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Unveiling evidence of natural and anthropogenic skin marks on baleen whales (Northwestern Iberian Peninsula coast)

ALGARVE UNIVERSITY

Faculty of Science and Technology

2021/2022

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Unveiling evidence of natural and anthropogenic skin marks on baleen whales (Northwestern Iberia Peninsula coast)

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Thesis authorship declaration

I declare to be the author of this thesis which is original and inedited. The authors and articles consulted are properly cited in the text and are included in the reference list.

(Joyce-Gabriela Azenha Neves)

Acknowledgments and Dedication

This thesis has been conducted in collaboration with the Bottlenose Dolphin Research Institute BDRI (represented by Bruno Díaz López) that has officially authorized me to use the data to elaborate this thesis. This is an unpublished thesis and is not prepared for further distribution. The author and the BDRI give the permission to use this thesis for consultation and to copy parts of it for personal use. Every other use is subject to the copyright laws; more specifically the source must be extensively specified when using results from this thesis.

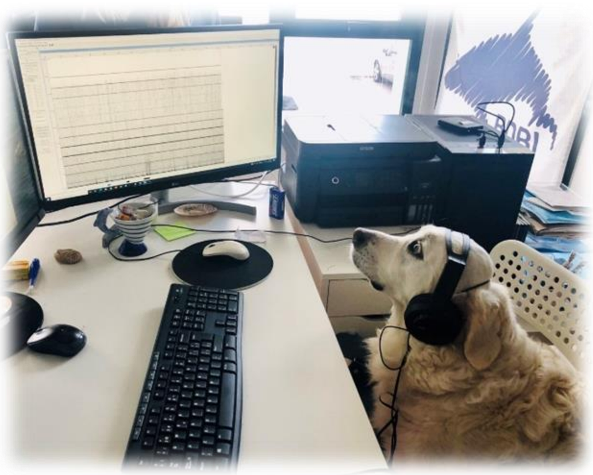
Funding for this research came from the Bottlenose Dolphin Research Institute BDRI. This study would not have been possible without the supervision of Dr. Bruno Díaz López and Dr. Séverine Methion, and the cooperation of Olga Mosca, Nathalie Dunel and all BDRI volunteers who gave generously of their time to help with laboratory and field work. Data collection complies with the current laws of the country in which it was performed, Spain.

Special thanks to Dr. Professor Ana Marçalo, Dr. Bruno Díaz López and Dr. Séverine Methion by the orientation, support, motivation, and patience.

Thanks to Olga Mosca and Nathalie Dunel for all the advice, support, and friendship. Your kindness is priceless!

I dedicate this work to my parents and sister who supported me in all my professional decisions and encourage me to go abroad; to my "bus driver" friend my biggest inspiration to work hard on my dreams and never give up until I got them; to Estela, Nikki and my mentors, my main source of motivation.

Finally, I dedicate this work to my favorite research investigator of the BDRI that inspired me with her skin marks, the only one capable to convince me to do breaks between my work schedule and shared with me the purest love ever. Thanks Ariel!



Resumo

Os Mysticeti enfrentaram um declínio drástico no passado, principalmente devido à caça à baleia na costa noroeste da Península Ibérica, levando quase à sua extinção na zona. No entanto, 40 anos mais tarde, as baleias estão a regressar à zona, utilizando-a principalmente como um local de alimentação. Mesmo com a proibição da caça à baleia nas águas da Galiza, ainda existem algumas ameaças que comprometem a saúde destes enormes viajantes. Para avaliar o estado de saúde de cetáceos em estado selvagem, são poucas as ferramentas que se pode utilizar sem se ser invasivo. A avaliação do estado da pele dos mamíferos marinhos tem sido utilizada para identificar indivíduos (foto-identificação) em todo o mundo e também pode fornecer informação sobre a saúde individual e populacional de uma espécie. As marcas cutâneas nos cetáceos podem ser diferenciadas em 2 tipos de causa: naturais (vírus, bactérias, fungos, ectoparasitas, tentativa de predação) e antropogénicas (interação com artes de pesca e colisões de embarcações), sendo utilizadas como uma base importante para monitorizar as alterações ao longo do tempo. Os dados de captura acessória, arrojamentos (encalhes na costa), ou captura-libertação são normalmente utilizados para avaliar o estado da pele em mamíferos marinhos, porém a foto-identificação aparece como uma ferramenta relativamente barata e não invasiva para avaliar o estado de saúde da pele, evolução, recorrência, prevalência de lesões ou identificar potenciais impactos sobre as espécies. Para explorar potenciais impactos sobre as baleias que se alimentam na costa noroeste de Espanha, foram realizadas saídas ao mar numa embarcação de 12 metros de cumprimento, entre os anos de 2017 a 2021 sempre com condições meteorológicas favoráveis. Quando avistadas as baleias, procedeu-se á aproximação das mesmas sempre com uma velocidade constante e lenta. Várias fotografias foram tiradas por dois investigadores experientes a um angulo perpendicular ao animal de forma a capturar cada uma das secções do corpo do animal (cabeça, corpo intermédio, barbatana dorsal e pedúnculo) de ambos os lados (direito e esquerdo). Mais tarde, todas as fotografias foram revistas e as de melhor qualidade (foco, luz, tamanho) foram selecionadas para representar cada secção do corpo de cada individuo de ambos os lados de maneira a reconstruir o corpo inteiro. Após uma análise detalhada, lesões epidérmicas visíveis, anomalias de coloração e ectoparasitas foram detetadas nas baleias azuis (*Balaenoptera musculus*), baleias-comum (*Balaenoptera physalus*) e baleias anãs (*Balaenoptera acutorostrata*). No total, foram documentados 36 tipos de marcas de pele incluindo lesões no contorno de barbatana, anomalias de pigmentação, manchas, dentadas, marcas

lineares, lesões, elevação cutânea, infeções e ectoparasitas associados. A lesão cutânea mais prevalecente foram as marcas negras em baleias azuis (95,2%), bolhas e marcas brancas em baleias-comum (74,2%) e bolhas em baleias-anãs (76,2%). As marcas brancas e marcas pretas englobam diferentes tamanhos e formas que não têm uma etiologia definida. Já as bolhas podem ter origem no aumento de exposição da pele a altos níveis de radiação UV. As lesões tipo herpes foram a marca cutânea mais severa e abundante encontrada nas baleias estudadas, caracterizadas por lesões punctiformes espalhadas geralmente por todo o corpo. Os resultados deste estudo mostraram que as espécies de baleias estudadas, que se alimentam na costa noroeste de Espanha, exibiram indicações de impactos causados por alterações climáticas e impactos antropogénicos. Em geral, as marcas cutâneas foram mais abundantes e prevalecentes nas baleias azuis, no entanto, mais severas nas baleias-comum. Já as baleias-anãs parecem ser a espécies mais vulnerável a enfrentar ameaças antropogénicas. Indentações e marcas lineares no pedúnculo podem estar fortemente associadas a emaranhamento em redes de pesca afetando 23.8% dos espécimes de baleia anã amostrados. Para além disto uma baleia-comum foi encontrada com uma rede de pesca presa ao redor do seu pescoço e espiráculo. Este indivíduo mostrou claras evidências corporais de marcas causadas por redes de pesca (possivelmente pesca fantasma) com uma possível infeção viral com origem nas mesmas. Marcas naturais como as causadas por dentes, não são tão frequentes em baleias como são em odontocetos, porém foram registadas provas de tentativas de predação por parte de orcas em baleias azuis e baleias-comum. Para além destas marcas de mordida, algumas espécies de ectoparasitas foram identificadas fixas ao hospedeiro como é o caso das diatomáceas, pequenos crustáceos comensais da família Cyamidae (*Isocyamus* sp.), copépodes parasitas (*Pennella balaenopterae*), cirrípedes (*Xenobalanus globicipitis*), possivelmente remoras (*Remora remora*) e lampreias (*Petromyzon* sp.). Outra espécie identificada como causadora de marcas ovais esbranquiçadas nas baleias amostradas é uma espécie de tubarão (*Isistius brasiliensis*), frequentemente encontrado em águas tropicais (destino final da rota de migração das baleias azuis e baleias-comum). Estas marcas de dentada de tubarão foram apenas encontras num estágio de cura avançado (cor esbranquiçada). Marcas recentes de dentada de tubarão (com sangue, rosadas com tecidos subcutâneos visíveis ou amareladas) nunca foram encontradas nas 3 espécies amostradas. Isto permitiu inferir que o processo de cura de feridas em baleias é possivelmente menor que 38 meses. No caso das baleias anãs, que são vistas durante todo o ano na área, também apresentaram

este tipo de marcas afetando 47.6% dos indivíduos, sugerindo que esta espécie possa estar a realizar migrações ou as marcas podem ser causadas pela espécie *Isistius plutodus*. Algumas novas marcas de pele foram documentadas pela primeira vez em todo o mundo nas baleias amostradas, como é o caso do: possível primeiro tumor encontrado numa baleia azul representado por uma aglomeração de elevações cutâneas na área do pedúnculo (diferente das bolhas encontradas na maioria das baleias possivelmente causadas por raios UV); primeiro caso de hypopigmentação com distribuição corporal anormal (diferente de vitiligo e albinismo parcial encontrado na literatura); e primeiro registo de possíveis cicatrizes provocadas por medusas que estão a proliferar cada vez mais nos oceanos, tirando vantagem do impacto das alteações climáticas. Herpes, marcas pretas e marcas brancas foram as lesões que mais contribuíram para a variabilidade das marcas de pele nas baleias, contudo nenhuma delas está particularmente relacionada com uma espécie ($p=0.520$, $df=2$, $F=0.288$) ou condição corporal específica ($p=0.951$, $df=1$, $F=0.0293$). Em suma, as nossas descobertas fornecem o catálogo mais completo de foto-identificação de marcas de pele para comparar entre espécies de baleias, explora ligações casuais entre a condição de pele e a saúde dos animais e também enumera as possíveis causas (naturais ou antropogénicas) associadas a cada lesão justificadas por revisão bibliográfica.

Palavras-chave: Marcas antropogénicas; baleia azul; baleia-comum; baleia anã; foto-identificação; doenças de pele.

Abstract

Mysticeti have faced a drastic decline in the past mainly due to whaling in the Northwestern coast of the Iberian Peninsula, leading to almost extinction in the zone. However, 40 years later, whales are returning to the area. Even with the ban of whaling in Galicia waters, some threats still exist that compromise the health of these huge travelers. To explore potential impacts on baleen whales feeding on the Northwestern coast of Spain, we analyzed photographs taken from 2017 to 2021. Epidermal lesions, coloration anomalies and ectoparasites were detected in blue (*Balaenoptera musculus*), fin (*Balaenoptera physalus*) and minke whales (*Balaenoptera acutorostrata*). Altogether, 36 types of skin marks were documented. The most prevalent skin lesion was black marks on blue whales (95.2%), blisters and white marks on fin whales (74.2%) and blisters on minke whales (76.2%). Herpes-like lesions were the most severe and abundant skin mark found in all species. Our results showed that skin marks were more abundant and prevalent on blue whales, however, more severe on fin whales. Minke whales appear to be the most vulnerable specie facing anthropogenic threats. Some novel skin marks were recorded for the first time, to our knowledge, on baleen whales worldwide as is the case of the possible first tumor in a blue whale specimen, first hypopigmentation case with abnormal body distribution and first records of possibly jellyfish stings. Herpes, black and white marks were the lesions that contributed the most for the variability of skin marks on whales, however none of those are particularly related to a specific specie or body condition. Therefore, our findings provide the most complete photo-identification catalogue of skin marks to compare across species of Mysticeti and explore casual links between skin condition and whales' health.

Keywords: Anthropogenic marks; Blue whale; Fin whale; Minke whale; Photo-identification; Skin disease.

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List of abbreviations

ai: mark abundance

BA: *Balaenoptera acutorostrata*

BDRI- Bottlenose Dolphin Research Institute

BM: *Balaenoptera musculus*

BP: *Balaenoptera physalus*

CePV-1: Cetacean Poxvirus 1

CePV-2: Cetacean Poxvirus 2

EM: Electron Microscopy

HV: Herpes virus

IUCN: International Union for Conservation of Nature

IWC: International Whaling Comission

li: mark severity

ni: total number of occurrences

OQS: obvious quality score

pi: mark prevalence

PVs: Papillomaviruses

Photo-ID: Photo-identification

Q: value score

ri: relative portion

SSWG: Ship Strike Working Group

TDS: Tattoo skin disease

UK: United Kingdom

UV: UltraViolet radiation

Chapter 1

1. Introduction

1.1. Marine Mammals Evolution

Mammals (re-)colonize marine environments 50 million years ago (Eocene epoch) as a diverse assemblage of at least seven distinct evolutionary linages (McGowen *et al*., 2014). Fully aquatic clades include Cetacea and Sirenia, both with extinct taxa that support the macroevolutionary transition from land to water (Gatesy *et al*., 2013; Springer *et al*., 2015). Semiaquatic clades are more numerous, and examples are found in a wide range of mammalian orders as is the case of Carnivora (pinnipeds and otters) or Cetartiodactyla (hippos) (Springer *et al*., 2021).

Whales, dolphins, and porpoises constitute the Cetacea which is an obligate aquatic mammals' clade (Thewissen *et al*., 2009). Cetacea includes a wide range of species with different body sizes that goes till 27 meters (blue whales) to 1.4 meters (vaquita) and different weights variating between 136.000 kg to 42 kg (Thewissen *et al*., 2009). In shape, all modern Cetacea are relatively similar. They have a horizontal tail fluke used for swimming; forelimbs that are flippers; they haven't external hind limbs; their neck is short; and their body is streamlined (Thewissen *et al*., 2009). Like other mammals and unlike other vertebrates, they nurse their calves, they have three ear bones that are involved in sound transmission and their lower jaws consist of a single bone (the dentary) (Thewissen *et al*., 2009). In spite of this, there are still some characteristics of terrestrial mammals in cetaceans, for instance, the presence of hair or fur (Thewissen *et al*., 2009). Modern cetaceans lost their hair coat as an adaptation to reduce friction and improve locomotion in the water (Thewissen *et al*., 2009). However, is still common to see some species that retained a few hairs on their face and some fetus has whiskers (Thewissen *et al*., 2009). Apart of that locomotory adaptations in cetaceans include hind limb loss, modification of the front limbs into flippers, and conversion of the tail into a powerful fluke (Springer *et al*., 2021). Other remarkable feature about cetaceans is the presence of lungs which means that they come to the surface to breath air, like other mammals and unlike fish. All these characteristics were used by scientists to conclude that cetaceans originated from land mammals (Fordyce & Muizon, 2001; Thewissen & Williams, 2002).

1.2. Cetacean's Skin

In their evolution, cetaceans also showed changes to their integument. The skin is a multi-layered organ that establishes a protective interface between the organism and the surrounding environment (Themudo *et al*., 2020) which makes it part of an important anatomical and physiological adaptation of mammals to the aquatic environment. Cetacea skin constantly interacts with dense, viscous, and thermally conductive water, which creates unique physical challenges to its outer surface (Springer *et al.*, 2021). Within cetaceans, the archetypal mammalian skin was drastically reshaped and remodeled, emerging as an impressive feature of their adaptation to aquatic environment (Themudo *et al*., 2020) and at the genome level, most cetacean skin-related innovations resulted from episodes of gene loss: spanning diverse processes such as skin keratinization and cornification, immunity, and inflammation or lubrification (Themudo *et al*., 2020). In terms of visual aspects, the skin pigmentation and colors in cetaceans are very diverse and clear evidence of evolution, in other words, speciation occurring over time.

In mammals, the skin provides a physical and immune barrier, while contributing to thermoregulation and water balance (Themudo *et al.*, 2020). The skin contains an outer layer (the epidermis), a middle layer (the dermis), and a deeper layer (the hypodermis) (Fig. 1), which forms the blubber when present. The dermis includes hair follicles, sebaceous and sweat glands, and claws (in pinnipeds, sea otters and polar bears). Cetacean’s skin is recognized by the absence of glands and hair, except for bristle-like hairs that occur around the mouth (Berta *et al*., 2015).

Cutaneous ridges have been described on the surface of the skin in many cetaceans. Although the function of these structures is still unknown, it has been suggested that they may play a role in tactile sensing or in the hydrodynamic characteristics of the animal or both (Shoemaker & Ridgway, 1991). Cetaceans’ integuments also have rete ridges on the underside of the epidermis that are oriented parallel to the body axis (Parry, 1949) forming slender flap-like projections between which dermal papilla are located (Fig. 1.1).

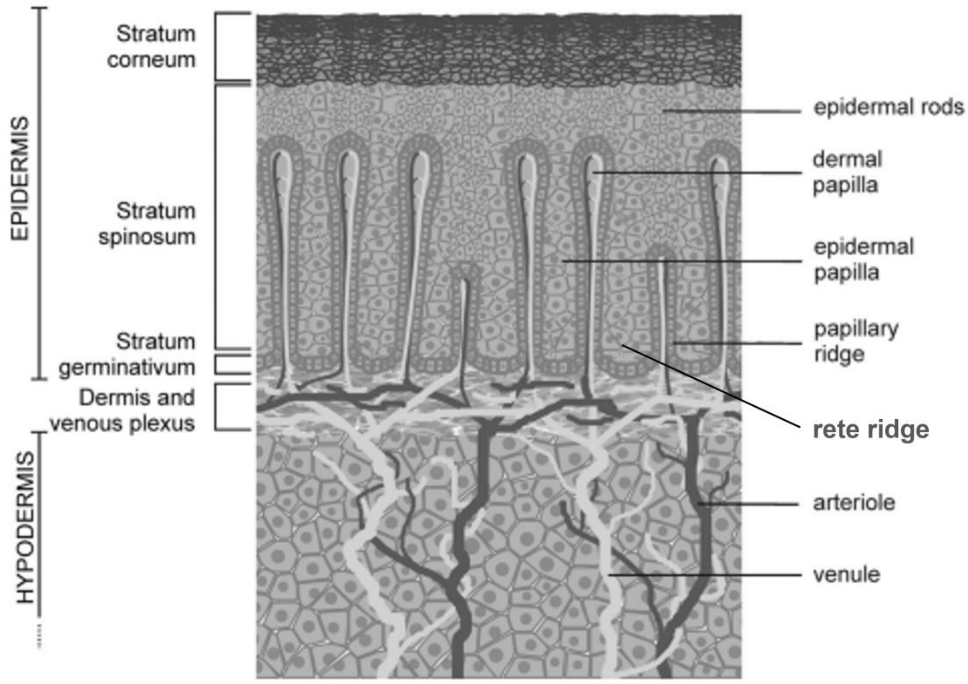


Figure 1.1- Cross section of cetacean skin (Adapted from Mouton & Botha, 2012).

1.2.1. Epidermis- The First Barrier of Defense

The stratum corneum of cetaceans is often referred to as the parakeratotic layer and composed of moderately flattened cells, characterized by retained elongated nuclei and prominent organelles, representing a form of parakeratosis (Spearman, 1972; Geraci *et al*., 1979). This last process was attributed to a type of cornification, associated with evolutionary hair follicle loss. The phospholipid-rich cornified layer presumably also aids in waterproofing the skin of these mammals (Spearman, 1972). Lipid droplets (storage organelles at the center of lipid and energy homeostasis) occurs in association with the nucleus, as well as an abundance of intra-nuclear inclusions like small fragments of cytoplasmic keratin (Mouton & Botha, 2012; Olzmann & Carvalho, 2019).

Keratins are scleroproteins that are responsible for mechanical support in the epithelial cells present in the surface of body skin (Schweizer *et al.*, 2006). These macromolecules are mechanically hard, chemically unreactive, insoluble, fibrous, and very tough (Sharma & Rajak, 2003). In cetaceans, the epidermis, is provided by lipokeratinocytes, responsible to produce keratin and lipid droplets (Menon *et al.*, 1986; Zabka & Romano, 2003). These lipids improve the capability of the lipokeratinocytes to act as physical barrier within a hypertonic environment, and contribute to buoyancy, streamlining, insulation and caloric characteristics of cetacean skin (Menon *et al*., 1986).

1.2.2. Dermis and Hypodermis- The Second and Third Barrier of Defense

While the first skin barrier of defense works to protect the organism against the environment, the second and third barrier, named dermis and hypodermis respectively, have present crucial immune components even more specific than the epidermis (Mouton & Botha, 2012). Microbial invaders are confronted by these barriers that evolved over time to eliminate invaders, digest invading cells into smaller antigens, and program lymphocytes to provide long term protection against microbes (Zabka & Romano, 2003).

Microbes that manage to penetrate the subcutaneous layers of the skin (dermis and hypodermis) will experience defenses mechanisms that involves the production of pro-inflammatory substances (e.g., chemokines by lipokeratinocytes). As a result, the immune cells, such as leucocytes or phagocytes, will migrate to the site of infection. After detection of the foreign proteins (potential bacterial, fungal, or parasitic invaders), Langerhans cells will phagocytize these antigens and migrate to adjacent lymph nodes. In the lymph nodes, the Langerhans cells will develop into mature dendritic cells which will process the antigen into smaller fragments to activate the lymphocytes (adaptive immune system) (Willey *et al*., 2011). On top of this chemical defense mechanism, non-specific antimicrobial substances such as lysozyme and the peptide β-defensin were found in cetacean integument (Meyer & Seegers, 2004). Lysozyme occurs between the layers of the stratum corneum, within cells of the stratum spinosum, dermis, and endothelial cells of the dermal blood vessels. The β-defensin was found to be concentrated in the upper five or six layers of the stratum corneum, as well as within the cells of the upper stratum spinosum (Mouton & Botha, 2012).

There is another important non-specific mechanism of defense present in the epidermis, called lymphocytes or white blood cells, that indicate the presence of the adaptative immune system on cetaceans. Lymphocytes are cells that circulate in the blood and can be found two main types, T cells and B cells (Marsili *et al.*, 2019). B cells produce antibody molecules that can latch on and destroy invading viruses or bacteria (Marsili *et al.*, 2019). In the other hand, T cells are direct fighters of foreign invaders. T cells have powerful cytolytic (produce cytokines) and immune-regulatory effects on antigens and will confine antigens that overcame the epidermis and dermis (Willey *et al.*, 2011). Zabka & Romano (2003) study was done on dolphin skin and revealed the presence of antigen cells (MHC II +) mostly located in the dermal papilla, along the epidermal-dermal border. The MHC II (+) cells were found to have a dendritic morphology with a pattern similar to Langerhans cells. The authors concluded that the cells were most likely Langerhans cells and not macrophages or dendritic cells. Zabka & Romano (2003) further suggested that pathogen invasion resulting from a wound way led to an inflammatory response, causing immune cells to migrate from the dermal papilla to the site of infection. However, the authors indicated that inflammation is normally absent in cetaceans, because this barrier is normally sufficient against small injuries sustained with interaction with other cetaceans. The authors further noted that wound healing in dolphins is not followed by scab formation. In these mammals' hydrophobic changes within in the stratum spinosum, causes rapid sloughing and replacement with cells of the stratum germinativum undergoing mitosis (Mouton & Botha, 2012).

1.2.3. Pigmentation

The pigmentation of the skin act mainly as a protection from sunlight exposure and plays an important role in social and sexual communication and camouflage from predators (Pawelek & Komer, 1982). In mammals, pigmentation is almost totally dependent on melanin pigment synthesis and distribution in the skin, hair, and eyes (Hearing & Tsukamoto, 1991). The variation in pigmentation may vary with the age of the individual, the season, and the exposure to sunlight (Pawelek & Korner, 1982). Melanin is a photoprotective pigment with the ability to absorb and disperse 50 to 70% of the solar ultraviolet (UV) radiation that penetrates the epidermis of the skin (Brenner & Hearing, 2008; Coelho *et al*., 2009). This photoprotective pigment is synthesized by melanocytes, which are specialized skin cells that produce two melanin variants. The range of coloration exhibited in mammals reflects the variation in the combination of these two types of melanin pigment present in the tegument: eumelanin, which are black or brown, and pheomelanin which are yellow or red (Morales-Guerrero *et al*., 2019). Their chemical structure and biosynthesis pathways are very different (Yamaguchi *et al*., 2007) as well as the photoprotective capacity of the two pigments. Brenner & Hearing (2008) reported that eumelanin confers better photoprotection than pheomelanin.

Now a days, the high level of solar Ultraviolet (UV) radiation is a serious threat to marine ecosystems especially to marine mammals' health. Martinez-Levasseur *et al*. (2013) reported that large whales have been shown to suffer sun-induced skin damage from continuous UV exposure. The UV exposure induces mitochondrial DNA damage in the skin of seasonally sympatric whales and accumulates with age, however, counteractive molecular mechanisms are significantly different between cetacean's species (Martinez-Levasseur *et al*., 2013). For example, sperm whales are a specie that remains for long periods in the surface, then they activate genotoxic stress pathways in response to UV exposure whereas the paler blue whale relies on increased pigmentation as the seasons progresses (Martinez-Levasseur *et al*., 2013).

It is known that the variation of typical coloration in species can influence social interactions between individuals and can be disadvantageous for survival (Pawelek & Korner, 1982; Hubbard *et al*., 2010). Abnormal pigmentation is rarely seen in the wild (Abreu *et al.*, 2013) and can lead to an intraspecific rejection, loss of opportunities to engage in social behavior, failure in attracting mates and increase of vulnerability to predation due to the lack of crypsis within the environment (Menthion & Díaz López, 2019). Hypopigmentation is a good example of a lack of pigment in a part or entire body that can occur in cetaceans' skin. This type of atypical pigmentation pattern can be acquired or congenital as a result of inheritance of mutations in pigment-related genes (Slominski *et al*., 2004). Vitiligo is an acquired atypical pigmentation pattern characterized by the development of depigmented patches on the skin where pigment-producing melanocytes of the epidermis have disappeared (Kemp *et al*., 2001). Albinism is an example of congenital atypical pigmentation characterized by the complete lack of pigmentation in the skin, hair, and eyes. In other hand, leucism and piebaldism are also congenital atypical but leucism is when a reduced pigmentation of hair is observed with colored skin and eyes and piebaldism is observed in individuals that have absence of pigment in localized parts of the body, forming irregular light patches in the skin (Slominski *et al*., 2004; Acevedo *et al*., 2009).

1.3. Skin Marks in Cetaceans

Skin marks and lesions on cetaceans have been reported since the 1950's (Simpson *et al*., 1958) and still occur frequently across the globe till these days (Leone *et al.*, 2019). The main causes can be considered as natural (predation marks, social/sexual interactions, viruses, bacteria, and fungi) (Baker, 1992; Van Bressem *et al.*, 1999a; Heithaus, 2001; Van Bressem *et al.*, 2003; Van Bressem *et al.*, 2007; Samarra *et al*., 2012), anthropogenic (habitat degradation, water pollution, fisheries, boat traffic) (Wilson *et al*., 1997; Wilson *et al*., 1999a; Van Bressem *et al*., 2007; Hart *et al.*, 2012), environmental (solar radiation and water salinity) (Wilson *et al.*, 1997; Martinez-Levasseur *et al.*, 2011; Hart *et al.*, 2012) or even all together.

1.3.1. Natural Causes

Natural marks have been used to investigate populations dynamics, demography, and social patterns of cetaceans (Wilson *et al.*, 1999b; Durban *et al.*, 2010; Robinson *et al.*, 2012). The visual examination through photo-ID of skin marks has also been used to gather evidence of potential threats (Robbins, 2011) and to provide a preliminary assessment of the overall health status of individuals and populations (Thompson & Hammond, 1992; Wilson *et al.*, 1999a; Van Bressem *et al.*, 2013). However, natural marks can be related to natural mortalities in cetaceans' populations and are known as diseases (caused by infectious agents like viruses, bacteria, and fungi), interspecific competition, and predation (Berta *et al.*, 2015). Cetaceans can also contract cancer, tuberculosis, herpes, and other diseases that leads to premature mortality (Berta *et al.*, 2015).

1.3.1.1. Viruses

The largest family of viruses known to cause skin diseases in cetaceans are Poxviridae (Bracht *et al.*, 2006). Among cetaceans, the odontocetes seems to be more susceptible to viruses than mysticetes (Fury & Reif, 2012). Is reported that pox viruses have been affecting mainly the specie *Tursiops truncatus*, *Leucopleurus acutus*, *Delphinus delphis*, *Lagenonorhychus albirostris*, *Stenella coeruleoalba*, *Cephalorhynchus hectori*, *Globocephala melaena* and porpoises' species (Geraci *et al.*, 1979; Baker, 1992; Bracht *et al.*, 2006; Fury & Reif, 2012; Barnett *et al.*, 2015). Dolphin pox is normally referred as "targets", "watered-silk", "ring", "pinhole", "circle" and "tattoo-like" (Figure 1.2) (Geraci *et al.*, 1979; Barnett *et al.*, 2015). These variety of lesions emerge as a single or overlapping circular grey spots. Later, these ring lesions may develop into black punctiform stippled patterns (Mouton & Botha, 2012). Studies revealed a thickened stratum corneum with ballooning degeneration, and eosinophilic inclusions containing viruses' particles, inside the cytoplasm of stratum intermedium cells (Mouton & Botha, 2012). Poxvirus particles in association with lesions have been demonstrated through electron microscopy (EM) and were previously classified as two new viruses, cetacean poxvirus 1 (CePV-1) and cetacean poxvirus 2 (CePV-2) (Bracht *et al*., 2006). Most recently, a retrospective study using archived material from United Kingdom (UK) in stranded cetaceans demonstrated also CePV-1 viruses in cetaceans (Barnett *et al*., 2015).

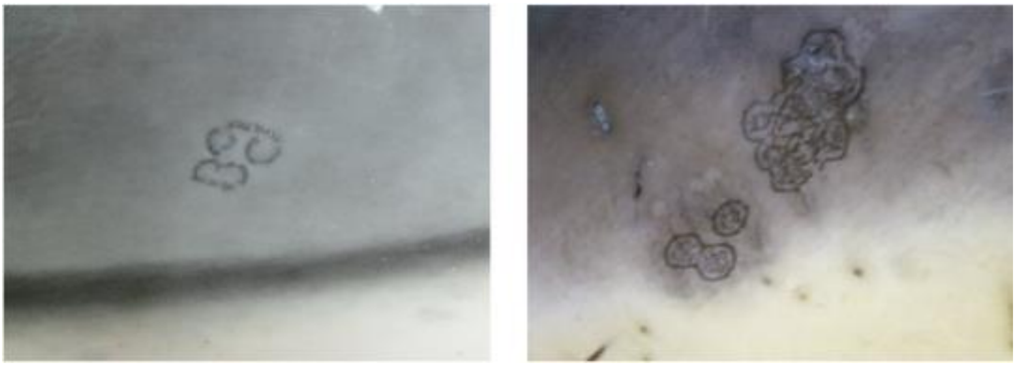


Figure 1.2- Examples of tattoo lesions observed in cetacean poxvirus-infected animals (Barnett *et al*., 2015).

Apart from Dolphin pox, three more virus types are important to highlight. First, Herpes Virus (HV), which is another type of virus that have been reported in cetaceans' skin since the late 1980s through EM analysis revealing HV-like particles in captive and free-swimming beluga whales (*Delphinapterus leucas*) (Martineau *et al*., 1988; Moeller, 2003). The lesions caused by HV appeared as multiple grey, raised, pale grey regions on the skin (Mouton & Botha, 2012) and available information on its epidemiology is limited. The capacity of HV to cause disease in cetaceans is unclear and may show variations depending on the different conditions between individuals and species (Arbelo *et al*., 2012). Histological analysis showed epithelial cells that underwent intercellular oedema, necrosis, and the development of microvesicles (Mouton & Botha, 2012).

Secondly, Papillomaviruses (PVs) are known as nonenveloped icosaedric DNA viruses with 55 nm in size approximately (Cruz *et al*., 2014). After infecting the skin, PVs indices proliferation of the stratified squamous epithelia, cause lesions (zur Hausen & de Villiers, 1994). This type of viruses has been reported in a wide range of cetaceans' skin and in some specific places of the body as the tongue, penis, pharynx, and first gastric compartment (Moeller, 2003). In 2005, it has been suggested that papillomas may result in malignant transformation of benign papillomatous lesions (Bossart *et al*., 2005). In free-ranging bottlenose dolphins, a high prevalence of papillomas has been documented, and the authors suggested that it may be related to high tumor prevalence (Bossart *et al*., 2005; Akritopoulou, 2014). Beyond bottlenose dolphins, narwhal (*Monodon monoceros*), manatee (*Trichechus manatus latirostris*), killer whale (*Orcinus orca*) and sperm whale (*Physeter catodon*) are also some examples that have been reported with Papillomavirus cutaneous infections according to Stoskopf (2015) and McAloose & Newton (2009).

At least, when considering the most consistent lesion in marine mammal skin, the vesicles are the most prevalent and in dolphins are associated with "tattoo" lesions and old scars (Stoskopf, 2015). Vesicles can have 1 mm to 3 cm in diameter (Stoskopf, 2015). They usually erode and leave shallow, fast-healing ulcers, but sometimes vesicles regress and leave plaque-like lesions (Stoskopf, 2015). The virus responsible for the vesicles, and the last type of virus in the list, is the calicivirus. Although there are just a few publications related to this virus in cetaceans, it is important to refer Smith *et al*. (1983) that reported a bottlenose dolphin with vesicular skin disease and Smith & Boyt (1990) that showed the presence of neutralizing bodies to several marine vesiviruses, including calicivirus.

1.3.1.2. Bacteria

Several bacteria have been identified from skin lesions in cetaceans. The majority are opportunistic, and some may have developed antibiotic-resistance due to the uncontrolled use of antibiotics in aquaculture in some countries (Cabello, 2004). In the worst-case scenario, bacteria can cause death of cetaceans (Buck *et al*., 1991). The most frequent bacteria identified in cetaceans' skin are ten (Mouton & Botha, 2012).

Starting with *Brucella,* this genus is a Gram-negative bacterial pathogen (Guzmán-Verri *et al*., 2012). The first discovery of a *Brucella* was in an aborted fetus of *Tursiops truncatus* (Ewalt *et al*., 1994) and since then the numbers of isolates have been increasing from different cetaceans (Guzmán-Verri *et al.*, 2012). Most of these isolates belong to different clusters but generally, the isolates from cetaceans are called *Brucella ceti* (Foster *et al*., 2007). This specie had been isolated from subcutaneous and skin lesions in cetaceans (Mouton & Botha, 2012) and the transmission seems to be through sexual intercourse, maternal feeding, aborted fetuses, placental tissues, mother-fetus transmission or through helminth reservoirs (Guzmán-Verri *et al*., 2012). The consequences of *B. ceti* in cetaceans has been associated with chronic disease, male infertility, neurobrucellosis, cardiopathies, bone and skin lesions, strandings and deaths (Guzmán-Verri *et al*., 2012).

Dermatophilus bacteria were for the first time reported in cetaceans by Mikaelian *et al*., (1999) when the authors studied six deceased beluga whales that were found in the St. Lawrence estuary. The lesion caused by *Dermatophilus*-like actinomycetes presented as a little depressed, round, and grey areas randomly distributed over the whole-body skin (Mouton & Botha, 2012). The *Dermatophilus* entered the stratum corneum and, the underlying stratum spinosum had marked spongiosis and vacuolar degeneration (Mikaelian *et al*., 1999). Minimal neutrophilic infiltration was present within the underlying dermal papillae (Mikaelian *et al*., 1999). This type of bacteria is known to be aerobic, gram-positive, and filamentous (Delano *et al*., 2002) that in some cases are responsible by streptothricosis, a subcutaneous bacterial infection more usually found in pinnipeds and polar bears (Stoskopf, 2015).

Escherichia is a recurrent and opportunistic pathogen genus (Schaefer *et al*., 2011) that can also be found in skin lesions of cetaceans. Buck *et al*. (1991) first isolated the gram negative and aerobic specie *Escherichia coli* from Atlantic bottlenose dolphins skin lesions from Florida and New York. Almost two decades later, Schaefer *et al*. (2011) studied the risk factors for colonization of *E. coli* in Atlantic bottlenose dolphins in the Indian River Lagoon (Florida). The last study confirmed that this bacteria colonization was higher in youngest individuals and in counties with higher cumulative rainfall. This discovery is also related with Lourenço *et al*. (2007) report, which the authors studied the environmental parameters and antimicrobial susceptibility of bacteria in the estuarine water, and they concluded that the *E. coli* specie was the most frequent found in water samples and they are responsible for the disease's transmitters by polluted waters.

The genus *Erysipelothrix* only had one pathogen specie associated which is the *Erysipelothrix rhusiopathiae* (Wang *et al*., 2010). This bacterium is a facultative anaerobe, non-spore-forming, Gram-positive and rod-shaped bacillus (Wang *et al*., 2010). This specie can persist for long times in marine environments and has been isolated from cutaneous slime of fish (Seibold & Neal, 1956; Lauckner, 1985; Kinsel *et al*., 1997). However, this specie is also the causative agent of erysipelas, a disease of many mammalians and avian species (Kinsel *et al*., 1997; Dunn *et al*., 2001, Wang *et al*., 2011). Cutaneous and acute septicemic forms have been reported in cetaceans as *Tursiops truncates* (Figure 1.3), *Stenella plagiodon*, *Globicepala melas*, *Tursiops aduncus*, *Lagenorhynchus albirosis*, *Lagenorhynchus obliquidens*, *Delphinapterus leucas*, *Grampus griseus*, *Orcinus orca* and *Phocoena phocoena* (Seibold & Neal, 1956; Chastel *et al*., 1975; Thurman *et al*., 1983; Buck & Spotte, 1986; Kinsel *et al*., 1997; Young *et al*., 1997; Boseret *et al*., 2002). The presence of gray, elevated rhomboid plaques with well-defined edges and diamond-shaped skin lesions, are the main signs of the presence of *E. rhusiopathiae* in cetaceans (Thurman *et al.*, 1983; Suer & Vedros, 1988; Kinsel *et al.*, 1997; Dunn *et al.*, 2001; Boseret *et al.*, 2002; Wang *et al.*, 2010; Melero et al., 2011). Cetaceans are the most susceptible marine mammal to this bacterial disease (Suer & Vedros, 1988; Higgins, 2000), including captive cetaceans that suffer with infections caused by dead fish not well preserved (Geraci *et al.*, 1966; Suer & Vedros, 1988; Higgins 2000) or injuries caused by the teeth of other cetaceans (St. Leger, 2007).

Figure 1.3- Example of a case of erysipelas, rhomboid-shaped skin lesions, observed in the specie *Tursiops truncatus* during external examination (Melero *et al*., 2011).

The genus *Klebsiella* is a gram-negative facultative anaerobic bacterium typically associated with animal infections (Clegg & Murphy 2016; Soto *et al*., 2017; Whitaker *et al.*, 2018). The specie *Klebsiella oxytoca* was isolated from skin lesions of an Atlantic bottlenose dolphin, while another *Klebsiella* sp. was obtained from a goose beak whale (*Ziphius cavirostris*), both from Florida (Buck *et al*., 1991). The studies referring the presence of this genus in cetaceans' skin are scarce.

Mycobacterium species have been associated with infections in cetaceans, as is the case of bottlenose dolphins, belugas and pseudorca's (Mouton & Botha, 2012). This genus has not a classification regarding being Gram-positive or Gram-negative, but they are more alike Gram-Positive coloration. The signs associated with this genus are non-healing, chronic cutaneous or subcutaneous lesions, granulomas in various organs, lymph nodes and pulmonary infections (St. Leger, 2007). The specie *M. marinum,* abundant in fresh and marine sediments, it was reported by Bowenkamp *et al*. (2001) as an opportunistic, infrequent pathogen of homeotherms. *M. marinum* was isolated from skin lesions that developed on the thorax and abdomen and near the genital folds of a captive beluga (Bowenkamp *et al*., 2001). On cut section they were epidermal ulcers with cores of purulent material that extended deep into the blubber (Van Bressem *et al*., 2008).

Belonging to the family *Pseudomonadaceae*, the genus *Pseudomonas* is an opportunistic pathogen, Gram-negative, motile, slender bacillus bacterium cells that normally occurs in terrestrial, salty and fresh water environments (Van Bressen *et al*., 2008; Mouton & Botha, 2012). These bacteria are known by colonize biofilm, wounds or can be found in a planktonic form (as a unicellular organism) (Khan *et al*., 2006; Van Bressen *et al*., 2008). Pseudomonas infections are invasive and toxicogenic that usually lead to septicaemia (Van Bressen *et al*., 2008; Wiley *et al*., 2011). In the 1970s, Diamond *et al*., (1979) reported that the specie *Pseudomonas aeruginosa* caused fatal bronchopneumonia and extensive dermatitis in an Atlantic bottlenose dolphin. It had hard, round, and raised dermal nodules with necrotic centers all over its body skin. The specie *P. aeruginosa* had been also reported to form large cutaneous ulcers in Atlantic bottlenose dolphins, penetrating deep into the tissue and leading to serious health problems (Mouton & Botha, 2012). Septicaemia develops when the bacteria proliferate into the walls of the blood vessels (St. Leger, 2007). Buck *et al*. (1991) also isolated *Pseudomonas sp*. and *Pseudomonas putrefaciens* from skin lesions in Atlantic bottlenose dolphins. In 1994, a female *L. obscurus* caught off Peru presented several nodules on the back and tailstock, lesions caused by *Pseudomonas* (Van Bressem *et al*., 2008).

Staphylococcus is a Gram-positive bacteria genus, member of the Family *Streptococcaceae*, that consists of a variety of opportunistic pathogens sometimes found in skin lesion of cetaceans (Mazzariol *et al*., 2018). *Staphylococcus delphini* is a specie that was isolated by Varaldo *et al*. (1988) from multiple suppurating skin lesions that responded well to antibiotic treatment in captive dolphins. Another unidentified specie was also isolated from the respiratory tract and the genitals of *Pontoporia blainvillei* and a *Eubalaena australis* from Brazil (Van Bressen *et al*., 2008). The specie *Staphylococcus aureus* has been occasionally reported in free-ranging Atlantic bottlenose dolphins (Schaefer *et al*., 2009; Morris *et al*., 2011; Stewart *et al*., 2014), short-finned pilot whales (Hower *et al*., 2013) and also isolated at the necropsy of a bottlenose dolphin maintained under human care (Faires *et al*., 2009).

The *Streptococcus* genus, member of the family *Streptococcaceae*, include Gram-positive diplococci bacteria, non-motile, catalase-negative, non-spore forming (Hardie & Whiley, 1995; du Toit *et al*., 1995; Numberger *et al*., 2021) which are common residents of cetacean’s skin and upper respiratory track (Mouton & Botha, 2012). This genus is an opportunistic bacterium responsible of cutaneous infections in stressed animals (Moeller, 2003). This genus is also present in cetaceans with septicaemia, metritis, and pneumonia (Mouton & Botha, 2012). Several species of cetaceans have been reported with streptococcal infections, however odontocetes have higher occurrences reported than mysticetes (Numberger *et al*., 2021). (St. Leger, 2007) reported a specific dermatological condition in river dolphins, commonly known as “golf ball disease”, caused by *Streptococcus iniae*. This specie can be noticed by the presence of slow-growing, nodular, subcutaneous abscesses in the cetaceans’ skin (St. Leger, 2007). The only case reported in mysticetes was in the specie *Eubalaena australis* calf that was dead and infected with the specie *Streptococcus dysgalactiae* (Bianchi *et al*., 2018).

Member of the *Vibrionaceae* Family, the *Vibrio* genus were referred as the most isolated bacteria from stranded cetaceans by Buck *et al*. (1991). The two species specifically associated with skin lesions were *V. alginolyticus* and *V. parahaemolyticus* both founded in Atlantic bottlenose dolphins. *Vibrio* species are known as Gram-negative, curved, obligate halophilic marine bacterium (Li *et al.*, 2018). Dhermain *et al*. (2002) study linked bacteria from this genus, in cases of cetacean septicemias. More recently, Di Renzo *et al.* (2017) reported a case of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* associated to the meningo-encephalitis in a bottlenose dolphin from the Adriatic coast of Italy.

1.3.1.3. Fungi

Cutaneous fungi are known to affect parts of the outermost skin layers of cetaceans (epidermis, stratum spinosum and stratum corneum), as well as mucocutaneous membranes, genitalia, or external ears (De Hoog *et al*., 2000). When fungi colonize the surface layers of cetaceans’ skin, they grow on compounds associated with the skin, including lipids and keratinous materials, without provoking an immune response (Reeb *et al*., 2010). These fungi are known as commensals. Other fungi only stay in cetaceans’ skin without growing or utilizing any host products as nutrients (Reeb *et al*., 2010). In these cases, the fungi are known as contaminants. Despite fungi being considered part of the normal skin microflora of cetaceans, they are also opportunistic and secondary invaders of skin tissues (Migaki & Jones, 1983). Fungal diseases have also been documented to cause death in captive and stranded cetaceans (Migaki & Jones, 1983; Buck *et al*., 1987; Higgins, 2000). However, infections from pathogenic fungi are less documented in free-ranging cetaceans, since diseased animals normally die and disappear to the deeper sea (Dhermain *et al*., 2002). At least four genera of fungi have been isolated and documented from skin lesions in cetaceans (Van Bressem *et al*., 2008; Mouton & Botha, 2012) which are: *Candida*, *Fusarium*, *Lacazia* and *Trichophyton*.

Species of *Candida* are normally associated with the mucous membranes of cetaceans in limited numbers, and occur mainly in the region of the blowhole, esophagus, vagina, and anal area (Migaki & Jones, 1983; Higgins, 2000). Infections caused by *Candida* mostly affect captive cetaceans (occurs secondary to stress) and are associated with immune-suppressed individuals, where the fungi infection may proliferate and cause serious local infection of the skin (Mouton & Botha, 2012). Normally in captive cetaceans, long-term antibiotic therapy, corticosteroid treatment or overtreatment of tank water are made and after sequenced by infections in the animals' skin (Higgins, 2000). These lesions can be identified as whitish, creamy plaques on the skin or mucosal surfaces (Van Bressem *et al*., 2008; Mouton & Botha, 2012). Histological examinations usually show colonies of pseudohyphae, septate hyphae and blastosphores (Migaki & Jones, 1983; De Hoog *et al*., 2000).

In cutaneous *Candida* infections, the skin or mucosal membranes, may suffer acanthosis (hyperplasia and thickening of the stratum spinosum) with pseudoepitheliomatous hyperplasia, with the fungus growing in the epithelial tissue (Mouton & Botha, 2012). These types of infections have been reported in captive cetaceans and varied from ulcerative dermatitis to inflammation without ulcers and healed ulcers (Mouton & Botha, 2012). The specie Candida albicans is known to caused extensive, granulating and sometimes ulcered skin lesions and oesophago-gastric ulcerations (Van Bressem *et al*., 2008). Cases of disseminated candidiasis caused the death of *Tursiops truncatus*, *Phocoena phocoena* and *Gobicephala melas* (Nakeeb *et al*., 1977; Dunn *et al*., 1982).

Fusarium species are known as opportunistic saprophytes pathogens that causes diseases in terrestrial plants and they also cause hyalohyphomycosis after traumatic inoculation in humans and animals (Frasca *et al*., 1996; Cabañes *et al*., 1997; De Hoog *et al*., 2000). In cetaceans, mycotic dermatitis caused by these fungi, was reported in a pygmy sperm whale, Atlantic white-sided dolphin, and captive beluga (Frasca *et al*., 1996; Bowenkamp *et al*., 2001). The characteristics observed in the skin were elevated, firm, erythematous, 2 to 5 mm nodules, cutaneous tubercles found mainly on the head, trunks, and caudal portions of the cetaceans' bodies (Frasca *et al*., 1996; Bowenkamp *et* *al.*, 2001). In 2012, a 10-year-old female false killer whale (*Pseudorca crassidens*) developed skin lesions in the left breast fin caused by *Fusarium solani* (Tanaka *et al*., 2012). Tanaka *et al.* (2012) reported about the case, and they studied the multiple granulomas spread diffusely into the deep dermis and bone which is very uncommon to happen considering that mycotic skin lesions by *Fusarium sp.* reported so far in marine mammals were regarded as superficial dermatitis.

Lacazia fungus are known to be a dimorphic pathogen belonging to the order Onygenales (Herr *et al*., 2001). The *Lacazia loboi* is a very known specie that causes invasive cutaneous lesions in cetaceans and humans (Dhermain *et al.*, 2002), also called lobomycosis, lacaziosis or keloidal blastomycosis (Higgins, 2000; Murdoch *et al.*, 2008; Kiszka *et al*., 2009). *L. loboi* cells found in *Tursiops truncatus* infected tissues are significantly smaller than those found in humans, suggesting that the organism react in different ways as they may not be identical in the two hosts (Haubold *et al*., 2000). This fungal infections in cetaceans' skin can be identified as grayish, whitish to slightly pink, verrucous lesions, most of the time in pronounced relief that may ulcerate (Migaki *et al*., 1971), although mainly on the head, flippers, abdomen, fin, back tail stocks and flukes (Migaki & Jones, 1983). Majority of the time, the disease evolves slowly, and it may lead to death (Simões-Lopes *et al*., 1993; Van Bressem *et al.*, 2007). The first case of *Lacazia loboi* was reported by Migaki *et al.* (1971) in Atlantic bottlenose dolphins. In 1973, De Vries & Laarman (1973) discover the same disease in a Guiana dolphin and more recently, some authors suggested that the incidence of this fungus might represent opportunistic infections in immune-compromised hosts (Murdoch *et al.*, 2008; Reif *et al*., 2008). The bioaccumulation of environmental contaminants in the affected dolphins was thought to contribute to vulnerability to this disease (Murdoch *et al*., 2008).

The last genus significant in the study of fungal skin infections in cetaceans is the *Trichophyton*. They belong to the dermatophytes group with the genera *Tinea*, *Epidermophyton* and *Microsporum* (De Hoog *et al*., 2000). Dermatophytes (*Epidermophyton*, *Microsporum* and *Trichophyton*) are generally considered as commensals because they are dependent on keratin as their source of carbon and nitrogen (Reeb *et al*., 2010). These group of fungi grow on the outermost layers of the slin, including muco-cutaneous membranes, genitalia, external ears, dead skin, or hair (Mouton & Botha, 2012). *Trichophyton* fungal infection was reported in cetaceans for the first time in the trunk of a captive Atlantic bottlenose dolphin, reported by Hoshina & Sigiura (1956). The occurrences and studies of this fungal infection in cetaceans are rare.

1.3.1.4. Ectoparasites

The term ectoparasite is referred to any type of organisms, ranging from algae to fish, that somehow clings or attaches to the surface of a marine mammal (Geraci & St. Aubin, 1987). Whose mode of attachment, feeding behavior, or relationship with the definitive host or transport animal (phoresis) are somehow obscure so that a parasitic origin cannot be excluded (Geraci & St. Aubin, 1987). Some organisms have evolved as real ectoparasites (mainly of large whales) and thus carry the potential to damage the host's epidermis. As an example, we can consider blue whales entering the cold waters. They acquire a yellowish film (sulfur bottoms) on their body skin resulting from diatom infestation (Hart, 1935). The species *Cocconeis ceticola* and *Navicola* spp. are good examples of ectoparasites that attach firmly to the cetaceans' skin surface by sucker-like valves (Hart, 1935). Sometimes they may penetrate the epidermis thereby becoming saprophytic ectoparasites (Hart, 1935).

Ectoparasite infections are normally caused by arthropods, which have adapted to the oceanic environment as the epizoic sessile barnacles (Félix *et al*., 2006). The species *Coronula diadema*, *C. reginae* and *Crytolepas rhachianecti* are epizoic sessile barnacles which attach so deeply onto their host skins that a pit remains when being removed or shed (Félix *et al*., 2006) that can make a scar (Scheffer, 1939) as it is the case of the specie *Xenobalanus globicipitis*. This last specie cause star-shaped scars on the epidermis of cetaceans (Bane & Zullo, 1980; Dreyer *et al*., 2020; Siliciano *et al*., 2020), however is not the most dangerous skin-invader for cetaceans. *Pennella balaenoptera* is one of the largest parasitic copepods in the ocean that actively penetrates the skin of mysticetes and anchors deeply in the bubbler, feeding on whale tissue and they also may cause local swelling of the skin and thin white scars (Clarke, 1966; Bertulli *et al*., 2012). Unfortunately, the vector-borne capacity of *P. balaenoptera* is poorly known, but it may play a pivotal role in the transmission of invasive pathogens (Hermosilla *et al*., 2015) as an invasive non-native species. This species can co-transport externally and internally other organisms including viruses, bacteria, and other eukaryotes, collectively referred to as the symbiome (Foster *et al*., 2021).

Whale lice (Cyamide; Amphipoda) are another parasitic group specific for cetaceans that include at least 11 different species (Leung, 1970; Lehnert *et al*., 2021). Whale lice have adapted to their marine environment in developing small, dorso-ventrally compressed body sizes with five pairs of legs ending in claw-like appendices and spines that help them to cling to their host (Rowntree, 1996; Lehnert *et al*., 2021). They are known to parasitizing the skin of sperm whales as euryxenous parasites (having a broad range of hosts) (Hermosilla *et al*., 2015). The exception is for example the specie *Cyamus balaenoptera* that tend to be more host-specific (Leung, 1970; Kaliszewska *et al*., 2005). Normally, cyamid ectoparasites reside in fissures and crevices of the skin and are often found close to sessile barnacles and on the callosities of the head (Scheffer, 1939; Leung, 1970). While slow-moving baleen whales have grooves and callosities for whale lice to attach to, they tend to aggregate in the genital slit, corners of the mouth or the blowhole (Rowntree, 1996) and in lesions with thickened edges in faster hydrodynamic odontocetes (Lehnert *et al*., 2021) (Figure 1.4). The worst-case scenario regarding the impact of these organisms is the appearance of dermatitis, when the host is heavily infested by the ectoparasites (Leung, 1970).

Figure 1.4- Whale lice in: (A) ulcerative lesion in harbour porpoise (*Phocoena phocoena*); (B) rake marks on pilot whale (*Gobicephala melas*) (Lehnert *et al*., 2021).

Lastly is important to refer the largest ectoparasites of cetaceans' skin (Pike, 1951; Geraci & St Aubin, 1987) known as Agnatha, an infraphylum of jawless fish (lamprey species). These species firmly attach to the skin of whales and are polyxenous ectoparasites. The feed on cetacean skin as a nutrient source amongst others. Even so, while being firmly attached by the teeth of their sucking disc, the lampreys can cause severe and deep hemorrhagic skin lesions (Pike, 1951). Bertulli *et al*. (2012) identified in their study a probable bite mark by sea lamprey (*Petromyzon marinus*) on minke whales as a fresh and healed lesion, greyish or pale colored, showing a slightly more circular than oval aspect and with more texture, including raised borders. Previous studies also reported *P. marinus* bites on minke whales and killer whales off Iceland (Ólafsdóttir *et al*., 2009; Samarra *et al*., 2012).

1.3.1.5. Intra-Interspecific interactions

Intra-interspecific interactions refer to interaction with individuals of the same species (intraspecific) or with other species (interspecific). When the interactions become aggressive it may lead to dangerous lesions and/or death of the animal (Patterson *et al*., 1998; Dunn *et al.*, 2002; Arbelo *et al*., 2013; Díaz-Delgado *et al*., 2018). When social intra-specific interactions occur, mild multifocal lesions over the skin body of the animal are seen. "Tooth-rake marks" are external linear and parallel erosions on the skin inflicted by teeth and can be observed in stranded or alive cetaceans (Puig-Lozano *et al*., 2020). However, when interactions became aggressive, tooth-rake marks could be severe and ulcerate the skin affecting the subcutaneous and muscle tissue (Puig-Lozano et al., 2020). Other examples of lesions that had been reported as intra-specific interactions include blunt traumas with subcutaneous focal/multifocal extensive hemorrhages, hematomas, tearing of the bubbler, vertebral ribs fractures, myonecrosis (specific to muscle tissues) and tearing of the parietal pleura with associated-pulmonary hemorrhage (Ross & Wilson, 1996; Jepson & Backer, 1998; Arbelo *et al*., 2013; Díaz-Delgado *et al*., 2018).

Aggressive encounters involving individuals of the same species (intra-specific interactions) and the formation of male alliances are described by some authors (Gerson & Hickie, 1985; Connor *et al*., 1992; Clapham, 1996; Parson *et al*., 2003; Wiszniewski *et al.*, 2012). Male alliances are responsible for violent kidnappings ("herding events") of non-pregnant females to increase their mating opportunities as well as of infanticides in different odontocetes species such as the bottlenose dolphin (*Tursiops* spp.) (Patterson *et al*., 1998; Dunn *et al*., 2002; Kaplan *et al*., 2009; Díaz López *et al*., 2018) and killer whales (*Orcinus orca*) (Towers *et al*., 2018). In the other hand, male humpback whales (*Megaptera novaeangliae*) have also been reported escorting receptive females and threatening other males by thrashing of their flukes or signings as communication signals in the context of male competition (Perrin *et al*., 2009). Although male coalitions have also been observed in whales, aggressive reactions are not usual, and fights rarely result in injuries or deaths (Clapham, 1996).

Interspecific interactions may occur as prey competition (Spitz *et al*., 2006), fight practice (Jepson & Backer, 1998), or predation or even territorial competition and sexual frustration (Ross & Wilson, 1996; Methion & Díaz López, 2021). For example, killer whales (*Orcinus orca*) have been observed attacking or pursuing different species of odontocetes and mysticetes (Baldridge, 1972; Silber *et al*., 1990; Jefferson *et al*., 1996; Ott & Danilewicz, 1996; Visser *et al.*, 2010; Saulitis *et al.*, 2015; Wellard *et al.*, 2016; Corsi *et al.*, 2021). More recently, Corsi *et al.* (2021) studied the different killer whale predatory scarring on mysticetes in the eastern North Pacific. The study found that gray whales had a relatively higher incidence of predatory scarring, humpback and gray whales had most of their rake marks on the trailing edge of the tail fluke and blue whales on the leading edge of the fluke. Of whales with scarring, blue whales were the most susceptible to fluke mutilation while humpback and gray whales were more likely to accumulate new rake marks over the years (Corsi *et al.*, 2021).

1.3.2. Anthropogenic Causes

The increase and the evolution of human activities in coastal and oceanic areas around the world has a massive pressure on marine biodiversity and ecosystems (Halpern *et al.*, 2012). Cetaceans are one of the most susceptible groups to human activity such as fishing interactions, vessel strikes and pollution (Reeves *et al.*, 2003). Most of the time, these three activities lead to individual markings on cetaceans. Individual marks are normally used to estimate the prevalence and source of injuries (or diseases) that are natural or anthropogenic in nature, and assess the potential differences within population, over time, or between populations in the rate of predatory or anthropogenic interactions (Baird *et al.*, 2014; Félix *et al.*, 2017). Although injuries caused by fisheries interactions (vessels and gears) are thought to be the most important management issue affecting cetaceans (Read, 2008; Moore, 2019) there is increasing concern about the impact of vessel strikes for cetaceans' species endangered or in recovery (Laist *et al.*, 2001; Van Waerebeek *et al.*, 2007; Luksenburg, 2014; Félix *et al.*, 2017).

Fishing gear interaction and vessel collisions are not always fatal (Van Waerebeek *et al.*, 2007; Bechdel *et al.*, 2009). The interactions may result in mutilated appendages, disfigured fins and cutting wounds that penetrate the muscle and sometimes reach the bone (Van Waerebeek *et al.*, 2007; Freitas *et al.*, 2008; Elwen & Leeney, 2010; Byard *et al.*, 2012). Photographic evidence has been taken through the years showing the evolution of large wounds, in the same individual, proving that cetaceans are highly resilient to those injuries (Zasloff, 2011; Bossley & Woolfall, 2014). However, when the population size is considered to evaluate the impact of fishing gears interaction and vessel collisions on coastal cetaceans, the smallest and the most discrete populations may suffer huge impacts (Parsons & Jefferson, 2000).

1.3.2.1. Fishing gear interaction

There is not much detailed information in the scientific literature about lesions or marks of fishing gear interaction (bycatch) on cetaceans. The impact of this activity on cetaceans has increased in the recent decades and is one of the main causes of dolphin mortality (Spencer *et al*., 2000). Interaction with fishing activities is defined as acute death of cetaceans after interaction with any type of fishing activity, whether by accidental capture (bycatch) or because of severe injuries caused by fishermen or fishing equipment (Kuiken *et al*., 1994). Arbelo *et al*. (2013) reported cases of anthropogenic lesions on cetaceans such as superficial cutaneous lesions caused by contact with fishing nets and ropes, wounds and fractures of the cranium and deep wounds made by fisheries instruments. Domiciano *et al*. (2016) also identified anthropogenic mortalities on cetaceans through gross skin lesions. The main gross lesions were superficial and deep bruises by contact with ropes or fishing nets encircling the body or extremities as mouth, fins, and tail (Domiciano *et al.*, 2016). Deep and linear wounds that penetrate the body cavity or were proximal to amputated fluke and fins were also observed (Domiciano *et al.*, 2016).

Some authors referred that Odontoceti as harbor porpoises, common dolphins and bottlenose dolphins are more frequently involved in interaction with fishing activities (Baker & Martin 1992, Kuiken *et al*. 1994, Kirkwood *et al.* 1997, Cox *et al.* 1998), however, skin lesions and mortality from entanglements in fixed fishing gear are also a significant problem for Mysticeti, in particular North Atlantic right whales (*Eubalaena glacialis*) and North Atlantic humpback whales (*Magaptera novaeangliae*) (Robbins & Mattila, 2000; Johnson *et al.*, 2005; Neilson *et al*., 2009; Robbins, 2009; Knowlton *et al*., 2012; Basran *et al*., 2019). Robbins & Mattila (2000) studied the specie *Magaptera novaeangliae* in the Gulf of Maine (USA) and they affirmed that the caudal peduncle is commonly implicated in humpback whales’ entanglements and consistently presented during the terminal dive. The authors also discovered that males were more likely than females to exhibit entanglement-related scars. Nine years later, Robbins (2009) published another article where it says that 97.2% of *Magaptera novaeangliae* individuals involved in eye-witnessed entanglements were independently scored as having a high probability of prior entanglement. The annual humpback whale mortality from entanglement was estimated at approximately 3% (Robbins, 2009). Knowlton *et al*., (2012) studied the same specie over 30 years (from Florida, USA, to Nova Scotia, Canada) and documented more than 1000 unique entanglement events. The results found by the authors showed that approximately 83% of the individuals photographed had been entangled at least once and juveniles were entangled at a higher rate than adults (Knowlton *et al.*, 2012). The annual percentage of whales observed with rope on the body increased suggesting that it is becoming even more difficult for the animals free themselves completely from fishing gears (Knowlton *et al.*, 2012).

1.3.2.2. Vessel collisions

The significant increase of maritime traffic around the world has contributed to a higher number of collisions with cetaceans in recent decades (Arbelo *et al.*, 2013). A vessel collision (or strike) is defined as any impact between any part of a watercraft and a live animal (Peel *et al*., 2018) or as a forceful impact between any part of the boat and a live animal resulting in death or physical trauma (Van Waerebeek *et al.*, 2007; Cates *et al.*, 2017). The effects of collisions on marine animals start to be a concerning when an extensive and growing utilization of the world's ocean by commercial and recreational vessels was verified. Between 1980 and 2018, the number of globally registered large commercial vessels increased from 11 108 to over 94 000 (Laist *et al.*, 2001; United Nations Conference on Trade Development, 2018). The largest increase in commercial vessels coincided with an increase in the amount of ship strikes fatal to mainly Mysticeti (Laist *et al*., 2001).

Arbelo *et al*. (2013) reported that species involved in collisions belonged to the deep-diving group, species that need a prolonged period of recuperation at the surface after one or more apneas. The authors identified consistent lesions with severe sharp trauma, deadly deep cuts and in some cases the body was completely sectioned (Arbelo *et al*., 2013). In some studies, sharp and blunt force injuries have been extensively described for whales (Moore *et al*., 2004; Campbell-Malone *et al*., 2008; Douglas *et al*., 2008; Hill *et al*., 2017). Sharp force injuries involve external gashes and several tail stocks or fins, principally originating from rotating propeller contact (Schoeman *et al*., 2020). Blunt force injuries typically originate from contact with the bow, hull, skeg, or rudder, and are classified as abrasions (removal of the epithelial layer of the skin), contusions (hemorrhages), laceration (tearing of the skin, and bone or skull fractures (DiMaio & DiMaio, 2001; Moore *et al*., 2013). Fortunately, in 2005, the Ship Strike Working Group (SSWG) was created to understand and reduce the threat of vessel strikes to cetaceans with the help of the "ship strike database" which contributed positively to monitor validated information on cetacean ship strikes worldwide (Schoeman *et al*., 2020).

1.3.2.3. Habitat degradation and water pollution

It is known that cetaceans can accumulate toxins and contaminants from the marine environment or consumed prey (Powell *et al*., 2018), acting as sentinels of ecosystem health (Wells *et al*., 2004; Van Bressem *et al*., 2015). The increasing prevalence of cetacean skin lesions can potentially be related with weakened immunity (Van Bressem *et al*., 2009), possibly as the result of stress from environmental perturbation (Fury & Reif, 2012), habitat degradation due to increasing anthropogenic effects (Stephens *et al*., 2012), concurrent infection (Schulman & Lipscomb, 1999), and the presence of pollutants in the environment (Wilson *et al*., 1999a; Reif *et al*., 2008; Stephens *et al*., 2012).

Harzen & Brunnick (1997) studied the resident bottlenose dolphins, from the Sado estuary (Portugal) and they found that 85% of the community showed signs of skin disorders. They compared these results to observations from other areas and concluded that habitat degradation played a significant role in these skin disorders since eutrophication seemed to be a serious problem in the estuary. Then, the apparent depressed immune systems were recognized by the authors as caused by stress, habitat degradation and pollution. The tattoo skin disease (TDS) may be associated with chemical and organic water pollution (Van Bressem *et al*., 2007).

Another study, conducted by Van Bressem *et al*., (2007), was done during 1984 till 2007 to investigate cases of skin and skeletal diseases in cetaceans. The overview reported tattoo skin diseases, lobomycosis-like disease, and other cutaneous infections with unknown aetiology. The authors suggested that anthropogenic factors like aquaculture, fish factories, untreated waste water, ballast water and chemical pollution, play a major role in the degradation of the habitats of these cetaceans, thus contributing to the poor health status of the population.

The Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) were studied by Kiszka *et al*. (2009) and the authors reported the presence of chronic mycotic disease of the skin and subdermal tissues. These dolphins showed numerous raised, greyish nodules on the head, flanks, dorsal fin, belly, back and tail. In some individuals, the lesions were more severe and some resembling other unknown fungal infections. Again, habitat degradation, especially along the coastal areas where urbanization, agricultural activities and untreated waste water are evident, was revealed as a contributing factor to the aetiology of the disease.

1.4. Photo-identification as a tool for evaluating the health of cetaceans

Visual health assessment methods as photo-identification (photo-ID) have been used to identify free-ranging individual cetaceans using markings of natural or anthropogenic origin (Pettis *et al.*, 2004; Ballance, 2018) through high resolution photographs taken from boats and aircraft (Moore *et al.*, 2021). Also crucial to investigations of population size and trends (Barlow *et al.*, 2011), scales of residency and ranging behavior (Fearnbach *et al.*, 2014; Mahaffy *et al.*, 2015; Gladilina *et al.*, 2018), demography (Aschettino *et al.*, 2012), social structure (Gero *et al.*, 2008), and habitat use (O'Brien *et al.*, 2020), photo-identification has been a valuable tool in understanding cetaceans and in the field of conservation science. Managing the recovery of endangered species relies on estimating population abundance and monitoring trends and diseases over time (Bogucki *et al*., 2019; Minton *et al*., 2020). Because it is rarely possible to simply count individuals, a common method for estimating abundance is mark recapture (Otis *et al.*, 1978; Seber, 1982). However, photo-ID shows up as a less invasive method in which natural or anthropogenic markings are used to differentiate between individuals without the stress of capture (Agler *et al*., 1990).

Photo-ID data can also be used to identify lesions and assess levels of skin disease, because it is not an invasive or expensive as other methods (Thompson *et al.*, 1992; Van Bressem *et al*., 2003; Pettis *et al.*, 2004; Van Bressem *et al*., 2007; Hart *et al*., 2012). This method helps assessing visually the health and injuries of cetaceans giving a distinctive, sequential perspective on the status and trend of each animal and aids in identifying sublethal impacts (Moore *et al*., 2021). For example, high-resolution drone images can provide information on skin condition, whale lice burdens, and the incidence of entanglement wounds, notably coupled with quantitative photogrammetry measurements from the same individual (Christiansen *et al.*, 2018; Soledade Lemos *et al*., 2020; Moore *et al*., 2021). Individual markings, such as scars or large wounds, can also be used to estimate the prevalence and source of disease or injuries (natural or anthropogenic), and assess whether there are potential differences within a population over time, or between populations in the rate of predatory or anthropogenic interactions (Baird *et al.*, 2014; Félix *et al*., 2017).

Injuries due to interactions with fisheries (vessel and gear) are thought to be the most important management issue affecting cetaceans (Moore, 2019). However, with low or absence of independent observer effort, poor reporting requirements for cetacean bycatch, and reduced conclusive necropsies of stranded animals, injurious or fatal interactions of cetaceans with fisheries are difficult to quantify (Williams *et al.*, 2011; Hines *et al.*, 2020). Photographic analysis of scars, presumed to be due to interactions with vessel or gear, offer valuable information on potential unaccounted sources of cryptic mortality and an opportunity to assess and monitor these anthropogenic impacts on wild populations (Leone *et al.*, 2019; Ramp *et al.*, 2021). Therefore, this method is considered essential in retrospective analysis for evaluating sub-lethal anthropogenic injury impact on health, informing annual injury determinations and estimates of human impact on cetacean's species, and predicting survival (Pettis *et al.*, 2004).

As all the tools used to identify animals, photo-ID also has their disadvantages and the biggest challenge of this method (to evaluate the health of a population and inform conservation) is that is time-consuming (Bogucki *et al.*, 2019). The digital photography has been increasing the volume of images submitted to catalogs around the world and has resulted in processing accumulations (Bogucki *et al.*, 2019). While natural and anthropogenic markings may be used for individual identification over periods from days to weeks, understanding which markings are permanent or will remain stable over the lifetime of the individual is essential for consistent long-term identification of individuals and causes of injury (Feyrer *et al.*, 2021). Misidentification due to loss or gain of markings can result in a Type I error (a false positive, incorrectly identifying an animal as a known animal) or Type II error (a false negative, incorrectly identifying a known animal as an unknown or new animal) (Feyrer *et al.*, 2021). Long term datasets require frequent re-evaluation to avoid Type I and II errors and to ensure distinctive marks are consistent and do not change or are not lost over the study period (Frasier *et al.*, 2009; Urian *et al.*, 2015; Feyrer *et al.*, 2021). However, recent advances in technology lined the way to automated image processing using neutral networks (Bogucki *et al.*, 2019). Exploiting this new technology will accelerate the speed at which these images can be matched to known individuals based on more accurate identification of skin lesions on cetaceans.

1.5. Cetaceans in the Northwestern Iberian Peninsula

Iberian Atlantic waters are known as highly productive and rich in marine resources (Wooster *et al.*, 1976), which are severely exploited by Spanish and

Portuguese fisheries. The high productivity of the Northwestern Iberian Peninsula (Galician shelf) can be explained by the northern limit of the east central Atlantic upwelling system (López *et al.*, 2004). The upwelling that occurs from April to September (Fraga, 1981) results in nutrient enrichment of the area (Blanton *et al.*, 1984) sustaining the high productivity and, consequently, leads to high biodiversity of species as fish and cephalopods (Solórzano *et al.*, 1988; Guerra, 1992; Giralt Paradell *et al.*, 2020). Following the sequence of predator/prey in food webs, all trophic levels can beneficiate of the "bottom-up control" (created by the upwelling in this ecosystem) making these waters a suitable place for predators and especially mysticetes (Spyrakos *et al*., 2011; Díaz López & Methion, 2019).

1.5.1. Myticetes

Mysticetes or baleen whales are large transient marine mammals, also referred as cosmopolitan species that are characterized by lack of teeth and presence of plates of corneal origin that allow them to filter the water and feed on small crustaceans or fish (Bannister, 2009; Marx *et al.*, 2019). On the Northwest Spanish coast, the presence of different species of whales from two different families, the Balenidae and Balaenopteridae families (Valdés, 2010; Aguilar, 2013) has been verified. The Balenidae family includes the bowhead and glacial right whale, the last one considered extinct in the East Atlantic. In the other hand, the Balaenopteridae family includes the so-called "rorquals" (Bannister, 2009). Rorquals have ventral folds that facilitate distension of the gular area and thus be able to filter a greater volume of water during feeding.

There are 5 species of rorquals documented in recent years in the northwest of the Iberian Peninsula (Aguilar, 2013; Díaz López & Methion, 2019; Methion & Díaz López, 2019; Díaz López *et al.*, 2021). These include the species fin whale (*Balaenoptera physalus*), blue whale (*Balaenoptera musculus*), common minke whale (*Balaenoptera acutorostrata*), humpback whale (*Megaloptera novaeanglidae*) and sei whale (*Balaenoptera borealis*) (Díaz López *et al.*, 2021). These whales have relatively short heads, less than a quarter of the body length (Bannister, 2009). In comparison with right whales, the baleen plates are short and wide, numerous ventral grooves are present, and there is a dorsal fin, sometimes quite small (Bannister, 2009). These species carry out seasonal migratory movements between areas of high primary productivity at high latitudes, during the warm months, and areas at lower and less productive latitudes where they reproduce during the winter months (Cooke, 2018). This seasonal migration appears to be less marked in the North Atlantic due to the presence of highly productive coastal upwelling areas that facilitate feeding and the Gulf Stream that favors winter breeding areas at relatively high latitudes (Aguilar, 2013; Díaz López & Methion, 2019).

Among the rorquals found in the Northwestern Iberian Peninsula waters, sei and fin whales are probably the most oceanic species and the fastest with 35 knots (>60 km/hr) and 20 knots (37 km/hr) respectively (Bannister, 2009). Blue whales can be found closer inshore, often associated with deep coastal canyons, and recognized as one of the most powerful swimmers, able to sustain speeds of over 15 knots (28 km/hr) for several hours (Bannister, 2009). The coastal rorqual is the humpback with long migrations between temperate/tropical breeding grounds and cold-water feeding grounds, with an average about 3-4 knots (5-7 km/hr) (Bannister, 2009). In the Northern Hemisphere, humpbacks are relatively more oceanic, but still coastal at some stage in their migrations. The minke whales are wide ranging, from polar to tropical waters in both hemispheres. Elsewhere minkes can often occur near shore, in bays and inlets, their migrations are less well-defined and predictable than the other migratory rorquals but as fin whales, this specie is known as fast swimmer that could reach more than 20 knots (Bannister, 2009).

These huge mammals are known to have high metabolic demands and a strong influence on marine ecosystems (before the advent of industrial whaling): as consumers of fish and invertebrates; as prey to other large predators; as reservoirs and vertical and horizontal vectors for nutrients; and as detrital sources of energy and habitat in the deep sea (Roman *et al*., 2014). These are the main reasons why baleen whales are recognized to have an important role as ecosystem engineers (Roman *et al*., 2014) because they can modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials (Jones *et al*., 1994; Berke, 2010). Their decline has likely altered the structure and function of the oceans (Roman *et al*., 2014) and for centuries, mysticeti have carried the impact of human greed, for products and profit (Bannister, 2009). Only the sperm whale (largest Odontoceti) has rivaled them as a whaling target (Mackintosh, 1965), then to regulate whaling and the uncontrolled hunting during the 20^{th} century that almost cause the extinction of many species of rorquals, it was created the International Whaling Commission (IWC) in 1949 (Sanpera & Aguilar, 1992; Valdés, 2010; Aguilar, 2013).

In the north coast of the Iberian Peninsula the whaling begun in the late Middle Ages but a clear decrease in catches, less dependence on the use of resources obtained from hunted whales, and a social reaction at the national and international level for the conservation of these species caused many countries (including Spain) to decide to end the hunt of whales (Aguilar, 2013; Díaz López *et al.*, 2021). However, the last vestiges of the Spanish whaling industry were concentrated on the Galician coast, a seasonal feeding area for whales and where the last terrestrial factories were active (Aguilar, 2013; Valdés, 2010). Between September 28 (1951), the date on which the first 24-meter pregnant fin whale was captured and October 21 (1985), the date on which the last specimen was captured (17-meter female fin whale), more than 5.000 whales were hunted in Galician waters, contributing significantly to depleting the populations of these large cetaceans in this region of the Atlantic Ocean (Valdés, 2010; Aguilar, 2013; López, 2014; Díaz López *et al*., 2021).

After almost 3 decades of regulation, some whale populations are beginning to show signs of recovery. Fin whale population that was considered "danger of extinction" by the IUCN in the past, now is considered as "vulnerable" and the population doubled in the last 40 years (Cooke, 2018). Despite this, there are other species such as the blue whale which are still in danger of extinction (Cooke, 2021) and whose presence in the Northwestern Iberian Peninsula waters is relatively rare (Aguilar, 2013; Díaz López & Methion, 2019). Since the summer of 2017, the last sighting of a specimen of this species in Galician waters, several specimens of blue whales were sighted again crossing these waters till these days (Díaz López *et al*., 2021), however, some traits still exist that compromise baleen whales occurrence and population health. Unfortunately, scientific information about skin health of mysticetes in the Northwest Iberian Peninsula is still poorly known and non-invasive techniques seem to be the best option to get information about species that are still recovering. Understanding the type, prevalence, and occurrence patterns of skin marks on whales can be a valuable aspect/tool to assess and study. It can provide insight into individual and population health, healing rates, vulnerability to infections, energic costs, important ecological associations (Barlow *et al*., 2019) and lead to some conclusions about possible anthropogenic impacts.

1.6. Study objectives

The main objective of this study is to investigate the ecology of baleen whales along the Northwestern Iberian Peninsula through the identification of skin marks and patterns of pigmentation.

First, using photo-ID data, we aim to assess skin marks on different species of whales present in the study area, describing the morphology and comment on their possible origins (natural or anthropogenic). Secondly the study aims to estimate the prevalence and the abundance of skin marks among the three whale's species. Lastly, we want to verify if the different skin marks are linked to a specific species or body condition by checking their relationship between the three whales' species (BM, BP and BA) and also with individual body condition (good and poor). These results can give an insight if human activity has a significant impact on skin health of baleen whales feeding in Northwestern Iberia Peninsula coast.

Chapter 2

2. Material and Methods

2.1. Study Area

Galicia´s coastline (about 1.200 km in length) is characterized by a relatively narrow continental shelf with a total surface area of 15.000 km^2 and some large coastal inlets (rías) (Fariña *et al.*, 1997). The region lies at the northern edge of one of the major upwelling areas in the world, the eastern boundary system off Northwest Africa and Southwest Europe (Wooster *et al*., 1976). The size and orientation of the rias affects the frequency and intensity of the seasonal upwelling events which increase this area's productivity (Goetz *et al*., 2013). The frequent upwelling of cold and dense North Atlantic Central Water (NACW) results in nutrient enrichment of the area (Blanton *et al*., 1984) and this area is among the most productive oceanic regions of the world (Spyrakos *et al.*, 2011). Upwelling reaches its highest intensity during summer (April to September) (Fraga, 1981; Prego & Bao, 1997).

The study area (Figure 2.1) extends along the southern coast of Galicia (Spain), covering the entire continental shelf from Muros (42.79º N, 9.15º W) to Cíes Islands (42.36º N, 8.94º W) encompassing 1300 km^2 of area with 92% corresponding to the continental shelf (depth <200 m) and the rest covering the continental slope down to a depth of 1050 m (Díaz López & Methion, 2019; Díaz López *et al*., 2019).

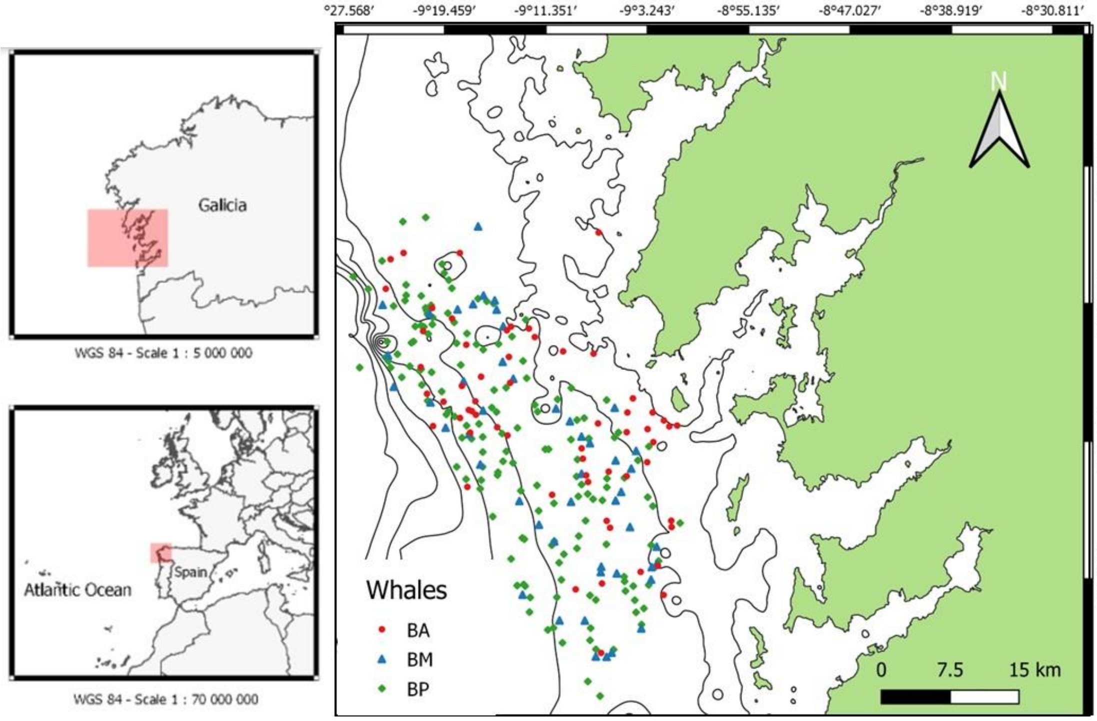


Figure 2.1- Map of the study area surveyed along the Northwestern coast of Spain, showing the distribution of the three species of baleen whales: minke whales (BA), blue whales (BM) and fin whales (BP).

2.2. Sampling strategy

Each sampling campaign was carried out during the day from vessel-based surveys at a constant speed of 6 knots, with the participation of 3 members of the scientific team (with extensive experience in the study of cetaceans) assisted by students who are doing their bachelor's or master's thesis at Bottlenose Dolphin Research Institute (BDRI). The team of observers was located on the flybridge (4 m above sea level) and on the bow and stern of the boat allowing 360-degree exploration of the sea surface in search of whales (naked eye or binoculars 10x50). Following the methodology used by the BDRI team in previous studies on the distribution of cetaceans (Díaz López & Methion, 2019; Díaz López *et al*., 2019; Díaz López *et al*., 2021), on each day instantaneous samples were collected every 20 minutes in which the time, position and speed of the vessel, the presence of cetaceans, anthropogenic and environmental data were recorded. The date, time, position, and depth were obtained using a GPS-Plotter probe associated with an 83-200 kHz echo sounder transducer. All the surveys were performed when the environmental conditions were favorable, which means and the visibility was not reduced by rain or fog, the sea conditions were less than 4 on the Beaufort wind force scale, and wave height smaller than 1.5 m (Díaz López & Methion, 2019).

During the surveys, every time a baleen whale was spotted, the researchers on board started immediately the sighting registration. The vessel slowly reached the area where the whale was spotted in order to minimize disturbance during the approach and allowing the monitoring of the animal at close distances (<50 m) (Methion & Díaz López, 2019). The total body length of the individuals was estimated by comparison to the vessel length when the whale was at less than 50 m distance (Methion & Díaz López, 2019). The date, starting and finish time, spatial coordinates (longitude and latitude), and environmental data as depth, sea surface temperature and sea surface salinity were recorded during the sighting. The different mysticetes individuals were photographed from the vessel-based surveys, using digital SLR cameras with zoom lenses. These data included more than 50000 photographs of whales' specimens collected between 2017 and 2021.

For an adequate identification of the individuals, photographs were taken at a perpendicular angle to the position of the animal to obtain the marks and pigmentation patterns present in the body of the whale. Due to the large size of these animals, two or three photographs of each flank were necessary, so the first covers the head and blowhole, up to the level of the pectoral fins, the second is the intermediate body, third includes the dorsal fin and forth show the caudal peduncle till the beginning of the tail (Figure 2.2). The tail was not considered in this study due to the lack of photographs in that respective section. The photographs were taken both right (DX) and left (SX) side of the body, whenever possible, to check if the pigmentation pattern and marks were identical in both sides in each individual. After all the pictures were taken, we leaved the animal and the vessel returned to the initial track. At the end of the survey, all the data collection was reviewed, extracted from the GPS and entered in a database.

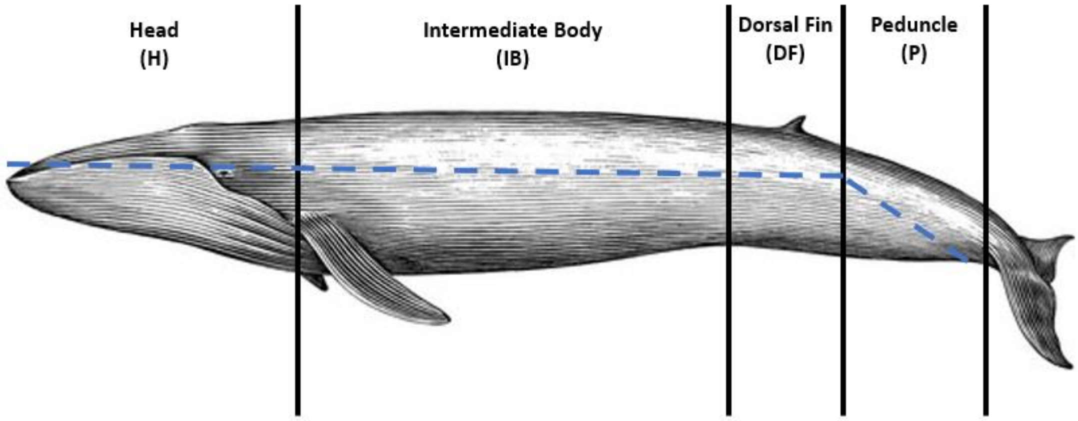


Figure 2.2- General representation of the whale body sections used to locate skin marks: Head (H); Intermediate Body (IB); Dorsal Fin (DF); and Peduncle (P). Only the areas above the dashed blue line were considered in this study.

2.3. Photograph analysis

To ensure correct identification of the species, some features were considered as key-clues. Blue whales can be identified and recognized at first by their strong blows but visually they are relatively bigger than fin and minke whales, lighter in coloration and they have unique pigmentation patterns along the body that do not change with time. Blue whales' dorsal fin is proportionally smaller than the other balaenopterids varying in shape (Gendron & De La Cruz, 2012). In the other hand fin whales are relatively darker than blue whales, they present a very characteristic white right lower jaw and a chevron brighter in the right side (Notarbartolo-Di-Sciara *et al*., 2003). Their dorsal fin is generally falcate in shape and higher than blue whales' dorsal fin (Aguilar & Garcia-Vernet, 2018). Finally, minke whales are the smallest in size and characterized by black or dark gray body with a light gray saddle patch in front of the dorsal fin and a well-defined white band located in the middle of their dark flippers. Generally, their dorsal fin is tall, curved, and pointier than blue and fin whales.

All photographs containing the dorsal fin section and intermediate body section were graded for quality and degree of distinctiveness to minimize misidentification and heterogeneity in capture probabilities (Urian *et al*., 2015). All photographs were given an absolute value score (1=low; 4=average; 10=high) for: (1) the perpendicular angle of the dorsal fin or intermediate body section to the camera; (2) the focus of the photograph being satisfactory to allow all features to be distinguished; (3) contrast and light intensity; and (4) the dorsal fin and intermediate body section being properly sized in the frame for all the feature to be clearly visible following Methion & Díaz López (2018). The individual scores for each category were summed to obtain an overall quality score (OQS). OQSs from 4-16 were considered poor quality; those from 19-25 were considered to be of average quality and those ≥28 were considered excellent (Methion & Díaz López, 2018).

The features referred above allowed us to organize all the photographs per species and the next step was identify individuals of each specie using standard photo-identification methods (Sears *et al.*, 1990). Individuals were included in a second distinctiveness category, based on the amount of information contained on the dorsal fin or intermediate body section, to ensure a homogeneous probability of being identified (Methion & Díaz López, 2018). Marked individuals were considered with distinct dorsal fins shape or single large notches. Unmarked individuals were characterized with no permanent features and no notches. Features such as natural/anthropogenic marks, body and dorsal fin scars or lesions were used as secondary characteristics, thereby reducing the possibility of false positives (Wilson *et al.*, 1999b; Tscherter & Morris, 2005). In case of blue whales, the skin pattern of coloration acted like a fingerprint (unique for each individual and never change over time) proving to be the most easy and accurate feature to identify blue whale individuals.

All the photo-ID process of each species of whales, was carried on during the years by BDRI volunteers and reviewed by three experienced researchers to avoid false positives and false negatives. The maturity of the whales was generally unknown with absence of dependent calves. Regarding this, only adults or juveniles were included in the photo-ID analysis in order to create an identification catalogue based on the dorsal fin shape, single large feature in the body or a matrix of evident marks (Würsig & Jefferson, 1990) or pattern of coloration (last one only applied for blue whales).

2.4. Body condition and skin mark analysis

Once having identified all the individuals of all sightings of all the surveys, the second step was the selection of the best pictures of each body section per individual, in each sighting, during the years 2017-2021 in order to use them to identify different types of skin marks. Photographs of both sides (DX and SX) containing the body sections necessary to the analysis were graded based on the quality of the picture (focus, light, and size) in order to minimize misidentification and heterogeneity in capture probabilities (Urian *et al.*, 2015; Methion & Díaz López, 2018). Following Díaz López *et al.*, (2017) methodology, all photographs, with more than 75 % of the body section visible, were given an absolute value score (0=low; 1=average; 2=high) for: (1) focus of the image being sufficient to allow all the skin marks to be distinguished; (2) light intensity and contrast; and (3) size of the body section in proportion to the whole picture. The individual scores for each category were summed to obtain an overall quality score which 0-2 were considered poor quality; those from 3-4 were average and those with 5-6 were considered good quality. Pictures with score of 0-2 were not considered in this study to ensure correct identification of the skin marks.

The best photographs from the intermediate body and dorsal fin were used to classify the body condition of all specimens of blue, fin and minke whales. The body condition classification (good and poor) used in this study was based on a visual evaluation of the shape of the bubbler along the spine. Healthy individuals, with a good body condition, presented a rounded shape along the spine while slim individuals presented a curvy shape with some ripples along the spine (Figure 2.3).

Figure 2.3- Example of minke whales in both categories of body condition. (A) Good body conditions, rounded sides and no vertebrae are visible. (B) Poor body condition, dorsal ridge and/or multiple vertebrae are visible. These categories are valid for all the species in this study. Photographs were taken by Bruno Díaz López and Séverine Methion (BDRI).

2.4.1. Skin mark abundance and prevalence

Among all the pictures with lesions, marks, or skin disorders, one picture of each body section with the highest value score (Q) were extracted and examined for each individual (per sighting). The disorders were classified based on their morphological features into 10 main categories, accordingly Bertulli *et al.* (2015) categories modified (Annex 1). A total of at least 36 different mark types were identified and counted, except for mottling, speckling, peeling, scratch patch, starburst, and diatoms, which only presence and absence were considered. For the other 30 mark types, the following parameters were calculated: (1) total number of occurrences for each mark *ni*: *i* is the type of the mark; (2) mark prevalence *pi*: frequency of individuals with the *i* mark; (3) mark severity *li*: mean number of marks of *i* type only on individual with *i* occurrences; (4) relative portion *ri* of each mark type to the total amount of marks *R*; and (5) mark abundance *ai*: mean number of the *i* mark per individual. Standard deviations were calculated for mark severity and mark abundance. These results allow us to verify which is the most frequent and prevalent type of skin mark and comment the possible origin (natural or anthropogenic). With this information we expect to understand if human activity (anthropogenic activity) has a significant impact for baleen whale population skin conditions in the Northwestern Iberia Peninsula.

2.5. Data Analysis

All the information and sightings of baleen whales collected on the sampling campaigns were entered into a database connected to a geographic information system using QGIS v. 3.16.5-Hannover software to assess the main spatial distribution of each sighting during the study period (see Figure 2.1).

2.5.1. Skin mark variability between species and body condition

To understand if there is any variability or relationship between the skin marks and the three species of baleen whales, and body conditions, all the quantitative data was converged by individual to have a total number of each type of skin mark for the full body (excluding the tail) and both sides (DX and SX). This procedure was done with the purpose to execute a Principal Component Analysis (PCA) using the factoextra package in v.1.0.7. through the statistic software R. PCA is usually used to explain the variance of a large set of irrelated variables and transforming it into a smaller set of uncorrelated variables called principal components (Hopke, 1985). The PCA objective is to reveal a more concealed set of skin marks that accounts the major pattern across all the original skin marks data, emphasizing the most significant parameters due to spatial and temporal variations. The PCA provided data reduction that described the whole data set excluding the less significant skin marks with minimum loss of original information (Vega *et al.*, 1988; Singh *et al.*, 2005).

Before the execution of the PCA, the best quality pictures were selected (Q>2) in order to create the full body of each individual (excluding tail). However, just a few individuals had enough good quality photos of all the body sections and both sides. To avoid bias, the presence of body sections (IB, DF and P) and sides (DX) with higher occurrences were considered. The test of normality, Shapiro-Wilk, was applied to verify if the data was normally distributed followed by the performance of the PCA with all the skin marks per individuals of each specie. A second PCA was applied for the three skin marks with higher values of variability which explains at least 80% of the data. A two-way PERMANOVA was performed in order to verify if there are any significative statistical differences between the dependent (skin marks) and independent variables (species and body conditions).

Chapter 3

3. Results

During the period 2017-2021, 121 boat based surveys were performed offshore in satisfactory conditions (up to 4 on the Beaufort wind force scale, wave height smaller than 1.5 m, and absence of rain or fog). During the study, 247 sightings of baleen whales involving more than 50000 photos in total were taken, which allowed us to identify 21 individual blue whales (BM), 160 individual fin whales (BP) and 30 individual minke whales (BA) seen on 68 different days at sea (56.2% of total number of courses). The body conditions were assessed and 86.67% of the baleen whales sampled were in good body condition and 13.33% were in poor body condition. These species were encountered along the shelf and offshore waters throughout the study area (see Figure 2.1). Blue whales were sighted between the months of July and October, fin whales were seen between the months of June and October and minke whales were seen year-round along the Galician coast. The size and composition of the baleen whales' aggregations were examined based on the total count of individuals observed at one time in the area. The group sizes ranged between 1 and 5 individuals of BM (mean = 1.45 ± 0.14, median = 1, n = 47), 1 and 16 individuals of BP (mean = 2.49 ± 0.19, median = 2, n = 147) and the BA ranged between 1 and 3 individuals (mean = 1.19 ± 0.07, median = 1, n = 53). For the group composition of each specie, all individuals were considered adults.

3.1. Skin mark abundance and prevalence

For the analysis of skin marks, 1285 photographs (Q>2) were used involving 21 individual blue whales, 124 individual fin whales and 22 individual minke whales. The proportions of body sections photographs captured per species ranged between 50% (BA head) till 100% (BM dorsal fin) of the total amount of individuals per species (N) (Table 3.1). The head was the body section with less percentage of photographs taken in all the species (Table 3.1). Considering the 4 body sections, a total of 23 different types of skin marks were found in BM, 36 types in BP and 25 on BA. All the mark types were distinguished and categorized into 10 different mark categories (Annex 2). Mottling, speckling, peeling, scratch patch, starburst and diatoms were considered as presence or absence due to the difficulty of counting. For that reason, these last categories were not included in the table of abundance and prevalence of skin marks to ensure the quality of the analysis.

Table 3.3- Proportions (%) of body section: Head (H), Intermediate Body (IB), Dorsal Fin (DF), and Peduncle (P) captured in photographs per species, *Balaenoptera musculus* (BM), *Balaenoptera physalus* (BP) and *Balaenoptera acutorostrata* (BA). N is the total amount of individuals.

	H	**IB**	**DF**	**P**	***N***
BM	90.48	95.24	100	95.24	21
BP	67.74	97.58	93.55	79.84	124
BA	50.00	90.91	90.91	81.82	22

3.1.1. *Balaenopera musculus*

A total of 100% of the BM population (21 individuals) showed at least 1 skin mark with a total of 6320 distinct marks identified. The most prevalent marks encountered were black marks (pi=0.952), blisters (pi= 0.810) and wounds (pi=0.762). The most abundant marks were herpes, blisters, and black marks with a mean value of *ai*=63.81 ± 22.01, ai=62.19 ± 8.91 and ai=59.67 ± 9.68 marks per individual, respectively. The most severe mark types were herpes, tooth-rakes, and black marks with a mean value of *li*=95.71 ± 88.61 marks per individual, *li*=11 ± 2.58 per individual and *li*=9.86 ± 16.99 per individual, respectively. Starburst, peeling, and diatoms were observed in blue whales affecting 19, 8 and 3 individuals respectively (Table 3.2).

3.1.2. *Balaenoptera physalus*

A total of 100% of the BP population (124 individuals) showed at least 1 mark with a total of 17799 distinct marks identified. The most prevalent marks found were white marks and blisters with *pi*=0.742 followed by scars of unknown origin with *pi*=0.637. The most abundant marks were herpes, white marks, and blisters with a mean value of *ai*=50.75 ± 39.59, *ai*=26.78 ± 14.33 and *ai*=14.74 ± 7.41 marks per individual, respectively. The most severe mark types were herpes (*li*=112.38 ± 90.75), tattoo-like (*li*=19 ± 19.76) and white marks (*li*=13.5 ± 22.14). Mottling, speckling, peeling, scratch patch, starburst and diatoms were all present in fin whales. Peeling and mottling were observed more frequently affecting 25 and 22 individuals respectively (Table 3.2).

3.1.3. *Balaenoptera acutorostrata*

A total of 100% of the BA population (22 individuals) showed at least 1 mark with a total of 1042 distinct marks identified. The most prevalent marks encountered were blisters (*pi*=0.727), white marks (*pi*=0.636) and wounds (*pi*=0.591). The most abundant marks were blisters, herpes, and cookie-cutter bite with a mean value of *ai*= 17.05 ± 10.40, *ai*=6.50 ± 9.74 and *ai*=4.50 ± 3.57 marks per individual, respectively. The most severe mark types were herpes, blisters, and cookie-cutter bite with a mean value of *li*= 71.50 ± 8.50 per individual, *li*=9.38 ± 16.02 per individual, *li*=6.6 ± 7.46 per individual respectively. Diatoms were observed in only one individual (BA016) (Table 3.2).

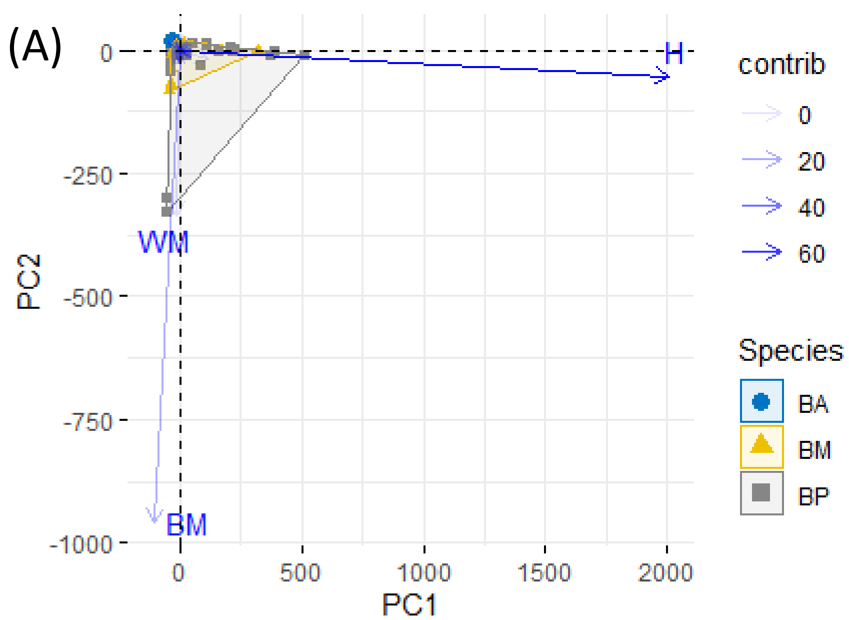
Table 3.4- Number of individuals with presence of i mark (BM, BP, BA) and mark prevalence (BM *pi*, BP *pi*, BA *pi*)

on baleen whales.

Mark type (Q<2)	BM	BM *pi*	BP	BP *pi*	BA	BA *pi*
Mottling	-	-	22	0.177	0	-
Speckling	0	-	2	0.016	0	-
Peeling	8	0.381	25	0.202	0	-
Scratch Patch	0	-	10	0.081	0	-
Starburst	19	0.905	1	0.008	0	-
Diatoms	3	0.143	3	0.024	1	0.048

3.2. Skin mark variability between species and body condition

Photographs of 90 selected individuals for skin marks variability analysis of baleen whales observed during 5 consecutive years had 34 skin mark types out of the 36 described earlier. Mottling, speckling, peeling, scratch patch, starburst and diatoms were present but discarded from the analysis (because we only had presence/absence data) remaining only with 29 different skin marks. A test of normality, Shapiro-Wilk, was performed for all the skin marks and showed that all the data is not normally distributed (all p<α) contributing for the rejection of the null hypothesis. When executed the first PCA (Figure 3.1.A) with all the skin marks data it was possible to verify that with just the summatory of the 2 first PCs, 88.8% of the total variance of the data was explained. The Eigenvalues were 10669 for the first component and 2720.77 for the second component. The skin marks that mainly contribute for the variability of the data were the herpes, black marks, and white marks. These 3 skin marks were used to perform the second PCA (Figure 3.1.B) and with only 2 components, 95% of the total variance of the data was explained. In PC1, H contribute with 0.99. In PC2, BM contribute with 0.94 and WM with 0.33. The Eigenvalues were 10620.7 for the first component and 2714.94 for the second component.



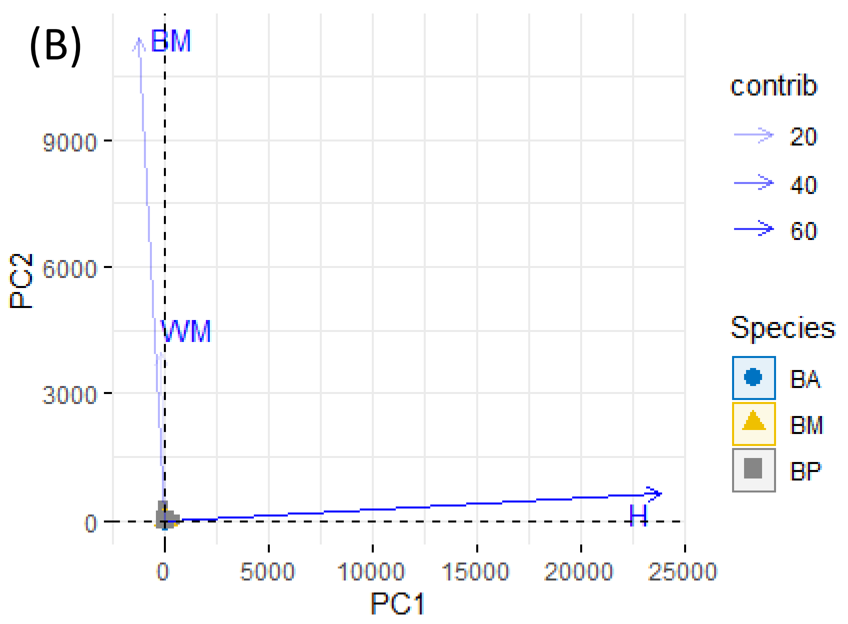


Figure 3.1- Principal Component Analysis (PCA): (A) of all the skin marks; (B) of only the skin marks with higher contributions herpes-like (H), black marks (BM) and white marks (WM) on the different species *Balaenoptera acutorostrata* (BA), *Balaenoptera musculus* (BM) and *Balaenoptera physalus* (BP).

The two-way PERMANOVA test showed that there are no significative statistical differences between the dependent and independent variables. Herpes, black and white marks are homogenous between baleen whale species (p=0.520, df=2, F=0.288) and between individual body condition (p=0.951, df=1, F=0.0293). We don't reject the null hypothesis (H0).

Chapter 4

4. Discussion

4.1. Skin mark abundance and prevalence

Although skin marks on blue, fin and minke whales have been discussed in previous studies (Bertulli *et al.*, 2015; Barlow *et al.*, 2019; Herr *et al.*, 2020) this is the first analysis of their abundance, prevalence, and detailed description of their morphology for the baleen whales feeding in the northwestern Iberia Peninsula coast. In this study, all epidermal skin marks and injuries were assessed and compared with previous studies published about skin anomalies (Bertulli *et al.*, 2012; Sanino *et al.*, 2014; Bertulli *et al.*, 2015; Barlow *et al.*, 2019; Herr *et al.* 2020; Toms *et al.*, 2020), epizoic infestations (Herr *et al.*, 2020; Flach *et al.*, 2021; Lehnert *et al.*, 2021), lesions and deformations (Hamilton & Marx, 2005; Bertulli *et al.*, 2015; Herr *et al.*, 2020), body condition (Wachtendonk *et al.*, 2022) and diseases (Van Bressem *et al.*, 2009), allowing us to create and develop the first skin mark catalogue for baleen whales present in the Northeastern Atlantic Ocean.

4.1.1. Cutaneous elevation

The results showed blisters, white marks, black marks as the most prevalent skin marks on baleen whales present in the North Atlantic. Prevalence has been proved to be a very useful tool to understand changes over time and infer population health (Hamilton & Marx, 2005). In our study blue whales showed a slightly higher prevalence of blisters (81%) comparing with fin (77.4%) and minke whales (72.7%). Depending on the size of the animal, the abundance of blisters can vary but can reach 100 blisters in one single section of the animal body. These results can be explained by a very famous threat to marine ecosystems, which is the high level of solar ultraviolet radiation (UV). Martinez-Levasseur *et al.*, (2013) study showed that continuous UV exposure induces mitochondrial DNA damage in the skin of cetacean species. When comparing individuals with contrasting skin colorations, as is the case of blue, fin and minke whales, darker skin colorations are known to have higher levels of melanin which are the responsible to confer protection against mtDNA UV-induced damage (Swalwell *et al.*, 2012). In this sense, the lighter pigmentation on blue whales can be the reason why they have higher prevalence and abundance of blisters comparing with fin and minke whales which are darker in coloration.

A special concerning case of blistering was observed in 1 blue whale individual (BM015) which was also suffering with skin loss (peeling) and found in poor body conditions (ribs visible). BM015 was detected with an agglomeration of elevations, greater than 20 cm, in the DX side of the peduncle (see Annex 23 A). By analyzing the shape-size of the agglomeration, the first impression was tumor presence. Geraci *et al.*, (1987) define tumors in cetaceans as neoplasms or abnormal growth of tissue in a part of the body, especially as a characteristic of cancer. A recent article revealed that whales and dolphins are less susceptible to cancer than humans and they had a high rate of gene mutation which leads to a high number of tumor suppressor genes (Tejada-Martinez et al., 2021). However, Newman & Smith (2006) defend that viral, fungal, or chemical clues can cause carcinogenesis in marine mammals. Potential causal agents of the disease are 2: *Alpha papilloma* virus (if coexistent with herpesvirus) and *Lacazia loboi* (Rehtanz *et al*., 2010; Ueda *et al*., 2013). Papillomaviruses or intranuclear virus-like particles are described as hyperplastic epithelial lesions revealed as prevalent wart forms (Van Bressem *et al*., 2008). This disease can be associated with immunologic dysfunctions (Bossart, 2007). In the other hand, Lobomycosis is a rare fungal disease affecting the skin and subcutaneous tissues, caused by *Lacazoi loboi*, sometimes referred to as zoonotic disease because is thought to affect only specific Delphinidae and humans (Reif *et al*., 2013). This last disease is described by literature as white-to-grayish nodules and verrucous plaques that can occur on dorsal and pectoral fins, head, peduncle, and tail (Migaki *et al*., 1971; Reif *et al*., 2006). Histologically, the lesions consist of multiple granulomas in the subepidermal tissue with inflammatory infiltrates (Reif *et al*., 2013). By visual comparisons, BM015 share similar type of elevations aggregated as Lobomycosis but the nodules still present a normal skin coloration. This is the first record of a strong alteration of the skin and subcutaneous tissues on baleen whales. It could be a tumor; however, it would be necessary to assess the evolution of the skin disease and/or biopsy information to conclude about the aetiology.

4.1.2. Patches

White and black marks form the category "patches" with the scratches. Despite sharing the same category, white and black marks don't share the same origin as scratch patches. Together, black and white marks affect all blue whale's individual sampled (100%), 84.7% of fin whales and 63.6% of minke whales sampled. Their abundance can reach more than 200 marks per body section as is the case of black marks in fin whales. Black marks considered in this study are a single or agglomeration of black small circular spots (sometimes punctiform but different than herpes-like) or big irregular shape pigmentation anomalies (see Annex 12). These types of marks were previously reported in minke whales (Bertulli *et al*., 2015), fin whales (Herr *et al*., 2020) and never in blue whales. The higher prevalence and abundance of black marks found on blue whales (95.2%) can be explained due to their lighter skin coloration that contrasts with the black marks and make them easier to spot comparing with other darker species (fin and minke whales). Sanino *et al*., (2014) observed similar black marks on the *specie Lagenorhynchus australis*, on the Chile's Aysén Region and defined the pigmentation anomaly as dark focal skin disease. Diseases are natural pathological processes that have a characteristic set of signs and symptoms that may affect the whole body or parts. In this case, black marks can be found in all body parts and their aetiology is unknown. So far is known that this disease doesn't affect the behavior of the individuals neither causes diving pattern alterations (Sanino *et al.*, 2014). Inside the black marks category is also important to highlight the presence of some black amorphous patches present in a small number of fin whales. This particular pattern of dark amorphous has similarities with blue whale coloration patterns which may be an indicator of hybridization between the two species (Bérubé & Aguilar, 1998). However, this study can't confirm that the individuals are hybrids only with visual data. Genetic tests are needed to prove this hypothesis.

Regarding white marks, small white dot lesions aggregated or singular in number were considered (different from bite marks in size), as well white pale patches of amorphous shape (different from hypopigmentation). White marks were more prevalent and abundant on fin whales with approximately 27 marks per individual that in extreme situations could reach more than 100 marks in a single body section. Herr *et al.*, (2022) found similar pale patches affecting approximately 21% of Antarctic fin whales sampled. When comparing with the whales sampled on the Northwestern Iberia Peninsula coast, a higher occurrence of white marks was verified affecting 57.1% of the blue whales sampled, 74.2% of the fin whales sampled and 63.6% of the minke whales sampled suggesting that maybe baleen whales in the North Atlantic are more exposed to probably viral infections (e.g., Poxvirus) or epibiotic barnacle attachment (e.g., *Xenobalanus globicipitis*). The loss rate of the white marks in general were reported to be zero on whales (Bertulli *et al.*, 2015) suggesting that they can accumulate over time making them good indicators to identify individuals. BP001 case is a very good example revealing a potential pathogenic disease (see Annex 11) very similar as "Cloudy White Spots" previously described by Toms *et al.*, (2020) on bottlenose dolphins in the Gulf of Mexico (North Atlantic). As this type of white marks had never been recorded in large baleen whales before, the potential causal agents are not discovered yet. However, BP001 is the only fin whale recorded with a fishing net entangled around her head. Several scratch patches along BP001 intermediate body were noticed with an agglomeration of "Cloudy White Spots" on the top. That may be a cause of infection originated by the fishing nets and possibly a case of severe "Rake Mark-Associated" (Toms *et al.*, 2020) described by the author as a possibly pathogenic-associated lesions that extends over fresh or healing rake marks including "Cloudy white Spots". Hart *et al.*, (2012) found for the same "Cloudy White Spots" as an association with herpes virus through a PCR test and curiously the cetaceans studied were from the North Atlantic as well. Even though all the possibility mentioned above, association with healing processes after trauma or even inflammation (Bossley & Woolfall, 2014) should not be discarded as a possible cause of white marks. Though, our discussion of the results are merely speculative, and genetic tests are needed to have a conclusion about the white marks aetiology.

4.1.3. Body and fin pigmentation

Despite the possible association of white marks with infections, pigmentation anomalies in cetaceans are not always necessarily pathogenic (Methion & Díaz López, 2019; Herr *et al*., 2022). Other types of white lesions in coloration were found and differentiated from white marks as is the case of hypo-pigmentation found in 5 fin whales feeding in the Northwestern Iberia Peninsula coast. Hypo-pigmentation have a prevalence of 4% and occurred with an average severity of li=11.64 mark per whale with the anomaly. The first record was BP007, previously reported by Methion & Díaz López (2019) as the first fin whale with atypical pigmentation pattern in the Atlantic Ocean. Hypo-pigmentation is the lack of pigment in a part or entire body either acquired (vitiligo) or congenital resulting from the inheritance of mutations in pigment related genes (albinism, leucism and piebaldism) (Methion & Díaz López, 2019). Methion & Díaz López (2019) strongly suggest that BP007 is a vitiligo case due to the position, irregular shape, and lack of inflammation of the white patches. The fact that vitiligo is acquired in early adulthood also supports this hypothesis. Furthermore, the other 3 individuals showed the same type of white-off lack of pigmentation located in the head and close the chevron but with a more rounded shape and sometimes single in quantity (different from white marks). Other supposition for these cases could be a possible burn-like injury as described by Bossley & Woolfall (2014) associated with wounds of unknown origin.

Another case of hypopigmentation registered during the study period was individual BP022 (see Annex 5). This individual revealed an excessively white coloration in the head comparing with the other fin whales. More than 50% of the top head, upper jaw and blowhole presented a lack of pigmentation (without shape or borders) becoming denser towards the right side of the head. The peduncle also showed a depigmented patch becoming denser towards the tail (right side) and no photographs of the tail were recorded. This fact is already incongruous to the usual well-defined borders presented by individuals with piebaldism and vitiligo (Herr *et al*., 2020; Methion & Díaz López, 2019). Despite the hypo-pigmentated coloration in the extremities of the body, several other marks were present along BP022 body, including multiple scrapes, 2 linear deep wounds (maybe caused by orcas), atypical lighter grey/whitish coloration of all body, and at least 2 ectoparasites attached in the chevron area. This atypical coloration could be due to the accumulation of scratches/lesions that removed the first layer of epidermis followed by intensive sunburns which may have contributed for the lack of pigmentation in these areas similar to those observed in Bossley & Woolfall (2008) study. However, that doesn't explain the lack of borders of the hypopigmented patches and the unusual lighter coloration of the full body, suggesting that this coloration is possibly not acquired but maybe genetic. In the literature, the most similar occurrence was reported by Toms *et al*., (2020) in bottlenose dolphins which was categorized as "Discolored Head/ Nuchal Patch". This category was described as hypopigmentation anomaly on melon and/or associated with post nuchal depression. However, the presence of other pigmentation anomalies associated with the "Discolored Head/ Nuchal Patch" in the same individual were not specified in the article and the potential causal agent was unknown.

In this study, no other hypo-pigmentation coloration was observed on blue or minke whales sampled during the present study. Hypo-pigmentation anomalies are very rare, worldwide 3 registers of blue whales with white anomalies were observed between the year 1997 and 2000 in the Northeastern Pacific Ocean (Fertl *et al.*, 2004). During the period 2008-2017, hypo-pigmentation was also observed at least in two different grey whale individuals in Laguna Ojo de Liebre (Northeastern Pacific Ocean), the more recent one was a calf with the body predominantly white and a series of black spots on both sides (Salinas-Zavala *et al*., 2017). The cases found on fin whales in the Northwestern Iberia Peninsula apparent to be distinct from the previous studies of hypo-pigmentation by the fact that fin whales presented the white coloration anomalies in minority comparing with the coverage of normal skin coloration or in the case of BP022, the huge patches of hypo-pigmentation didn't exhibited defined borders. For the present study there is no clear evidence that the hypopigmentation occurrences are congenital or acquired since these individuals were photographed in the adult state. Moreover, there is no other published evidence of white anomalies on fin whales in other oceans making the topic hard to evaluate and study without genetic data. The

North Hemisphere appear to be a special area of interest regarding further studies of atypical pigmentation.

4.1.4. Bite marks

Another important category marks differentiated from the "white marks" category were the bite marks. Bite marks when healed, they present grey/white coloration. Cookie-cutter bite or ectoparasite bite of unknown origin differ from white marks particularly because they are not aggregated in specific areas of the body or in pale patches. Sometimes cookie-cutter and unknown ectoparasite bite presented a slightly depression or elevation (possibly associated with inflammation). Usually, they are found more spread through all body and the margins can be oval /rounded and well-defined in shape (see Annex 14). Cookie-cutter were previously reported in migratory species as blue whales (Barlow *et al.*, 2019), fin hales (Herr *et al.*, 2022) and minke whales (Bertulli *et al.*, 2015) but also can be commonly found in other marine species as swordfish (Papastamatiou *et al*., 2010), large sharks (Hoyos-Padilla *et al*., 2013), pinnipeds (Gallo-Reynoso & Figuerona-Carranza, 1992) and Odontoceti (Heithaus, 2001; Van Utrecht, 1959).

Bite marks were affecting 61.9% of the blue whales sampled, 30.6% of the fin whales sampled and 50% of the minke whales sampled in the Northwestern Iberian Peninsula coast. Our results showed fewer blue whales affected by ectoparasite bites than the 96.6% registered for blue whales present by in New Zealand waters (Antarctic Ocean) (Barlow *et al*., 2019). These results can be explained by the fact that, when bite marks are completely healed, they form dents (Herr *et al.*, 2022) or slightly depressions in the skin (Barlow *et al*., 2019) which were considered in this study as wounds, due to the difficulty of identifying the real causal agent. Wounds were affecting 76.2% of blue whale sampled, 54,0% of fin whales sampled and 59.1% of the minke whales sampled. This procedure maybe had leaded to an underestimation of bite marks, however, also leaded to a more accurate and still realistic classification of the skin marks.

According to the literature, 2 out of the 4 phases described by Barlow *et al.*, (2019) were found in this study which represents the last two healed stages of cookie-cutter bites. Phase 1 are fresh wounds with subdermal pink tissue visible; In phase 2 the coloration is more yellowish/brownish. Neither phase 1 nor 2 were noticed in the baleen whale's species in this study. Assuming that blue and fin whales do vertical migration to tropical waters, place of residence of *Isistius brasiliensis* (Quoy & Gaimard, 1824)

(Compagno, 1984) and even when they return from high latitudes, they were never captured in photo with cookie-cutter bites in phase 1 or 2, this may indicate a faster healing process than the 38 months suggested by Barlow *et al.*, (2019).

Another interesting result was the cookie-cutter bites severity on minke whales showing 6.60 marks per individual when they are considered present in the Galician coast during all the seasons (unpublished data). Similar results were found in the transient blue and fin whales with respectively 5.51 and 6.92 marks per individual. That can be evidence of a possible migration of some minke whale individuals similar as described by Risch *et al.*, (2014) in the western North Atlantic. Apparently, some minke whale individuals migrate from the western North Atlantic to the Caribbean (tropical waters) during winter (Risch *et al.*, 2014) and the same could be happening for the minke whales present in the eastern North Atlantic coast. Another possible explanation for the high severity value can be due to the presence of another specie of cookie-cutter shark along the Iberia Peninsula (Zidowitz *et al.*, 2004), the *Isistius plutodus* (Garrick & Springer, 1964). The presence of this other species of cookie-cutter shark in the study area, reinforce the idea of faster healing process due to an expected higher probability of occurrence of phase 1 or 2 of cookie-cutter bites on baleen whales on the Northwestern Iberia Peninsula coast, which was not observed in our study. Even though cetaceans have been reported to possess great healing capacity (Bossley & Woolfall, 2014; Zasloff, 2011), is important to highlight the differences in the rate of granulation tissue formation and wound contraction between species (Su *et al.*, 2022). Even if they are closely related like dogs and cats, different species can develop slower or faster healing processes (Su *et al.*, 2022). These differences could be related to variability in cutaneous blood supply during the healing process (Bohling *et al.*, 2004) and should be taken in consideration for further studies on the subject.

Regarding severity of bite marks in general, the values found in the Northwestern Iberian Peninsula baleen coast were very similar for the three species (6.69 marks per blue whale, 6.03 marks per fin whale and 5.67 marks per minke whale). The complete loss rate of the marks is pointed to be low resulting in a very small probability of loss over time (Bertulli *et al.*, 2015). The category ectoparasite bite of unknown origin was created as part of the bite marks because of the uncertainty around the potential causal agent. However, at least two marine species are in the list of potential causal agents: the sea lamprey *Petromyzon marinus* (Linnaeus, 1758) and the remora *Remora australis* (Bennett, 1840).

4.1.5. Ectoparasite attached possible linked with bites

Ectoparasite bites of unknown origin appear to affect more blue whales (38.1%) than fin and minke whales (~14% both), however not significantly (G=14.41, df=14, P>0.005). The higher abundance of this marks found on blue whales (ai= 10.05 ± 3.84) can be explained by a possible high variety of ectoparasites attachment on their larger body size when comparing with fin and minke whales. Despite the variety of mark shapes found in this category, there is at least 2 types that resemble the shape of the mouth of 2 different species found attached in the present study. One of these cases is the specie *Petromyzon marinus,* pointed as the potential causal agent of perfect circular marks on several baleen whales' skin, sometimes showing a sliding pattern (e.g., Annex 15A). Commonly found on the Northeast Atlantic (Kottelat & Freyhof, 2007), the lampreys tend to attach to their hosts with an oral disc and rasp through tissue with a tongue-like piston in order to have access to blood, their primary food (Farmer, 1980; Hardisty & Potter, 1971). The lamprey's attachment and scars has been noticed in some baleen whales as for example, North Atlantic right whales, fin whales, Pacific humpback whales and minke whales (Pike, 1951; Nemoto, 1955; Nichols & Hamilton, 2004; Nichols & Tscherter, 2011). Apart of that, in the North Atlantic, the lampreys have been noticed more frequently specially in Icelandic waters attached to minke whales (Bertulli *et al*., 2012; Astthorsson & Palsson, 2006). Considering this information and the vertical migrations done by blue and fin whales, is expected that the species showed up signs of lamprey bites as they possibly use these areas during summer as feeding grounds (Sears & Perrin, 2018). Nichols & Tscherter (2011) identified minke whale individuals with lamprey attached and the scaring result after detaching. The photographs documented by the author allowed us to verify a strong similarity in shape with the marks documented in the present study. The possibility of being a *Petromyzon marinus* bite also can explain the sliding marks associated with the circular marks, which are assumed to result from lamprey skidding over the whale's skin to reattach at a more favorable location (Nichols & Tscherter, 2011). Additionally, some ectoparasites classified as "unknown" due to the medium quality of the pictures (see Annex 33), revealed an anguilliform body grading from gray bluish dorsally to silver white ventrally which match with previous description of newly transformed lampreys (Rochard & Ellie, 1994; Renaud, 2011). A reddish/pink coloration was also observed in the site of the attachment spreading along the ectoparasite body resembling blood and again reinforcing the hypothesis of *Petromyzon marinus* as the causal agent of some circular marks.

As mentioned before, photograph records are a useful tool to study skin marks however, most of the time the potential causal agent is unknown, the process of marking are rarely photographed. In this sense animal-borne video recordings can be useful to capture the origin of a mark. Animal-borne video records from blue whales (Flammang *et al*., 2020) help to demystify the parasitic fish attached to 2 fin whales sampled in the in the present study, and possibly related with the mysterious marks with raised borders (bigger than cokkie-cutter bites) on some baleen whales. The commonly called "whalesuckres" or remoras (family Echeneidae) are known to inhabit in all tropical waters including the Equatorial Atlantic Ocean (Wingert *et al*., 2021) which is the possible breeding ground of some blue and fin whales that pass through Iberian Peninsula waters. The specie *Remora australis* has been described as a symbiotic specie that tend to adhere exclusively to cetacean's skin using a modified dorsal fin (Flammang *et al*., 2020; Wingert *et al*., 2021). The reason behind the attachment selection on a host's body is unknown but, in this study, remoras were found in the SX side of the dorsal fin section and in the DX side of the intermediate body of fin whales (see Annex 32 A & B). Despite the idea of attachment, remoras tend to move freely along the whale body's using swimming and sliding behaviors (Flammang *et al*., 2020) suggesting that one single remora individual can cause multiple marks along the host body when coming to the surface. The relationship between whales and remoras are still poorly known, however, some studies support the theory that remoras feed on parasites on the host surface which increase the host health (Cressey & Lachner, 1970) and in return remoras take vantage of reduced locomotor costs and predation risk (Muir *et al*., 1967; Silva-Jr & Sazima, 2003). Despite the validation of remora attachment to fin whales in Galician waters, there is no bibliographic evidence that confirm the marks with raised borders were made by remoras.

4.1.6. Infection lesions

Another type of circular marks found in this study were dark fringe and white fringe (see Annex 28). In total 4 blue whales were affected by dark fringe and white fringe were absent. The fin whales were the most affected by dark fringe with 25 individuals affected and 2 individuals with white fringe. Minke whale were not affected by dark fringe, but one individual showed 1 white fringe. Dark fringe lesions were categorized as infection lesions, and Poxvirus has been suggested as the possible cause of lesions on bottlenose dolphins, sperm whales and killer whales (Herr *et al*., 2020; Toms *et al*., 2020). Hart *et al.*, (2012) tested dark fringe lesions using PCR tests and all samples were negative for poxvirus and herpesvirus. However, histological analysis provided evidence of a prior viral infection on the individuals sampled which indicated that the virus could manifest as dark fringe (Hart *et al*., 2012).

In the other hand white fringe has been noticed on bottlenose dolphins (Leone *et al*., 2019) and viral infections are also pointed as the main cause. Hart *et al.*, (2012) used a sample of white fringed lesions to do a phylogenetic analysis and the results indicated similarities with Delphinid Herpesvirus 3. So far, there is no other studies confirming the aetiology of white fringe lesions. Most of the literature indicate minor infections, calicivirus or ectoparasites perhaps worsened by pollution and/or by negative environmental conditions as the main causal agent of white fringe lesions (Hart *et al.*, 2012; Harzen & Brunnick, 1997, Mariani *et al.*, 2016). There are no scientific records of dark and white fringe occurring on baleen whales till now. One of the reasons can be due to the small size of the mark which makes them more difficult to spot or also can be related with the lack of research on the skin health of mysticeti.

Herpes-like lesions were the most abundant, severe, and small skin mark found on blue and fin whales. More frequently found in the dorsal fin and peduncle, no significative relationship between herpes-like lesions and body condition of the individuals were detected. This type of lesions appeared to be homogeneous between blue, fin and minke whales. Despite extensive literature review only two reports of the lesions were found in Mysticeti. Bertulli *et al*., (2012) and Bertulli *et al.*, (2015) found the same small black dot lesions on minke whales resembling those associated with herpesvirus capsids observed by transmission electron microscopy in skin samples of Peruvian dusky dolphins (*Lagenorhynchus obscurus*) (Van Bressem *et al.*, 1994). Another study focused on *Lagenorhynchus australis* skin marks, found size-wise similar skin lesions called "Dark Focal Skin Disease" (Sanino *et al.*, 2014). This disease appeared to not affect the individual's behavior neither their normal diving pattern (Sanino *et al*., 2014). This is the first-time herpes-like lesions were reported in blue and fin whales worldwide. More epidemiological, genetic, and clinical research are needed to understand the aetiology and pathogenesis of the lesions.

The other 2 important components of the category infections lesions are the tattoo-like lesions and wart-like lesions. Between the two types, the wart-like lesions were rarer. Wart-like lesions affected one fin whale (BP004) and 4 minke whales in total. No blue whales were affected by wart-like lesions. Wart-like hyperplasia were found for the first time by Bertulli *et al*., (2012) on minke whales demonstrating similarities with the warts documented in harbour porposises (*Phocoena phocoena*) in the North Atlantic (Van Bressem *et al*., 1999b). The North Atlantic Ocean is once more, a focus of attention regarding new discoveries of skin marks with unknown aetiology. However, changes in the water salinity and temperature can be pointed as a potential cause of wart-like lesions.

Considering the 3 Rias present in the study area, some features like thermal inversion from November to February, intense upwelling events from April to September, and input of warm water mass in October are known to happen (Alvarez *et al*., 2005). Slightly differences in temperature and salinity can be observed in the "mouth" of Arousa, where water is fresher in winter and upwelling events are less frequent in summer (Alvarez *et al*., 2005). In the Northwestern coast of Spain, minke whales are usually sighted in shallow waters (53 m of depth) close to the shore and close to the edge of the continental platform (250 m of depth) (Díaz López *et al*., 2021). This means that minke whales can be exposed to these differences of temperature and salinity which are known to be highly correlated with epidermal diseases on cetaceans (Wilson *et al*., 1999). Populations from areas of low water temperature and low salinity exhibit higher lesions prevalence and severity. The differences of temperature and salinity may impact the skin epidermis integrity or produce more general physiological stress, potentially making animals more vulnerable to natural infections (Wilson *et al*., 1999).

In this study, 47.6% blue whales, 9.7% fin whales and 9.5% of minke whales sampled were affected by tattoo-like lesions. Only exceeded by herpes, tattoo-like lesions were the second most severe mark type found in baleen whales. Although there are fewer studies for mysticetes, tattoo lesions have been described in fin whales (Baker, 1992), bowhead whale (Henk & Mullan, 1996), southern right whale (Reeb, 2001), north Atlantic right whale (Hamilton & Marx, 2005) and blue whales (Brownell *et al*., 2007). This type of infection lesions is commonly associated with poxvirus (Van Bressem & Van Waerebeek, 1996). Sanino *et al*., (2014) noticed tattoo skin disease, on *Lagenorhynchus australis,* very narrow and thin in the beginning, then with time, the marks significantly increased in width becoming lighter in the center with dark border. This discovery demand precaution when analyzing linear and rounded black marks and dark fringe as they can be involved as a healing step of tattoo skin disease. Some authors also suggest that tattoo skin disease may be related with degrading or stressful environment (Van Bressem *et al.*, 2003; Viddi *et al.*, 2005) leading to infection with secondary pathogens and water quality (Hamilton & Marx, 2005).

4.1.7. Injuries, fin outliner and linear marks

Injuries, fin outline and linear lesions were considered in this study separately as different categories but at the end they form the group of marks more probably related with human activity interactions or even linked to climate change. Our results showed that marks in the fin outline affected more the minke whales (40.9%), followed by fin whales (21.8%) and blue whales (9.5%), however not significant comparing minke and blue whales ($G=2.2314$, $df=3$, $P>0.005$). Some features found as notches trailing and leading edge on whales exhibit a diverse variety as described by Luksenburg (2014) such as non-linear severed amputation (see Annex 22A), opposing cuts (see Annex 6A), parallel cuts (see Annex 6B), cut-like indentation (see Annex 7A), or round cut (see Annex 7B and 7C). Between those categories, 3 are possibly related with anthropogenic causes and other 2 with natural causes. Non-linear severed amputations and rounded cuts, as observed in BP052, are probably a result of intra-specific interaction or possibly shark attacks (Van Waerebeek *et al.*, 2007; Kiszka *et al.*, 2008; Heithaus, 2001). Opposing cuts can be associated with fishing lines which could have been wrapped around the dorsal (Wells *et al.*, 2008; Read & Murray, 2000). Parallel cuts in the leading edge can be likely a result of a propeller interaction (Read & Murray, 2000) and cut-like indentation can be caused by either fishing gear (Read & Murray, 2000) or propeller (Wells *et al.*, 2008). The possibility of human activity impact on whale's fin outliner lesions are supported by the high fishing activity along Galician coast with a high potential for cetacean-fisheries interactions including bycatch that appears to be high during the night in water depths of 100-300 m (Goetz *et al.*, 2014). According to the results of the present study, the smallest specie that is seen year-round closer to the shore (see Figure 2.1) showed more evidence of notches in the dorsal fin than the other 2 relatively large migratory species (blue and fin whales). This can be explained by the fact that minke whales share the same area as fisheries during all the year increasing the probability of lesions occurrence when comparing with other migratory species that mostly come to feed in more deep waters. Another important aspect that can explain more fin outliner lesions on minke whales is their diet. North Atlantic minke whales exploit a variety of prey species such as krill, herring, and capelin in different areas according to availability (Skaug *et al*., 1997). Therefore, interaction with fisheries and competition with other species for the same preys can be a possible explanation for the fin outliner lesions.

Regarding linear marks of unknown origin our results pointed fin whales as the most affected (77.4%), followed by blue whales (66.7%) and minke whales (54.5%) with medium scrapes as the most abundant type of linear mark for all the species. These results were mostly associated to superficial abrasions rather than severe or deep scrapes found in all the body sections. The high prevalence of linear marks on baleen whales are not totally understood. Fin whales sampled in the Antarctic showed similar linear marks but no conclusions about the origin were made (Herr *et al*., 2022). Medium and fine scrapes were analyzed by Bertulli *et al*., (2015) on Icelandic minke whales and the prevalence of both scrapes were very low (5% and 17.7%, respectively) comparing with the 40.9% of medium scrapes and 22.7% of fine scrapes on minke whales found in the Northwestern coast of Spain. In general, linear lesions can be associated with the distribution of prey availability close to human impacted areas or the consequence of travelling in areas with high fishing effort occurring, as is the case of the North Atlantic Ocean (Kroodsma *et al*., 2018). Apart of that, other novel discoveries in cetaceans' behavior such as specie/individual "personality" (Díaz López, 2020) could lead some individuals to interact more with other cetaceans' species and/or fisheries, increasing the probability of contact and consequent lesions. Despite the speculations, the origin of this type of skin marks cannot be determined by visual assessment. Biopsy data or live records of the formation of linear marks are needed to conclude about the topic.

It is known that interactions between species can leave several distinct marks very recognizable. For example, evidence of tooth-rake marks on Odontoceti are very common mainly due to intraspecific interactions (Connor *et al*., 2005), however, when observed in baleen whales, intraspecific interactions are refuted due to their lack of teeth. In our results minke whales sampled on the Northwestern Iberia Peninsula coast appear to have absence of tooth-rake marks. Another population also inhabiting the Atlantic Ocean, the Icelandic minke whale, exhibited tooth-rakes on the flank and tail-stock (Bertulli *et al*., 2012). Killer whale's predation attempt was pointed as the possible origin (Bertulli *et al*., 2012). In the Antarctic Ocean, killer whales have been observed feeding on minke whales (*Balaenoptera bonaerensis*) (Pitman & Ensor, 2003; Pitman & Durban, 2011) which can indicate that possibly minke whales are more susceptible to killer whale predation depending on the permanence of predators in the area or can depend on the killer whale type of diet, prey preferences or reduced prey availability *in situ*.

Contrary to minke whales sampled in the study area, evidence of tooth-rake marks from killer whales' predation attempt were documented for the first time in blue and fin whales in the North Atlantic Ocean. In the present study, a total of 2 blue whale and 7 fin whales were observed with multiple healed parallel linear marks in the dorsal fin and intermediate body. Martinez-Levasseur *et al*., (2011) and Corsi *et al.*, (2021) also recorded the presence of killer whale tooth-rakes on blue whales in the North Pacific Ocean and Totterdell *et al.,* (2021) documented for the first time 3 events of killer whale killing and eating blue whales. By visual comparison between the photographic data published by Totterdell *et al*., (2021) and our photographs, the similarities between the pattern, shape, and length of the marks are high. This suggest that blue whales in the North Atlantic also have been approached or simply suffered a predation attempt by killer whales during their migratory routes. There is no scientific data reporting about the migration routes of the killer whales along the Iberia Peninsula coast however, blue, fin and killer whales appear to use the same area to travel. The killer whale population occurrence in the Mediterranean Sea is apparently related to the migration of their main prey, the bluefine tuna (*Thunnus thynnus*) (Esteban *et al*., 2016). The bluefine tuna migrates from the Mediterranean Sea to the North Sea (Cort & Nøttestad, 2007) and was often seen during the surveys of the present study. This reinforces the idea of possible killer whale predation attempt on baleen whales in the North Atlantic Ocean.

Apart of predations attempts of natural causes, other injuries marks were documented probably linked to anthropogenic causes. One blue whale (BM003) was found with not very deep propeller cuts along de dorsal spine, and other 2 blue whales, 3 fin whales and 5 minke whales showed back indentations. A recent study in the Northwest Atlantic revealed that back indentations on humpback, fin and blue whales can be strongly related with fishing net entanglement (Ramp *et al*., 2021). Ropes and nets can become attached to various body parts including mouth, around head plus pectoral fins (e.g., BP001), dorsal fin, peduncle and tail leading, normally, to nicks, holes, amputations, back indentation, scrapes, and scratches formations (Robbins & Mattila, 2004; Johnson *et al*., 2005). Usually, back indentation occurs along the peduncle and tail, and estimations showed that entanglement rates can be underestimated when using photo-ID images. This is explained by the fact that baleen whales, such as blue, fin and minke whales, rarely reveal the end of the peduncle or flukes as they dive. The entanglement rates found by Ramp *et al*., (2021) using photo-ID images was 6.5% for fin and 13.1% for blue whales, however when using drone images, the entanglement rates increased to 80% on fin whales and 60% on blue whales. Our results of back indentation revealed relatively low prevalence on the baleen whales sampled. Minke whales were the most affected (22.7%), followed by blue whales (14.3%) and fin whales (2.4%). This results, even though the possibility of underestimation, suggest that species with a more coastal distribution, such as minke whales, are apparently more affected due to the higher density of fishing effort along the coast (Pauly, 2009). This confirms the little evidence of injuries (anthropogenic impacts) present in baleen whales sampled on the Northwestern coast of Spain. The increase of fisheries activity and boat traffic in the ocean is clearly a concern for the health of baleen whales and is probably one of the main causes of few skin marks found in this study.

A new type of scars identified for the first time in whales, capture our attention by resembling artistic draws of non-parallel lines tattooed in the skin. Included in the category injuries, scars appear to be more prevalent in fin whales affecting 63.7% of the individuals sampled. These marks are thought to be associated with severe jellyfish stings. Thaikruea & Siriariyaporn (2016) documented several cases of high magnitude of severe box jellyfish in humans and the pattern of the skin marks resemble a lot the one's found on the baleen whales sampled. Supporting this hypothesis, is known that recent year's evidence indicates that Cnidaria (jellyfish) have increased in abundance throughout the world's oceans and blooms occur more frequently (Purcell *et al*., 2007). In the Northeast Atlantic, jellyfish have increased in frequency since 2002, mostly during winter (Licandro *et al.*, 2010). They are distributed widely from coastal to oceanic waters and their outbreak appear to be associated with warm winters and surface hydrography (Licandro *et al.*, 2010). Predictions of climate change suggest that the Northeast Atlantic will continue to warm (IPCC, 2007) contributing for the proliferation of species like *Pelagia noctiluca* and consequently, increase the probability of encounters (stings) between jellyfish aggregations and baleen whales. However, other studies suggest that jellyfish may also benefit from anthropogenic pressures on the marine environments (Richardson *et al*., 2009; Purcell, 2012) such as overfishing, habitat modification, aquaculture, and translocation (Brotz *et al*., 2012). Despite all the coincidences, our discussion of the results is merely speculative and the different type of skin reactions to stings in mammals, jellyfish abundance and distribution, global sea surface temperature, changes of salinity and pH in the ocean should be considered to make a conclusion about the topic.

4.1.8. Ectoparasites attached

When whales suffer injuries or severe cuts exposed in the epidermis, they are susceptible to other problems like parasitism (Lehnert *et al.*, 2021). One occurrence of possibly whale lice parasitism was recorded on BP126 (see Annex 29). The individual presented a linear deep wound going from half of the intermediate body down in the SX side till maybe the peduncle area. The origin of this wound is unknown but does not appear to be natural predation attempt due to the singularity of the sliding wound. In between the wound, two white "elevations" can be seen resembling the *Isocyamus* sp. reported by Lehnert *et al*., (2021) on harbour porpoises. This cyamids spend their entire life on their host, without swimming stages (Leger *et al.*, 2018) and they can be used as indicators for social structure and behavior of their hosts because they can reveal information about species interactions or migrations of their hosts (Balbuena & Raga, 1991; Balbuena & Raga, 1995; Fraija-Fernández *et al*., 2017). Off the Galician coast, some cetaceans were already recorded infected with *Isocyams deltobranchium* including True's beaked whale (*Mesoplodon mirus*), a common dolphin (*Delphinus delphins*) and a harbour porpoise stranded in Spain (Martinez *et al*., 2008). This fact supports a possible whale lice proliferation between BP126 and other cetaceans, maybe explained by the inter-specific social behavior exhibited by cetaceans in the Galician coast (Boxshall *et al*., 2005). During the surveys, baleen whales were several times sighted in aggregation with other species of Mysticeti and/or Odontoceti but this information was not relevant for the main purpose of this study.

Regarding all the ectoparasites found in the baleen whales' skin, some epizoa were recognized by their morphological characteristics, such as the commensal semi-stalked barnacle *Xenobalanus globicipitis*, the parasitic copepod *Pennella balaenopterae*, and the parasitic crustacean *Isocyamus* sp., previously referred as whale lice. The epibiotic barnacle *X. globicipitis* can be found in tropical and temperate waters in several free-living larval stages followed by a non-feeding, short-lived cyprid stage which is specialized to settle in a suitable substrate (Kautek et al., 2019; Siciliano *et al.*, 2020; Dreyer *et al*., 2020). The skin of cetaceans seems to be one of those suitable places for the settlement of the specie *X. globicipitis* that commonly attach to fins and flukes delighting of transportation and feeding by filtering the surrounding waters (Moreno-Colom *et al*., 2020). The *X. globicipitis* was found in the three species of whale's studied, showing a prevalence of 61.9% in blue whales, 30.6% on fin whales and 4.8% on minke whales. This discrepancies in the results can be explained by the host swimming speed and diving depth which can reduce the likelihood of cyprid settlement and adult survival (Kane *et al*., 2008). Blue whales are relatively slower swimmers than fin and minke whales (Bannister, 2009). The spatial distribution of the whale's species is also crucial for *X. globicipitis* attachment on the hosts. Only blue and fin whales are confirmed to travel to tropical waters, different from minke whales that are present in Galician waters during all the seasons. However, high water temperature and primary production might influence larval survival and increase their densities in the water (Dreyer *et al.*, 2020) suggesting that the settlement of *X. globicipitis* could also occur in temperate waters during whale coastal aggregations, when euphausiids feeding could be supported by a high coastal primary production in the Northwestern coast of Spain (Dreyer et al., 2020; Díaz López & Methion, 2019).

Another distinct case of ectoparasite attachment is *P. balaenopterae*. This ectoparasite need a suitable substrate to settle where can grow and reach 32 cm in length, and ovisacs can extend an additional 40 cm (Abaunza *et al*., 2001). Differing from *X. globicipitis*, *P. balaenopterae* can be found laterally spread along the host body with their heads embed into the host blubber causing local swelling of the skin or small white scars plus slightly depression on the skin when they detach (Gill *et al.*, 2000). This mesoparasitic copepod has a broad biogeographical distribution and are commonly seen on sei and minke whales (Dailey & Vogelbein, 1991; Olafsdóttir & Shinn, 2013; Uchida *et al*., 1998). In our study area, this ectoparasite was found in 1 blue whale, 4 fin whales and 2 minke whales sampled in the study area. Unlike other studies that relate the presence of ectoparasites to thin individuals (e.g., Flach *et al.*, 2021), this study proved that occurrence of ectoparasites was not significantly related with poor body conditions of the whales. However, long-term cumulative tendencies of ectoparasite infection can be associated with a challenged whale's immune system (Vecchione & Aznar, 2014) and emergence of ectoparasite infestation on hosts skin can be associated with immunosuppressive effects of viral infections such as morbillivirus outbreak, and unusual heavy loads of pollutants on the animal (Aznar *et al.*, 1994; Aznar *et al.*, 2005;

Flach *et al.*, 2021). Furthermore, more research on these relationships are needed to understand better ectoparasites attachments on baleen whales.

4.2. Skin mark variability between species and body condition

Skin marks and ectoparasites identified among the 3 whales species found in the Northwestern coast of Spain showed no significant incidence in a specific species or body condition. Individuals with poor body conditions were observed along the Galician coast representing 14.29% of the blue whales, 15.32% of the fin whales and 19.05% of the minke whales sampled representing a minority of concerning cases of unhealthy individuals probably linked with the sex of the whale and coincidences with oceanographic conditions (Wachtendonk *et al.*, 2022). However, is important to highlight that the high occurrence of skin marks in general and atypical changes of skin coloration on baleen whales may have impacts on social interactions between individuals and can be unfavorable for survival and mating selection of the species (Methion & Díaz López, 2019). Despite that, the body condition is known to have a direct influence on the survival and reproductive success of individuals as well (Gaillard *et al.*, 2000; Clutton-Brock & Sheldon, 2010). Therefore, the development of more studies in the future about the whales' body condition considering oceanographic conditions can provide important insights for ecophysiological studies and contribute as a proxy for fitness in a population (Christiansen *et al.*, 2020) helping to improve the knowledge about this species.

Chapter 5

5. Conclusion

Monitoring the health of wild animals such as free-ranging baleen whales is challenging, particularly when live-capture options and laboratory resources are not available or limited. In contrast, this study proved that is possible, through photo-ID, record the number of skin marks from a distance reducing the impact on these animals that are still recovering. In further studies would be interesting to maybe try another type of photographic methodology as adopted by Ramp *et al*., (2021) and Herr *et al*., (2022), to avoid the possible undercounting of skin marks and also to have a more detailed view of the full body of the animal (including tail).

In further studies we recommend developing more research regarding the ecology of the baleen whales present in Galician waters such as distribution along the coast and the effect of environmental drivers on their distribution. Moreover, it would be interesting to investigate the prevalence, abundance, and severity of blisters on, specially, blue whales because they are more vulnerable to UV radiation and differentiate them in different categories of skin coloration as is suggested by Gendron & De La Cruz (2012). As blue whales have a very diverse and unique skin pattern of coloration, making them lighter or more mottled, this probably suggest that between blue whale's individuals, lighter skin colorations are more vulnerable to blisters than mottled individuals. Secondly, another important aspect to study could be the distribution of blisters on the different body section and conclude which body section is more affected or subjected to UV radiation and relate that information with their diving behavior and the typical surface feed behavior that they frequently execute during summer in the Northwestern Iberia Peninsula.

In conclusion, baleen whales feeding in the Northwestern coast of Spain exhibited skin marks indicators of global problematics such as climate change (jellyfish scars) and anthropogenic impacts (some injuries, fin outliners and linear lesions). Skin marks were more abundant and prevalent on blue whales, however, more severe on fin whales. Minke whales appear to be the most vulnerable specie facing anthropogenic threats, reflected in the possible fishing gear lesions found as back indentation. Evidence of predation attempts by killer whales were recorded on blue and fin whales' skin sampled in Galicia waters. Herpes, black and white marks were the lesions that contributed the most for the variability of skin marks on whales, however this study conclude that none of those are particularly related to a specific specie or body condition.

Chapter 6

6. References

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Annex

Annex

Annex 1

Table- Categories and respective mark types, description, possible origin, colour, body location, estimate sizes and references to assess through photo-ID of mysticetes species.

Categories	Mark type	Description	Possible Origin	Colour	Body location	Estimate size	References
1) Fin outline	a) Notch Leading Edge	Semicircular, triangular, squared, identation in shape	Natural predation attempt or anthropogenic	Skin	Leading edge of the fin	Vary in size	Bertulli *et al.*, 2015
	b) Notch Trailing Edge	Semicircular, triangular, squared, identation in shape	Natural predation attempt or anthrophogenic	Skin	Trailing edge of the fin	Vary in size	Bertulli *et al.*, 2015
	c) Protruding piece	Piece of tissue protruding	Natural predation attempt or anthropogenic	Skin	Trailing edge of the fin	<1 cm	Bertulli *et al.*, 2015
	d) Hole	Circular and hollow punctures	Anthropogenic	-	Trailing and leading edge of the fin	Vary in size	Barlow *et al.*, 2019
2) Body and fin pigmentation	a) Mottling	Circular or small oval marks	Natural	Dark grey, black	Flank, peduncle	<5 cm wide	Bertulli *et al.*, 2015
	b) Speckling	Ovoid marks	Natural	Dark grey contrasting colour as on the skin	Behind eye, flank, peduncle	<1 cm	Bertulli *et al.*, 2015
	c) Hypo-pigmentation	Irregular hypo-pigmented patches	Natural	Off-white	All body parts	Vary in size	Bertulli *et al.*, 2015;Methion & Díaz López, 2019
	d) Peeling	Patches of skin loss	Natural	Skin	All body parts	Vary in size	Bertulli *et al.*, 2015
3) Patches	a) White marks	Irregular, circular or punctiform patches	Natural	White	All body parts	Punctiform to vary in size	Bertulli *et al.*, 2015
	b) Black marks	Irregular, circular or punctiform patches	Natural	Black	All body parts	Punctiform to vary in size	Bertulli *et al.*, 2015
	c) Scratch Patch	Aglometarion of multiple linear scratches fading and impossible to count. Can be caused by fishing nets	Anthropogenic	Light grey to White	All body parts	Vary in size	Auger-Méthé & Whitehead, 2007
4) Bite marks	a) Cookie-cutter bite	Oval shaped scars or crater-like wounds. Usually *Isistus sp.* bite	Natural	Grey, light grey.	Flank, peduncle, back	4-5 cm wide	Bertulli *et al.*, 2015; Barlow *et al.*, 2019
	b) Ectoparasite bite	Circular scars with texture and raised borders. Unknown specie bite	Natural	Grey, with/without dark outline.	All body parts	Vary in size	Bertulli *et al.*, 2015
5) Linear marks	a) Skidding	Parallel, sinuous or linear sliding marks	Natural parasitism or anthropogenic	Light grey	Flank, peduncle, dorsum, back.	>1 m long	Bertulli *et al.*, 2015
	b) Fine scrape	1 or 2 parallel linear marks	Natural predation attempt or anthropogenic	Off-white	All body parts	<1 m long and <1 cm of thickness	Bertulli *et al.*, 2015
	c) Medium scrape	1 or 2 parallel linear marks	Natural predation attempt or anthropogenic	Off-White	All body parts	<1 m long and >1 cm of thickness	Bertulli *et al.*, 2015
	d) Tooth-rake	Multiple parallel linear marks made by interspecific interaction	Natural predation attempt	Light or dark grey	All body parts	<1 cm thickness	Bertulli *et al.*, 2015
6) Injuries	a) Wounds	Unknown origin. Can present a slightly depression on the skin, circular or linear	Natural predation attempt or anthropogenic	White, grey, pink or red	Dorsum, flank, peduncle	Vary in size	Bertulli *et al.*, 2015
	b) Scars	Unknown origin. Non-paralell and sinuous linear marks. Can be jellyfish scars	Natural or anthropogenic	Dark grey, grey to skin colour	All body parts	Vary in size and thickness	-
	c) Back indentation	Deep or semicircular indentation in the skin and propeller cuts	Natural predation attempt or anthropogenic	-	Dorsal ridge and peduncle	<2 cm thickness	Bertulli *et al.*, 2015
	d) Amputation	Significant losses of tissue/ mutilation	Natural predation attempt or anthropogenic	-	Fin, snout	Vary in size	Bertulli *et al.*, 2015
	e) Deformation	Change of normal shape and form of body tissues	Natural or anthropogenic	-	Fin	Vary in size	Bertulli *et al.*, 2015

Categories	Mark type	Description	Possible Origin	Colour	Body location	Estimate size	References
7) Cutaneous elevation	a) Blisters	Air or fluid-filled elevation of the epidermis, usually round in shape. Singular, disperse or aggregated.	Natural	Whitish to dark grey	All body parts (except for fin and ventrum)	Vary in size	Bertulli *et al.*, 2015; Barlow *et al.*, 2019
8) Infections lesions	a) Tattoo-like	Irregular hyperpigmented marks with a dark outline, evoking a stippled pattern	Natural	Dark grey, grey	Dorsum, flank	Vary in size	Bertulli *et al.*, 2015
	b) Herpes-like	Small black dot lesions	Natural	Black	Flank	Punctiform	Bertulli *et al.*, 2015
	c) Wart-like	Hyperplastic lesions	Natural	Light grey	All body parts (except fin)	<7 cm wide	Bertulli *et al.*, 2015
	d) Starburst	Mark with a clear central origin and tendrils that extend outward, away from the origin	Natural	White or light coloured	All body parts (except for fin and ventrum)	<5 cm wide	Barlow *et al.*, 2019
	e) Dark fringe	Circular, pale areas surrounded by a dark halo	Natural	Darker than the colour of the skin	All body parts	<5 cm	Toms *et al.*, 2020
	d) White fringe	Hypopigmented halos surrounding small circles of normal coloured or black skin	Natural	Lighter than the colour of the skin	All body	<5 cm	Toms *et al.*, 2020
9) Ectoparasites attatched	a) Diatoms	Irregular, circular or jagged patches	Natural attachment	Orange, green, brown	All body parts	Patches vary in size	Toms *et al.*, 2020
	b) Whale lice	Small cyamids, dorso-ventrally compressed body with 5 pairs of legs ending in claw-like appendices and spines . Usually *Isocyamus* sp.	Natural attachment	White organisms	Wounds, fissures, crevices and around sessile barnacles	Mean size =7 mm	Rowntree, 1996
	c) *Pennella balaenopterae*	Mesoparasite copepods	Natural attachment	Dark, brown or redish	All body parts (except tail, flippers and dorsal fin)	8-32 cm	Vecchione & Aznar, 2014
	d) *Xenobalanus globicipitis*	Sessile barnacles	Natural attachment	Dark	Dorsal fin and flukes	≤5 cm	Flach *et al.*, 2021
	e) Fish	Different parasitic fish species that attatch on the skin	Natural attachment	Various	All body parts	Vary in size	Bertulli *et al.*, 2015
	f) Unknown	Ectoparasites not identifiable	Natural attachment	Various	All body parts	Vary in size	-
10) Miscellaneous	a) Miscellaneous	All other marks of unkown etiology	Natural or anthropogenic	Various	All body parts	Vary in size	Bertulli *et al.*, 2015

Annex 2

Table- Mark prevalence and abundance: (A) blue whales; (B) fin whales and (C) minke whales. For each mark type were calculated: (1) the total number of each mark ni during the study period: i is the type of mark; (2) mark prevalence pi: frequency of individuals with the i mark; (3) the mark severity li: mean number of marks of i type only on individual with i occurrences; (4) relative portion ri of each mark type to the total amount of marks R; (5) mark abundance ai: mean number of the i mark per individual. (6) Maximum and minimum number of i marks ni in a singular individual. Standard deviations are in parentheses.

(A) Mark type (Q > 2)	ni	pi	li	ri	ai	Ai range

Notch Leading Edge	0	-	-	-	-	-	-	-
Notch Trailing Edge	5	0,095	2,500	(1,50)	0,001	0,238	(0,19)	0-4
Protruding Piece	0	-	-	-	-	-	-	-
Hole	0	-	-	-	-	-	-	-
Total Fin Outliners	**5**	**0,095**	**2,500**	**(0,10)**	**0,001**	**0,238**	**(0,31)**	**0-4**
Hypo-pigmentation	0	-	-	-	-	-	-	-
Total Body and Fin Pigmentation	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
White Marks	237	0,571	7,406	(7,44)	0,038	11,286	(2,64)	0-36
Black Marks	1253	0,952	9,866	(16,99)	0,198	59,667	(9,68)	0-116
Total Patches	**1490**	**1,000**	**9,371**	**(7,17)**	**0,236**	**70,952**	**(15,58)**	**0-116**
Cookie-cutter bite	204	0,429	5,514	(5,07)	0,032	9,714	(2,22)	0-24
Ectoparasite Bite	211	0,381	8,440	(14,80)	0,033	10,048	(3,84)	0-74
Total Bite Marks	**415**	**0,619**	**6,694**	**(3,13)**	**0,066**	**19,762**	**(10,28)**	**0-74**
Skidding	13	0,286	1,625	(1,11)	0,002	0,619	(0,26)	0-4
Fine Scrape	41	0,286	2,412	(3,13)	0,006	1,952	(0,74)	0-14
Medium Scrape	110	0,667	2,391	(1,80)	0,017	5,238	(0,89)	0-10
Tooth-rake	66	0,095	11,000	(2,58)	0,010	3,143	(1,25)	0-15
Total Linear Marks	**230**	**0,667**	**2,987**	**(0,86)**	**0,036**	**10,952**	**(3,20)**	**0-15**
Wounds	929	0,762	7,432	(9,94)	0,147	44,238	(5,95)	0-56
Scars	8	0,048	8,000	0,00	0,001	0,381	(0,36)	0-8
Back identation	14	0,143	1,273	(0,45)	0,002	0,667	(0,20)	0-2
Amputation	1	0,048	1,000	0,00	<0,001	0,048	(0,05)	0-1
Deformation	0	-	-	-	-	-	-	-
Total Injuries	**964**	**0,762**	**6,986**	**(2,81)**	**0,153**	**45,905**	**(9,62)**	**0-56**
Blisters	**1306**	**0,810**	**9,262**	**(14,85)**	**0,207**	**62,190**	**(8,91)**	**0-98**
Tattoo-like	89	0,476	4,450	(4,57)	0,014	4,238	(1,28)	0-20
Herpes-like	1340	0,286	95,714	(88,61)	0,212	63,810	(22,01)	0-300
Wart-like	0	-	-	-	-	-	-	-
Dark Fringe	80	0,190	6,667	(5,78)	0,013	3,810	(1,38)	0-21
White Fringe	0	-	-	-	-	-	-	-
Total Infections lesions	**1509**	**0,571**	**47,156**	**(9,94)**	**0,239**	**71,857**	**(64,34)**	**0-300**
Whale Lice	0	-	-	-	-	-	-	-
Pennella balaenopterae	3	0,048	3,000	0,00	<0,001	0,143	(0,14)	0-3
Xenobalanus globicipitis	397	0,619	4,179	(3,89)	0,063	18,905	(8,04)	0-19
Fish	0	-	-	-	-	-	-	-
Unknown	1	0,048	1,000	0,00	<0,001	0,048	(0,05)	0-1
Total Ectoparasites attatched	**401**	**0,762**	**4,134**	**(3,72)**	**0,063**	**19,095**	**(3,86)**	**0-19**
Miscellaneous	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total marks	6320	1,000	13,058	(20,85)	1,000	300,952	(5,13)	0-300

ni pi li ri ai Ai range

(B) Mark type (Q > 2)	**ni**	**pi**	**li**		**ri**	**ai**		**Ai range**
Notch Leading Edge	5	0,040	1,000	(0,00)	<0,001	0,040	(0,10)	0-1
Notch Trailing Edge	52	0,202	1,576	(1,05)	0,003	0,419	(0,43)	0-4
Protruding Piece	2	0,016	1,000	(0,00)	<0,001	0,016	(0,06)	0-1
Hole	1	0,008	1,000	(0,00)	<0,001	0,008	(0,04)	0-1
Total Fin Outliners	**60**	**0,218**	**1,463**	**(0,97)**	**0,003**	**0,484**	**(0,23)**	**0-4**
Hypo-pigmentation	163	0,040	11,643	(11,62)	0,009	1,315	(2,30)	0-44
Total Body and Fin Pigmentation	**163**	**0,040**	**11,643**	**(11,62)**	**0,009**	**1,315**	**(2,31)**	**0-44**
White Marks	3321	0,742	13,500	(22,14)	0,186	26,782	(14,33)	0-156
Black Marks	1548	0,460	13,119	(30,96)	0,087	12,484	(13,47)	0-263
Total Patches	**4869**	**0,847**	**13,376**	**(25,45)**	**0,273**	**39,266**	**(14,00)**	**0-263**
Cookie-cutter bite	422	0,202	6,918	(9,90)	0,024	3,403	(3,50)	0-64
Ectoparasite Bite	54	0,137	3,000	(3,31)	0,003	0,435	(0,71)	0-14
Total Bite Marks	**476**	**0,306**	**6,025**	**(9,00)**	**0,027**	**3,839**	**(2,55)**	**0-64**
Skidding	231	0,403	2,511	(2,33)	0,013	1,863	(1,19)	0-12
Fine Scrape	78	0,266	1,773	(1,31)	0,004	0,629	(0,54)	0-7
Medium Scrape	276	0,532	2,208	(1,87)	0,015	2,226	(1,16)	0-14
Tooth-rake	62	0,056	5,636	(2,67)	0,003	0,500	(0,77)	0-10
Total Linear Marks	**647**	**0,774**	**2,379**	**(2,13)**	**0,036**	**5,218**	**(0,97)**	**0-14**
Wounds	768	0,540	5,774	(8,15)	0,043	6,194	(4,22)	0-57
Scars	1356	0,637	7,175	(9,32)	0,076	10,935	(5,76)	0-103
Back identation	3	0,024	1,000	(0,00)	<0,001	0,024	(0,07)	0-1
Amputation	19	0,105	1,056	(0,23)	0,001	0,153	(0,18)	0-2
Deformation	5	0,032	1,250	(0,43)	<0,001	0,040	(0,10)	0-2
Total Injuries	**2151**	**0,798**	**6,199**	**(8,68)**	**0,121**	**17,347**	**(3,32)**	**0-103**
Blisters	**1828**	**0,742**	**8,705**	**(10,62)**	**0,103**	**14,742**	**(7,41)**	**0-68**
Tattoo-like	475	0,097	19,000	(19,76)	0,027	3,831	(5,11)	0-82
Herpes-like	6293	0,202	112,375	(90,75)	0,353	50,750	(39,59)	0-400
Wart-like	2	0,008	1,000	(0,00)	0,000	0,016	(0,05)	0-1
Dark Fringe	108	0,202	3,000	(3,52)	0,006	0,871	(1,03)	0-19
White Fringe	26	0,016	8,667	(4,11)	0,001	0,210	(0,62)	0-14
Total Infections lesions	**6904**	**0,669**	**56,590**	**(80,85)**	**0,387**	**55,677**	**(18,25)**	**0-400**
Whale Lice	2	0,008	2,000	(0,00)	<0,001	0,016	(0,08)	0-2
Pennella balaenopterae	22	0,032	2,000	(3,46)	0,001	0,177	(0,63)	0-14
Xenobalanus globicipitis	597	0,306	9,476	(11,86)	0,033	4,815	(7,12)	0-64
Fish	2	0,016	1,000	(0,00)	<0,001	0,016	(0,05)	0-1
Unknown	27	0,056	1,688	(1,40)	0,002	0,218	(0,33)	0-6
Total Ectoparasites attatched	**650**	**0,347**	**6,989**	**(10,27)**	**0,036**	**5,242**	**(3,27)**	**0-64**
Miscellaneous	**86**	**0,032**	**21,500**	**(33,94)**	**0,005**	**0,694**	**(2,75)**	**0-73**
Total marks	17799	1,000	25,104	(30,04)	1,000	143,540	(8,72)	0-400

(C) Mark type (Q > 2)								
Notch Leading Edge	2	0,045	2,000	(0,00)	0,002	0,091	(0,19)	0-2
Notch Trailing Edge	14	0,364	1,400	(0,49)	0,013	0,636	(0,45)	0-2
Protruding Piece	1	0,045	1,000	(0,00)	0,001	0,045	(0,96)	0-1
Hole	0	-	-	-	-	-	-	-
Total Fin Outliners	**17**	**0,409**	**1,417**	**(0,50)**	**0,016**	**0,773**	**(0,26)**	**0-2**
Hypo-pigmentation	0	-	-	-	-	-	-	-
Total Body and Fin Pigmentation	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
White Marks	119	0,636	4,103	(4,26)	0,114	5,409	(2,79)	0-19
Black Marks	39	0,318	2,600	(2,19)	0,037	1,773	(1,21)	0-7
Total Patches	**158**	**0,636**	**3,591**	**(3,76)**	**0,152**	**7,182**	**(2,20)**	**0-19**
Cookie-cutter bite	99	0,455	6,600	(7,46)	0,095	4,500	(3,57)	0-26
Ectoparasite Bite	3	0,136	1,000	(0,00)	0,003	0,136	(0,17)	0-1
Total Bite Marks	**102**	**0,500**	**5,667**	**(7,12)**	**0,098**	**4,636**	**(2,58)**	**0-26**
Skidding	3	0,136	1,000	(0,00)	0,003	0,136	(0,19)	0-1
Fine Scrape	15	0,227	2,500	(2,50)	0,014	0,682	(0,82)	0-8
Medium Scrape	19	0,409	1,462	(0,75)	0,018	0,864	(0,55)	0-3
Tooth-rake	0	-	-	-	-	-	-	-
Total Linear Marks	**37**	**0,545**	**1,682**	**(1,52)**	**0,035**	**1,682**	**(0,51)**	**0-8**
Wounds	102	0,591	3,923	(4,98)	0,098	4,636	(2,88)	0-23
Scars	3	0,045	1,500	(0,50)	0,003	0,136	(0,21)	0-2
Back identation	11	0,227	1,375	(0,48)	0,011	0,500	(0,38)	0-2
Amputation	2	0,091	1,000	(0,00)	0,002	0,091	(0,16)	0-1
Deformation	12	0,227	1,500	(1,00)	0,012	0,545	(0,55)	0-4
Total Injuries	**130**	**0,682**	**2,826**	**(3,97)**	**0,125**	**5,909**	**(1,41)**	**0-23**
Blisters	**375**	**0,727**	**9,375**	**(16,02)**	**0,359**	**17,045**	**(10,40)**	**0-75**
Tattoo-like	31	0,091	6,200	(5,27)	0,030	1,409	(1,74)	0-16
Herpes-like	143	0,045	71,500	(8,50)	0,137	6,500	(9,74)	0-80
Wart-like	21	0,182	3,500	(2,75)	0,021	1,000	(1,49)	0-9
Dark Fringe	0	-	-	-	-	-	-	-
White Fringe	1	0,045	1,000	(0,00)	0,001	0,045	(0,10)	0-1
Total Infections lesions	**196**	**0,318**	**16,333**	**(24,02)**	**0,188**	**8,909**	**(4,49)**	**0-80**
Whale Lice	0	-	-	-	-	-	-	-
Pennella balaenopterae	4	0,091	2,000	(1,00)	0,004	0,182	(0,32)	0-3
Xenobalanus globicipitis	1	0,045	1,000	(0,00)	0,001	0,045	(0,10)	0-1
Fish	0	-	-	-	-	-	-	-
Unknown	7	0,091	2,333	(1,89)	0,007	0,318	(0,50)	0-5
Total Ectoparasites attatched	**12**	**0,227**	**2,000**	**(1,53)**	**0,012**	**0,545**	**(0,27)**	**0-5**
Miscellaneous	**15**	**0,136**	**3,000**	**(4,00)**	**0,014**	**0,682**	**(1,07)**	**0-11**
Total marks	1042	1,000	9,738	(10,66)	1,000	47,905	(2,96)	0-80

Annex 3

Table- The most prevalent (pi) and abundant (ai) types of skin marks per specie (BM, BP, BA).

pi	B	BM	WM	W	S	H	Cb
BM	0,810	0,952	0,571	0,762	0,048	0,286	0,429
BP	0,742	0,460	0,742	0,540	0,637	0,202	0,202
BA	0,727	0,318	0,636	0,591	0,045	0,045	0,455

ai	B	BM	WM	W	S	H	Cb
BM	62,190 ± 8,91	59,667 ± 9,68	11,286 ± 2,64	44,238 ± 5,95	0,381 ± 0,36	63,810 ± 22,01	9,714 ± 2,22
BP	14,742 ± 7,39	12,484 ± 13,47	26,782 ± 14,33	6,194 ± 4,22	10,935 ± 5,76	50,750 ± 39,59	3,403 ± 3,50
BA	17,045 ± 10,40	1,773 ± 1,21	5,409 ± 2,79	4,636 ± 2,88	0,136 ± 2,21	6,500 ± 9,74	4,500 ± 3,57

Annex 4

Table- Percentage of each specie (BM, BP, BA) with poor and good body condition.

Body Condition	BM (%)	BP (%)	BA (%)
Poor	14,29	15,32	18,18
Good	85,71	84,68	81,82

Annex 5

Figure- Sighting of BP022 (21/08/2018) showing clear evidence of hypopigmentation (different from vitiligo found in BP007) in the head and terminal peduncle. Unknown aetiology.

Annex 6

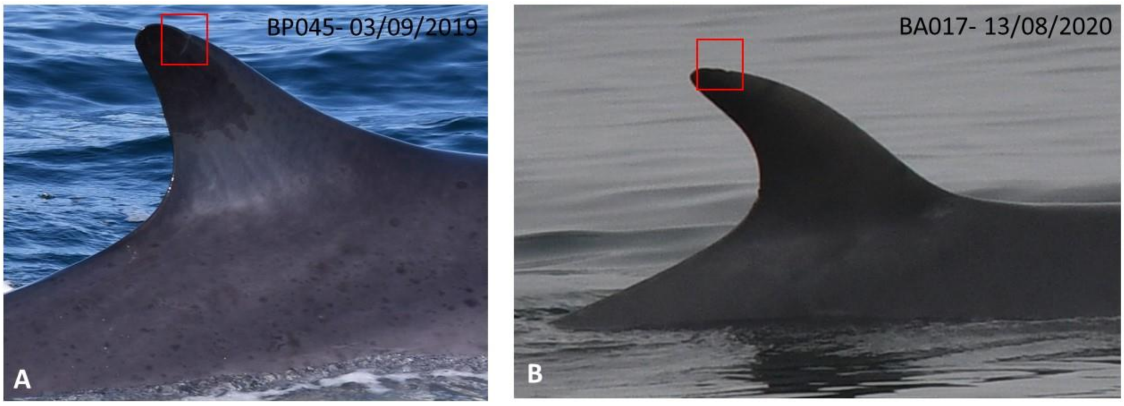

Figure- (A) Fin whale with notch leading edge and (B) minke whale with 2 notches leading edge probably caused by interaction with human activity.

Annex 7

Figure- (A) Minke whale with small notch trailing edge, (B) blue whale probably hybrid with huge notch trailing edge and (C) fin whale with big notch trailing edge probably caused by interaction with human activity.

Annex 8

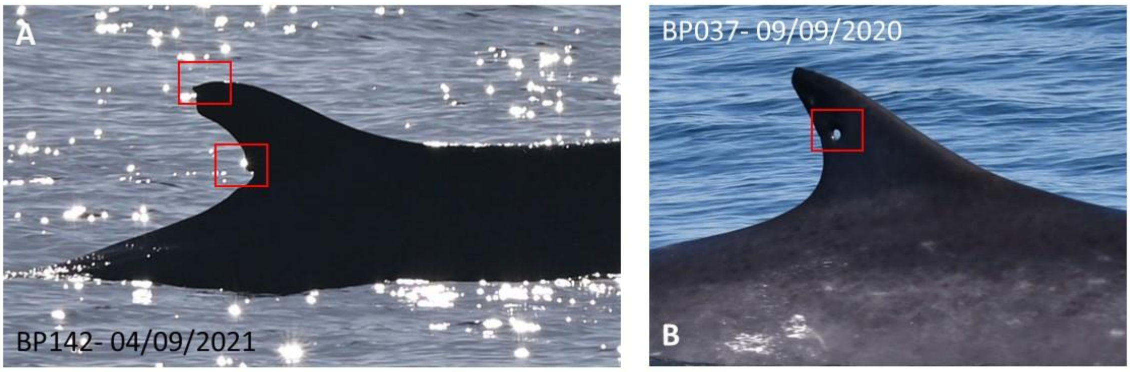

Figure- (A) Fin whale with 2 protruding piece and (B) fin whale with a hole probably caused by interactions with human activity.

Annex 9

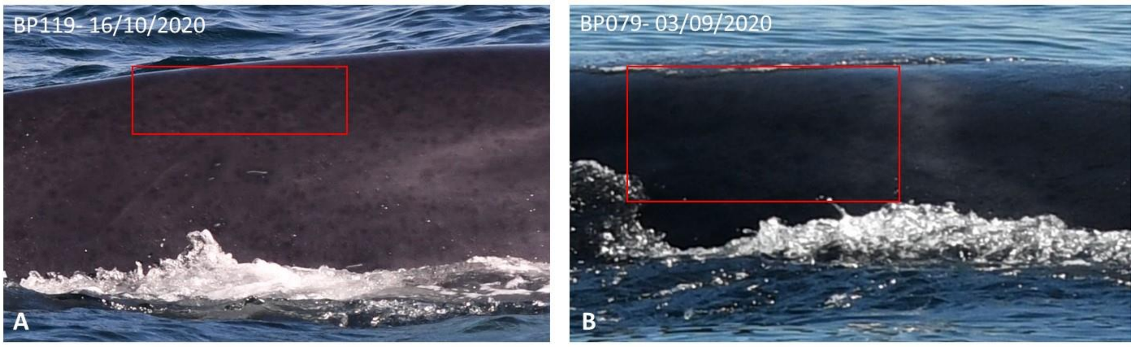

Figure- (A) Fin whale very mottled and (B) fin whale a little mottled (natural cause).

Annex 10

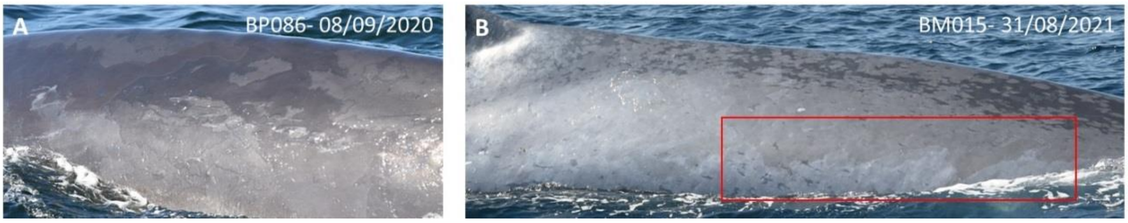

Figure- (A) Fin whale showing evidence of peeling and (B) blue whale found with a possible tumor in the peduncle is also experiencing skin loss.

Annex 11

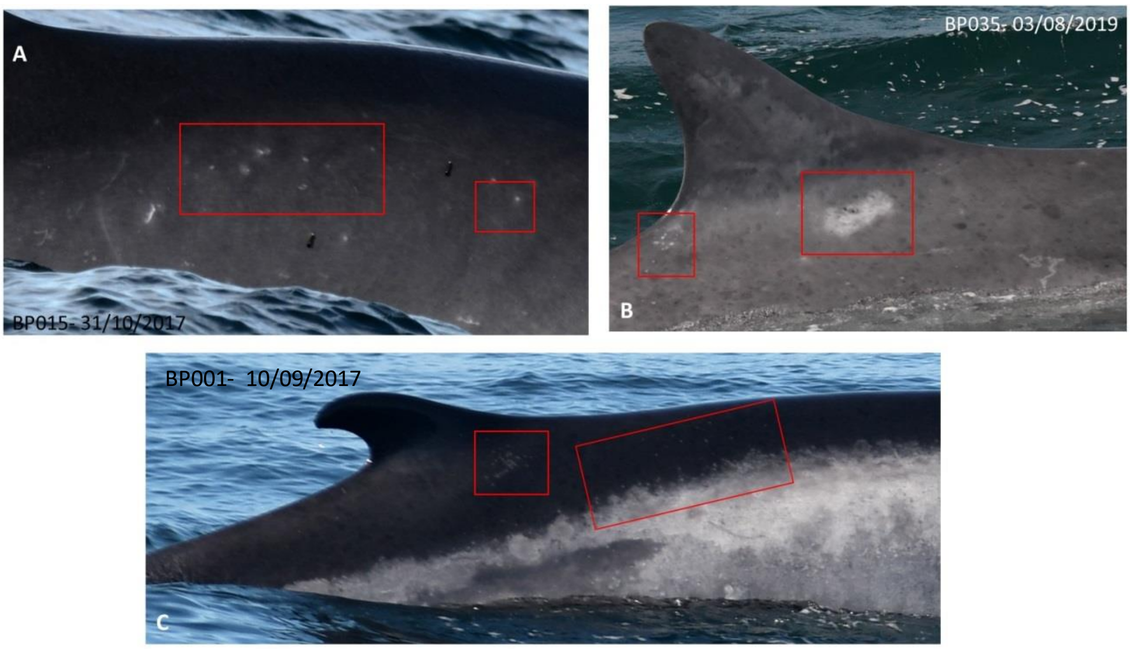

Figure- White marks: (A) Fin whale with white marks possibly caused by *Xenobalanus globicipitis*, (B) fin whale showing white amorphous and white spots, (C) BP001 was found entangled in a fishing net and the scars along the body showed signs of infection ("Cloudy White Spots") possibly caused by herpesvirus.

Annex 12

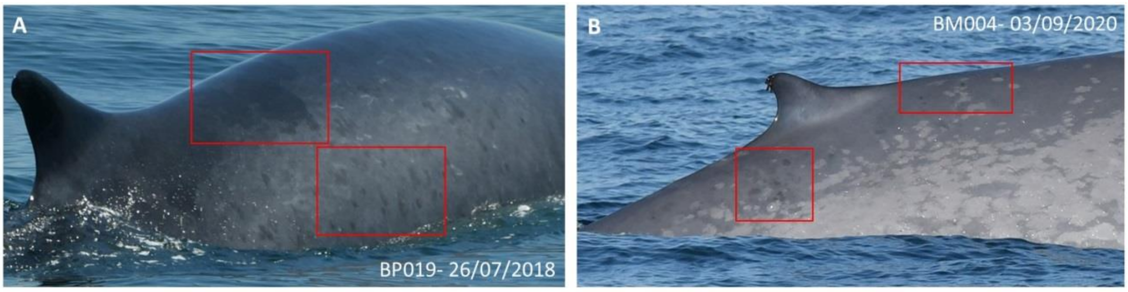

Figure- Black marks: (A) fin whale showing black amorphous marks and black spots, (B) blue whale with black spots. Unknown aetiology.

Annex 13

Figure- (A) Fin whale found with a fishing net attached to her head, evidence of fishing net lesions infected and fishing net scratch patch.

Annex 14

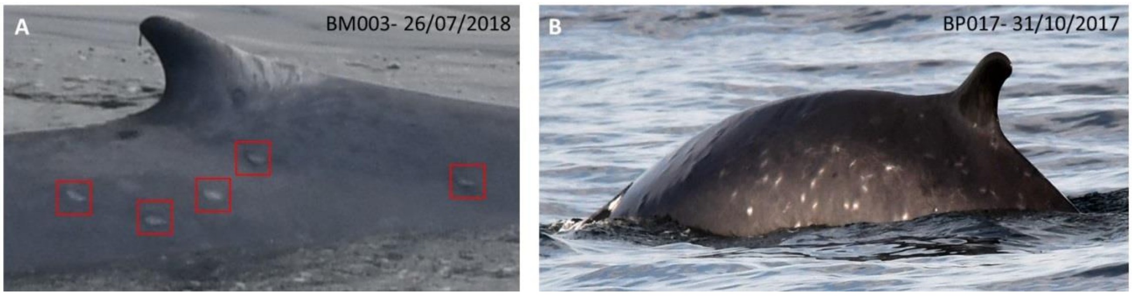

Figure- (A) Blue whale with a few cookie-cutter bites and (B) fin whale full of cookie-cutter bites.

Annex 15

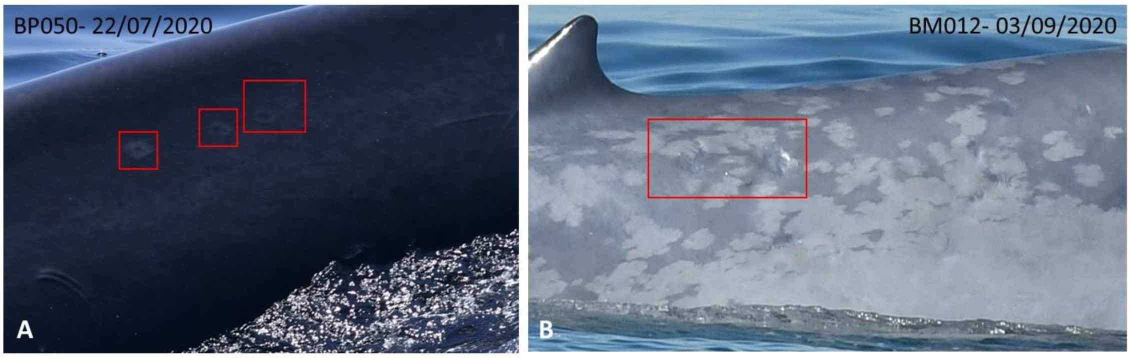

Figure- Ectoparasite bites no confirmed origin: (A) fin whale possibly with several sliding lamprey bites and (B) blue whale possibly with remora bites.

Annex 16

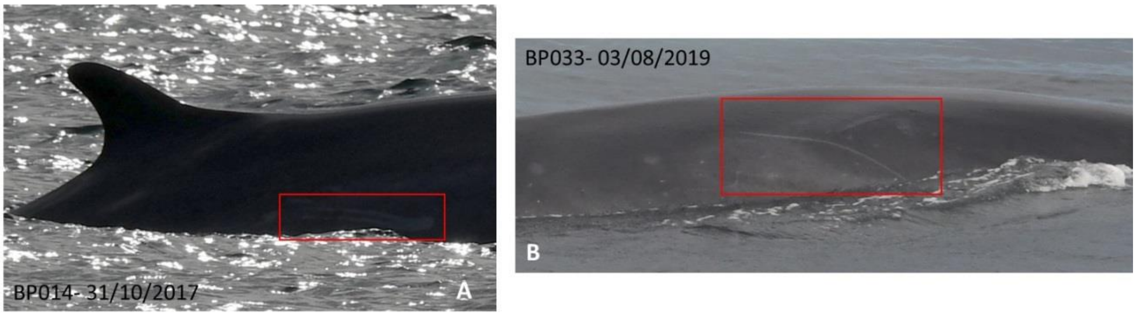

Figure- (A) Fin whale with skidding possibly originated by an ectoparasite and (B) fin whale with skidding of unknown origin.

Annex 17

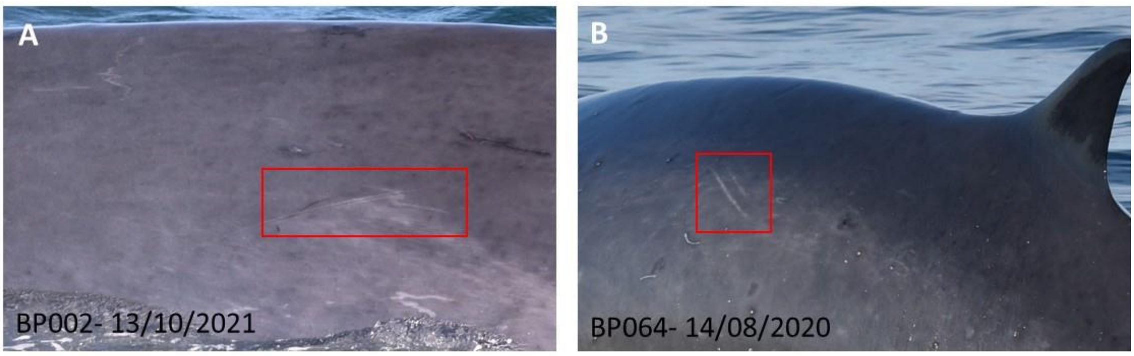

Figure- Scrapes: (A) Fin whale with two fine scrapes and (B) fin whale with 2 medium scrapes of unknown origin.

Annex 18

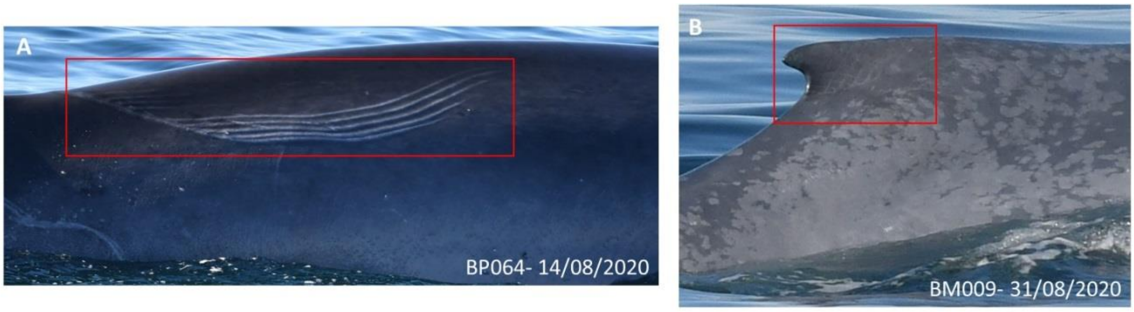

Figure- Killer whale tooth-rakes: (A) fin whale with tooth-rake marks in the intermediate body and (B) fin whale with tooth-rakes marks in the dorsal fin.

Annex 19

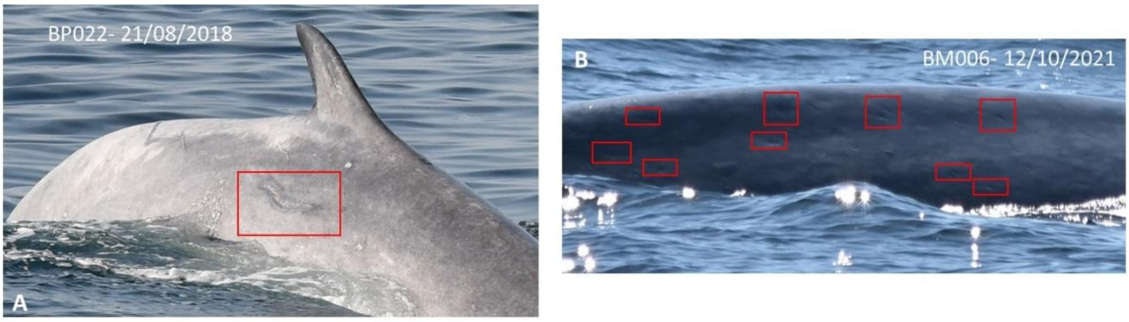

Figure- (A) fin whale with deep wound not healed, possibly linked to predation attempt, and (b) blue whale with several skin depressions possibly associated with cookie-cutter or ectoparasites bites already healed.

Annex 20

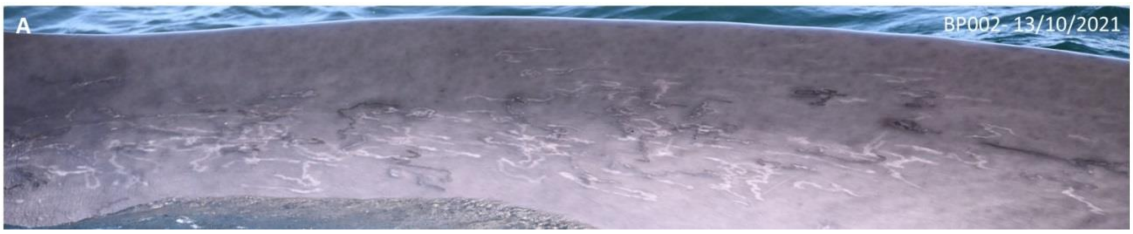

Figure- (A) fin whale showing scars probably originated by jellyfish stings. Previously undocumented marking.

Annex 21

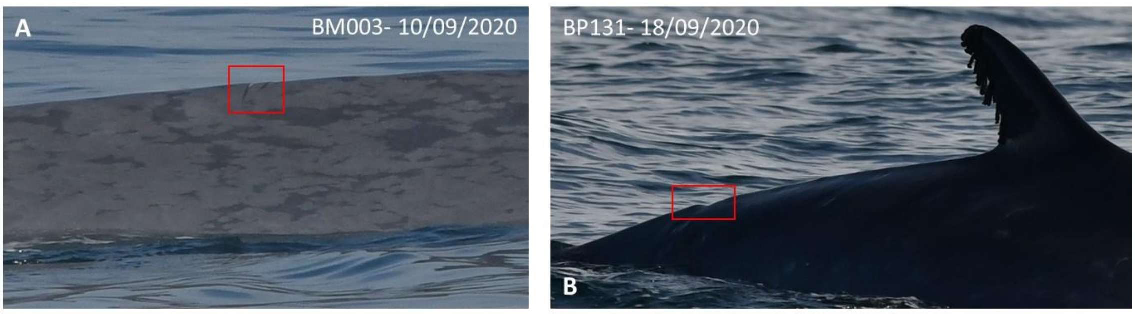

Figure- (A) Blue whale with 2 propeller cuts and (B) fin whale with a back indentation probably caused by fishing net entanglement.

Annex 22

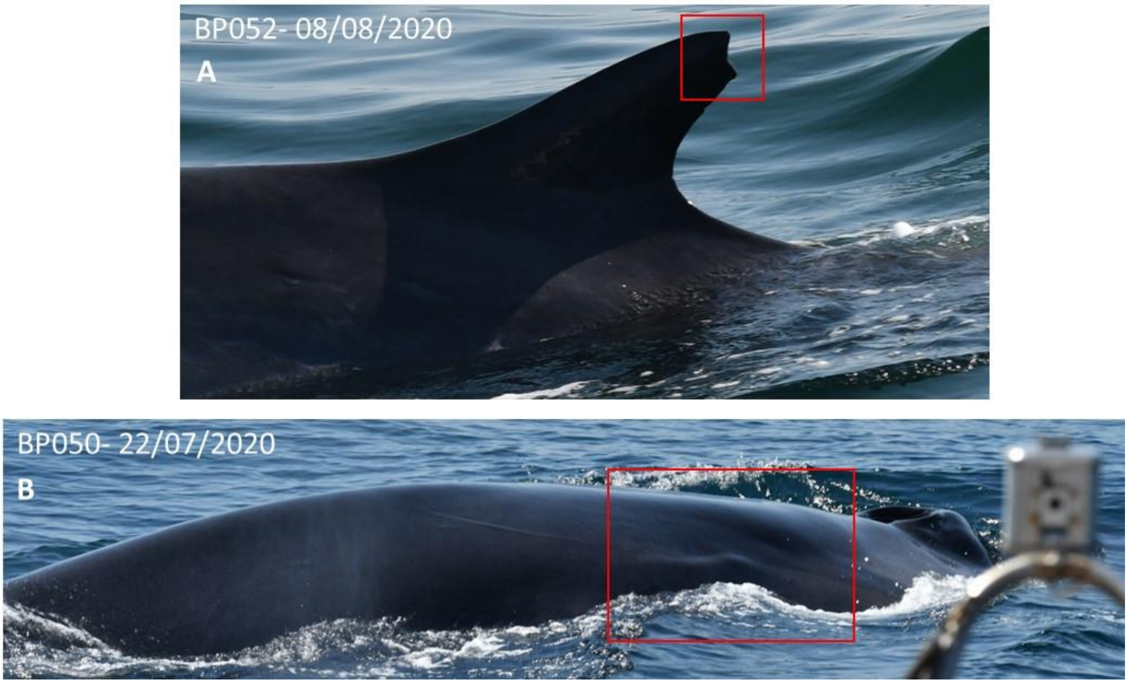

Figure- (A) Fin whale with an amputation in the dorsal fin and (B) fin whale with a deformation in the intermediate body probably caused by interaction with human activity.

Annex 23

Figure- (A) Blue whale with a possible tumor, (B) fin whale with several blisters of different sizes and (C) minke whale full of uniform-size blisters possibly linked with UV radiation exposure.

Annex 24

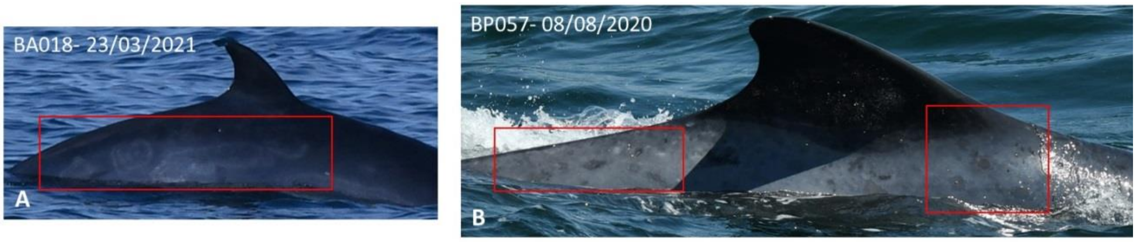

Figure- Tattoo-like lesions: (A) minke whale with big tattoo-like lesions and (B) fin whale full of small tattoo-like lesions possibly Poxvirus or as a result of, probably, habitat degradation combined with pathogen infection and water pollution.

Annex 25

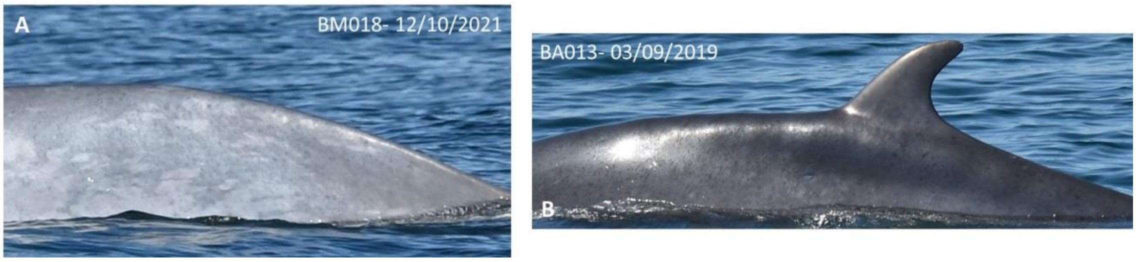

Figure- (A) Blue whale with herpes-like lesions in the peduncle and (B) minke whale full of herpes-like lesions. Unknown aetiology.

Annex 26

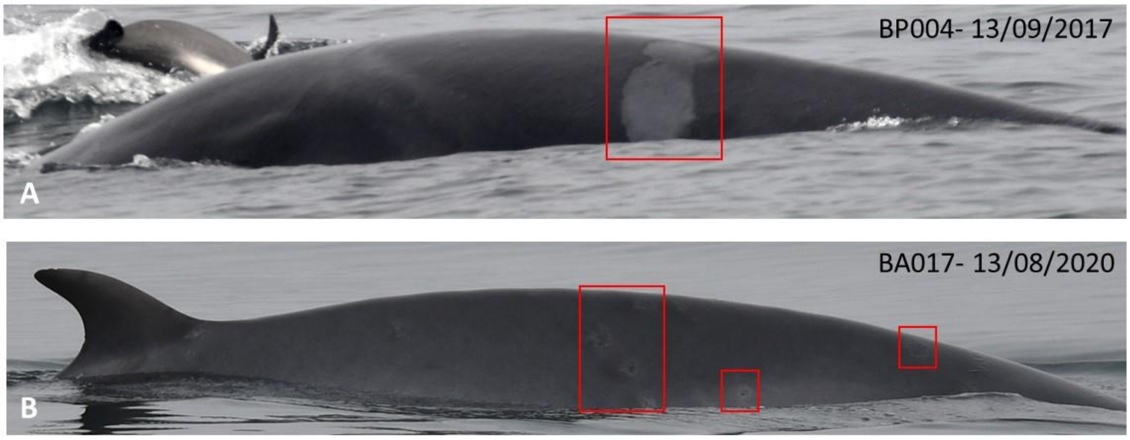

Figure- (A) Fin whale with a big wart-like lesion and (B) minke whale with small wart-like lesions. Unknown aetiology.

Annex 27

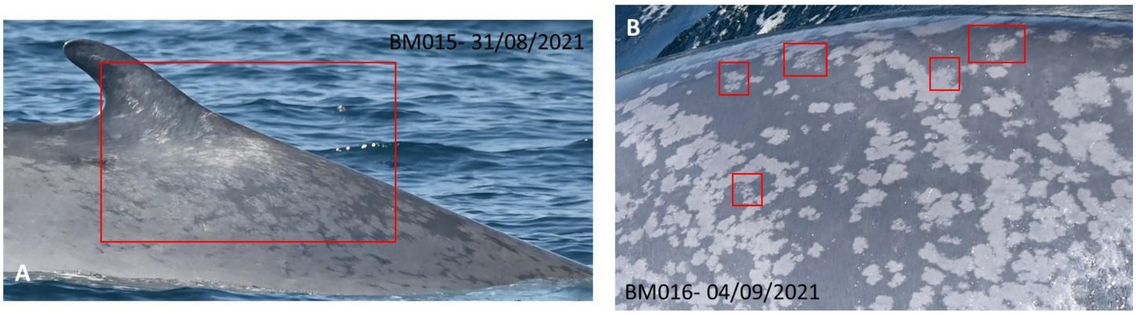

Figure- (A) Blue whale with a lot of starbursts in the dorsal fin section and (B) blue whale with less starburst in the intermediate body associated with rupture of blisters.

Annex 28

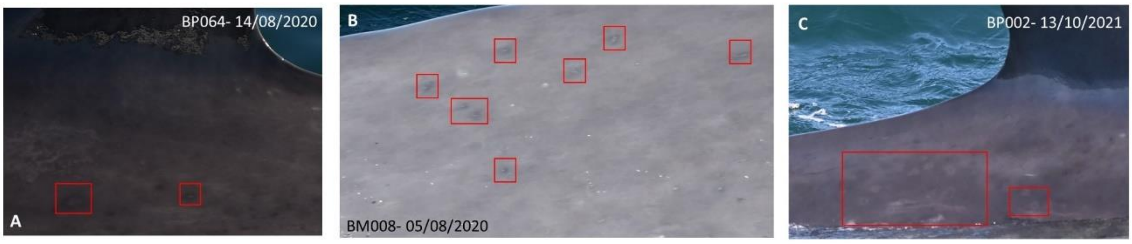

Figure- (A) Fin whale with dark fringe, (B) blue whale with several dark fringe possible caused by Poxvirus and (C) fin whale with several white fringe possibly associated with Delphinid Herpesvirus 3.

Annex 29

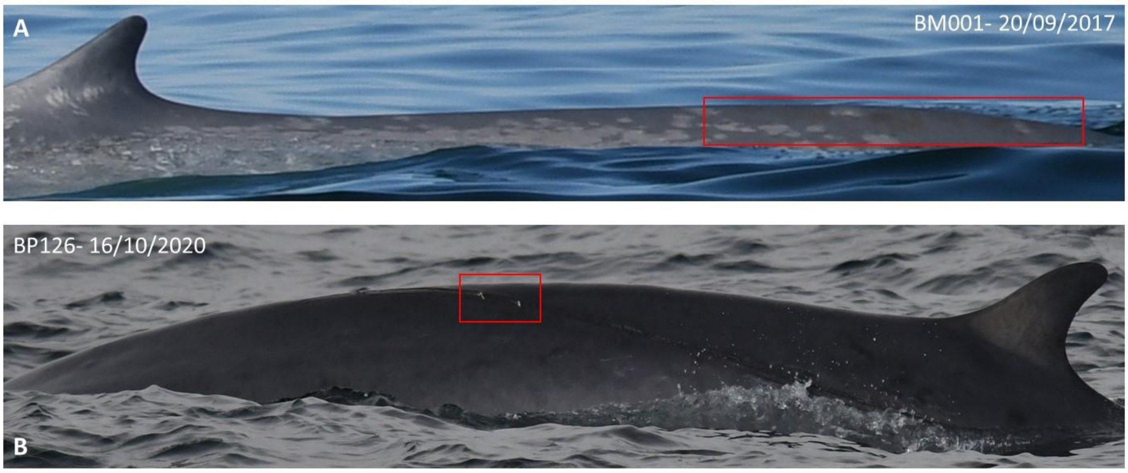

Figure- (A) Blue whale with patches of diatoms attached to the peduncle and (B) fin whale injured with possibly whale lice inside the wound.

Annex 30

Figure- (A) Fin whale infested with the ectoparasite *Pennella balaenopterae* laterally along the body and (B) fin whale with only one attachment of *Pennella balaenopterae*.

Annex 31

Figure- (A) Blue whale full of big *Xenobalanus globicipitis* attached, (B) fin whale with medium and big *Xenobalanus globicipitis* and (C) fin whale full of medium and big *Xenobalanus globicipitis* attached in the dorsal fin.

Annex 32

Figure- (A) Fin whale with a big remora attached in the dorsal fin section and (B) fin whale with a relatively small remora attached in the intermediate body.

Annex 33

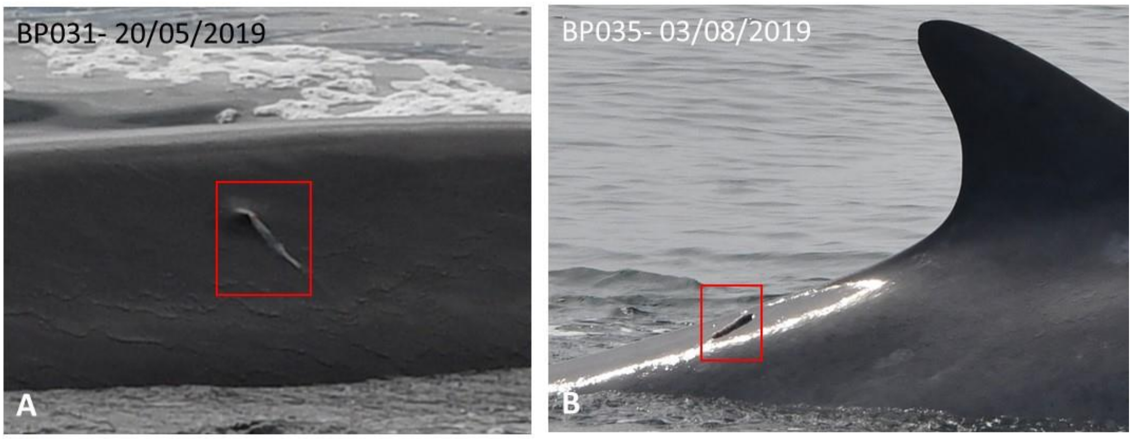

Figure- Unknown ectoparasites attached: (A) fin whale with possibly a lamprey attached and (B) fin whale with an unknown ectoparasite attached.

Annex 34

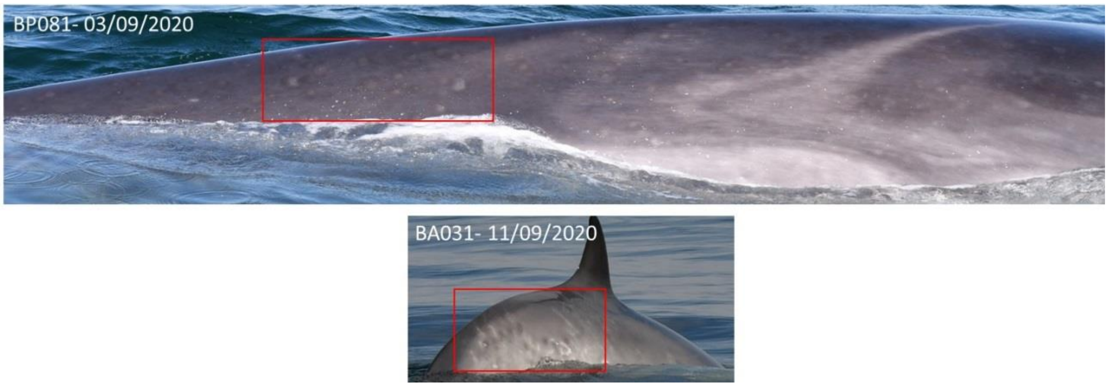

Figure- Examples of skin marks in the miscellaneous category: (A) fin whale with unknown circular white patches maybe slightly elevated similar to mottled and (B) minke whale with scars in the peduncle some are parallel and others irregular forming a swell elevation.