KÁTIA REGINA GROCH

Interação antropogênica e sanidade de baleias-jubarte (*Megaptera novaeangliae*) na costa brasileira

SÃO PAULO

2014

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Tese apresentada ao Programa de Pós-Graduação em Patologia Experimental e Comparada da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências

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Prof. Dr. José Luiz Catão-Dias

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FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que o Projeto intitulado "Interação antropogênica e sanidade de Baleias-Jubarte (*Megaptera novaeangliae*) na Costa Brazileira", protocolado sob o nº 2212/2011, utilizando 350 (trezentos e cinquenta) Baleias-Jubarte, sob a responsabilidade do Prof. Dr. José Luiz Catão Dias, está de acordo com os princípios éticos de experimentação animal da "Comissão de Ética no uso de animais" da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo e foi aprovado em reunião de 04/5/2011.

We certify that the Research "Anthropogenic interactions and health aspects of humpback whales in the Brazilian Coast", protocol number 2212/2011, utilizing 350 (three hundred and fifty) Humpback whales, under the responsibility Prof. Dr. José Luiz Catão Dias, agree with Ethical Principles in Animal Research adopted by "Ethic Committee in the use of animals" of the School of Veterinary Medicine and Animal Science of University of São Paulo and was approved in the meeting of day 05/04/2011.

São Paulo, 3 de abril de 2014.

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DEDICATÓRIA

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RESUMO

GROCH, K. R. **Interação antropogênica e sanidade de baleias-jubarte (*Megaptera novaeangliae*) na costa brasileira*.*** [Anthropogenic interactions and health aspects of humpback whales (*Megaptera novaeangliae*) in the Brazilian coast]. 2014. 139 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2014.

A exposição das baleias-jubarte (*Megaptera novaeangliae)* aos impactos antropogênicos está aumentando devido à crescente recuperação da população posterior à proibição da caça comercial, associado ao incremento das atividades humanas nas áreas de reprodução e migração da espécie. No entanto, pouco se sabe sobre saúde e processos patológicos em baleias-jubarte no Brasil. O presente estudo investigou o estado de saúde da população de baleias-jubarte que migra sazonalmente para a costa sudeste e nordeste do Brasil, buscando identificar eventuais enfermidades letais e não letais, por meio de análises histopatológicas e estudo de osteopatias, assim como, quantificar as ocorrências das interações antropogênicas prejudiciais à espécie. A avaliação dos efeitos decorrentes das ações antropogênicas foi realizada a partir de imagens obtidas de baleias foto-identificadas em vida livre ao longo de 5 anos (2008-2012), por meio das quais foram caracterizadas as lesões cutâneas e quantificadas as marcas decorrentes de interações antropogênicas, predatórias ou intraespecíficas. Dessa forma, obteve-se uma estimativa dos indivíduos que sobreviveram a emalhes em apetrechos de pesca, colisões com embarcações e/ou interações com predadores. A prevalência das lesões cutâneas foi estimada utilizando-se uma abordagem conservadora, porém sistemática, e poderá ser utilizada como base para o uma avaliação contínua do estado de saúde desta população de baleias-jubarte. A investigação de processos patológicos em animais encalhados foi realizada valendo-se da análise histopatológica dos tecidos de 19 animais encontrados em bom estado de conservação, enquanto as osteopatias foram estudadas por meio da análise macroscópica do tecido esquelético de 49 animais necropsiados. Análise imuno-histoquímica foi utilizada em casos selecionados para pesquisa de Morbillivirus. Os achados histopatológicos demonstraram que grande parte dos filhotes que encalham apresenta sinais de distúrbios neonatais ou perinatais, além de serem suscetíveis a processos infecciosos. O estudo de lesões esqueléticas em animais encalhados demostrou que as baleias-jubarte são suscetíveis a processos degenerativos, infecciosos, traumáticos e malformações do desenvolvimento. Este estudo apresenta uma primeira visão sobre condições sanitárias e processos patológicos em baleias-jubarte no Brasil e pode direcionar os estudos futuros nesta área. Através do monitoramento da ocorrência de lesões não-letais bem como através da investigação dos processos patológicos e causas de morte das baleias-jubarte, este estudo apresenta indicadores das condições do ambiente onde estes animais vivem, podendo contribuir para as ações de manejo e conservação da espécie.

Palavras-chave: Cetáceo. Enfermidade. Patologia. Análise fotográfica. Osteopatologia.

ABSTRACT

GROCH, K. R. **Anthropogenic interactions and health aspects of humpback whales (*Megaptera novaeangliae*) in the Brazilian coast***.* [Interação antropogênica e sanidade de baleias-jubarte (*Megaptera novaeangliae*) na costa brasileira]. 2014. 139 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2014.

The exposure of humpback whales (*Megaptera novaeangliae*) to anthropogenic impacts is increasing due to population recovery following the legal ban on commercial whaling, together with the increment of human activities at the species breeding grounds and migration routes. However, little is known about the health and pathologic processes in humpback whales in Brazil. The present study investigated the health status of the population of humpback whales that seasonally migrate to the southeastern and northeastern Brazilian coast, aiming to identify possible lethal and nonlethal diseases, through histopathological analysis and study of osteopathies, as well as to quantify the occurrence of anthropogenic interactions, detrimental to the species. The evaluation was carried out through image analysis from photo-identified whales in the wild, during 5 years (2008-2012), through which skin lesions and scars were characterized, and anthropogenic, predatory or inter-specific marks were quantified. An estimate on individuals who survived to entanglement in fishing gear, collisions with vessels and/or interactions with predators was obtained. The investigation of pathologic processes in stranded animals performed by means of histopathological analysis of tissues from 19 animals found in good condition, while osteopathies were studied through macroscopic analysis on skeletal tissue of 49 necropsied animals. Immunohistochemical analysis was carried out in selected cases to search for Morbillivirus. The prevalence of skin lesions was estimated using a conservative, but systematic, approach and may be used as the basis for a long term assessment of the health status of this humpback whale population. Histopathological findings showed that most stranded calves had signs of perinatal or neonatal disorders, and are susceptible to infections. The study of skeletal lesions in stranded animals has shown that humpback whales are susceptible to degenerative, infectious and traumatic processes, and malformations. Finally, data obtained in this study present a first overview on health conditions and pathological processes in humpback whales in Brazil and may guide future studies in this area. By monitoring the occurrence of nonlethal lesions, as well as investigating pathological processes and causes of death of humpback whales, this study provides the environmental conditions in which these animals live and can contribute for the management and conservation actions for the species.

Keywords: Cetacean. Diseases. Pathology. Photographic analysis. Osteopathology.

SUMÁRIO

INTRODUÇÃO

1 INTRODUÇÃO

A baleia-jubarte (*Megaptera novaeangliae*) pertence à ordem Cetartiodactyla, subordem ou superfamília Mysticeti, constituída pelas baleias-verdadeiras ou baleias sem dentes (COMMITTEE ON TAXONOMY, 2013). É uma espécie cosmopolita com hábitos costeiros, que realiza migrações sazonais, utilizando as regiões tropicais com águas rasas e mornas para reprodução e cria dos filhotes durante o inverno (NOWAK, 2003). A espécie foi alvo da caça comercial, praticada intensivamente, em especial em meados do século XX. Estima-se que a população foi reduzida a 10% do seu tamanho original (CLAPHAM; YOUNG; BROWNELL JR, 1999), tornando essa atividade insustentável. A caça comercial foi proibida pela Comissão Internacional Baleeira (CIB) no final da década de 60, no entanto a União Soviética caçou ilegalmente quase 100.000 baleias no hemisfério sul até 1973 (CLAPHAM; BAKER, 2009). Ao total, durante o século XX, mais de 200.000 baleias foram caçadas no hemisfério sul (FINDLAY, 2001).

Estimativas apontam que a população de baleias-jubarte que se reproduz na costa do Brasil era de aproximadamente 25 mil animais antes da modernização da caça, e chegou a cerca de 500 animais na década de 1950, ou seja, 2% da população original (ZERBINI et al., 2011a). No Brasil, a caça comercial de baleias foi proibida em 18 de dezembro de 1987, pela Lei Federal nº 7.643. Em anos recentes, a população tem mostrado evidências de recuperação (ANDRIOLO et al., 2010), e em 2006 chegou a 27% do tamanho pré-caça (ZERBINI et al., 2011a). Atualmente a baleia-jubarte consta na lista do IBAMA de espécies da fauna brasileira ameaçadas de extinção, no apêndice I da CITES – Convenção sobre o Comércio Internacional de Espécies Ameaçadas da Flora e Fauna Selvagens, e na categoria de menor preocupação ("*least concern*") da IUCN (União Internacional para a Conservação da Natureza e dos Recursos Naturais).

Apesar da recuperação, pouco se sabe sobre as condições de saúde, ameaças e impactos que esta espécie pode estar sofrendo atualmente, e de que forma os possíveis impactos podem afetar essa recuperação. Com a intensificação das atividades antropogênicas como a pesca e a exploração de óleo e gás natural nas zonas costeiras, o cenário atual nas áreas de ocorrência das baleias-jubarte é completamente diferente do cenário pré-caça, resultando em maior risco de interações prejudiciais à espécie.

Avaliar a saúde de cetáceos em vida livre é uma tarefa difícil, sendo que a maior parte do conhecimento existente provém de animais em cativeiro ou encalhados. Entretanto, recentemente, um crescente número de estudos foi desenvolvido para avaliar e monitorar a saúde dos cetáceos, utilizando como base de dados as imagens obtidas para foto-identificação dos indivíduos (WILSON; THOMPSON; HAMMOND, 1997b; PETTIS et al., 2004; HAMILTON; MARX, 2005; VAN BRESSEM et al., 2007; VAN WAEREBEEK et al., 2007; STEIGER et al., 2008; BRADFORD et al., 2009; NEILSON et al., 2009). A foto-identificação é uma técnica que permite o reconhecimento individual dos cetáceos a partir de marcas naturais e padrões de coloração ou formato da nadadeira dorsal (KATONA; WHITEHEAD, 1981). No caso das baleias-jubarte, o emprego de imagens obtidas de animais foto-identificados permite a investigação de lesões cutâneas, uma vez que a pele das mesmas é predominantemente lisa e escura. Injúrias que promovem solução de continuidade cutânea aparentemente cicatrizam sem melanócitos, gerando cicatrizes esbranquiçadas (ALBERT et al., 1980). Essa característica proporciona um registro persistente das lesões sofridas pelo animal (ROBBINS; MATTILA, 2001)[1].

Evidências de injúrias decorrentes de emalhe em redes ou linhas de pesca (KRAUS, 1990; BRADFORD et al., 2009; NEILSON et al., 2009), colisão com embarcações (KRAUS, 1990; BRADFORD et al., 2009) e/ou predação por orcas (*Orcinus orca*) (STEIGER et al., 2008), entre outras, têm sido inferidas através das lesões e cicatrizes encontradas nas baleias. A ocorrência de desordens dermatológicas com padrões semelhantes à candidíase cutânea, lacaziose e poxvirose, previamente relatadas em golfinhos encontrados encalhados ou mantidos em cativeiro (VAN BRESSEM; WAEREBEEK, 1996), têm sido propostas através da análise de imagens de foto-identificação de cetáceos de diversas espécies (VAN BRESSEM; GASPAR; AZNAR., 2003; BEARZI et al., 2009). Sendo assim, através da avaliação criteriosa das imagens obtidas dos espécimes foto-identificados, é possível caracterizar lesões cutâneas e quantificar as escaras antropogênicas em baleias em vida livre, proporcionando, por exemplo, a obtenção de informações valiosas sobre os indivíduos que sobreviveram a emalhes, predação ou colisões com embarcações. Além disso, este modelo de estudo

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[1] ROBBINS, J.; MATTILA, D. K. **Monitoring entanglements of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine on the basis of caudal peduncle scarring.** Hammersmith, London: International Whaling Commission, Paper SC/53/NAH25 presented to the IWC Scientific Committee (unpublished), 2001.

permite inferir sobre a ocorrência de processos infecciosos cutâneos em mamíferos marinhos e, desta maneira, lançar as bases para investigações futuras nesta área.

Concomitantemente, pelo fato das informações obtidas através da análise das imagens oriundas da foto-identificação não estarem relacionadas aos casos fatais, o exame de animais encontrados mortos nas praias, ou que encalham vivos e acabam vindo a óbito, torna-se parte essencial da avaliação de saúde da população uma vez que pode elucidar aspectos importantes relacionados às causas de morte das baleias-jubarte (WILEY et al., 1995). A preocupação é crescente em função do aumento de doenças infecciosas emergentes que vêm afetando diversas populações de cetáceos ao redor do mundo, como por exemplo, a morbilivirose dos cetáceos, que atinge o sistema nervoso e respiratório além de causar uma diminuição da resistência imunológica, resultando na morte de centenas de animais durante os surtos (DOMINGO et al., 1990; LIPSCOMB et al., 1994; DUIGNAN et al., 1996; KENNEDY, 1998). É possível que a ocorrência de boa parte das doenças emergentes esteja relacionada ao aumento da pressão antropogênica e degradação do habitat. Entretanto, informações sobre processos patológicos e causas de encalhe e morte de baleias-jubarte são escassas na literatura. Isto se deve principalmente pelas limitações durante o exame dos animais, que é bastante prejudicado em função do estado de decomposição em que chegam à costa e dificuldades logísticas.

A revisão de literatura que se segue resume aspectos sobre a biologia das baleias-jubarte com enfoque na população que ocorre em águas brasileiras e apresenta brevemente os objetivos deste estudo, os quais serão desenvolvidos nos capítulos subsequentes.

REVISÃO DE LITERATURA

2 REVISÃO DE LITERATURA

A baleia-jubarte (*Megaptera novaeangliae,* Borowski, 1781) pertence à ordem Cetartiodactyla, que inclui os artiodáctilos e os cetáceos. A espécie possui dois orifícios respiratórios no topo da cabeça e placas córneas chamadas barbatanas no lugar dos dentes, o que caracteriza as baleias-verdadeiras, e a classifica como Mysticeti[2] juntamente com outras 13 espécies de baleias. Os misticetos estão divididos em quatro famílias, sendo que as baleias-jubarte fazem parte da família Balaenopteridae (COMMITTEE ON TAXONOMY, 2013).

O gênero *Megaptera* deriva do grego *mega*, que significa grande, e *ptera*, que significa asa, e refere-se às longas nadadeiras peitorais, que distinguem a baleia-jubarte dos outros balenoperídeos. De fato, desde a cabeça do úmero até a ponta, a nadadeira peitoral chega a medir de 28,3 a 34,1% do comprimento total do corpo, ou seja, o equivalente a cerca de um terço do tamanho do animal (TRUE, 1904). O bordo anterior da nadadeira peitoral, o rostro e as laterais da região mandibular possuem protuberâncias chamadas tubérculos, diferentemente dos outros misticetos (CLAPHAM; MEAD, 1999). As baleias-jubarte possuem 207 a 400 barbatanas pretas ou parcialmente brancas presas em cada lado da maxila. Apresentam 14 a 22 pregas ventrais, características da família Balaenopteridae, que se estendem desde a mandíbula até o umbigo (TRUE, 1904; CLAPHAM; MEAD, 1999). A coloração na região dorsal do corpo é preta em todos os indivíduos, enquanto na região ventral varia entre branco e preto, em vários padrões de combinação (TRUE, 1904; MATTHEWS, 1937; PIKE, 1953; MIKHALEV, 1997). O bordo posterior da cauda é serrilhado e sua porção ventral possui variados padrões de coloração desde toda branca até toda preta, o que distingue uma baleia de outra permitindo sua identificação individual (KATONA; WHITEHEAD, 1981). O formato e tamanho da nadadeira dorsal é bastante variável, desde baixa e arredondada até alta e falcada (KATONA; WHITEHEAD, 1981; CLAPHAM; MAYO, 1990). As fêmeas em geral são maiores do que os machos. As únicas diferenças anatômicas entre machos e fêmeas, visíveis externamente, são a presença de uma protuberância arredondada ao final da fenda genital das fêmeas, chamada de lobo hemisférico, que é ausente nos machos (GLOCKNER, 1983); e a distância entre a fenda genital e o orifício anal, que é menor nas fêmeas (TRUE, 1904). A coluna vertebral das baleias-jubarte é constituída por sete vértebras cervicais, 14 torácicas, 10 ou 11

[2] A classificação taxonômica de Cetacea e Mysticeti encontra-se no momento em avaliação (COMMITTEE ON TAXONOMY, 2013).

lombares e 21 caudais, totalizando 52 ou 53 vértebras. Nove ou dez arcos hemais (ossos *chevron*) articulam-se ventralmente com as primeiras vértebras caudais. A escápula das baleias-jubarte é diferente de todas as outras baleias por não possuir o processo acrômio e apresentar o processo coracóide extremamente rudimentar (TRUE, 1904).

A baleia-jubarte é encontrada nos maiores oceanos, comumente em zonas costeiras ao longo das plataformas continentais, a não ser durante a migração, quando passa por áreas com grande profundidade (DAWBIN, 1966; CLAPHAM; MATTILA, 1990). Se alimentam durante o verão em altas latitudes e no inverno migram para áreas de reprodução e cria de filhotes em águas tropicais e subtropicais, onde geralmente encontram-se associadas a ilhas e sistemas recifais (MACKINTOSH, 1942; DAWBIN, 1966; WHITEHEAD; MOORE, 1982). A única exceção ao padrão migratório sazonal típico é a população que habita o mar da Arábia, que parece alimentar-se e reproduzir-se em águas tropicais (MIKHALEV, 1997).

A reprodução das baleias-jubarte é marcadamente sazonal. As fêmeas entram no estro durante o inverno e os machos exibem um aumento na espermatogênese neste período (CHITTLEBOROUGH, 1958). O período de gestação é de 11 a 12 meses e a maior parte dos partos ocorre no início do mês de agosto do ano seguinte (no caso das populações do hemisfério sul). Há registros de gestação de gêmeos, porém sem confirmação em relação ao sucesso na cria destes. Geralmente nasce apenas um filhote com tamanho entre 4,5 e 5 metros (CHITTLEBOROUGH, 1958). A lactação se estende por cerca de 10 meses e meio, porém a partir do sexto mês o filhote começa a se alimentar de forma independe. O ciclo estral recomeça logo após o nascimento do filhote, quando há perda deste ou após o fim da lactação (CHITTLEBOROUGH, 1958; CLAPHAM; MAYO, 1990). A maioria dos filhotes separa-se da mãe durante ou logo antes do seu segundo inverno, entretanto alguns permanecem associados por dois anos (CLAPHAM; MAYO, 1990; BARAFF; WEINRICH, 1993).

A maturidade sexual é atingida com cerca de 5 anos (CHITTLEBOROUGH, 1965; CLAPHAM, 1992) e a maturidade física é atingida entre 8 e 12 anos após a maturidade sexual. Em geral a fêmea tem um filhote a cada dois anos, porém há registros de gestação anual, inclusive simultânea ao período de lactação (CHITTLEBOROUGH, 1958). Durante a estação reprodutiva os machos cantam, e considera-se que um dos possíveis propósitos deste comportamento seja o de atrair as fêmeas. Porém, sabe-se que o canto também promove a interação com outros machos (PAYNE; MCVAY, 1971; TYACK, 1981). Nessa época grupos formados por vários machos podem exibir comportamento agressivo, disputando por potenciais fêmeas (TYACK; WHITEHEAD, 1983; BAKER; HERMAN, 1984).

No hemisfério sul as baleias-jubarte se alimentam principalmente de *krill,* pequenos crustáceos da espécie *Euphausia superba* (MATTHEWS, 1937). A expectativa de vida é incerta. A idade máxima estimada através da contagem de camadas no tampão laminar do ouvido médio de uma baleia-jubarte foi 48 anos, considerando que as baleias acumulavam duas camadas por ano (CHITTLEBOROUGH, 1965). No entanto, estudos recentes com base na história de avistagens de baleias-jubarte e que após a morte tiveram os tampões do ouvido médio analisados, demonstram que as camadas são depositadas anualmente e que, portanto, a idade máxima estimada até hoje para uma baleia-jubarte é 96 anos (GABRIELE et al., 2010).

A Comissão Internacional Baleeira reconhece sete áreas reprodutivas e seis áreas de alimentação para as baleias-jubarte no Hemisfério Sul. A população que utiliza a costa brasileira como área de reprodução é conhecida como "estoque reprodutivo A" (IWC, 1998). Se concentra principalmente na região do Banco dos Abrolhos (ANDRIOLO et al., 2010), e se alimenta próximo às ilhas Georgia do Sul e Sanduíche do Sul, na região Antártica (STEVICK et al., 2006; ZERBINI et al., 2006; ENGEL; MARTIN, 2009).

As populações de baleias-jubarte têm sido estudadas no mundo inteiro. Parâmetros ecológicos sobre a população de baleias-jubarte que utiliza a costa brasileira têm sido estudados desde o final da década de 80, através de investigações comportamentais, bioacústica, análises genéticas a partir de amostras de pele, e foto-identificação pelo padrão de coloração na região ventral da cauda das baleias-jubarte (eg. MARTINS et al., 2001; MORETE et al., 2003b; MORETE et al., 2003a; MORETE; BISI; ROSSO, 2007b, a; ENGEL et al., 2008; MORETE et al., 2008; ROSSI-SANTOS et al., 2008; SOUSA-LIMA; CLARK, 2008; CYPRIANO-SOUZA et al., 2010; WEDEKIN et al., 2010b), além dos estudos por meio de monitoramento aéreo (e.g. ANDRIOLO et al., 2006; ANDRIOLO et al., 2010) e telemetria satelital (ZERBINI et al., 2006; ZERBINI et al., 2011b) .

Entretanto, o conhecimento sobre a saúde das baleias-jubarte é escasso. Pouco se sabe sobre processos patológicos que afetam animais desta espécie. Casos de infecção parasitária pelo nematoda *Crassicauda boopis* já foram relatados e podem causar arterite e eventual insuficiência renal (BAYLIS, 1920). Cinco casos de alterações ósseas encontram-se descritos na literatura (STEDE, 1994; PATERSON; VAN DYCK, 1996; KOMPANJE, 1999; FÉLIX; HAASE; AGUIRRE, 2007; HELLIER; HUFTHAMMER; LISLEVAND, 2011). Estes casos serão detalhados e discutidos no capítulo 3. Causas de morte de baleias-jubarte geralmente são diagnosticadas quando há lesões proeminentes e/ou visíveis externamente, como marcas por emalhamento em redes de pesca ou colisão com embarcações (WHITEHEAD, 1987; LIEN, 1994; WILEY et al., 1995). Entre as causas naturais de injúrias inclui-se a predação. Existem relatos de baleias-jubarte sendo atacadas por orcas (*Orcinus orca*), porém sem evidência clara de morte da presa (CHITTLEBOROUGH, 1953; WHITEHEAD; GLASS, 1985; FLOREZ-GONZALEZ; CAPELLA; ROSENBAUM, 1994). Tais ataques são registrados apenas ocasionalmente, entretanto cicatrizes deixadas pela marca dos dentes de orcas ou falsas-orcas (*Pseudorca crassidens*) no corpo e na cauda das baleias indicam que as interações são relativamente frequentes (KATONA; WHITEHEAD, 1981). Apesar da morte de adultos ser pouco provável a partir da predação, e este tipo de ameaça ser transitória, os filhotes são aparentemente mais suscetíveis (FLOREZ-GONZALEZ; CAPELLA; ROSENBAUM, 1994). O conhecimento sobre causas de morte relacionadas a interações antropogênicas e interações com predadores será detalhado nos capítulos subsequentes desta tese.

Assim como a maioria dos cetáceos, os encalhes de baleias-jubarte geralmente consistem de apenas um indivíduo por vez, de forma isolada. Há apenas um registro de mortalidade em massa em que 15 baleias-jubarte encalharam mortas em Cape Cod, Massachusetts, Estados Unidos da América, em um período de seis semanas em 1987-1988. A mortalidade foi associada à intoxicação por satitoxina, após ingestão de peixes conhecidos como cavala do Atlântico (*Scomber scombrus*) (GERACI et al., 1989).

Na costa do Banco dos Abrolhos, o encalhe de baleias-jubarte tem sido monitorado de forma sistemática pelo Instituto Baleia Jubarte desde 2002. Os primeiros resultados deste monitoramento são apresentados nos capítulos 2 e 3, entretanto a caracterização da ocorrência e distribuição dos encalhes ao longo da costa estão fora do escopo desta tese.

O objetivo do presente estudo foi investigar o estado de saúde da população de baleias-jubarte que migra sazonalmente para as costas sudeste e nordeste do Brasil, identificar eventuais enfermidades letais e não letais, e quantificar a ocorrência de interações antropogênicas prejudiciais à espécie. Abaixo segue uma breve descrição dos objetivos específicos abordados em cada capítulo desta tese.

O capítulo 1 descreve a avaliação realizada a partir de imagens de foto-identificação obtidas de baleias em vida livre, por meio das quais serão caracterizadas as lesões cutâneas e quantificadas as escaras antropogênicas encontradas, proporcionando informações sobre os indivíduos que sobreviveram a emalhes em apetrechos de pesca, colisões com embarcações e/ou interações com predadores. Este capítulo foi elaborado com a perspectiva de submissão à revista PloS One.

O capítulo 2 descreve alterações histopatológicas em tecidos de baleias encontradas encalhadas na costa brasileira e investiga a presença de Morbillivirus através de análise imuno-histoquímica. Este capítulo foi redigido com vistas à submissão ao periódico *Diseases of Aquatic Organisms* ou *Journal of Comparative Pathology*.

O capítulo 3 descreve as alterações esqueléticas encontradas em baleias-jubarte encalhadas na costa do Banco dos Abrolhos através de análise macroscópica. Este capítulo foi submetido para publicação na revista *Diseases of Aquatic Organisms*.

Este estudo constitui o primeiro esforço sistemático para avaliar o estado sanitário e quantificar a ocorrência de interações antropogênicas prejudiciais à baleia-jubarte na costa brasileira. Espera-se que os resultados deste estudo forneçam importantes indicadores dos desafios naturais e antrópicos que as baleias-jubarte enfrentam e das condições sanitárias do meio onde vivem. Dessa forma, esperamos que os dados aqui apresentados possam contribuir para embasar ações de manejo e conservação da espécie.

CAPÍTULO 1

Survey of skin lesions on humpback whales from the Abrolhos Bank, Brazil, using photographic analysis

3 CAPÍTULO 1 - SURVEY OF SKIN LESIONS ON HUMPBACK WHALES FROM THE ABROLHOS BANK, BRAZIL, USING PHOTOGRAPHIC ANALYSIS

3.1 INTRODUCTION

The exposure of humpback whales (*Megaptera novaeangliae*) to anthropogenic impacts is increasing in Brazil due to the increment of human activities at the species breeding ground (MARTINS et al., 2013). The humpback whales migrate from Antarctic waters to the southeastern and northeastern Brazil looking for the exceptional conditions in the Abrolhos Bank (16°40'–19°30'S, 37°25'-39°45"W) for their reproduction (MARTINS et al., 2001; ANDRIOLO et al., 2006; ANDRIOLO et al., 2010). Evidences indicate the feeding area for this population locate on waters around South Georgia and South Sandwich Islands (STEVICK et al., 2006; ZERBINI et al., 2006; ENGEL; MARTIN, 2009), which are neither occupied by human populations nor are commercially explored with the same intensity as are the coastal waters off Brazil. Thus, the main risks for anthropogenic impacts for whales are possibly concentrated on their breeding area and possibly on their migratory routes (MARTINS et al., 2013).

The remarkable increase in human activities in the reproductive area for humpback whales in Brazil, particularly regarding to ports' modernization, fishing, oil exploration, and traffic of large ships, possibly result in a higher risk of deleterious effects of anthropogenic stressors, such as acoustic disturbance, entanglement in fishing gear and collisions with boats. Large whales are also subject to natural threats, including predators such as killer whale (*Orcinus orca*) and a variety of shark species (HEITHAUS, 2001; WELLER, 2009). All these interactions can favor the development of debilitating conditions of the individual and may affect the reproductive success as well as facilitate the emergence of infectious diseases (VAN BRESSEM et al., 2009). As a consequence, the health and conservation of humpback whales are of great concern.

Analysis of photographs gathered from photo-identification studies is a valuable tool to assess the potential impacts of natural and anthropogenic threats to cetaceans. Fishing gear, vessel collisions, predation and intraspecific agonistic behavior often leave distinctive wounds or scars, which can be identified from photographs (MARTIN; SILVA, 2006; VAN WAEREBEEK et al., 2007; STEIGER et al., 2008; WELLS et al., 2008; BRADFORD et al., 2009; NEILSON et al., 2009; LUKSENBURG, 2014). Consequently, the presence of such marks helps to identify what human activities marine mammals are exposed to and to what degree. On the other hand, the occurrence of dermatologic disorders has been evaluated by means of analysis of photo-identification images of several cetacean species (HARZEN; BRUNNICK., 1997; VAN BRESSEM; GASPAR; AZNAR., 2003; BEARZI et al., 2009; KISZKA; VAN BRESSEM; PUSINERI, 2009). Individual humpback whales can be identified from their ventral fluke pigmentation pattern (KATONA et al., 1979; KATONA; WHITEHEAD, 1981). For this reason it is possible to quantify the prevalence of injuries within a population. Therefore, through careful evaluation of the images it is possible to characterize skin lesions and quantify anthropogenic scars on whales in the wild, providing valuable information on the individuals who survived from entanglement or collisions with vessels and allowing inferences about the occurrence of infectious skin processes (PETTIS et al., 2004; HAMILTON; MARX, 2005; VAN WAEREBEEK et al., 2007; BRADFORD et al., 2009; NEILSON et al., 2009).

Little is known about the health of large whales. Studies on baleen whales have successfully quantified anthropogenic interactions and characterized skin lesions through retrospective evaluation of data and images recorded during photo-identification surveys (KRAUS, 1990; STEIGER et al., 2008; BRADFORD et al., 2009; NEILSON et al., 2009). The aim of this study was to investigate through photographic analysis the skin conditions of humpback whales in the Abrolhos Bank, its main breeding area in Brazil. The specific objectives were to characterize and quantify the occurrence of predator, intraspecific and anthropogenic interactions, and possible cutaneous diseases.

3.2 MATERIALS AND METHODS

3.2.1 Study area

The main study area comprises the region of the Abrolhos Bank (16°40’- 19°30’S and 37°25’- 39°45’W), which is an extension of the continental shelf in the southern Bahia and northern Espírito Santo states, in Brazil. The Abrolhos Bank consists of a vast shallow region encompassing extensive coral reef systems, the biggest and most biologically diverse coral reef concentration of the South Atlantic Ocean (LEÃO; KIKUCHI; TESTA, 2003). This area includes three protected areas: Abrolhos National Marine Park (ANMP), Ponta da Baleia/Abrolhos Marine Protected Area, Corumbau Marine Extractive Reserve. The local economy is mainly based on artisanal fishery, eucalyptus culture and tourism; however, the anthropogenic activities that pose major risk of disturbance in the region include ships traffic and those related to oil and gas exploration (MARTINS et al., 2013).

3.2.2 Data collection

Boat based surveys were conducted in the Abrolhos Bank, during the humpback whales breeding season from July to November in a five year period, from 2008 to 2012. Observations were made from diesel powered boats of 14 – 18 m in length. Boat cruises typically lasted 1 to 6 days and surveys were carried out during the daytime in Beaufort Sea Scale 4 or less, at an average speed of 7 nautical miles per hour. A more detailed description of the adopted methodology for the boat cruises is given in Wedekin et al. (2010b). Whales were photographed with digital SLR cameras equipped with 70 to 400 mm zoom lenses, at distances ranging from a few to several hundreds of meters. When whales were sighted, an attempt to obtain images of the ventral fluke surface and sequences seeking the maximum photographic coverage of the animal's body was made, according to their exposure on the surface (Figure 1). To avoid bias in representing predominantly animals with lesions, photographers were oriented to acquire images of all the whales that came up under the appropriate conditions, disregarding the presence or absence of visible lesions. Individuals were identified using natural marks and pigmentation patterns of the ventral surface of the fluke (KATONA; WHITEHEAD, 1981). Standardized field sheets were used to collect general sighting data, such as date, geographic location, group composition, fluke pigmentation pattern and any information that would help the association of body images to ventral fluke identification of animals (Annex A).

Figure 1 - Sequence of photographs for analysis of humpback whales' skin conditions in the Abrolhos Bank, Brazil

Fonte: (GROCH, 2012).

3.2.3 Image analysis

All photographs taken between 2008 and 2012 of each individual were screened for skin lesions and marks on the basis of the macroscopic aspect. Photographs of individually identified animals resighted within a month period were grouped and analyzed as a single sighting. Photographs judged to have appropriate quality based on distance to the whale, focus, angle and lighting were considered suitable for analysis. Lesions and marks were classified in categories adapted from previous studies on baleen whales (KRAUS, 1990;

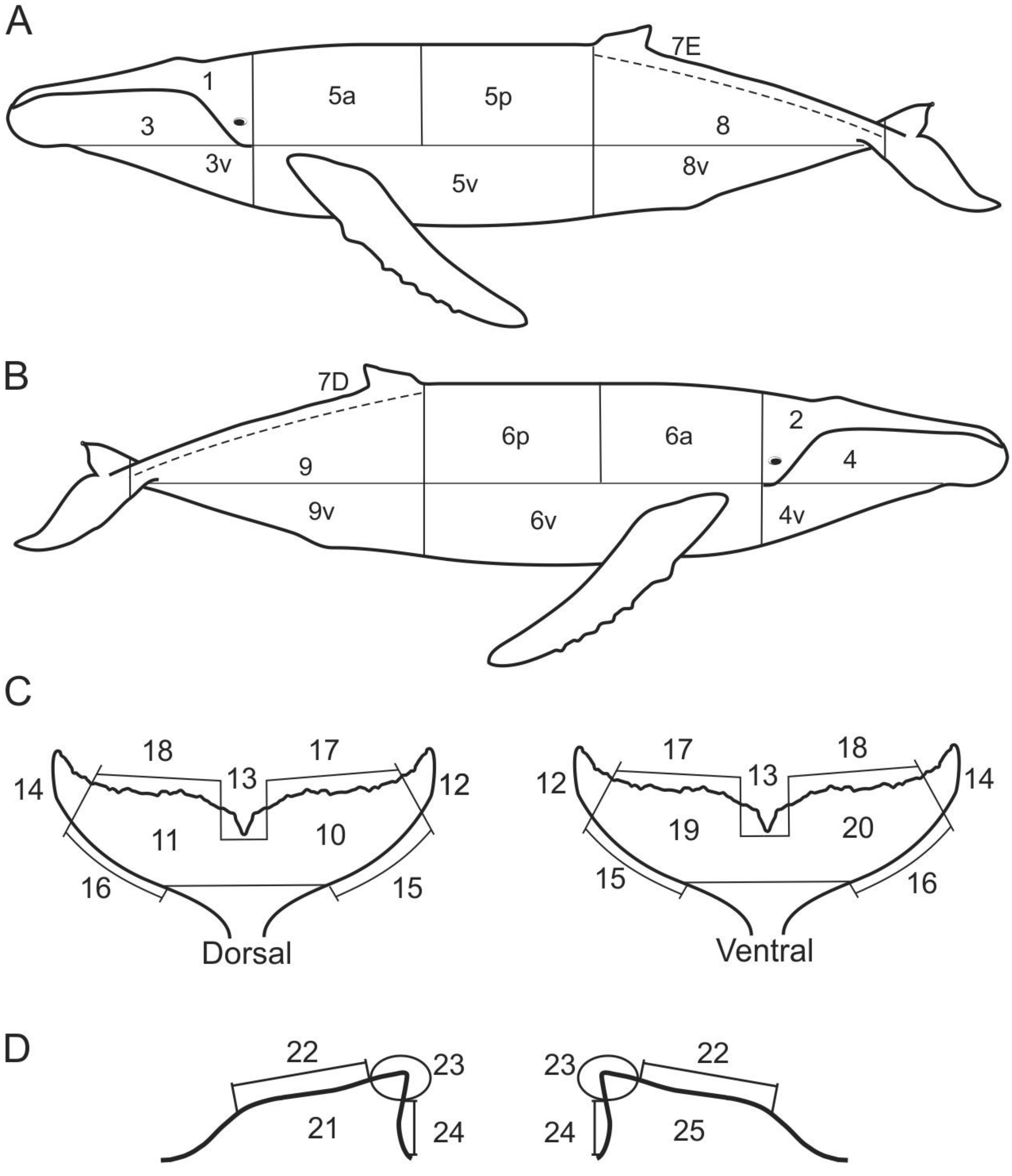
ROBBINS; MATTILA, 2001[3]; HAMILTON; MARX, 2005; STEIGER et al., 2008; BRADFORD et al., 2009; NEILSON et al., 2009) where codes were assigned to the presence or absence of lesions or scars, and to its characteristics (Table 1). The images were grouped by individual and codes were attributed to each of 25 topographic regions of the whale's body (Figure 2), that was adequately photographed. Pectoral fins and ventral region were only occasionally inspected when exposed in the surface and were not included in the systematic evaluation.

Table 1 - Summary of lesion or mark codes used for analysis of humpback whales topographic regions, after Bradford et al., (2009); Robbins and Mattila, (2001)[3] and Steiger et al., (2008)

Code	Definition	Description
N	No lesion/mark	No visible lesion or mark
C	Epibionts	Divided in the following categories: CR – Presence of barnacles; CI – Presence of cyamids.
F	Wound	Divided in the following categories: F1) Open, fresh or partially healed; F2) Completely healed.
I	Intraspecific interaction	Presence of superficial linear marks or scars in several directions
E	Entanglement	Divided in the following categories: E1) Linear or wide scars which appeared to wrap around the feature; E2) At least one visible linear notch or indentation (on the dorsal or ventral peduncle); E3) Extensive tissue damage and deformation of the feature.
V	Vessel collision	Lesion or scar that appears the result of propeller wounds or severe physical trauma.
S	Predation by cookie cutter shark (*Isistius* sp).	Superficial round to oval ulcer or scar.
T	Predation by shark (unknown species)	Presence of tissue damage with a semilunar shape (> 5 cm in diameter).
O	Predation by Killer whale (*Orcinus orca*)	At least 3 parallel linear marks compatible to killer whale rake marks (applied mostly to fluke analysis). Divided in the following categories: O1 - Rake marks associated to damage to the integrity of fluke; O2 – Severe scarring (3 or more sets of rake marks); O3 – 1 or 2 sets of rake marks present.
B	Bulla or nodule	Presence of circumscribed elevations of the skin (> 1 cm in diameter). Number of visible lesions were counted and divided in the following categories: 1) Discrete (1-5 lesions); 2) Moderate (5-10 lesions); 3) Severe: (More than 10 lesions).
U	Ulcers	Cutaneous ulcers (not resembling marks inflicted by *Isistius* sp.)
W	White to gray areas	Presence of discolored or pale areas. Divided in the following categories: 1) Irregular, with variable extension; 2) Coalescent and extensive areas; 3) Multifocal pin-points; 4) Extensive multifocal to coalescing pin-points or irregular patches.
D	Unknown lesion	Lesion of unknown origin that does not fit into any other categories
A	Association of lesions	Used in combination to other codes to denote association of lesions or marks.
P	Partially visible	Used in combination to other categories to denote that the topographic region is only partially visible (partially underwater, or too dark to completely assess scarring).
X	Not visible	The topographic region was not photographed or the image quality was not enough to evaluate the presence of lesions or marks.

[3] ROBBINS, J.; MATTILA, D. K. **Monitoring entanglements of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine on the basis of caudal peduncle scarring.** Hammersmith, London: International Whaling Commission, Paper SC/53/NAH25 presented to the IWC Scientific Committee (unpublished), 2001.

Figure 2 - Diagrams of topographic regions of the humpback whale analyzed for the presence of lesions or marks



Legend. (A) Left lateral view, (B) right lateral view, (C) dorsal and ventral views of tail, (D) left and right views of dorsal fin. The topographical regions are numbered 1 to 25, wherein the number 7 is divided into left and right, the numbers 3, 4, 5, 6, 8 and 9 divided into dorsal and ventral and numbers 5 and 6 anterior and posterior (adapted from BRADFORD et al., 2009).
Fonte: (GROCH, 2014).

Entanglement scar coding followed Robbins and Mattila (2001)[4] and Neilson et al. (2009). High likelihood of past entanglement cases were considered those presenting at least two regions assigned with codes E1 to E3, which are equivalent to codes S3 to S5 used by Robbins and Mattila (2001)[4] and Neilson et al. (2009). Prevalence of killer whale interaction was evaluated through the analysis of ventral flukes of identified humpback whales; however, marks in other body regions were also recorded. The analysis followed the categories proposed by Steiger et al. (2008). Whales with presence of lesions or scars were classified as "positive whales". The analysis and coding of images was conducted by a single person (KRG), as recommended by Neilson et al. (2009), in order to obtain diagnostic consistency. The prevalence of skin conditions was estimated considering the number of positive whales versus the number of examined whales, as well as considering the number of whales with lesions or marks in a specific topographic region versus the number of whales in which the topographic region in question was examined. The sex was determined by genetic analysis of collected skin samples (CYPRIANO-SOUZA et al., 2010). Simple linear regression model was applied to compare prevalence between different years. Chi-square test was used to examine independence between sexes.

3.3 RESULTS

Data were collected during 61 boat based surveys, covering 7272 nautical miles of track line in the Abrolhos Bank and adjacent waters, from 2008 to 2012. During 182 days of surveys, 2765 animals were sighted. We examined over 21000 images from 909 individuals which were photo-identified through their unique ventral fluke coloration patterns, including 7 calves. The sex determination from 151 animals was available, 59% (89/151) were males and 41% (62/151) were females. Fluke photographs were used to systematically score predation by orca or sharks and propeller wounds (n = 909). Images from 68.4% (622/909) of the photo-identified whales were of appropriate quality and coverage of at least part of dorsal region of the body (regions 5a, 5p, 6a, 6p, 8, 9) thus being subjected to complete score protocol for analysis of skin

[4] ROBBINS, J.; MATTILA, D. K. **Monitoring entanglements of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine on the basis of caudal peduncle scarring.** Hammersmith, London: International Whaling Commission, Paper SC/53/NAH25 presented to the IWC Scientific Committee (unpublished), 2001.

conditions. From these 622 whales, 21 (3.4%) were seen in two different years (n=18) or two different boat cruises in the same year (n = 2), and 10 animals had photographs covering the dorsal body region in both sightings allowing evaluation of lesion progression or regression.

Table 2 - Summary of sampling effort and total photo-identified animals during boat expeditions in the Abrolhos Bank, Brazil, from 2008 to 2012

	2008	2009	2010	2011	2012	Total	Annual Mean	SD
No. of boat surveys	17	15	14	7	8	**61**	12	4.4
Days of surveys	57	37	40	24	24	**182**	36	13.6
Effort (Nautical miles)	2342	1523	1588	932	887	**7272**	1454	592.7
Effort (Hours)	402	251	282	153	169	**1257**	251	100.1
No. of groups of whales approached	322	190	248	198	185	**1143**	229	57.9
No. of whales sighted	701	466	607	470	521	**2765**	553	100.4
No. of calves sighted	104	72	121	75	111	**483**	97	22.0
No. photo-identified whales	258	167	173	117	215	**930***	186	53.2

Legend. SD = Standard deviation; * = number include resighted whales.

3.3.1 Skin conditions without apparent relation to anthropogenic interaction

From 622 animals that had images from the dorsal region of the body, 334 (53.7%) had circumscribed, elevated lesions interpreted as bulla or nodules of variable sizes and unbroken integument (Figure 3A). Mean prevalence was 0.54 ± 14.5, peaking at 0.71 in 2011. Lesions presented a positive significant relationship between prevalence and years ($F = 20.2$, $p=0.018$, $R^2=0.84$). From those individuals with sex determination, 51.8% (42/81) were males and 48.1% (39/81) were females (Table 3), with no significant difference between genders ($p > 0.05$). Calves were not observed to have bullae or nodules. In 32.6% (109/334) of the animals the condition was classified as severe (more than 10 lesions visible in a body region) (Figure 3B).The lesions were disseminated, also observed in the flukes, lateral and ventral regions of the body (Figure 4A). In 6% (20/334) of the cases the lesions were associate with skin discoloration (Figure 4B). Bulla or nodules were present in 5 (50%) of the 10 resighted individuals that had dorsal photographic coverage in both occasions. No change in the severity and macroscopic aspect between sightings was noted, except for one individual in which the lesions appear to evolve to discoloration when the animal was resighted three years later

(Figure 5). Depressed, round, normal pigmented lesions of similar size of bullae or nodules were observed in 12.4% (77/622) of the animals (Figure 6A).

Table 3 - Prevalence of cutaneous bullae or nodules in humpback whales from Abrolhos Bank, Brazil

	2008	2009	2010	2011	2012	Male	Female	Total*
No. of PI whales with dorsal body images	149	110	111	73	189	75	58	**622**
No. Positive	53	51	57	52	125	42	39	**334**
Prevalence (%)	35.6	46.4	51.4	71.2	66.1	56	67	**53.7**
B < 5	22	16	15	11	33	10	11	**97**
B 5 ≥ 10	15	22	31	17	47	14	17	**131**
B > 10	16	13	11	24	45	18	11	**106**

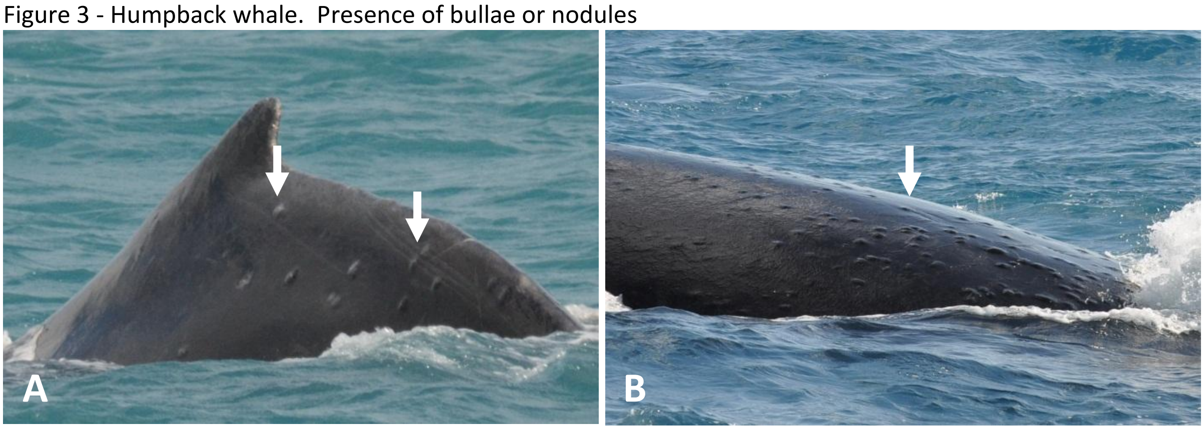
Legend. No. of PI whales = number of photo-identified whales examined; * = numbers exclude resighted whales.

Skin discoloration of variable distribution was observed in the majority of the animals (82.6%, 514/622). From 115 whales with known sex, 64 (55.6%) were male and 51 (44.3%) were female), with no significant difference between them (p>0.05). It was presented as irregular multifocal patches in 69.1% (430/622) of the cases (Figure 7), while velvety white patches in the dorsal fin extending to the dorsal region of the flank were observed in 17.2% (107/622) (Figure 8). Disseminated pale pinpoints were observed in 33% (205/622) of the animals, and in 1.8% (11/622) the pinpoints were intensely white (Figure 9). In 24.2% (124/622) of the cases discoloration patterns seemed to have coalesced leading to focally extensive or diffuse discoloration of the body. Occasionally, pairs of mother and calves were observed with similar pale patterns in the skin (Figure 10).

Multiple superficial linear excoriations running in different directions were on the dorsum of 59.5% (370/622) of the cases. From these, 40.8% (151/370) were intensely marked. Considering these intensely marked individuals, 97% (31/32) of those with known sex were male; and 15% (56/371) presented linear marks associated to pale patches (Figure 11A). Variably healed wounds were observed on salient regions of the body, such as the dorsal fin. Open or partially healed wounds were present in the leading or trailing edges of the dorsal fin in 26.5% (165/622). Partial amputation or deformation of the dorsal fin was observed in 2.9% (18/622) of the individuals (Figure 11B). In 67% (12/18) of these the lesion was completely healed (Figure 12A) and in 33% (6/18) the lesion was recent, open or partially healed (Figure 12B). The leading edges of dorsal fin and fluke had marked linear white scars or healed superficial wounds in 4.3% (44/622) and 23.2% (33/142) of cases, respectively (Figure 13A). Seven animals (1.1%, 7/622) presented open or partially healed wounds of uncertain origin in the sides of the dorsal fin (Figure 13B).

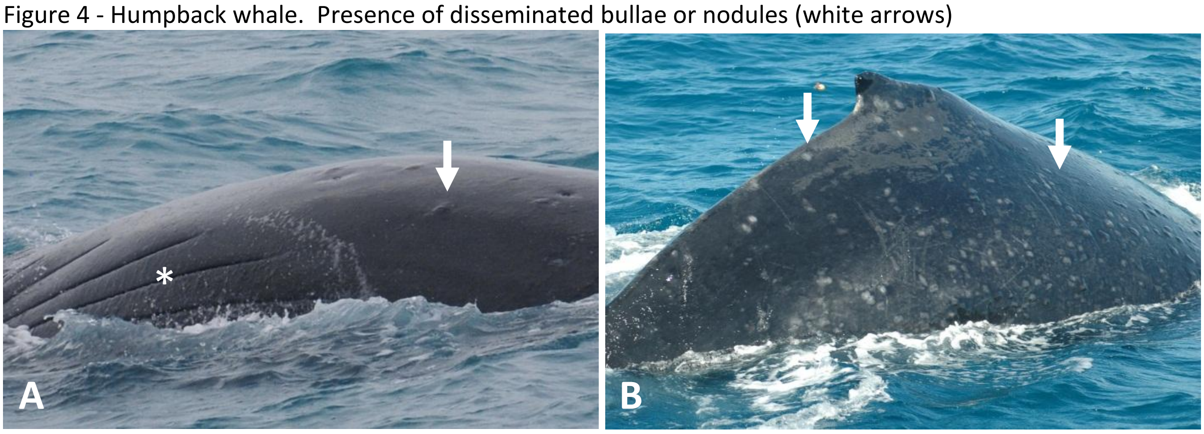
On rostral region, 25.2% (59/228) of animals showed multiple ulcers immediately on top of the natural projections called tubercles (Figure 14A). In 4.8% (11/228) of the cases, barnacles were observed over the tubercles (Figure 14B). Barnacle were also observed in the cervical region, just behind the blow hole in 6% (28/463) of the animals, and in at least 18% (5/28) of these, they were associated to discolored patches in the skin (Figure 15).

Figure 3 - Humpback whale. Presence of bullae or nodules



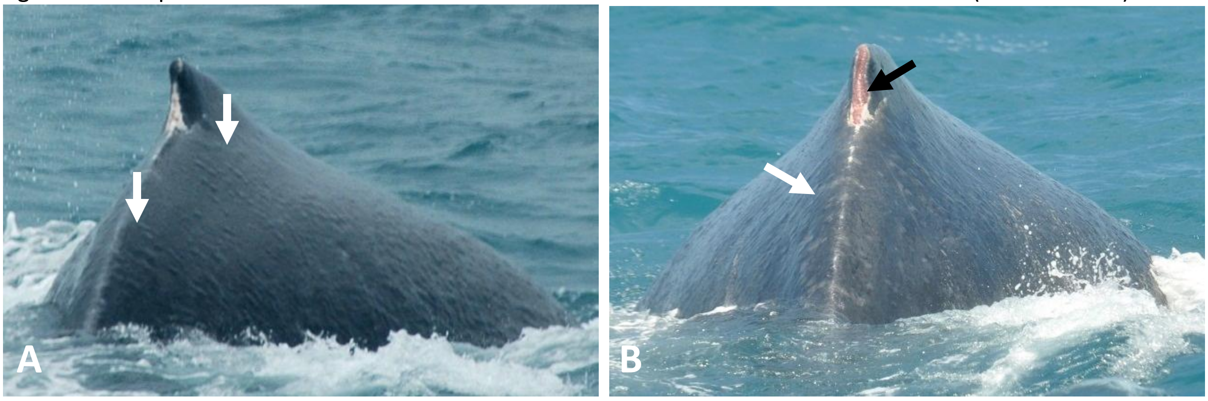
Fonte: (GROCH, 2012). Fonte: (MARCONDES/IBJ, 2011).
Legend A) Case 269, left lateral view. Moderate. B) Case 1254, dorsal view. Disseminated, severe.

Figure 4 - Humpback whale. Presence of disseminated bullae or nodules (white arrows)



Fonte: (GROCH, 2012). Fonte: (NEVES/IBJ, 2009).
Legend A) Case 129, ventral view. Note the ventral groves of the animal (asterisk). B) Case 702, right lateral view. Severe. Note lesions discoloration.

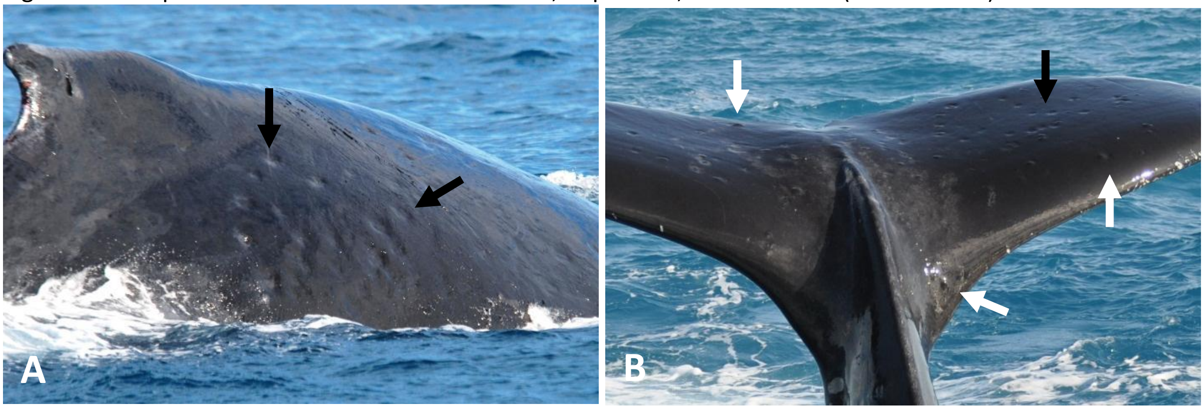
Figure 5 - Humpback whale. Case 544. Presence of severe disseminated bullae or nodules (white arrows)



Fonte: (MARCONDES/IBJ, 2008). Fonte: (GROCH/IBJ, 2011).

Legend A) November 13, 2008. B) October 30, 2011. In the second sighting the lesions were associated to skin discoloration and the animal showed a focally extensive wound in the dorsal fin (black arrow).

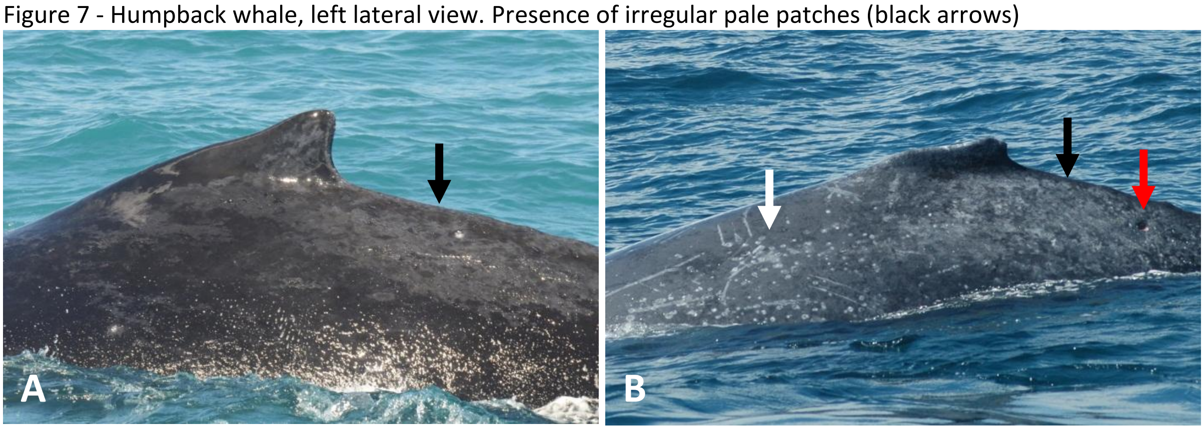
Figure 6 - Humpback whale. Presence of multifocal, depressed, round lesions (black arrows)



Fonte: (WEDEKIN/IBJ, 2011). Fonte: (WEDEKIN/IBJ, 2011).

Legend. A) Case 1326, right lateral view. B) Case 1254, fluke dorsal view. Note the presence of bullae or nodules (white arrows).

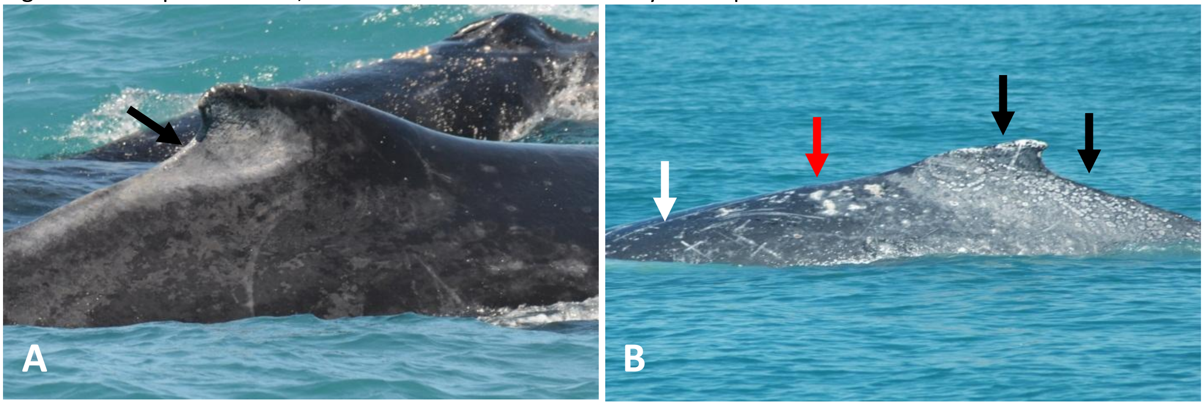
Figure 7 - Humpback whale, left lateral view. Presence of irregular pale patches (black arrows)



Fonte: (GROCH/IBJ, 2012). Fonte: (D'AGOSTINI/IBJ, 2010).

Legend. A) Case 226. B) Case 781. Note irregular white patch (black arrow), pin-point lesions (white arrow) and a round ulcer (red arrow).

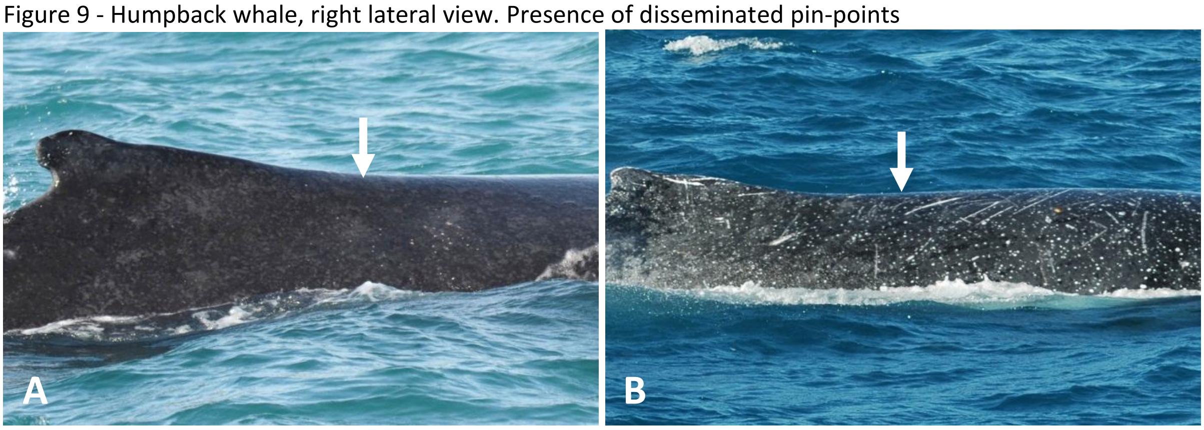
Figure 8 - Humpback whale, lateral view. Presence of velvety white patches



Fonte: (GROCH/IBJ, 2012). Fonte: (GROCH/IBJ, 2010).

Legend. A) Case 238, right lateral view. Focally extensive while velvety white patches in the dorsal fin extending to the dorsal region of the flank (black arrow). B) Case 822, left lateral view. Extensive white velvety areas (black arrow), multifocal ulcerations with proliferative reactive appearance (red arrow), white linear marks and white pin-point lesions (white arrow).

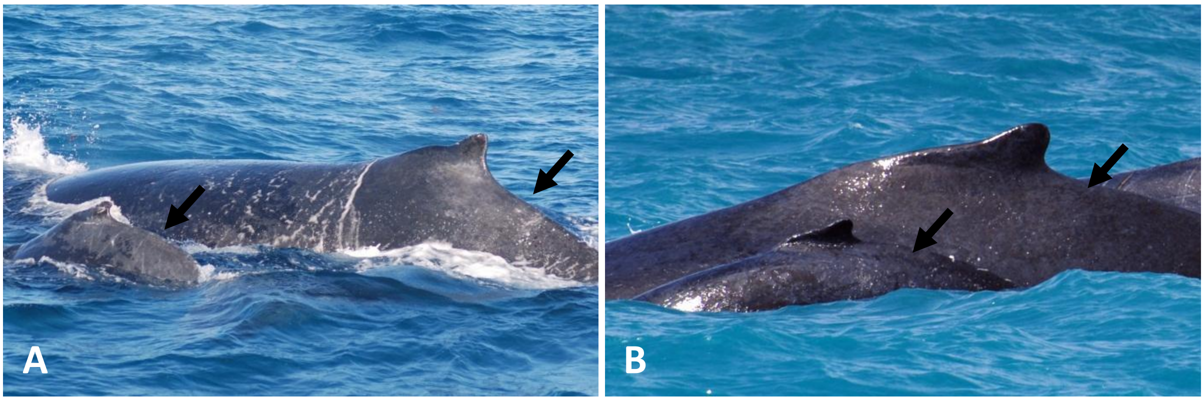
Figure 9 - Humpback whale, right lateral view. Presence of disseminated pin-points



Fonte: (GROCH/IBJ, 2012). Fonte: (MARCONDES/IBJ, 2010).

Legend. A) Case 240, pale lesions. B) Case 805, white lesions.

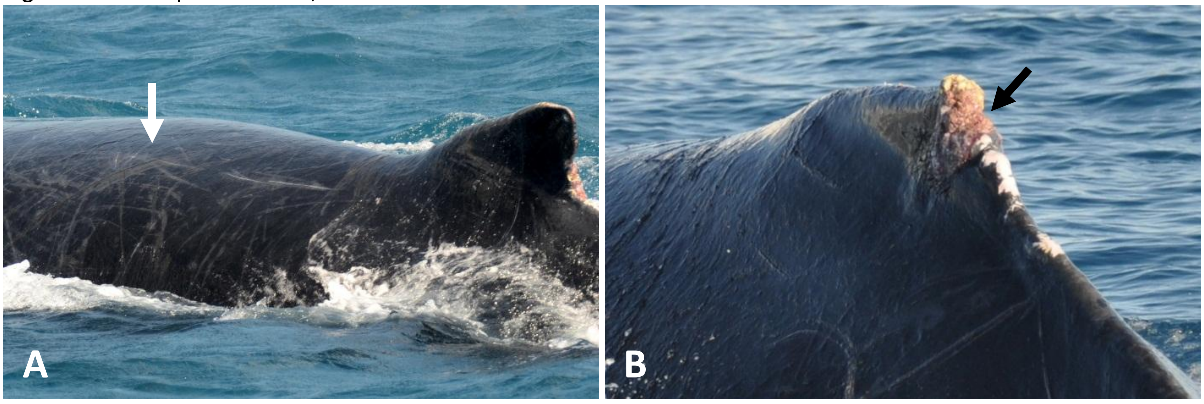
Figure 10 - Humpback whale, left lateral view. Presence of extensive pale patches on flanks of mother and calves



Fonte: (NEVES/IBJ, 2009). Fonte: (GROCH/IBJ, 2012).

Legend. A) Case 698. B) Case 130.

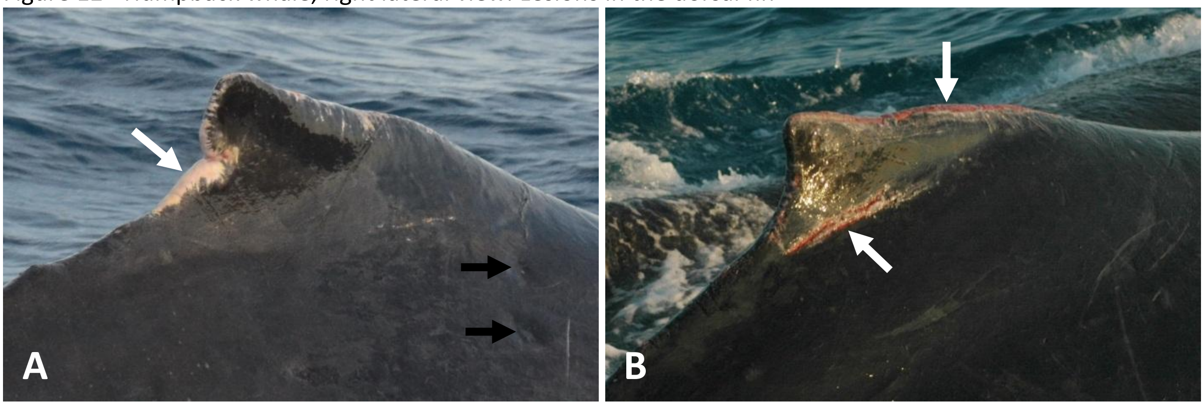
Figure 11 - Humpback whale, left lateral view



Fonte: (GROCH/IBJ, 2012). Fonte: (GROCH/IBJ, 2011).

Legend. A) Case 186. Presence of linear marks in various directions associated to pale patches. Note partially healed wound in the trailing edge of the dorsal fin. B) Case 1250. Partially healed wound in the dorsal fin presenting granulation tissue.

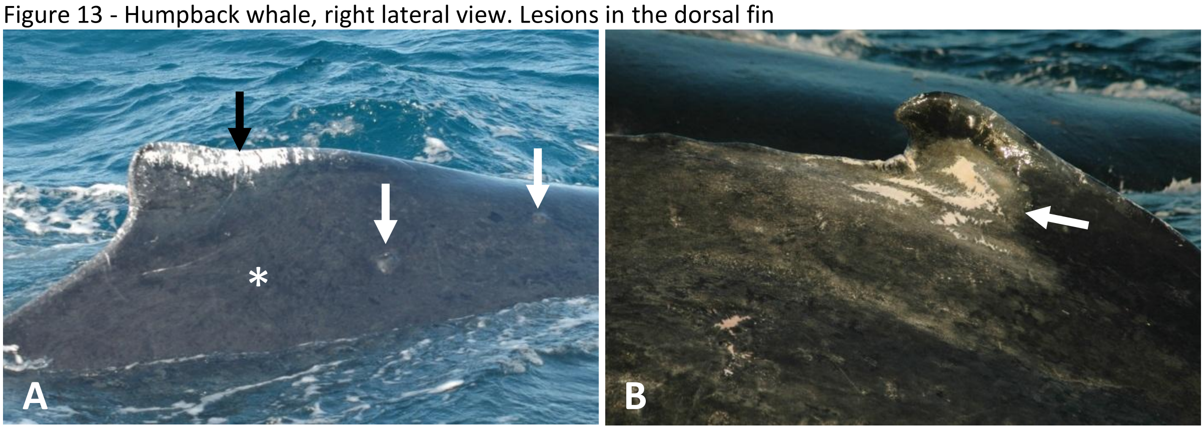
Figure 12 - Humpback whale, right lateral view. Lesions in the dorsal fin



Fonte: (GROCH/IBJ, 2012). Fonte: (ENGEL/IBJ, 2009).

Legend. A) Case 256. Completely healed wounds (white arrows). Note also depressed round marks (black arrows). B) Case 737. Presence of open wounds.

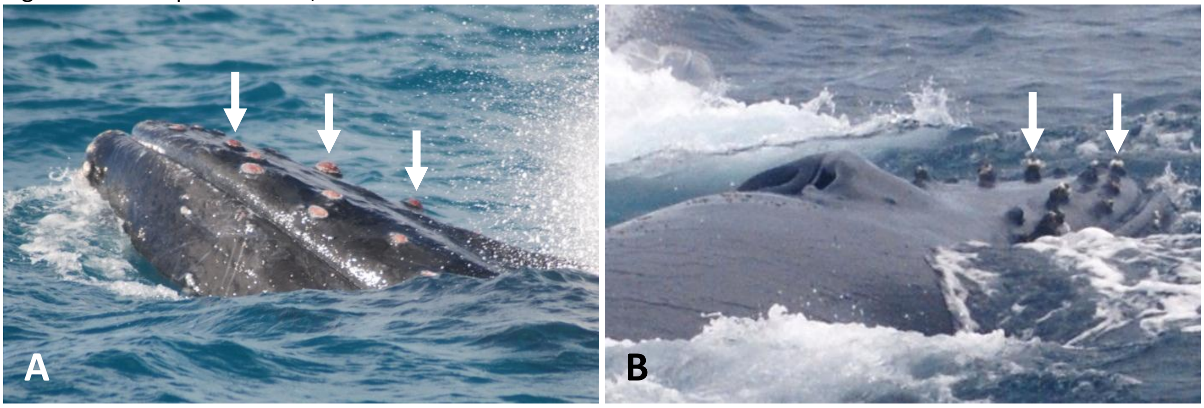
Figure 13 - Humpback whale, right lateral view. Lesions in the dorsal fin



Fonte: (NEVES/IBJ, 2008). Fonte: (WEDEKIN/IBJ, 2008).

Legend. A) Case 406, white scars in the leading edge (black arrow). Note that the animal also presents a diffuse pale skin pattern (asterisk) and bullae or nodules (white arrows). B) Case 518. White scar of unknown origin associated to pale patches (white arrow).

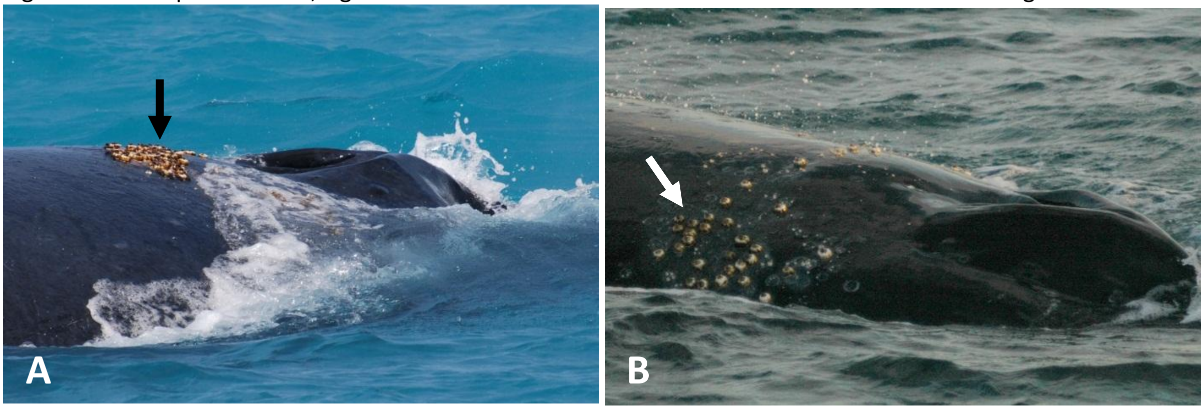
Figure 14 - Humpback whale, rostrum. Lesions in the tubercles



Fonte: (NEVES/IBJ, 2009). Fonte: (GROCH/IBJ, 2012).

Legend. A) Case 675. Presence of ulcerated tubercles. B) Case 119. Presence of barnacles over the tubercles.

Figure 15 - Humpback whale, right lateral-dorsal view. Presence of barnacles in the cervical region



Fonte: (GROCH/IBJ, 2012). Fonte: (NEVES/IBJ, 2008).

Legend. A) Case 130. B) Case 435. Note a pale patch associated to barnacles (white arrow).

3.3.2 Skin conditions associated to anthropogenic interaction

In total, 17.7% (26/147) of individuals with lateral peduncle photographs had a high likelihood of prior entanglement (Figure 16). There was no significant relationship between prevalence and year ($p > 0.05$). Marks suggestive of interaction with fishing gear were also observed in other 9 animals affecting the dorsum, dorsal fin and peduncle (Figure 17A). In one case, a constrictive cable was observed embedded in a wound in the dorsal region (Figure 17B).

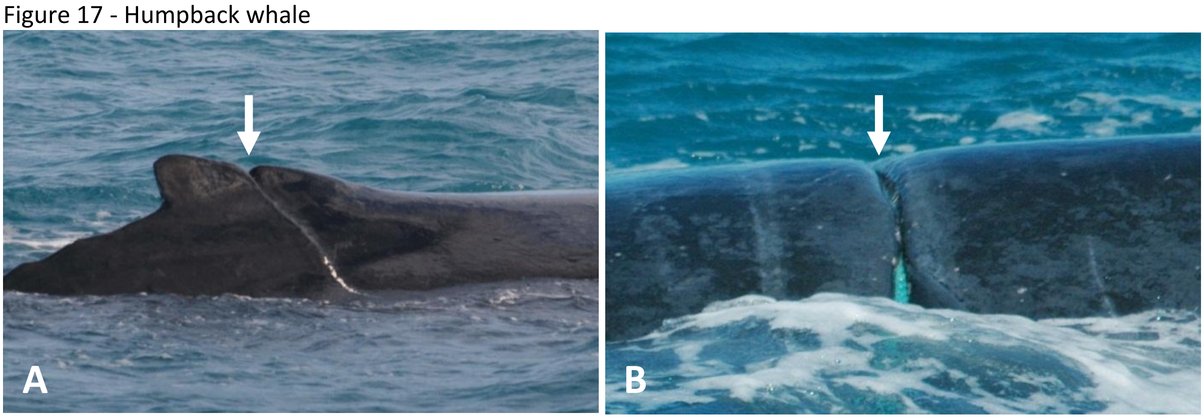
Injuries resulting from collisions with watercraft occur when there is a meeting of the keel or propeller of the vessel with the surface of the animal's body, causing deep and/or serial cuts. When appendages or extremities are affected, the trauma can lead to amputation (ROMMEL et al., 2007). In the present study wounds suggestive of being caused by a propeller were observed in 0.9% (6/622) of the cases subjected to complete score protocol. Considering total number of animals analyzed, 0.4% (4/909) had straight cuts leading to partial amputation of the fluke (Figure 18A), consistent with vessel collision. Combining all cases, a total of 9 animals had propeller wounds (in 1 case both dorsal fin and fluke had lesions). Four were sighted in 2008, two in 2009 and 3 in 2012. In 55% (5/9) of the cases the lesions were located in the dorsal fin (Figure 18B) or dorsum (Figure 19), in 44% (4/9) were in the tail fluke, and in 11% (1/9) of the cases the peduncle was involved.

Figure 16 - Humpback whale, right view of the peduncle

Fonte: (WEDEKIN/IBJ, 2011). Fonte: (GROCH/IBJ, 2011).

Legend. A) Case 1297. Wrapping scars and partially healed wounds dorsally in the peduncle and in the leading margin of fluke. B) Case 1274. Indentation and wrapping wound dorsally in the peduncle. Note the presence of cyamids.

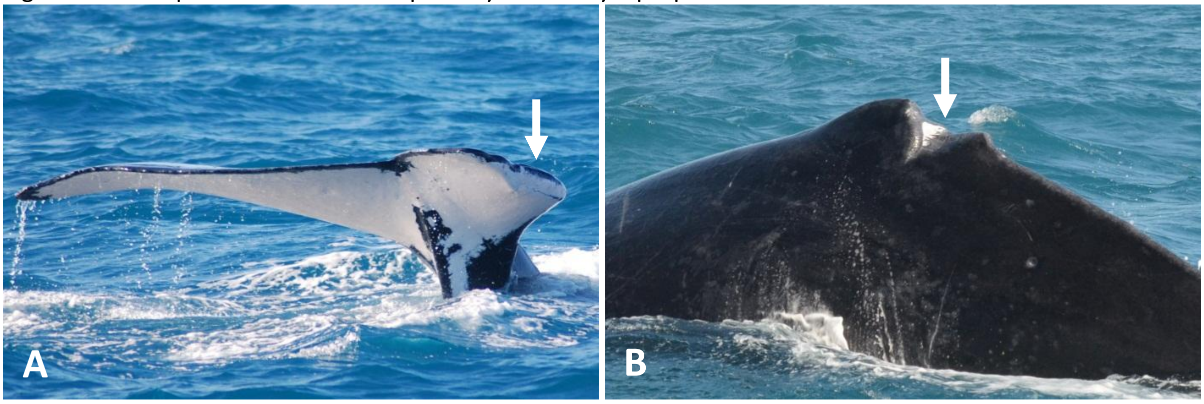
Figure 17 - Humpback whale



Fonte: (GROCH/IBJ, 2012). Fonte: (GROCH/IBJ, 2008).

Legend. A) Case 132, right lateral view. Deformation in the dorsal fin, possibly result from entanglement in fishing gear. B) Case 341, left view of the dorsum. Presence of a green cable deeply embedded in the skin.

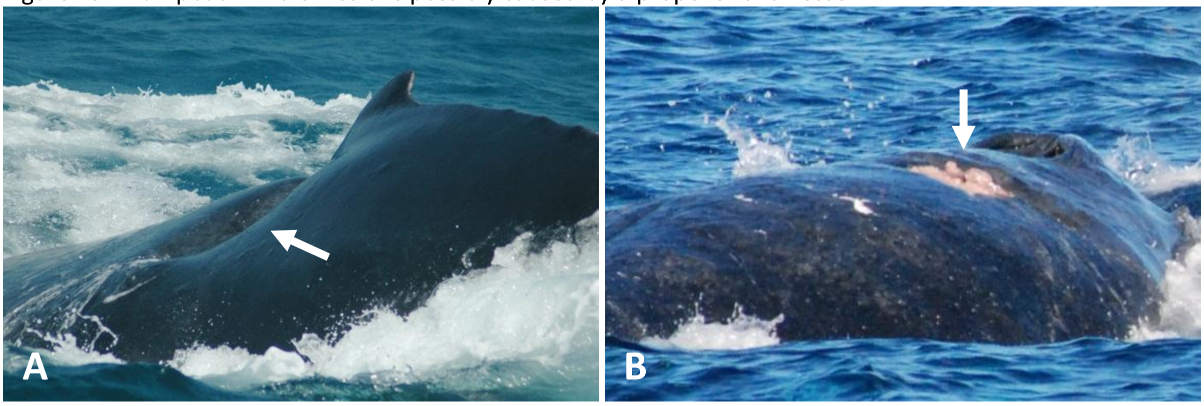
Figure 18 - Humpback whale. Lesions possibly caused by a propeller of a vessel



Fonte: (NEVES/IBJ, 2009). Fonte: (GROCH/IBJ, 2012).

Legend. A) Case 678. Partial mutilation of the fluke with a healed straight cut. B) Case 200, left lateral view. Deep incisive vertical cut in the dorsal fin. Note the exposure of conjunctive tissue (white).

Figure 19 - Humpback whale. Lesions possibly caused by a propeller of a vessel



Fonte: (NEVES/IBJ, 2008). Fonte: (GROCH/IBJ, 2009).

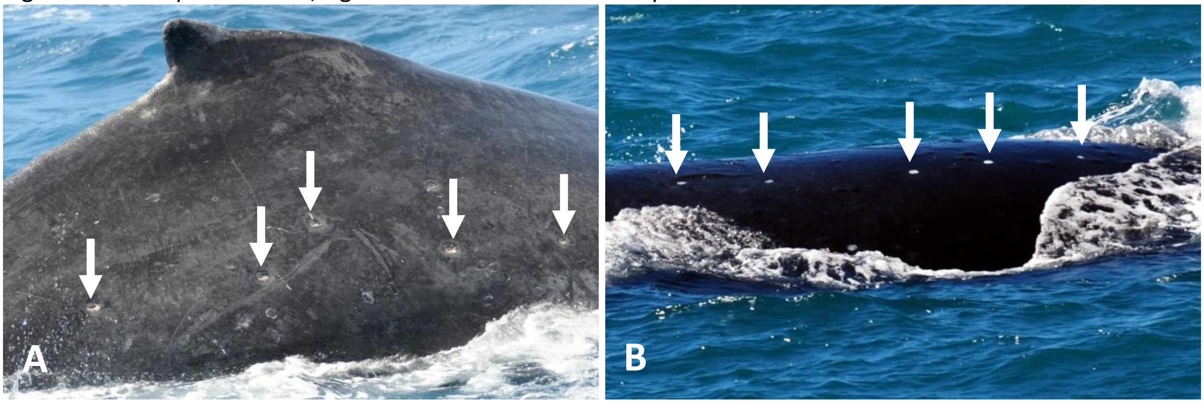
Legend. A) Case 480, left lateral view. Presence of a deep wound leading to deformation of the dorsum. B) Case 606, right lateral view of the dorsum. Presence of extensive linear open cut. Note the exposure of blubber (light red tissue).

3.3.3 Skin conditions associated to predation

A total of 34% (212/622) of the animals showed cookie cutter shark (*Isistius* sp.) wounds, which were observed in all regions of the body, including the rostrum in 17% (36/212) of the cases (Figure 20). The prevalence neither differed among years ($p > 0.05$) nor between sexes ($p > 0.05$), with 40% (30/75) males and 57% (33/58) females. Characteristic crescent-shape wounds, apparently from unknown species of sharks and leading to loss of tissue in the trailing edge of the fluke, were observed in 0.5% (5/909) of the cases (Figure 21).

Marks consistent with killer whale interaction (*O. orca*) are characterized by at least three parallel lines, 2.5 to 5.0 cm, equally distant from each other, consistent with teeth spacing of individuals of this species, and occurs primarily in the margins of the fluke (GEORGE et al., 1994). Overall, 4.7% (43/909) of fluke photographs had unambiguous, discernable parallel marks consistent with killer whale interaction. Of these, 58% (25/43) had 1 or 2 sets of rake marks usually in the extremities of the fluke (Figure 22), and 37.2% (16/43) had damaged flukes with severed tissue associated with the rake marks (Figure 23A). One of these animals presented severely deformed fluke with curled extremities (Figure 23B).

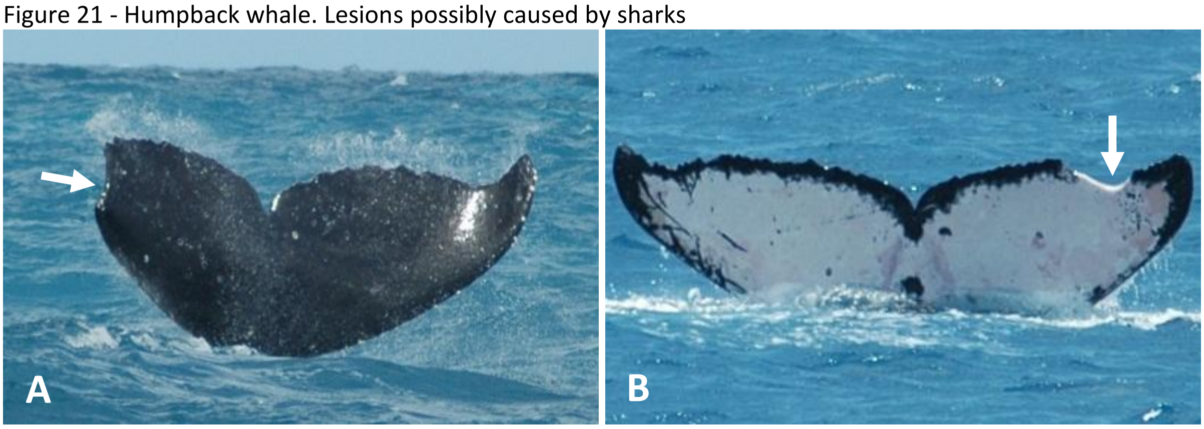
Figure 20 - Humpback whale, right lateral view. Lesions compatible with cookie-cutter shark interaction



Fonte: (GROCH/IBJ, 2012). Fonte: (GROCH/IBJ, 2012).

Legend. A) Case 1399, right lateral view of the dorsum. Presence of multiple partially healed round to oval ulcerations. B) Case 107, multiple white oval scars. Note the presence of bullae or nodules.

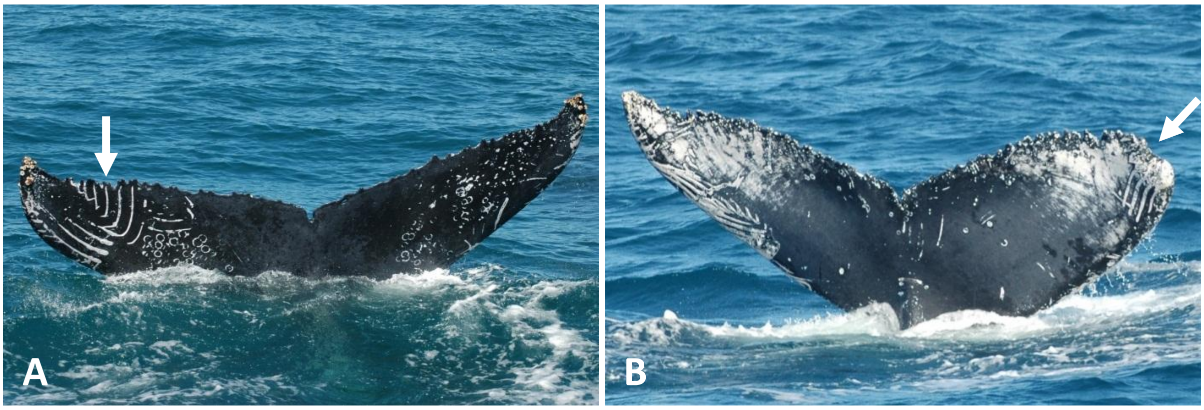
Figure 21 - Humpback whale. Lesions possibly caused by sharks



Fonte: (D'AGOSTINI/IBJ, 2010). Fonte: ENGEL/IBJ, 2008).

Legend. A) Case 863. Injury leading to partial amputation of the fluke. B) Case 533. Injury in the trailing edge of the fluke, with crescent-shape.

Figure 22 - Humpback whale. Ventral view of the fluke showing parallel marks consistent with killer whale interaction



Fonte: (WEDEKIN/IBJ, 2008). Fonte: (NEVES/IBJ, 2008).

Legend. A) Case 328. B) Case 406, intense scarring and damage of the fluke extremity.

Figure 23 - Humpback whale. Latero-ventral view of the fluke showing parallel marks consistent with killer whale interaction

Fonte: (NEVES/IBJ, 2008). Fonte: (NEVES/IBJ, 2009).

Legend. A) Case 559, intense scarring and open wound in the leading edge of the fluke of a calf. B) Case 711, intense scarring leading to damage and deformation of the fluke.

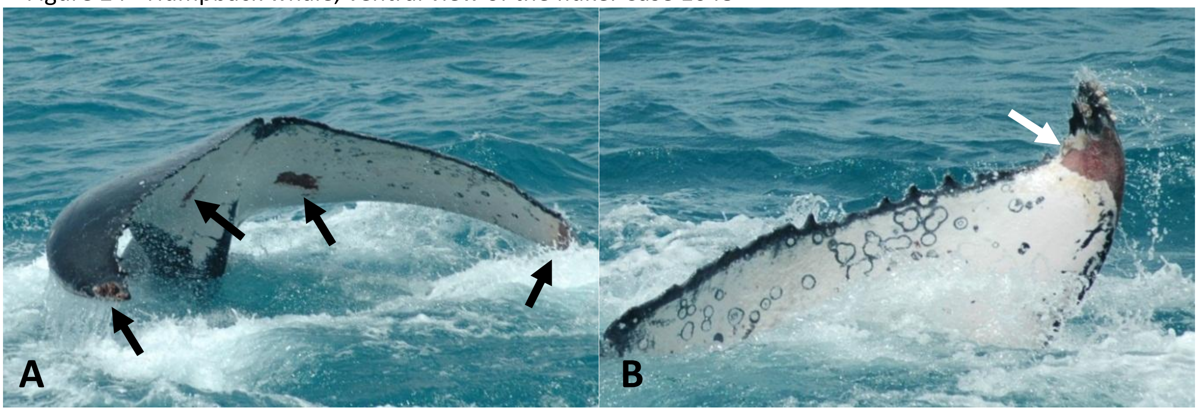
3.3.4 Other noteworthy cases

One animal presented bending fluke with unusual multiple ulcers in the ventral surface of the fluke (Figure 24).

A distinct lesion was noted in the ventral side of the flukes and was coded as “D” (unknown lesion). It appeared as a multifocal to coalescing, red, well demarcated erosions. Since this lesion could only be observed in the white skin, prevalence was calculated including only good quality pictures of the ventral flukes with a pigmentation pattern of more than 60% white (P1 and P2, according ROSENBAUM et al. (1995)). An animal sighted in different occasions let us observe that the lesions are covered by a yellow or orange film within a few days (Figure 25). A total of 14.8% (58/392) of the individuals presented red lesions and/or orange film. The mean annual prevalence was 15.6 ± 4.47, range 10.3 - 22.7%. There was no relationship between prevalence and year ($p > 0.05$).

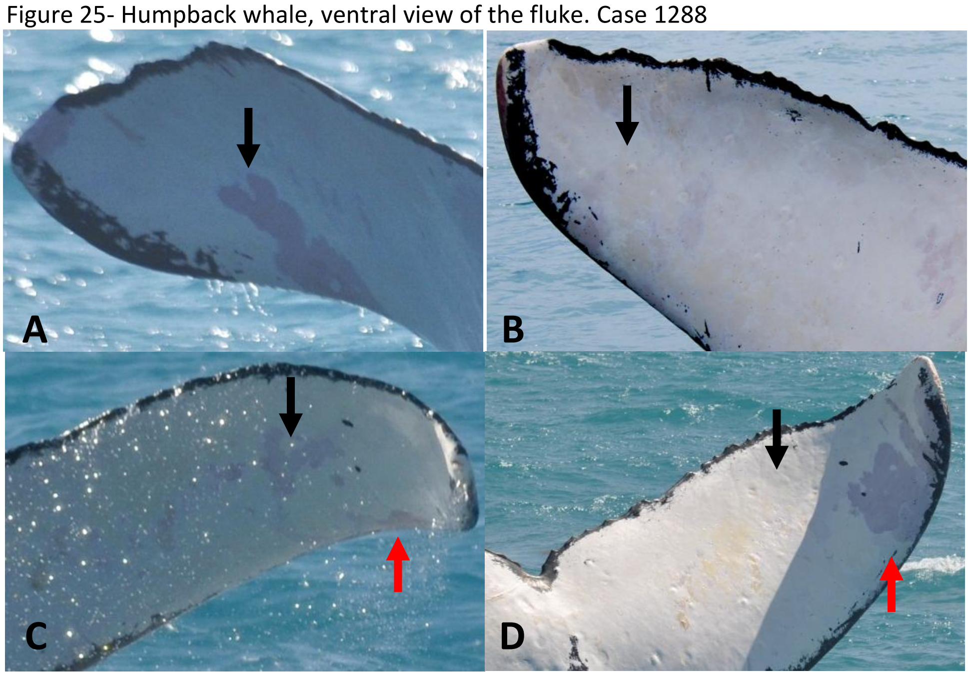
Four animals (without ventral fluke identification) presented focal, round, partially healed, deep wounds dorso-laterally in the body. The wound let a white scar in one occasion and leaved a cavity in the skin and blubber in 3 cases, with granulation tissue observed in 2 cases (Figure 26).

Figure 24 - Humpback whale, ventral view of the fluke. Case 1048



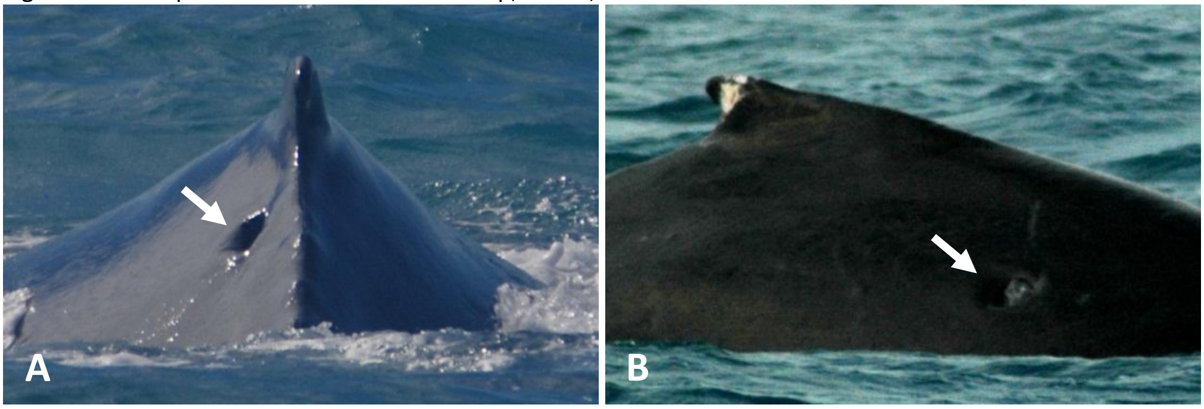
Fonte: (D'AGOSTINI/IBJ, 2010). Fonte: (D'AGOSTINI/IBJ, 2010).
Legend. A) Partial bending of the fluke and multiple ulcerative wounds. B) Loss of epidermis in the extremity of the fluke.

Figure 25- Humpback whale, ventral view of the fluke. Case 1288



Fonte: (GROCH/IBJ, 2011).
Legend. A) Multifocal to coalescing red lesions in the skin. A and C) October 29, 2011. The lesion appears depressed in the epidermis. B) November 8, 2011. Note the lesion is covered by a yellow film; D) October 30, 2011. The lesion appears have expanded (red arrow) and covered by yellow film (black arrow).

Figure 26 - Humpback whale. Presence of deep, round, wounds



Fonte: (GROCH/IBJ, 2012). Fonte: (D'AGOSTINI/IBJ, 2010).

Legend. A) Left lateral view of a whale photographed on August 25, 2012. B) Right lateral view of a whale photographed on August 12, 2010. Note the presence of a pale granulation tissue deeply in the wound.

Table 4 shows a summary of the main skin conditions found.

Table 4 - Main macroscopic findings obtained through the analysis of marks and lesions on the skin of humpback whales in the wild

Main macroscopic findings	**Total positive/ total examined**	**Prevalence (%)**
Skin conditions without apparent relation to anthropogenic interaction		
Presence of bullae or nodules	334/622	53.7%
Discoloration	514/622	82.6%
Intense scarring of the body	151/370	40.8%
Open or partially healed wounds in dorsal fin	165/622	26.5%
Deformation of dorsal fin	18/622	2.9%
Ulcerations at tubercles	59/228	25.2%
Barnacles over tubercles	11/228	4.8%
Barnacles over the cervical region	28/463	6.0%
Red lesion and orange film	58/392	14.8%
Skin conditions associated to anthropogenic interactions		
Entanglement in fishing gear (peduncle and fluke)	26/147	17.7%
Vessel collision (tail fluke)	4/909	0.4%
Vessel collision (dorsal region and peduncle)	6/622	0.9%
Skin conditions associated to predation		
Cookie-cutter shark wounds (*Isistius* sp.)	212/622	34%
Shark wound in the fluke (unknown species)	5/912	0.5%
Killer whale interaction (*Orcinus orca*)	43/912	4.7%

3.4 DISCUSSION

This study is the first analysis of prevalence and detailed morphology description of skin lesions on humpback whales from the south western Atlantic Ocean population. We found a highly prevalent cutaneous lesion characterized by disseminated nodules or bullae (53.7%, ranging from 35.6% in 2008 to 71.2% in 2011), with a significant positive tendency over the years. The origin of the lesions and their significance for the health of humpback whales are unknown. Similar lesions were recently reported in Irrawaddy dolphins (*Orcaella brevirostris*) from Malaysia, India and Bangladesh with prevalence ranging from 2.2% to 13.9% (VAN BRESSEM et al., 2014). Multifocal ovoid raised lesions were reported in 76.4% (52/68) of blue-whales (*Balaenoptera musculus*) off the coast of Isla Grande de Chiloe, Chile (BROWNELL et al., 2007)[5]. However, the lesions were strictly ovoid in shape and of the size of a cookie cutter shark bite, differing from our findings where lesions were also round and of variable sizes. In adult humpback whales, similar lesions were observed in 98% (50/51) of the animals in American Samoa, 73.9% (54/73) of the animals in Hawaii and 4.1% (9/222) of the animals in Gulf of Maine, in the United States (MATTILA; ROBBINS, 2008)[6]. Blister-like lesions have been reported in 17.3% of the North Atlantic right whales (*Eubalaena glacialis*) (HAMILTON; MARX, 2005), however, the lesions were found to progress to rupture of the epidermis, which was not observed in our study.

Van Bressem et al. (2014) reported animals presenting the condition several years, progressing with an increment on the number of visible nodules and not resighted after having developed a high number of lesions. In our study, from the 7 animals sighted in two occasions presenting lesions, only one case showed progression with discoloration associated to these lesions, while the remaining 6 presented unchanged. A humpback whale with visible raised nodules photographed in 1979 in the Gulf of Maine still presented it in 2008 (MATTILA; ROBBINS, 2008). Therefore, this evidence implies a more chronic condition and further photo-

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[5] BROWNELL, R. L.; CARLSON, C. A.; VERNAZZANI, B. G.; CABRERA, E. **Skin lesions on blue whales off southern Chile**: possible conservation implications? Anchorage, USA: International Whaling Commission, Paper SC/59/SH21 presented to the IWC Scientific Committee (unpublished), 2007.

[6] MATTILA, D. K.; ROBBINS, J. **Incidence of raised and depressed ovoid skin lesions on humpback whales of American Samoa.** Santiago, Chile: International Whaling Commission, Paper SC/60/DW3 presented to the IWC Scientific Committee (unpublished), 2008.

identification studies may help to elucidate the impact of cutaneous nodules on long term health of affected humpback whales.

The lesions sampled from a dead Irrawaddy dolphin were characterized histopathologically as fibropapillomas, consisting of hyperplasic epithelium, and thick collagen bundles in the dermis (VAN BRESSEM et al., 2014). It is unknown if the lesions observed in the present study are fibropapillomas, and if a viral etiology is involved as in other fibropapillomatous conditions, as reported in sea turtles (Herpesvirus) and terrestrial mammals (Papillomavirus). Skin bullae were associated to Calicivirus in bottlenose dolphin (*Tursiops truncatus*) (SMITH; SKILLING; RIDGWAY, 1983). Reaction to the adhesion sites of remora (*Entosphenus tridentatus*) is also one of the causes raised for this kind of injury in whales (MATTILA; ROBBINS, 2008)[7], however, viral, bacterial, fungal infection, allergic reaction or neoplastic diseases cannot be ruled out.

A high percentage of whales (82.6%) presented lesions with a pale or lighter aspect compared to the normal skin. It is not known if the discoloration observed in our study represents different stages of a single lesion. In many cases they co-occurred and in some cases the irregular patches seemed to transition to pinpoints and to extensive or diffuse discoloration of the skin, however, further studies are necessary to clarify this aspect. White lesions were observed in 51.7% of North Atlantic right whales (HAMILTON; MARX, 2005). Skin lesions similar to the irregular patches and white velvety lesions reported in our study were observed in small cetaceans from South America (VAN BRESSEM et al., 2007) and California coast (MALDINI et al., 2010). Irregular gray patches were observed in one (1.47%, 1/68) blue whale and described based on macroscopic appearance as "tattoo-like skin lesions" suggesting a possible viral etiology related to poxviruses (BROWNELL et al., 2007)[8]. Pathological conditions of undefined origin with similar patterns to cutaneous candidiasis, lacaziosis and poxviruses were previously reported in stranded cetaceans or dolphins maintained in captivity (DUNN, 1982; VAN BRESSEM; WAEREBEEK, 1996). In the present study, we did not find lesions that clearly suggest a specific infectious agent. However, the etiology of conditions such as bulla or

[7] MATTILA, D. K.; ROBBINS, J. **Incidence of raised and depressed ovoid skin lesions on humpback whales of American Samoa.** Santiago, Chile: International Whaling Commission, Paper SC/60/DW3 presented to the IWC Scientific Committee (unpublished), 2008.

[8] BROWNELL, R. L.; CARLSON, C. A.; VERNAZZANI, B. G.; CABRERA, E. **Skin lesions on blue whales off southern Chile**: possible conservation implications? Anchorage, USA: International Whaling Commission, Paper SC/59/SH21 presented to the IWC Scientific Committee (unpublished), 2007.

nodules, irregular pale patches and multifocal white pinpoints are still to be elucidated and its significant prevalence in the studied population may be of concern.

The coalescing erosions found in the ventral fluke of 15.6% of the humpback whales in this study are new to the literature and the causes are unknown. Orange films have been reported in small odontocetes (WILSON; THOMPSON; HAMMOND, 1997a; VAN BRESSEM et al., 2007; BEARZI et al., 2009; MALDINI et al., 2010; RIGGIN; MALDINI, 2010). Diatoms or other epifauna embedded in the skin are the most likely cause of the yellow or orange coloration. When comparing bottlenose dolphins from the same population using different areas, it is suggested that a lower prevalence occurs in warmer waters (with temperatures ranging from 13.8°C to 20°C). Despite we do not have data from the feeding grounds where the animals experience lower temperatures, we present evidence that orange films are formed during the period that humpback whales are in the Abrolhos Bank, where the temperatures range from 22°C to 24°C in the winter (MARTINS et al., 2001).

During the breeding season, humpback whales constitute mating groups of three to eight whales that can temporarily aggregate forming groups of more than 12 animals. When males dispute for a female, typical surface activity with agonistic behavior, such as investing and avoiding, can be observed. Greater physical contact between the animals leading to several superficial cutaneous injuries or marks may result from these interactions. Because body appendices are prone to injury during intraspecific interaction, superficial skin wounds or scars found in the fluke, dorsal fin and protuberances such as the rostral tubercles were most likely a result from intraspecific interaction. The amount of scarring may indicate social rank and the intensity of aggressive interactions an animal has been exposed during its lifetime (SCOTT et al., 2005). The majority (60%) of the humpback whales presented excoriations of different degrees. From those of known sex that were intensely marked, the majority was male (97%). This finding corroborates behavior observations of mating groups (TYACK; WHITEHEAD, 1983). Discoloration of the skin has been observed in association to excoriations and possibly reflects the skin response to superficial injuries.

Humpback whales are commonly colonized by various epizoonts. Sessile crustaceans (family Cirripedia), the acorn barnacles (*Coronula diadema* and *Coronula reginae*), are commonly found attached to humpback whales fluke, pectoral fins and ventral surfaces (FERTL, 2002). Barnacles attached to the tip of tubercles and dorsally in the cervical region, observed in 4.8% and 6% of the whales, respectively, are not usually described in the literature. It is possible that its attachment in the neck is facilitated by the low water flow in that region, especially in cases where individuals exhibit a lower mean swim speed, as expected for mother and calf pairs. The presence of these epibionts over tubercles may be facilitated by the irregular surface generated at the ulcerated skin. One may consider also that the deployment of these barnacles due to rubbing during male competition could also result in damage to the skin.

Characteristic wounds resulting from interaction with fishing artifacts are generally linear and contour a body surface, leaving indentations or compression set of the filament or cable over the region (ROBBINS; MATTILA, 2004)[9]. For systematic non-lethal entanglement estimates, photographs of the lateral view of caudal peduncle, taken in a parallel and slight forward approach of each whale during fluke-up dives, were selected. Scar or marks coding were used according to previously validated protocols with cases with known history of entanglement (ROBBINS; MATTILA, 2001)[10], which include evaluation of dorsal and ventral distal peduncle and leading margins of the fluke. However, since not all humpback whale entanglement cases involve the caudal peduncle, all available images of individual whales were also examined for the presence of fishing gear or entanglement scars. It is important to note that while scars and indentations on the leading edge of the fluke and dorsal peduncle are believed to be probably the result of human activities such as fisheries, scars on the leading edges of fluke and dorsal fin with no lesions on the peduncle were considered a result from intraspecific interaction.

The percentages of photoidentified whales presenting evidences of previous anthropogenic interaction (17.7% for entanglement through peduncle analysis and 0.4% for vessel collision through fluke analysis) were similar to those determined for western grey whales (*Eschrichtius robustus)*, estimated in 18.6% and 2.0%, respectively (BRADFORD et al., 2009). The prevalence of collision with vessel found in hunted Bowhead whales (*Balaena mysticetus*) was 1% (GEORGE et al., 1994). In contrast, even considering the most conservative estimates, other baleen whale studies found comparatively higher prevalence. For example, 61.6% and 6.4% of photo-identified North Atlantic right whales (*Eubalaena glacialis*) presented

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[9] ROBBINS, J.; MATTILA, D. K**. Estimating humpback whale (*Megaptera novaeangliae*)entanglement rates on the basis of scar evidence.** Provincetown: Final report to the US National Marine Fisheries Service, order number 43ENNF030121 (unpublished), 2004.

[10] ROBBINS, J.; MATTILA, D. K. **Monitoring entanglements of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine on the basis of caudal peduncle scarring.** Hammersmith, London: International Whaling Commission, Paper SC/53/NAH25 presented to the IWC Scientific Committee (unpublished), 2001.

scars from entanglement and ship strikes, respectively (HAMILTON; MARX; KRAUS, 1998)[11]. Regarding other humpback whale populations, estimates for entanglement rates of photo-identified individuals were 56% in the Gulf of Maine and 52% in the northern southeast Alaska (ROBBINS; MATTILA, 2001[12]; NEILSON et al., 2009).

The majority of the entanglement scars were detected on evaluation of lateral view of caudal peduncle and tail fluke (26 cases). Considering all images available from body regions, only two cases showed evidence of entanglement in the dorsal region. Therefore, this study corroborates the finding that the caudal peduncle and fluke are adequate target regions for a systematic evaluation and comparison to other studies. On the other hand, the majority of vessel collisions wounds or scars were found in the dorsum and dorsal fin followed by the tail fluke and peduncle, as observed in other studies (BRADFORD et al., 2009). The presence of a vertical incisive scar on the top of a dorsal fin, probably the result from a propeller hit was reported in killer whale (VISSER; FERTL, 2000) and a false killer whale (*Pseudorca crassidens*) from Southern Caribbean waters (LUKSENBURG, 2014). It is known that collisions between whales and vessels can cause severe injuries and are often fatal. Therefore, the fact that little evidence of marks resulting from boat collisions was observed may indicate that there is a low incidence of whale and vessel encounters or that the majority of whales do not survive after collision with boats (KRAUS, 1990).

The case of chronic entanglement found in our study, in which the whale had a rope embedded in the skin, may be of concern. Despite the animal apparently did not show restrictions to swimming, it is known that the gear that remains attached to the whale's body can increase drag by towing accessory gear and substantially affect the energy budget of an entangled large whale through time (MOORE; HOOP, 2012). Furthermore, there are cases where laceration and consequent infection lead to death by secondary bronchopneumonia (CASSOFF et al., 2011). Cases reported in North Atlantic right whales show that entanglement can persist for multiple years; however, in most severe cases entangled right whales tend to die over periods of about 6 months (CASSOFF et al., 2011; MOORE; HOOP, 2012). Unfortunately no further sightings were reported for this case.

[11] HAMILTON, P. K.; MARX, M. K.; KRAUS, S. D. **Scarification analysis of North Atlantic right whales (*Eubalaena glacialis*) as a method of assessing human impacts.** Boston: Report to the Northeast Fisheries Science Center, National Marine Fisheries Service, Contract No. 46EANF60004 (unpublished), 1998.

[12] ROBBINS, J.; MATTILA, D. K. **Monitoring entanglements of humpback whales (*Megaptera novaeangliae*) in the Gulf of Maine on the basis of caudal peduncle scarring.** Hammersmith, London: International Whaling Commission, Paper SC/53/NAH25 presented to the IWC Scientific Committee (unpublished), 2001.

Rates of individuals that survived to anthropogenic interactions are expected to vary between populations and may reflect differences of the anthropogenic pressure in each geographic region. Our data suggest lower rates on entanglement marks (17.7%) and vessel collisions (0.4%) when comparing with the highly threatened North Atlantic right whales and similar rates when comparing to western grey whales, indicating that the conflict does exist. Given that the study covers animals that survived such interactions, and that in many cases these interactions are fatal to whales (KRAUS, 1990; ROBBINS; LANDRY; MATTILA, 2008) especially in case of vessel collisions, it is fair to assume this is a conservative estimate, but still an important parameter for the initial survey of the threats to this population. Furthermore, the exploitation of the Brazilian coast is increasing and if the activities are not controlled and ordered, the scenario may quickly change. Part of the main breeding site of the species in the Brazilian coast is located in protected areas (Abrolhos National Marine Park, Ponta da Baleia/Abrolhos Marine Protected Area, Corumbau Marine Extractive Reserve), however the area range used by a humpback whale is extremely large (WEDEKIN et al., 2010b), therefore the contribution of these protected areas to minimize the incidence of anthropogenic interactions may be limited.

Shark-inflicted wounds and scars are, in many cases, the only evidence of interactions between sharks and cetaceans. The frequency of such wounds has been used as an indirect measure of the threat that sharks pose to dolphins and whales (HEITHAUS, 2001). The cookie cutter sharks (*Isistius* spp.) are opportunistic, epipelagic, small sharks of the Dalatiidae family that occur on the continental slop and are known to molest cetaceans, pinnipeds and oceanic fishes. When they feed, may cause oval or circular injuries in the skin of whales, known as cookie cutter wounds (HEITHAUS, 2001). Depressed ovoid lesions consistent with wounds caused by cookie cutter sharks were found in 67.3% of humpback whales in American Samoa, 61.2% in Hawaii and 0.5% in Gulf of Maine (MATTILA; ROBBINS, 2008)[13]. In our study, at least 34% of the whales had wounds likely caused by cookie cutter sharks and 0.5% had part of the tail fluke damaged by sharks of larger sizes of unknown species.

Craterous eruptions have been reported in the North Atlantic right whales with a papillomatous core (MOORE et al., 2004; HAMILTON; MARX, 2005). Similar proliferative

[13] MATTILA, D. K.; ROBBINS, J. **Incidence of raised and depressed ovoid skin lesions on humpback whales of American Samoa.** Santiago, Chile: International Whaling Commission, Paper SC/60/DW3 presented to the IWC Scientific Committee (unpublished), 2008.

reaction was observed in 2 individuals in our study that had single deep wounds possibly caused by puncturing artifacts such as harpooning or tag implantation (Figure 26).

About five percent of the humpback whales photo-identified in the Abrolhos Bank are affected by killer whale predation attempts. It is unknown if these interactions occur in the feeding or in the breeding area, or even during migration. Despite killer whales are not often sighted in the humpback whales main concentration area in the Brazilian coast, injuries found in the fluke of a calf (Figure 23A), born in that year, demonstrates that killer whale attacks occur in the wintering ground. In fact, most attacks appear to occur when whales are calves (CLAPHAM, 1996; NAESSIG; LANYON, 2004; MEHTA et al., 2007) and may be considered a source of calf mortality (STEIGER et al., 2008). Killer whale rake marks rates represent unsuccessful attacks, however, it may reflect the degree of predatory pressure in a given population (STEIGER et al., 2008). The rates found in our study were lower than those found in North Atlantic humpback whales in the feeding grounds (range 5 – 20%, mean 15%) and wintering grounds (range 6 - 31%, mean 17%) (STEIGER et al., 2008), and for the western North Atlantic humpback whales (mean 14%)(KATONA et al., 1988). In a study analyzing cutaneous marks in 195 bowhead whales hunted in Alaska (Bering Seas, Chukchi and Beaufort) between 1976 and 1992, George et al. (1994) found a prevalence of 4.1% (8/195) of animals with scars attributed to the interaction with killer whales. Baldridge (1972) described the attack of a killer whale to a gray whale (*Eschrichtius robustus*) where the blubber from ventral region, between the genital and neck portion was consumed, indicating that it is possible that this may be a preferred area of predation. Moreover, Rice and Wolman (1971) reported that gray whales examined during hunting seasons on the Saint Lawrence island had killer whale interaction marks in the pectoral fins and fluke, suggesting that killer whales try to immobilize the prey damaging the fins. Therefore, the estimated occurrence of interaction with killer whales obtained in the present study may be underestimated as it was difficult to assess the pectoral fins and the ventral region of the animals. It is also important to consider that the scars observed in this study may represent only harassment of animals and ineffective attempts to predation, as commented by George et al., (1994). However, considering the specie's potential powerful fluke, killer whales would not submit themselves to substantial risk associated to attacks on humpback whales without the benefit of a fair amount of success. Cases of killer whale and other marine mammals killed by large whales by hitting with the flukes have been reported (ESCHRICHT, 1866; MAZZONE, 1987). For this reason, we agree with Steiger et al.

(2008) that these encounters are likely predatory attacks. The lower prevalence found in the present study may be explained by the hypothesis that: 1) killer whales are much less abundant in the south western Atlantic; 2) killer whales have a broader diet in this area, in comparison to areas assessed in other studies. Also, if one of the major selective advantage of migration for pregnant baleen whales is to reduce the risk of killer whale attacks on newborn calves (CORKERON; CONNOR, 1999), it may have been successful and it is a contributor factor for this humpback whale population recovery, since the numbers are increasing (ANDRIOLO et al., 2010).

There is no evidence that the lesions described in this study are indicative of a fatal condition for humpback whales, but they may represent sublethal conditions that can act synergistically impacting the overall health of the individuals. In fact, skin homeostasis disruption incites inflammatory reactions, raising energy requirements. Injuries also may generate continuity solutions which may serve as a gateway to opportunistic microorganisms, which ultimately may cause infection of internal organs or tissues leading to general debilitation in a long term (ACKERMANN, 2013).

Characterizing and quantifying skin conditions through photographs have some difficulties and limitations inherent to the method. Photo quality, lighting and angle can significantly hinder the interpretation (MATTILA; ROBBINS, 2008)[14]. Estimates presented here likely underestimate the true proportion of humpback whales that have experienced anthropogenic interactions, predation or cutaneous diseases. Although only good quality photographs were selected to the analysis, image coverage was not comprehensive and homogeneous for every individual whale. Images were limited to the dorsal region of animals, precluding analysis of ventral body, which are known preferred regions for predation (RICE; WOLMAN, 1971). Also, the feature targeted for photo-identification excludes the portion of the body found ideal for evaluation of entanglement related lesions or scars (JOHNSON et al., 2005). It is also necessary to consider that not all previous events may leave recognizable scars and that some of the scars or wounds observed are inconclusive if caused by predation, entanglement or vessel collision. Therefore, our results offer conservative estimates of the actual scenery for the health of this humpback whale population and must be interpreted with

[14] MATTILA, D. K.; ROBBINS, J. **Incidence of raised and depressed ovoid skin lesions on humpback whales of American Samoa.** Santiago, Chile: International Whaling Commission, Paper SC/60/DW3 presented to the IWC Scientific Committee (unpublished), 2008.

caution since the exploitation of natural resources has been intensified along the Brazilian coast leading to an increment of the anthropogenic pressure over the marine ecosystem.

The characterization and quantification of anthropogenic impacts such as fisheries, vessel interactions and other health parameters of cetacean populations are recommended in the Brazilian Action Plan for Conservation of Aquatic Mammals (ICMBIO, 2011). Furthermore, diseases are important factors in the recovery of previously exploited populations and should be taken into consideration in action plans for the conservation of the species. Here we address these health parameters of humpback whales based on the analysis of photographs taken from 2008 to 2012. This study constitutes the first overview of skin conditions of a live population of large whales in the Brazilian coast. By determining the main patterns of skin lesions in this population of humpback whales we provide baselines for continuing evaluation of their health status. The skin conditions may be used as indicators of environmental health where the humpback whales live and contribute to guide management and conservation actions for the species.

3.5 ACKNOWLEDGMENTS

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CAPÍTULO 2

Histopathological findings in humpback whales stranded along the Brazilian coast

4 CAPíTULO 2 - HISTOPATHOLOGICAL FINDINGS IN HUMPBACK WHALES STRANDED ALONG THE BRAZILIAN COAST

4.1 INTRODUCTION

Humpback whales (*Megaptera novaeangliae*) occur in all oceans of both hemispheres and migrate between winter breeding grounds and summer feeding destinations (CLAPHAM, 2009). The southwestern Atlantic Ocean population migrates annually from the feeding areas in Antarctic waters to the Brazilian coast to breed and nurse their calves in the first months of their lives. Major concentration occurs in the Abrolhos Bank (16° 40’−19° 30’ S, 37° 25’−39° 45’ W), an enlargement of the continental shelf (MARTINS et al., 2001; ANDRIOLO et al., 2006; ANDRIOLO et al., 2010). Population size was estimated at 10,160 (95% CI: 6,607-17,692) whales in 2011 (JULIÃO, 2013). Strandings have been recorded along the whole Brazilian coast and efforts to gather information on pathological conditions and cause of death through post-mortem examination have increased in the last decade. To date, a total of 191 humpback whales have been single stranded from 2004 to 2013 in the Abrolhos Bank and adjacent waters, where the majority (~55%) was calves[15].

Investigation of cause of strandings and death is necessary to provide clues on the natural challenges and anthropogenic impacts these animals face. However, information on pathological changes and causes of mortality of humpback whales is scarce. Post mortem examination is extremely difficult, especially in large and decomposed carcasses. Most information available is limited to cases with massive and/or prominent lesions and externally visible injuries, such as entanglement in fishing gear and collision with vessel. In a study of 38 humpback whales which stranded in the eastern cost of United States (US) between 1985 and 1992, 20 animals were sufficiently preserved and examined to investigate the cause of death. In 60% (12/20) of the cases there were signs of anthropogenic factors that might have contributed or been directly responsible for their death. From these 30% (6/20) were attributed to ship strike, 25% (5/20) to entanglement in fishing gear and one animal presented wounds and marks compatible with both conditions. The remaining 8 cases (40%) were attributed to a unspecified natural condition; however, details such as macro or microscopic

[15] Obtained from database of Instituto Baleia Jubarte (unpublished data).

findings were not provided (WILEY et al., 1995). In Ecuador, 4 humpback whales adults and calves stranded between 1994 and 2002 had marks or remnants of gear suggesting entanglement in fishing nets, other 2 calves were by-caught (FELIX et al., 1997; SCHEIDAT et al., 2000; ALAVA et al., 2005), and one had signs of predation by shark or killer whale (ALAVA et al., 2005). In Colombia, from 24 strandings registered between 1986 and 2000, 58% (14/24) of the animals presented signs of anthropogenic interaction, of which 10 whales were entangled in fishing gear and 3 individuals were killed by vessel strikes. One animal exhibited marks consistent with a hunting attempt and in 1 case the cause was considered natural (not specified). In the other 9 cases the cause of death could not be determined (CAPELLA ALZUETA; FLOREZ-GONZALEZ; FERNANDEZ, 2001). A few strandings were registered in Netherlands between 2003 and 2006, including a by-caught calf, a juvenile with a rope deeply embedded around the head which lead to strangulation, and a juvenile female presumably hit by a ship's propeller (CAMPHUYSEN, 2007). In Queensland, Australia, two humpback whale calves stranded alive, in 1989 and 1991, and subsequently died presenting a large wound in the lateral of the thorax and several excoriations along the body, both consistent with shark predation (PATERSON; DYCK, 1991; PATERSON; QUAYLE; DYCK, 1993).

The only event of humpback whales mass stranding occurred in a feeding area in Cape Cod Bay and Nantucket Sound, US, between November 1987 and January 1988. In this case, 14 whales died after consuming Atlantic mackerel (*Scomber scombrus*) containing saxitoxin, which is a neurotoxin that blocks the entry of sodium ions to nerve and muscle cells (KOGURE et al., 1988). The toxin was detected in the kidneys, livers and stomach contents. Histopathologic analyses on 3 animals revealed no significant lesions (GERACI et al., 1989). Tumors reported for the species include cerebral lipoma and papilloma in the tongue (PILLERI, 1966; STOLK, 1952[16] apud GULLAND; LOWENSTINE; SPRAKER, 2001, p. 525).

In Brazil, a total of 58 humpback whales were recorded stranded in the southeastern region from 1981 to 2011. The cause of stranding was consistent with entanglement in fishing gear in 3 cases (2 dead calves and one juvenile that was released alive). Bacteriological survey of 3 whales stranded alive retrieved Vibrionaceae (7 species) and Aeromonadaceae (5 species) agents. Samples were collected from blowhole, lung, eye, tongue, mouth, and anus (MOURA et al., 2013). In 1987 a 4.7m female calf was reported to have been caught incidentally in a net set about 700m from shore at Vila Velha, Espírito Santo state (BARROS, 1991). In 2007 a

[16] STOLK, A. Some tumors in whales. **Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen**, v. 55, p. 275-278, 1952.

humpback whale calf was found close to shore alone and exhibiting an unusual behavior, repeatedly diving to the bottom, keeping the rostrum towards the sand and no avoidance to people approaches. The animal was not sighted in the following days. One week later, a decomposed carcass of a humpback whale calf was found with propeller wounds and was thought to be the same animal (NETO et al., 2008). Despite increasing effort on carcass examination, most of the times lesions are not externally evident and the diagnosis is impaired by decomposition and limitations regarding logistics and size. Therefore, any opportunities to gather good quality samples for histopathology are of great value. The aims of this study are to describe pathological changes and investigate potential causes of death of humpback whales stranded along the Brazilian coastline between 2004 and 2013, through histopathological analysis.

4.2 MATERIALS AND METHODS

The main study area comprise approximately 500 km of coast line at the Abrolhos Bank and adjacencies, between municipalities of Belmonte-BA (15°44S; 38°53W) and Santa Cruz-ES (20°00S; 40°09W). Data from stranded animals, other than that which we obtained (IBJ), included necropsy reports and formalin fixed tissues provided by other institutions that are part of the national stranding network: Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (AQUASIS); Fundação Mamíferos Aquáticos (FMA), Centro de Recuperação de Animais Marinhos (CRAM), Universidade Federal do Rio Grande (FURG); Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal Rio Grande do Sul (UFRGS).

The majority of whales necropsied were found dead on the beach after being washed ashore. Live stranded whales that could not be rescued and subsequently died or were euthanized were also investigated. Necropsy was carried out using a standardized protocol according to Geraci and Lounsbury (GERACI; LOUNSBURY, 2005). Selected tissues were collected during necropsy and fixed in 10% buffered formalin for histologic and immunohistochemical analyses. Fixed tissue samples were trimmed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for examination by light microscopy. Additional histochemical techniques i.e., Periodic Acid Schiff (PAS), Gram, Reticulin and Congo Red (PROPHET et al., 1992) were carried out to better characterize specific lesions.

For immunohistochemistry, a monoclonal antibody against the nucleoprotein of Canine Distemper Virus (MoAb CDV-NP, VMRD, Inc., Pullman, WA, USA) known to react with cetacean morbillivirus was used as primary antiserum. Immunohistochemical analysis was performed on selected samples of brain, spinal cord, intestinal, pulmonary and lymphoid tissues according to Raga et al. (2008).

The information obtained from postmortem examination and collected tissues varied according to field conditions, size and decomposition state of the animals. Three age categories were used, calf <8 m, juvenile 8−11.6 m, and adult >11.6 m, based on the standard body length for the species (MAZZUCA; ATKINSON; NITTA., 1998). The nutritional status of each animal was classified as good, moderate or poor based on dorso-axial muscular mass, prominence of ribs, scapula or axial skeleton, and amount of intrathoracic and abdominal fat. The state of decomposition was classified as very fresh, fresh, moderate autolysis, advanced autolysis or very advanced autolysis (GERACI; LOUNSBURY, 2005). Whenever possible, causes of death were classified as anthropogenic or natural (non-anthropogenic), according to Arbelo et al. (2013).

Cardiac blood and swabs from blowhole and pulmonary fluid were collected from 1 individual (case 345), and submitted for hematological analysis and microbiological culture, respectively.

4.3 RESULTS

A total of 191 whales stranded at the Abrolhos Bank region between 2004 and 2013. Tissues from 37 animals were examined by light microscopy. From these, 11 were of appropriate quality for histopathologic analysis and included in this study, while 24 were too decomposed or lacked samples from the vital organs. Samples from another 8 whales were collected in the northeastern coast (Ceará [n=2, 03°32’S/38°48’W; 02°53’S/41°16’W] and Sergipe state [n=4, 10°40’S/36°44’W; 11°16’S/37°14’W; 11°14’S/37°13’W]) and in the southern coast of Brazil (Rio Grande do Sul state [n=2, 29°41’S/49°58’W; 30°15’S/50°14’W]). The final set of 19 animals included in this study comprised 11 (58%) males, 7 (37%) female and 1 whale of unknown sex. The majority (n=15, 79%) were calves, 3 (16%) were juvenile and 1 (5%) was adult. Fifteen (79%) animals stranded alive, 3 of which were euthanized, while 4 (21%) were found dead. Details for individual cases are presented in tables 5 and 6.

A few relevant notes on history and macroscopic findings of selected cases may be mentioned: case 254 presented a history of exposure to sun along 1 day of stranding which lead to a severe extensive loss of skin due to sun burn. Case 578 had hemothorax and severe hemorrhage associated with two recently fractured ribs, without evidence of bone repair; case 333 presented an abnormally heavy infestation by whale lice (*Cyamus* sp) covering more than 60% of the skin and severe infectious spondylitis in two caudal vertebrae (case detailed in chapter 3); case 482 had intracranial hemorrhage; case 520 had superficial cutaneous lesions possibly caused by contact with fishing nets and ropes; cases 397 and 470 presented a poor body condition with axial muscle atrophy; blood-tinged frothy fluid was frequently present in the airways. Hematological analysis of cardiac blood from case 345 showed low hemoglobin values (9.5 g/dL), hematocrit (29%) and total leukocytes (1700/µl). From the same case, pure culture of *Escherichia coli* was isolated from blowhole and abundant cultures of E. coli and *Aeromonas hydrophila* were isolated from lung.

Causes of death could be established in 89.4% (16/19) of the cases and were classified as neonatal or perinatal in 57.8% (11/19) comprising most of the calves (11/15, 73.3%). Two calves died due to trauma from intra- or interspecific interaction leading to multisystemic hemorrhage (case 588) and rib fractures (case 578). In the later, trauma coexisted with neonatal pathology. In one case the cause of death was associated with predation by sharks (case 271). One calf (case 345) had findings supportive of acute septicemia by *E. coli* and *A. hydrophila* (pathology associated with good nutritional status) and a juvenile (case 333) had septicemia of unknown origin. Two animals (cases 397 and 470) had an unknown condition associated with significant loss of body weight. One calf (case 520) had anthropogenic pathology associated with interaction with fishing net.

Major histopathological findings by organic systems observed in the 19 examined whales are described below.

The most common finding in the lung was the presence of keratin squames (12/15) in the alveolar and bronchiolar spaces in calves, occasionally completely obliterating the alveolar spaces (Figure 27A). Hyaline membrane formation was observed in 5 out of 15 calves (Figure 27B). From 18 animals that had lung examined, histopathological findings included atelectasis (11/18), alveolar hystiocytosis (4/18), pneumocyte type II hyperplasia (3/18), lymphoplasmacytic bronchitis (2/18), emphysema (2/18), bronchiolar epithelial hyperplasia (1/18) and thromboembolism (1/18, Figure 28A).

E. coli and *A. hydrophila* were isolated from lung of case 345. This individual had a suppurative bronchopneumonia (Figure 28B) with type II pneumocyte hyperplasia, alveolar hemorrhage, aspirated granular amorphous material, and occasional keratin squames. Multiple intravascular Gram negative bacterial emboli were observed in different organs. Additionally, hemorrhagic enteritis with lymphoplasmacytic and neutrophylic infiltrate in the apical zone of the mucosa (Figure 29A,B), acute hepatocellular necrosis with hemorrhage predominantly periportal and midzonal (Figure 30), acute tubular necrosis, and necrotic and ulcerative dermatitis with neutrophilic infiltrate, hemorrhage, edema and espongiosis were noted.

Histopathological changes observed in the heart included, interstitial edema (11/18) hemorrhage (7/18), contraction band necrosis (6/18, Figure 31), myocardial degeneration (2/18) and lymphoplasmacytic and neutrophilic myocarditis (1/18).

Among the main histopathological findings in the stomach were lymphoplasmacytic and neutrophilic gastritis (4/15), edema of submucosa and lamina propria (4/15)*, a*cute apical hemorrhage (2/15) in the mucosa of the glandular stomach compartment and gastric-associated lymphoid tissue hyperplasia in the glandular and pyloric stomach compartments (1/15)*.* In the intestine*,* epithelial desquamation (3/15), and intestine-associated lymphoid tissue hyperplasia (2/15), apical hemorrhagic enteritis with lymphoplasmacytic and neutrophilic infiltrate (1/15), lymphoplasmacytic and eosinophilic enteritis (1/15) were noted. Findings in the liver included intracytoplasmic hepatocellular hyaline globules with pink points (9/13, Figure 32A), hepatocellular necrosis (4/13, Figure 32B), hemorrhage (3/13), periportal (centroacinar) lymphoplasmacytic hepatitis (3/13), vacuolar degeneration (lipid type) (8/13, Figure 33), ductal hyperplasia (2/13), and cholestasis (1/13).

Histopathological findings in the kidney comprised acute tubular necrosis (7/18), multifocal capsular hemorrhages (7/18), lymphoplasmacytic infiltrate (2/18), mesangiocapillary glomerulonephropathy (1/18) and tubular dilatation with proteinosis (1/18). Cystitis (2/11) and *m*ucosa-associated lymphoid tissue hyperplasia (1/11) were seen in the urinary bladder. In the genital system*,* perimetritis with multifocal hemorrhage (1/4) and testicular hemorrhage (1/4) were noted.

Histopathological findings in the spleen included lymphoid depletion (6/12) with occasional absence of periarteriolar sheat (2/12), lymphocytolysis, and capsular hemorrhage

(2/12). Changes observed in lymph nodes comprised lymphoid depletion (4/8), sinus hystiocytosis (2/8), follicular hyalinosis (1/8) and reactive lymphoid hyperplasia (1/8). Extramedullary hematopoiesis was observed in every spleen (n=12), lymph nodes (3/8), liver (3/8) and thymus (2/8). Lymph nodes showed an inverse architecture with central location of lymphoid follicles.

The main findings in the skeletal muscle included edema (4/10), lymphoplasmacytic and neutrophilic myositis (2/10, Figure 34) and muscular degeneration (1/10). Histopathological findings in the skin comprised generalized pyodermatitis with necrosis, intralesional bacterial colonies and cyamids (1/14, Figure 35A), ulcerative dermatitis with necrosis associated with cookie cutter shark bite and intralesional bacteria (1/14), focally extensive necro-ulcerative dermatitis (1/14) and suppurative epidermitis (1/14). Other findings in the skin included intra and/or intercellular edema (5/14, Figure 35B), dermal hemorrhage (4/14), dermo-epidermal separation (4/14) ballooning degeneration (2/14), neutrophilic and hystiocytic infiltrate in the deep dermis (1/14).

Brain and/or spinal cord were available from 5 animals. The main histologic findings were perivascular hemorrhage (3/5), congestion (3/5) and perineuronal edema (1/5).

Tissues from 13 whales were tested by immunohistochemistry (Cases 207, 237, 260, 271, 301, 333, 341, 345, 385, 396, 397, 482, 520) and did not reveal evidence of morbillivirus infection.

Table 5 - Individual cases of humpback whales stranded in Brazil, examined through histopathology

Specimen (serial)	Date of stranding (dd/mm/yy)	Location	Federal Unit	Sex	SL (m)	Age class	Stranding condition	Body condition	Conservation status at necropsy
207	07-Oct-05*	Caravelas	BA	ND	4.14	Calf	Dead	Not determined	Moderate autolysis
237	17-Sep-06	Aracaju	SE	M	4.06	Calf	Alive	Moderate	Moderate autolysis
254	04-Aug-07	Belmonte	BA	M	10.1	Juvenile	Alive (euthanized)	Good	Moderate autolysis
260	21-Aug-07	São Mateus	ES	M	4.37	Calf	Dead	Moderate	Moderate autolysis
271	08-Sep-07	Pirambu	SE	F	4.87	Calf	Alive	Moderate	Moderate autolysis
293	04-Jul-08	Alcobaça	BA	M	3.83	Calf	Alive	Moderate	Moderate autolysis
301	14-Aug-08	Conceição da Barra	ES	M	3.9	Calf	Alive	Moderate	Moderate autolysis
333	04-Jul-09	Guriri	ES	F	9.18	Juvenile	Alive	Good	Moderate autolysis
341	21-Aug-09	Bitupita	CE	F	10	Juvenile	Alive	Good	Fresh
345	08-Sep-09	São Mateus	ES	F	4	Calf	Alive (euthanized)	Moderate	Fresh
385	19-Aug-10	Itaporanga	SE	F	4.63	Calf	Dead	Good	Fresh
397	22-Aug-10	Capão da Canoa	RS	M	12.5	Adult	Alive	Poor	Moderate autolysis
396	04-Sep-10	São Gonçalo do Amarante	CE	M	3.94	Calf	Alive	Moderate	Fresh
470	14-Jul-11	Balneário Pinhal	RS	F	7.73	Calf	Alive (euthanized)	Poor	Moderate autolysis
482	10-Sep-11	Linhares	ES	M	3.27	Calf	Alive	Good	Moderate autolysis
520	06-Sep-12	Aracaju	SE	F	4.5	Calf	Dead	Moderate	Fresh
578	08-Sep-13	Barra do Riacho	ES	M	3.5	Calf	Alive	Good	Moderate autolysis
588	30-Sep-13	Prado	BA	M	4.0	Calf	Alive	Good	Moderate autolysis
593	10-Oct-13	Santa Cruz de Cabrália	BA	M	4.5	Calf	Alive	Good	Moderate autolysis

Legend: * = Date of examination for specimens with unknown stranding date; ND = Not determined; M = Male; F = Female; SL = Standard length; BA = Bahia; SE = Sergipe; ES = Espírito Santo; CE = Ceará; RS = Rio Grande do Sul.

Table 6 - Individual cases of humpback whales stranded in Brazil: histopathological findings

(Continue)

Specimen (serial)	Most significant pathological findings	Etiologic diagnosis	Pathological entity
207	Pulmonary atelectasis and emphysema with intra-alveolar squames.	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
237	Pulmonary edema and intra-alveolar squames; alveolar histiocytosis; lymphoid depletion; multisystemic serosal hemorrhage (heart, stomach, kidney); dermo-epidermal separation with granular basophilic material accumulation.	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
254	Pulmonary edema; bronchiolar and alveolar hemorrhage; atelectasis; myocardial contraction band necrosis, diffuse moderate vacuolar hepatocellular degeneration; lymphoplasmacytic hepatitis; multisystemic interstitial hemorrhage (lung, heart, testicle).	ND	ND
260	Pulmonary edema; intra-alveolar squames and hyaline membrane; alveolar histiocytosis; atelectasis; perivascular and perineuronal cerebral edema.	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
271	Severe edema; occasional intra-alveolar squames; multisystemic serosal hemorrhage (heart, ovary, kidney).	Predation. Fetal distress. Mother-calf separation	Natural (Predation and neonatal or perinatal pathology)
293	Pulmonary edema, alveolar histiocytosis associated with intra-alveolar aspirated material and squames; atelectasis; myocardial contraction band necrosis.	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
301	Pulmonary edema; lymphoid depletion; hepatocellular necrosis; myocardial contraction band necrosis; multisystemic serosal hemorrhage (heart, esophagus, stomach, liver, lung, trachea).	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
333	Lymphoplasmacytic bronchitis, interstitial pneumonia and alveolar histiocytosis; myocardial degeneration, moderate to severe hepatocellular vacuolar degeneration; hepatocellular necrosis; lymphoplasmacytic pericholangitis; moderate necrotic lymphadenitis; sinus histiocytosis; mesangiocapillary glomerulonephropathy.	Unknown origin septicemia	Natural (Pathology associated with good nutritional status: septicemia)
341	Multifocal eosinophilic and lymphoplasmacytic enteritis.	ND	ND
345	Suppurative bronchopneumonia; pulmonary edema, atelectasis; type II pneumocyte hyperplasia; occasional intra-alveolar squames; acute tubular necrosis; hemorrhagic enteritis and gastritis; lymphid depletion; acute hepatocellular necrosis and hemorrhage.	Septicemia associated with *Escherichia coli* and *Aeromonas hydrophila* infection	Natural (Pathology associated with good nutritional status: septicemia)
385	Pulmonary edema; intra-alveolar squames; lymphoid depletion; myocardial contraction band necrosis; multisystemic hemorrhage (heart, adrenal, spleen, stomach, tongue).	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)

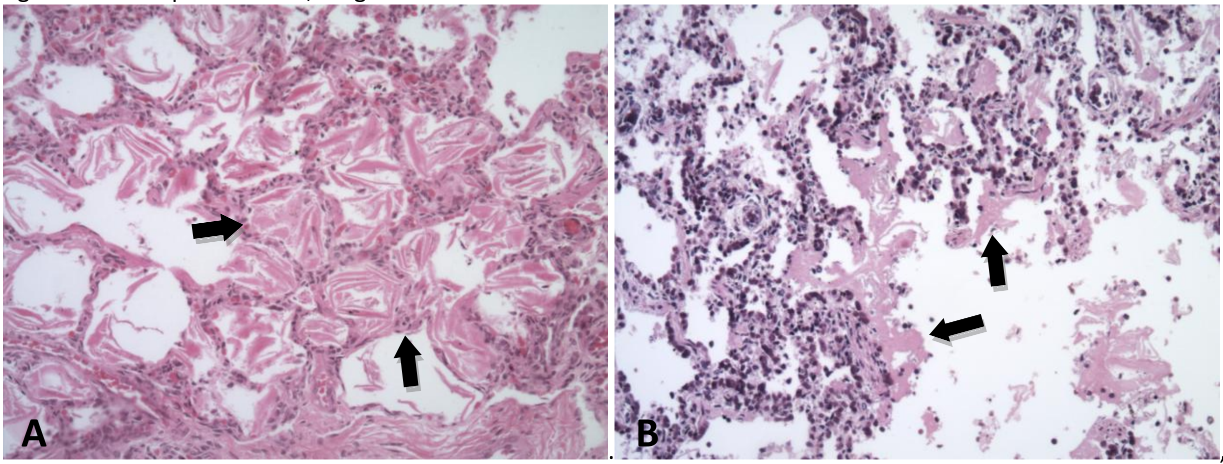
Table 6 - Individual cases of humpback whales stranded in Brazil: histopathological findings

(Conclusion)

Specimen (serial)	Most significant pathological findings	Etiologic diagnosis	Pathological entity
397	Lymphoplasmacytic bronchitis, atelectasis; lymphoid depletion with follicular hyalinosis; lymphoplasmacytic periportal hepatitis; marked vacuolar hepatocellular degeneration.	Emaciation	Natural (Pathology associated with significant loss of nutritional status)
396	Pulmonary edema; intra-alveolar squames and hyaline membrane; atelectasis; marked desquamative enteritis; multisystemic serosal hemorrhage (heart, kidney, lymph node, spleen).	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)
470	Lymphoplasmacytic bronchitis; bronchial epithelium hyperplasia; lymphoid depletion; hepatocellular vacuolar degeneration.	Emaciation	Natural (Pathology associated with significant loss of nutritional status)
482	Cerebral hemorrhage, pulmonary edema, interstitial pneumonia and intra-alveolar meconium, hyaline membrane and squames; myocardial degeneration and contraction band necrosis.	Trauma. Fetal distress. Mother-calf separation	Natural (Trauma from unknown origin and neonatal or perinatal pathology)
520	Pulmonary edema; interstitial pneumonia; intra-alveolar squames and hyaline membrane and bacteria; atelectasis; thromboembolism in the lung; multifocal hepatic necrosis; lymphoid depletion.	Entanglement. Fetal distress. Mother-calf separation	Anthropogenic pathology (interaction with fishing activities)
578	Marked pulmonary hemorrhage; pulmonary edema; intra-alveolar squames, hyaline membrane and bacteria; emphysema; atelectasis; marked multisystemic hemorrhage (kidney, lung, trachea, heart, liver, stomach, epididymis).	Trauma. Fetal distress. Mother-calf separation	Natural (Traumatic intra-or interspecific interaction and neonatal or perinatal pathology)
588	Severe pulmonary hemorrhage and edema; lymphoplasmacytic rabdomyositis; multisystemic hemorrhage (cerebrum, lung, stomach, spleen, heart, kidney, bladder, skeletal muscle).	Trauma	Natural (Traumatic intra- or interspecific interaction)
593	Presence of intra-alveolar squames in the lung; bronchiolar epithelium hyperplasia; multisystemic serosal hemorrhage (cerebrum, lung, heart, skeletal muscle, trachea).	Fetal distress. Mother-calf separation	Natural (Neonatal or perinatal pathology)

Legend: ND = Not determined.

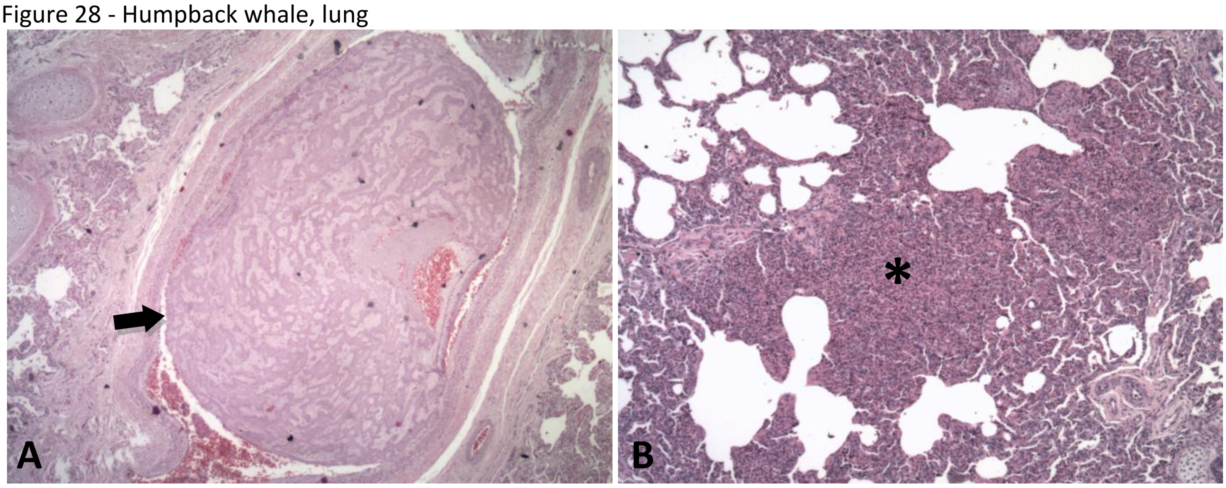
Figure 27 - Humpback whale, lung



Fonte: (GROCH, 2013). Fonte: (GROCH, 2013).

Legend: A) Presence of intra-alveolar keratin squames. Case 520. HE 10X. B) Presence of intra-alveolar hyaline membrane. Case 578. HE 10X.

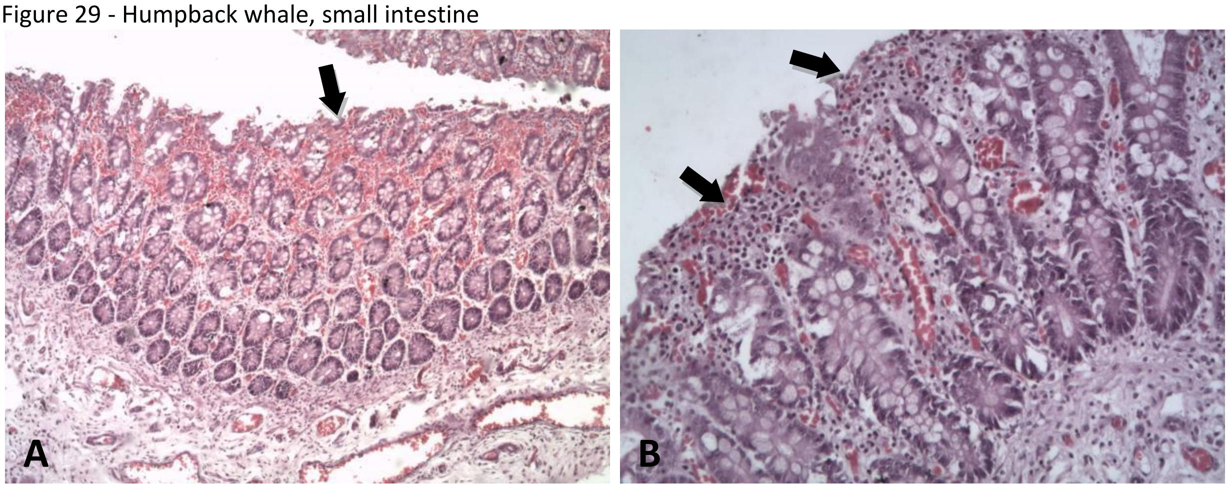
Figure 28 - Humpback whale, lung



Fonte: (GROCH, 2013). Fonte: (GROCH, 2013).

Legend: A) Complete occlusive mural thrombosis. Case 333. HE 2X. B) Suppurative bronchopneumonia. Note consolidation areas and inflammatory exudate. Case 345. HE 20X.

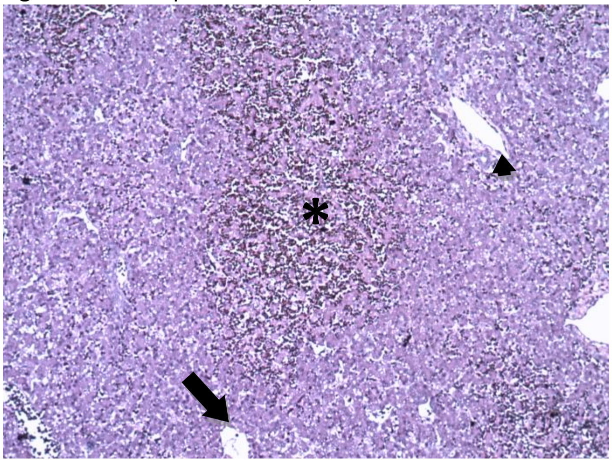
Figure 29 - Humpback whale, small intestine



Fonte: (GROCH, 2013). Fonte: (GROCH, 2013).

Legend: A) Apical mucosa hemorrhage. Case 345. HE 4X. B) Lymphoplasmacytic infiltrate in the apical zone of the mucosa. Case 345. HE 10X.

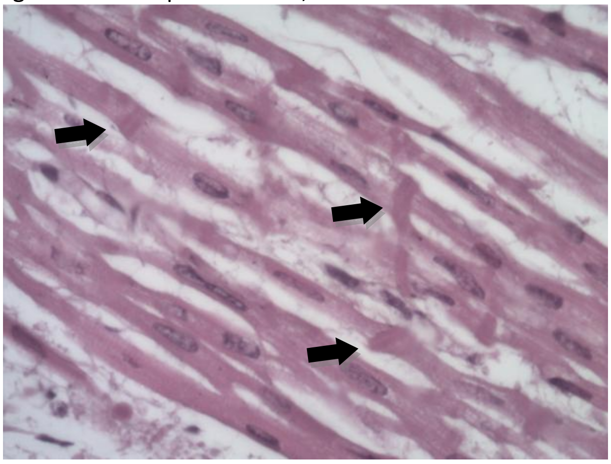
Figure 30 - Humpback whale, liver



Fonte: (GROCH, 2013).

Legend: Hepatocellular necrosis with periportal and midzonal hemorrhage (asterisk). Note centrilobular vein (arrow) and portal space (arrowhead). Case 345. HE 4X.

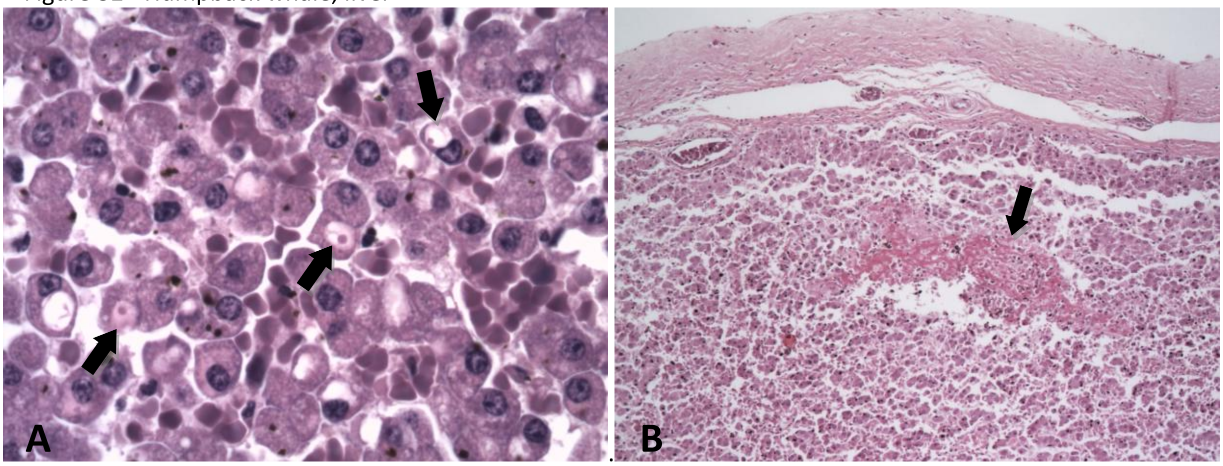
Figure 31 - Humpback whale, heart



Fonte: (GROCH, 2013).

Legend: Endomysial edema and contraction band necrosis (arrows). Case 482. HE 40X.

Figure 32 - Humpback whale, liver

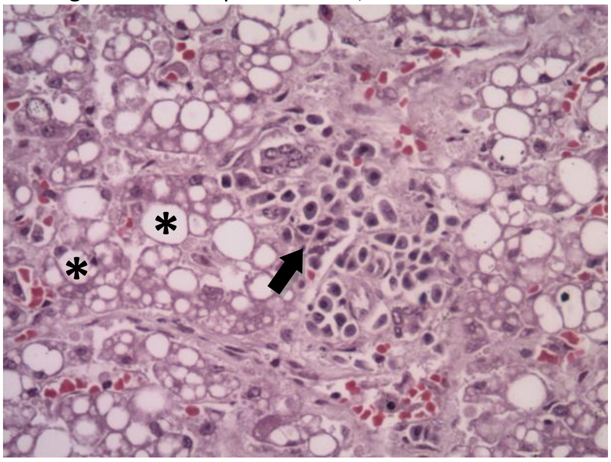


Fonte: (GROCH, 2013).

Fonte: (GROCH, 2013).

Legend: A) Presence of hyaline globules with pink point in the cytoplasm of hepatocytes. Case 396. HE 40X.
B) Hepatocellular coagulation necrosis (arrow). Case 520. 4X.

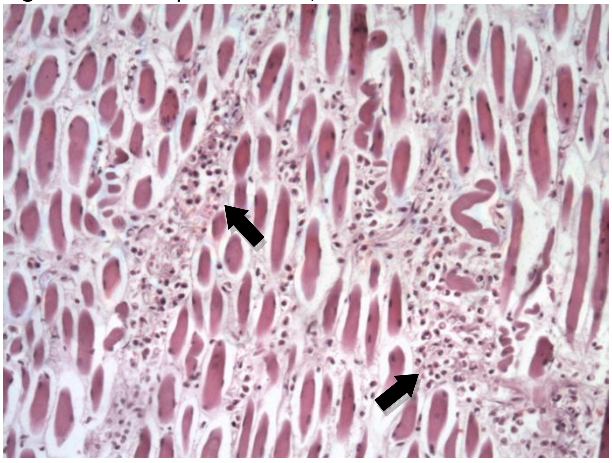
Figure 33 - Humpback whale, liver



Fonte: (GROCH, 2013).

Legend: Hepatic lipidosis (asterisk) and periportal lymphoplasmacytic infiltrate (arrow). Case 333. HE 20X.

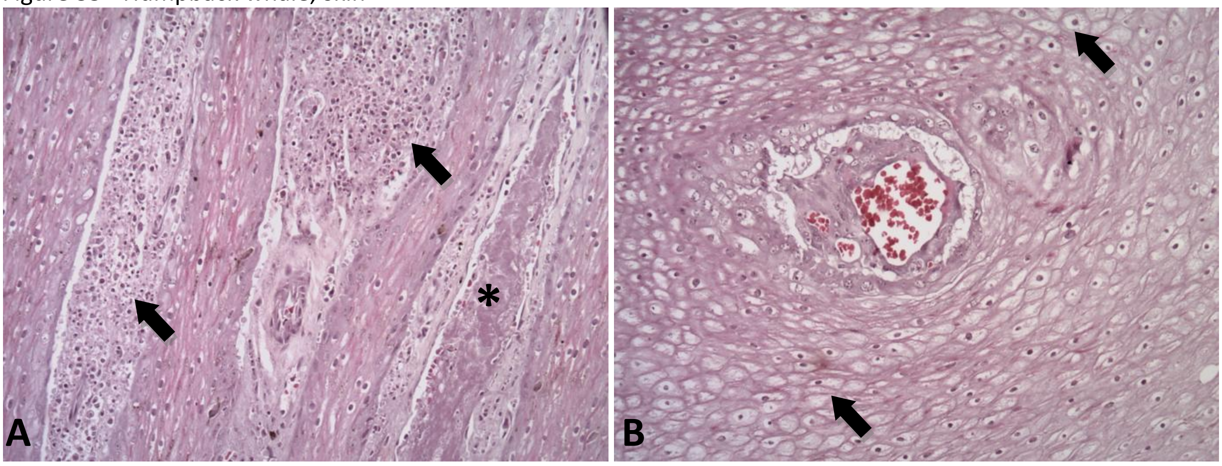
Figure 34 - Humpback whale, skeletal muscle



Fonte: (GROCH, 2013).

Legend: Multifocal lymphoplasmacytic and neutrophilic myositis. Case 482. HE 10X.

Figure 35 - Humpback whale, skin



Fonte: (GROCH, 2013).

Legend: A) Lymphoplasmacytic, macrophagic and neutrophilic infiltrate in the dermal papilla (arrow). Note the presence of bacteria (asterisk). Case 333. HE 10X. B) Intracellular edema of keratinocytes in the *stratum intermedium*. Case 333. HE 20X.

4.4 DISCUSSION

In this study we examined 19 humpback whales found stranded in Brazil. The pathological entity most frequently encountered was neonatal or perinatal pathology in which morphological and etiological characteristics are possibly related to problems in gestation, birth, nursing or behavior (ARBELO et al., 2013). Pulmonary atelectasis with squamous epithelial cells, intralveolar fibrin, hyaline membrane formation, edema, and hemorrhage were common findings in these cases. Keratin squames are filamentous eosinophilic structures, sometimes with central nucleus in the lumina of alveoli, consistent with sloughed epithelial cells from the oronasal region and amniotic fluid (CASWELL; WILLIAMS, 2007). Hyaline membranes are aggregates of fibrin, other serum proteins, and cell debris that appear as linear masses of discrete, densely eosinophilic material lining alveolar septa. Injury of type I pneumocytes or endothelial cells in the alveolar septa often lead to loss of interstitial fluid into the alveolus (edema) and formation of hyaline membranes. These lesions may be associated with interstitial pneumonia, which is usually seen in acute respiratory distress syndrome (ARDS). This syndrome is a clinically defined condition with an acute onset of bilateral pulmonary disease characterized by hypoxemia but no evidence of left atrial hypertension. Diffuse alveolar damage with proliferation of type II pneumocytes can be observed in less fulminant cases (CASWELL; WILLIAMS, 2007). Surfactant dysfunction, for example, by inhibition of the surfactant function by serum proteins that flood the alveoli and/or injury to surfactant producing type II pneumocytes, provokes atelectasia and abnormal surface tensions in the alveoli that physically damage the type I pneumocytes. Other causes of diffuse alveolar damage would include pulmonary infections by virus, bacteria, parasites, trauma, shock, disseminated intravascular coagulation, pancreatitis, uremia (CASWELL; WILLIAMS, 2007).

Presence of intra-alveolar keratin squames and formation of hyaline membranes are suggestive of dystocia or fetal distress, which also is observed in humans and domestic animals (LOPEZ; BILDFELL, 1992; CASWELL; WILLIAMS, 2007). In cetaceans, concentric layers of hyaline membranes were seen occluding bronchioles of harbor porpoises (*Phocoena phocoena*) (JAUNIAUX et al., 2002). A case of meconium aspiration syndrome was reported in a bottlenose dolphin (*Tursiops truncatus*) neonate who died immediately after birth. At necropsy, a knot was found in the umbilical cord and may have led to hypoxia (TANAKA et al., 2014). Studies involving humans, primates and domestic animals demonstrated that aspiration of meconium and squamous cells is not a normal consequence of parturition (GOODING et al., 1971). Therefore, our findings suggest that perinatal asphyxia or respiratory distress should be taken into account as a possible cause of neonatal mortality in humpback whales as seen in other cetacean species (TANAKA et al., 2014). Comparison with lungs of healthy calves or animals that died due to distinct conditions may be useful to assess the significance of this condition.

In case 345, an acute septic process associated with *E. coli* and *A. hydrophila* infection was the most plausible cause of stranding and death. The animal presented acute bronchopneumonia with bacterial colonies in the heart, intestines and blood vessels. Colisepticemia occurs commonly in bovine calves, and sporadically in young animals of other domestic species. Reduced transfer or absorption of maternal colostral immunoglobulin, or intercurrent disease or debilitation is usually prerequisite for *E. coli* septicemia. Most likely portal of entry in neonates includes navel, upper respiratory tract and possibly tonsils and intestines (BROWN; BAKER; BARKER, 2007). In the present case, *E. coli* was cultured from blowhole and bronchial exudate. It is possible that the pathologic agent entered via the respiratory tract; however, navel is also a plausible route of entry and cannot entirely be discarded. The animal also presented anemia and lymphopenia, which may be expected in a debilitated animal with acute septicemia. *E. coli* septicemia was associated with lack of maternally acquired immunity in a captive bottlenose calf that died 82 hours after birth. No nursing was observed from birth to death. The animal presented multiple hemorrhages in the subcutaneous tissue, head and cervical region, pulmonary edema and congestion, synovitis of the atlanto-occipital joint, hepatic hemorrhage and necrosis, and intravascular Gram negative bacteria in multiple organs (VAN ELK et al., 2007). Pneumonia was associated with *E. coli* in 2 cases and *A. hydrophila* in one case in harbour porpoises (JAUNIAUX et al., 2002). In our case, the findings included pneumonia, hepatocellular necrosis, clumps of small bacilli in vessels through the body and serosal hemorrhages, which are suggestive of an endotoxemic process (BROWN; BAKER; BARKER, 2007).

One whale (case 520) had marks suggestive of entanglement, on the basis of recent skin lacerations and presence of subcutaneous hemorrhages. Interaction with fishing activities usually lead to acute death by accidental capture (bycatch) or as result of severe injuries caused by fishermen or fishing equipment (KUIKEN et al., 1994; MOORE et al., 2013). Animals are usually in good body condition and show abundant stomach content (ARBELO et al., 2013). Histopathological findings include pulmonary congestion and edema, multifocal subpleural and intra-alveolar hemorrhages, alveolar emphysema and intracytoplasmic hyaline globules in the hepatocytes and may be interpreted as evidence of hypoxia (JEPSON et al., 2000; ARBELO et al., 2013). In the present case, the animal also had keratin squames and hyaline membranes in the alveolar spaces, suggestive of a concomitant respiratory distress.

Accidental capture or entanglement of cetaceans in fishing gear has increased in recent decades and is currently one of the main threats to cetaceans worldwide (PERRIN; DONOVAN; BARLOW, 1994; CASSOFF et al., 2011; KNOWLTON et al., 2012. Many cases are reported in the literature showing that humpback whales are susceptible to entanglement, especially calves (WILEY et al., 1995; FELIX et al., 1997; SCHEIDAT et al., 2000). Collision with vessel is another potential anthropogenic threat to cetaceans. The accident is usually fatal especially in case of propeller-induced wounds, which produce sharp deep incisions that communicate the internal cavities with the extern, or even produce one deadly deep cut; in some cases completely sectioning the animal's body. Blunt trauma due to collision with boats can cause multiple fractures and subcutaneous and internal hemorrhages (ARBELO et al., 2013; MOORE et al., 2013). Collision with vessel was not observed in our study and entanglement in fishing net was not as common as reported in humpback whales in the north hemisphere (WILEY et al., 1995) yet it is important to consider that in our study we included only animals with samples of enough quality for histopathological analysis, assuming a bias since mostly those whales that stranded alive (79%) where contemplated. In both type of anthropogenic interaction (entanglement and vessel collision) the animals may die at sea and the carcass may sink or wash to shore after many days, and be found in an advanced decomposition state (WILEY et al., 1995; CASSOFF et al., 2011; MOORE et al., 2013). Therefore, the fact that we did not diagnose high rates of entanglement or cases of vessel collision in the present study does not necessarily mean that they do not occur.

Two animals presented pathology associated with significant loss of nutritional status (cases 397 and 470). Case 470 was a calf and the poor body condition may indicate starvation after maternal separation. In many cases where cetaceans strand with evidence of emaciation it is difficult to establish whether the condition results from a single or multiple etiopathogenic mechanisms (ARBELO et al., 2013). High prevalence of parasites is a common finding and its etiopathogenic value is conditional on the functional importance of the affected organs. Immunodepression associated with viral infections (e.g. morbillivirus) and bacterial pulmonary infections are also reported (JEPSON et al., 1999; ARBELO et al., 2013). In fact, case 397 was a live stranded adult that had several species of Vibrionaceae and Aeromonadaceae bacteria isolated from open orifices of the body. *Vibrio mediterranei* was isolated from the right lung (MOURA et al., 2013). Histopathological lesions found in this individual include lymphoplasmacytic bronchitis and atelectasis and lymphoid depletion. The latter might reflect immunosuppression what might have favored bacterial proliferation. The animal also presented hyalinosis in lymph nodes, which is a common age-related finding in cetaceans (ARBELO et al., 2013).

Traumatic interactions possibly caused by individuals of the same species (intraspecific), or with other cetacean species (interspecific) were observed in two cases (cases 578 and 588). Lesions may vary from superficial skin marks to severe traumatic lesions represented by fractured ribs and subcutaneous and pulmonary hemorrhages (ARBELO et al., 2013), as observed in case 578, which had fractured ribs with multisystemic hemorrhage. Intraspecific interaction seems a plausible explanation for these cases since one of the characteristics of the reproduction behavior of humpback whales is the aggressive intrasexual competition among males during the breeding season. In an attempt to access a female, calves can be injured (TYACK; WHITEHEAD, 1983). Traumatic injuries from the infanticide behavior of adult dolphins have also been documented in bottlenose dolphin calves (PATTERSON et al., 1998; DUNN et al., 2002). Differential diagnosis includes dystocic parturition. Cranial and bilateral rib injuries consistent with compression during parturition have been reported in a humpback whale and a minke whale (*Balaenoptera acutorostrata*) (PATERSON; VAN DYCK, 1996). Regarding interaction with other marine animals, traumatic lesions due to shark bites were observed in a live stranded calf (case 271) and were interpreted as predation. In this case the animal also presented signs of respiratory distress.

Death of stranded cetaceans occurring in the absence of recognized injury has been attributed to stress or “alarm reaction”. In this situation, the animals show a wide range of lesions in the myocardium, liver and kidney attributed to catecholamine injury or vasospasm, including spasm of cardiac arteries, with ischemia and reperfusion (COWAN; CURRY, 2008). The lesions are seen in the organs and tissues of beach-stranded, net-caught or captive animals and include contraction band necrosis of cardiac and smooth muscles, smooth muscle spasm, ischemic injury to the intestinal mucosa, acute tubular necrosis and hepatocellular hyaline globules (JABER et al., 2004; COWAN; CURRY, 2008). Myocardial contraction band necrosis and acute renal tubular necrosis were also found in our study (8/18 and 7/18, respectively). Hepatocellular hyaline globules were seen in of 9 out of 13 humpback whales. These inclusions typically had an eccentric densely staining core (pink point) as described in other cetaceans and are known to contain acute phase glycoproteins (fibrinogen and alpha-1-antitrypsin) (KENNEDY et al., 1993; JABER et al., 2004). The hepatocellular hyaline globules are associated with stress and acute hemodynamic change, as consequence of hepatocellular hypoxia. The hypothesis is that when a cetacean actively strand, compression over thoracic and abdominal cavities hinder blood flow, ultimately causing congestion and hypoxia (JABER et al., 2004). In fact, most animals (79%) included in our study stranded alive and may have undergone changes related to stress or "alarm reaction", as suggested by the findings of the above lesions.

Besides its function in the immune response, it has been hypothesized that cetaceans' spleen function may not primarily be a blood storage and may have a hematopoietic role (ARVY; PILLERI, 1970). In most domestic mammals, hematopoiesis occurs in the fetal yolk sac, liver and spleen, until the development of bone marrow which usually assumes the task. Hematopoiesis occurring in organs outside the bone marrow is called extramedullary hematopoiesis (EH) and may occur, for example, during active immune responses to pathogens (KIM, 2010). Cowan and Smith (COWAN; SMITH, 1999) found megakaryocytes in the spleens of 100% (n = 50) examined bottlenose dolphins, suggesting that at least that component of hematopoiesis takes place in the spleen in those species. In our study, all examined spleens presented megakaryocytes and blastic lymphoid cells, also occasionally observed in the liver, thymus and lymph nodes. This finding supports the hypothesis that extramedullary hematopoiesis may be a normal finding in some cetacean species, including humpback whales.

Morbillivirus (family *Paramyxoviridae*, genus *Morbillivirus*) outbreaks have caused a high number of deaths among pinnipeds and cetaceans in the Northern Hemisphere. Two morbillivirus species are known to affect aquatic animals: *Phocine distemper virus* (PDV) and *Cetacean morbillivirus* (CeMV). PDV has been isolated from pinnipeds, and 3 strains of CeMV (porpoise morbillivirus [PMV], dolphin morbillivirus [DMV], and pilot whale morbillivirus [PWMV]) have been isolated from dolphins and pilot whales (BARRETT et al., 1993; TAUBENBERGER et al., 2000). The susceptibility of Mysticetes to morbillivirus infection has been confirmed in two fin whale (*Balaenoptera physalus*) calves from Belgium and France (JAUNIAUX et al., 2000) and one adult fin whale from the Mediterranean Sea, which presented a *Toxoplasma gondii* and DMV coinfection (MAZZARIOL et al., 2012). In humpback whales the only evidence of exposure is seropositivity against DMV and PMV antibodies in one individual from Gulf of Maine, United States (US) (ROWLES et al., 2011). An ongoing unusual mortality event in the east coast of US have caused the death of 1163 bottlenose dolphin from

July 2013 until March 2014 (mean of strandings in previous years was 169) and the tentative cause is being attributed to CeMV, based on preliminary diagnostic testing (NOAA, 2014). During this outbreak, the worst ever recorded, it was published in the news that three stranded humpback whales tested positive for DMV (UPI, 2013). In our study humpback whales tested negative for morbillivirus; however, we recently reported the first lethal case in a Guiana dolphin (*Sotalia guianensis*) that inhabits the coastal waters of Abrolhos Bank (GROCH et al., 2014, Annex B). These finding shows that morbillivirus is extant in the Brazilian waters and that Guiana dolphin calves are susceptible to infection. This raises concern on the epidemiology of morbillivirus in cetaceans from the Abrolhos Bank region, especially on the susceptibility of humpback whales, which are born in this area and might be exposed to the virus. It is unknown whether subclinical infection occurs in Guiana dolphins or other cetaceans, or whether the virus has undergone species-adaptive changes, as proposed for PWMV (TAUBENBERGER et al., 2000). The sequence data from our study suggest that the virus from the Guiana dolphin calf is a novel strain of the CeMV species. This conclusion is supported by phylogenic analysis and geographic distribution of the virus and by its distinct host. Further studies are required to determine the epidemiology of morbillivirus infection in this and other cetacean species and to assess the risk for epizootic outbreaks among South Atlantic cetaceans.

Determination of the cause of death is difficult in free-ranging wild animals, particularly cetaceans (SIEBERT et al., 2001). The challenge seems to grow according to the size of the animal. They may become stranded when severely ill or can die in the sea and be washed ashore several days later. In the present study we examined a relatively small portion of the total of stranded humpback whales in Brazil (10%, 19/191). We believe these findings may contribute to the understanding of pathological processes in humpback whales and add valuable information for comparative pathology. Furthermore, *E. coli* septicemia has not been previously reported in humpback whales. The diagnosis was based on detection of Gram negative bacterial colonies associated with hemorrhage and neutrophilic infiltration in multiple organs, associated with pure culture of *E. coli.* Complementary studies on the characterization of the *E. coli* strain may give further insights on its pathogenesis in cetaceans.

Our data herein presented are far from exhaust the theme; instead, it was the first view on pathology of a case series of humpback whales. Complementary diagnostic techniques such as microbiological, immunohistochemical and molecular analysis may provide further understanding on the etiology and pathogenesis of conditions found in this study. General information on frequency of strandings and mortality along the entire breeding area, comprising the cases not included in the present study, may be useful for any inference of disease impact at a population level. We expect that our results may help to improve and guide management of stranded animals, examination and sampling collection in future cases.

4.5 ACKNOWLEDGMENTS

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CAPÍTULO 3

Skeletal abnormalities in humpback whales stranded in the Brazilian breeding ground

5 CAPíTULO 3 - SKELETAL ABNORMALITIES IN HUMPBACK WHALES STRANDED IN THE BRAZILIAN BREEDING GROUND

5.1 INTRODUCTION

Every winter, humpback whales *Megaptera novaeangliae* of the southwestern Atlantic Ocean population migrate from feeding grounds in Antarctic waters to tropical waters on the Brazilian coast to breed and calve. They concentrate mainly in the Abrolhos Bank (16°40’–19°30’S, 37°25’–39°45’W), an enlargement of the continental shelf (ANDRIOLO et al., 2006). A total of 9330 whales was estimated for the Brazilian coast in 2008 (WEDEKIN et al., 2010a). Strandings commonly occur; from 2002 until 2011, there were 153 single stranding cases along the Abrolhos Bank seashore and adjacent waters. In 91% of these cases[17], the whale died at sea and was washed ashore.

Full necropsy and histopathological analysis of soft tissues are often impaired by decomposition state and necessary logistics related to the size of the animal. Analysis of skeletal changes, which are relatively resistant to decomposition, may provide valuable information on the life history and pathological conditions of the animal. However, information on skeletal abnormalities in humpback whales is scarce: 5 single cases have been reported (STEDE, 1994; PATERSON; VAN DYCK, 1996; KOMPANJE, 1999; FÉLIX; HAASE; AGUIRRE, 2007; HELLIER; HUFTHAMMER; LISLEVAND, 2011). Stede (1994) reported osteoperiostitis ossificans hypertrophicans in the caudal vertebra of a humpback whale calf from Germany. Paterson and Van Dyck (1996) described a humpback whale fetus from Australia with injuries consistent with trauma during parturition, such as bilaterally fractured ribs and superficial focally extensive cystic osseous lesions associated with periosteal new bone formation in the supra-orbital region. Hellier et al. (2011) reported the presence of a remodeled lesion ventrally in the maxilla, possibly related to trauma to the baleen plates and/or adjacent soft tissue in a 13.5-m-long humpback whale. The animal also presented bony bridging in the left aspect of the neural arches of 2 cervical vertebrae, and multiple localized areas of reactive bone growth in the vertebral column (HELLIER; HUFTHAMMER; LISLEVAND, 2011). Spondyloarthritis was suspected

[17] Obtained from database of Instituto Baleia Jubarte (unpublished data).

to occur in caudal vertebrae of a specimen from Denmark (KOMPANJE, 1999). Severe infectious spondylitis affected 7 lumbar and 4 caudal vertebrae of a 7.25-m-long humpback whale from Ecuador (FÉLIX; HAASE; AGUIRRE, 2007). The goal of the present study was to investigate the occurrence of skeletal pathologic processes in humpback whales, as well as to evaluate their potential to compromise health and contribute to the stranding event or death.

5.2 MATERIALS AND METHODS

The total coastal area covered consists of approximately 500 km between the municipalities of Belmonte, state of Bahia (15°44’S, 38°53’W), and Santa Cruz, State of Espírito Santo (20°00’S, 40°09’W), Brazil, including the coastline of Abrolhos Bank and Royal Charlotte Bank.

One hundred and fifty-three humpback whales were found stranded from 2002 until 2011[18]. For each animal, a specimen number was issued and information regarding date and location of stranding, body length and sex were recorded. Bones from 49 animals that stranded alive and subsequently died, or were found dead, were examined in situ. Some examinations were performed opportunistically when the team was helping section carcasses to facilitate removal. Bones were cleaned during dissection and the remaining soft tissue was removed through maceration in water and/or washing and drying. A rib with extensive bone outgrowth and remodeling (Specimen 256) was radiographed and scanned using computed tomography (CT) at 7 mm slice thickness, 80 kV and 240 mA, with a 2-channel helical scanner (GE Medical Systems) at Centro de Imagem São Paulo in Teixeira de Freitas, Bahia. Images were processed using K-PACS (www.k-pacs.net) and Vitrea (www.vitalimages.com) software.

All bones found with pathological changes were digitally photographed, collected along with samples of apparently normal bones, identified by specimen number and archived at IBJ in Caravelas, Bahia. The exceptions are the skeletons of 3 animals that are currently on exhibition at the IBJ, in Praia do Forte, Bahia (Specimen 122), and at the Frans Krajcbergof Ecological Museum, in Nova Viçosa, Bahia (Specimens 242 and 334). Due to field logistical difficulties, only

[18] Obtained from database of Instituto Baleia Jubarte (unpublished data).

2 ribs with the most severe lesions were collected from Specimen 358, which had pathological changes in 13 ribs; the remaining ribs were photographed at the stranding site.

Age classes were assigned based on the standard body length following the classification used in Mazzuca et al. (1998), i.e. calves <8 m, juveniles 8–11.6 m and adults >11.6 m. Estimated length based on measurements of incomplete carcasses was used to estimate maturity whenever possible. Sex was determined by gross examination at the time of stranding or by genetic analysis of skin (ENGEL et al., 2008; CYPRIANO-SOUZA et al., 2010).

In adult animals, bones were removed from the carcass for examination. Vertebrae of younger animals had most soft tissue removed and the vertebral body and the ossified portions of the arch and transverse processes were examined. Intervertebral disks were inspected for narrowing of the disc space between the 2 vertebrae, disk protrusion and abnormal ossification. The gross examination of each bone included assessment of erosion, cavities or eburnation on joint surface, exostosis, anomaly, fusion and fracture. Pathological changes were further classified as resulting from developmental malformation, and degenerative, infectious and inflammatory, or traumatic lesion. In the present study, pathological conditions were categorized based on what was considered the nature of the early lesion or pathogenesis. For degenerative and inflammatory diseases, the classification proposed by Kompanje (1999) and Thompson (2007) was used.

During necropsies, dissection of thoracic and abdominal cavities was prioritized. Skulls were occasionally found a few meters apart from carcasses, partially buried in sand, with post mortem fractures of rostrum or damages due to shark scavenging. Examination was limited to the position that they were found in (usually with dorsal side down) and precluded a reliable assessment; thus, skull examination was not included in this study.

5.3 RESULTS

Of the 153 whales that stranded on the Abrolhos and Royal Charlotte Banks region between 2002 and 2011, 49 animals were examined for skeletal disorders. Of these, 22.4% (11/49) were females, 28.6% (14/49) were males and 49% (24/49) whales were of undetermined sex; 38.8% (19/49) were calves, 20.4% (10/49) were juveniles, 26.5% (13/49) were adults and 14.3% (7/49) were of undetermined age (Table 7).

Table 7 - *Megaptera novaeangliae.* Totals of animals examined by sex and age class and prevalence of pathological changes

	No. of animals examined		Prev. (%)
	Total	**With pathology**	
Sex			
Male	14	5	35.7
Female	11	1	9.1
Undetermined	24	6	25.0
Age class			
Calf	19	0	0
Juvenile	10	3	30.0
Adult	13	6	46.2
Undetermined	7	3	42.9

Pathological changes were observed in 24.5% (12/49) of the examined animals. Table 8 shows incidence by skeletal region. In the vertebral column, 28.6% (4/14) of the individuals presented lesions in the cervical region, 10% (2/20) presented pathological changes in the thoracic region and 15.8% (3/19) presented lesions in the caudal region. None of the 16 animals from which lumbar vertebrae were examined had lesions. Ribs of 17.1% (7/41) of the animals presented pathological changes. In the appendicular skeleton, the scapulohumeral joints of 3.7% (1/27) of the animals presented lesions; In the appendicular skeleton, considering the carcasses that had at least one scapular glenoid fossa and/or head of humerus examined, the scapulohumeral joints of 3.7% (1/27) of the animals presented lesions.In the distal articulation of the humerus, 9.1% (1/11) of the animals had pathological changes. Radii of 14.3% (1/7) of the animals presented lesions. No scapular blades (n = 23), ulnar articular facets (n = 6) or mandibles (n = 8) of the animals showed signs of fractures or other lesions.

Table 8 - *Megaptera novaeangliae.* Prevalence of pathological changes in skeletons of humpback whales

Skeleton region	No. of animals examined		Prev. (%)
	Total	With pathology	
Vertebral column			
Cervical	14	4	28.6
Thoracic	20	2	10.0
Lumbar	16	0	0
Caudal	19	3	15.8
Ribs	41	7	17.1
Scapulohumeral joint	27	1	3.7
Humerus, distal joint	11	1	9.1
Radius	7	1	14.3
Ulna	6	0	0.0

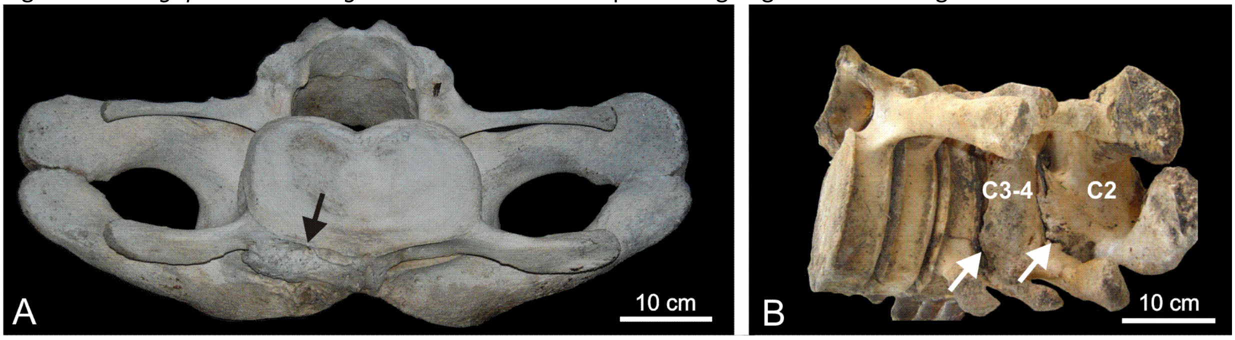
Degenerative and developmental malformations were observed with higher frequency (10.2%; 5/49), followed by inflammatory and traumatic lesions (8.2%; 4/49). The description and general data of specimens with lesions are shown in table 9.

5.3.1 Degenerative diseases

Spondyloarthrosis resulting from degenerative changes in the intervertebral disk was found in 3 whales. In Specimen 242, erosion and spondylophytes were observed in 2 cervical vertebrae and prominent spondylophytes were observed in 2 thoracic and 2 caudal vertebrae. Specimen 238 presented syndesmophyte formation ventrally in the cervical region leading to ankylosis of C2 and C3 (Figure 36A). Specimen 122 presented degenerative changes on cervical vertebrae C1 to C4. Erosion was observed in the caudal epiphyses and spondylophytes in the right lateral margin of C1 and C2. Ankylosis of C3 and C4 was observed due to syndesmophyte formation in the right lateral of the vertebral body (Figure 36B).

Arthrotic changes were observed in 13 ribs of Specimen 358. The articular facet of ribs presented variable degrees of erosion and/or cavities and multiple areas of irregular periosteal new bone formation around the neck (Figure 37A). The third rib on each side presented the most severe lesions with lytic changes and exostosis, leading to deformation of articular facets (Figure 37B). Unfortunately, vertebrae were not present in the carcass and could not be examined.

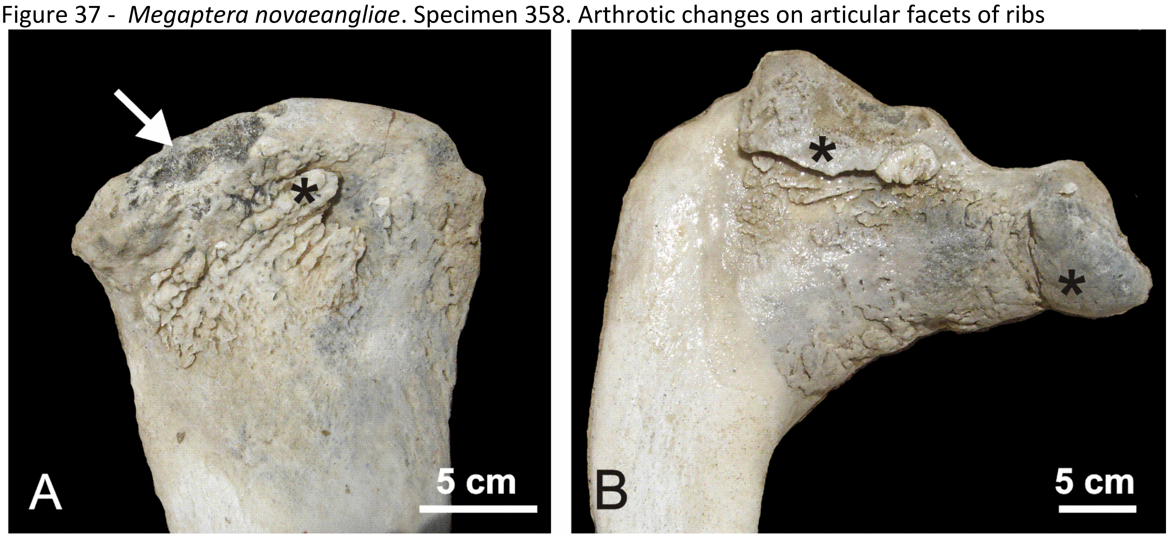
Figure 36 - *Megaptera novaeangliae*. Cervical vertebrae presenting degenerative changes



Fonte: (GROCH, 2011).

Legend. (A) Specimen 238. Frontal view of cervical vertebrae C2 and C3 with ventral syndesmophyte formation (arrow). (B) Specimen 122. Right lateral view of cervical vertebrae with smooth bony outgrowths resembling bridges between adjacent vertebrae C2 and C4, leading to ankylosis of C3 and C4

Figure 37 - *Megaptera novaeangliae*. Specimen 358. Arthrotic changes on articular facets of ribs



Fonte: (GROCH, 2011).

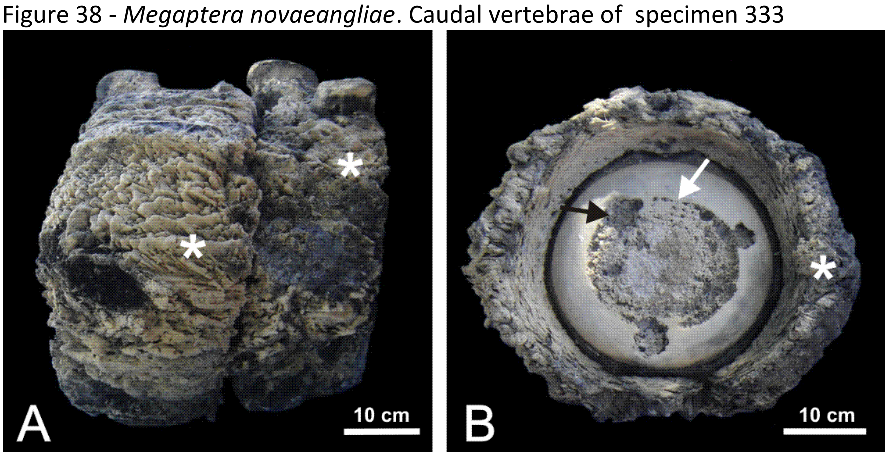
Legend. (A) Cavities (arrow) and exostosis (asterisk) in one of the ribs. (B) Lateral view of the third left rib showing extensive exostosis (asterisks)

5.3.2 Infectious and inflammatory diseases

Primary inflammatory diseases were observed in 4 whales. Partial destruction of the distal articular surface of the radius and periosteal proliferative reaction was observed in Specimen 238 (Figure 38A). In the vertebral column, Specimen 382 presented extensive erosion of the right fossa of the atlantooccipital joint, with extensive irregular exostosis along the whole right lateral aspect of the vertebral body (Figure 38B). In one juvenile whale (Specimen 333), 2 caudal vertebrae had extensive erosion and cavities on adjoining end plates and exuberant irregular bony proliferation on the entire circumferences of their vertebral bodies, but the neural canal did not appear to be compromised (Figure 38). The animal was found stranded with an abnormally high incidence of whale lice (*Cyamus* sp.) covering more than 60% of the skin. No evidence of infection or a specific cause of death was found during partial necropsy. Lesions in the skeletal system were found 1 wk later, when the team returned to the stranding site to examine bones that had not been carried away by the tide. At that time, there were only 5 vertebrae remaining, the last 2 being those affected.

Specimen 122 presented exuberant irregular exostosis around the body of 6 caudal vertebrae, Ca6 to Ca9 and Ca11 to Ca12, clearly more developed on the left side. Vertebrae Ca7 to Ca9 and at least one chevron bone were almost completely ankylosed (Figure 39). Severe disk impairment is evident due to diminished intervertebral space between these vertebrae. Severe erosion and cavities on the caudal epiphysis of Ca6 and Ca11 and on the cranial epiphysis of Ca7 and Ca12 were observed, with complete destruction of the cranial end plate of Ca12. This finding indicates that there was a disruption of the intervertebral disks between Ca6 and Ca7, and between Ca11 and Ca12. It seems that 2 separate processes have taken place in the animal, interspersed by an unaffected vertebra (Ca10). In the same specimen, inflammatory changes were also observed in the right scapulohumeral joint, with erosion in the head of the humerus and focally extensive exostosis in the margin of the scapular glenoid fossa (Figure 40).

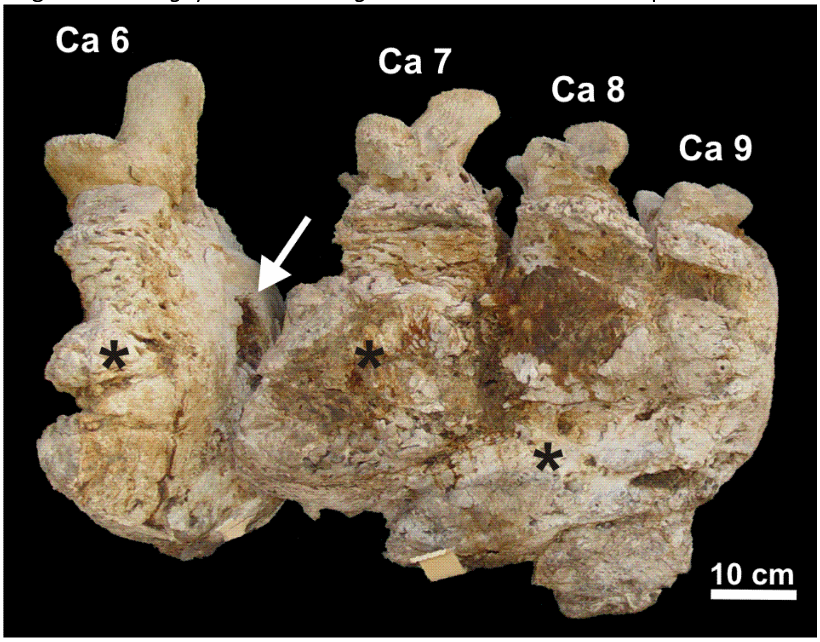
Figure 38 - *Megaptera novaeangliae*. Caudal vertebrae of specimen 333



Fonte: (GROCH, 2011).

Legend. (A) Lateral view of 2 caudal vertebrae with irregular bony proliferation (asterisks). (B) Vertebral epiphysis of the left vertebra shown in A presenting severe erosion, cavities (black arrow) and eburnation (white arrow). Note the thick layer of exuberant exostosis over the whole circumference of the vertebral body (asterisk)

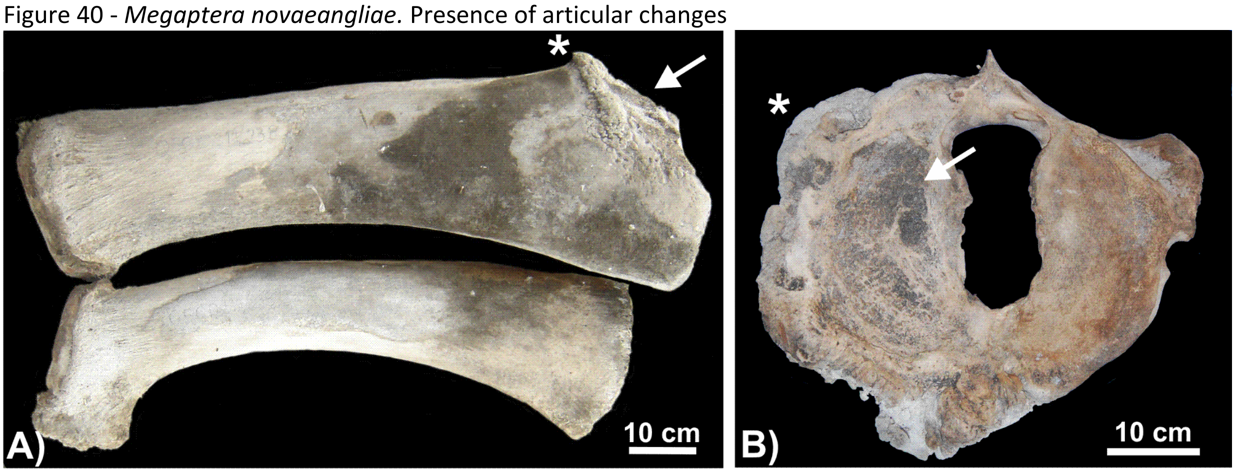
Figure 39 - *Megaptera novaeangliae*. Caudal vertebrae of specimen 122



Fonte: (GROCH, 2011).

Legend. Lateral view of the caudal vertebrae showing severe erosion and cavities on distal epiphysis of Ca6 (arrow) and exuberant irregular exostosis (asterisks) leading to ankylosis of Ca7 to Ca9 and the chevron bone

Figure 40 - *Megaptera novaeangliae.* Presence of articular changes



Fonte: (GROCH, 2011).

Legend. (A) Radius and ulna, Specimen 238. Arthritis on the distal articular surface of radius showing its partial destruction (arrow) and proliferative reaction on the margins of the lesion (asterisk). (B) Atlas, Specimen 382. Extensive erosion of the right glenoid fossa of atlas (arrow) and irregular exostosis on the whole right lateral of the vertebral body (asterisk)

5.3.3 Developmental malformations

Fusion of the distal portion of spinal processes of 2 thoracic vertebrae was found in one specimen (334). Spina bifida, a developmental anomaly in which the neural arch fails to fuse in the midline, leaving an opening dorsally in the medullar canal, was found in Specimen 122 on cervical vertebrae C3 to C5 (Figure 41).

Fusion of the ipsilateral first 2 ribs was found in 4 whales. The fusion was bilateral in 2 specimens and unilateral in the other 2 specimens. The fusion pattern differed between individuals as well as in the same individual. Specimen 320 had bilateral rib fusion forming bicipital ribs, where most of the length of the rib was fused into one mass, forming an enlarged plate distally with 2 well-differentiated heads proximally, which articulate with the respective transverse processes. In Specimen 391, the mid to proximal region of the right first and second ribs was fused, leaving distinct distal regions. Proximally, a small underdeveloped head of the first rib was partially fused to a well-developed head of the second rib, which seems to be articulated with the transverse processes of the juxtaposed thoracic vertebrae. The contralateral first 2 ribs were almost completely fused, forming a wide plate distally and 2 heads proximally, one well differentiated and another underdeveloped (Figure 42). Of whales that had unilateral rib fusion, Specimen 333 presented with fusion in the mid to proximal region with well-differentiated distal portions of the ribs, one well-developed head and another head poorly differentiated. Specimen 238 presented unilateral rib fusion with a distinct bicipital proximal region.

Figure 41 - *Megaptera novaeangliae.* Cervical vertebrae of specimen 122

Fonte: (GROCH, 2011).
Legend. Dorsal view of the cervical vertebrae C2 to C7 showing open neural channel on C3 to C5 (arrow).

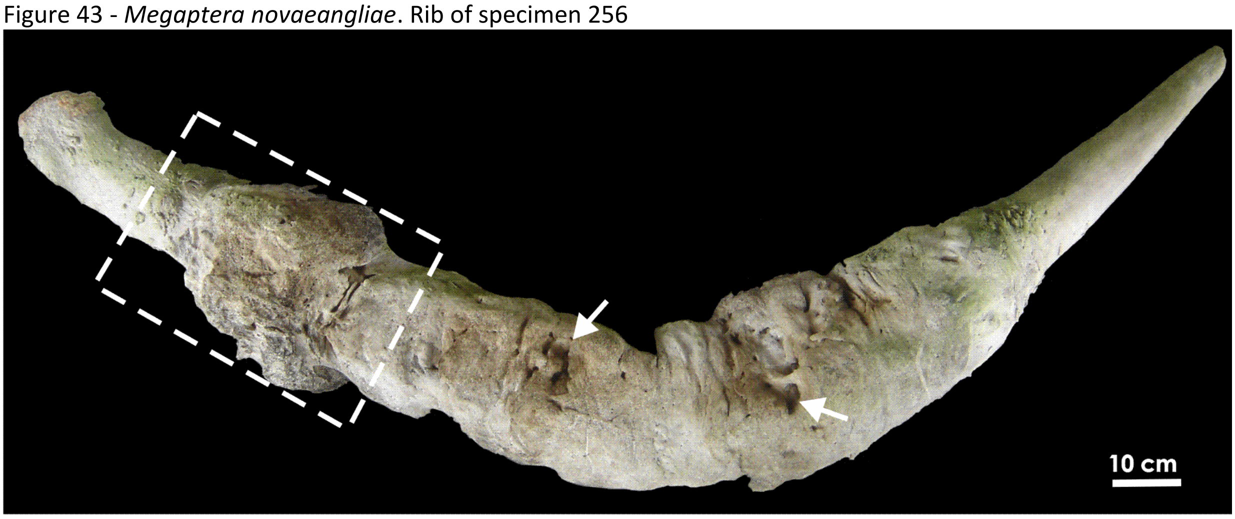
Figure. 42 - *Megaptera novaeangliae*. Ribs of specimen 391

Fonte: (GROCH, 2011).
Legend. Bilateral fusion of the first 2 ribs showing different patterns distally and 2 heads proximally, one well differentiated and the other underdeveloped (arrows).

5.3.4 Traumatic lesions

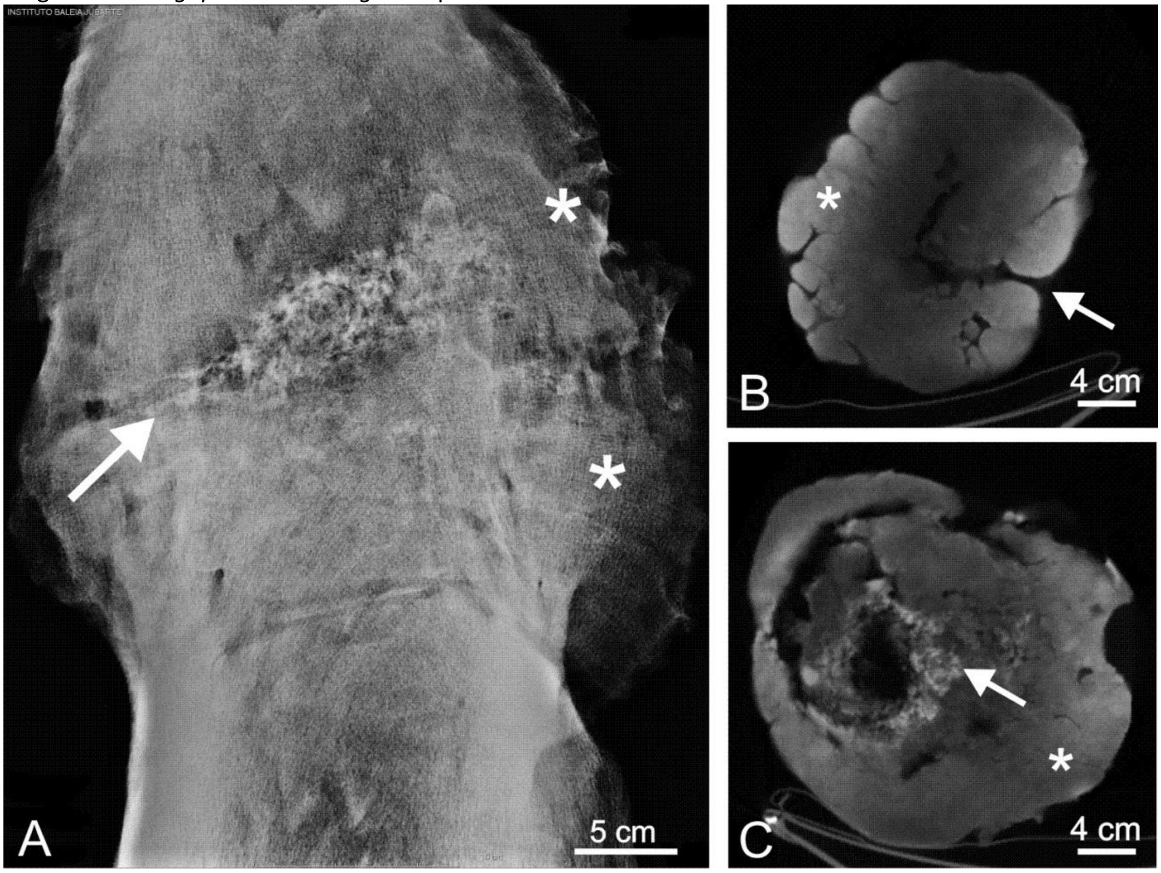
Well-consolidated fractures were observed in the ribs of 3 whales. Specimen 164 presented a clear osseous callus on one rib and an ossified periosteal reaction on 4 additional ribs. Specimens 238 and 358 had evident osseous callus on one rib. The skin and subcutaneous tissue overlying the fracture site was evaluated only in specimen 238 and did not show open wounds or scars. Specimen 256 presented extensive exostosis and remodeling, covering approximately two-thirds of the mid portion of the rib, almost doubling its expected diameter compared with the normal appearance of the extremities (Figure 43). The rib presented with an accentuated curvature and a misalignment compared with other examined ribs. No lesions were observed in the other 12 ribs examined from this animal. The carcass was buried and the other bones could not be accessed. Radiographs and CT scans illustrated a chronic osteomyelitis associated with periosteal proliferation and subperiosteal new bone formation. In most areas, destruction of the normal rib architecture was observed, and the periosteal new bone invaded the medullary cavity. In cross-sections, 3 regions with linear radiolucency over most of the full rib thickness were observed and indicate regions of fractures. In one of these regions, the prominent exostosis resembled an osseous callus (Figure 44A). The radiolucent areas form cavities and fistulae lined by material with higher density (Figure 44B,C).

Figure 43 - *Megaptera novaeangliae*. Rib of specimen 256



Legend. Extensive bony growth and remodeling of one rib. Note the presence of osseous callus (dashed rectangle) and multifocal cavities (arrows). Normal appearance of the rib is observed in both extremities. Fonte: (GROCH, 2011).

Figure 44 - *Megaptera novaeangliae.* Specimen 256



Fonte: (GROCH, 2011).

Legend. (A) Radiograph of the rib region marked by the dashed rectangle in Figure 43. Note the linear radiolucent area (arrow) surrounded by large osseous callus (asterisks). (B,C) Computed tomographs of the rib. Note periosteal exostosis (asterisks), fistulae (B, arrow) and cavities lined with material with higher density (C, arrow).

Table 9 - *Megaptera novaeangliae.* Skeletal lesions in humpback whales

Specimen (serial)	Date of stranding (dd/mm/yy)	Sex	SL (m)	Age class	Bones examined	Type of lesion	Lesion description and location on bone
122	6/6/02	M	13.7	Adult	Complete skeleton	Developmental malformation; degenerative; inflammatory	Spina bifida on cervical vertebrae C2–C4; erosion and spondylophytes in cervical vertebrae C1 and C2; syndesmophyte formation laterally leading to ankylosis of C3–C4; diskospondylitis on caudal vertebra from Ca6 to Ca9 and Ca11–Ca12 with ankylosis of Ca7–Ca9; arthritis on right scapulohumeral joint.
164	26/7/04	M	13.7[b]	Adult	5 ribs, 31 vertebrae, 1 scapula, 2 humeri	Traumatic	One rib with osseous callus, 4 ribs showing ossified periosteal reaction.
238	30/9/06	M	14.63	Adult	8 ribs, 10 vertebrae, 1 scapula, 1 humerus, 2 radii, 1 ulna	Developmental malformation; traumatic; degenerative; inflammatory	First 2 ribs fused on right side; 1 rib with osseous callus on left side; syndesmophyte formation ventrally on cervical vertebrae leading to ankylosis of C2–C3; arthritis on distal articular surface of left radius.
242	26/10/06[a]	M	15.42	Adult	23 ribs, 28 vertebrae, 2 scapulae, 1 humerus, 2 radii	Degenerative	Erosion and spondylophytes on 2 cervical vertebrae; spondylophytes on 2 thoracic and 2 caudal vertebrae.
256	6/8/07	F	14[b]	Adult	13 ribs, 2 scapulae, 2 humeri	Traumatic	Multiple fractures with extensive exostosis and remodeling associated to osteomyelitis on 1 rib.
320	28/10/08	M	12.15	Juvenile	20 ribs, 1 scapula	Developmental malformation	First 2 ribs fused bilaterally.
333	4/7/09	Indet	9.18	Juvenile	22 ribs, 5 vertebrae, 1 scapula, 1 humerus	Developmental malformation; inflammatory	First 2 ribs fused on right side; diskospondylitis on 2 caudal vertebrae (chevron bearing region).
334	2/7/09	Indet	Indet	Indet	24 ribs, 45 vertebrae, 1 scapula, 1 humerus, 2 mandibles	Developmental malformation	Fusion of spinous processes of 2 thoracic vertebrae.
358	16/10/09[a]	Indet	Indet	Indet	18 ribs, sternum	Inflammatory; traumatic	Erosion and hyperostosis on articular facets of 13 ribs; 1 rib with osseous callus.
382	17/8/10	Indet	9.0[b]	Juvenile	29 ribs, 16 vertebrae, 2 scapulae, 2 humeri	Inflammatory	Arthritis on glenoid fossa of cervical vertebra C1 with exuberant exostosis on vertebral body.
391	20/8/10	Indet	11	Juvenile	18 ribs, 17 vertebrae, 1 scapula	Developmental malformation	First 2 ribs fused bilaterally.
499	14/9/06[a]	Indet	Indet	Indet	2 humeri	Degenerative	Osteophytes on distal articulation of humerus.

Legend. Specimens (n = 12) are ordered by serial field number. SL: standard length; Indet: indeterminate. Type of lesion according to Kompanje (1999) and Thompson (2007);
[a]Date of examination for specimens with unknown stranding date; [b]estimated length.

5.4 DISCUSSION

After the protection of humpback whales in Brazil (Brazil Federal Decree no. 211, 28 Feb 1962) and the statutory ban of commercial whaling in the end of the last century (Brazil Federal Law no. 7.643, 18 Dec 1987), the population of humpback whales has shown evidence of recovery (ANDRIOLO et al., 2010). Despite increases in the population, little is known about the health conditions, threats and impacts on this species, and how these factors may affect its recovery.

However, assessing the health of large whales in the wild is a difficult task. Stranded humpback whales are often found dead, in states of advanced decomposition, with only skeletal remains available for diagnostic examination. The gross evaluation of bone may contribute to the understanding of pathologic processes that affect the health of these animals. In fact, like other systems, the skeletal system reacts to injury and is susceptible to circulatory, inflammatory, neoplastic, metabolic and developmental disorders (ROSENBERG, 2005). Evidence of infectious and degenerative diseases for example, has been inferred through osseous changes in cetaceans (COWAN, 1966; DE SMET, 1977; MORTON, 1978; KINZE, 1986; KOMPANJE, 1995c, 1999; SWEENY et al., 2005; FÉLIX; HAASE; AGUIRRE, 2007; SIMÕES-LOPES; MENEZES; FERIGOLO, 2008).

Skeletal abnormalities have been documented in several species of cetaceans; however, published information on osseous pathology in humpback whales is scarce. Five single cases have been reported (STEDE, 1994; PATERSON; VAN DYCK, 1996; KOMPANJE, 1999; FÉLIX; HAASE; AGUIRRE, 2007; HELLIER; HUFTHAMMER; LISLEVAND, 2011). Analysis usually relies on museum specimens, or stranded animals dissected with the purpose of collecting the skeleton. To our knowledge, this is the first report on bone pathology of humpback whales from the southwestern Atlantic Ocean and the first study on skeletal abnormalities based on the systematic analysis of skeletal remains of stranded whales.

Skeletal changes were found in at least 24.5% (12/49) of the humpback whales examined. A higher percentage was found in males than in females. However, it is important to consider that in most cases, the sex was not determined (due to absent distinctive features or tissue sample suitable for genetic analysis) and a prolapsed penis in decomposed carcasses facilitates identifying males. In this case series, calves stranded with higher frequency; however, skeletal lesions were observed only in juveniles and adult animals. A higher incidence of stranded calves is expected, since the region is used for calving. The low incidence of skeletal abnormalities in calves may be explained because of the grossly recognizable bony changes that may take some time to be established and may not be readily identified in the first months after birth. However, traumatic injuries to bones from the infanticide behavior of adult dolphins have been documented in bottlenose dolphin *Tursiops truncatus* calves (PATTERSON et al., 1998; DUNN et al., 2002). Injuries consistent with compression during parturition have been reported in a humpback whale and a minke whale *Balaenoptera acutorostrata* (PATERSON; VAN DYCK, 1996). Furthermore, a condition described as osteoperiostitis ossificans hypertrophicans was recognized in the caudal vertebra of a 6.9 m female humpback whale calf from Germany (STEDE, 1994), demonstrating that skeletal changes may be recognized in any stage of life.

The skeletal survey of large whales is quite challenging due to difficulties related to the animal's size, necessary logistics and limitations imposed by tidal variations. These limitations unfortunately precluded reliable skull examination in most cases in the present study; however, skulls of 4 animals presented with scavenging or post mortem damage. The number of bones examined per animal varied from 2 to the entire postcranial skeleton. These results are therefore conservative estimates of the prevalence of lesions as it was not possible to address the condition of all bones in the majority of the carcasses.

In the vertebral column, inflammatory conditions were found in the cervical region of one whale involving the atlantooccipital joint (Specimen 382) and in the caudal region of 2 other whales, involving 2 vertebrae of a juvenile whale (Specimen 333) and 6 vertebrae of an adult whale (Specimen 122), on which the lesions led to ankylosis of 3 vertebrae and chevron bones. In the case of Specimen 382 (Figure 40B), the lesion observed in the atlas with severe extensive articular surface destruction and exuberant irregular bone reaction suggests an inflammatory etiology, considering that in degenerative conditions the exostosis would generally be more regular, well defined and smooth. Pathological changes in the atlantooccipital joint have been diagnosed as degenerative joint disease in 3 bottlenose dolphins, one pantropical spotted dolphin *Stenella attenuata* and one pygmy sperm whale *Kogia breviceps*, while infectious inflammatory arthritis was reported in 3 specimens of bottlenose dolphin from the western coast of the Gulf of Mexico (TURNBULL; COWAN, 1999).

The deformations observed in the caudal vertebrae of Specimens 333 and 122 (Figures 38 and 39, respectively) are presumably due to a chronic bacterial infection affecting intervertebral disks and adjacent vertebral bodies (spondylodiscitis or infectious spondylitis). Differential diagnosis includes spondyloarthritis, a group of inflammatory diseases related to genetic and environmental factors (including ankylosing spondylitis, Reiter's syndrome, and reactive arthritis, among others), known to occur in humans predominantly with positive Human Leukocyte Antigen b27 (TURNBULL; COWAN, 1999). This process was suspected to occur in fin whales *Balaenoptera physalus* and in one humpback whale from Denmark (KOMPANJE, 1999), as well as in one humpback whale from Ecuador (FÉLIX; HAASE; AGUIRRE, 2007). Spondyloarthritis is grossly characterized by ossification of perivertebral ligaments and connective tissues outside the annulus fibrous forming bony bridging between consecutive vertebral bodies without evidence of intervertebral disk degeneration, as well as by the presence of paradiscal ossicles and changes of zygapophyseal joints (KOMPANJE, 1999). In Specimens 122 and 333, injury of the intervertebral disk was evident and diagnosis of diskospondylitis or spondylodiscitis seems to be appropriate. Kompanje (1999) reported similar lesions in the bowhead whale *Balaena mysticetus*, the blue whale *Balaenoptera musculus* and Bryde's whale *Balaenoptera edeni*. In odontocete cetaceans, lesions with the characteristics of spondylodiscitis have been reported in the long-finned pilot whale *Globicephala melas* (COWAN, 1966), the bottlenose dolphin (MORTON, 1978; ALEXANDER; SOLANGI; RIEGEL, 1989; KOMPANJE, 1999), the Guiana dolphin *Sotalia guianensis* (SIMÕES-LOPES; MENEZES; FERIGOLO, 2008), Sowerby's beaked whale *Mesoplodon bidens*, the harbor porpoise *Phocoena phocoena* (KOMPANJE, 1995b, 1999), the white-beaked dolphin *Lagenorhynchus albirostris*, the Atlantic white-sided dolphin *Lagenorhynchus acutus*, the short-beaked common dolphin *Delphinus delphis*, the false killer whale *Pseudorca crassidens*, the killer whale *Orcinus orca* and the Irrawaddy dolphin *Orcaella brevirostris* (KOMPANJE, 1999).

Infectious agents are capable of compromising joints in humans and domestic animals. Arthritis may also be a manifestation of systemic infection. Certain infectious agents may initiate an immune-mediated reaction to nonviable bacterial components in response to infection at sites distant from the joint (THOMPSON, 2007). In the juvenile specimen (Specimen 333), a male of 9.18 m body length, external examination did not show evidence of injury or a penetrating wound that could cause the direct implantation of infectious agents from the skin surface into the joint. Hematogenous infection of vertebrae is common in domestic animals as a sequel to neonatal bacteremia or septicemia following omphalophlebitis or infections of the gastrointestinal tract or lungs (THOMPSON, 2007). Inadequate transfer of colostrum immunoglobulins is a predisposing factor. In this case, it is possible that the lesion occurred secondarily to an infection elsewhere in the body. Unfortunately, necropsy was not performed on the adult specimen (Specimen 122); however, in adult domestic animals, infection of joints is often induced by external and internal traumatic events.

Externally, Specimen 333 also showed a relatively high infestation of whale lice. An adult humpback whale with marked scoliosis in the caudal region was found almost motionless and heavily parasitized by whale lice off the coast of Maui (OSMOND; KAUFMAN, 1998). The young humpback whale with lesions on lumbar and caudal vertebrae reported by Félix et al. (FÉLIX; HAASE; AGUIRRE, 2007) also showed an abnormally high cyamid infestation. Cyamids are epibionts specialized in feeding on whale skin, normally found in low quantities over the skin of whales. The infestation level is possibly controlled by the removal rates during swimming and surfacing behaviors (i.e. breaching and tail slapping). An abnormally high infestation is usually associated with debilitated animals and may be a consequence of decreased mobility (PFEIFFER, 2009). Like other cetaceans, the movement of whales is produced through the up-and-down movement of the peduncle and propulsion of the fluke (SLIJPER, 1946). As suggested by Osmond and Kaufman (1998) and Félix et al. (2007), the infestation may be a consequence of severe lesions in the vertebral column. Therefore, it is possible that the lesions found in the axial skeleton of Specimen 333 may have reduced its mobility and fitness, leading to an increase in ectoparasites. Since the maneuverability and the tail are also the major defenses against predators such as sharks and killer whales (CHITTLEBOROUGH, 1953), lesions compromising mobility may predispose the animal to predation. Furthermore, chronic inflammatory lesions may be detrimental to the general health of these animals. For these reasons, we believe that the lesions found in the caudal vertebrae of Specimens 122 and 333 may have contributed to their stranding and death.

Two animals showed lesions in the flipper. Degenerative changes were found in the scapulohumeral joint of Specimen 122 and severe arthritic changes were observed in the radius of Specimen 238 (Figure 40A). A similar inflammatory lesion on the right radius with bony lysis and periosteal reaction was reported in a North Atlantic right whale *Eubalaena glacialis* associated with entangled fishing gear (MOORE et al., 2004). In the present case, there was no external evidence of entanglement; however, the pectoral fin was found completely sectioned at the lesion level with distal structures missing, and the entanglement hypothesis cannot be ruled out. Differential diagnosis includes penetrating and blunt trauma from unknown origin and infection of adjacent tissues.

Spondyloarthrosis was found in the cervical region of 3 whales (Specimens 242, 238 and 122; Figure 36) and also in the thoracic and caudal regions of 1 whale (Specimen 242). Primary degenerative joint disease occurs in older animals without any apparent predisposing cause. Secondary degenerative joint disease is caused by direct damage, poor conformation, and instability or abnormal directional forces in the joint or supporting structures, predisposing the animal to degenerative changes, and can occur in adult and in immature animals (THOMPSON, 2007). Differential diagnosis includes discospondylitis and spondyloarthritis (KOMPANJE, 1999; THOMPSON, 2007). Although it was not possible to verify the condition of congruent endplates of ankylosed cervical vertebrae, erosion was present in non-ankylosed vertebrae, paradiscal ossicles were not observed and zygapophyseal joints were normal. The diagnosis of intervertebral disk degeneration leading to spondyloarthrosis was judged more appropriate in these cases. The condition is also known as discarthrosis, spondylosis deformans or spondylitis deformans. Similar lesions have been reported in the Guiana dolphin (FURTADO; SIMÕES-LOPES, 1999; SIMÕES-LOPES; MENEZES; FERIGOLO, 2008), the white-beaked dolphin (VAN BREE; NIJSSEN, 1964; KOMPANJE, 1995c; GALATIUS et al., 2009), the harbor porpoise (KINZE, 1986), the beluga *Delphinapterus leucas* and the bottlenose dolphin (FURTADO; SIMÕES-LOPES, 1999), the false killer whale, the short-finned pilot whale *Globicephala macrorhynchus*, the northern bottlenose whale *Hyperoodon ampullatus*, Hector's beaked whale *Mesoplodon hectori* and True's beaked whale *Mesoplodon mirus* (KOMPANJE, 1999), and the rough-toothed dolphin *Steno bredanensis*, the Atlantic spotted dolphin *Stenella frontalis*, the clymene dolphin *Stenella clymene* and the long-beaked common dolphin *Delphinus capensis* (FURTADO; SIMÕES-LOPES, 1999).

Bilateral, erosive and remodeling lesions on subchondral rib surfaces were observed in 13 ribs of Specimen 358 (Figure 37). Similar lesions have been reported in sperm whales *Physeter macrocephalus* and were diagnosed as osteonecrosis, possibly resulting from dysbaric stress (MOORE; EARLY, 2004). Differential diagnosis to this process includes infection and autoimmune inflammatory reaction. At present, there is no evidence that humpback whales can undergo lesions associated with dysbaric stress and complementary studies would be necessary to clarify this hypothesis. In the present case, the pathological changes observed on ribs are likely of degenerative etiology.

The presence of osseous callus was observed in the ribs of 3 whales (Specimens 164, 238 and 358), with evidence of fracture or fissure repair. Fractures in large whales have been reported in ribs of an 18.9 m fin whale (MOORE; EARLY, 2004), in the mandible of a 11.4 m bowhead whale (PHILO; HANNS; GEORGE, 1990) and in the radius and ulna of a skeletally immature fin whale (OGDEN et al., 1981) of unknown origin. Bilaterally fractured ribs and superficial focally extensive cystic osseous lesions associated with periosteal new bone formation dorsolaterally in the supra-orbital region were observed in a humpback whale and a minke whale calf from Australia; these lesions were consistent with trauma during parturition (PATERSON; VAN DYCK, 1996). Fractures on the mandible, skull and thoracic transverse process of North Atlantic right whales were attributed to propeller cuts or massive blunt trauma from ship collision (MOORE et al., 2004). In odontocete cetaceans, rib fractures are apparently common (COWAN, 1966; DE SMET, 1977; KOMPANJE, 1995a; DUNN et al., 2002; SIMÕES-LOPES; MENEZES; FERIGOLO, 2008). In our cases, the causes of fractures were not apparent. The fracture may be pathological or a result of blunt trauma. A pathological fracture could have been the result of neoplasia, osteomyelitis or degenerative bone disease. Blunt trauma could have occurred from ship collision, a result of aggressive intraspecific or interspecific behavior, or interaction with predators (DE SMET, 1977; PHILO; HANNS; GEORGE, 1990; ROSS; WILSON, 1996; PATTERSON et al., 1998; DUNN et al., 2002; MOORE et al., 2004). In cases found with a well-formed osseous callous, there was no macroscopic evidence to support the diagnosis of pathological fracture and a traumatic event was considered to be more likely. However, histological and radiological studies would be necessary to confirm this hypothesis.

Specimen 256 (Figure 43) presented extensive bony growth and remodeling that covered approximately two-thirds of the rib, yet both extremities were relatively normal. The rib showed accentuated curvature and a misalignment. Radiological images evidenced multiple sites of possible fractures (Figure 44). Our hypothesis is that one or more fractures triggered the infection leading to osteomyelitis; however, it is uncertain whether osteomyelitis was already present, predisposing the bone to pathological fractures. The formation of the osseous callus was chronically stimulated, leading to extensive diffuse exostosis and osseous remodeling. The overall radiological appearance was similar to the infectious form of a condition known in humans as diffuse sclerosing osteomyelitis (DSO), with extensive sclerosis and periosteal new bone formation associated with infection (GROOT; VAN MERKESTEYN; BRAS, 1996). In this case, it is possible that the secondary infection of multiple complicated fractures in the rib induced a chronic reparative reaction, producing features similar to those of DSO. Lesions with grossly similar appearance, attributed to several healed fractures with complicating osteomyelitis, were found in one rib of killer whale found in Wieringen Island, Holland (KOMPANJE, 1991).

Fusion of the first 2 cranial ribs was found in 4 whales, both unilaterally (Specimens 333 and 238) and bilaterally (Specimens 320 and 391; Figure 42). A similar condition has been mentioned in literature as a bicipital, bifid or double-headed rib (TURNER, 1871; ALLEN, 1916; NISHIWAKI; KASUYA, 1971; OMURA et al., 1981; DELYNN et al., 2011). This developmental abnormality is characterized as the fusion of the first 2 ribs with a cleft in the vertebral end, originating 2 distinct processes that may articulate with the last cervical and first thoracic vertebra or with the first 2 thoracic vertebrae, which has occasionally been observed in large whale skeleton museum specimens, including species such as the North Atlantic right whale (ALLEN, 1916), the southern right whale *Eubalaena australis*, the blue whale (TURNER, 1871), the sei whale *Balaenoptera borealis* (SLIJPER, 1936; NISHIWAKI; KASUYA, 1971) and Bryde's whale (SLIJPER, 1936; OMURA et al., 1981). In the present study, bicipital ribs were found unilaterally and bilaterally in humpback whales. Additionally, another form of abnormality with fusion of ribs forming 1 or 2 distinct processes in the vertebral end and 2 processes in the sternal end has been found. To our knowledge, this form of fusion has only been described bilaterally in a blue whale by Slijper (1936). Unilaterally fused fifth and sixth ribs with distinct extremities, associated with a deformed sternum and rib cage distortion, have been reported in the bottlenose dolphin (DELYNN et al., 2011). The specimen also presented with bilateral bicipital ribs and severe developmental malformations in the axial skeleton (DELYNN et al., 2011). In the present study, this condition was found unilaterally and bilaterally in specimens with either normal or bicipital rib fusion of the first 2 ribs in the contralateral side.

Spina bifida was observed in 3 cervical vertebrae of Specimen 122 (Figure 41). Spina bifida results from defective closure of dorsal vertebral laminae in a vertebral column segment (THOMPSON, 2007). Two categories of spina bifida malformations may be considered in the present case: spina bifida occulta, without protrusion of the spinal cord or meninges, and spina bifida cystic, in which there is protrusion through the defective vertebral arch of a cystic swelling, involving the meninges, spinal cord or both (DORLAND, 1997). Defective arch closure was reported in the long-finned pilot whale, mostly involving thoracic vertebrae (COWAN, 1966). In domestic animals, it has been reported in canine, feline, bovine, equine and ovine specimens (WILSON et al., 1979; LECOUTEUR; GRANDY, 2010) and may be associated with various soft tissue defects including defective innervations of muscle groups, kidney fusion and uterine aplasia (THOMPSON, 2007). Degenerative changes were observed in the spinal column of an 18-yr-old bottlenose dolphin with multiple developmental abnormalities in the axial skeleton (DELYNN et al., 2011). In the present case, the cervical vertebrae showing spina bifida also had spondyloarthrosis, leading to ankylosis. It is unclear whether the lesions are associated, but it should be considered. Overall, because it was an adult animal, the condition may not have caused major disabilities which would compromise its survival and may have manifested as the occult form (spina bifida occulta).

The developmental malformations documented herein are apparently incidental findings of no major concern to the health of these individuals; however, it is unknown whether they were associated with abnormalities in adjacent soft tissues and organs. Therefore, further studies should be carried out to elucidate whether skeletal abnormalities affect innervations, vasculature pathways and tendon insertions or predispose animals to degenerative changes.

This study contributes to a better understanding of the skeletal disorders in humpback whales, adding new information from 12 cases out of 49 animals, which shows that approximately one-quarter of the animals had at least one type of lesion. This is a conservative estimate in view of the limitations on the number of bones examined per animal. Therefore, efforts to examine skeletal tissues during necropsy are encouraged and provide additional insights into the life history and morbid conditions of the animals found in advanced stage of decomposition. Complementary diagnostic techniques are needed to better understand and differentiate pathologic conditions affecting bones. Diagnostic imaging techniques are valuable tools (SWEENY et al., 2005). Bacteriology and molecular techniques are needed for the survey of possible etiologic agents involved on infectious conditions. Histopathological and immunohistochemical analyses of bone and adjacent soft tissue may provide further understanding on the etiology and pathogenesis of these conditions.

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CONSIDERAÇÕES FINAIS

6 CONSIDERAÇÕES FINAIS

Este estudo consiste no primeiro levantamento sobre aspectos sanitários das baleias-jubarte no Brasil. Por meio da análise de imagens obtidas de animais em vida livre ao longo de 5 anos (2008-2012) foi possível caracterizar os principais padrões de lesões cutâneas nesta população e quantificar marcas decorrentes de interação com predadores, intraespecíficas e antropogênicas, tais como interação com artefatos de pesca e colisão com embarcações.

As taxas de indivíduos que sobreviveram a interações antrópicas demostram a existência de conflitos e refletem a pressão oriunda da atividade humana sofrida pelas baleias nas suas áreas de uso. Da mesma forma, as marcas decorrentes de interação com potenciais predadores podem indicar o grau ocorrência deste tipo de interação na população.

As baleias-jubarte apresentam alta prevalência de lesões nodulares ou bolhosas e diversos padrões de manchas claras ao longo do corpo, além de outras lesões. Apesar de não se ter encontrado evidências de condições fatais, as lesões observadas podem representar condições subletais que ao atuar sinergisticamente podem levar ao desequilíbrio nas defesas do organismo e impactar a saúde dos indivíduos.

A prevalência das lesões encontradas nesta população de baleias-jubarte foi estimada utilizando-se uma abordagem conservadora, porém sistemática, e permite ser utilizada como base para uma avaliação contínua do estado de saúde desta população.

Os achados histopatológicos em animais encalhados permitiram elucidar aspectos importantes relacionados às causas de morte das baleias-jubarte. Grande parte dos filhotes estudados apresentou alterações compatíveis com distúrbios neonatais ou perinatais, além de, em alguns casos, apresentarem processos infecciosos significativos.

O estudo de lesões esqueléticas em animais encalhados demostrou que as baleias-jubarte são suscetíveis a processos degenerativos, infecciosos, traumáticos e malformações do desenvolvimento.

A caracterização macroscópica dos processos cutâneos em baleias em vida livre, bem como a das alterações histopatológicas em diversos órgãos e tecidos e a identificação de processos patológicos no sistema esquelético das baleias-jubarte na costa brasileira lançam bases para investigações futuras acerca da saúde desta população. Além disso, os dados apresentados neste trabalho contribuem para o entendimento dos processos patológicos em grandes baleias, aportando valiosa informação em termos de patologia comparada de cetáceos. No entanto, mais estudos são necessários para elucidar as causas e o significado patológico das diversas condições observadas neste estudo. Técnicas diagnósticas complementares tais como análises microbiológicas, imuno-histoquímicas e moleculares poderão proporcionar um melhor entendimento quanto a etiologia e patogênese destas condições.

Enfim, os dados obtidos neste estudo apresentam uma primeira visão sobre condições sanitárias e processos patológicos em baleias-jubarte no Brasil e, cremos, poderão direcionar os estudos futuros nesta área. Valendo-se do monitoramento da ocorrência de lesões não-letais, bem como por meio da investigação dos processos patológicos e causas de morte das baleias-jubarte, será possível inferir sobre as condições do ambiente onde estes animais vivem, e desta forma, contribuir para o aprimoramento das ações de manejo e conservação da espécie.

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ANEXOS

ANEXO A - FICHA DE OBSERVAÇÃO DE BALEIAS-JUBARTE

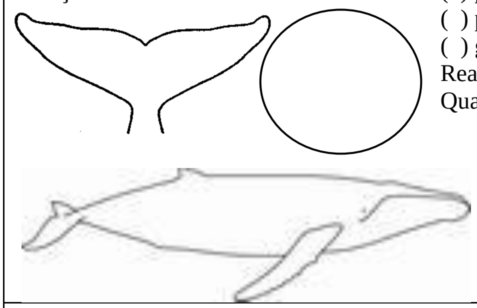
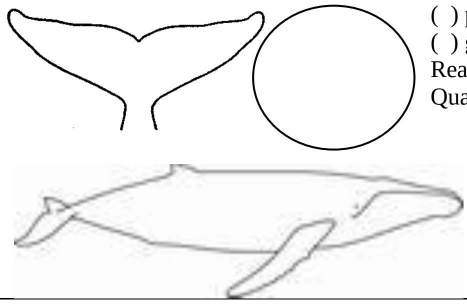
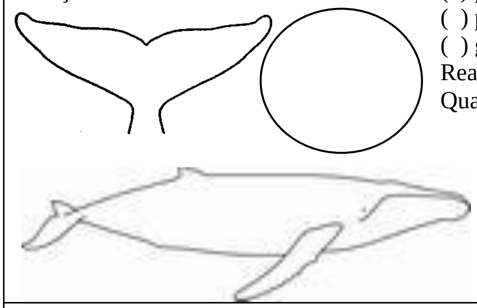
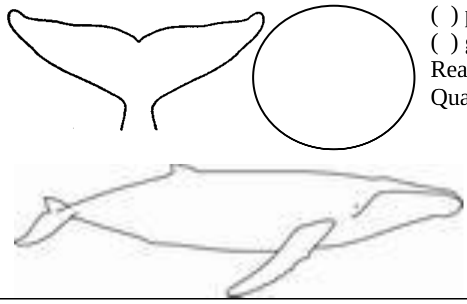
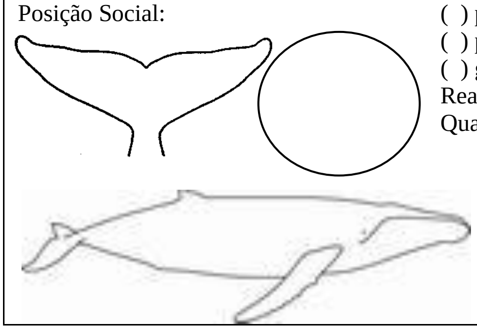
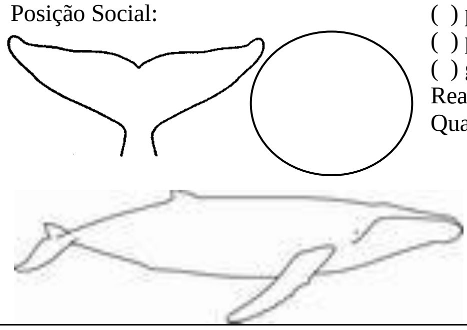
INSTITUTO BALEIA JUBARTE
FICHA DE OBSERVAÇÃO DE BALEIAS-JUBARTE

Ficha nº :______ Cruzeiro nº :______ Data: _____/_____/_______	Nº Grupo: ______	Registrador: ________________	
Hora de início e término da avistagem:____________ às ____________ (Total __________________ minutos)			
Horário de aproximação : _____________			
Pos. geogr inicial:______________ º S	Pos. geogr.final:	______________ º S	Profundidade: ________
______________ º W		______________ º W	Fotógrafo:___________

Nro. de indivíduos: ___________ Composição social: ________________ GC: sim ( ) não ( )

Comportamentos observados: [ ] antes da aproximação ( ) após aproximação { } filhote

[ ] ( ) { } repouso (boiada)	[ ] ( ) { } batida de cabeça	[ ] ( ) { } salto _______________
[ ] ( ) { } deslocamento (rumo)	[ ] ( ) { } espiar	[ ] ( ) { } exp. caudal em mergulho__
[ ] ( ) { } canto		[ ] ( ) { } batida de cauda ________
[ ] ( ) { } exp. caudal parada	[ ] ( ) { } salto/golpe de caudal	[ ] ( ) { } batida de peitorais ______
	[ ] ( ) { } exposição ventral	
	[ ] ( ) { } serpentear	[ ] ( ) { } emissão de ruído
	[ ] ( ) { } merg. em deslocamento	[ ] ( ) { } exalação de bolhas

ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:	ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:
ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:	ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:
ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:	ID Padrão: Posição Social:  Biópsia n◦: ( ) pele em álcool ( ) pele em formol ( ) gordura Reação: S( ) Ñ( ) Qual:

Observações:

ANEXO B - MANUSCRITO PUBLICADO NA REVISTA EMERGING INFECTIOUS DISEASES

C. cayetanensis and these closely related species in monkeys; what additional monkey host species, especially on Bioko Island, may harbor *Cyclospora* spp.; and what other as yet unrecognized species of *Cyclospora* may be infecting primates.

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Novel Cetacean Morbillivirus in Guiana Dolphin, Brazil

To the Editor: Since 1987, morbillivirus (family *Paramyxoviridae*, genus *Morbillivirus*) outbreaks among pinnipeds and cetaceans in the Northern Hemisphere have caused high rates of death (*1*,*2*). Two morbillivirus species are known to affect aquatic animals: *Phocine distemper virus* (PDV) and *Cetacean morbillivirus* (CeMV). PDV has been isolated from pinnipeds, and 3 strains of CeMV (porpoise morbillivirus [PMV], dolphin morbillivirus [DMV], and pilot whale morbillivirus [PWMV]) have been isolated from dolphins and whales (*3*,*4*).

Serologic surveys indicate that morbilliviruses infect marine mammals worldwide (*5*); however, only 1 fatal case in a bottlenose dolphin (*Tursiops truncatus*) has been confirmed in the Southern Hemisphere (in the southwestern Pacific Ocean) (*6*). Positive DMV-specific antibody titers in 3 Fraser's dolphins (*Lagenodelphis hosei*) stranded off Brazil and Argentina in 1999 indicate the exposure of South Atlantic cetaceans to morbillivirus (*7*). We report a case of lethal morbillivirus infection in a Guiana dolphin (*Sotalia guianensis*), a coastal marine and estuarine species that occurs off the Atlantic Coast of South and Central America.

A female Guiana dolphin calf (108 cm in total body length) (*8*) was found stranded dead in Guriri (18°44′S; 39°44′W), São Mateus, Espírito Santo State, Brazil, on November 30, 2010; the dead calf was severely emaciated. Postmortem examination of the animal showed multifocal ulcers in the oral mucosa and genital slit, diffusely dark red and edematous lungs, and congested and edematous brain. Samples of selected tissues were collected, fixed in buffered formalin, and processed according to routine histopathologic methods. By microscopy, the most noteworthy lesions included marked lymphoplasmacytic and neutrophilic meningoencephalitis, optic nerve perineuritis, and hypophysitis. Lungs showed moderate acute diffuse lymphoplasmacytic and neutrophilic interstitial pneumonia; severe multicentric lymphoid depletion and multifocal necrotizing hepatitis were also observed.

Immunohistochemical analysis was performed by using CDV-NP MAb (VMRD, Inc., Pullman, WA, USA), a monoclonal antibody against the nucleoprotein antigen of canine distemper virus that cross-reacts with cetacean morbilliviruses (*9*). Known positive and negative control tissues and test sections with omitted first-layer antibody were included. Viral antigen was detected in neurons in the brain, bronchiolar epithelium and macrophages in the lungs, bile duct epithelium in the liver, and macrophages and lymphocytes in lymph nodes.

We extracted RNA from frozen lung samples by using TRIzol Reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions and amplified a 374-bp conserved fragment of the phosphoprotein (P) gene by reverse transcription PCR. The following *Morbillivirus* spp.–specific primers were used for PCR: 5′-ATGTTTATGATCACAGCGGT-3′ (forward) and 5′-ATTGGGTTGCACCACTTGTC-3′ (reverse) (*3*). MEGA5 (http://megasoftware.net/) was used to construct a neighbor-joining phylogenic tree based on the sequenced amplicon from this study (GenBank accession no. KF711855) and 12 other GenBank sequences that represent the 6 morbillivirus species already described in the literature. The analysis placed the Guiana dolphin strain at the CeMV clade, but segregated it from the already described dolphin morbillivirus strains PMV, DMV, and PWMV (Figure). The sample shared 79.8% nt and 58.4% aa identity with PMV, 78.7% nt and 56.6% aa identity with DMV, and 78.7% nt and 57.1% aa identity with PWMV. Within the *Morbillivirus* spp., PDV shared the lowest sequence identity (51.1% nt and 26.8% aa).

In summary, sequence analysis of the morbillivirus from the dead Guiana dolphin suggests that the virus is a novel strain of the CeMV species; this conclusion is supported by phylogenic analysis and geographic distribution of the virus and by its distinct host. Emaciation, marked lymphoid depletion, interstitial pneumonia, and meningoencephalitis are common findings in morbillivirus-infected animals (*1*,*2*). Together with antigenic and genomic evidence, our findings indicate that morbillivirus infection is extant in Guiana dolphins in the waters off Brazil.

Morbillivirus outbreaks have caused a high number of deaths among pinnipeds and cetaceans and are a major risk to previously unexposed nonimmune populations of aquatic mammals (*1*,*2*). A high number of morbillivirus-related deaths have not yet been reported among aquatic mammals in the waters off Brazil, but our findings shows that Guiana dolphin calves are susceptible to infection. Subclinical morbillivirus infection with immune suppression has been reported in bottlenose dolphins in Florida (*10*). It is unknown whether subclinical infection occurs in this host population or whether the virus has undergone species-adaptive changes, as proposed for PWMV (*4*). The sequence data from our study suggest that the virus from the Guiana dolphin calf is the fourth member of the CeMV group and is closer to the root of the CeMV clade than to that of DMV, PMV, or PWMV. Further studies are required to determine the epidemiology of morbillivirus infection in this and other cetacean species and to assess the risk for epizootic outbreaks among South Atlantic cetaceans.

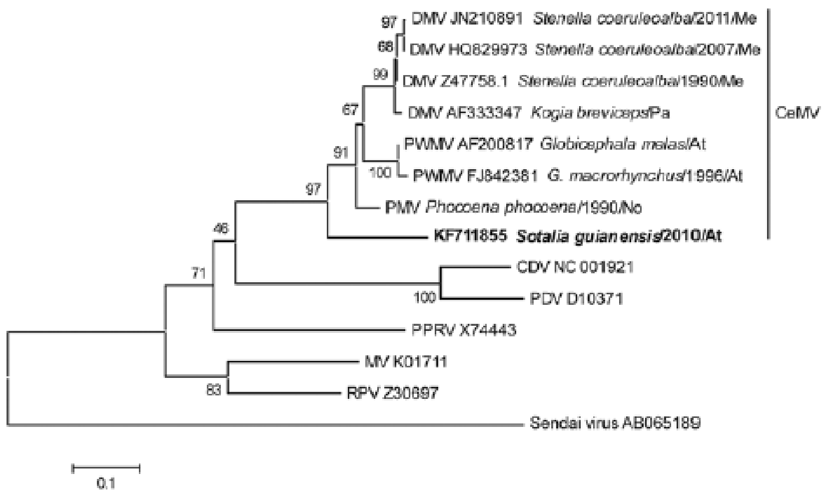

Figure. Phylogenetic tree of a 374-bp conserved region from the phosphoprotein gene of a cetacean morbillivirus isolated from a Guiana dolphin (in boldface; GenBank accession no. KF711855) and 12 other previously described morbilliviruses. Sendai virus was added as an outgroup member. Sequences were aligned and a neighbor-joining tree with 1,000 bootstrap replications was generated by using MEGA5 (http://megasoftware.net/). For comparison, recognized viruses of the *Morbillivirus* spp. (*Measles virus* [MV], *Rinderpest virus* [RPV], *Peste-des-petits ruminants virus* [PPRV], *Canine distemper virus* [CDV], and *Phocine distemper virus* [PDV]) were included, as were the 3 *Cetacean morbillivirus* (CeMV) strains: porpoise morbillivirus (PMV), dolphin morbillivirus (DMV), and pilot whale morbillivirus (PWMV). Sequence names are followed by species of cetacean, year of stranding (when available), and the abbreviation for the geographic area. Me, Mediterranean Coast; Pa, Pacific Ocean; At, Atlantic Ocean; No, North Sea. The sequence for PMV strain *Phocoena phocoena*, is from Barrett et al. (*3*). The scale bar indicates

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