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Departemento de Biologia

Sara de Almeida Simões

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Dissertation presented to the University of Aveiro for the fulfilment of the necessary requisites to obtain the degree of Master in Marine Biology, developed under the scientific guidance of PhD Investigator Catarina Isabel da Costa Simões Eira, Research assistant at the Biology Department of University of Aveiro & CESAM, and under the scientific guidance of PhD Bruno Díaz López, Founder and Chief Biologist of the Bottlenose Dolphin Research Institute.

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palavras-chave *Tursiops truncatus,* cicatrizes, interações sociais, marcas de parasitas, doenças de pele, saúde, indicadores de comportamento, noroeste da Espanha.

resumo

Os cetáceos exibem, frequentemente, marcas corporais que podem ser causadas por doenças, parasitas e interações intraespecíficas e com atividades humanas. Neste estudo, foi usado um ano de dados de identificação fotográfica de golfinhos roazes residentes na Ría de Arousa (NW Espanha). Os objetivos deste estudo foram avaliar os tipos de marcas corporais encontrados nos golfinhos roazes e compará-los entre machos e fêmeas adultos. Quatro seções do corpo (anterior, ventral, central e posterior) foram definidas para avaliar a prevalência, abundância, riqueza e distribuição das marcas. Foram realizadas 65 saídas (dias) de barco, entre janeiro e outubro de 2017, num período de 352 horas, com 218 grupos de golfinhos observados durante 98 horas. Foram identificados 178 indivíduos e 21 indivíduos adultos, cujo sexo foi determinado (12 homens e 9 mulheres), foram utilizados para a análise. Observaram-se marcas sociais (arranhões, cortes e marcas lineares), condições de pele (doenças) e marcas causadas por parasitas. Nenhum dos indivíduos apresentou marcas induzidas por humanos. Todas as marcas corporais, exceto marcas causadas por parasitas, foram encontradas em todos os indivíduos. A abundância de marcas foi maior nos machos (média ± DP = 130,7 ± 20,7) do que nas fêmeas (média ± DP = 88,3 ± 17,5). A riqueza em arranhões foi significativamente maior nos machos, enquanto a riqueza em marcas de doenças de pele foi significativamente maior nas fêmeas. Os tipos de marcas de corporais dependiam da secção do corpo, com a secção posterior a exibir mais marcas de arranhões e a seção anterior mais marcas de doenças. As diferenças de comportamento entre os sexos, com os comportamentos mais agressivos e agonistas dos machos, em comparação com as fêmeas, podem explicar o facto de os machos apresentarem uma maior incidência de marcas induzidas por interações sociais. Estes resultados mostram que a análise das marcas corporais em cetáceos é uma técnica de investigação que pode ser usada para avaliar a saúde e o comportamento social de uma população.

key-words *Tursiops truncatus*, scars, social interactions, parasitic marks, skin disorders, health status, behavioural indicators, NW Spain.

abstract

Cetaceans frequently display skin marks which can be caused by diseases, parasites, and interactions among conspecifics and with human activities. For the present study, we used one year of photo-identification data for resident bottlenose dolphins of the Ría de Arousa (NW Spain). The aims of this study were to assess the types of skin marks found on bottlenose dolphins and compare them between adult males and females. Four body sections (anterior, ventral, central, and posterior) were defined to assess the prevalence, abundance, richness, and distribution of the marks. Boat-based surveys were carried out on 65 days between January and October 2017 for a duration of 352 hours, with 218 groups of dolphins observed during 98 hours. A total of 178 individuals were identified, and 21 adult sexed individuals (12 males and 9 females) were used for the analysis. Social-induced marks (tooth-rakes, nicks, and linear marks), skin disorders, and parasitic marks were observed, but none of the individuals showed human-induced injuries. All skin marks, except parasitic marks, were found on all the individuals. The abundance of marks is similar between males (mean ± SD = 130.7 ± 20.7) and females (mean ± SD = 88.3 ± 17.5) (Mann-Whitney, $z = 1.53$, $p > 0.05$). The richness in tooth-rakes was significantly higher in males, while the richness in skin disorders was significantly higher in females. Skin mark types were dependent on body section, with the posterior section exhibiting more tooth-rake marks and the anterior section exhibiting more skin disorder marks. The behavioural variation between sexes, with males exhibiting more aggressive and agonistic behaviour than females, may explain the fact that males have a higher incidence of social-induced marks. These results show that the analysis of cetacean skin marks is a research technique that can be efficiently used to assess the health and social behaviour of a population.

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Chapter I - Introduction

1.1. Common Bottlenose Dolphin

The common bottlenose dolphin (*Tursiops truncatus,* hereafter bottlenose dolphin) (Montagu, 1821) is a long-lived (males up to 48 years and females more than 57 years) (Reid & Wilson, 1995; Mann et al., 2000; Wells & Scott, 2005), large-brained (Mann et al., 2000), apex predator that feeds at a high trophic level, being considered a sentinel of coastal ecosystems (Mallette et al., 2016). Since bottlenose dolphins are a cosmopolitan Delphinidae (Wells & Scott, 2005) that typically resides close to shore, there is a significant amount of knowledge about their society (Reid & Wilson, 1995; Weir & Stockin, 2001; Bearzi, 2005; Wells & Scott, 2005; Díaz López & Shirai, 2008; Moreno & Acevedo-Gutiérrez, 2016). For the same reason, this species is the most popular coastal species (Müller et al., 1998), and is subject to a broad range of anthropogenic impacts (Díaz López, 2006, 2012; Lemon et al., 2006; Bearzi et al., 2008; Mallette et al., 2016).

Figure 1: Bottlenose dolphin (*Tursiops truncatus*) - countershaded coloration (Weir & Stockin, 2001).

Bottlenose dolphins are recognized by their robust body, a sharp demarcation between the melon and the short rostrum, and dark coloration in a countershaded fashion, ranging from a dark grey on their backs to a pale cream on the belly, and flippers and flukes are also dark grey (Reid & Wilson, 1995; Wells & Scott, 2005) (fig.:1). The length of an adult individual can range from 2.5 m to about 3.8 m, and can vary with the geographic location due to the water temperature (inversed variation) (Wells & Scott, 2005). Older dolphins are often scarred and pale around their mouth and on the tip of the rostrum (Reid & Wilson, 1995).

Information transfer relies on social interaction patterns among individuals (Titcomb et al., 2015; Moreno & Acevedo-Gutiérrez, 2016). Bottlenose dolphins are complex social animals (Wells et al., 1987; Reid & Wilson, 1995; Mann et al., 2000; Constantine et al., 2004; Díaz López & Shirai, 2008; Moreno & Acevedo-Gutiérrez, 2016). Social bonds influence essential fitness traits such as calving success, juvenile male survival and adult male mating success (Smith et al., 2016). The group composition of bottlenose dolphins is very dynamic (Connor et al., 2000) varying in size from 2-15 individuals to networks of hundreds of individuals (Mann et al., 2000) during one day, having also long-term and stable social bonds (Díaz López & Shirai, 2008; Titcomb et al., 2015; Carnabuci et al., 2016; Moreno & Acevedo-Gutiérrez, 2016; Smith et al., 2016). Sex, age, reproductive maturity, familial relationships, and social bond histories seem to be important factors in determining the group composition (social units: nursery groups; mixed sex groups of juveniles; individual adult males or strongly bonded pairs of males) (Mann & Smuts, 1998; Connor et al., 2000; Wells & Scott, 2005). This type of plasticity of association among bottlenose dolphins is called fission-fusion (Connor et al., 2000). The group size depends on habitat and activity (Shane, 1990; Wells & Scott, 2005). Adding the fact that they are acoustically specialized animals (Díaz López, 2011; Titcomb et al., 2015; Carnabuci et al., 2016; Luís et al., 2016), which allows them to have a social structure thus maximizing their learning, feeding, reproduction, defence and communication abilities (Müller et al., 1998; Guevara-Aguirre & Gallo-Reynoso, 2016), the last one being defined as any action that affects the behaviour of another individual – either by altering or maintaining it (Díaz López, 2011). All these abilities permit them to explore a wide variety of habitats (Reid & Wilson, 1995; Müller et al., 1998; Guevara-Aguirre & Gallo-Reynoso, 2016). Bottlenose dolphin's wide distribution, associated with local behaviour, tends to produce a noticeable external differentiation among populations (particularly morphometric differences), also as a consequence of local selection pressure and genetic drift (Carnabuci et al., 2016).

In some cases, boundaries between populations can be demarcated by physiographic features, such as the abrupt change in water depth (Wells & Scott, 2005).

Despite of being apex predators, bottlenose dolphins may be preyed upon, particularly by sharks such as bull sharks (*Carcharhinus leucas*), tiger sharks (*Galeocerdo cuvier*), great white sharks (*Carcharodon carcharias*), and dusky sharks (*Carcharhinus obscurus*), and they may be occasionally preyed upon by killer whales (*Orcinus orca*) (Heithaus, 2001). Mothers and calves appear to be the most affected by shark predation due to the higher incidence of bites and the predominance of young dolphin remains in shark's stomachs (Mann, 1999).

1.1.1. Taxonomy

The Cetacea has been divided into two suborders: the Mysticeti (baleen whales) and the Ondontoceti (toothed whales) (Price et al., 2005). This order includes the bottlenose dolphin (*Tursiops truncatus*) (Price et al., 2005); Tursio from the Latin meaning dolphin, -ops from the Greek meaning appearance, and trunco from the Latin meaning truncated (Reid & Wilson, 1995). Bottlenose dolphins belong to the genus *Tursiops* from the Delphinidae family that belongs to the Odontoceti suborder from the order Cetacea (Price et al., 2005).

1.1.2. Habitat

The bottlenose dolphin is a species with high capabilities of dispersal and it is globally found in the Atlantic, Pacific and Indian Oceans in temperate and tropical waters, inhabiting pelagic and coastal waters (Reid & Wilson, 1995; Connor et al., 2000; Weir & Stockin, 2001; Wells & Scott, 2005; Santos et al., 2007; Bearzi et al., 2008; Methion & Díaz López, 2018). They show a high variability of near-shore habitats (Bearzi & Politi, 1999; Guevara-Aguirre & Gallo-Reynoso, 2016; La Manna et al., 2016), including bays, tidal creeks and estuarine systems, even ranging into rivers as these are places characterised by high levels of primary productivity and prey abundance (Connor et al., 2000; Santos et al., 2007; Bearzi et al., 2008; Díaz López & Methion, 2017). The range of this species appears to have limits related to temperature through the distribution of prey (Weir & Stockin, 2001; Wells & Scott, 2009). There exist two types of populations of bottlenose dolphins: large transient pelagic/offshore populations living in cold, deep waters, and smaller resident coastal/nearshore populations living in warm, shallow waters, also called pelagic ecotype and coastal ecotype, respectively (Wells & Scott, 2009). Larger group sizes in deeper waters may benefit from cooperative feeding on patchy food resources, and protection against predation (Bearzi, 2005).

In some locations, bottlenose dolphins show high site fidelity in coastal areas with protection from predators and with high prey availability and variety (Bearzi et al., 2008; La Manna et al., 2016; Zanardo et al., 2016; Díaz López et al., 2017). Long-term residency has been reported for the coastal dolphins and has been described by relatively permanent home range or repeated occurrence of the dolphins in the same place for many years (Wells & Scott, 2009). Resident coastal populations of bottlenose dolphins have been described in Moray Firth (Scotland), Cardigan Bay (Wales), the Shannon Estuary (Ireland), the Sado Estuary (Portugal), and Galician waters (Spain) (Reid & Wilson, 1995; Bearzi et al., 2008; Augusto et al., 2011; Díaz López, 2012; Methion & Díaz López, 2018).

Genetic differentiation is apparent between offshore and coastal populations due to strong site fidelity, and resource specialization as a consequence of different behavioural strategies (Fernández et al., 2011a).

1.1.3. Diet

Bottlenose dolphins became generalists, capable of taking advantage of a variety of prey items (see Appendix I), such as demersal, pelagic, epipelagic, mesopelagic, sciaenid fish, cephalopods, and crustaceans (Reid & Wilson, 1995; Bearzi & Politi, 1999; Santos et al., 2001; López et al., 2004; Santos et al., 2007; Rosel et al., 2009;

Guevara-Aguirre & Gallo-Reynoso, 2016). In spite of being generalists, they can have some dietary preferences (Santos et al., 2001; Lusseau et al., 2004; Wells & Scott, 2005). Also, individuals within a population may show some degree of specialization depending on the sex and size (Wells & Scott, 2005) with the size being probably related to age, which reflects increased experience, improved diving and prey catching abilities, and can also be related to increased stomach capacity (Fernández et al., 2011a).

1.1.4. Reproduction

Cetaceans produce precocial offspring that are heavier and have long gestation periods (Mann, 1997). Resulting from selective pressures, this development is advantageous to them since they must be able to swim and breathe independently from the moment of birth, and must nurse from a constantly moving mother (Mann & Smuts, 1998).

In the species bottlenose dolphin males are sexually active throughout the year (Reid & Wilson, 1995; Wells & Scott, 2005) with prolonged elevation of testosterone concentrations when females are in ovulation (Wells & Scott, 2005). The females become sexually mature between 5 and 8 years of age (Connor et al., 2000) and are seasonally polyoestrous (Wells & Scott, 2005; De Francesco & Loy, 2016), producing 2-7 ovulations per year (De Francesco & Loy, 2016). The males become sexually mature later than females when they reach between 8 and 12 years (Connor et al., 2000). Births occur at any time of the year, with females having a tendency to give birth during a particular season (Reid & Wilson, 1995; Smith et al., 2016), depending on the cycles of prey availability, predation pressure (Reid & Wilson, 1995; Mann et al., 2000), and water temperature (Wells et al., 1987; Reid & Wilson, 1995; Mann et al., 2000; Smith et al., 2016). Females up to 48 years old have successfully given birth and raised calves (Reid & Wilson, 1995; Wells & Scott, 2005), and therefore they do not exhibit reproductive senescence (Reid & Wilson, 1995). When impregnated, they give birth to a single offspring after a gestation of 12 months (Mann et al., 2000; Wells & Scott, 2009).

The offspring's length depends on the geographic region and can range from about 84 to 140 cm (Connor et al., 2000; Wells & Scott, 2009). The interbirth interval for females with surviving young ranges between 4 to 5 years (Mann & Smuts, 1998; Scott et al., 2005), with a maternal care of 3 to 6 years (Mann, 1997; Mann & Smuts, 1998; Wells & Scott, 2009). The separation between mother and calf often coincides with the birth of another calf (Grellier et al., 2003; Wells & Scott, 2009). In some cases, maternal care can reach up to 8 years, including an expanded period of lactation lasting multiple years (Smith et al., 2016). This can happen when foetal loss occurs (Mann et al., 2000). Normally the period of lactation is 18 months and nutritional independence occurs around 18 to 20 months of age (Mann & Smuts, 1998; Mann et al., 2000; Mallette et al., 2016). Despite nutritionally independent, calves entirely depend on their mothers during the first 2 to 3 years (Wells et al., 1987; Speakman et al., 2010). When a female experiences a premature loss of her offspring she can quickly become sexually receptive again, reducing the general interbirth interval (Hrdy, 1979; Díaz López et al., 2017).

Bottlenose dolphins develop sexual behaviour very early, many years before sexual maturity (Wells et al., 1987). It can occur between individuals of different species, between individuals of the same sex, and involving immatures, occurring in reproductive and non-reproductive contexts (Wells et al., 1987).

1.1.5. Impact of human activity

Bottlenose dolphins have been facing several threats caused by anthropogenic disturbance (physical and acoustic) (Goodwin & Cotton, 2004; Lemon et al., 2006; Pennino et al., 2016), such as fishery activities and habitat modification/degradation (Fertl & Leatherwood, 1997; Díaz López, 2006; Díaz López et al., 2019), especially individuals that live near the coast (Methion & Díaz López, 2018). The fact that this species has a long life span and low reproductive rates, turns it particularly vulnerable to anthropogenic impacts (Constantine et al., 2004; Gridley et al., 2015), being among the most threatened cetacean species (Methion & Díaz López, 2018). The impact of fisheries is translated in bycatch, overfishing, and boat strikes (Bearzi et al., 2008). The habitat degradation includes environmental contamination by pollutants and can affect reproduction and health (Bearzi et al., 2008).

1.1.5.1. Bottlenose dolphin conservation status

The bottlenose dolphin is listed in annex II and IV of the EU Habitats Directive (Directive 92/43/CEE, www.ec.europa.eu accessed 15 October 2017). For species listed in the annex II, member states are required to designate Special Areas of Conservation (SACs) and their respective management plans (Fernández et al., 2011b; Santos et al., 2011; Spyrakos et al., 2011; Louis et al., 2014). This species has been classified as vulnerable in Mediterranean waters and is present in the ACCOBAMS (Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous Atlantic area) region (Pennino et al., 2016). It is listed in Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention; www.coe.int, accessed 15 October 2017).

In the UK, two Special Areas of Conservation (SACs) under Annex II have been designated for this species, one in Cardigan Bay (West Wales), and another in the inner Moray Firth (Scotland) (Weir & Stockin, 2001). In British waters, bottlenose dolphins are also protected under the Fisheries Act (1987), Wildlife and Countryside Act (1981, 1986), Convention on the Conservation of Migratory Species of Wild Animals (the Bonn Convention, 1979), and the Agreement on the Conservation of Small Cetaceans of the Baltic and North Seas (ASCOBANS, 1979) (Reid & Wilson, 1995).

In Spain, several international protocols of marine conservation that affect particularly whales and dolphins have been made; it is the case of the 4th Protocol of “Convenio de Barcelona” about conservation of marine biodiversity (habitats and species) in the Mediterranean sea, bottlenose dolphin is listed in its Annex II; the

OSPAR agreement and its Annex V about the same subject in the north Atlantic, and the already mentioned ACCOBAMS. Every cetacean species in Spanish waters are included in the “Listado de Especies Silvestre en Régimen de Protección Especial” – R.D. 139/2011, where the bottlenose dolphin is considered vulnerable. The national “Real Decreto” 1727/2007 controls all marine activities, especially recreational, restricting the approach of boats with engines to any cetacean species (500m of distance) (http://www.boe.es/diario_boe/txt.php?id=BOE-A-2008-516).

1.2. Social structure and inter-individual interactions

The study of animal social structure gives important information on ecological relationships between animals and their conspecifics (Whitehead, 1997; Díaz López & Shirai, 2008). It is established by interactions between individuals, which in turn affect the individual fitness, disease transmission, genetic structure, viability of populations (Titcomb et al., 2015), as well as information flow (Titcomb et al., 2015; Rankin et al., 2016).

Social structure of bottlenose dolphins (*Tursiops truncatus*) varies between locations, depending on prey availability (Reid & Wilson, 1995; Moreno & Acevedo-Gutiérrez, 2016), habitat, and/or foraging specialization (Díaz López & Shirai, 2008; Titcomb et al., 2015), but it has general trends (Moreno & Acevedo-Gutiérrez, 2016).

1.2.1. Social behavioural patterns - Intraspecific interaction

Odontocetes tend to use teeth in aggressive intraspecific interactions (Heyning, 1984; Scott et al., 2005) with the tooth rakes being characteristic of the species’ dentition, and good indicators of intra-specific interactions (McCann, 1974; Scott et al., 2005).

MacLeod (1998) hypothesized that scars indicate male quality in dominance and/or fitness to other males during aggressive interactions. For bottlenose dolphin this hypothesis does not apply since their scars regain pigmentation over time (Lockyer

& Morris, 1990; MacLeod, 1998; Scott et al., 2005). This way, they could indicate relative rates and timing of received aggression (Scott et al., 2005). The most severe forms of aggression expressed by bottlenose dolphins involve contact through body slamming, ramming, and biting (Samuels & Gifford, 1997; Connor et al., 2000; Scott et al., 2005). Biting is the abrupt contact between one dolphin's teeth and another dolphin's body, resulting in long, thin, parallel, or almost parallel tooth rake marks on the skin (Samuels & Gifford, 1997; Scott et al., 2005).

Competitive behaviour usually differs between sexes within a species, corresponding to differences in the resources for which males and females compete (Samuels & Gifford, 1997).

1.2.1.1. Male-male competition

Aggression is more frequent among adult males, with competition for mates within and outside of an alliance resulting in aggressive encounters (Scott et al., 2005), and competition for access to females (Samuels & Gifford, 1997). In addition, aggressive sociosexual behaviour occurs in non-reproductive context among them (Scott et al., 2005).

Males form long-term strong first-order alliances between two or three individuals, and second- and third-order alliances, which are temporary unions of two or three alliances, respectively (Connor et al., 2000). These bonds between males facilitate defence, and cooperation for female acquisition (Reid & Wilson, 1995; Mann et al., 2000; Chilvers & Corkeron, 2001; Scott et al., 2005; Rosel et al., 2009; Moreno & Acevedo-Gutiérrez, 2016). The fact that they share copulatory access to a female reduces an individual's reproductive success but it is better than no copulatory success at all (Reid & Wilson, 1995).

Sexual coercion appears to be most common in gregarious and polygynous species where females encounter not one but several males that compete for access to them (Connor et al., 2000; Scott et al., 2005). Male bottlenose dolphins use aggressive coercion against single females (Wells & Scott, 2009). To maintain consortships, observed aggression from male bottlenose dolphins towards females include head jerks (sharp movements of the head), chasing, tail hitting, charging, biting or jawing, and body slamming (Scott et al., 2005).

1.2.1.2. Female-female relations

Female bottlenose dolphins show remarkably low rates of aggression (Samuels & Gifford, 1997; Scott et al., 2005; Marley et al., 2013). They are considered to be tolerant (Sterck et al., 1997) with low contest competition, forming egalitarian relationships and rarely forming alliances (Scott et al., 2005). Females typically compete for access to resources necessary for reproduction (Samuels & Gifford, 1997) (fig.2). When they individually hunt for mobile prey, they do not need to compete for, nor share, food (Scott et al., 2005).

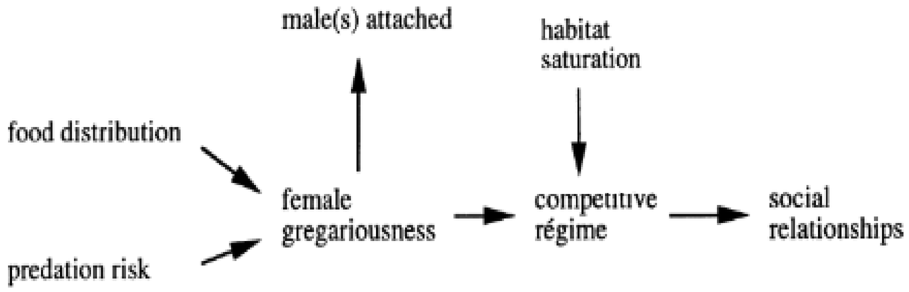

Figure 2: Flow diagram showing female gregariousness determined by opposing pressures from predation risk and food distribution, the distribution of males depending of female gregariousness, all combined determine social relationships (Sterck et al., 1997).

Females form loose associations with many individuals, forming female bands (groups of reproducing females and calves), and their social structure is more similar between behavioural states than the one of males (Connor et al., 2000; Wells & Scott, 2009). Females use these bands of many loose connections for defence from male harassment when sexual conflict is present in the population, calf protection, social development and assistance, reducing the maternal investment (Connor et al., 2000). The reduction in maternal investment happens when there is alloparental care, referring to adult females other than the biological mother taking care of the calves for a period of time (Shane, 1990). Mating promotes mixed-sex groups and it involves increased coercive behaviour from males that preferentially associate with receptive females (Connor et al., 2000). Either males or females have the opportunity to copulate with multiple mates in a single breading season (Reid & Wilson, 1995). Associations between immatures and adults occur sometimes, but age segregations appear to be the general rule (Wells et al., 1987). Immatures tend to be seen with other immatures more frequently than with adult classes (Wells et al., 1987).

1.3. Importance of studying skin marks

Cetaceans are affected by various injuries and skin disorders that can be caused by intra- and interspecific interactions (Scott et al., 2005; Marley et al., 2013), human activities (Wells et al., 2008; Bertulli et al., 2012; Díaz López et al., 2017; Leone et al., 2019), parasitic copepods and fish, or infectious agents (Bertulli et al., 2012; Leone et al., 2019). Biological and chemical contaminants may also contribute to the appearance of skin disorders on cetaceans (Leone et al., 2019). All these causes lead to different types of skin marks that are considered to be good indicators of inter-individual interactions occurrence (Heyning, 1984; Visser, 1998; Scott et al., 2005; Marley et al., 2013), general population health, changes in environmental conditions, and exposure to pollutants (Bertulli et al., 2012; Leone et al., 2019). The use of such indicators is possible due to the delicate nature of cetacean skin that gets easily injured (Heyning, 1984; MacLeod, 1998) since, unlike other mammals, they lack hair coverage (MacLeod, 1998).

Persistent characteristics that can last throughout an individual's lifetime, such as scars and natural markings have been used to identify delphinids (Würsig & Würsig, 1977; Lockyer & Morris, 1990; Scott et al., 2005; Speakman et al., 2010). Dolphins bite marks (also referred to as nicks) are primarily found on the dorsal fin, flippers, flukes, and peduncle, which are areas narrow enough for a dolphin to grasp entirely in its mouth (Heyning, 1984). Bottlenose dolphins' wounds tend to heal rapidly due to a buffer layer of degenerating cells that replace the open wound, and protects the new growth of tissue (Lockyer & Morris, 1990; Scott et al., 2005). Scars such as tooth rakes (intertooth distances: bottlenose dolphin - 10.97-12.32 mm (Díaz López et al., 2017); harbour porpoise (*Phocoena phocoena*) - 3.36-3.87 mm; common dolphin (*Delphinus delphis*) – 4.46-4.95 mm; risso's dolphin (*Grampus griseus*) – 15.28-17.67 mm; killer whale (*Orcinus orca*) – 28.64-35.1 mm (Ross & Wilson, 1996)) tend to not damage deep tissue, disappearing after 5 to 20 months (Lockyer & Morris, 1990; MacLeod, 1998; Scott et al., 2005) due to the melanocytes that reside deep in the epidermis and give rise to columns of pigmented cells (Lockyer & Morris, 1990; Scott et al., 2005). Other types of scars that result from a significant loss of tissue, such as shark bites, can heal completely, but are permanent and not pigmented (Heithaus, 2001; Lockyer & Morris, 1990; Scott et al., 2005).

Females show remarkably low rates of aggression, even towards their calves (Scott et al., 2005). However, mother-offspring conflict can happen prior to separation, leading to the infliction of tooth rakes on the calf (Wells et al., 1987).

Tooth rake marks can help inferring attempted predation by matching the dentition of predators such as killer whales (*Orcinus orca*; (George et al., 1994)), and sharks (Heithaus, 2001; Scott et al., 2005). Aggressive encounters don't occur only from other species towards bottlenose dolphins (Díaz López et al., 2017). Bottlenose dolphins are known to be aggressive towards other smaller species of delphinids such as harbour porpoises (*Phocoena phocoena*, Linnaeus 1758), belonging to the family Phocoeniidae from the superfamily Delphinidae (Díaz López et al., 2017).

The impacts of human activities on skin marks of bottlenose dolphin are represented by different sources, such as boat traffic (boat strikes, contact with spinning propellers) and fisheries (Lockyer & Morris, 1990; Wells et al., 2008). Entanglement in line or nets (fishing gears), in addition to scarring consistent with wounds from lines can also lead to the loss of appendages (Well et al., 2008).

1.4. Objectives

Taking into consideration that skin marks are indicators of social interaction, population health, and exposure to human activities and pollutants (Bertulli et al., 2012; Díaz López et al., 2017; Leone et al., 2019) this thesis aims to describe what types of skin marks are present on the bottlenose dolphins of Ría de Arousa (Galicia, NW Spain). In particular, this study aims to assess the quantity and richness of the skin marks and if there are differences between males and females.

Chapter II - Methods

2.1. Ría de Arousa

2.1.1. Geographic description

Galicia is situated in the northwest of the Iberian Peninsula (Evans & Prego, 2003; Díaz López et al., 2017). With a coastline of 1195 km, there are a series of ancient drowned tectonic valleys that were taken by the sea, known as 'rías' (Prego et al., 1999; Evans & Prego, 2003; Díaz López et al., 2017; Díaz López & Methion, 2018) (fig.:4). There are five rías along the western coastline oriented in a northeast-southwest direction (Figueiras et al., 2002; Alvarez et al., 2005). They are about 30 km long and 6 km wide in their middle part (Figueiras et al., 2002). These rías behave as partially mixed estuaries with positive circulation where the partial stratification is maintained by the river discharge in winter, and by solar heating and coastal upwelling in summer (Alvarez et al., 2005). They are divided into zones that show an external oceanic area (outer area) and an internal estuarine area (inner area) with important tidal influence (Evans & Prego, 2003).

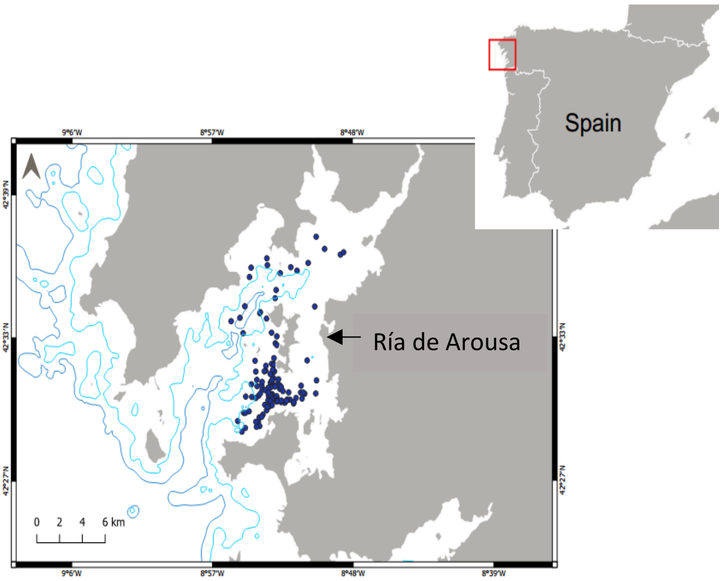

Figure 3: Map made with the QGIS software. Ría de Arousa on the left and its' position on the map of Spain on the right. Each point of the ría represents one sighting where at least one bottlenose dolphin used on this study was observed.

Of the five rías, Ría de Arousa is the largest (Mora, 1982; Figueiras et al., 2002) (fig.:3), with an area of 240 km² (Prego et al., 1999), and an average depth of 19m (Díaz López & Methion, 2017). The entire system is subjected to a semidiurnal and mesotidal tide regime, ranging from 1.1 m during neap tides to 3.5 m during spring tides (Alvarez et al., 2005; Díaz López & Methion, 2018; Methion & Díaz López, 2018). This ría is connected to the open sea by two entrances due to the existence of an island called Sálvora at the outermost part (Alvarez et al., 2005). Its freshwater runoff mostly originates from the Ulla and Umia rivers (Alvarez et al., 2005).

Galicia is the most important fishing region of Spain and one of the most important in the world (López et al., 2003, 2004), with 87 fishing ports (López et al., 2004), about 2900 commercial fishing boats operate in inshore waters, using traps, trawls, purse seines, gill-nets and longlines (Freire & Garcia-Allut, 2000; Santos et al., 2007), and shellfish aquaculture, more specifically mussels and oysters (Díaz López & Methion, 2018; Methion & Díaz López, 2018). The five rías support a high density of mussel floating rafts (Figueiras et al., 2002) producing annually 300 000 tons (98% of the total Spanish, 50% of the European and almost 13% of the world production) (Díaz López & Methion, 2017) of *Mytilus galloprovincialis* (Fuentes et al., 2000). Ría de Arousa is the one with the highest density, where 2292 rafts (69% of the total rafts in Galicia) cover 27 km², which represents 11% of the total surface of the ría (Figueiras et al., 2002). This culture began in Ría de Arousa in 1946 with 125 m² rafts with each raft having 500 hanging ropes 5m long (Figueiras et al., 2002). Now, the area that rafts occupy is 500m² with the ropes having 12 m long (Figueiras et al., 2002).

The ría where bottlenose dolphins are mostly sighted is Ría de Arousa (Pierce et al., 2010; Methion & Díaz López, 2018). Due to the very heterogeneous fleet operating in this area, they are subjected to several threats, such as marine traffic, bycatch (López et al., 2003), and overfishing (Freire & García-Allut, 2000).

2.1.2. Biodiversity

The Galician shelf lies at the northern limit of the east central Atlantic upwelling system (López et al., 2004) or Canary upwelling system, one of the four major upwelling regions in the world (Díaz López & Methion, 2018) that occurs from March to October (Figueiras et al., 2002; López et al., 2003, 2004; Torres & Barton, 2007; Spyrakos et al., 2011), penetrating the Ría de Arousa (Prego et al., 1999; Díaz López & Methion, 2018). During this event, surface waters move offshore, and cold, saltier, nutrient-rich waters move onshore and upward (Alvarez et al., 2005). The rest of the year, there is a dominance of downwelling conditions (Figueiras et al., 2002), during which warmer, fresher, nutrient-depleted surface waters move inshore and downward reducing the strength of exchange flow (Alvarez et al., 2005). The upwelling system sustains a high primary productivity (Prego et al., 1999; Alvarez-Salgado et al., 2000; López et al., 2002, 2003, 2004; Alvarez et al., 2005; Santos et al., 2007, 2011; Díaz López & Methion, 2018; Methion & Díaz López, 2018) due to the presence of nutrient-rich water masses that upwell towards surface layers (Alvarez et al., 2005; Díaz López & Methion, 2018). The primary production caused by this upwelling system is almost twice that in the global coastal zone (Alvarez-Salgado et al., 2000; López et al., 2004), and is expressed in high biodiversity (López et al., 2002, 2003, 2004; Santos et al., 2007; Pierce et al., 2010), which includes 300 species of fish (López et al., 2003, 2004; Santos et al., 2007), more than 78 species of cephalopods (López et al., 2003, 2004), and at least 19 species of marine mammals (16 cetaceans and 3 pinnipeds) (López et al., 2002, 2003, 2004; Santos et al., 2007). Resident marine mammal species include the common dolphin (*Delphinus delphis*), the harbour porpoise (*Phocoena phocoena*), and the bottlenose dolphin (*Tursiops truncatus*) (López et al., 2002, 2003).

2.1.2.1. Population of Ría de Arousa

The bottlenose dolphin (*Tursiops truncatus*) is the second most abundant species of marine mammals in Galician waters (López et al., 2004) with an estimation of at least 660 specimens (López et al., 2003; Santos et al., 2007), being present year-round (Methion & Díaz López, 2018). It is the most abundant species in the coastal waters, where they inhabit the series of rías along the coastline, in which residency patterns have been observed (López et al., 2003; Santos et al., 2007; Pierce et al., 2010; Fernández et al., 2011b; Díaz López & Methion, 2018; Methion & Díaz López, 2018). In Galician waters, bottlenose dolphins have a varied diet that includes 23 families of fish and 12 species of cephalopods, although their main prey seem to be blue whiting (*Micromesistius poutassou*) and hake (*Merluccius merluccius*) (Santos et al., 2011). In these waters they are vulnerable to several human impacts, such as boat traffic, bycatch (López et al., 2003), overfishing (Freire & García-Allut, 2000), and aquaculture industry (Díaz López & Methion, 2017). Despite of these threats, there is a core of resident bottlenose dolphins in Ría de Arousa with some other individuals coming and going at different periods of time (Methion & Díaz López, 2018). For this ría the abundance estimate for this species is of 56 individuals in autumn and 144 individuals in winter, indicating some level of emigration depending on the season (Methion & Díaz López, 2018). The group size within the bottlenose dolphin population can range from 1 to 64 individuals with most encountered groups (81%) being formed by less than 20 individuals (Díaz López & Methion, 2017). In this area there is a core of resident bottlenose dolphins, about 22 males and 4 females (Díaz López & Methion, 2017). According to Díaz López & Methion (2017) it is possible that resident males of Ría de Arousa have a smaller home range than females, showing strong site fidelity. Other individuals of the population show different levels of site fidelity with fluctuations in temporary emigration rates by coming in and out of this area for different periods of time. The fact that females have larger home ranges in Ría de Arousa may be explained by the absence of natural predators.

In this area, bottlenose dolphins are frequently observed using areas of shellfish production and shallow waters, which shows a fine-scale pattern of habitat selection (Díaz López & Methion, 2017). It appears that shellfish farms have an effect on the distribution of bottlenose dolphins with a preference to waters in their vicinity, as a result of large aggregations of fish around the rafts, providing higher concentration and quality of prey (i.e. families Gadidae and Carangidae) for the dolphins (Díaz López & Methion, 2017). This reduces their time spent searching for prey and the energy required to feed (Methion & Díaz López, 2018).

2.2. Data collection

While working on the field, a 12 m long research vessel “Tyba III” powered by two 180 hp inboard engines was used (fig.:4). On the vessel the observers were responsible for scanning the sea surface (360º) with naked eye and/or binoculars (10 x 50) with the main goal of searching for bottlenose dolphins. There was always the presence of at least one experienced photographer and at least 3 experienced observers on the flying bridge located 4 m above the sea level. Boat based observations depend on weather conditions. They were performed during daylight hours when there was no rain or fog and when the sea conditions were up to 3 on the Douglas sea force scale (approximate equivalence to the Beaufort wind force scale) (Díaz López & Methion, 2018) (see Appendix III).

Figure 4: The three main observation positions on the boat by BDRI.

Every time bottlenose dolphins were spotted searching effort ceased and a sighting began with the recording of the date, initial and final time, location (Universal

Transverse Mercator, UTM, coordinates), and group size and composition. A group of dolphins was considered to be solitary dolphin when alone, or aggregation of dolphins when there was close spatial cohesion with interactions between individuals and all participating in the same activities (Methion & Díaz López, 2018). The group size was assessed based on the count of different individuals that were observed at one time in the area. During sightings, continuous photographic information was taken with digital single lens reflex (SLR) cameras equipped with a 35 to 300 mm telephoto zoom lens for posterior analysis in the laboratory. Group sizes were then reajusted according with the photographs taken during field work by increasing the number of individuals present if more individuals were photographed. Individual's age class was also estimated during the sightings by following the definitions for newborn, immature and adult of Díaz López & Methion (2017). Newborns were defined as dependent dolphins, until 1.5 m in length, with foetal marks, and swimming in the infant position below the mother (Methion & Díaz López, 2018). Immatures were considered to be the ones with few rake marks and skin lesions, two-thirds maximum the length of adults, and never observed in the infant position (Methion & Díaz López, 2018). Adults were considered to have a length > 2.5 m, darker skin colouration, and can be marked or unmarked (Methion & Díaz López, 2018).

2.3. Photo-identification

In the laboratory the photographs taken in each sighting from each course were organized into their respective date and sighting folder in order to facilitate the photo-id analysis. Photo-id analysis was carried out following Methion & Díaz López (2018). All the photo-id was reviewed independently by two experienced researchers. Bottlenose dolphins were identified based on the size, location, and patterns of nicks on the trailing edge of the dorsal fin and surrounding areas (Würsig & Jefferson, 1990). The individuals (dolphins) from a sighting were organized in different folders. For example the folder 1 A4 is the first folder created in the sighting for the individual called A4.

Figure 5: Interns working together on photo-identification in the institute, by BDRI.

After searching for individual A4 through all the photographs from the same sighting, other folders (inside folder 1 A4) will hold the best photograph of both sides of the dorsal fin, if possible, body photographs to identify skin marks, photographs with views of the head, genital area and fluke, in case there are photographs of them, and photographs of the animal's behaviour, in case there is a good sequence representing a behaviour. After this procedure, a second individual is identified, and the same steps are repeated for this individual.

During photo-ID it is important to identify the sex of the individuals, the age category, and determine which calf belongs to which female. The identification of the sex was possible by direct observations and/or by photographs of the genital region following Díaz López (2012). The males were sexed by the observation of an erection or by the characteristic gap (>2.5 cm) between the genital and anal slits. The females were sexed by the observation of mammary slits and absence of gap between the genital and anal slits.

Figure 6: Dorsal fins of three different dolphins with different characteristics, by BDRI.

Every photo of identified individuals was re-examined for false positives and false negatives, and to avoid human error caused by fatigue during photo-id analysis, the matching of the individuals was made with a catalogue including dolphins in the area since 2014 (Methion & Díaz López, 2018) and was confirmed by the use of the software DARWIN (ver. 2.22 (Eckerd College, Petersburg, FL, USA)).

2.4. Analysis of the photographs

In this study data collected during one year period were used to perform the detection of skin marks on the body of the identified individuals. By following a modified design of Marley et al. (2013) the dolphin's body was divided into four sections: anterior, ventral, central, and posterior (fig.:7). In each section the presence/absence, and the total number of the different types of marks (i.e. tooth rakes, skin disorders, etc.) were considered for each individual for posterior statistical analysis (see Appendix II).

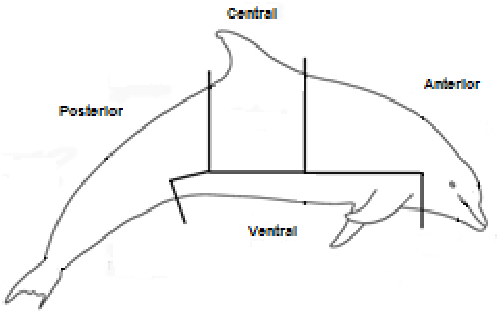

Figure 7: Design of the dolphin's body divided into four sections (modified from Marley et al., (2013)).

All photographs of the body of each individual were graded for quality in order to minimize misidentification of skin marks and heterogeneity in detection probabilities (i.e. pictures out of focus could limit the probability to detect certain types of skin marks). Following Díaz López et al. (2017) and Methion & Díaz López (2018), photographs were given an absolute value score (1, low; 4, average; 10, high) for: (1) the focus of the image being sufficient to allow all skin marks to be distinguished; (2) light intensity; and (3) the body being suitably sized in the frame for all skin marks to be clearly visible. The individual scores for each category were summed to obtain an overall quality score (OQS). OQSs < 12 were considered low quality; those from 12 to 18 were considered to be of average quality and those > 18 were considered excellent. To ensure correct detection of body marks, only average and excellent quality photographs were considered good enough to ensure correct identification.

2.5. Statistical analysis

Two parameters were calculated to assess the type and quantity of skin marks on the body side and on each body section: the prevalence (proportion of individuals with each type of skin mark) (Bertulli et al., 2016) and the richness of skin marks (relative proportion of each mark type to the total amount of marks) (based on Leone et al. (2019)). A third parameter was calculated for the full body side: the abundance of skin marks in the whole population (mean number of skin marks per individual).

The normal distribution of the parameters was checked using the Shapiro-Wilk test. The data were not normally distributed ($p < 0.05$). The richness and abundance of skin marks were then compared between males and females with the help of Mann-Whitney U-test (Bertulli et al., 2016; Leone et al., 2019).

To assess the distribution of the skin marks among the body sections for all individuals, and for males and females separately a Pearson's Chi-square test was used (Scott et al., 2005). Different combinations of variables were used to create contingency tables: 1. body section – number of skin marks for each mark type including all individual; 2. body section – number of skin marks for each mark type for males, and females separately; 3. sex – number of skin marks for each mark type considering the full body side; 4. sex – number of skin marks on each body section; 5. sex – number of skin marks for each mark type for the anterior, ventral, central, and posterior sections separately.

The data was compiled on Excel files and the analysis was done with the software PAST (Hammer et al., 2001).

Chapter III – Results

Boat-based surveys were carried out on 65 days between January and October 2017 over 352 hours, with 218 groups of dolphins observed during 98 hours. A total of 178 identified individuals were seen, and 21 adult individuals (12 males and 9 females) were used for the analysis.

The total number of photographs per individual varied between 50 and 390 with an average of 172 photographs per individual. They had an average of 0.6 high quality photographs and 19 average quality photographs. 57% of the individuals had pictures of all 4 body sections, 38% of 3 body sections, and 5% of 2 body sections (Fig.: 8). Not all the individuals had photographs of both sides of the body, therefore only one side of the body was analysed for each individual.

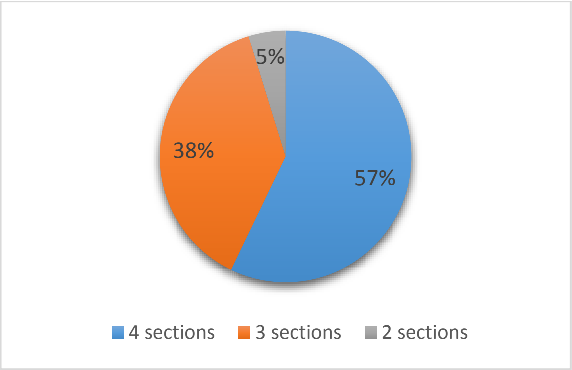

Figure 8: Percentage of individuals with 4, 3, or 2 body sections visible on photographs.

3.1. Types of marks present

Social-induced marks (tooth-rakes, nicks, and linear marks), skin disorders, and parasitic marks were observed, and none of the individuals showed human-induced marks. Therefore, these were not included in further analyses. No nicks were detected in the anterior section of the body in any of the observed individuals.

3.2. Prevalence of skin marks in the population

All the individuals presented skin disorders and social-induced marks with the exception of the parasitic marks (62% of the individuals). Males had a prevalence of 67% of parasitic marks and females had a prevalence of 56%. There were no differences on the prevalence of each type of mark between males and females considering the full body side ($X^2 = 0.09$, df = 4, $p > 0.05$) (fig.9). Overall, 85.7% of the individuals presented the tattoo skin disease, 52.4% presented light focal skin disease, and 28.6% presented dark focal skin disease. Within males, 91.7% presented the tattoo skin disease, and 41.7% and 33.3% presented the light and the dark skin diseases, respectively. The tattoo skin disease was present on 77.8% of the females, and the light and the dark focal skin diseases were present on 66.7% and 11.1% of the females, respectively.

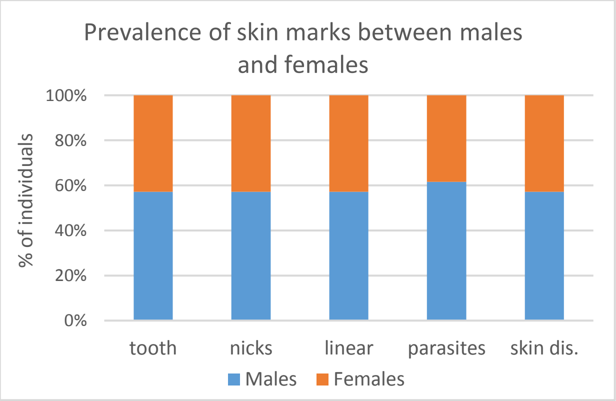

Figure 9: Prevalence of each skin mark type between males and females on the full body side.

All the body sections had a higher prevalence of tooth rake marks, ranging from 83% to 100% for all individuals, from 88% to 100% for males, and from 75% to 100% for females. Furthermore, they showed a lower prevalence of parasitic marks, ranging from 17% to 62% for all individuals, from 13% to 67% for males, and from 11% to 56% for females. On the ventral section the prevalence of nicks was lower as well with 25% for all individuals, 13% for males, and 50% for females (see Appendix IV).

For each of the four body sections, the prevalence of the five skin mark types did not show differences between sexes (anterior: $X^2 = 1.89$, df = 3, $p > 0.05$; ventral: $X^2 = 1.99$, df = 4, $p > 0.05$; central: $X^2 = 0.58$, df = 4, $p > 0.05$; posterior: $X^2 = 0.83$, df = 4, $p > 0.05$) (fig.:10) (see Appendix IV).

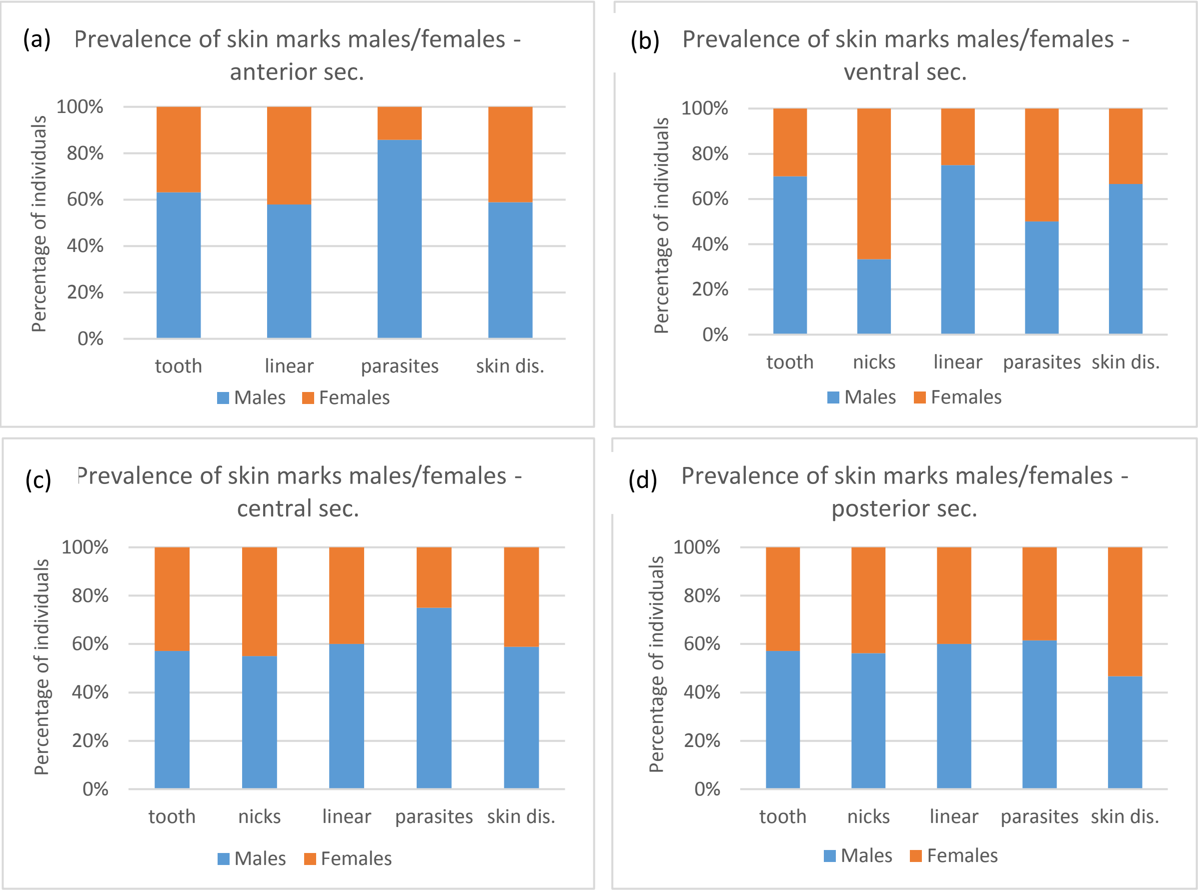

Figure 10: Percentage of males and females with each type of skin mark on the (a) - anterior section, (b) - ventral section, (c) - central section, and (d) - posterior section.

3.3. Abundance of skin marks

The abundance of skin marks was assessed only with individuals with the four sections of the body photographed. The abundance obtained was of 136 marks per individual (mean ± SE = 136.2 ± 21.3) for the full body side. When considered separately the abundance of skin marks was not significantly different between males (mean ± SE = 151.0 ± 27.7, n=8) and females (mean ± SE = 106.5 ± 31.1, n=4) (Mann-Whitney test, $z = 0.76$, $p > 0.05$).

3.4. Richness of skin marks

The richness of tooth-rake marks was the predominant for 62% of the individuals followed by the skin disorders being predominant for the rest of the 38% of the individuals. When comparing the full side of the body between males and females, males had a significantly higher richness of tooth-rake marks ($z = 2.17$, $N_1 = 12$, $N_2 = 9$, $p < 0.05$), while females had a significantly higher richness of skin disorders ($z = 2.31$, $N_1 = 12$, $N_2 = 9$, $p < 0.05$).When comparing each body section, for the anterior and central sections the richness of tooth-rake marks was significantly higher in males than females (anterior: $z = 3.05$, $N_1 = 12$, $N_2 = 8$, $p < 0.05$; central: $z = 2.42$, $N_1 = 12$, $N_2 = 9$, $p < 0.05$). There were no significant differences between sexes on the ventral section. On the posterior section the richness of skin disorders was significantly different between males and females with females having a significantly higher richness ($z = 2.23$, $N_1 = 12$, $N_2 = 9$, $p < 0.05$).

3.5. Distribution of skin marks

The skin mark type was highly significantly dependent of the body section both when all individuals were combined ($X^2 = 447.93$, df = 12, $p < 0.01$) and when sexes were considered separately (males: $X^2 = 349.13$, df = 12, $p < 0.01$; females: $X^2 = 184.74$, df = 12, $p < 0.01$) (fig.:11; see Appendix V). With all individuals combined the anterior section showed more skin disorders, while the posterior section had more tooth-rake marks (fig.:11(a)). The central section had a more homogeneous distribution of skin marks (fig.:11(a)). When considered separately, males and females also showed more skin disorders on the anterior section and more tooth-rake marks on the posterior section than on the other body sections (fig.:11(b), (c)).

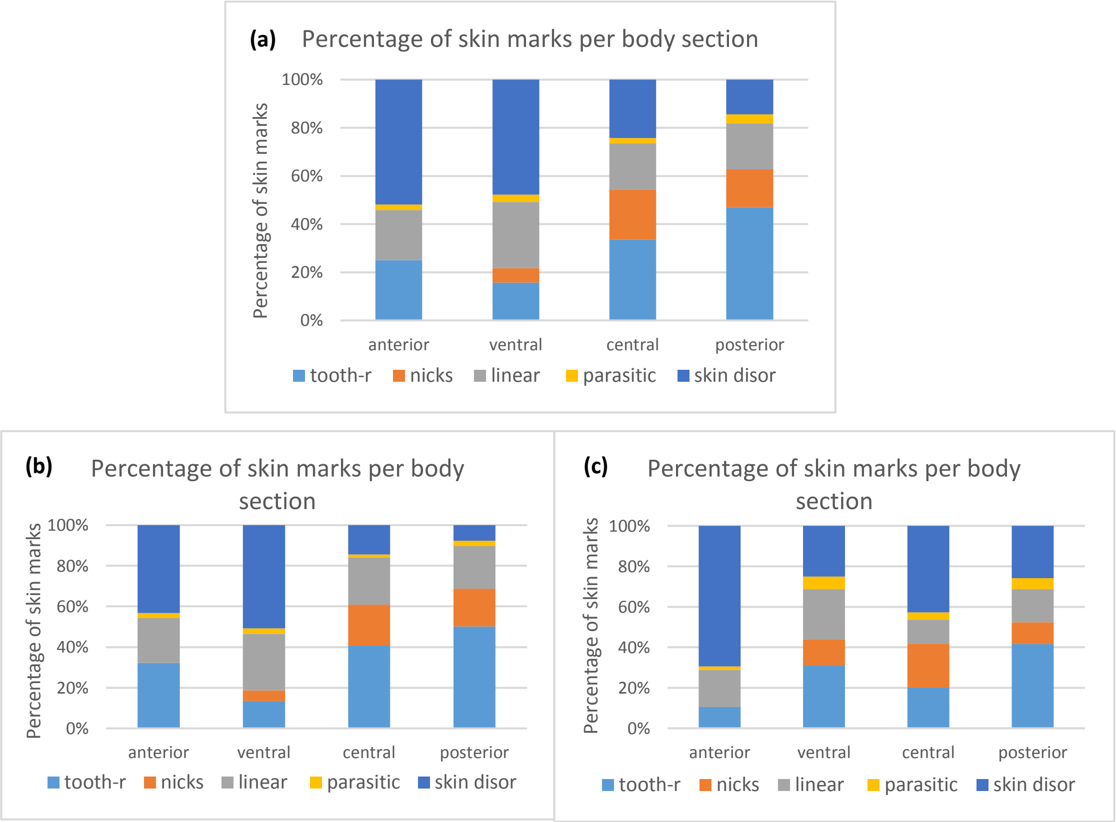

Figure 11: Distribution of each skin mark type on the body (a) - among all individuals; (b) - among males; (c) - among females.

When comparing the skin mark types and the body sections between males and females both were highly significantly dependent of the sex (skin mark type: $X^2 = 107.71$, df = 4, $p < 0.01$; body sections: $X^2 = 33.22$, df = 4, $p < 0.01$) (fig.:12; see Appendix VI). Males showed to have more tooth-rake marks on the body, while females had more skin disorder marks in comparison to the other types of skin marks (fig.:12(a)). Looking into the amount of skin marks on each body section, males had more marks on the ventral section than females (fig.:12(b)).

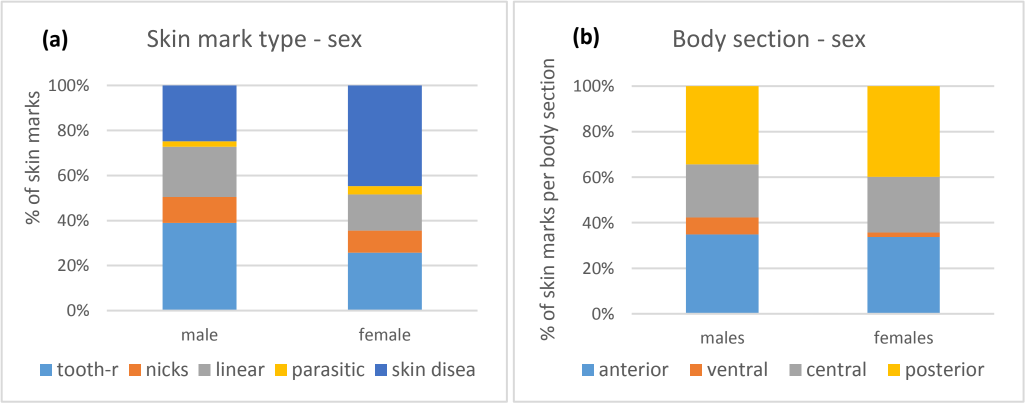

Figure 12: Graphs comparing (a) the amount of skin mark types and (b) the amount of skin marks on each body section between males and females.

When comparing each body section separately, the skin mark types were highly significantly associated with the sex of the individuals (anterior: $X^2 = 59.37$, df = 3, $p < 0.01$; central: $X^2 = 69.46$, df = 4, $p < 0.01$; posterior: $X^2 = 62.69$, df = 4, $p < 0.01$) (fig.:13; Appendix VI). However, concerning ventral sections, the number of observations in this body section was very low to allow for statistical comparisons. On the anterior, central, and posterior sections, males had more tooth-rake marks present than the other types of marks, while females had more skin disorder marks (fig.:13).

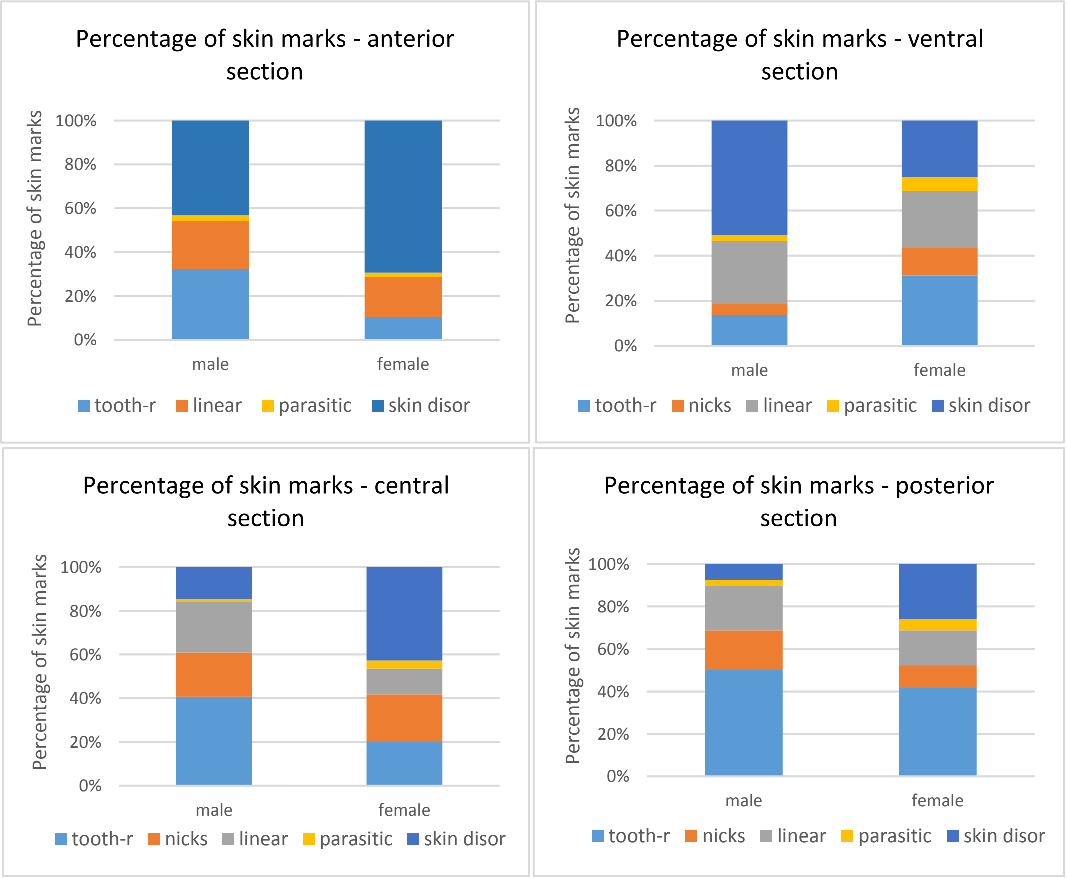

Figure 13: Distribution of skin mark types on males and females for each body section.

Chapter IV - Discussion

The aims of this study were to know which types of skin marks bottlenose dolphins from the Ría de Arousa exhibit and to assess the differences in the number and richness of the different skin mark types between adult male and female individuals.

4.1. Skin marks prevalence of bottlenose dolphins of the Ría de Arousa

The individual bottlenose dolphins studied in the Ría de Arousa showed a very high prevalence of all the skin mark types observed (disorders, tooth rakes, linear, nicks - 100%; parasitic - 62%). In other studies, the prevalence of skin disorders and other skin lesions is proved to be high in bottlenose dolphins (Wilson et al., 1999; Leone et al., 2019). The study published by Wilson et al. (1999) used bottlenose dolphins of ten different populations (Scotland, Wales, England, France, New Zealand, Croatia, Portugal, North Carolina, and Florida) in order to assess the prevalence of skin lesions among them. They verified that skin disorders were common in all the populations studied with between 63% and 100% of the individuals being affected. Leone et al. (2019) also verified that over 60% of the individuals studied were affected by skin disorders (diseases and parasitic) in the central Mediterranean Sea. Both of these studies are in accordance with our results, with the individuals having a high prevalence of skin marks.

All the types of skin marks observed in this study were also observed on other species of cetaceans such as the common minke whale (*Balaenoptera acutorostrata*), the white-beaked dolphin (*Lagenorhynchus albirostris*) in Icelandic waters (Bertulli et al., 2016), and the Indo-Pacific humpback dolphin (*Sousa chinensis*) at the Pearl River Estuary in Hong Kong (Chan & Karczmarski, 2019).

4.2. Skin disorders

Cetaceans have a relatively thick, keratin-rich skin that works as a physical barrier against injury and pathogens by having present immune cells from the immune system (Mouton & Botha, 2012; Van Dolah et al., 2015). Despite this barrier, skin disorders are globally common in several species of cetaceans (Van Bressem & Van Waerebeek, 1996; Wilson et al., 1999; Leone et al., 2019), including bottlenose dolphins (Van Bressem et al., 2007; Leone et al., 2019), depending on environmental (i.e temperature, salinity, etc.) and anthropogenic factors (i.e. habitat degradation, pollutants, etc.) of the different locations (Leone et al., 2019; Methion & Díaz López, 2019b). In this study, skin disorders were present on all the individuals, and were the second type of mark with the highest richness among individuals, with the highest among females. These marks usually suggest that the general population might not be in perfect health (Van Bressem et al., 2007), although some marks may result from congenital inheritance of depigmentation (Methion & Díaz López, 2019b). Health problems reflected by skin disorders may be caused by the presence of stressors such as chemical and biological contaminants in the environment (Van Bressem et al., 2007; Blacklaws et al., 2013). These and injuries may affect the natural skin barriers leading to disease (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2008).

Anthropogenic stressors may influence the course of diseases (Van Bressem et al., 2007) such as the release or use of sewage sludge for fertilizing purposes and wastewater effluents (Cobelo-Garcia & Prego, 2004; Filgueiras & Prego, 2007; Álvarez-Vásquez et al., 2016). These can lead to the increase of potential pathogens, eutrophication, and to higher concentrations of heavy metals into the ecosystems as observed at some rías of Galicia (Ferrol, and Vigo) (Cobelo-Garcia & Prego, 2004; Filgueiras & Prego, 2007; Mouton & Botha, 2012; Álvarez-Vásquez et al., 2016). As in those rías, untreated wastewater effluents from the canning industry can be a source of water contamination in Ría de Arousa. Moreover, bottlenose dolphins as top predators tend to accumulate high levels of chemicals and heavy metals in their tissues possibly turning them more susceptible to disease

(Wilson et al., 1999). Cobelo-Garcia & Prego (2004) compared the mean concentrations of heavy-metals in the Ría de Ferrol with the mean concentrations of heavy-metals in other water systems of Galícia and other places of the world. They verified that in Galicia these concentrations are important, which might be one of the reasons why in this study all the individuals were affected by skin disorders, which, according to the available literature, has not been verified in other areas.

Lower water temperature and salinity could lead to a higher incidence and severity of skin disorders since the first may limit blood flow to the skin blocking immune protection or limiting the rate of cell regeneration, and the second may cause cellular damage to the epidermis weakening its ability to protect the animal from infectious agents (Wilson et al., 1999; Mouton & Botha, 2012; Van Bressem et al., 2015). The Ría de Arousa is strongly influenced by the Ulla and Umia rivers and by oceanic waters, the salinity of the surface waters (5 m) range between 32 and 36 ppm reaching values as low as 3 ppm when runoffs occur during raining seasons with the temperature ranging between 11 and 20 °C (Macho et al., 2010). This study area has a relatively big range of salinity values and temperature that can affect the occurrence of skin disorders on the bottlenose dolphins studied, as it has been observed by Wilson et al. (1999). They verified at Moray Firth, Scotland, that the epidermal condition was poor with up to 66% of skin covered by epidermal lesions with a prevalence of 98.5%, where bottlenose dolphins experience the lowest water temperature and salinity (9.2 °C and 34.27 psu). Here, males and females had a higher richness of skin disorders on the anterior section of the body compared to other body sections. This can be explained by the fact that this section is more exposed to air and sunlight when they breathe. This was observed in other species of cetaceans such as southern right whales (*Eubalaena australis*) (Harzen & Brunnick, 1997) and on Peale's and Chilean dolphins (Sanino et al., 2014). The exposure to wind and sun turns the skin drier, becoming darker and cracked and blisters can appear when exposed for a long period of time (Sweeney & Ridgway, 1975).

Social-induced skin marks such as tooth-rakes and nicks can also be a route of infection, leading to skin disorders (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2015). It was observed on dusky dolphins (*Lagenorhynchus obscurus*) from the eastern South Pacific that tattoo skin disease marks were sometimes associated with scars (Van Bressem & Van Waerebeek, 1996).

In this study, the origin of skin disorders is not known with certainty as the data were analysed visually with photographs. Here, it was possible to recognise different types of skin disorders such as focal skin disease and tattoo skin disease (Appendix II). Tattoo skin disease (TSD) has been well studied in odontocetes and has been proven to be an effective way of visual diagnosis of poxvirus infections (Blacklaws et al., 2013). Poxvirus infections can be detected by tattoo skin disease lesions (Harzen & Brunnick, 1997). These virus belong to the genus *Cetaceanpoxvirus* of the subfamily Chordopoxvirinae from the family Poxviridae (Bracht et al., 2006). Three types of morphology were observed by Blacklaws et al. (2013) depending on the phase of infection: the acute phase is represented by dark, irregular or rounded lesions with a stippled pattern, the healing phase is represented by coalesced, circular, light grey patches with a darker outline, and the last phase is represented by grey rounded marks surrounded by a darker ring. Some skin disorders such as TSD may persist for several months, or even years, or may heal and sometimes return (Sanino et al., 2014). Since it is easily distinguished from other skin disorders, it is considered to be a potential general health indicator for cetaceans (Blacklaws et al., 2013; Bossart et al., 2015). Skin disorders have been associated with moments of higher stress levels in marine mammals, climate events, degraded environmental conditions, and compromised general health (Harzen & Brunnick, 1997; Bossart et al., 2015). TSD and pale dermatitis were observed on bottlenose dolphins in Sado Estuary (Portugal) by Van Bressem et al. (2003) and in Sepetiba Bay (Brazil) by Van Bressem et al. (2015), respectively.

Apart from the TSD mark that has been effective in the diagnosis of poxvirus infections, the use of visual identification of skin marks caused by disease is a limited method to determine the exact cause. Histological analysis together with the analysis of photographs can be used to understand if other skin disorder marks are an effective way of diagnosis for other infection origins (Bertulli et al., 2012; Leone et al., 2019). Skin disorders caused by bacteria are rare, usually happening after viral, parasitic, or traumatic lesions (Harzen & Brunnick, 1997).

According to Díaz López & Methion (2017), bottlenose dolphins are the most commonly observed cetacean species in the study area. Some years ago, more specifically on the 13th November 2002, a tanker called Prestige that transported 77 000 tons of heavy fuel-oil, broke in two and sunk at the Galician bank offshore and affected more than 800 km of the NW Spanish coast (González et al., 2006; Penela-Arenaz et al., 2009). This accident released polycyclic aromatic hydrocarbons (PAHs), and other toxic components into the aquatic environment (González et al., 2006; Penela-Arenaz et al., 2009). These components are believed to contribute to the severity of viral infections through toxicity in the immune system (Ross, 2002). The spill of oil leads to different ways of exposure for cetaceans such as dermal absorption, inhalation of volatile compounds above the surface, and ingestion of contaminated food (Robineau & Fiquet, 1994; Schwacke et al., 2014; Lane et al., 2015). The release of PAHs and other components in the environment may be one reason for the high prevalence of skin disorders in these bottlenose dolphins due, for example, to the ingestion of contaminated prey.

Some studies such as the one conducted by Lane et al. (2015) found that the reproductive success rate decreased and that the mortality rate increased in the population of bottlenose dolphins from Barataria Bay (Louisiana, USA) after the Deepwater Horizon (DWH) oil spill. The exposure of bottlenose dolphins to oil spills and consequent petroleum hydrocarbons led to increased incidence of adrenal gland and lung diseases, and immunosuppression (Lane et al., 2015; Van Dolah et al., 2015). As it has been shown in other populations, such as the one from Barataria Bay, petroleum hydrocarbons could have led to immunosuppression in the individuals studied thus inducing skin disorders.

Marine mammals, especially those with a coastal distribution, are at risk from persistent organic pollutants (POPs) that include polychlorinated biphenyls (PCBs) and pesticides via food (Aguilar et al., 1999; Pierce et al., 2008; Méndez-Fernandez et al., 2017). PCBs are lipophilic pollutants and, in cetaceans, their higher concentrations are found in blubber (Pierce et al., 2008; Méndez-Fernandez et al., 2014a, 2017). They are very common in industrialized regions (Ricca et al., 2010) and can lead to immunosuppression and reproductive impairment in mammals by being hormone and endocrine disrupters (Aguilar et al., 1999; Murphy et al., 2010; Jepson et al., 2016). The immunosuppression increases the susceptibility to disease and presence of parasites (Jepson et al., 2016). Among the most common cetacean species in the NW of Spain, the bottlenose dolphin exhibits the highest concentrations of PCBs (Méndez-Fernandez et al., 2014a; Jepson et al., 2016; Méndez-Fernandez et al., 2017). Jepson et al. (2016) found high mean concentrations of more than 100.0mg/kg lipid weight for bottlenose dolphins off Iberian Peninsula (NE Atlantic) with the highest marine mammal PCB toxicity threshold being 41mg/kg lipid weight. They also observed that adult males had a higher concentration than adult females. However, when considering immature individuals, they observed that females had a concentration of PCBs slightly higher than males. The high concentrations of PCBs found on bottlenose dolphins from the NE of the Iberian Peninsula may explain the high prevalence of skin disorders on the bottlenose dolphins observed in this study. Méndez-Fernandez et al. (2014b) studied the concentration of different heavy metals on five species of small cetaceans in the NW of the Iberian Peninsula, including common dolphin (*Delphinus delphis*), striped dolphin (*Stenella coeruleoalba*), long-finned pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), and bottlenose dolphin, taking into account also the sex. It was shown that bottlenose dolphins exhibited detectable concentrations of toxic elements such as arsenic (As), mercury (Hg), and cadmium (Cd) probably due to higher exposure through diet and indirectly the environment, and did not find any relationship between heavy metal concentrations and sex (Méndez-Fernandez et al., 2014b). Both concentration of PCBs and levels of toxic elements could have contributed to the development of skin disorders on the individuals in this study area.

Bottlenose dolphins are the only cetacean species to have been observed within the aquaculture zones of Ría de Arousa, using these areas very frequently (Díaz López & Methion, 2017). The abundance estimation is of 56 individuals in autumn and 144 individuals in winter (Methion & Díaz López, 2018) with a core resident population of about 22 individuals (Díaz López & Methion, 2017). Its coastal distribution makes this species more exposed to pollutants (Aguilar et al., 1999; Méndez-Fernandez et al., 2017). Apart from other xenobiotics, shellfish aquaculture can lead to water pollution due to the use of antifouling paint biocides that contain toxic compounds (Kannan et al., 1996; Methion & Díaz López, 2019a). The fact that the bottlenose dolphins are commonly found within shellfish farms, exposes them to these compounds, which can make them more vulnerable to infections due to immunosuppression (Kannan et al., 1996), which in turn might lead to skin disorders.

4.3. Sex differences in skin disorders

Differences on the richness of skin disorders between sexes are not commonly observed in odontocetes but it has been observed in Burmeister's Porpoises (*Phocoena spinipinnis*) near Peru, with males having a higher prevalence of TSD marks than females (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009). The aggressive interactions between males was suggested to be the reason of that difference (Van Bressem et al., 2009). In this study, male bottlenose dolphins exhibited indeed more social-induced marks than females. Social-induced marks are thought to be one of the reasons why males have more skin disorders than females in all the other studies done on skin mark differences between sexes (Van Bressem et al., 2015). However, even though males had more skin marks induced by social interaction than females, there were no differences on the amount of skin disorder marks between both sexes. This way, the "theory" that more social skin marks lead to more skin disorders does not apply to the bottlenose dolphins in this study area.

4.4. Social-induced marks

All the individuals had social-induced skin marks showing that these bottlenose dolphins have a high level of physical interactions between individuals regardless of sex. However, males had more social-induced skin marks than females suggesting behavioural differences between sexes. Conspecific aggression between male bottlenose dolphins often involves several individuals due to competition between alliances for access to females (Connor et al., 2000; Scott et al., 2005), which seems to be the principal reason for the difference in the amount of this type of skin mark. In the Mediterranean Leone et al. (2019) also verified that bottlenose dolphin males have more social-induced marks than females.

Males showed more tooth-rake marks on the central and posterior sections. Females had more tooth-rake marks on the posterior section than on the other body sections. It appears that the central and posterior sections are more likely to show scarring when individuals interact, possibly because they use their dorsal side and peduncle (less vulnerable) as protection from attacks (Heithaus, 2001).

Scott et al. (2005) used photographs to analyse the prevalence of tooth rake marks and they found that wild Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) males had more marks than females. Marley et al. (2013) had the same results with differences between sexes. Since in bottlenose dolphins marks regain pigmentation they could indicate relative rates and timing of received aggression.

4.5. Parasitic marks

In this study 62% of the individuals showed skin marks caused by parasites, and most of them are suspected to have been originated by lampreys. In this area, the species of lamprey existing is the sea lamprey (*Petromyzon marinus*) (Silva et al., 2013). These ectoparasites live several years in freshwater as larvae (Lança et al., 2013; Silva et al., 2013). Then they undergo metamorphosis that allows them to migrate to the sea and begin hematophagous feeding (feed on blood) to reach adult size and sexual maturation (Lança et al., 2013; Silva et al., 2013, 2014). In the case of this study area, the first months of the hematophagus feeding occur during the downstream migration from the rivers and the residence in the Ría de Arousa (Silva et al., 2013). The sea lamprey has a varied diet feeding on fishes, such as sturgeon (*Acipenser sturio*), herring (*Clipea harengus*), haddock (*Melanogrammus aeglefinus*), Atlantic salmon (*Salmo salar*) (Maitland, 1980), and sometimes they feed on cetaceans (Maitland, 1980; Lança et al., 2013; Silva et al., 2014). They use the cetacean skin as a source of nutrients and attach with the help of their teeth of its sucking disc (Samarra et al., 2012). When attached the lampreys use a rasping tongue to abrade the epidermis down to the blubber (Pike, 1951; Samarra et al., 2012). They leave a typical white or greyish oval-shaped scar with a pit in the middle (Pike, 1951). These type of scars can also be a way of infection by micro-organisms (Pike, 1951).

4.6. Human-induced marks

In spite of Ría de Arousa being an area with high levels of marine traffic (Freire & García-Allut, 2000; López et al., 2003) no human-induced injuries were found on any individual. This type of mark was absent from the individuals used in this study probably because only a few individuals (adult sexed individuals) were included in the analysis. A study made by Covelo & Martinez (2001) analysed the number of cetacean strandings in Portugal and along the NW coast of Spain between 1996 and 1998. They verified that 53% of the strandings caused by bycatch, including bottlenose dolphin strandings, were observed in Galicia. Lopez et al. (2002) studied the number of strandings and by-catch in Galician waters from 1990 to 1999 and observed that for bottlenose dolphins 14% of the strandings recorded were caused by by-catch.

As mentioned before, bottlenose dolphins are the only cetacean species to have been observed inside of the shellfish aquaculture zone, using these areas frequently (Díaz López & Methion, 2017). Methion & Díaz López (2019a) showed that this population uses these areas to feed due to the high biodiversity and abundance of fish, making it less energetically costly for the dolphins. Despite of shellfish aquaculture in Ría de Arousa having an impact in terms of food resource, this activity can pose threats to this population such as increased collisions with vessels working in the area, habitat loss, and noise and water pollution (Díaz López, 2012; Methion & Díaz López, 2019a).

Even though no human-induced marks were observed on this study, other studies proved that human activities have an impact (López et al., 2003; Methion & Díaz Lópes, 2019a). In this area, bottlenose dolphins are subjected to several threats, such as marine traffic, bycatch (López et al., 2003), and overfishing (Freire & García-Allut, 2000). Long term studies will allow the use of a higher number of individuals belonging to a wider range of age categories giving more accurate results.

Chapter V – Conclusions

In conclusion, this study gives important information on how severely bottlenose dolphins from Ría de Arousa are affected by skin disorders, since they are the second type of mark, right after the social-induced marks, with the highest richness among individuals. This study also confirms the higher incidence of social-incidence marks on male bottlenose dolphins from Ría de Arousa, as shown elsewhere in the world. The visual use of skin disease marks gives an idea of the variety of diseases for which origin is unknown showing the need for histological research to confirm the causes. In the future, it would be interesting to make a more detailed study on skin mark differences between males and females combined with age, sexual coercion of females, and individual reproductive status. More long-term studies need to be done worldwide.

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Appendix I

Table 1: Examples of prey species used by bottlenose dolphins along their global range.

Squid (*Sepioteuthis australis , Illex illecebrosus, Ornithoteuthis antillarum, Todarodes sagittatus, Illex condetii, Loligo gahi*) - (Walker et al., 1999; Zanardo & Parra, 2016; Santos et al., 2007a)
Trevally (*Pseudocaranx spp.*) - (Zanardo & Parra, 2016)
Garfish (*Hyporhamphus melanochir*) - (Díaz López, 2012; Zanardo et al., 2016)
Yellow-eyed mullet (*Aldrichetta forsteri*) - (Zanardo & Parra, 2016)
Horse mackerel (*Trachurus trachurus*) - (Bearzi & Politi, 1999)
Gilt sardine (*Sardinella aurita*) - (Bearzi & Politi, 1999)
Sardine (*Sardina pilchardus*) - (Bearzi & Politi, 1999)
Pacific sardine (*Sardinops sagax*) - (Bearzi, 2004; Santos et al., 2007a, 2007b)
Seabass (*Atractoscion nobilis*) - (Bearzi, 2004)
Splitnose rockfish (*Sebastes diploproa*) - (Bearzi, 2004)
Slender conger (*Uroconger lepturus*) - (Condet & Dulau-drouot, 2016)
Kaup's arrowtooth eel (*Synaphobranchus kaupii*) - (Condet & Dulau-drouot, 2016)
Striped mullet (*Mugil cephalus*) - (Acevedo, 1991; Irvine et al., 1980; Walker et al., 1999; Shane, 1990)
Spotted seatrout (*Cynoscion nebulosus*) - (Walker et al., 1999; Shane, 1990)
Sand seatrout (*Cynoscion arenarius*) - (Walker et al., 1999; Shane, 1990)
Needlefish (*Strongylura notate*) - (Irvine et al., 1980; Shane, 1990)
Hardhead catfish (*Arius felis*) - (Irvine et al., 1980; Shane, 1990)
Gafftopsail catfish (*Bagre marinus*) - (Shane, 1990)
Snook (*Centropomus undecimalis*) - (Shane, 1990)
Balao (*Hemiramphus balao*) - (Shane, 1990)
Ladyfish (*Elops saurus*) - (Shane, 1990)
Silver perch (*Bairdiella chrysoura*) - (Shane, 1990)
Cods (Gadidae) - (Santos et al., 2007a, 2007b; Santos et al., 2001; Weir & Stockin, 2001)
Curled octopus (Eledone cirrhosa) - (Reid & Wilson, 1995; Santos et al., 2007a; Santos et al., 2001; Weir & Stockin, 2001)
Salmon (*Salmo salar*) - (Reid & Wilson, 1995; Santos et al., 2007a; Santos et al., 2001; Weir & Stockin, 2001)
Spot (*Leiostomus xanthurus*) - (Walker et al., 1999)
Atlantic croaker (*Micropogon undulates*) - (Walker et al., 1999)
Anchovy (*Engraulis encrasicholus*) - (Bearzi & Politi, 1999)
Sprat (*Sprattus sprattus*) - (Bearzi & Politi, 1999)
Hake (*Merluccius merluccius*) - (Bearzi & Politi, 1999; Fernández et al., 2011; López et al., 2004; Pierce et al., 2010; Spyrakos et al., 2011)
Blue whiting (*Micromesistius poutassou*) - (Fernández et al., 2011; López et al., 2004; Pierce et al., 2010; Spyrakos et al., 2011)
Whiting (*Merlangius merlangus*) - (Santos et al., 2007a, 2007b; Santos et al., 2001)
Saithe (*Pollachius virens*) - (Santos et al., 2007a, 2007b; Santos et al., 2001)
Scad (*Trachurus trachurus*) - (Santos et al., 2007a)
Black scabbard fish (*Aphanopus carbo*) - (Santos et al., 2007a)
Haddock (*Melanogrammus aeglefinus*) - (Santos et al., 2001)
Ballyhoo (Hemiramphus brasiliensis) - (Lewis, 2003)

Appendix II

Table 2: Definitions and figures representing the different types of marks that are going to be focused on this study.

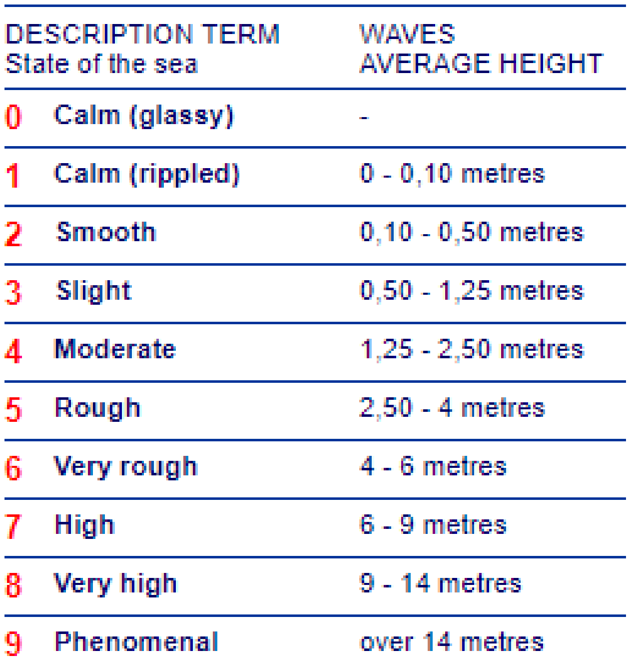
Type of mark	Definition	Figure
Social origin	**Nicks** Bites (missing pieces of tissue) on the dorsal fin, flippers, flukes and peduncle caused by interaction with conspecifics, or entanglement. (Heyning, 1984; Wells et al., 2008; Würsig & Würsig, 1977)	
	Tooth rakes Long, thin, parallel or nearly parallel scratches caused by teeth that penetrate the skin and often the blubber tissues. (Lockyer & Morris, 1990; Marley et al., 2013; Samuels & Gifford, 1997)	
	Linear marks Scrapes on the skin of unknown origin (Bertulli et al., 2016)	

Infectious origin	**Tattoo skin disease** TSD is characterised by very typical, irregular, grey, black or yellowish, stippled cutaneous lesions referred to as tattoos. (Van Bressem et al., 2009)	
	Dark focal skin disease Characterized by numerous small dark rounded marks distributed in an almost uniform pattern, which aetiology is unclear (Sanino et al., 2014).	
	Light focal skin disease Characterized by numerous small light rounded marks distributed in an almost uniform pattern, which aetiology is unclear (Sanino et al., 2014).	

Parasitic origin	**Lamprey** **(***Petromyson marinus***)** Typical white or greyish oval-shaped scar with a pit in the middle, including raised borders. (Pike, 1951; Bertulli et al., 2016)	

Appendix III

Table 3: Douglas sea state scale taken from http://www.eurometeo.com/english/docs.



DESCRIPTION TERM State of the sea		WAVES AVERAGE HEIGHT
0	Calm (glassy)	-
1	Calm (rippled)	0 - 0,10 metres
2	Smooth	0,10 - 0,50 metres
3	Slight	0,50 - 1,25 metres
4	Moderate	1,25 - 2,50 metres
5	Rough	2,50 - 4 metres
6	Very rough	4 - 6 metres
7	High	6 - 9 metres
8	Very high	9 - 14 metres
9	Phenomenal	over 14 metres

Appendix IV

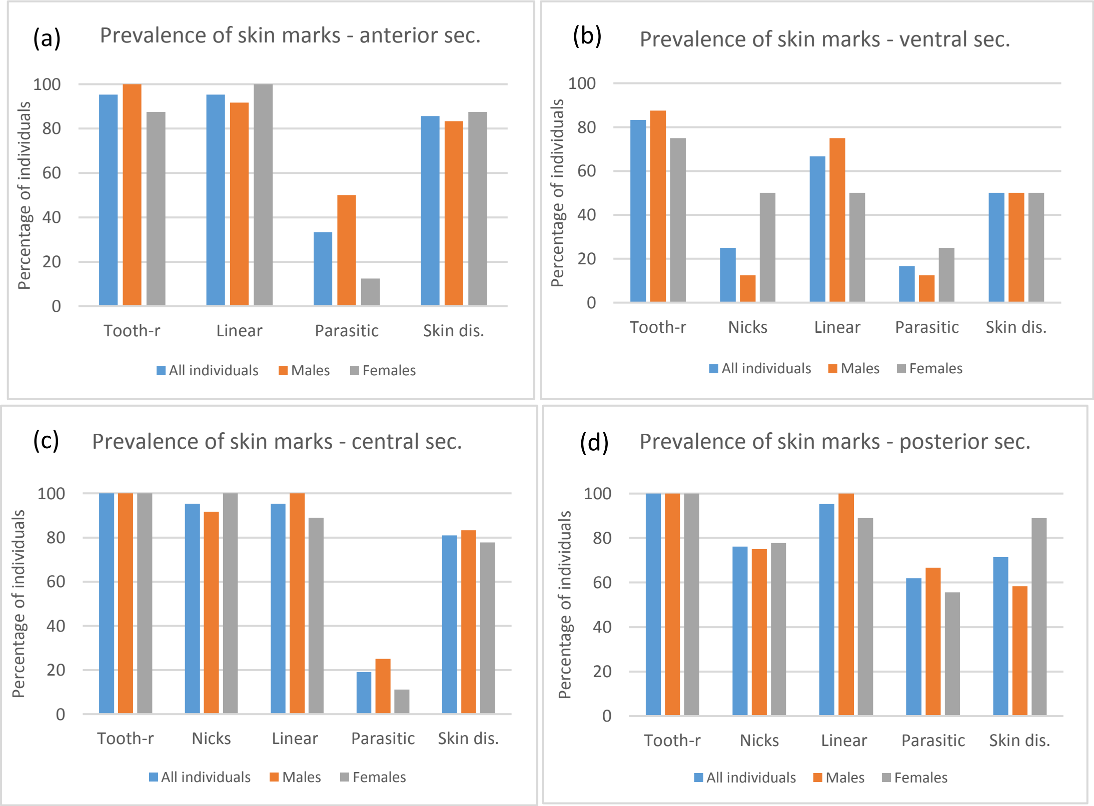


Figure 14: Prevalence of skin marks among all individuals, males and females on the (a) - anterior section, (b) - ventral section, (c) - central section, and (d) - posterior section.

Appendix V

(a)

	anterior	ventral	central	posterior	total
tooth-r	204	21	188	402	815
nicks	0	8	116	134	258
linear	169	37	108	164	478
parasitic	19	4	12	32	67
skin dis.	422	64	136	123	745
total	814	134	560	855	2363

(b)

	anterior	ventral	central	posterior	total
tooth-r	176	16	149	270	611
nicks	0	6	74	100	180
linear	120	33	85	112	350
parasitic	14	3	5	15	37
skin dis.	236	60	53	41	390
total	546	118	366	538	1568

(c)

	anterior	ventral	central	posterior	Total
tooth-r	28	5	39	132	204
nicks	0	2	42	34	78
linear	49	4	23	52	128
parasitic	5	1	7	17	30
skin dis.	186	4	83	82	355
total	268	16	194	317	795

Figure 15: Contingency tables between skin mark types and body sections (a) - among all individuals, (b) - among males, (c) - among females.

Appendix VI

(A)

(a)

	male	female	total
tooth-r	611	204	815
nicks	180	78	258
linear	350	128	478
parasitic	37	30	67
skin dis.	390	355	745
total	1568	795	2363

(b)

	males	females	total
anterior	546	268	814
ventral	118	16	134
central	366	194	560
posterior	538	317	855
total	1568	795	2363

(B)

(a)

	male	female	total
tooth-r	176	28	204
linear	120	49	169
parasitic	14	5	19
skin dis.	236	186	422
total	546	268	814

(b)

	male	female	total
tooth-r	16	5	21
nicks	6	2	8
linear	33	4	37
parasific	3	1	4
skin dis.	60	4	64
total	118	16	134

(c)

	male	female	total
tooth-r	149	39	188
nicks	74	42	116
linear	85	23	108
par.fish	5	7	12
skin dis.	53	83	136
total	366	194	560

(d)

	male	female	total
tooth-r	270	132	402
nicks	100	34	134
linear	112	52	164
par.fish	15	17	32
skin dis.	41	82	123
total	538	317	855

Figure 16: Contingency tables between (A) males/females and (a) skin mark types, and (b) body sections; (B) skin mark types and male/female for each body section (a) anterior, (b) ventral, (c) central, and (d) posterior.

Skin mark patterns of bottlenose dolphins (*Tursiops truncatus*) in the Ría de Arousa, Galicia (Spain)

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INTRODUCTION

Cetaceans frequently display skin marks that can be caused by diseases, parasites, and interactions among conspecifics and with human activities. In the present study, we used one year of photo-identification data for resident bottlenose dolphins. The aims of this study were to assess the types of skin marks present on resident bottlenose dolphins from Ría de Arousa (NW Spain) (Methion & Díaz López 2018) (Figure 1) and compare them between adult males and females.

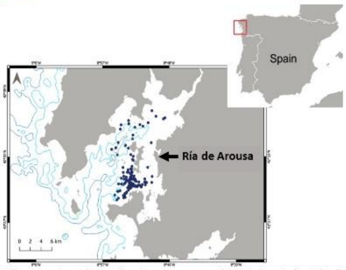

Figure 1: The Ría de Arousa (NW Spain). Circles represent sightings of bottlenose dolphins used for this study.

METHODS

Boat-based surveys were carried out between January and October 2017. Photographs from 21 adult sexed individuals (12 males and 9 females) were used to assess the prevalence of the different types of skin marks (skin disorders, social-induced marks, parasitic marks) present on the four body sections (Figure 2).

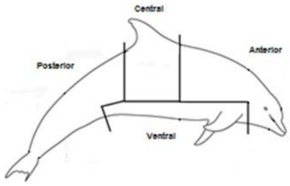

Figure 2: Design of the dolphin's body divided into four sections (modified from Marley et al., (2013)).

RESULTS

We monitored 218 groups of bottlenose dolphins during 98 hours among 65 daily boat-based surveys. A total of 178 bottlenose dolphins were identified through photo-identification, and 21 adult individuals were sexed (12 males and 9 females). Skin disorders and social induced marks had a prevalence of 100%, and the parasitic marks had a prevalence of 62% (Figure 3). There were no differences on the prevalence of each type of skin mark between sexes, considering the full body side ($X^2 = 0.09$, df = 4, $p > 0.05$) and each body section (anterior: $X^2 = 1.89$, df = 3, $p > 0.05$; ventral: $X^2 = 1.99$, df = 4, $p > 0.05$; central: $X^2 = 0.58$, df = 4, $p > 0.05$; posterior: $X^2 = 0.83$, df = 4, $p > 0.05$) (Figure 3).

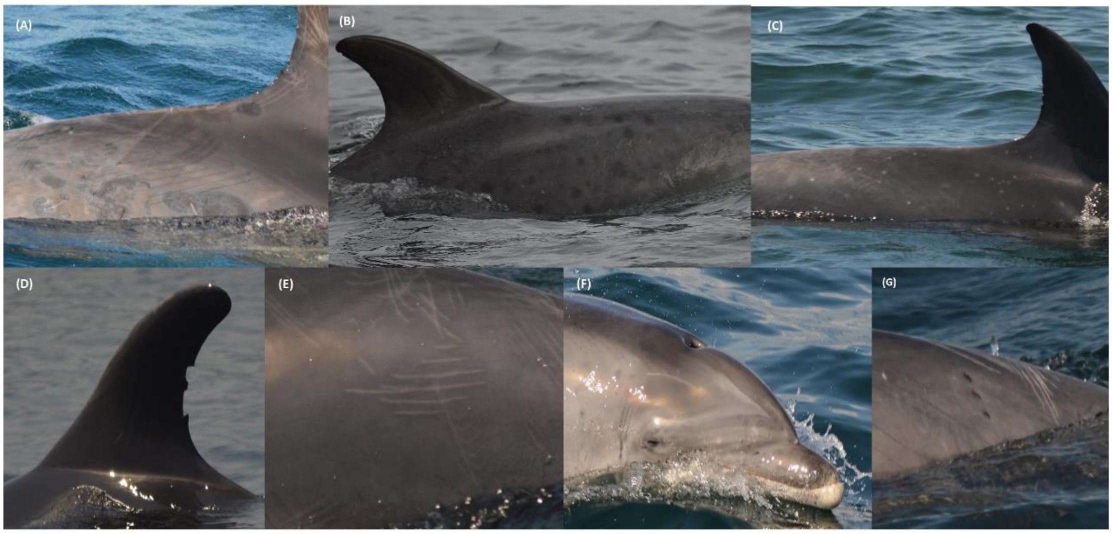

Figure 3: The different types of skin marks observed:

- Skin disorders: (A) tattoo skin disease (TSD); (B) dark focal skin disease; (C) light focal skin disease;
- Social-induced marks: (D) nicks; (E) tooth rakes; (F) linear marks;
- Parasitic marks: (G) lamprey bites.

CONCLUSIONS

The individual bottlenose dolphins studied in the Ría de Arousa showed a very **high prevalence** of all the skin mark types observed. **Environmental and anthropogenic stressors** can be affecting the occurrence of skin disorders.

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