yes**
PR	No		No	Yes	Yes	No	No	No	**Yes**	**Yes**	No	**Yes**	**Yes**
SP (Baix)	No	No		Yes	Yes	No	No	No	**Yes**	**Yes**	No	**Yes**	**Yes**
SP (Can)	**Yes**	**Yes**	**Yes**		No	**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
SP (Ubat)	**Yes**	**Yes**	**Yes**	No		**Yes**	No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
RJ (Guan)	No	No	No	Yes	Yes		No	No	**Yes**	**Yes**	No	**Yes**	**Yes**
RJ (Quis)	No	No	No	No	No	No		No	**Yes**	**Yes**	No	**Yes**	**Yes**
ES	No	Yes	No	Yes	Yes	No	No		**Yes**	**Yes**	No	**Yes**	**Yes**
BA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		No	**Yes**	**Yes**	**Yes**
PB	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No		Yes	No	No
RN	No	No	No	Yes	Yes	No	No	No	**Yes**	**Yes**		**Yes**	**Yes**
CE	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes		No
PI	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	

Yes: significat different; prevalence was greater in colum compared to line. Yes: significat different and prevalence was lower in colum compared to line No: not significant . $p<0.05$

5. ARTIGO B*

*Manuscrito submetido ao periódico Marine and Freshwater Research

Short Communication

Running head: Fibropapillomatosis and health in *Chelonia mydas*

Fibropapillomatosis among juvenile *Chelonia mydas* at their southwestern Atlantic limit: implications for population conservation

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Abstract. Considered panzootic among *Chelonia mydas*, fibropapillomatosis manifests during their neritic recruitment; a period involving extrinsic and intrinsic stressors associated with habitat and dietary shifts. This study sought to contribute towards understanding fibropapillomatosis implications for *C. mydas* populations through prevalence comparisons and haematological and biochemical analyses of juveniles off southern Brazil. Forty-three juvenile *C. mydas* (31.9–51.5 cm curved carapace length–CCL) were caught and had their body condition index (BCI) determined, while 42 specimens had blood profiles (36 parameters) evaluated. Tumours were observed on ten specimens (~23%) (mean CCL and mass ± SD and BCI ± SE; 43.51 ± 4.42 cm, 10.42 ± 2.81 kg and 1.24 ± 0.03) while 33 had none (41.06 ± 4.66 cm, 8.58 ± 2.97 kg and 1.21 ± 0.02). Significantly lower levels of cholesterol and urea, but greater numbers of eosinophils, were observed in tumour-afflicted animals. These observations were attributed to possible digestive and metabolism efficiency and/or differences in diet associated with temporal-recruitment bias and disease development, and greater nonspecific immune stimulation. While most animals had adequate body condition independent of disease, longer-term studies are required to elucidate any protracted population effects including direct mortalities, and the extent to which negative anthropogenic activities influence disease severity and indirect mortalities.

Additional keywords: blood, coastal area, sea turtles, South America, tumours.

5.1 Introduction

Seven species of marine turtles exist; all with varying global distributions, but a common ecological importance in maintaining oceanic ecosystems (Spotila 2004). Owing to pollution, habitat degradation, climate change and fishing mortality, most species have population statuses collectively listed as either ‘Vulnerable’, ‘Endangered’ or ‘Critically Endangered’ (IUCN 2015). Of particular concern is the cosmopolitan green turtle, *Chelonia mydas* (Linnaeus 1758), which mostly frequents neritic zones following oceanic recruitment and transition from an omnivorous to predominantly herbivorous diet (Gama *et al*. 2016). Their near-shore proximity renders all *C. mydas* susceptible to anthropogenic threats—which are exacerbated off industrialised coasts.

One such area of concern in Brazil is the Paranaguá estuarine complex–PEC (25°20’ to 25°35’S, 48°17’ to 48°42’W), which is an important foraging ground for juvenile *C. mydas* (Gama *et al*. 2016). Although a World Heritage site (UNESCO), the PEC is heavily impacted by artisanal fisheries, harbours, urbanization and pollution. These factors have been demonstrated to affect the morbidity and mortality of various marine sentinels, as stated previously for small cetaceans in the same area (Domiciano *et al*. 2016). Potential consequences for *C. mydas* range from mortality to less obvious acute and chronic sublethal impacts encompassing various diseases, including fibropapillomatosis–FP (Jones *et al*. 2016).

Fibropapillomatosis is a panzootic disease associated with a viral aetiological agent (Chelonid herpesvirus 5), and is characterised by external and internal tumours that potentially can impair swimming, feeding, reproduction and internal-organ function (Jones *et al*. 2016). The few studies assessing live FP-afflicted *C. mydas* (via blood profiles) off Hawaii and in the South Atlantic Ocean imply variable immunosuppression, anaemia, uraemia, low cholesterol, lymphocytopenia, and hypoproteinaemia (Aguirre *et al*. 1995; Aguirre and Balazs 2000; Santos *et al*. 2015). Such physiological variation possibly reflects extrinsic variables, including spatio-temporal differences in diet and environmental conditions and/or intrinsic features like gender, nutritional and life stages, and concomitant disease (Aguirre and Balazs 2000).

Uncertainty concerning the key extrinsic or intrinsic mechanisms supports the wide-scale monitoring of divergent populations of *C. mydas* (Aguirre and Balazs 2000). More specifically, broad assessments of inflicted populations might facilitate a clearer understanding of the implications of FP among *C. mydas* and progress hypotheses concerning likely causal factors, and therefore mitigation.

Considering the above, our primary aim was to detect the prevalence of FP and compare the blood profiles of *C. mydas*, with and without FP tumours at the unassessed southern range of their Brazilian distribution in the PEC. Using this information, our secondary aim was to propose strategies for future research and monitoring the broader effects of FP among *C. mydas* populations as a precursor to examining direct or indirect causal relationships with environmental factors.

5.2 Materials and methods

Chelonia mydas were targeted during seven days in April 2014 using a surface-set gillnet (300-mm mesh) diurnally deployed in the PEC (Fig. 1). Each *C. mydas* was disentangled and manually restrained onboard while blood (2–3 ml) was withdrawn from the occipital sinus, using a heparinised 20-gauge needle and 3.0-ml syringe within <60 min of capture. Glucose (mg dL^{-1}) was immediately measured in the whole blood sample using the One Touch®

Ultra® 2 glucometer, before the remainder was placed into two 5-ml tubes, containing either lithium heparin anti-coagulant or gel and stored on ice prior to laboratory processing.

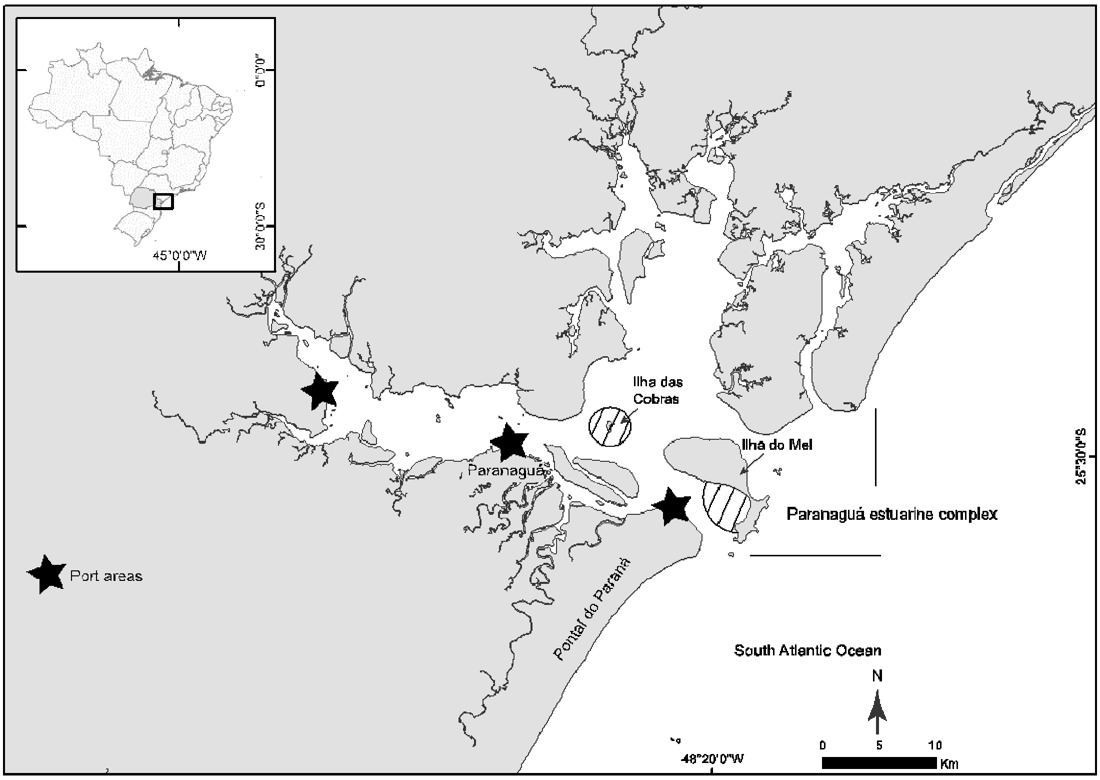

Fig. 1. Map of the Paranaguá estuarine complex, Brazil showing the areas where *Chelonia mydas* (n = 43) were intentionally caught in April 2014.

All specimens were measured for curved carapace length (CCL to the nearest 0.1 cm) and weighed (body mass; BM to the nearest 0.1 kg). Condition was estimated and classified as ‘good’, ‘fair’ or ‘’poor’ (based on the plastron, eyes, muscular mass and fat deposits) following Torezani *et al*. (2010). Additionally, the body condition index (BCI) was derived (Labrada-Martagón *et al*. 2010). Any skin FP tumours were noted, biological samples were collected and specimens accordingly designated as either diseased or not. Individuals were flipper-tagged (National Band and Tag Co., USA; style 681) and released.

Heparinised blood samples were analysed for total erythrocyte (He-10^6 µL^{-1}) and leukocyte (WBC-10^3µL^{-1}) counts within 10 h using Natt-Herrick's diluents (1:200) and a Neubauer hemocytometer. Packed cell volume (PCV-%) was determined by centrifuging heparinised blood in microhematocrit tubes (14,800 × g for 5 min, model H-240 DIG; Centribio). Plasma proteins (g dL^{-1}) were detected using a refractometer (Vet 360 TS meter, Reichert Analytical Instruments). Blood smears were fixed and stained with Diff-Quick (Laborclin©) and the differential white blood-cell count was determined via optical microscope lens. Thrombocytes were counted by 100 leukocytes.

Serum-gel tubes with whole blood were centrifuged (1,800 × g for 10 min, model 80-2B; Centribio) and the serum frozen (-20°C) for three months. Serum parameters were measured with commercial kits (Siemens) and processed using the Dimension® Clinical Chemistry System for: alanine aminotranferase (ALT) (U L^{-1}), aspartate aminotransferase (AST) (U L^{-1}), creatine kinase (CK) (U L^{-1}), gamma glutamyl transpeptidase (GGT) (U L^{-1}), alkaline phosphatase (ALP) (U L^{-1}), albumin (g dL^{-1}), urea (mg dL^{-1}), creatinine (mg dL^{-1}), uric acid (mg dL^{-1}), lactate (mmol L^{-1}), cholesterol (mg dL^{-1}), triglycerides (mg dL^{-1}), magnesium (mg dL^{-1}), calcium (mg dL^{-1}), and phosphorus (mg dL^{-1}). Electrolytes were measured by the electrode ion selective method, using a Cobas® b 121 gas analyser, including: sodium (mmol L^{-1}), potassium (mmol L^{-1}), chloride (mmol L^{-1}), and ionized calcium (iCa; mg dL^{-1}).

All physiological variables and CCL, BM and BCI, were separately analysed using linear mixed models (LMM) that included 'sampling day' as random and 'FP tumours' (present/absent) as fixed (CCL was a covariate in all physiological models). Absolute data were checked for normality (Q-Q plots) and log-transformed as required, while percentage data were arcsine square-root transformed. The significance of blood parameters between turtles with or without tumours was determined using a Wald-*F*. All LMMs were fitted using ASReml in R.

5.3 Results

Forty-three *C. mydas* were caught: ten (~23 %, mean CCL and BM ± s.d. of 43.51 ± 4.42 cm and 10.42 ± 2.81 kg) had mostly small (<1.5 cm) external tumours; and 33 (41.06 ± 4.66 cm CCL and 8.56 ± 2.97 kg) had no visible FP. Neither CCL nor BM significantly varied between groups (LMM, $P > 0.05$). All except one (which had no tumours and was 'fair'), were in good body condition, and BCI remained similar between individuals without (mean ± s.e. 1.21 ± 0.02) or with tumours (1.24 ± 0.03) (LMM, $P > 0.05$; Table 1).

Blood samples were collected from all except one *C. mydas* (tumour free) (Table 1). Compared to tumour-free individuals, those with tumours had significantly lower cholesterol (mean ± SE; 85.66 ± 6.74 vs 57.10 ± 9.24 mg dL^{-1}) and urea (37.85 ± 8.59 vs 7.80 ± 0.99 mg dL^{-1}), but greater absolute numbers and percentages of eosinophils (0.88 ± 0.26 vs 3.10 ± 1.1 % and 1.73 ± 0.45 vs 5.80 ± 1.76 %) (LMM, $P < 0.05$; Table 1). Irrespective of tumours, CCL also was significantly and positively associated with total protein, albumin, albumin-to-globulin, and PCV, and negatively associated with chloride (LMM, $P < 0.05$)—possibly reflecting items consumed by *C. mydas* with different sizes or ages .

Table 1. Summaries of Wald-*F* statistics from linear mixed models assessing the importance of the fixed effect of ‘fibropapillomatosis (FP) tumours’ in *Chelonia mydas,* for explaining variability among blood-physiology parameters, and the observed means (±SE) in the absence (n=32 for blood chemistry, but 33 for body condition index) or presence (n = 10) of tumours. Some data were ^{1}log- or ^{2}arcsine square-root transformed prior to analyses. *$P < 0.05$; **$P < 0.01$.

		FP not present		FP present	
Variable	Wald *F*	Mean (SE)	Range	Mean (SE)	Range
Body condition index (BCI)	0.05	1.21 (0.02)	0.93–1.35	1.24 (0.03)	1.13–1.39
Cholesterol (mg dL^{-1})	6.16*	85.66 (6.74)	19.00–175.00	57.10 (9.24)	24.00–105.00
Triglyceride (mg dL^{-1})	0.07	51.47 (5.67)	0.00–124.00	55.90 (16.65)	4.00–173.00
Urea^1 (mg dL^{-1})	9.72**	37.85 (8.59)	0.20–208.00	7.80 (0.99)	2.00–12.00
Creatinine^1 (mg dL^{-1})	4.07	0.30 (0.02)	0.10–0.60	0.25 (0.05)	0.10–0.60
Uric acid^1 (mg dL^{-1})	0.34	0.98 (0.65)	0.10-21.00	0.32 (0.05)	0.20–0.70
Total protein (mg dL^{-1})	1.42	2.68 (0.15)	1.00–3.90	2.42 (0.34)	1.20–4.60
Albumin (g dL^{-1})	0.74	0.35 (0.03)	0.10-0.80	0.30 (0.06)	0.10–0.70
Globulin (g dL^{-1})	1.38	2.33 (0.12)	0.90–3.40	2.12 (0.28)	1.10–3.90
Albumin:Globulin	0.20	0.14 (0.01)	0.04–0.26	0.13 (0.02)	0.06–0.21
Alanine aminotranferase (ALT)^1 (U L^{-1})	0.24	2.88 (0.53)	1.00–18.00	2.00 (0.21)	1.00–3.00
Aspartate aminotransferase (AST)^1 (U L^{-1})	1.13	70.13 (8.64)	19.00–238.00	48.50 (5.28)	19.00–74.00
Alkaline phosphatase (ALP)^1 (U L^{-1})	0.94	24.16 (2.58)	7.00–84.00	19.80 (2.85)	7.00–34.00
Creatinokinase (CK)^1 (U L^{-1})	2.16	451.16 (121.99)	0.00–3,151.00	144.90 (44.80)	5.00–414.00
Na^1 (mmol L^{-1})	0.80	156.36 (1.87)	123.70–197.70	159.40 (2.76)	149.50–178.80
K (mmol L^{-1})	0.60	3.95 (0.07)	3.19–5.08	4.03 (0.16)	3.25–4.83
Cl^1 (mmol L^{-1})	0.03	114.72 (1.66)	87.70–149.00	115.00 (2.01)	104.30–127.70
Ionized calcium (iCa) (mg dL^{-1})	3.56	1.87 (0.07)	1.22–2.72	2.19 (0.20)	1.30–3.13
Ca (mg dL^{-1})	0.71	6.26 (0.17)	4.36–8.22	6.59 (0.22)	5.69–7.87
Phosphorus (mg dL^{-1})	3.05	5.85 (0.37)	2.20–8.90	4.73 (0.67)	2.20–9.40
Ca:P	3.55	1.25 (0.10)	0.53–2.83	1.62 (0.21)	0.84–2.91
Mg (mg dL^{-1})	2.78	11.32 (0.74)	4.52–20.75	9.37 (0.75)	6.37–13.01
Gamma glutamyl transpeptidase (GGT) (U L^{-1})	0.27	6.28 (0.36)	2.00–13.00	5.90 (0.59)	1.00–7.00
Lactate^1 (mmol L^{-1})	0.29	7.14 (0.72)	1.20–17.10	8.30 (1.82)	2.00–19.40
Glucose (mg dL^{-1})	0.60	50.19 (2.34)	24.00–88.00	45.89 (1.49)	43.00–55.00
He^1 (10^6 µL^{-1})	0.11	0.15 (0.03)	0.01–0.94	0.13 (0.02)	0.03–0.27
PCV (%)	2.22	26.22 (1.48)	8.00–38.00	22.60 (2.40)	10.00–37.00
Leucocytes (10^3 µL^{-1})	0.82	43.7 (3.10)	17.5–80.5	50.2 (5.6)	22.2–86.2
Heterophils ^{2} (%)	0.22	71.70 (2.51)	42.00–93.00	70.80 (3.6)	46.00–86.00
Eosinophils ^{2} (%)	5.90*	1.73 (0.45)	0.00–10.00	5.80 (1.76)	0.00–17.00
Lymphocytes^2 (%)	0.68	24.43 (2.41)	5.00-55.00	20.70 (2.62)	6.00–32.00
Thrombocytes^1 (%)	0.80	161.26 (23.04)	17.00–502.00	247.71 (60.52)	35.00–494.00
Monocytes^1 (%)	0.04	1.93 (0.46)	0.00–8.00	2.70 (1.24)	0.00–9.00
Heterophils (10^3 µL^{-1})^1	0.95	31.25 (2.57)	13.42–67.42	34.86 (3.50)	17.70–51.70
Eosinophils (10^3 µL^{-1})	6.89*	0.88 (0.26)	0.00–6.18	3.10 (1.09)	0.00–10.35
Lymphocytes (10^3 µL^{-1})^1	0.26	11.18 (1.56)	1.50–37.61	10.82 (1.93)	2.22–24.15
Monocytes(10^3 µL^{-1})	1.29	0.97 (0.23)	0.00–5.08	1.44 (0.68)	0.00–5.34

5.4 Discussion

The results from this study contribute towards understanding the health implications of FP among juvenile *C. mydas* in the southwestern Atlantic Ocean, including some of the distinguishing physiological features. Monitoring this cohort is a valuable conservation tool because post-recruits have been reported at greater risk of disease than their larger, immature conspecifics; possibly owing to stress associated with their habitat and diet shift (from open-ocean to near shore) and disparate environmental threats (Flint *et al*. 2010). The observations can be discussed with previous assessments according to possible intrinsic and extrinsic variability, and ultimately used to direct future work.

The FP prevalence (~23%) here was lower than that among individuals caught off the more northern Rio de Janeiro (~43 %; n = 246; Tagliolatto 2015) and Espírito Santo (~34 %; n = 640 and 58 %; n = 163; Torezani *et al*. 2010; Santos *et al*. 2010), but greater than to the south, off Santa Catarina (~10 %; n = 82; Proietti *et al*. 2007). These differences might imply a latitudinal environmental gradient, although previous studies have shown FP prevalence can vary substantially in time and space, even among immediately adjacent regions—the reasons for which are not fully understood (Jones *et al*. 2016).

Greater consistency was observed among the general presentation of tumours here and throughout previous regional studies. Supporting this statement, most FP-affected animals had apparent good health, and like Santos *et al*. (2010) and Work *et al*. (2004) we failed to observe a significant difference in BCI among non-afflicted and afflicted *C. mydas*—although the utility of BCI for such assessments is by no means definitive (Work *et al*. 2004).

In terms of evaluated parameters, the significantly lower urea and cholesterol, but elevated eosinophils among FP-afflicted individuals, both conflict and support previous assessments. Specifically, Santos *et al*. (2015) attributed elevated blood urea nitrogen (BUN) in severely FP-afflicted *C. mydas* to greater protein catabolism or dehydration. Off Hawaii, elevated BUN, but lower cholesterol among diseased *C. mydas* were observed concomitant with poorer body condition and starvation (Aguirre *et al*. 1995; Aguirre and Balazs 2000).

Considering the homogenous body condition among *C. mydas* here, any differences in urea and cholesterol might simply reflect divergent metabolic, digestion and absorption of protein and cholesterol in diseased animals (Stacy and Boylan 2014). An alternative hypothesis involves temporal bias in neritic recruitment and spatial dietary segregation. Specifically, during recruitment, juveniles transition from an omnivorous (oceanic) to herbivorous diet with concomitantly lower urea and cholesterol (Whiting *et al*. 2007). Possibly, the FP-afflicted individuals here simply were earlier neritic recruits (longer exposed to the etiological agent and/or environmental factors) than more recently arrived non-afflicted conspecifics (from disease-free oceanic waters) (Ene *et al*. 2005).

Unlike cholesterol or urea, increased eosinophils in tumoured *C. mydas* is more easily attributed to disease. Eosinophils are phagocytic cells particularly involved in destroying parasites (Work *et al*. 1998) and so their elevation might imply ancillary diseases. However, high eosinophils concentrations also occur with tissue repair, cancer, and allergic and antitumor activity in mammals (Carretero *et al*. 2015). No relationships between tumours and immune reaction via eosinophils have been investigated for sea turtles, but these potentially warrant investigation in future research.

Notwithstanding the apparent consistent health among FP-afflicted and non-afflicted individuals, the observed variation above supports ongoing assessments to provide prognostic information about the protracted fate of diseased animals and strategies for minimising impacts globally. One example of the utility of such an approach occurred off Hawaii where *C. mydas* strandings were monitored for 26 years (from 1982) and with a population decline primarily attributed to FP (28% of specimens). The rate of infection stabilised during the mid 1990s, possibly due to: (i) partial herd immunity (to the aetiological agent); and/or (ii) mitigation strategies, involving removing both diseased animals and a tumour-inducing environmental insult involved in the pathogenesis (Chaloupka *et al*. 2008; 2009).

Beyond altered levels of blood parameters, key concerns among *C. mydas* with advanced FP are lesions in the eyes, mouth, cloaca and inguinal and axillar areas which could affect foraging, reproduction and migration (Aguirre and Balazs 2000; Work *et al*. 2004). Other potentially more direct consequences might include greater mortalities owing to fisheries interactions. Specifically, although few data are available (Foley *et al*. 2005; Chaloupka *et al*. 2008), the morphological discontinuities of tumours could increase the possibility of entanglement in gillnets and trawls (both of which are intensely used off South America) and/or the removal time and therefore handling. Considering commercial fishing is a major source of mortality among marine turtles globally, even marginal increases would have considerable negative impacts on populations. Other less obvious consequences of FP include sublethal effects associated with lipid mobilization of bioacumulated contaminants, which could exacerbate the immunosuppression observed in severely tumoured, emaciated animals (Keller *et al*. 2014).

The implications of the various impacts above support FP being considered a threat for *C. mydas* conservation in the southwestern Atlantic Ocean, where the prevalence can reach >50% of individuals in some populations (Santos *et al*. 2010; Zwarg *et al*. 2014). Ongoing studies are required to facilitate a more holistic overview of the various intra- and inter-population effects of the disease among *C. mydas* along with any correlations between environmental factors and anthropogenic activities. Doing so should help define priorities not only for regional, but also global disease mitigation.

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6. CONCLUSÃO

- A prevalência de fibropapilomatose foi semelhante ao longo do período avaliado no litoral do Brasil, porém, os valores variaram significativamente entre estados (latitude), possivelmente devido a co-fatores ambientais;

- Os estados do Rio de Janeiro, Paraná e Santa Catarina apresentaram prevalências da doença semelhantes, porém, menores em relação ao nordeste do Brasil, indicando grandes grupos e objeto de estudo a serem explorados quanto as diferenças/semelhanças para melhor entendimento da fibropapilomatose;

- A avaliação da doença sob o ponto de vista da pirâmide epidemiológica demonstrou ser importante para o entendimento da fibropapilomatose. Dessa forma, fatores ambientais, como variações latitudinais, como também biológicos como o tamanho corpóreo da tartaruga-verde, a caracterização dos tumores e a relação com as estirpes virais, são relevantes para avaliação epidemiológica;

- Os tumores apresentaram alterações compatíveis com fibropapilomas e os diferentes graus de degeneração nas camadas da epiderme reforçam a etiologia viral, assim como a detecção de variantes de ChHV5 e raras inclusões virais, compatíveis com a descrição de “superspreaders”;

- As variantes de ChHV5 e agrupamentos geográficos das mesmas demonstram a complexidade para o entendimento da infecção e capacidade migratória da tartaruga-verde;

- Os parâmetros clínicos laboratoriais são diferentes em populações de tartarugas-verde geograficamente distintas, portanto, além de indicadores do estado de saúde e resposta a doenças (ex. fibropapilomatose), são possíveis indicadores de mudanças de habitat e dieta dos animais.

7. PERSPECTIVAS

A avaliação da fibropapilomatose ainda é escassa em tartarugas-verde resgatadas mortas ou capturadas vivas no Brasil. Dessa forma, são necessárias informações para o melhor entendimento da fibropapilomatose sobre:

- Valores de prevalência;
- Gravidade da doença;
- Análises moleculares e histológicas dos tumores;
- Forma de transmissão;
- Genética dos animais afetados;
- Taxa de regressão e mortalidade da doença.