PRIFYSGOL BANGOR UNIVERSITY

Causes of spatio-temporal trends in skin lesions of Welsh bottlenose dolphins

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“There’s no question dolphins are smarter than humans as they play more.”

Albert Einstein

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List of Abbreviations

AFT: Abraded fin tip

ANOVA: Analysis of Variance

BND: bottlenose dolphin

CCC Ceredigion County Council

CCW Countryside Council for Wales

CW: Casual Watch

DS: Dedicated search

GAM: General additive model

GLM: General linear model

ID: Photo-identification

IO: Independent observer

Km: Kilometres

LT: Line transects

m: meters

NE: Northeast

NLT: Non-line transects

NRW: Natural Resources Wales

PO: Primary observer

POPs: Persistent organic pollutants

SAC: Special Area of Conservation

SWF: Sea Watch Foundation

TSD: Tattoo skin disease

Abstract

Diseases in the marine environment and their tendency for potential outbreaks pose a major threat for a variety of ocean-dwelling animals, including marine mammals such as cetacean species. As apex predators, cetaceans are capable of accumulating contaminants both from the consumed prey and the environment, reflecting in that way ecosystem’s health. The bottlenose dolphin (*Tursiops truncatus*) is an ideal bio-indicator in terms of detection of diseases due to its coastal distribution and sociality. Sociality seems to play a key role in the transmission of diseases. Evaluation of epidermal skin abnormalities (skin lesions) on bottlenose dolphins is an effective way to determine the occurrence of a disease. The most common way of such evaluation is through visual inspection with the use of photo-ID techniques. In the majority of the studies, skin lesions have been categorised according to their colour and texture, and their occurrence appears to be linked to both anthropogenic and environmental factors. Cardigan Bay is the largest embayment in the UK with the highest abundance of semi-resident bottlenose dolphin populations. The aims of the study were: i) to investigate if there is a temporal pattern in the prevalence of skin lesions in Welsh bottlenose dolphins and ii) whether there is a difference in the prevalence of these lesions among groups of individuals for the period 2011-2018, using photo-ID techniques. Overall, 213 individuals (182 adults & 31 calves) were analysed for four main categories of lesions. Two of these categories were caused by viral diseases and the other two by a combination of intra-specific interactions and anthropogenic factors (e.g. fishing gears). Over the years, tattoo lesions and wounds showed an increase in prevalence. Among groups of individuals, discolouration and wounds were found to be more prevalent in adult males, while punctiform (herpes-like) marks were found to be more prevalent in calves. With many concerns and questionings raising regarding potential increases in human intervention and impacts in coastal areas along with the climate change, conservation and sustainability of bottlenose dolphin populations in Cardigan Bay and in general, are of high importance. Hence, conduction of further quantitative studies focusing on the causation of skin lesions and their transmission along with management plans and mitigation measures would be essential and extremely invaluable.

1. Introduction

1.1. Skin diseases in the ocean

In marine life, the evidence of the existence of diseases, along with scientific statements and suggestions pointing out a tendency for potential proliferation and dispersal over the years (Ward & Lafferty, 2004), have raised concerns about a possible deterioration in ocean health (Lafferty *et al.*, 2004, 2015; Schuldt *et al.*, 2016). Potential outbreaks of diseases are capable of altering the function and the structure of marine ecosystems, affecting by this way a great variety of ocean-dwelling taxa, including vertebrates (e.g. fish, mammals, turtles), invertebrates (e.g. corals, cnidaria, crustaceans, echinoderms) and seagrasses (Lafferty *et al.*, 2004; Lafferty & Hofmann, 2016). Many of these organisms can serve as hosts for a great variety of microparasites and pathogens including bacteria and viruses (Van Bressem *et al.*, 1999; Lafferty *et al.*, 2004). Such pathogens, along with a variety of biotoxins, have been reportedly found to be attributed with increased risks of mortalities of the hosts, which in the worst cases, combined with other factors, are able to threat populations to small-scale extinctions (Geraci, 1999; Harwood, 2001; Birkun Jr, 2002; de Vere *et al.*, 2018).

Although marine environment is characterised by a higher complexity and uncertainty in the understanding of its mechanisms comparatively to terrestrial ecosystems, there is mounting evidence concerning the factors that contribute to the occurrence and dispersal of diseases, since many sources of causation have been reported to favour their generation and development (Reno, 1998; Harvell *et al.*, 1999; Ward & Lafferty, 2004; Savage *et al.*, 2015; Schuldt *et al.*, 2016). The majority of these causes have anthropogenic origin, with pollution, climate change/warming and introduced species being the most notorious and common ones (Torchin *et al.*, 2002; Ward & Lafferty, 2004; Maynard *et al.*, 2015; de Vere *et al.*, 2018). Some of the most characteristic examples, concerning the penetration of microparasites and pathogens in the aquatic environment and the display of an indirect evidence that reflects a possible degradation of the ecosystem, include: i) coral diseases, which are thought to be associated with an apparent decline in coral reefs and the climate warming (Hughes *et al.*, 2018), ii) diseases occurring in loggerhead sea turtles (*Caretta caretta*) associated also with climate warming (Herbst, 1994) and prevalence of viruses (Stacey *et al.*, 2008), iii) bioaccumulation of toxicants in marine mammals such as cetaceans (Mouton & Botha, 2012; de Vere *et al.*, 2018) and many more. Among the aforementioned categories, marine mammals seem to be the category of animals that require further attention and monitoring in terms of understanding and evaluating a marine habitat's health and viability. As apex predators, marine mammals and especially cetaceans, have been characterised as "ecosystem sentinels" (Wells *et al.*, 2004; Moore, 2008; Powell *et al.*, 2018) due to their capability of accumulating toxins from both the environment and the prey the consume, reflecting in that way ecosystem's health. As a result, evaluation of their health condition is, undoubtedly, vital in terms of drawing important and valuable conclusions regarding the overall health of the area they inhabit.

1.2 Skin diseases in cetaceans

Cetacean species have the privilege to be equipped with a "keratin-rich" skin that acts as an effective barrier against pathogens under natural conditions (Pfeiffer & Jones, 1993; Mouton & Botha, 2012). Characterised by unusual thickness when compared to human skin (approximately 20 times thicker (!)), skin of cetaceans consists of a variety of layers in the epidermis, the dermis and the hypodermis, thus providing three "lines of defence" against potential infectious invaders (Jones & Pfeiffer, 1994) (Figure 1). Hicks *et al.* (1985) stated that there is a large capacity in the layers occurring in the epidermis of bottlenose dolphins (*Tursiops trancatus*), which contain large populations of cells that may contribute on the cicatrization of traumata on the skin. The aforementioned facts along with the smooth surface of cetaceans' skin due to the absence of pelage and the rapid sloughing of the epidermal layer (Hicks *et al.*, 1985) enhance the probability of limitation of microorganism attachment and subsequent penetration on cetaceans' skin.

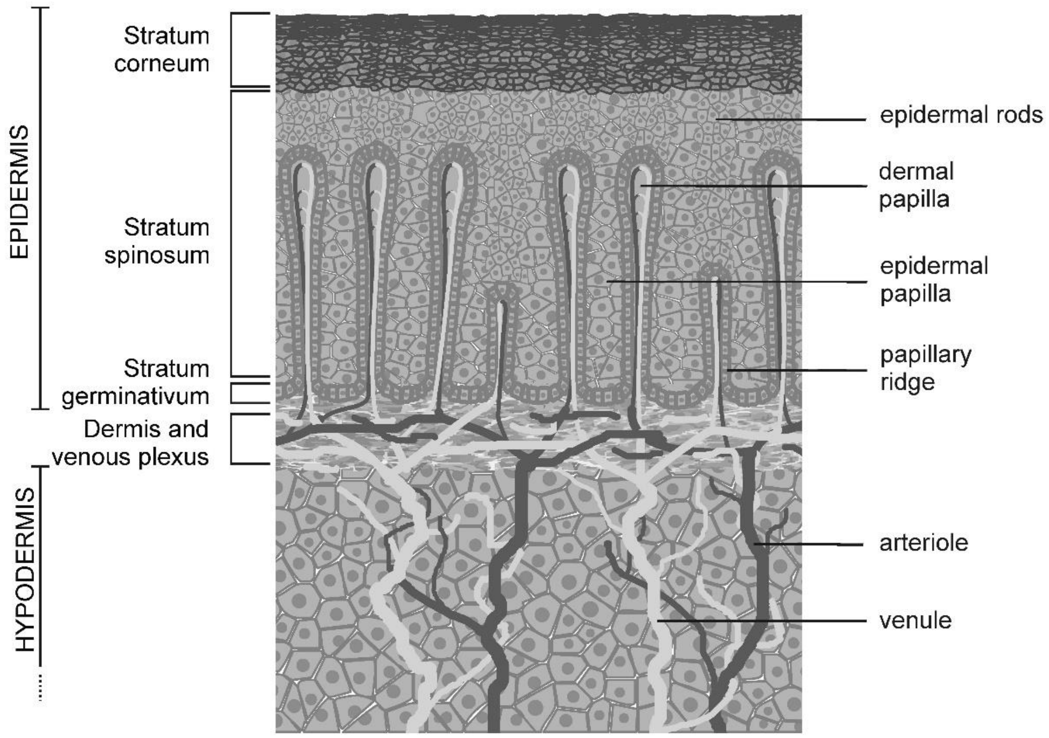


Figure 1. A cross sectional area of cetacean skin depicting the general anatomy of dermal, epidermal and hypodermal layers (Source: Mouton & Botha, 2012).

Although these formidable defence mechanisms enhance the impediment of pathogen penetration on the skin, studies revealed numerous cases of skin lesions worldwide during the last decades. Such studies, that focus on skin lesions on a variety of cetacean species, have been conducted since the 1950s (Simpson *et al.*, 1958; Sweeney & Ridgway,1975; Thomson & Hammond, 1992; Wilson *et al.*, 1997, 1999, 2000; Mouton & Botha, 2012) with the frequency of these studies steadily increasing over the years. Natural causes such as fluctuations in temperature, salinity and solar radiation (Wilson *et al.*, 1999; Martinez-Levasseur *et al.*, 2010) and ectoparasites (Ólafsdóttir, *et al.*, 2013) are considered key factors in skin lesions development. Nonetheless, anthropogenic factors are also considered major determinants, both in terms of generation and establishment of conditions that favour their outspread. Pollution, entanglement in fishing gears, collisions (vessel strikes) and interaction with fisheries (entanglement) have been reported to be the most important and widely-known ones (See de Vere *et al.* (2018) for details).

1.3 Skin lesions in the bottlenose dolphin (*Tursiops truncatus*)

1.3.1 Categories of skin lesions found on bottlenose dolphins through photo-identification

Photo-identification (hereafter photo-id) is one of the most popular and widely used methods used in numerous studies focusing on identification of skin deformities (Wilson *et al.*, 1997,1999,2000; Maldini *et al.*, 2010; Hart *et al.*, 2012; Gonzalvo *et al.*, 2015; Hupman *et al.*, 2017; Leone *et al.*, 2019). The popularity and the wide use of this method are related to the fact that photo-id is a relatively cheap and non-invasive method of evaluating body condition and skin lesions occurrence (Pettis *et al.*, 2004). As for bottlenose dolphins, assessment through photo-id is relatively easier and more accurate since they tend to acquire marks such as nicks and notches on their dorsal fin allowing in that way to identify them as unique animals (Würsig & Jefferson, 1990; Levesque *et al.*, 2016). Photo-id techniques reveal the minimum value of skin disease prevalence since, most of the times, only the upper part body parts of the animals are visible (Hart *et al.*, 2012). Other methods focusing on skin disease assessment on free-ranging bottlenose dolphin populations include data from capture-release methods, bycatch, and data from stranding individuals (Baker, 1992; Van Bressem *et al.*, 2006).

Initial studies focused on epidermal skin abnormalities on bottlenose dolphins (Thompson and Hammond, 1992; Wilson *et al.*, 1997) stated that classification of skin lesions is based on the colour and the texture of the lesions. More specifically:

- Thompson and Hammond (1992) in their study described four basic categories of skin lesions which include dark lesions (example in Figure 2), ring lesions, depigmentation (example in Figure 3) and Injuries/Deformities.

- Wilson *et al.* (1997) in their study followed a different pattern in terms of classification of skin lesions, using however Thompson and Hammond (1992) classification as a baseline, enhanced with more categories. Their new classification included black lesions (B), white lesions (W), dark-fringed spots (DFS), abraded fin tip (AFT), cream lesions (C), white-fin fringe (WFF), orange patches (OP), cloudy lesions (CL), lunar lesions (LUN) and white-fringed spots (WFS) (See examples in Figure 4) (brackets contain the abbreviations used then).

Classification made by Wilson *et al.* (1997) was used as a baseline for subsequent relevant studies and is still used until now.

Occurrence of such lesions is linked to a broad spectrum of causes including environmental parameters such as solar radiation, low salinity and fluctuations in water temperature (as stated above) (Wilson *et al.*, 1999; Martinez-Levasseur *et al.*, 2011), injuries caused by parasitic copepods and shark bites (Corkeron 1987b; Wilson *et al.*, 1997; Ribeiro *et al.*, 2010; Samarra *et al.*, 2012) and injuries caused by interaction with fisheries (e.g.fishing gears and net entanglement) (de Vere *et al.*, 2018; Leone *et al.*, 2019)

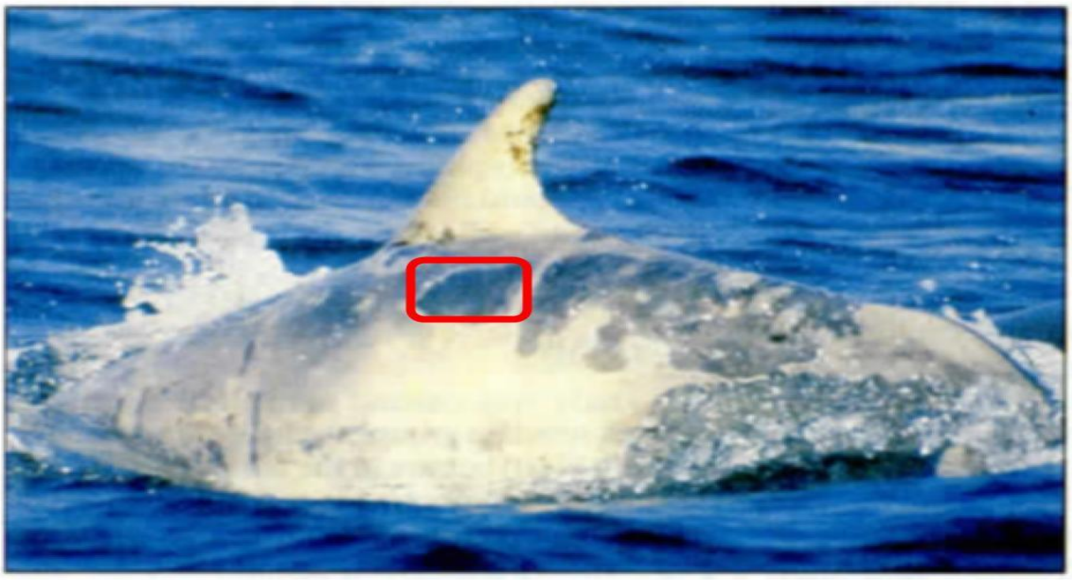
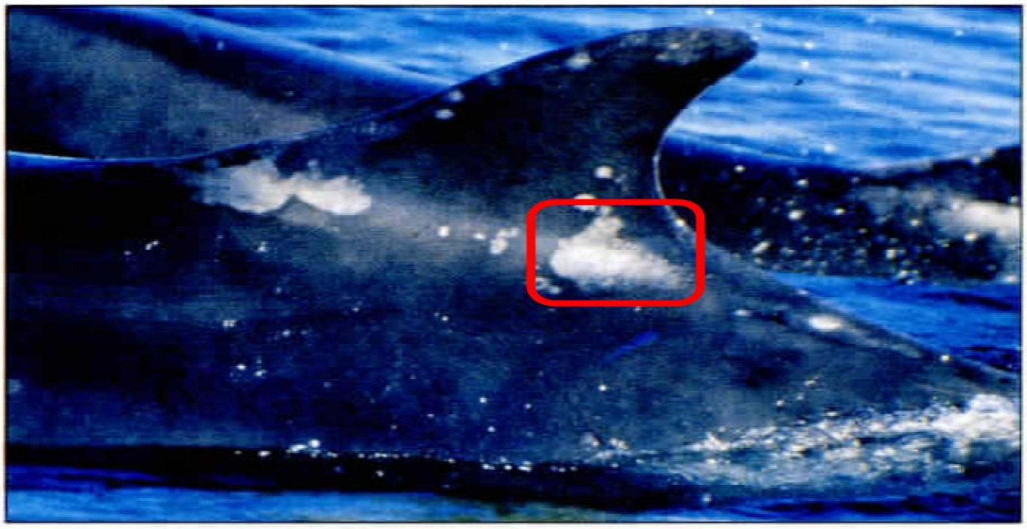


Figure 2. A bottlenose dolphin individual in Moray Firth (Scotland) exhibiting dark lesions (Thompson and Hammond, 1992 – modified photo)



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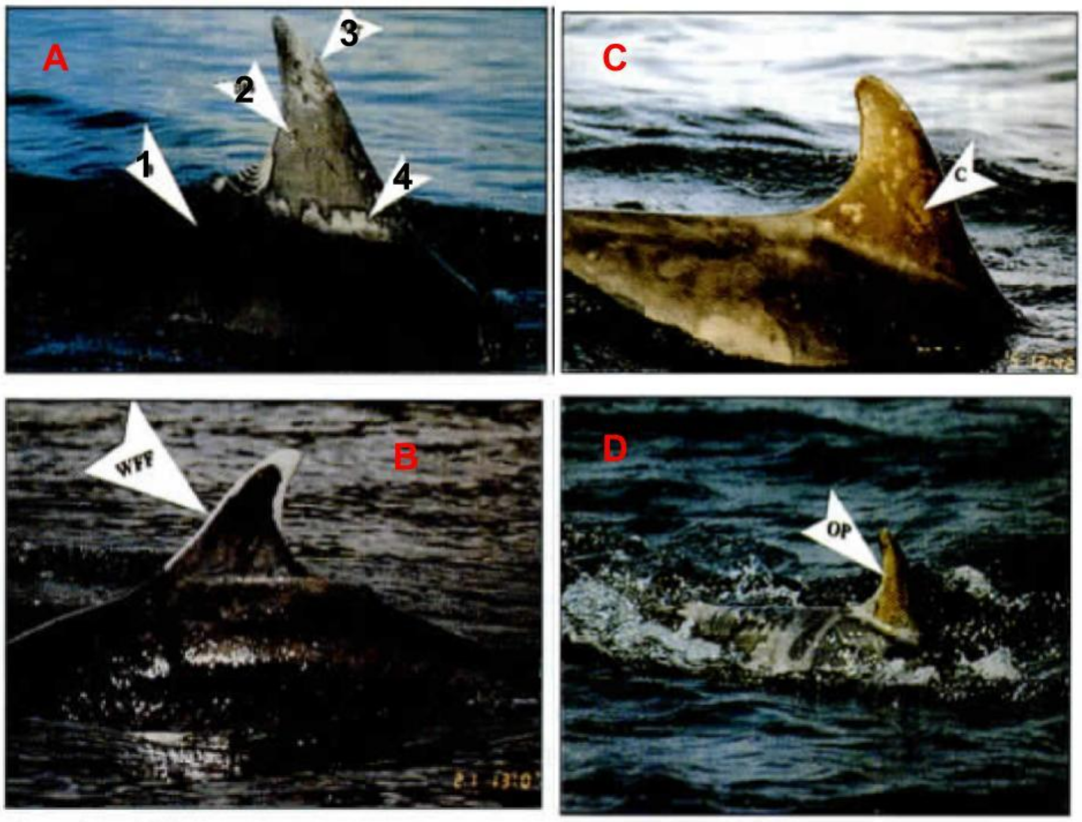

Figure 4. A) Black lesions (1), Dark-fringed spots, (2) Abraded fin tip (3), and White lesions (4), B) white-fin fringe, C) Cream lesions and D) Orange patches (Wilson *et al.*, 1997-modified photographs)

1.3.2 Sociality in bottlenose dolphins – A key factor in the transmission of diseases?

Bottlenose dolphins (*Tursiops truncatus*) are the most common members of the family *Delphinidae* that can be found, literally, in every ocean worldwide, both in coastal and offshore areas (Connor *et al.*, 2000) (Figure 5). Bottlenose dolphins are extremely social species (Feingold & Evans, 2014a), capable of forming large aggregations up to hundreds of individuals (Connor *et al.*, 2000). They live in dynamic fission-fusion societies which means that their social group compositions are characterised by both spatial and temporal variability over years, days and even minutes (Wells *et al.*, 1987; Connor *et al.*, 2000). Social associations among groups of bottlenose dolphins do not occur randomly. In fact, they are driven by social features such as social hierarchy, sexual maturity and kinship (Felix *et al.*, 2019) including biological features as well, such as reproductive and foraging strategies (Mann *et al.*, 2000; Mann & Karniski, 2017). Such features indicate considerable differences between male and female bottlenose dolphins in terms of the establishment of group composition, and in lifestyle to a further extent. Males are known to form affiliations with one or two males known as “alliances” (Mann *et al.*, 2000). Alliances enable males to increase their chances to gain and maintain access to estrus females. Males tend to defend females from other male alliances through cooperation with the other member of their alliance (Mann *et al.*, 2000). Successful establishment of tight alliances among males is characterised by great stability and can last for many years (Mann *et al.*, 2000). Females tend to create bigger networks than males. Formation of female social networks is thought to be related to protection from potential predators and the safeguard of habitat resources (Smolker *et al.,* 1992). Nevertheless, cases where stable male-female associations have been observed over the years, do also exist (Baker *et al.,* 2018)

Social behaviour in gregarious and "socially-complexed" animals is a key factor in terms of spreading of infectious diseases, due to the high contact rates existing among individuals (Kappeler *et al.,* 2015; Sah *et al.,* 2018). Moreover, the fact that pathogens that are characterised by high transmissibility are able to cause prolonged disease outbreaks in such species (Sah *et al.,* 2018), implies an increased vulnerability among their populations.

In case of bottlenose dolphins, intra-specific interactions are the main representative activities that reflect their sociability. Such interactions involve aggressive, both intra- and inter-sexual, conflicts among individuals (Scott *et al.,* 2005; Marley *et al.,* 2013). Intra-sexual interaction occurs mainly among male individuals that exhibit aggressive behaviour towards each other, aiming to increase their possibilities of mating opportunities (Marley *et al.,* 2013). On the other hand, aggressive behaviour among females exist to a much lower rate (Marley *et al.,* 2013). Inter-sexual conflicts are usually expressed by male individuals towards females through intimidation and, sometimes, through sexual coercion, which is a common phenomenon in cases where a female tries to escape when surrounded by male alliances (Marley *et al.,* 2013). Both intra- and inter-sexual interactions are usually expressed by tail slapping, chasing and biting that lead to the production of scratches, known as teeth rakes (Samuels & Gifford, 1997; Scott *et al.,* 2005). If caused by infected individuals, teeth rakes may assist the establishment and, subsequently, the propagation of a disease as they allow pathogens penetrate the epidermis (Mouton & Botha, 2012). Interspecific competition with other delphinids does also occur. Both types of competitions increase as a result of habitat degradation and prey depletion, caused mainly by human pollutants and fisheries respectively (Lane *et al.,* 2014). In that way, stress among individuals is also increasing, resulting in further undermining of their defence mechanisms making them even more prone to disease transmissions (Mouton & Botha, 2012; Lane *et al.,* 2014).

Distribution patterns on bottlenose dolphins are characterised by extreme variability (Mann *et al.,* 2000; Scott *et al.,* 2005) with some populations exhibiting migratory behaviour and some others being more resident to confined ranges (Wilson *et al.,* 1997). Such variability is principally attributed to the different reproductive and foraging strategies among populations resulting, occasionally, in associations among individuals, resident to different areas (Mann *et al.,* 2000). Interactions between dolphins of different residency may increase the probability of a disease transmission, especially if one of these populations inhabit a polluted or degraded area.

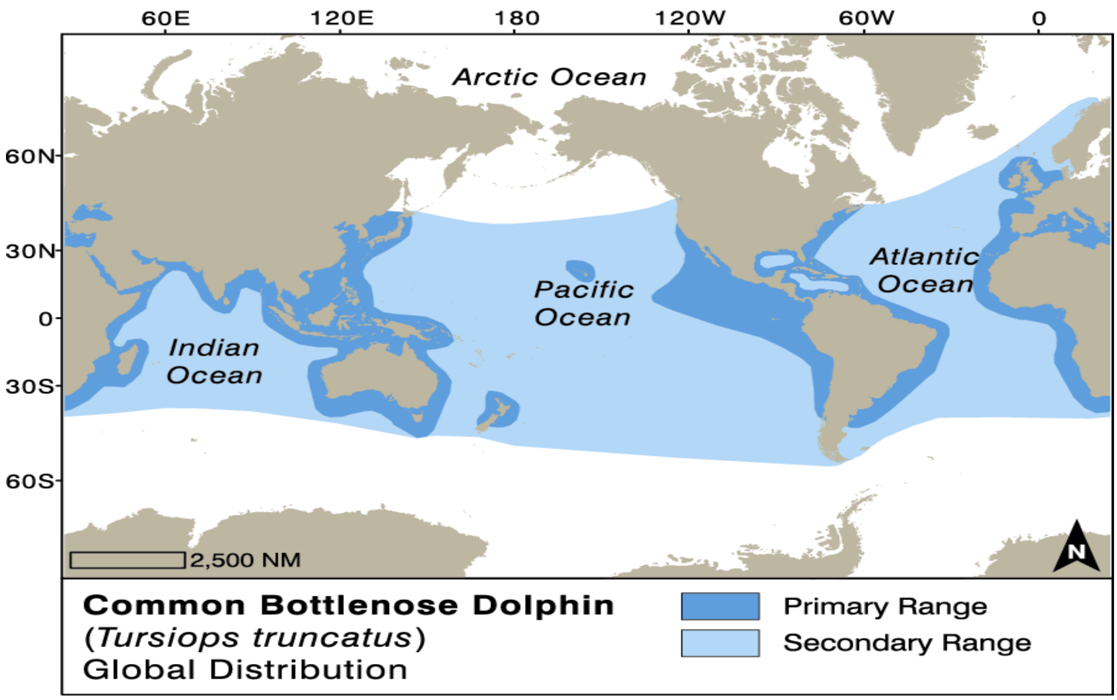

Figure 5. The global distribution of the common bottlenose dolphin *Tursiops truncatus* (Source: Jefferson *et al.*, 2011)

1.4 Classification of skin lesions according to the host micro-organism – A brief overview

According to the host-microorganism, skin lesions are divided into four categories: Bacteria, Fungi, Parasites and Viruses (Van Bressem *et al.,* 2008). Here, there are presented some of the more important and wide-spread ones.

BACTERIA

A great variety of bacteria has been isolated from skin lesions in cetaceans. In some areas, where aquacultures exist nearby, aquatic bacteria are likely to develop antibiotic resistance due to the uncontrolled use of antibiotics by humans there (Cabello, 2006). Some of the most common bacteria are presented below:

Aeromonas spp.

Aeromonas species are responsible for pneumonia and ulcerative dermatitis in cetaceans including the common bottlenose dolphin *Tursiops truncatus.* Cusick and Bullock (1973) first reported the aforementioned diseases in a bottlenose dolphin individual in North Carolina (USA) back in 1970s. Additionally, there are concerns that *Aeromonas* spp. may be responsible for skin diseases occurring among cetacean populations in South Africa (Van Bressem *et al.*, 2008).

Erysipelothrix rhusiopathiae

E. rhusiopathiae is a bacterium which is common contaminant in fish. As a result, the major pathway of transmission to cetaceans is through ingestion of such fish. In cetaceans, it can be also transmitted through biting by conspecifics. *Erysipelothrix* *rhusiopathiae* is responsible for subacute disease in the initial stages, but it may result in chronic dermatological disease and septicemia, mostly in cetaceans kept in captivity (Geraci *et al.,* 1966).

***Pseudomonas* spp.**

Although it is a bacterium common in terrestrial and freshwater environments, *Pseudomonas* spp. can also be found in marine environments. It is responsible for the infection and death of a variety of plant species, animals and humans (Khan *et al.*, 2006). Regarding cetaceans, it has been reported that a bottlenose dolphin individual, off the coast of Florida, was infected by *Pseudomonas* spp. and died within 70 days due to extensive dermatitis and bronchopneumonia (Diamond *et al.,* 1979). Other fatal diseases caused by *Pseudomonas spp.* include osteomyelitis, septicemia and skin necrosis (Van Bressem *et al.,* 2008).

***Vibrio* spp.**

The presence of *Vibrio* spp. in marine environments is related to environmental factors such as salinity and temperature. Known species of the *Vibrionaceae* family encountered in marine environments include *V. alginolytus, V. damsela, V. parahaemolyticus* and *V. vulnificus*. These species have been detected in slow-healing ulcers and wounds in different body parts of cetaceans held in captivity (Pereira *et al.*, 2007).

FUNGI

Some of the most common species of fungi that have been detected and isolated from cetaceans include *Candida albicans*, *Fusarium* spp., *Lacazia loboi* and *Trichophyton* spp. *L. loboi* is the most well studied species among them and seems to be the most widespread as well. In terms of fungal diseases in cetaceans, candidiasis and lobomycosis are the ones most often reported (Murdoch *et al.,* 2008).

Candidiasis

Candidiasis is a disease mainly found in captive cetaceans and is caused by the fungus *Candida albicans*. Dissemination of candidiasis has been linked with deaths of harbour porpoises (*Phocoena phocoena*), bottlenose dolphins (*Tursiops truncatus*) and long-finned pilot whales (*Globicephala melas*) individuals (Nakeeb *et al*., 1977; Dunn *et al*., 1982).

Lobomycosis

Lobomycosis is a chronic granulomatous disease of the skin and subcutaneous tissue that affects (only) humans and dolphins under natural conditions (Murdoch *et al.*, 2008). Regarding its phenotype, it usually appears as whitish, greyish or pinkish lesions (Figure 6) that may often lead to ulcerations (Migaki *et al.*, 1971; Murdoch *et al.*, 2008). The expand of this disease in not rapid (Rodriguez,1993); however, it might lead to death in extreme cases (Van Bressem *et al.,* 2007a).

Figure 6. Infection by *Lacazia loboi* on a bottlenose dolphin from the Indian River Lagoon (Murdoch *et al.*, 2008)

PARASITES

It has been reported that a variety of cetacean species kept in captivity, including common dolphins (*Delphinus delphis*), bottlenose dolphins (*Tursiops truncatus*) and killer whales (*Orcinus orca*), exhibit extensive dermatitis associated with invasive ciliates (Choi *et al.*, 2003). Infection from parasites is characterised by the presence of distinguishable ulcers and subcutaneous necrosis (Schulman and Lipscomb, 1999).

VIRUSES

Caliciviruses

Cetacean calicivirus derives from the genus *Vesivirus* and was first detected and isolated in the early 1980s from old scars and pustular tattoo skin lesions in two bottlenose dolphins native to the Atlantic Ocean (Van Bressem *et al.*, 2008). According to Smith *et al.* (1983), this virus generated vesicles that were rapidly corroded and, as a result, shallow ulcers were produced on the skin of one of the two individuals. Transmission of the virus to other aquatic animals is not clear but it is probably achieved through direct contact between animals (Smith and Boyt, 1990).

Herpesviruses

Corpuscles of a herpes-like virus were detected and isolated from beluga whales (*Delphinapterus leucas*) and bottlenose dolphins (*Tursiops truncatus*) (Barr *et al.*, 1989; Smolarek Benson *et al.*, 2006). Size of lesions caused by *Herpesvirus* is variable and is determined, mainly, by their abundance. If a large number of lesions is present, then their diameter generally will not exceed 2 cm; only a few may be present with a diameter of up to 30 cm (Van Bressem *et al.*, 2008). A characteristic type of herpesviruses is the alpha-herpesvirus, which is responsible for dermal and systemic infections and may result even in death of the affected organism in extreme cases.

Papillomaviruses

Endonuclear particles of *Papillomaviruses* were identified on a harbour porpoise stranded in German waters (Figure 7) and on a killer whale (*Orcinus orca*) individual held in captivity (Van Bressem *et al.,* 2008). At least six known types of *Papillomaviruses* that could potentially infect bottlenose dolphins. Such infection may result in malignant transformation of benign papillomatous lesions (Bossart *et al.,* 2005)

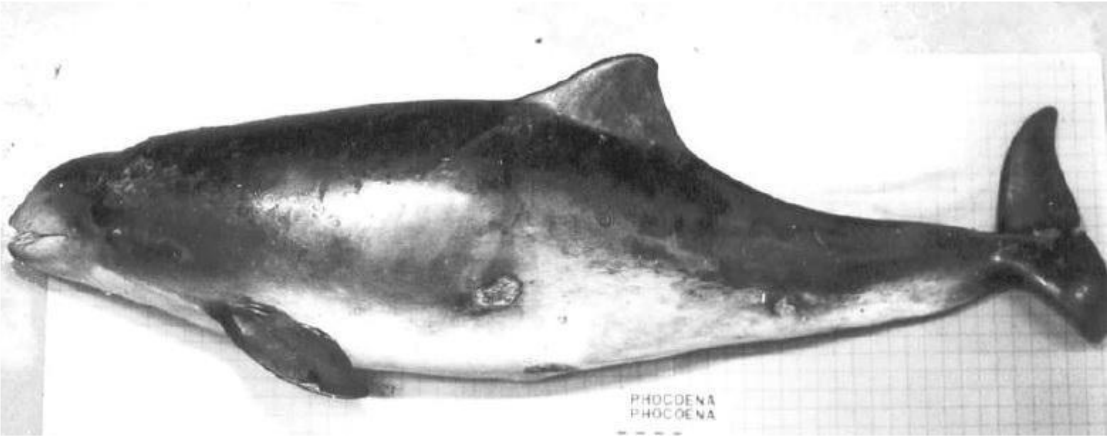

Figure 7. Infection by *Papillovirus* on a harbour porpoise in Germany (Source: Van Bressem *et al.,* 2008)

Poxviruses

Poxviruses are linked directly with causes of tattoo skin diseases (TSD). Tattoo like lesions are stippled, irregular lesions that are characterised by grey, black and occasionally yellowish pigmentation (Pearce *et al.,* 2008; Powell *et al.,* 2018). Since recent studies (Powell *et al.,* 2018; Leone *et al.,* 2019) indicate a higher prevalence of TSD in coastal areas where the environment is more contaminated relatively to offshore areas, tattoo-like lesions may be used as a health indicator for both the cetaceans themselves and the area they inhabit.

1.5 GAPS IN KNOWLEDGE

Skin lesions on bottlenose dolphins (BNDs) and cetacean species in general have been well studied across many regions worldwide (Greenwood *et al.,* 1974; Baker, 1992; Wilson *et al.,* 1999; Van Bressem *et al.,* 2007; Bearzi *et al.,* 2009; Maldini *et al.,* 2010; Hart *et al.,* 2012; Leone *et al.,* 2019). However, studies concerning spatio-temporal trends using photo-identification are scarce and limited. A first categorization of skin lesions from BND populations in Cardigan Bay was made back in 2006 by Edita Magileviciute. She pointed out in her study that the distribution of frequencies of skin lesions found in social network components were not random. Later, in 2014, Elena Akritopoulou studied the spatio-temporal trends of skin lesions and showed that a high proportion (73%) of Cardigan Bay BNDs were infected by at least one lesion. Studies concerning skin lesions are with no doubt extremely important for BND conservation since transmission of some diseases may threaten their populations, even to small scale extinction (Van Bressem *et al.,* 1999). It is also well documented that sociability of the species promotes diseases' transmission and spread (Van Bressem *et al.,* 2009; Felix *et al.,* 2019). Gaps in knowledge are primarily linked to the understanding of BNDs social networks and the investigation of the sources that generate and assist the establishment of these lesions on BNDs. Over the last years, more and more studies are conducted, attempting in monitoring BNDs populations, social structure and patterns of disease transmission. Use of recent studies focusing on stable BNDs populations monitoring (e.g. Baker *et al.,* 2018; Leone *et al.,* 2019) combined with earlier studies, enhance the probability of getting more accurate and precise estimations of the complex life of these animals and hence, of the disease transmission mechanisms. New data from studies using photo-id techniques and focusing on anthropogenic and environmental parameters that are thought to be responsible for a potential transmission of diseases, are key factors in attempting to fill gaps in knowledge. So, gaps in knowledge are also related to the limited availability of data used in the past studies.

1.6 Aims and objectives

The main aim of the study was to collate and record the existing scientific information regarding skin lesions in bottlenose dolphins and link them with possible sources of causation. These sources might have either natural origin (e.g. temperature fluctuations, interaction with conspecifics) or could be related to anthropogenic impacts (e.g. pollution, entanglement, vessel strikes etc.). To achieve this, a categorization based on a compilation of scientific publications was made and two main questions – objectives were addressed. These questions – objectives were the following:

- Is there a temporal pattern in the prevalence of lesions?
- Is there a difference in the prevalence of lesions among groups of individuals (e.g. sex and maturity)?

2. Study Area

Cardigan Bay is the largest bay along the British Isles and is located in the west coast of Wales. It measures over 100km across its westernmost extent and covers an area of 4,986.86 km^2 in total from the western tip of the Llŷn Peninsula in the north (52° 47' 45'' N, 004° 46' 00'' W) to St. David's Head in the south (51° 54' 10'' N, 005° 18' 54'' W) (Lohrengel *et al.,* 2017). It is a relatively shallow embayment with an average water depth of 40m (Evans, 1995). The maximum depth in the bay does not exceed 60m, with depth becoming shallower from west to east and is characterised by very gentle slopes (Evans, 1995).

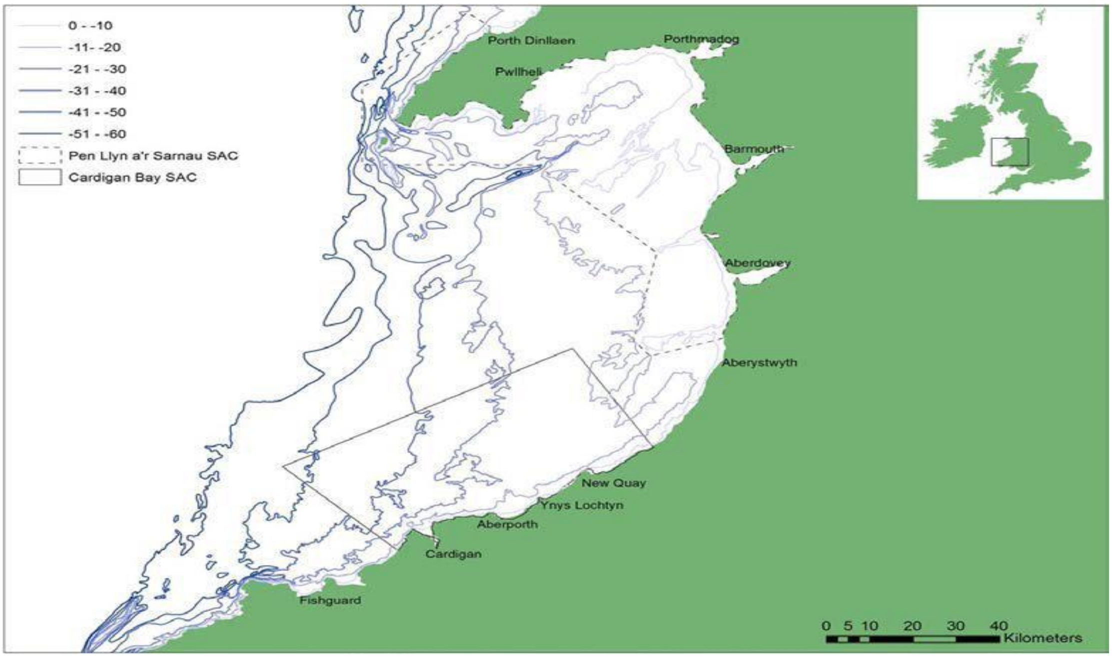

Figure 8. The study area, Cardigan Bay including Pen Llyn a'r Sarnau SAC (indicated by hatched boundary lines) and Cardigan Bay SAC (indicated by continuous boundary lines) (Source: Feingold and Evans, 2014a).

Although the occurrence of temperature fluctuations, especially in the shallow waters of the bay, the mean annual sea water temperature in the area seems to be stable at just above 11°C. More specifically, seasonal temperature fluctuations in Cardigan Bay have a range between a minimum of 5°C, both in inshore and offshore waters during February/March, and a maximum of 16°C and 20°C in offshore and inshore waters respectively during August/September (CCC, 2001a). Seasonal fluctuation occurs in salinity as well, mainly due to fresh water inputs from rivers and rainfalls. The salinity in the area ranges from 33.3‰ in the winter to 34.2‰ in the summer (CCC, 2001a). Noteworthy fact is the presence of Aeron, Dyfi and Teifi rivers in the local area and their continuous contribution in fresh water input in the bay that clearly affects not only the salinity but also the local water temperature and quality (CCW, 2005).

Cardigan Bay is characterised by an open coastline which indicates a clear exposure to prevailing winds, mainly of western and south-western origin, that most of the times exceeds 3 on the Beaufort scale (Evans, 1995). As a result, conduction of surveys might not be effective and hence, limited opportunities of such surveys may exist even when sea conditions are favourable. Tides are semi-diurnal and enter the bay via the St. George Channel, reaching up to over 5 meters (17 ft.) at extreme springs (Evans, 1947). The seabed distribution is strongly linked with the tidal current speed. In offshore areas, where high energy currents occur, the dominant sediments are cobbles and gravel while the inshore ones, where lower energy currents occur, are characterised mainly by a strong presence of thinner sediments such as silt, mud and finer sand.

According to Annex II of the EU Habitats and Species Directive, the bottlenose dolphin requires spatial protective measures (Council Directive 92/43/EEC). In addition, bottlenose dolphin is also listed under Annex IV of the Directive, which requires austere protection for all European cetacean species using wider measures. The aforementioned facts indicate the importance of the conservation of the species' populations in Cardigan Bay. As a result, two marine Special Areas of Conservation (SACs) have been established there: 1) Cardigan Bay SAC, which encompasses an area of 958.65km^2 and the species was the major reason for the designation of the area and 2) Pen Llŷn a'r Sarnau SAC which encompasses an area of 1460.35km^2 and the species here is a qualifying feature (Feingold and Evans, 2014). Small numbers of bottlenose dolphin populations are present and could be encountered in other coastal areas around the British Isles too (Evans and Hammond, 2004; Brereton *et al.*, 2018); however, there is only one other SAC for bottlenose dolphin in UK waters which is established in Moray Firth, NE Scotland (Wilson *et al.*, 1999; Butler *et al.*, 2011).

3. Methods

3.1 Field Data Collection

The appropriate data were collected during boat surveys conducted in Cardigan Bay from March to October during the years 2001-2018 focusing, mainly, on the coastal area (up to no more than 6 miles from land), including the outermost limits of the bay as well. In general, two types of surveys were conducted: (1) dedicated line transect surveys (LT) and (2) dedicated non-line transect or dedicated photo-identification (NLT), also referred as *ad libitum* surveys.

Line Transects (LT)

Dedicated line transect surveys (LT) of the study area were conducted since 2001 using the vessels listed in Table 1. During the implementation of LT surveys, vessels were maintaining a constant speed, although the average speed varied between each vessel (See Table 1). Surveys were highly depending on weather and initiated only when environmental conditions where favourable: sea state ≤3 on the Beaufort scale, visibility > 1.5km, and no precipitation. If conditions changed during the surveys, data collected in sub-optimal or non-optimal conditions were not taken into consideration for the subsequent analysis part. Transect lines used for the study area (Cardigan Bay SAC & northern Cardigan Bay) were pre-designed. As a result, if local environmental conditions (f.e. visibility) were significantly worsened, then an alternative transect line was chosen. In rare cases where conditions became completely inappropriate, the survey was abandoned.

Boat name	Year	Length (m)	Eye height (m)	Speed (kn)	Engine type	No. of trips	No. of km	Area
Ocean Breeze	2001	9	3.5	7.5	200 hp diesel	30	2,180	CB SAC
Sulaire	2002-06	10	3.0	8	380 hp turbo diesel	298	4,614	CB SAC
Celine	2005-07	10.6	2.0	6	30 hp diesel	41	2,897	NCB
Scorpius	2007	9	2.4	10	230 hp diesel	2	338	CB SAC
Dunbar Castle II	2005-17	9.7	3.5	7	120 hp diesel	276	11,606	CB SAC
Pedryn	2006-17	11.7	3.0	10	Twin 350hp diesel	41	8,762	NCB
MaChipe	2007-17	10	4.5	10	Twin 220hp diesel	55	7,215	NCB
Highlander	2015	10	4.0	10	Twin 370hp diesel	1	145	NCB
Severn Guardian	2016	18.3	5.5	9	Twin Volvo D9 MH	2	295	CB SAC
*Bay Explorer**	2011-17	10	2.5	varies	Twin 200hp petrol	14	409	CB SAC

Table 1. Vessels used in surveys for the data collection. Details regarding the years in which they were used, length, eye height on the observer platform, mean boating speed, engine type, total number of trips, number of kilometres covered and survey area, for which the boats were used are included in the table. CB SAC = Cardigan Bay Special Area of Conservation, NCB = Northern Cardigan Bay, * Used only for Non-line-transect surveys

During the conduction of the line transects, 2 primary observers (POs) were allocated on the roof of the vessels (except *Pedryn* where only one PO was allocated), each one scanning with the naked eye from abeam (90°) on their side to 10° on the opposite side. Investigation and confirmation of the spotted animals (including their ID) was made with the use of binoculars that were provided to the observers. Independent observers (IOs) were recruited for each survey as well and their tasks included the scanning of the track line ahead (with the use of binoculars) and the detection of marine mammals. When a cetacean was spotted, both POs and IOs had to record immediately the sighting, the distance and the angle of the animal to the boat, and the boat position, with the help of a handheld GPS unit.

Figure 9. The "*Dunbar Castle 2*" vessel. It is the main vessel used for surveys and data collection in the Cardigan Bay SAC area from 2005 and onwards.

Along with sightings, effort data were collected as well. Effort data included the effort status (e.g. linear transect line, dedicated survey etc.) along with environmental variables (visibility, sea state, swell, transect leg, boat position) and were recorded at 15 minutes intervals throughout the survey, or whenever changes on the weather conditions were observed. Both sightings and effort data where recorded on specially designed forms, following the protocol of the Sea Watch Foundation (SWF) (See Appendix 1).

During the surveys four types of effort were recorded: 1) Line transect survey (LT) where the vessel followed a pre-defined transect line with both POs and IOs searching for sightings, 2) dedicated search (DS) where the boat did not follow a specific transect line and only POs were scanning for sightings, 3) casual watch (CW), where non-dedicated observers were scanning for sightings, usually when weather conditions where not optimal and 4) photo-identification (ID) when the vessel stopped temporarily following the transect line and managed to approach a group of animals in close range so as to obtain the desirable and appropriate photos for the photo-ID process.

Photo-ID process

As mentioned above, the photo-ID procedure initiated when the survey vessel deviated (temporarily) from the transect line to get closer to the pod of the dolphins once they were sighted. In the meantime, changes in effort type were noted on the effort form since this deviation from the transect line was considered as a "new line of effort" (Lohrengel *et al.*, 2017). Once the animals were sighted, the boat approached them slowly and, preferably, on a parallel course in order to start the photo-ID sampling. The approach of the individuals was to between 20m and 50m, under species licence granted by Natural Resources Wales (NRW), in compliance with protocols laid out in the Photo-ID licence agreement, in order to minimise potential disturbance. Such disturbance could lead to signs of abnormal behaviour of the animal such as deliberate avoidance, continuous tail slaps and prolonged dives. In such cases, the encounters were brought to an end and the vessel returned back to the last spot which it last left on the transect line and continued the LT survey.

Photographs of the dolphins were taken by two photographers based on the bow of the boat, focusing mainly on the dorsal fin of the animal. Ideally, whenever it was possible, photographs of other body parts of the individuals (head, back, peduncle, flanks, flukes) were taken as well. The presence of at least two photographers was essential in order to ensure that all individuals were photographed and, also, to increase as much as possible the reliability of the data. The photographic equipment used for the collection of the images included mainly Canon EOS 7D and Canon 40D cameras with 18-200mm or 75-350mm zoom lenses. However, there were a few (not very often though) cases where other camera types were used as well, including Canon EOS 600D, 1000D & 1100D, Canon 550D, Canon EOS REBEL T3, T5 & T6, PENTAX K-x and NIKON D3200. A photo-ID encounter lasted for approximately between 35-40 minutes (maximum) as the photo-ID licence indicates, and the whole process officially ended once both sides of the dorsal fin were properly photographed.

Definition of parameters such as the age and the gender of the individuals was made, mainly, according to physical characteristics. For instance, age was determined according to individual's body size, closeness to an adult individual and skin colour (Smolker *et al.,* 1992; Feingold and Evans, 2014a). On the contrary, definition of the gender was not always feasible and accurate. Precise estimations and subsequent confirmations of the gender were made only whenever aerial behaviour or bow-riding of the dolphins was observed and, hence, their genital area was obvious. However, due to the rareness of such images, the definition of the gender in the majority of the cases followed a different pattern: individuals accompanied by a calf were classified as adult females and individuals with large and, most of the times, marked body were considered potential males.

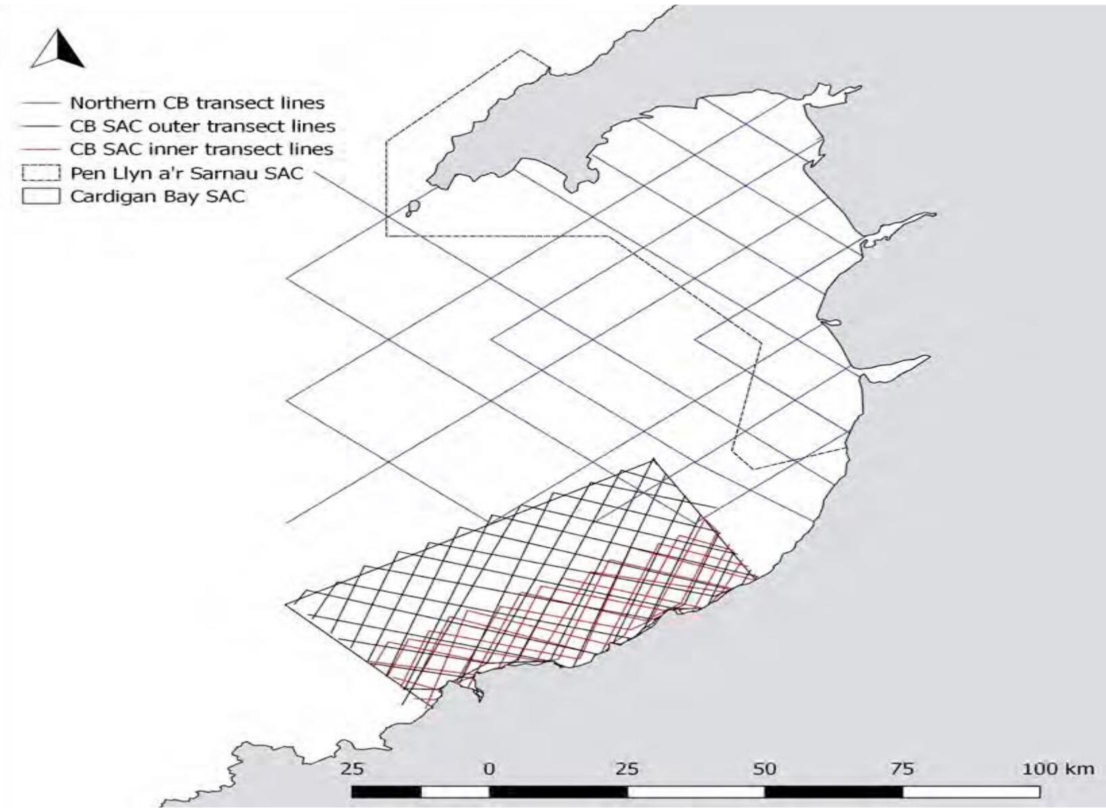

Figure 10. Transect patterns followed during line transect surveys in Cardigan Bay (Source: Lohrengel *et al.,* 2017).

Non-line Transects (NLT)

Dedicated non-line transects were conducted in order to obtain additional data and they were undertaken, most of the times, when weather conditions were insufficiently favourable or sub-optimal for the whole day of the survey, or whenever the vessel was available for a short period of time only. The approach in terms of data collection was similar to LTs with the main difference being the utilisation of POs only. Recorded effort types during NLTs included CW, DS and ID.

3.2 Selection of the Images & Quality Rating

Photos obtained in the field are contained in the SWF photo-ID catalogue which, currently, holds an amount of at least 388 bottlenose dolphin individuals (Lohrengel, *pers. comm.*). Each individual, contained in the catalogue, is characterised by a unique reference code number which is related to the extend and the level of the mark; "W" stands for well-marked individuals while "S" and "U" stand for slightly marked and unmarked individuals respectively. Similarly, the letters "R" and "L" indicate photos of the dolphin where the exhibition of marks is present on the dorsal fin, on the right and on the left side respectively. The code number of an image does also contain information regarding the date, the vessel and the photographer (See example below (Figure 11)).

Figure 11. Picture showing the right side of the dorsal fin of individual 181-06W. Full reference code number of the individual: 181-06W_R_180805_022_dunbar_KLO_013_1. According to the code number, this is a picture of the individual 181-06W showing the right side of the fin, it's the 13th picture taken during the first set of dolphins during the 22nd encounter on Dunbar research vessel in the 2018 season by Katrin Lohrengel (KLO), taken on the 5th August 2018 (photo: © Sea Watch Foundation, 2018).

The selection of the images was made according to the grading system described in Rosso *et al.*, (2011), where quality rating was divided into six stages (See Figure 12) based on the distance, the focus and the body area that is being showed. Photographs that were chosen for the analysis were of $Q \geq 5$ in order to avoid mistakes as much as possible in description and, subsequently, in the identification of body marks (Gowens & Whitehead, 2001). Once the appropriate images were selected, they were imported into an excel spreadsheet which contained information regarding the reference code number and the quality rating of the image, the location and the photograph equipment that was used and, also, columns that were used for a brief description of the image along with different categories of skin marks.

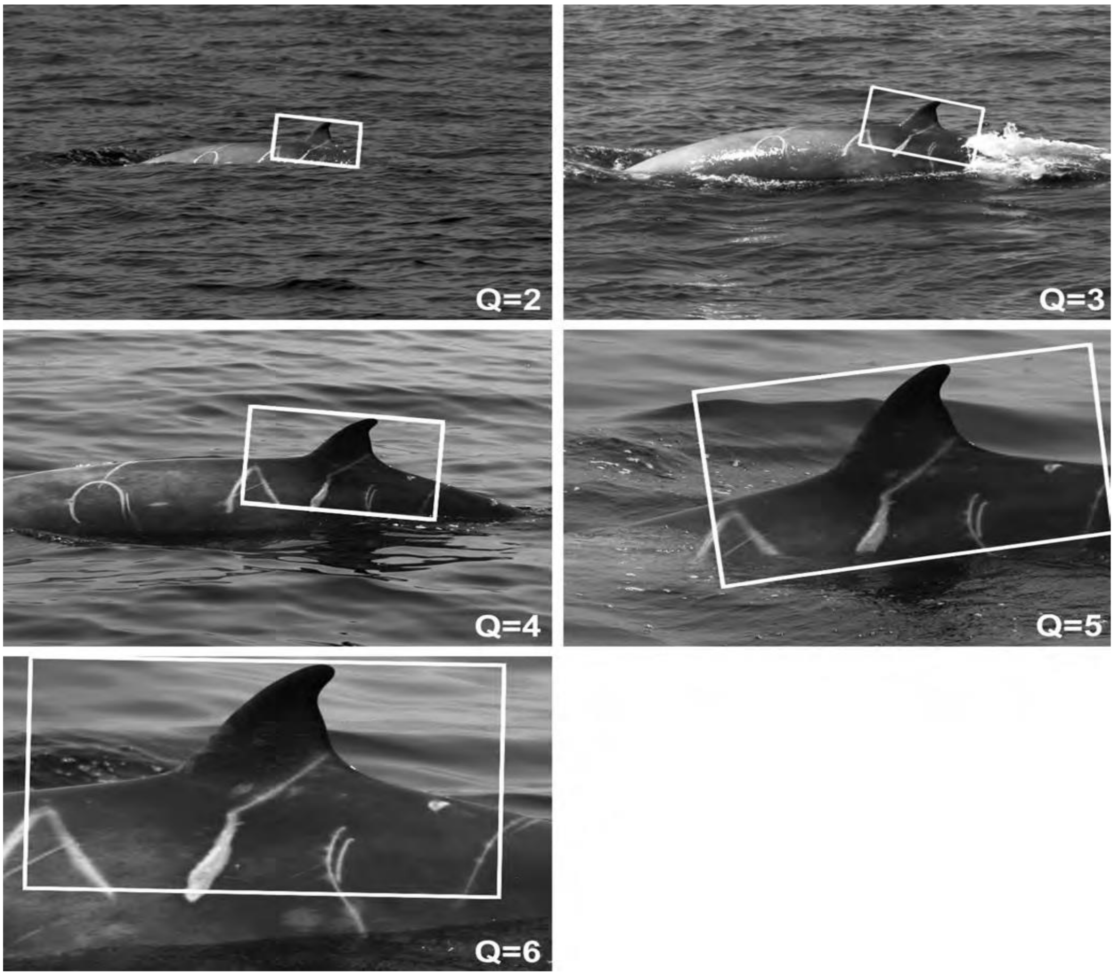

Figure 12. Example of the dorsal fin area (defined by a white rectangle) of a Curvier's beaked dolphin (Z*iphius cavirostris)* in different photos, according to the quality; Q=2: A very distant shoot showing the whole area, Q=3: A distant shoot showing a partial area, Q=4: A distant shoot showing the entire area, Q=5: Close and well-focused photo with a good representation of the area, Q=6: Very close and well-focused photo showing the whole area (Source: Rosso *et al.*, 2011)

3.3 Data Analysis

For the estimation of the prevalence of skin lesions across years, a linear regression analysis was performed. More specifically, a general linear model (GLM) with continuous variable was used. The continuous variable in the present analysis was the "year".

Similarly, a GLM with a categorical variable was used in order to estimate the prevalence of lesions among groups of individuals. Since there were occasions were more than one photo depicted the same individual, a general additive model (GAM) was used in order to reduce duplicates in the sample and define the prevalence of skin lesions according to individuals instead of the photographs. The categorical variables used here were the sex, the maturity and the period when the individuals were photographed.

Finally, analysis of variation (ANOVA) was used in order to investigate whether there were significant differences, both in the estimation of the probability of skin lesions across years and the prevalence of lesions among groups of individuals.

Individuals were divided into females, males and unknown according to their sex and into adults and calves according to their level of maturation. Individuals in the catalogue defined as "possible females" and "possible males" were treated as "females" and "males" respectively. Similarly, in terms of maturation, juvenile individuals were classified as calves. The whole period, in which photographs were taken, was divided into three categories: P1 (early summer period), which included March, April and May, P2 (mid-summer period), which included June and July and P3 (late summer period) which included August, September and October.

All the statistical analyses were conducted in R v3.6.0 and were performed for four different categories of skin deformities. These categories were namely "Tattoos", "Punctiform marks", "Discolouration" and "Wounds".

4. Results

4.1 Skin mark categorisation

A total of 476 images depicting 213 individuals (182 adults & 31 calves) were selected and assessed. Categorisation of skin marks took place after the consummation of the images' selection. Classification of skin lesions in bottlenose dolphin, and in cetaceans in general, varies among scientific publications and, hence, an attempt was made to categorise the lesions found in the present study according to "key" scientific published papers, with an emphasis on the most recent ones. For the images assessed here, skin lesions were grouped into eight categories (See Table 2). Examples showing some of these categories are presented below (See Figures 13-20).

Skin marks	Description	References
Punctiform (herpes-like) marks	Small black dotted marks caused by herpes-viruses	Bertulli *et al.*, 2016b
Pox-like marks / Ring lesions	Circular (mainly) & irregular shaped lesions caused by pox-viruses. Colouration varies and ranges from total hyper-pigmented lesions to pale and dark-fringe and vice versa.	Thompson & Hammond, 1992; Maldini *et al.*, 2010; Leone *et al.*, 2019
Tattoo & Tattoo-like lesions	Hyperpigmented skin lesions of irregular shape characterised by a dark outline and a stippled pattern	Van Bressem *et al.*, 2003
Discolouration / Hypopigmentation	Whitish or paler colouration in the affected area	Maldini *et al.*, 2010; Bertulli *et al.*, 2016b
(Linear) Wounds	Presence of lacerations on the epidermis of the individual.	Bertulli *et al.*, 2016b; Leone *et al.*, 2019
Orange hues	Small but dense spots of orange colouration	Wilson *et al.*, 1997; Maldini *et al.*, 2010
Cutaneous elevations	Numerous or single skin elevations (papules & nodules)	Bertulli *et al.*, 2012; Van Bressem *et al.*, 2014; Bertulli *et al.*, 2016b

Miscellaneous marks	Marks that were not found to match with any of the other categories. Such marks vary both in shape and colouration	Maldini *et al.*, 2010; Bertulli *et al.*, 2016b

Table 2. Types of skin lesions found in bottlenose dolphins from Cardigan Bay, Wales, UK. A brief description of the marks and the scientific papers where information was taken and previous descriptions of skin lesions were made, are contained in the second and the third column respectively.

Figure 13. 025-01W calf exhibiting black punctiform (herpes-like) marks (photo: © Sea Watch Foundation, 2013).

Figure 14. The individual 066-10L showing tattoo lesions on the left flank (photo: © Sea Watch Foundation, 2012)

Figure 15. Ka (113-06R) exhibiting discolouration on the dorsal fin (photo: © Sea Watch Foundation, 2018)

Figure 16. Zorro (083-01W) presenting a linear white wound along with discolouration on the leading edge of the dorsal fin (photo: © Sea Watch Foundation, 2018).

Figure 17. The calf 091-08S covering with orange hues, almost on its entire body parts depicted here. (photo: © Sea Watch Foundation, 2013)

Some of the other skin marks identified here included lesions, probably caused by ectoparasites (e.g. barnacles), always according to the (visual) assessment of the shape of the mark. (Figure 20). However, the level of confidence regarding the origin of these marks is not high.

Figure 18. The individual 036-06W showing an ectoparasite (probably *Pennella spp.*) attached on the leading edge of its dorsal fin. Linear marks (probably caused by conspecifics) are also present in the dorsal fin (photo: © Sea Watch Foundation, 2012)

Figure 19. 069-01S calf suffering from miscellaneous marks that have covered almost its entire body. A hole in the middle of these polygonical marks suggest that they might have caused by attachments of barnacles. These lesions are quite similar to those described as "Polygons" in Maldini *et al.* (2010) (photo: © Sea Watch Foundation, 2013)

Figure 20. The individual 145-04W exhibiting discolouration on its dorsal fin (among other marks). This type of discolouration might be attributed to the attachment of ectoparasites according to the shape of these white patches (photo: © Sea Watch Foundation, 2018)

As mentioned above, four types of skin marks were used in the subsequent statistical analyses: a) Tattoo & tattoo-like lesions, b) punctiform (herpes-like) marks, c) discolouration and d) wounds. These types of skin deformities were the most common and notorious ones found in the sample assessed here and, hence, they were selected for the analysis part. It is worth to be noted though that these categories include some other categories that were merged for the purposes of the analysis (f.e. pox-like marks were classified as tattoo lesions due their common source of origin (pox-like viruses)).

4.2 Probability of skin marks across years

The results of the analysis indicate that there was a significant increase in prevalence of tattoo lesions across years (Intercept = -355+122, slope =0.18 ± 0.06, x^2 =80.34, p=0.004.) (Figure 21).

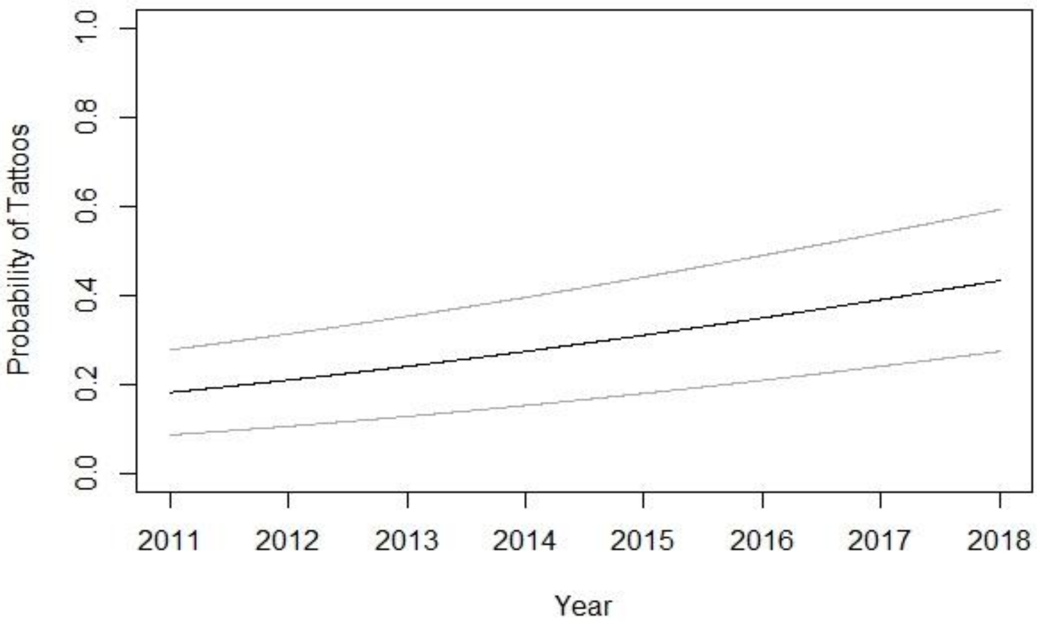

Figure 21. Probability of tattoo marks across years for the period 2011-2018

On the contrary, there was no change in prevalence of punctiform marks across years (Intercept =152+164, slope = -0.07 ± 0.08, x^2=94.12, p = 0.35) (Figure 22)

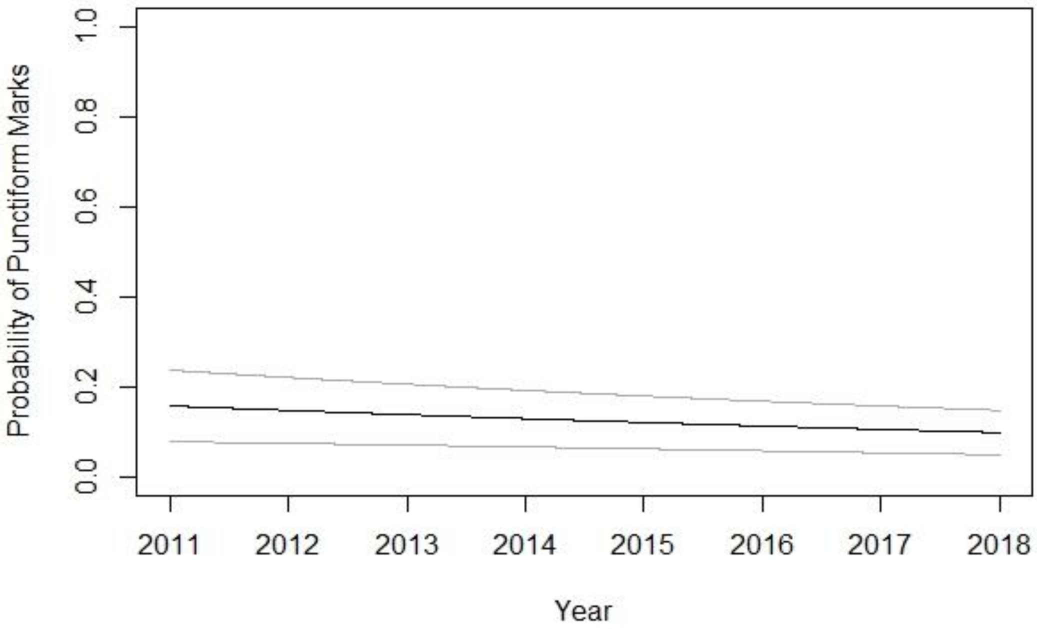

Figure 22. Probability of punctiform marks across years for the period 2011-2018

Similarly to punctiform marks, no significant change in the prevalence of discolouration has been observed across years (Intercept= -34+131, slope =0.02 ± 0.06, x^2 =147.5, p =0.8) (Figure 23).

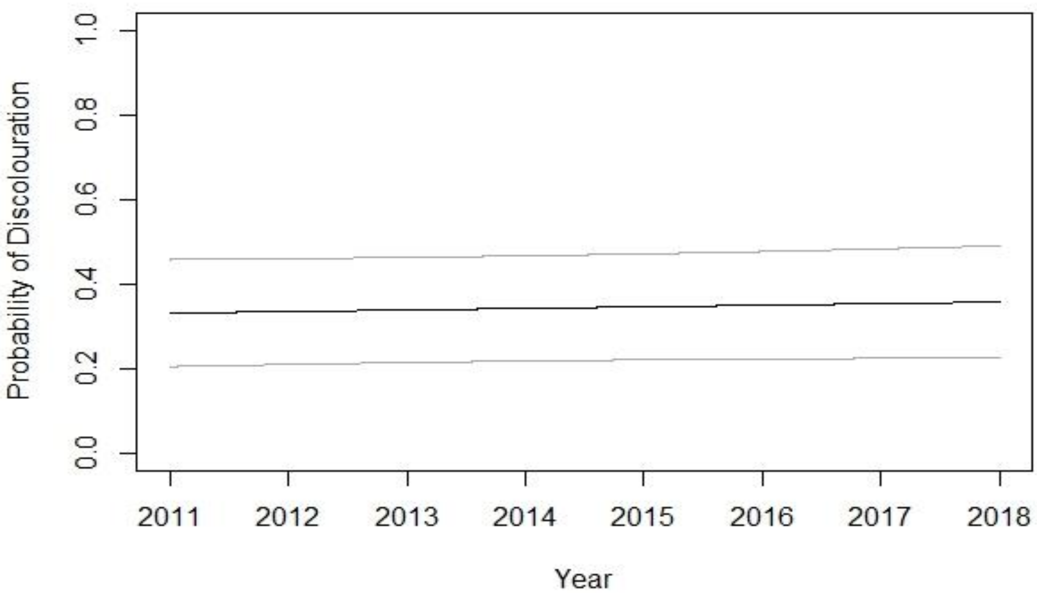

Figure 23. Probability of discolouration across years for the period 2011-2018

Finally, wounds seemed to be changing over the years, as the results of the analysis indicate a significant increase in their prevalence over the years. (Intercept= -475+140, slope=0.24 ± 0.07, x^2=53,47, p = 0.003.) (Figure 24).

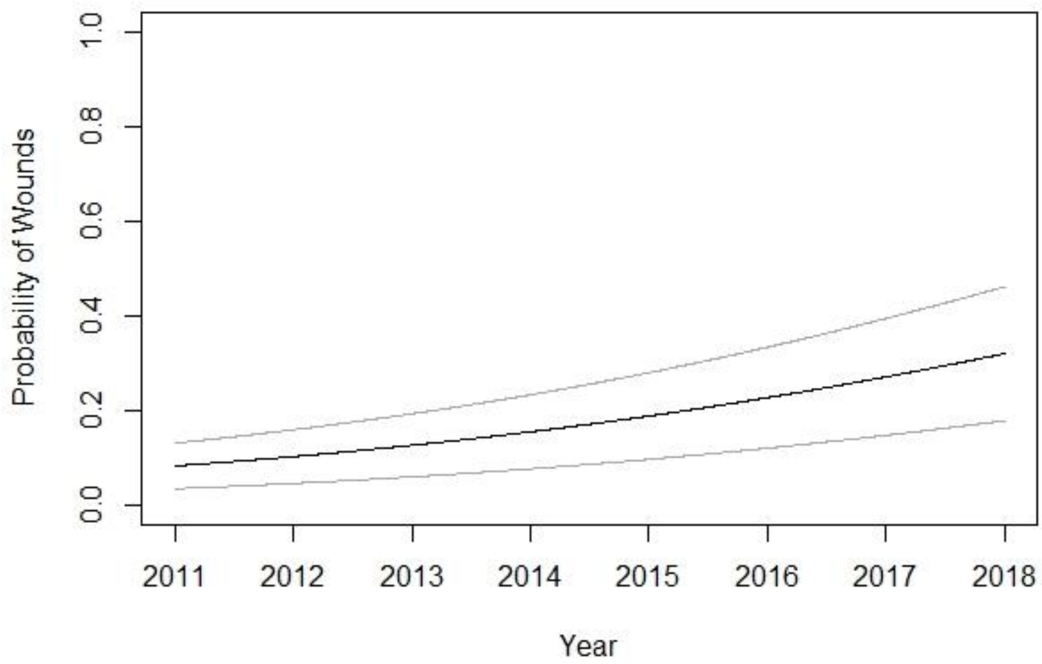

Figure 24. Probability of wounds across years for the period 2011-2018

4.3 Prevalence of lesions among groups of individuals

Tattoos

Overall, tattoo lesions did not show a significant change in prevalence among groups of individuals (sex and maturity) and periods.

More specifically, there was no significantly higher prevalence (x^2=584.93, p=0.34) of tattoos in males (0.35) and unknowns (0.33) than in females (0.27) (Figure 25).

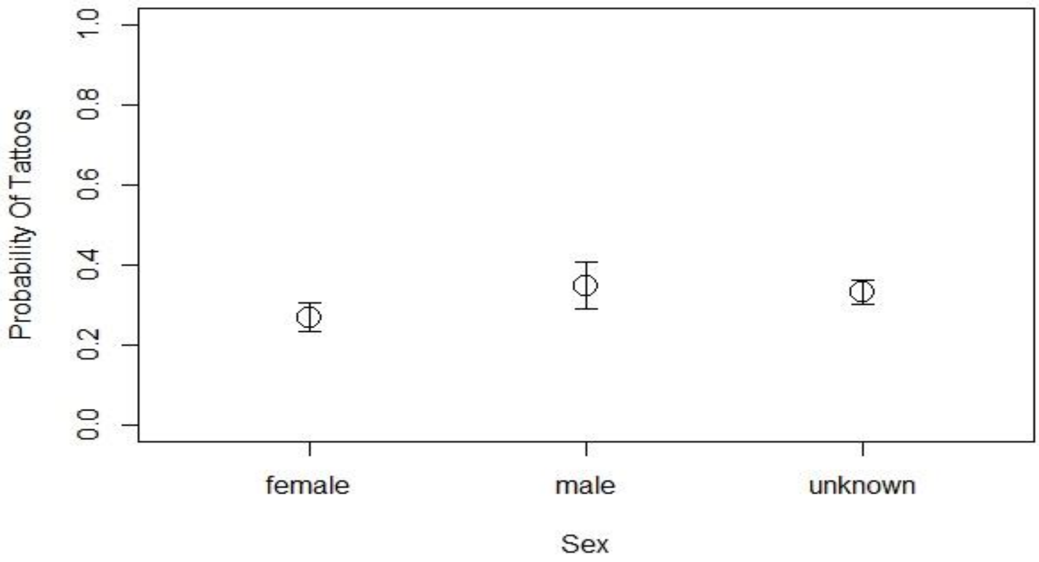

Figure 25. Probability of tattoo lesions between groups according to their sex

As for the level of maturation, there was also no significant difference (x^2=587.08, p=0.8) in the prevalence of tattoo lesions between adults (0.31) and calves (0.32) (Figure 26).

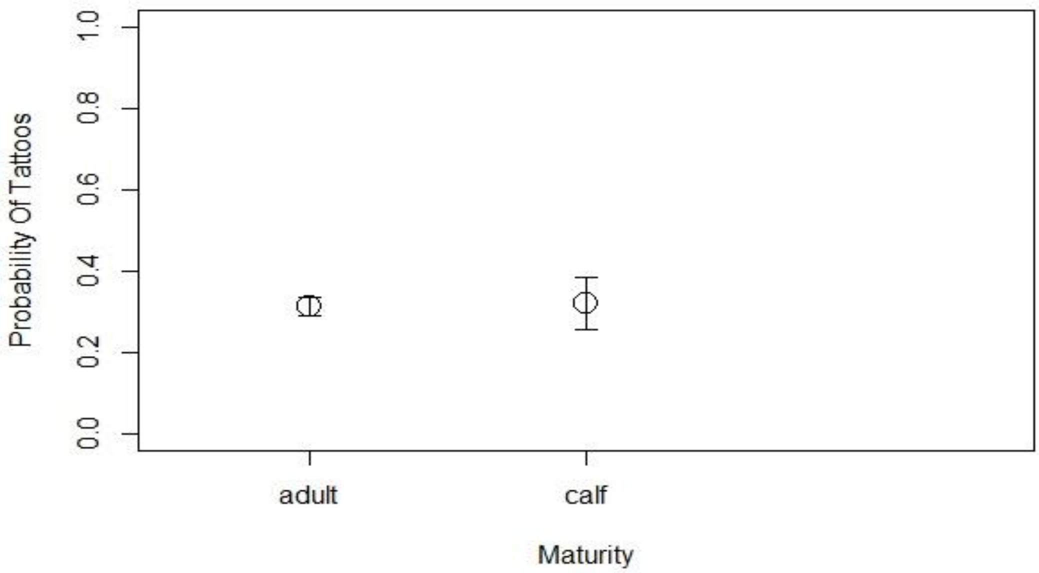

Figure 26. Probability of tattoo lesions between groups according to their level of maturation.

Finally, there was no significant difference (x^2=584.34, p=0.25) in the prevalence of tattoo lesions between P1 (0.26), P2 (0.35) and P3 (0.32) periods (Figure 27).

Mean values of the probability of tattoo lesions per year, per groups of individuals (i.e. sex & maturity) and per period are presented below (See Table 3.).

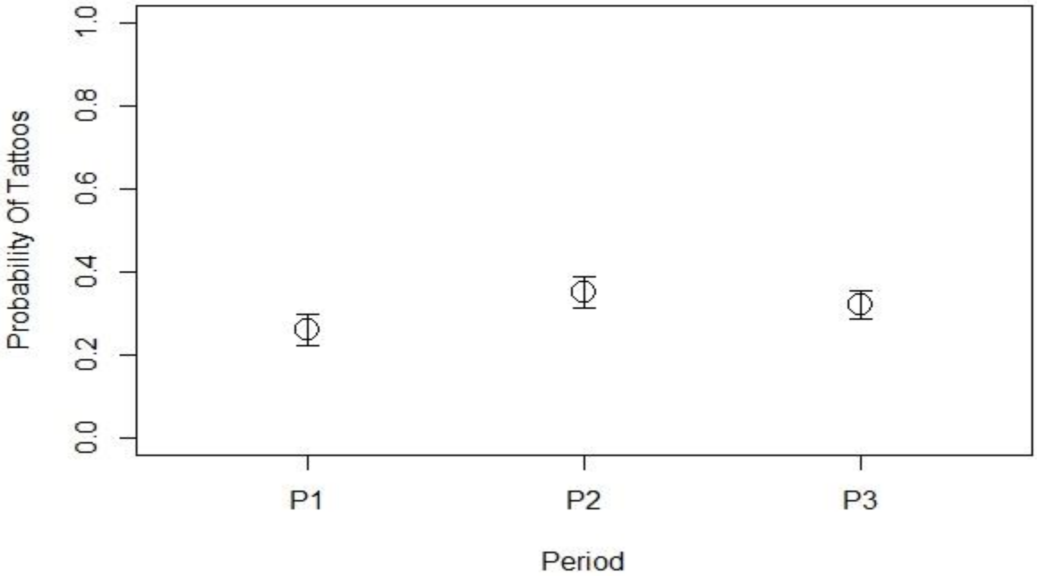

Figure 27. Probability of tattoo lesions according to the period when individuals were photographed.

TATTOOS								
Year	2011	2012	2013	2014	2015	2016	2017	2018
Mean	0.67	0.24	0.31	0.27	0.22	0.36	0.44	0.55

Period	**P1**	**P2**	**P3**
Mean	0.26	0.35	0.32

Maturity	**Adult**	**Calf**
Mean	0.31	0.32

Sex	**Female**	**Male**	**Unknown**
Mean	0.27	0.35	0.33

Table 3. Aggregation of the overall probabilities of tattoo lesions. Probabilities are expressed by a mean value per year, period and group of individuals (sex and maturity).

Punctiform (Herpes-like) Marks

There was no significant difference in prevalence (x^2=79.69, p=0.051) of punctiform marks between females (0.09), males (0.15) and unknowns (0.17) (Figure 28).

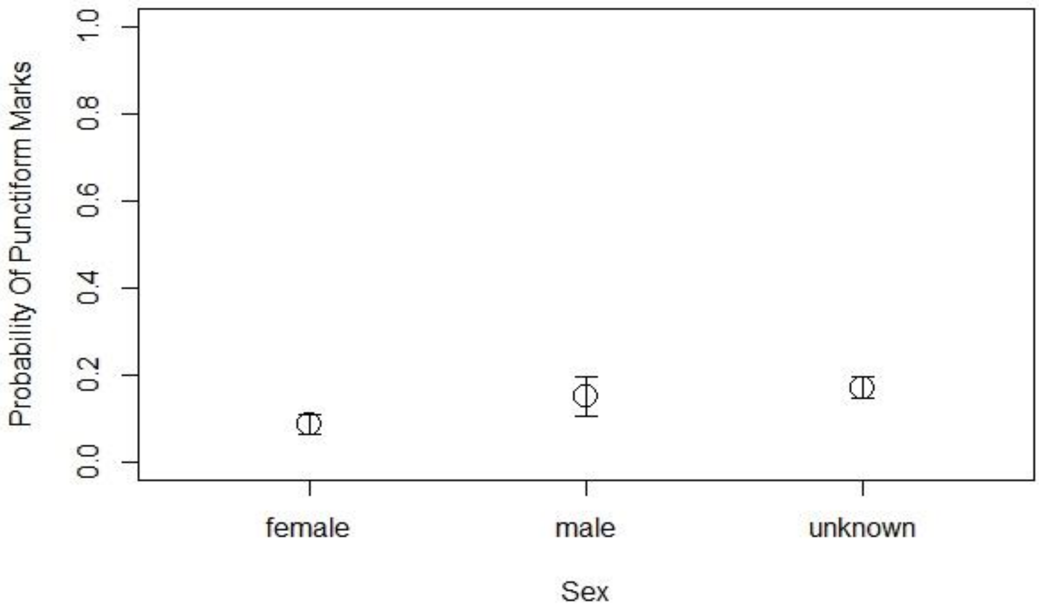

Figure 28. Probability of punctiform marks between groups according to their sex.

On the contrary, there was a significantly higher prevalence (x^2=364.42, p=0.01) of punctiform marks in calves (0.38) than in adults (0.11) (Figure 29).

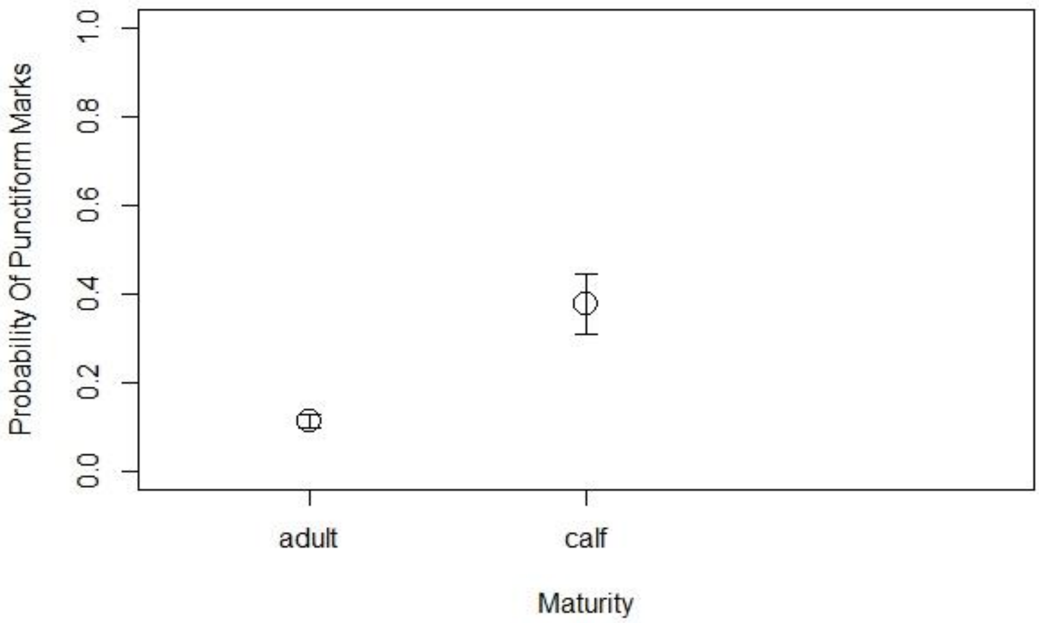

Figure 29. Probability of punctiform marks between groups according to their level of maturation.

As for the periods, there has been observed a significant increase (x^2 =370.87, p=0.0006) in prevalence of punctiform marks during P1 (0.17) and P2 (0.21) comparatively to P3 (0.07) (Figure 30).

Mean values of the probability of punctiform marks per year, groups of individuals (i.e. sex & maturity) and period are presented below (See Table 4.).

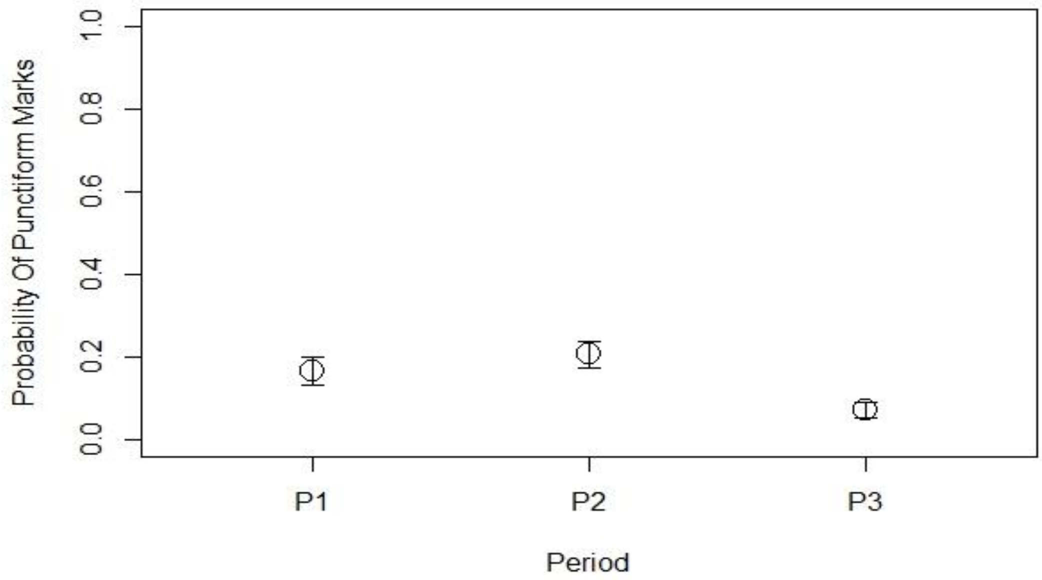

Figure 30. Probability of punctiform marks according to the period when individuals were photographed.

PUNCTIFORM MARKS

Year	2011	2012	2013	2014	2015	2016	2017	2018
Mean	0.33	0.20	0.24	0.45	0.06	0.11	0.08	0.25

Period	**P1**	**P2**	**P3**
Mean	0.17	0.21	0.07

Maturity	**Adult**	**Calf**
Mean	0.11	0.38

Sex	**Female**	**Male**	**Unknown**
Mean	0.09	0.15	0.17

Table 4. Aggregation of the overall probabilities of punctiform marks. Probabilities are expressed by a mean value per year, period and group of individuals (sex and maturity).

Discolouration

There was a significantly higher prevalence (x^2=590.26, p=0.0004) of discolouration in males (0.68) comparatively to females (0.25) and individuals of unknown sex (0.38) (Figure 31).

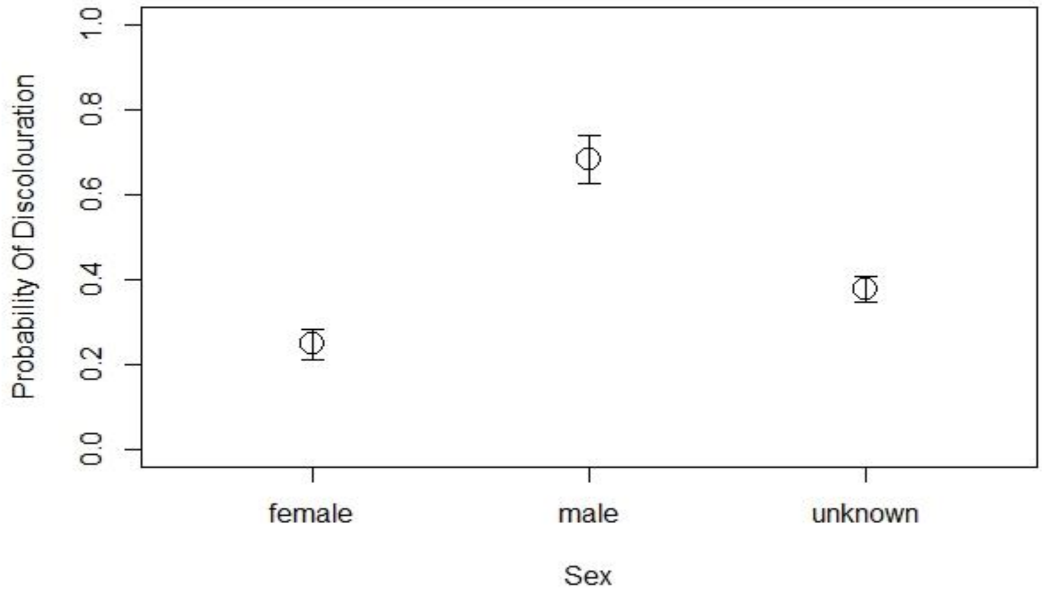

Figure 31. Probability of discolouration between groups according to their sex.

Regarding maturation level, there was also a significant difference (x^2=618.44, p=0.004) in prevalence of discolouration with adults (0.40) being affected more than calves (0.21) (Figure 32).

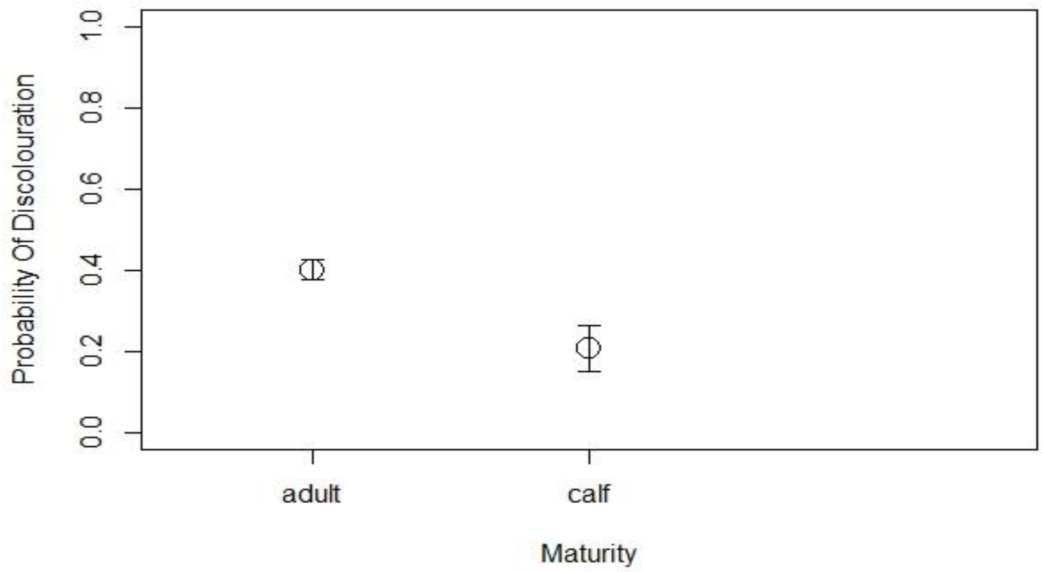

Figure 32. Probability of discolouration between groups according to their level of maturation.

As for the periods, there was no significant difference (x^2=624.29, p=0.33) in prevalence of discolouration between P1 (0.43), P2 (0.37) and P3 (0.35) (Figure 33).

Mean values of the probability of discolouration per year, per groups of individuals (i.e. sex & maturity) and per period are presented below (See Table 5.).

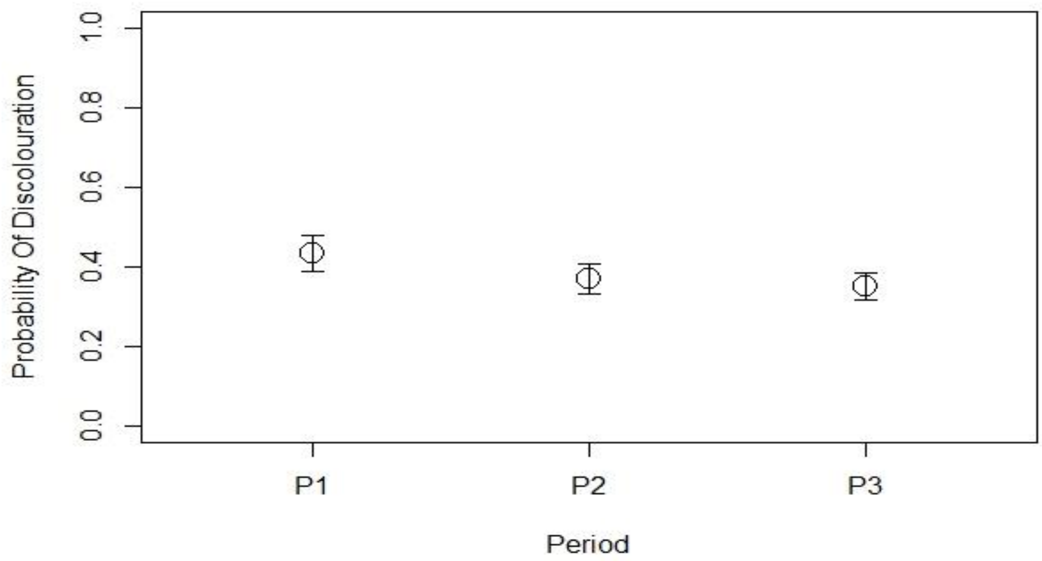

Figure 33. Probability of discolouration according to the period when individuals were photographed.

DISCOLOURATION

Year	2011	2012	2013	2014	2015	2016	2017	2018
Mean	0.33	0.40	0.41	0.45	0.30	0.38	0.32	0.53

Period	**P1**	**P2**	**P3**
Mean	0.43	0.37	0.35

Maturity	**Adult**	**Calf**
Mean	0.40	0.21

Sex	**Female**	**Male**	**Unknown**
Mean	0.25	0.68	0.38

Table 5. Aggregation of the overall probabilities of discolouration. Probabilities are expressed by a mean value per year, per period and per group of individuals (sex and maturity).

Wounds

Probability of exhibition of wounds was found to be significantly increased (x^2=449.53, p=0.05) in males (0.35) comparatively to females (0.10) and individuals of unknown sex (0.21) (Figure 34).

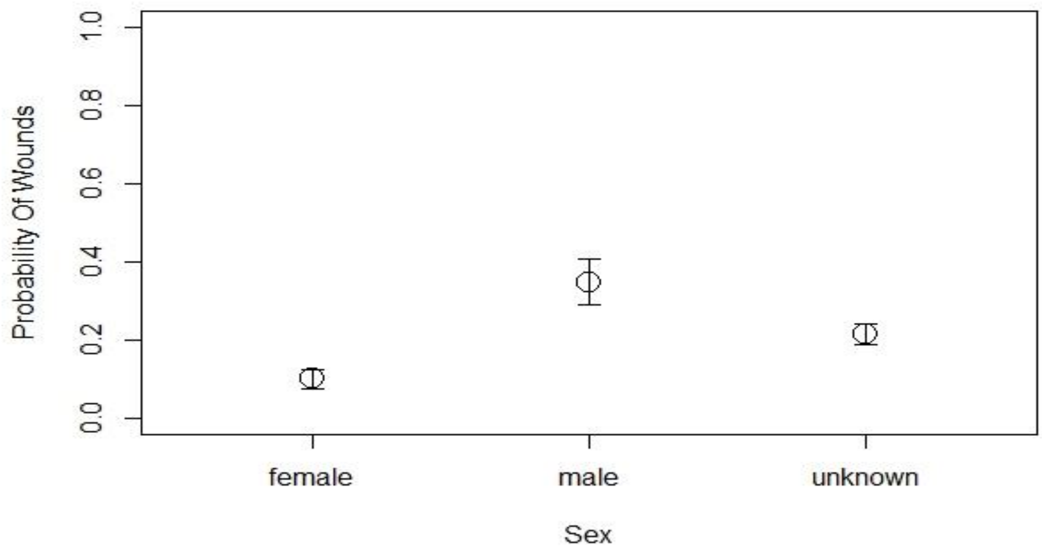

Figure 34. Probability of wounds between groups according to their sex.

In terms of maturity, there was also a significant increase (x^2=451.01, p=0.02) in prevalence of wounds in adult individuals (0.22) than in calves (0.02) (Figure 35).

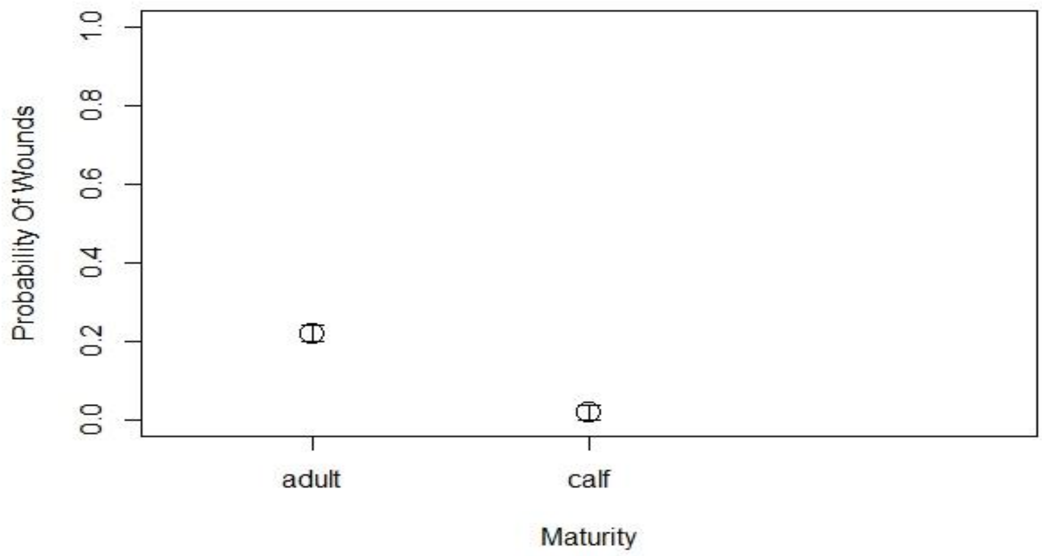

Figure 35. Probability of wounds between groups according to their level of maturation.

Finally, there was no significant difference (x^2=468.13, p=0.84) in probabilities of wounds between P1 (0.20), P2 (0.21) and P3 (0.18) periods (Figure 36).

Mean values of the probability of wounds per year, per groups of individuals (i.e. sex & maturity) and per period are presented below (See Table 6.).

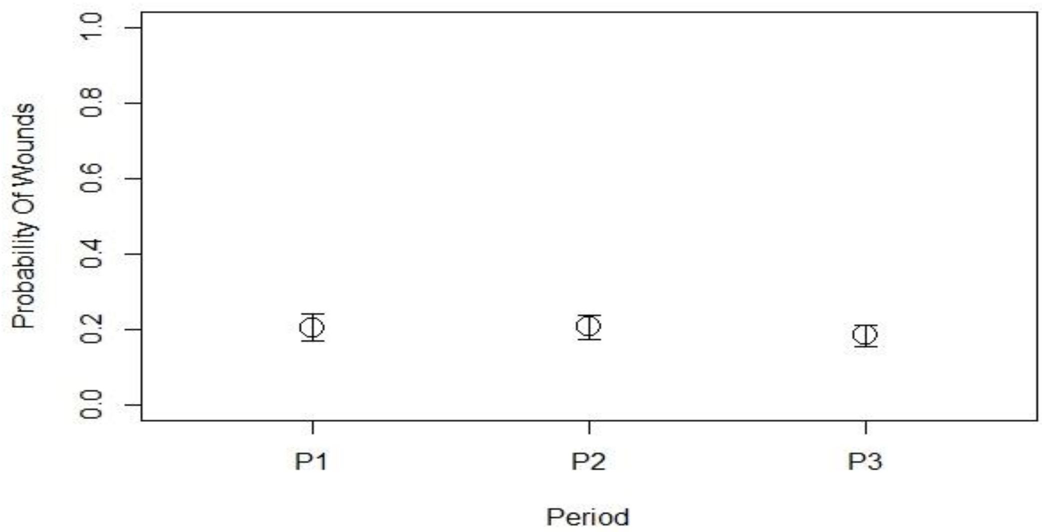

Figure 36. Probability of wounds according to the period when individuals were photographed.

WOUNDS								
Year	2011	2012	2013	2014	2015	2016	2017	2018
Mean	0.67	0.13	0.15	0.23	0.08	0.30	0.52	0.29

Period	**P1**	**P2**	**P3**
Mean	0.20	0.21	0.18

Maturity	**Adult**	**Calf**
Mean	0.22	0.02

Sex	**Female**	**Male**	**Unknown**
Mean	0.10	0.35	0.21

Table 6. Aggregation of the overall probabilities of wounds. Probabilities are expressed by a mean value per year, per period and per group of individuals (sex and maturity).

5. Discussion

5.1 General discussion

Previous studies focusing on free-ranging delphinid populations worldwide pointed out that prevalence of skin lesions varied, ranging between 48% and 100% in general, and between 63% and 100% for bottlenose dolphins specifically (Wilson *et al.,* 1999; Bearzi *et al.,* 2009; Maldini *et al.,* 2010; Hart *et al.,* 2012; Leone *et al.,* 2019). In the UK, studies focusing on the investigation of skin lesions date back to 1992 when Thompson & Hammond triggered the idea of monitoring a group of wild bottlenose dolphins, resident in Moray Firth (NE Scotland), with the use of photo-ID techniques. Results of their study lead to the first categorisation of skin marks in bottlenose dolphins in UK waters. Five years later, a more systematic research on that population was conducted by Wilson and his colleagues (1997) who proposed a new, more accurate classification of skin marks. This classification was used as a baseline for a number of similar studies conducted later (See for example Hart *et al.*, 2012 and Gonzalvo *et al.,* 2015). Investigation of skin lesions concerning the Welsh bottlenose dolphin population were not the exception to the rule since two relevant studies were implemented in the past. The first one took place back in 2006 by Magileviciute who studied the social network of bottlenose dolphins and attempted to categorise the skin marks found on them according to Wilson's classification. A more systematic study targeting, exclusively, on the investigation of skin lesions in Welsh bottlenose dolphins and their spatio-temporal patterns was conducted in 2014 by Akritopoulou, who interestingly showed that at least 73% of the individuals she assessed, were suffering by at least one type of lesion. In general, photographic methods have been proven to be a useful, cheap and effective measure in terms of investigation of epidermal skin marks on cetaceans. Most of the times, assessment of such deformities equals to accurate evaluation of the health condition of cetacean populations, since lesions that are present on their body reflect their health status (Wilson *et al.,* 1999; Van Bressem *et al.*, 2009;

Mouton & Botha, 2012; Hart *et al.*, 2012; Sanino *et al.*, 2014; Hupman *et al.*, 2017; Powell *et al.,* 2018; Chan & Karczmarski, 2019).

The present study was focused on four specific categories of skin lesions that had known source of causation. It is worth to be noted here that wounds were also considered as a skin lesion as they are highly related with teeth rakes. Teeth rakes were used in previous studies (Thompson & Hammond, 1992; Maldini *et al.,* 2010; Hupman *et al.,* 2017) as "lesions" as they could be utilised as a pathway that allows pathogens to penetrate the epidermis and form actual lesions (Van Bressem *et al.*, 2009). Moreover, the "tattoo" category here, included marks that exhibit a high macroscopic similarity with tattoo skin disease (TSD) described in Van Bressem *et al.* (2009), but it is not certain if they are actually "tattoos", since confirmation of aetiology of such marks could be not confirmed, due to the unfeasibility of conducting biopsy sampling. In such cases, it is common phenomenon to add the suffix "-like" to those lesions (f.e. tattoo -"like" disease) (Van Bressem *et al.,* 2009; Bertulli *et al.,* 2012)

5.2 Skin lesion prevalence across years

Results of this study showed an increasing tendency in prevalence of tattoo lesions and wounds over the years. Tattoo skin disease (TSD) is a well-studied type of lesion (Van Bressem *et al.,* 1999, 2003, 2009, 2015) and its increasing prevalence on Welsh bottlenose dolphin individuals over time, might indicate a poor health condition of the infected dolphins (Wilson *et al.,* 1999, 2000; Maldini *et al.*, 2010). Moreover, as many studies witness (Wilson *et al.,* 1999; Powell *et al.,* 2018; Chan & Karczmarski, 2019), TSD might also reflect a possible environmental degradation in the area overtime. In Cardigan Bay, recreational activities become more frequent over the years. More specifically, boat disturbance has been steadily increasing due to the increased use of boats for recreational activities, showing signs of disturbance on bottlenose dolphin social networks, which could have important implications on the transmission of diseases, causing skin lesions (Bristow & Rees, 2001; Richardson, 2012). Bearing in mind the routinely appearance of bottlenose dolphins in coastal areas along with the strong presence of the aforementioned recreational activities and the potential anthropogenic impacts in these areas (e.g. chemical pollution, active fisheries etc.) that may lead to a certain degradation of the water quality and the environment in general (Lane *et al.,* 2014; de Vere *et al.*, 2018), the results of the study regarding tattoo lesions were not surprising ones. The occurrence and outspread of viral diseases (including TSD amongst others) has been shown to be associated with the amount of pollutants occurring in an area such as heavy metals, plastics and persistent organic pollutants POPs (See Mouton & Botha, 2012 for details). Coastal areas are undoubtedly more susceptible to human intervention/impacts comparatively to offshore marine areas and so, the likelihood of encountering viral diseases such as TSD is greater.

Regarding the wounds, results were also expectable. This category included skin marks caused, mainly, by intra-specific interactions and, to a lower extent, by anthropogenic activities. As for the latter, the most notorious ones were marks caused by fishing lines. However, linear marks of unknown origin were also detected. Interaction with conspecifics is evident with the presence of teeth rakes over the dolphin's body. In the present study, only teeth rakes resulted in lacerations in the epidermis and slight penetration in the dermis (Leone *et al.*, 2019) were used here and classified as "wounds". A reasonable explanation on the increase of the prevalence of wounds across years lies again to the fact stated above, concerning the increasing use of boat for fishing purposes or other recreational activities. Increased number of boats might enhance the probability of a boat strike or might result in the entanglement of one or more dolphins in fishing gears (Lane *et al.,* 2014; Dolman & Moore, 2017), verifying in that way the interactions existing between bottlenose dolphins and fisheries (de Vere *et al.,* 2018). Another explanation might probably be linked to various agonistic interactions existing within social groups (Marley *et al.*, 2013) which are primarily determined by the sex of the individuals (Connor *et al.,* 1992; Samuels & Gifford, 1997) (See also § 5.3 below).

5.3 Skin lesion prevalence among groups of individuals

Results of the present study regarding skin lesion prevalence among groups of individuals showed that there were significant differences in three of the four categories analysed here. Although having showed an upward trend over the years, tattoos were the only skin lesion category that did not exhibit variation neither between individuals of a different sex and maturity, nor between periods where photographs of the dolphins were taken. On the contrary, punctiform marks, discolouration and wounds presented significant differences among groups. A summarisation of the outputs of the analysis regarding skin mark categories and the variables that were tested is presented below in Table 7.

Test	**Tattoos**	**Puncformation**	**Discolouration**	**Wounds**
Sex	NONE	NONE	Male +	Male +
Age	NONE	Calf +	Adult +	Adult +
Period	NONE	P1 and P2 +	NONE	NONE
Year	Increase +	NONE	NONE	Increase +

Table 7. A summarisation of the variables along with the skin marks categories that were tested in the present study. A significant increase (+) in the prevalence of tattoo lesions over the years has been observed. Punctiform marks were significantly higher in calves during the early and mid-summer periods (P1 and P2). Discolouration seemed to be significantly higher in adults and more specifically in male adults. Wounds showed an increase over the years with adult males being the individuals that were affected the most. “NONE” was used in cases when non-significant change has been observed among groups of individuals or across periods or years.

Punctiform – Herpes-like marks

Although these marks were not found to present a significant increase or decrease across years, they seemed to be more prevalent during the early and the mid-summer period. These results could probably raise questionings since such change was not expected and there was found no relevant information in the literature that could support these findings. However, there is a possible explanation for this case and is linked, directly, with the data used in the study, since a greater number of photographs of individuals suffering from punctiform marks were taken during the early and the mid-summer periods (P1 & P2). Another possible explanation could be related to the increase of individuals occurring during summer months (Ugarte & Evans, 2006;

Feingold & Evans, 2014a) but in general, the level of certainty in terms of explaining these differences is below average.

As for the prevalence among groups of individuals and specifically between maturation levels, the results were surprising, indicating a significantly higher prevalence in calves than in adults. A first, possible explanation here is the difference in the number of individuals assessed here since there were used photographs of 182 adults and only 31 calves for the statistical analysis. Higher prevalence of punctiform marks in calves was also surprising due to the fact that calves are likely to be protected from the transmission of viral (mainly endemic though) diseases through maternal immunity (Van Bressem *et al.,* 2009; Maldini *et al.,* 2010; Powell *et al.,* 2018). However, transmission of the disease to calves is not always inevitable as it has been reported to pass into calves through suckling (Van Bressem *et al.,* 1994), and this might be the most reasonable explanation in terms of interpretation of the results here.

Discolouration & Wounds

Discolouration and wounds seemed to follow a similar pattern in terms of prevalence among groups of individuals, as both of them were found to be more prevalent in adult males. Discolouration is linked with a broad range of causes including environmental causes (e.g. fluctuations in water temperature, salinity and solar radiation (Wilson *et al.,* 1999; Martinez-Levasseur *et al.,* 2010; Hart *et al.,* 2012)), intra- (Scott *et al.,* 2005; Marley *et al.,* 2013) and inter-specific interactions (Corkeron *et al.,* 1987b; Heithaus, 2001) and attachment of ectoparasites such as *Xenobalanus globicipitis* on dolphins' several body parts (mostly on the dorsal fin) (Ribeiro *et al.,* 2010; Samarra *et al.,* 2012). Interaction with conspecifics or antagonistic species and predators is also a major source of epidermal scars. Such scars could be subsequently turned into lacerations or even small shallow indentations (Leone *et al.,* 2019), which consist a category of wounds. In some cases, discolouration might be a result of successive scarring from a wound of traumatic origin (Maldini *et al.,* 2010) which, clearly, indicates a possible relationship between discolouration and wounds. Discolouration on cetaceans could be exhibited either as patches or uniformly. Hence, an explanation for the similarity in trend with wounds is probably the fact that many (not all though) wounds showed a different, usually paler, pigmentation in the area where they were present. Indeed, in several cases, where individuals were found to carry such marks, they were included in both the "discolouration" and "wound" categories in the analysis.

The fact that male individuals showed an increased prevalence, both in discolouration and wounds, was an expected one, especially for the latter category. In terms of differences in prevalence of wounds according to sex, evidence exists in the literature, with studies, focusing on sex differences in terms of social interactions, pointing out a higher probability of scarring in male than in female individuals (Scott *et al.,* 2005; Marley *et al.,* 2013). Adult males are known to form affiliations with one or two more dolphins known as "alliances" (Smolker *et al.,* 1992; Connor *et al.,* 2010). Formation of alliances between males enhance the probability of getting access to females and, also, defend them against other males (Connor *et al.,* 2010). Higher probability of scarring and wounds on males might be highly depending on differences in social group formations between males and females (See §1.3.2) and might also be connected to differences in reproductive strategies between sexes. Reproductive strategies in males seem to clearly focus on getting and maintaining access to females during oestrus periods while female reproductive strategies are probably centered on access to resources necessary for reproduction and food and on the protection of their calves from conspecifics and predators (Samuels *et al.,* 1997; Mann *et al.,* 2000; Sprogis *et al.,* 2016). Moreover, adult males have been reported to be involved in intersexual conflicts as they tend to exhibit sexual coercion towards females during the breeding season (Scott *et al.,* 2005; Lee *et al.,* 2019) indicating the exhibition of a more aggressive and violent behaviour (comparatively to females)). In the present study, interactions among individuals were probably entirely intra-specific since no marks were found to be caused by antagonists (e.g. other delphinid species) or predators such as the killer whale (*Orcinus orca*).

As mentioned above, results of the study indicate a possible relationship between wounds and discolouration as they seem, most of the times, to be associated with each other and they tend to follow a similar pattern in terms of prevalence between sexes. To a further extent, these results verify the fact that adult male bottlenose dolphins do probably have a different lifestyle from females. Indeed, bottlenose dolphins exhibit a variety of behavioural differences between sexes which are related to social associations, reproductive and feeding strategies, foraging tactics, size of home range and habitat selection (Weaver, 2015). For example, Smith *et al.* (2013) reported that in terms of foraging and mating opportunities, male bottlenose dolphins may range over larger areas. Similarly, Sprogis *et al.* (2016) suggested that female bottlenose dolphins had smaller home ranges, delimited mainly in coastal, sheltered water habitats, presumably for the safety of their calves from predators and conspecifics. Leone *et al.* (2019) study on bottlenose dolphin populations in the central Mediterranean verified that there is a tendency of females to prefer such safer areas. They also pointed out that males preferred habitats with higher probability of spotting and potentially capturing food, as they were spotted to wander nearby areas with trammel nets. Another possible explanation concerning the increased number of wounds on males could be related to potential interactions with their prey. As males tend to roam larger areas (as stated above), and hence deeper waters than females, there is an increased possibility of selecting larger preys. Interaction with larger preys (e.g. larger fish or squids) could possible lead to natural skin marks as well. For example, Mariani *et al.* (2016) in their study described natural marks caused by squids on Risso's dolphins (*Grampus griseus*) in the Mediterranean Sea. However, this hypothesis seems to lack of certainty, since no such documented reports on bottlenose dolphins exist.

Although behavioural responses in wild bottlenose dolphins are characterised by extreme complexity and variability (Weaver, 2015), bearing in mind the aforementioned differences in lifestyle and behavioural responses between male and female bottlenose dolphins, it looks like this study may, at least partially, verify the fact that these differences make male bottlenose dolphins more susceptible to wounds and discolouration. Differences between sexes in terms of susceptibility on skin marks, along with the understanding of their behavioural patterns in general, should be further examined in future studies in order to gradually fill the gap of uncertainty and draw precise conclusions in terms of the lifestyle and, specifically, the sociality of these incredible and mesmerising animals.

6. Limitations and Future Recommendations

Limitations of the present study involved mainly the quality and quantity of the data for the period examined, the skin lesion categories used for the analysis and the time frame for investigating and sorting out the data. Selection of the images assessed here was according to relatively strict criteria as images were selected not only according to the quality and resolution but, also, according to the angle of which they were taken. Detection and evaluation of skin lesions according to photo-ID might result into inaccurate and imprecise estimations and hence, parameters concerning the photographs should be seriously taken into consideration in order to reduce the probability of an incorrect estimation as much as possible. Photo-ID is undoubtedly an effective, non-invasive technique when it comes to investigation of the prevalence of body marks in wild cetacean populations. Nevertheless, since photo-ID is able to capture images only of a relatively small proportion of an individual's body, underestimation of skin lesions prevalence is a common phenomenon; photo-ID techniques do actually reveal the smallest proportion that could have potentially been affected (Hart *et al.,* 2012).

The use of digital photography in Cardigan Bay for cetacean monitoring purposes initiated back in 2007 and hence, the initial target in the present study was to investigate the trends of skin lesions for the period 2007-2018. However, due to the limited time frame for the implementation of the project along with the time-consuming process of the evaluation of the images, the time frame had to be reduced to eight years (2011-2018). Moreover, photographs taken in the field were targeting mainly the dorsal fin, according to the photo-ID protocols. As a result, other body areas affected by skin lesions, including the back, the flanks, the peduncle etc., might not be adequately representative in terms of the aforementioned evaluation of skin lesions.

Another limitation was the number of categories of skin marks used in the present study. For the purposes of the analysis, there was a merge of some categories described in previous studies (Thompson and Hammond, 1992; Wilson *et al.,* 1997; Van Bressem *et al.,* 2009, 2015; Leone *et al.,* 2019). For instance, categories that were characterised by a paler pigmentation and described previously as abraded fin tip (AFT) and white fin-fringe (WFF) (See Wilson *et al.,* 1997 & Gonzalvo *et al.,* 2015), pale lesions (See Hart *et al.,* 2012) and pale skin patches (PSP) (See Sanino *et al.,* 2014) were all included in a new category called "discolouration". This category did also contain lesions previously described with the same name (discolouration), in a similar way (See Maldini *et al.,* 2010; Bertulli *et al.,* 2016). Samewise, pox-like, tattoo-like and tattoo marks were all utilised as "tattoos" in the analysis, due to their common source of origin (they are caused by pox-like viruses, See Van Bressem *et al.,* 2009 & 2015 for details), while skin marks described as scratches, linear wounds and small shallow indentation (SSI) (See Leone *et al.,* 2019) were utilised here as "wounds". The latter category included skin marks caused by both interaction with conspecifics and anthropogenic impacts (e.g. fishing gears). As for some other categories identified here and were not used in the analysis (e.g. orange hues), this happened due to the very limited number of images (and individuals) that exhibiting such lesions in comparison with the ones used in the analysis.

The last limitation of the study is linked to the number of individuals assessed here, as there were used only individuals of known identity (with a known reference code number), all of which were marked. A potential use of unmarked individuals could have resulted in overestimation of the prevalence due to pseudoreplication. For the analysis among groups, individuals were separated into calves and adults and into females, males and unknown in terms of maturity and sex respectively. Again, for the purposes of the analysis (simplification of the dataset, easiness to run the code in R etc.), individuals belonging in the juvenile stage were also classified as calves, while "possible female" and "possible male" individuals were classified as females and males respectively.

The Sea Watch Foundation holds a database (catalogue) that, currently, contains images of 388 identified individuals and is updated every year, which may result in more photographic data and more confirmed genders of dolphins. As a result, more systematic and quantitative analyses, relevant to the extent and prevalence of different skin lesion categories could be implemented, with a much higher probability of entire precision and accuracy on the estimates.

The population of Welsh bottlenose dolphins is the largest coastal population occurring in British waters with resident individuals consisting a proportion between 52% and 63% of the entire population of the species in UK (Feingold & Evans, 2014a). This fact clearly indicates the importance for systematic monitoring of the population. Based on the results and the limitation of the present study, a number of recommendations is proposed, aiming in future studies regarding the Welsh bottlenose dolphin population and, more specific, in the assessment of the population's health condition according to evaluations on skin marks found on them. Future recommendations include the following:

- Conduction of studies that will also include dolphin populations resident to other coastal areas in UK such as the Moray Firth (Scotland). Such studies could provide invaluable information in terms of monitoring and comparing bottlenose populations and, also, evaluate their health status in a wider, regional scale.

- Creation of a new database relevant to health assessment of the dolphins where information regarding skin lesions and overall body condition per individual is contained.

- Conduction of more opportunistic surveys in order to acquire larger amount of data.

- Conduction of systematic studies focusing exclusively on the investigation of seasonal rates of skin marks acquired from intra-specific interactions in order to determine and elucidate aggression levels (f.e. during the breeding season) among individuals, as they have been proven to be a source of causation of skin marks (scratches, scars).

- Formation of new software packages aiming at the measurement and the calculation of the extent and prevalence of skin marks identified on bottlenose dolphin (and marine mammal in general) individuals.

- Close collaboration with institutes specialising in detection of skin lesions via microbiological techniques (e.g. molecular techniques, biopsies, PCR etc.) as they could provide invaluable information regarding microorganisms that are responsible for the cause of such dermal lesions (e.g. viruses, bacteria, parasites etc.).

7. Conclusions

The present study aimed to identify trends in prevalence of skin lesions of Welsh bottlenose dolphins focusing mainly on four categories, namely “tattoos”, “punctiform marks”, “discolouration” and “wounds”. In general, categorisation of skin lesions, in most of the studies that have been conducted in the past, followed a similar pattern which is based on classifications made in key publications (e.g. Wilson *et al.,* 1997) which were made according to the colour and the texture of the lesions (Thompson & Hammond, 1992). However, there is no strict pattern in terms of classification since it is up to the author at some extent. Here, the focus was given almost entirely on the origin of the skin conditions and the assessment was made using photo-id techniques. More specifically, skin conditions assessed here were caused from viruses (pox-viruses and, probably, herpes-viruses), intraspecific interactions and anthropogenic activities (fishing gears).

Viral infections are among the most notorious and well-studied ones in terms of infectious diseases (Sweeney & Ridgway, 1975; Baker, 1992; Van Bressem *et al.,* 2008; Mouton & Botha, 2012). In the present study, Welsh bottlenose individuals carrying pox-like and herpes-like marks were detected. Pox-like marks are expressed phenotypically either as circular light pigmentated marks with a darker outline and vice versa (Maldini *et al.,* 2010), or as larger irregular light or dark coloured lesions with a stippled pattern, commonly known as “tattoo” and “tattoo-like” lesions (Van Bressem *et al.,* 2003, 2009). On the other hand, infection by herpes viruses result in existence of black dots on the skin, known as herpes-like lesions or punctiform marks (Barr *et al.,* 1989; Bertulli *et al.,* 2016b). Both pox-viruses and herpes-viruses are known to be associated with suppression of host’s immune system (Van Bressem *et al.,* 2003, 2008, 2009; Powell *et al.,* 2018) and seem to be more prevalent in individuals exposed to polluted areas (Mouton & Botha, 2012; Jepson *et al.,* 2016). Such exposure is mainly related with POPs, plastics and heavy metals. In Cardigan Bay, significant increase in probability of tattoo lesions over the years showed in this study, is probably attributed to the degradation of the area, probably through anthropogenic activities such as pollution and recreational activities (Richardson, 2012; de Vere *et al.,* 2018).

Prevalence of skin lesions in bottlenose dolphins has been extensively used as a health indicator both in terms of individual and ecosystem's health (Pettis *et al.,* 2004; Maldini *et al.,* 2010; Powell *et al.,* 2018), since a variety of pathogens has been isolated and examined from such lesions (Geraci 1966; Smith *et al.,* 1983; Baker, 1992; Smolarek Benson *et al.,* 2006; Van Bressem *et al.,* 2008). Moreover, as a top predator, the bottlenose dolphin has the ability to bioaccumulate toxins both from the environment that inhabits and its consumed prey and, hence, it is considered an ideal bioindicator in terms of an ecosystem's health (Wells *et al.,* 2004; Powell *et al.,* 2018). Such apex predators are also termed "ecosystem sentinels" (Wells *et al.,* 2004). Utilisation of the "sentinel species animal model" (Bossart & Duignan, 2018) in terms of an aquatic ecosystem's health evaluation is, with no doubt, an extremely effective method.

Welsh bottlenose dolphin population is the largest semi-resident population along the British Isles. Results of the study in terms of viral infection indicated a potential exposure to degraded areas. Part of the Cardigan bottlenose dolphin population is known to range into the NE Irish Sea in winter (Feingold & Evans, 2014a), including the Liverpool Bay, which is known of being a polluted area (Law *et al.,* 1991). "Dolphin-visits" in Liverpool Bay enhance the chances of being infected and, hence, transmit the disease to conspecifics through their social interactions (Evans, *pers. comm.*). As a result, studies focusing on prevalence of skin lesions in terms of residency would be essential in order to draw more accurate conclusions in terms of such disease transmissions in a spatial scale.

The present study followed a different approach than Magileviciute (2006) and Akritopoulou (2014) studies in terms of assessing bottlenose dolphin skin conditions, since the classification here followed different patterns. More specifically skin conditions were classified mainly according to their source of causation (e.g. viral, anthropogenic etc.) and as a result, some categories previously described in these two studies were merged and were used as one category (as mentioned above). This study could provide important information for assessing the health of Welsh bottlenose populations suffering from specific skin disorders, mainly of viral and anthropogenic origin. Results are focusing both in prevalence across years and among groups of individuals and can be easily used as a baseline for future monitoring in terms of spatial and temporal trends.

The bottlenose dolphin (*Tursiops truncatus*) is listed under Annex II of the EU Habitats and Species Directive, as well as under Annex IV of the Directive (Council Directive 92/43/EEC), indicating the importance of the requirement of strict, large-scale spatial protective measures. Infectious diseases have raised major concerns in terms of conservation and sustainability of bottlenose dolphin populations (Smith *et al.,* 2009). Impacts of transmission of disease caused by viruses, which in some cases include pox-viruses that have been detected here, are able to cause even death of individuals and, to a further extent, potential small-scale extinctions among populations (Smith *et al.,* 2008; Van Bressem *et al.,* 1999, 2008). Anthropogenic pressures are able to alter biological, physical and chemical features of aquatic ecosystems. Potential increase of these pressures contributes to the exacerbation of such environmental alterations (Schuldt *et al.,* 2016) resulting in direct threats on bottlenose dolphin populations. Further conduction of systematic and quantitative studies focusing on skin lesion prevalence over time along with population dynamics and social networks of the species and the determination of their immunogenic structure would lead not only to the accurate understanding of the various disease transmissions, but also to better and wiser management towards the conservation and sustainability of the species.

8. References

Baker, I., O'Brien, J., McHugh, K., Ingram, S. N., & Berrow, S. (2018). Bottlenose dolphin (Tursiops truncatus) social structure in the Shannon Estuary, Ireland, is distinguished by age-and area-related associations. *Marine Mammal Science*, *34*(2), 458-487.

Baker, J. R. (1992). Skin disease in wild cetaceans from British waters. *Aquatic Mammals*, *18*(1), 27-32.

Barr, B., Dunn, J. L., Daniel, M. D., & Banford, A. (1989). Herpes-like viral dermatitis in a beluga whale (Delphinapterus leucas). *Journal of Wildlife Diseases*, *25*(4), 608-611.

Bearzi, M., Rapoport, S., Chau, J., & Saylan, C. (2009). Skin lesions and physical deformities of coastal and offshore common bottlenose dolphins (Tursiops truncatus) in Santa Monica Bay and adjacent areas, California. *AMBIO: A Journal of the Human Environment*, *38*(2), 66-72.

Bertulli C.G., Cecchetti A., Van Bressem M.-F. and Van Waerebeek K., (2012). Skin disorders in common minke whales and white-beaked dolphins off Iceland, a photographic assessment. *Journal of Marine Animals and Their Ecology*. 5(2): 29-40.

Bertulli, C. G., Rasmussen, M. H., & Rosso, M. (2016). An assessment of the natural marking patterns used for photo-identification of common minke whales and white-beaked dolphins in Icelandic waters. *Journal of the Marine Biological Association of the United Kingdom*, *96*(4), 807-819.

Birkun Jr, A. (2002). Natural mortality factors affecting cetaceans in the Black Sea. *Cetaceans of the Mediterranean and Black Seas: state of knowledge and conservation strategies. A report to the ACCOBAMS Secretariat, Monaco*, 16.

Bossart, G. D., & Duignan, P. J. (2018). Emerging viruses in marine mammals. *health*, *2*, 5.

Bossart G.D., Ghim S.J., Rehtanz M., Goldstein J.D., Varela R.A., Ewing R., Fair P.A., Lenzi R., Joseph B., Hicks C., Schneider L., McKinnie C.J., Reif J.S., Sanchez R., Lopez A., Novoa S., Bernal J., Goretti M., Rodriguez M., Defran R.H., Jenson A.B., (2005). Orogenital neoplasia in Atlantic bottlenose dolphins (*Tursiops truncatus*). *Aquatic Mammals*. 31(4): 473–480.

Brereton, T., Jones, D., Leeves, K., Lewis, K., Davies, R., & Russel, T. (2018). Population structure, mobility and conservation of common bottlenose dolphin off south-west England from photo-identification studies. *Journal of the Marine Biological Association of the United Kingdom*, *98*(5), 1055-1063.

Bristow, T., & Rees, E. I. S. (2001). Site fidelity and behaviour of bottlenose dolphins (Tursiops truncatus) in Cardigan Bay, Wales. *Aquatic Mammals*, *27*(1), 1-10.

Butler, J. R., Middlemas, S. J., Graham, I. M., & Harris, R. N. (2011). Perceptions and costs of seal impacts on Atlantic salmon fisheries in the Moray Firth, Scotland: implications for the adaptive co-management of seal-fishery conflict. *Marine Policy*, *35*(3), 317-323.

Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environm Microb 8: 1137-1144

CCC (Ceredigion County Council), Countryside Council of Wales, Environment Agency Wales, North Western and North Wales Sea Fisheries Committee, Pembrokeshire Coast National Park Authority, Pembrokeshire County Council, South Wales Sea Fisheries Committee, Trinity House, and Dŵr Cymru Welsh Water (2001a) *Cardigan Bay cSAC Management Plan*. Ceredigion County Council, Aberystwyth. 203pp.

CCW (Countryside Council for Wales) (2005) *Draft advice provided by the Countryside Council for Wales in fulfilment of Regulation 33 of the Conservation (Natural Habitats, &c.) regulations 12994 for Pen* Llŷn *a'r Sarnau / Lleyn Peninsula and the Sarnau European Marine Site.* Countryside Council for Wales, Bangor. 76pp.

Chan, S. C., & Karczmarski, L. (2019). Epidermal Lesions and Injuries of Coastal Dolphins as Indicators of Ecological Health. *EcoHealth*, 1-7.

Connor, R. C., Smolker, R. A., & Richards, A. F. (1992). Two levels of alliance formation among male bottlenose dolphins (Tursiops sp.). *Proceedings of the National Academy of Sciences*, *89*(3), 987-990.

Connor, R. C., Watson-Capps, J. J., Sherwin, W. B., & Krützen, M. (2010). A new level of complexity in the male alliance networks of Indian Ocean bottlenose dolphins (Tursiops sp.). *Biology Letters*, *7*(4), 623-626.

Connor R. C., Wells R. S., Mann J. and Read A. J., (2000). The bottlenose dolphin. social relationships in a fission-fusion society. In Mann J., Connor R.C., Tyack P.L. and Whitehead H (eds) pp. 91 – 126. *Cetacean Society. Field studies of dolphins and whales.* University of Chicago Press. Chicago and London

Corkeron PJ, Morris RJ, Bryden MM. Interactions between bottlenose dolphins and sharks in Moreton Bay, Queensland. Aquat Mamm. 1987b; 13: 109–113.

Cusick, P.K. and Bullock, B.C. 1973. Ulcerative dermatitis and pneumonia associated with Aeromonas hydrophila infection in the bottle-nosed dolphin. JAVMA 163: 578-579

de Vere, A. J., Lilley, M. K., & Frick, E. E. (2018). Anthropogenic impacts on the welfare of wild marine mammals. *Aquatic Mammals*, *44*(2), 150-180.

Diamond, S.S. Ewing, D.E. and Caldwell,G.A. 1979. Fatal Bronchopneumonia and dermatitis caused by Pseudomonas aeruginosa JAVMA 175: 984-987

Dolman, S. J., & Moore, M. J. (2017). Welfare implications of cetacean bycatch and entanglements. In *Marine Mammal Welfare* (pp. 41-65). Springer, Cham.

Dunn, J.L., Buck, J.D. and Spotte, S. 1982.Candidiasis in captive cetaceans 1982. JAVMA 181:1316

Evans C.D.R., (1995). Offshore Environment. In Barne J.H., Robson C.F., Grellier K., Arnold H., Thompson P., Wilson B., *Management Recommendations for the Cardigan Bay Bottlenose Dolphin Population.* A report to the Countryside Council for Wales by University of Aberdeen, Dept. of Zoology, Cromarty. Contract Science Report 134, 68p.

Evans, P. G., & Hammond, P. S. (2004). Monitoring cetaceans in European waters. *Mammal review*, *34*(1-2), 131-156.

Evans, Ronald G. "The intertidal ecology of Cardigan Bay." *The Journal of Ecology* (1947): 273-309.

Feingold, D., & Evans, P. G. H. (2014). Bottlenose Dolphin and Harbour Porpoise Monitoring in Cardigan Bay and Llŷn a'r Sarnau Special Areas of Conservation 2011–2013. *Natural Resources Wales Evidence Report Series*, (4).

Félix, F., Van Bressem, M. F., & Van Waerebeek, K. (2019). Role of social behaviour in the epidemiology of lobomycosis-like disease (LLD) in estuarine common bottlenose dolphins from Ecuador. *Diseases of aquatic organisms*, *134*(1), 75-87.

Geraci, J., J. Harwood, & A. Lounsbury. 1999. Marine mammal die-offs: causes, investigations and issues. Pp. 367–395 in Conservation and management of marine mammals (J. Twiss and R. Reeves, eds.). Smithsonian Institution Press, Washington, D.C.

Geraci, J.R., Sauer, R.M. and Medway, W. 1966. Erysipelas in dolphins. Am J Vet Research 27: 597-606.

Gonzalvo, J., Giovos, I., & Mazzariol, S. (2015). Prevalence of epidermal conditions in common bottlenose dolphins (Tursiops truncatus) in the Gulf of Ambracia, western Greece. *Journal of Experimental Marine Biology and Ecology*, *463*, 32-38.

Gowans, S., & Whitehead, H. (2001). Photographic identification of northern bottlenose whales (Hyperoodon ampullatus): sources of heterogeneity from natural marks. *Marine Mammal Science*, *17*(1), 76-93.

Greenwood AG, Harrison RJ, Whitting HW. Functional and pathological aspects of the skin of marine mammals. In: Harrison RJ, editor. Functional anatomy of marine mammals. New York: Academic Press; 1974. pp. 73–110.

Hart, L. B., Rotstein, D. S., Wells, R. S., Allen, J., Barleycorn, A., Balmer, B. C., ... & McFee, W. (2012). Skin lesions on common bottlenose dolphins (Tursiops truncatus) from three sites in the Northwest Atlantic, USA. *PloS one*, *7*(3), e33081.

Harwood, J. (2001). Marine mammals and their environment in the twenty-first century. *Journal of Mammalogy, 82*(3), 630-640.

Heithaus MR. Shark attacks on bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia: Attack rate, bite scar frequencies and attack seasonality. Mar Mamm Sci. 2001; 17: 526–539.

Herbst, L. H. (1994). Fibropapillomatosis of marine turtles. *Annual Review of Fish Diseases*, *4*, 389-425.

Hicks BD, St. Aubin DJ, Gerach JR, Brown WR. Epidermal growth in the bottlenose dolphin, *Tursiops truncatus.* The Journal of Investigative Dermatology 1985; 85 60-63.

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., ... & McWilliam, M. J. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492.

Hupman, K. E., Pawley, M. D., Lea, C., Grimes, C., Voswinkel, S., Roe, W. D., & Stockin, K. A. (2017). Viability of Photo-Identification as a Tool to Examine the Prevalence of Lesions on Free-Ranging Common Dolphins (Delphinus sp.). *Aquatic Mammals*, *43*(3).

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (2011). *Marine mammals of the world: a comprehensive guide to their identification*. Elsevier.

Jepson, P. D., Deaville, R., Barber, J. L., Aguilar, À., Borrell, A., Murphy, S., ... & Cunningham, A. A. (2016). PCB pollution continues to impact populations of orcas and other dolphins in European waters. *Scientific reports*, *6*, 18573.

Jones, F. M., & Pfeiffer, C. J. (1994). Morphometric comparison of the epidermis in several cetacean species. *Aquatic Mammals*, *20*, 29-29.

Kappeler, P. M., Cremer, S., & Nunn, C. L. (2015). Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies.

Khan, N.H., Ishii, Y., Kimata-Kino, N., Esaki, H., Nishino, T., Nishimura, M., and Kogure, K. 2006. Isolation of Pseudomonas aeruginosa from open ocean and comparison with freshwater, clinical, and animal isolates. Microb Ecol DOI: 10.1007/s00248-006-9059-3.

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., ... & Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual review of marine science*, *7*, 471-496.

Lafferty, K. D., & Hofmann, E. E. (2016). Marine disease impacts, diagnosis, forecasting, management and policy.

Lafferty, K. D., Porter, J. W., & Ford, S. E. (2004). Are diseases increasing in the ocean?. *Annu. Rev. Ecol. Evol. Syst.*, *35*, 31-54.

Lane, E. P., De Wet, M., Thompson, P., Siebert, U., Wohlsein, P., & Plön, S. (2014). A systematic health assessment of Indian ocean bottlenose (Tursiops aduncus) and Indo-Pacific humpback (Sousa plumbea) dolphins incidentally caught in shark nets off the KwaZulu-Natal coast, South Africa. *PloS one*, *9*(9), e107038.

Law, R. J., Fileman, C. F., Hopkins, A. D., Baker, J. R., Harwood, J., Jackson, D. B., ... & Morris, R. J. (1991). Concentrations of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. *Marine Pollution Bulletin*, *22*(4), 183-191.

Lee, H. H., Wallen, M. M., Krzyszczyk, E., & Mann, J. (2019). Every scar has a story: age and sex-specific conflict rates in wild bottlenose dolphins. *Behavioral Ecology and Sociobiology*, *73*(5), 63.

Leone, A. B., Ferraro, G. B., Boitani, L., & Blasi, M. F. (2019). Skin marks in bottlenose dolphins (Tursiops truncatus) interacting with artisanal fishery in the central Mediterranean Sea. *PloS one*, *14*(2), e0211767.

Levesque, S., Reusch, K., Baker, I., O'Brien, J., & Berrow, S. (2016, January). Photo-identification of bottlenose dolphins (Tursiops truncatus) in Tralee Bay and Brandon Bay, Co. Kerry: A case for SAC boundary extension. In *Biology and Environment: Proceedings of the Royal Irish Academy* (Vol. 116, No. 2, pp. 109-118). Royal Irish Academy.

Lohrengel, K., Evans, P.G.H., Lindenbaum, C.P., Morris, C.W., Stringell, T.B. (2017) Bottlenose dolphin monitoring in Cardigan Bay 2014-2016, NRW Evidence Report No: 191, 163pp, Natural Resources Wales, Bangor

Maldini, D., Riggin, J., Cecchetti, A., & Cotter, M. P. (2010). Prevalence of epidermal conditions in California coastal bottlenose dolphins (Tursiops truncatus) in Monterey Bay. *Ambio*, *39*(7), 455-462.

Mann, J., & Karniski, C. (2017). Diving beneath the surface: long-term studies of dolphins and whales. *Journal of Mammalogy*, *98*(3), 621-630.

Mann, J., Connor, R. C., Barre, L. M., & Heithaus, M. R. (2000). Female reproductive success in bottlenose dolphins (Tursiops sp.): life history, habitat, provisioning, and group-size effects. *Behavioral Ecology*, *11*(2), 210-219.

Mariani, M., Miragliuolo, A., Mussi, B., Russo, G. F., Ardizzone, G., & Pace, D. S. (2016). Analysis of the natural markings of Risso's dolphins (Grampus griseus) in the central Mediterranean Sea. *Journal of Mammalogy*, *97*(6), 1512-1524.

Marley, S. A., Cheney, B., & Thompson, P. M. (2013). Using Tooth Rakes to Monitor Population and Sex Differences in Aggressive Behaviour in Bottlenose Dolphins (Tursiops truncatus). *Aquatic Mammals*, *39*(2).

Martinez-Levasseur, L. M., Gendron, D., Knell, R. J., O'Toole, E. A., Singh, M., & Acevedo-Whitehouse, K. (2010). Acute sun damage and photoprotective responses in whales. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1711), 1581-1586.

Migaki, G., Valerio, M.G., Irvine, B.A and Graner, F.M. 1971. Lobo's disease in an Atlantic bottle-nosed dolphin. JAVMA 159: 578-582.

Maynard, J., Van Hooidonk, R., Eakin, C. M., Puotinen, M., Garren, M., Williams, G., & Harvell, C. D. (2015). Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. *Nature Climate Change*, *5*(7), 688.

Moore, S. E. (2008). Marine mammals as ecosystem sentinels. *Journal of Mammalogy*, *89*(3), 534-540.

Mouton, M., & Botha, A. (2012). *Cutaneous lesions in cetaceans: an indicator of ecosystem status* (pp. 123-150). InTech.

Nakeeb, S., Targowski, S.P. and Spotte, S. 1977. Chronic cutaneous candidiasis in bottle-nosed dolphins. JAVMA 171: 961- 965.

Ólafsdóttir, D., & Shinn, A. P. (2013). Epibiotic macrofauna on common minke whales, Balaenoptera acutorostrata Lacépède, 1804, in Icelandic waters. *Parasites & vectors*, *6*(1), 105.

Pearce, G., Blacklaws, B.A., Gajda, A.M., Jepson, P., Deaville, R. and Van Bressem, M-F. 2008. Molecular identification and phylogenetic relationships in poxviruses from cetacean skin lesions. Conference of the European Cetacean Society, The Netherlands, 10-12 March 2008.

Pereira, C.S., Amorim, S.D. Santos, A.F.M., Siciliano, S., Moreno, I.M.B., Ott, P.H. and Rodrigues, D.P. 2007.Vibrio spp. isolados de mamíferos marinhos capturados na região litorânea do sudeste ao sul do Brasil. Pesq Vet Bras 27:81-83

Pettis HM, Rolland RM, Hamilton PK, Brault S, Knowlton AR, et al. (2004) Visual health assessment of North Atlantic right whales (Eubalaena glacialis) using photographs. Canadian Journal of Zoology 82: 8–19.

Pfeiffer, C. J., & Jones, F. M. (1993). Epidermal lipid in several cetacean species: ultrastructural observations. *Anatomy and embryology*, *188*(3), 209-218.

Powell, S. N., Wallen, M. M., Bansal, S., & Mann, J. (2018). Epidemiological investigation of tattoo-like skin lesions among bottlenose dolphins in Shark Bay, Australia. *Science of the Total Environment*, *630*, 774-780.

Reno, P. W. (1998). Factors involved in the dissemination of disease in fish populations. *Journal of Aquatic Animal Health*, *10*(2), 160-171.

Ribeiro, F. B., Carvalho, V. L., Bevilaqua, C. M. L., & Bezerra, L. E. A. (2010). First record of Xenobalanus globicipitis (Cirripedia: Coronulidae) on Stenella coeruleoalba (Cetacea: Delphinidae) in the oligotrophic waters of north-eastern Brazil. *Marine Biodiversity Records*, *3*.

Richardson, H. (2012). The effect of boat disturbance on the bottlenose dolphin (Tursiops truncatus) of Cardigan Bay in Wales. *University College London*.

Rodriguez-Toro G (1993) Lobomycosis. International Journal of Dermatology 32:324–332

Rosso, M., Ballardini, M., Moulins, A., & Würtz, M. (2011). Natural markings of Cuvier's beaked whale Ziphius cavirostris in the Mediterranean Sea. *African Journal of Marine Science*, *33*(1), 45-57.

Sah, P., Mann, J., & Bansal, S. (2018). Disease implications of animal social network structure: a synthesis across social systems. *journal of animal ecology*, *87*(3), 546-558.

Samarra, F. I., Fennell, A., Aoki, K., Deecke, V. B., & Miller, P. J. (2012). Persistence of skin marks on killer whales (Orcinus orca) caused by the parasitic sea lamprey (Petromyzon marinus) in Iceland. *Marine Mammal Science*, *28*(2), 395-401.

Samuels, A., & Gifford, T. (1997). A quantitative assessment of dominance relations among bottlenose dolphins. *Marine Mammal Science, 13*(1), 70-99.

Sanino, G. P., Van Bressem, M. F., Van Waerebeek, K., & Pozo, N. (2014). Skin disorders of coastal dolphins at Añihue Reserve, Chilean Patagonia: a matter of concern. *Boletín del Museo Nacional de Historia Natural, Chile, 63*, 127-157.

Savage, A. E., Becker, C. G., & Zamudio, K. R. (2015). Linking genetic and environmental factors in amphibian disease risk. *Evolutionary applications*, *8*(6), 560-572.

Schuldt, J. P., McComas, K. A., & Byrne, S. E. (2016). Communicating about ocean health: theoretical and practical considerations. *Philosophical Transactions of the Royal Society B: Biological Sciences, 371*(1689), 20150214.

Schulman, F.Y. and Lipscomb, T.P. 1999. Dermatitis with Invasive Ciliated Protozoa in Dolphins That Died During the 1987–1988 Atlantic Bottlenose Dolphin Morbilliviral Epizootic. Vet Pathol 36:171–174

Scott, E. M., Mann, J., Watson-Capps, J. J., Sargeant, B. L., & Connor, R. C. (2005). Aggression in bottlenose dolphins: evidence for sexual coercion, male-male competition, and female tolerance through analysis of tooth-rake marks and behaviour. *Behaviour*, *142*(1), 21-44.

Simpson, C.F., Wood, F.G. and Young F. 1958. Cutaneous lesions on a porpoise with Erysipelas. JAVMA 133: 558-560

Smith A.W. and Boyt, P.M. 1990. Calicivirus of ocean origin: a review. J Zoo Wildl Med 21:3-23

Smith, K. F., Acevedo-Whitehouse, K., & Pedersen, A. B. (2009). The role of infectious diseases in biological conservation. *Animal conservation*, *12*(1), 1-12.

Smith, H. C., Pollock, K., Waples, K., Bradley, S., & Bejder, L. (2013). Use of the robust design to estimate seasonal abundance and demographic parameters of a coastal bottlenose dolphin (Tursiops aduncus) population. *PloS one*, *8*(10), e76574.

Smolarek Benson, K.A., Manire, C.A., Ewing, R.Y., Saliki, J.T., Townsend, F.I., Ehlers, B. and Romero, C.H. 2006. Identification of novel alpha- and gammaherpesviruses from cutaneous and mucosal lesions of dolphins and whales. J Virol Methods 136:261-6.

Smolker, R. A., Richards, A. F., Connor, R. C., & Pepper, J. W. (1992). Sex differences in patterns of association among Indian Ocean bottlenose dolphins. *Behaviour*, *123*(1-2), 38-69.

Sprogis, K. R., Raudino, H. C., Rankin, R., MacLeod, C. D., & Bejder, L. (2016). Home range size of adult Indo-Pacific bottlenose dolphins (Tursiops aduncus) in a coastal and estuarine system is habitat and sex-specific. *Marine Mammal Science*, *32*(1), 287-308.

Stacy, B. A., Wellehan, J. F., Foley, A. M., Coberley, S. S., Herbst, L. H., Manire, C. A., ... & Jacobson, E. R. (2008). Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (Caretta caretta). *Veterinary microbiology*, *126*(1-3), 63-73.

Sweeney, J. C., & Ridgway, S. H. (1975). Common diseases of small cetaceans. *J. Am. Vet. Med. Assoc*, *167*(7), 533-540.

Torchin, M. E., Lafferty, K. D., & Kuris, A. M. (2002). Parasites and marine invasions. *Parasitology*, *124*(7), 137-151.

Ugarte, F. and Evans, P.G.H. (2006) *Monitoring of marine mammals in the Cardigan Bay SAC: surveys from May 2003 to April 2005.* Marine Monitoring Report No. 23. Species Challenge Report No. 05/01/04. Countryside Council for Wales, Bangor. 38pp.

Van Bressem, M. F. E., Flach, L., Reyes, J. C., Echegaray, M., Santos, M., Viddi, F., ... & Van Waerebeek, K. (2015). Epidemiological characteristics of skin disorders in cetaceans from South American waters. *Latin American Journal of Aquatic Mammals*, *10*(1), 20-32.

Van Bressem, M. F., Raga, J. A., Di Guardo, G., Jepson, P. D., Duignan, P. J., Siebert, U., ... & Aguilar, A. (2009). Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. *Diseases of aquatic organisms*, *86*(2), 143-157.

Van Bressem, M. F., Van Waerebeek, K., Aznar, F. J., Raga, J. A., Jepson, P. D., Duignan, P., ... & Di Beneditto, A. P. (2009). Epidemiological pattern of tattoo skin disease: a potential general health indicator for cetaceans. *Diseases of Aquatic Organisms*, *85*(3), 225-237.

Bressem, M. F. V., Waerebeek, K. V., Garcia-Godos, A., Dekegel, D., & Pastoret, P. P. (1994). Herpes-like virus in dusky dolphins, *Lagenorhynchus obscurus*, from coastal Peru. *Marine Mammal Science, 10*(3), 354-359.

Van Bressem, M. F., Van Waerebeek, K., & Raga, J. A. (1999). A review of virus infections of cetaceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. *Diseases of aquatic organisms*, *38*(1), 53-65.

Van Bressem, M.-F., Van Waerebeek, K., Reyes, J.C., Félix, F., Echegaray, M., Siciliano, S., Di Beneditto, A.P., Flach, L., Viddi, F., Avila, I.C., Herrera, J.C., Tobón, I.C., Bolaños, J., Moreno, I.B., Ott, P.H., Sanino, G.P., Castineira, E., Montes, D., Crespo, E., Flores, PAC, Haase, B., Mendonça de Souza, S.M.F., Laeta, M. and Fragoso, A.B. 2007a. A preliminary overview of skin and skeletal diseases and traumata in small cetaceans from South American waters.

Van Bressem, M. F., Van Waerebeek, K., Montes, D., Kennedy, S., Reyes, J. C., Garcia-Godos, I. A., ... & Alfaro-Shigueto, J. (2006). Diseases, lesions and malformations in the long-beaked common dolphin Delphinus capensis from the Southeast Pacific. *Diseases of Aquatic Organisms*, *68*(2), 149-165.

Van Bressem M-F, Van Waerebeek K, Reyes JC, Felix F, Echegaray M, et al. (2007) A preliminary overview of skin and skeletal diseases and traumata in small cetaceans from South American waters. Latin American Journal of Aquatic Mammals 6: 7–42.

Ward, J. R., & Lafferty, K. D. (2004). The elusive baseline of marine disease: are diseases in ocean ecosystems increasing?. *PLoS biology*, *2*(4), e120.

Weaver, A. (2015). Sex difference in bottlenose dolphin sightings during a long-term bridge construction project. *Animal Behavior and Cognition*, *2*(1), 1-13.

Wells, R. S., Rhinehart, H. L., Hansen, L. J., Sweeney, J. C., Townsend, F. I., Stone, R., ... & Rowles, T. K. (2004). Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth*, *1*(3), 246-254.

Wilson, B., Arnold, H., Bearzi, G., Fortuna, C. M., Gaspar, R., Ingram, S., ... & Schneider, K. (1999). Epidermal diseases in bottlenose dolphins: impacts of natural and anthropogenic factors. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1423), 1077-1083.

Wilson, B., Grellier, K., Hammond, P. S., Brown, G., & Thompson, P. M. (2000). Changing occurrence of epidermal lesions in wild bottlenose dolphins. *Marine Ecology Progress Series*, *205*, 283-290.

Wilson, B., Thompson, P. M., & Hammond, P. S. (1997). Skin lesions and physical deformities in bottlenose dolphins in the Moray Firth: population prevalence and age-sex differences. *Ambio (Sweden)*.

Wood, S. N. (2001). mgcv: GAMs and generalized ridge regression for R. *R news*, *1*(2), 20-25.

Würsig B. and Jefferson T. A., (1990). Methods of photo-identification for small cetaceans. In: Hammond P.S., Mizroch S.A. and Donovan G.P. (eds) Individual Recognition of Cetaceans: Use of Photo-identification and other Techniques to Estimate Population Parameters. *Report of the International Whaling Commission. Special Issue.* 12: 43 – 52.

9. Appendices

Appendix 1. Sea Watch Foundation (A) effort and (B) sightings forms

(A)

Page .... of ....

sea watch FOUNDATION

VESSEL-BASED EFFORT RECORDING FORM

Date (dd/mm/yyyy): .... **Vessel:** .... **Contact Name/Address:** ....

Tel/E-mail: .... **Observer names:** ....

Start Time .... **GMT / BST** **End Time** .... **Total Time** ...... **Observer Height Above Sea Level (m)** .... **Field of View:** 180° fwd; 90°L; 90°R; 360° (tick)

TIME GMT/BST	LATITUDE (degrees, decimal minutes)	LONGITUDE (degrees, decimal minutes)	BOAT COURSE	SPEED (knots)	EFFORT TYPE	SEA STATE	SWELL HEIGHT	VISIBILITY	BOAT ACTIVITY	SIGHT. REF.

DATA DEFINITIONS: *Use categories provided below where possible*
Time: 24-hour clock; specify GMT or BST. **Location:** Record latitude and longitude (deg., decimal min. preferred) every 15 minutes or when course changes, if lat/long unavailable, note location in relation to local landmarks. **Boat course:** Record course as vessel heading not course over ground (as deg. magnetic). **Speed:** Record in knots, if available. **Effort Type:** OFF = end of effort or not watching; CASW = casual watching; DEDS = dedicated search; LINE = line transect. **Sea State:** 0 = mirror calm; 1 = slight ripples, no foam crests; 2 = small wavelets, glassy crests, but no whitecaps; 3 = large wavelets, crests begin to break, few whitecaps; 4 = longer waves, many whitecaps; 5 = moderate waves of longer form, some spray; 6 = large waves, whitecaps everywhere, frequent spray; 7 = sea heaps up, white foam blows in streaks; 8 = long, high waves edges breaking, foam blows in streaks; 9 = high waves, sea begins to roll, dense foam streaks. **Swell Height:** Light = 0-1 m; Moderate = 1-2 m; Heavy = >2 m. **Visibility:** <1 km; 1-5 km; 6-10 km; >10 km. **Boat Activity:** Record No of each and type: NB = No boats, VE = unspecified vessel, YA = yacht, RB = row boat or kayak, JS = jet ski, SB = speed boat, MB = motor boat, FI = fishing boat, FE = ferry, LS = large ship , SV = seismic vessel, WS = warship. **Sighting Reference:** Refer to number(s) on Sighting Record Form.

(B)

Page .... of ....

VESSEL-BASED SIGHTINGS RECORDING FORM

sea watch FOUNDATION

Date (dd/mm/yy) .... Contact name / address .... Phone ....

E-mail: .... Boat name .... Journey Description ....

Sea State .... Swell Height .... Visibility .... Trip Start Time .... GMT / BST End Time .... Observer Height Above Sea Level (m)

Field of View: 180° fwd; 90°L; 90°R; 360° (tick)

Ref. No.	TIME BST/GMT	LOCATION (Latitude & longitude if possible)	SPECIES	CONF.	TOTAL NO.	NO. CALVES	NO. JUVES	BEARING ANIMAL	DIST. TO ANIMAL	BEHAVIOUR	REACTION	ANIMAL HEADING	ASSOC. SEABIRDS	OBSERV NAME

DATA DEFINITIONS: *Use categories provided where possible.*

Sea State: 0 = mirror calm; 1 = slight ripples, no foam crests; 2 = small wavelets, glassy crests, but no whitecaps; 3 = large wavelets, crests begin to break, few whitecaps; 4 = longer waves, many whitecaps; 5 = moderate waves of longer form, some spray; 6 = large waves, whitecaps everywhere, frequent spray; 7 = sea heaps up, white foam blows in streaks; 8 = long, high waves edges breaking, foam blows in streaks; 9 = high waves, sea begins to roll, dense foam streaks. **Swell Height:** Light = 0-1 m; Moderate = 1-2 m; Heavy = >2 m. **Visibility:** <1 km; 1-5 km; 6-10 km; >10 km. **Reference No.:** Number each sighting sequentially to allow for cross-reference with effort or additional notes. If a repeat sighting, use the same number as for the first sighting of the group. **Time:** 24-hour clock; circle BST or GMT. **Location:** Record latitude and longitude (deg., decimal min. preferred), if lat/long unavailable, note location in relation to local landmarks. **Species:** Give the best judgement of species ID; use general categories if unsure (e.g. dolphin species). **Confidence:** Definite; Probable; Possible. **Total No.:** Give range if unsure of exact number. **Calves/Juveniles:** Estimate counts of different–sized animals relative to adult body size (calves up to 50% adult size, juveniles 50-75%). **Bearing:** Degrees (magnetic). **Distance to animal:** Metres. **Behaviour:** Surfacing; Normal Swim; Fast Swim; Blowing; Feeding; Leap/breaching; Tail slap; Spy-hop; Bow-ride; Rest/Milling; Aggression; Sexual. **Reaction:** POS (attracted to boat); NEG (avoided boat); NON (no response observed). **Animal heading:** Note general direction of movement, or whether direction is variable. **Seabirds:** Note seabirds closely associated with the animals; record species of bird, if known, and number of birds.

Appendix 2. Codes used in the analysis & statistical outputs (conducted in R v3.6.0)

TATTOOS

```
> mean(Data$Tattoos)
0.3135593


> aggregate(Data$Tattoos,by=list(Data$Year),FUN=mean)
  Group.1         x
1    2011 0.6666667
2    2012 0.2365591
3    2013 0.3148148
4    2014 0.2727273
5    2015 0.2196970
6    2016 0.3586957
7    2017 0.4400000
8    2018 0.5490196
> aggregate(Data$Tattoos,by=list(Data$Period),FUN=mean)
  Group.1         x
1      P1 0.2598425
2      P2 0.3500000
3      P3 0.3189189
> aggregate(Data$Tattoos,by=list(Data$Maturity),FUN=mean)
  Group.1         x
1   adult 0.3126492
2    calf 0.3207547
> aggregate(Data$Tattoos,by=list(Data$Sex),FUN=mean)
  Group.1         x
1  female 0.2684564
2    male 0.3484848
3 unknown 0.3307393
```

Continuous Explanatory Variable (Year) -Run Model and Check

M1<-glm(Tattoos~Year,data=Data,family="binomial")

M1<-gam(Tattoos~Year+s(Name,bs="re"),data=Data,family="binomial")

Summary M1: Parametric coefficients:

```
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -355.41706  122.41663  -2.903  0.00369 **
Year          0.17599    0.06076   2.897  0.00377 **
```

Approximate significance of smooth terms:

```
         edf Ref.df Chi.sq p-value
s(Name) 58.23    242  80.34 0.00042
```

<u>ANOVA (M1):</u> Parametric Terms:

```
df Chi.sq p-value
Year  1   8.39 0.00377
```

Approximate significance of smooth terms:

```
          edf Ref.df Chi.sq p-value
s(Name)  58.23 242.00  80.34 0.00042
```

SEX

M1<-glm(Tattoos~Sex,data=Data,family="binomial")

Deviance Residuals:

```
   Min       1Q   Median       3Q      Max
-0.9257  -0.8962  -0.7907   1.4876   1.6218
```

Coefficients:

```
           Estimate Std. Error z value Pr(>|z|)
(Intercept)  -1.0025     0.1849  -5.423 5.87e-08 ***
Sexmale       0.3768     0.3177   1.186    0.236
Sexunknown    0.2976     0.2275   1.308    0.191
```

anova(M1,test="Chisq")

```
    Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                  471     587.09
Sex   2    2.165       469     584.93   0.3388
```

MATURITY

M2<-glm(Tattoos~Maturity,data=Data,family="binomial")

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.8795  -0.8659  -0.8659   1.5249   1.5249

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.78776    0.10538  -7.475 7.71e-14 ***
Maturitycalf  0.03746    0.31258   0.120    0.905
```

anova(M2,test="Chisq")

```
         Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                       471     587.09
Maturity  1  0.01431       470     587.08   0.9048
```

PERIOD

M3<-glm(Tattoos~Period,data=Data,family="binomial")
summary(M3)

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.9282  -0.8764  -0.7758   1.4490   1.6418

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -1.0468     0.2023  -5.173  2.3e-07 ***
PeriodP2      0.4277     0.2616   1.635    0.102
PeriodP3      0.2880     0.2566   1.123    0.262
```

anova(M3,test="Chisq")

```
       Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                     471     587.09
Period  2   2.7548       469     584.34   0.2522
```

PUNCTIFORM MARKS

```
> mean(Data$Punctiform)
[1] 0.1419492
> aggregate(Data$Punctiform,by=list(Data$Year),FUN=mean)
  Group.1          x
1    2011 0.33333333
```

```
2    2012 0.20430108
3    2013 0.24074074
4    2014 0.04545455
5    2015 0.06060606
6    2016 0.10869565
7    2017 0.08000000
8    2018 0.25490196
> aggregate(Data$Punctiform,by=list(Data$Period),FUN=mean)
  Group.1          x
1      P1 0.16535433
2      P2 0.20625000
3      P3 0.07027027
> aggregate(Data$Punctiform,by=list(Data$Maturity),FUN=mean)
  Group.1         x
1   adult 0.1121718
2    calf 0.3773585
> aggregate(Data$Punctiform,by=list(Data$Sex),FUN=mean)
  Group.1          x
1  female 0.08724832
2    male 0.15151515
3 unknown 0.17120623
```

Continuous Explanatory Variable (Year) -Run Model and Check

M1<-glm(Punctiform~Year,data=Data,family="binomial")

M1<-gam(Punctiform~Year+s(Name,bs="re"),data=Data,family="binomial")

Summary(M1)

```
Parametric coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) 152.42986  164.12415   0.929    0.353
Year         -0.07663    0.08147  -0.941    0.347

Approximate significance of smooth terms:
         edf Ref.df Chi.sq  p-value
s(Name) 60.17    242  94.12 1.62e-06 ***
```

<u>Anova</u>

```
Parametric Terms:
     df Chi.sq p-value
Year  1  0.885   0.347

Approximate significance of smooth terms:
          edf Ref.df Chi.sq  p-value
s(Name)  60.17 242.00  94.12 1.62e-06
```

SEX

```
M1<-glm(Punctiform~Sex,data=Data,family="binomial")
> summary(M1)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6128  -0.6128  -0.5732  -0.4273   2.2086

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -2.3477     0.2903  -8.087 6.11e-16 ***
Sexmale       0.6249     0.4496   1.390   0.1645
Sexunknown    0.7706     0.3342   2.306   0.0211 *
```

anova(M1,test="Chisq")

```
     Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                   471     385.61
Sex   2   5.9163       469     379.69  0.05191 .
```

MATURITY

```
> M2<-glm(Punctiform~Maturity,data=Data,family="binomial")
> summary(M2)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.9734  -0.4878  -0.4878  -0.4878   2.0918



Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)   -2.0687     0.1548 -13.364  < 2e-16 ***
Maturitycalf   1.5680     0.3229   4.856  1.2e-06
```

> anova(M2,test="Chisq")

```
         Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                       471     385.61
Maturity  1   21.194       470     364.42 4.151e-06
```

PERIOD

```
M3<-glm(Punctiform~Period,data=Data,family="binomial")
> summary(M3)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6797  -0.6797  -0.3817  -0.3817   2.3045

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -1.6189     0.2389  -6.778 1.22e-11 ***
PeriodP2      0.2712     0.3086   0.879  0.37943
PeriodP3     -0.9636     0.3739  -2.577  0.00996 **
```

anova(M3,test="Chisq")

```
       Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                     471     385.61
Period  2   14.739       469     370.87 0.0006302
```

DISCOLOURATION

```
mean(Data$Discolouration)
[1] 0.3792373
> aggregate(Data$Discolouration,by=list(Data$Year),FUN=mean)
  Group.1         x
1    2011 0.3333333
2    2012 0.3978495
3    2013 0.4074074
4    2014 0.4545455
5    2015 0.2954545
6    2016 0.3804348
7    2017 0.3200000
8    2018 0.5294118
> aggregate(Data$Discolouration,by=list(Data$Period),FUN=mean)
  Group.1         x
1      P1 0.4330709
2      P2 0.3687500
3      P3 0.3513514
> aggregate(Data$Discolouration,by=list(Data$Maturity),FUN=mean)
  Group.1         x
1   adult 0.4009547
2    calf 0.2075472

> aggregate(Data$Discolouration,by=list(Data$Sex),FUN=mean)
  Group.1         x
1  female 0.2483221
2    male 0.6818182
```

3 unknown 0.3774319

Continuous Explanatory Variable (Year) -Run Model and Check

```
M1<-glm(Discolouration~Year,data=Data,family="binomial")
> M1<-gam(Discolouration~Year+s(Name,bs="re"),data=Data,family="binomial")
> summary(M1)
```

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	**-34.00878**	**131.26857**	**-0.259**	**0.796**
Year	**0.01656**	**0.06516**	**0.254**	**0.799**

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(Name)	95.96	242	147.5	8.7e-07	***

anova (M1)

Parametric Terms:

	df	Chi.sq	p-value
Year	1	0.065	0.799

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value
s(Name)	95.96	242.00	147.5	8.7e-07

SEX

```
M1<-glm(Discolouration~Sex,data=Data,family="binomial")
> summary(M1)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.5134	-0.9736	-0.7556	1.3960	1.6691

Coefficients:

```
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -1.1076     0.1896  -5.841 5.19e-09 ***
Sexmale       1.8697     0.3253   5.748 9.01e-09 ***
Sexunknown    0.6071     0.2292   2.649  0.00807 **
---
```

anova(M1,test="Chisq")

```
     Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                   471     626.52
Sex   2   36.258       469     590.26 1.339e-08
```

MATURITY

```
M2<-glm(Discolouration~Maturity,data=Data,family="binomial")
summary(M2)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-1.012  -1.012  -1.012   1.352   1.773

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.40149    0.09968  -4.028 5.63e-05 ***
Maturitycalf -0.93829    0.35307  -2.658  0.00787 **
```

anova(M2,test="Chisq")

```
Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                       471     626.52
Maturity  1   8.0833       470     618.44 0.004467
```

PERIOD

```
M3<-glm(Discolouration~Period,data=Data,family="binomial")
summary(M3)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.0654  -0.9592  -0.9304   1.4125   1.4464

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.2693     0.1791  -1.504    0.133
PeriodP2     -0.2683     0.2427  -1.105    0.269
```

```
PeriodP3     -0.3438     0.2362  -1.455    0.146
```

anova(M3,test="Chisq")

```
Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                   471     626.52
Period  2   2.2308       469     624.29   0.3278
```

WOUNDS

```
> mean(Data$Wounds)
[1] 0.1970339
> aggregate(Data$Wounds,by=list(Data$Year),FUN=mean)
  Group.1          x
1    2011 0.66666667
2    2012 0.12903226
3    2013 0.14814815
4    2014 0.22727273
5    2015 0.07575758
6    2016 0.30434783
7    2017 0.52000000
8    2018 0.29411765
> aggregate(Data$Wounds,by=list(Data$Period),FUN=mean)
  Group.1         x
1      P1 0.2047244
2      P2 0.2062500
3      P3 0.1837838
> aggregate(Data$Wounds,by=list(Data$Maturity),FUN=mean)
  Group.1          x
1   adult 0.21957041
2    calf 0.01886792
> aggregate(Data$Wounds,by=list(Data$Sex),FUN=mean)
  Group.1         x
1  female 0.1006711
2    male 0.3484848
3 unknown 0.2140078


> M1<-glm(Wounds~Year,data=Data,family="binomial")
> M1<-gam(Wounds~Year+s(Name,bs="re"),data=Data,family="binomial")
> summary(M1)

Parametric coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept) -475.96501  140.48334  -3.388 0.000704 ***
Year           0.23549    0.06972   3.378 0.000731 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value
s(Name) 40.46    242  53.47 0.00348 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.174   Deviance explained = 22.4%
UBRE = -0.049378  Scale est. = 1         n = 472
```

anova (M1)

```
Parametric Terms:
     df Chi.sq  p-value
Year  1  11.41 0.000731

Approximate significance of smooth terms:
           edf Ref.df Chi.sq p-value
s(Name)  40.46 242.00  53.47 0.00348
```

SEX

```
M1<-glm(Wounds~Sex,data=Data,family="binomial")
```

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.9257  -0.6940  -0.6940  -0.4607   2.1429

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -2.1898     0.2722  -8.043 8.74e-16 ***
Sexmale       1.5641     0.3753   4.168 3.08e-05 ***
Sexunknown    0.8889     0.3118   2.850  0.00437 **
```

anova(M1,test="Chisq")

```
     Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                   471     468.47
Sex   2   18.942       469     449.53 7.705e-05
```

MATURITY

```
M2<-glm(Wounds~Maturity,data=Data,family="binomial")
> summary(M2)
```

```
Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.7042  -0.7042  -0.7042  -0.1952   2.8179

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)    -1.268      0.118  -10.75  < 2e-16 ***
Maturitycalf   -2.683      1.016   -2.64  0.00829 **
```

> anova(M2,test="Chisq")

```
         Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
NULL                       471     468.47
Maturity  1   17.458       470     451.01 2.938e-05
```

PERIOD

```
M3<-glm(Wounds~Period,data=Data,family="binomial")
> summary(M3)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6797  -0.6797  -0.6373  -0.6373   1.8406

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.357024   0.219915  -6.171  6.8e-10 ***
PeriodP2     0.009344   0.294176   0.032    0.975
PeriodP3    -0.133895   0.290511  -0.461    0.645
```

anova(M3,test="Chisq")

```
       Df Deviance Resid. Df Resid. Dev Pr(>Chi)
NULL                     471     468.47
Period  2  0.34083       469     468.13   0.8433
```