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**Investigation of spatio-temporal trends in skin lesions of bottlenose dolphins in Wales**

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## **DECLARATION**

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## **Abstract**

### **Investigation of spatio-temporal trends in skin lesions of bottlenose dolphins in Wales**

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Over the last 20 years, skin lesions in different populations of bottlenose dolphins have been studied worldwide via photo-ID techniques. The classification of skin lesions on bottlenose dolphins have been categorised according to their colour and texture in several studies. Climate change and anthropogenic activities seem to contribute in the appearance and development of skin lesions and diseases. The prevalence of skin lesions on the species has been used among others as a health indicator. The Welsh population of bottlenose dolphins is larger than the populations from the Moray Firth and Shannon Estuary. Cardigan Bay is one out of two main UK coastal areas used by semi-resident bottlenose dolphin populations and with the highest abundance. The aim of this study was to investigate the spatio-temporal trends of skin lesions on the Welsh dolphins for the period 2001-14 using photo-ID techniques, mainly in Cardigan Bay. The possible effect of age, gender, residency and Sea Surface Temperature (SST) on skin lesion prevalence and extent was explored. In overall, 260 individuals were analysed for 15 skin lesion categories, out of which nine of them were mainly observed over time. Tooth rakes/scars (84%), white lesions (43.8%) and cloudy lesions (23.4%) were some of them. Additionally, 73% of the individuals were affected by at least one type of lesion and 56% of the population by more than two different types. The females were more prevalent to skin lesions during the period 2010-14 than males. In contrast to other studies, calves were more prevalent in skin lesions than adults. Also, no significant association was found in skin lesion prevalence between SST, different areas, and between resident, visitors and transient individuals. The presence of DFS and WFS (lesions, out of which pox viruses and herpesviruses have been isolated in other studies) and the analysis of photographic data indicated possible presence of pox-viruses and/or tattoo lesions in the Welsh dolphins. Therefore further systematic and quantitative study of the prevalence and extent of skin lesions is needed in order to assess better the patterns of skin lesions on this population. Accurate evaluation is essential for effective management towards the sustainability of this important population.

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“Endless forms most beautiful and most wonderful have been and are being evolved”

Charles Darwin, Origin of the species

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## **List of Abbreviations**

AFT: Abraded fin tip

BL: Black lesions

CL: Cloudy lesions

CR: Cream lesions

DBD: Dark black dot lesions

DFS: Dark fringed spots

MDS: Multidimensional Scaling

OL: Orange lesions

SAC: Special Area of Conservation

SST- Sea Surface Temperature

WFF: White fin fringe

WFS: White fringed spots

WL: White lesions

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# 1. Introduction

## 1.1 General Biology

The common bottlenose dolphin (*Tursiops truncatus*) is included within the cetacean sub-order of odontocetes and the family *Delphinidae*. Due to the fact it is a cosmopolitan species, it can be found in every ocean worldwide, occurring in a wide range of habitats such as tropical and temperate waters, coastal areas, continental areas, exposed pelagic waters, lagoons and estuaries (Shane and Wells, 1986; Connor *et al.*, 1998; Wells & Scott, 1999; Reynolds *et al.*, 2000). Bottlenose dolphins have been recorded around UK coastal waters, with semi-resident populations around North and west Wales (including Cardigan Bay) and eastern Scotland (including the Moray Firth), and more ephemeral groups in Cornwall, Devon and the Hebrides (UKBAP, 2002; Reid *et al.*, 2003; Evans *et al.*, 2003; Evans & Hammond, 2004; Hammond, 2008).

The final body size attained by adult common bottlenose dolphins is determined by sea water temperature and varies between 2.20m and 4.10m in different geographical marine areas (Reynolds *et al.*, 2000). However, it is uncertain whether variations in body size are generated by the ability of the individuals to adapt to diet habits or sea water temperature. Although bottlenose dolphins feed mostly on different squid and fish species, it appears that scombrids, sciaenids and mugilids are more preferable prey families (Wells & Scott, 2002). The body colouration of the bottlenose dolphin is characterised by a combination of several shades of grey and lighter pigmentation in the abdominal area (Wells *et al.*, 1987).

According to dental analyses, the life span of a male bottlenose dolphin can reach approximately 45 years and in females exceeds 50 years (Wells & Scott, 1999). Female individuals mature sexually and physically between their 5<sup>th</sup> and 13<sup>th</sup> year of life and the males between their 9<sup>th</sup> and 14<sup>th</sup> year of life. The reproduction period of the female bottlenose dolphin can last on average for 48 years with calving intervals ranging from 3 to 6 years (Wells *et al.*, 1987). In general, the average calving interval for bottlenose dolphins has been assessed at 3 years, and has been found also in the Cardigan Bay population (Wells & Scott, 1999; Cornell *et al.*, 2005; Feingold & Evans, 2014). After a gestation period of 12 months, calf growth has been recorded at between 0.8m to 1.4m length (Wells *et al.*, 1987).

## 1.2 Social behavior of bottlenose dolphins

Bottlenose dolphins show highly social behavior (Connor, 2000; Pesante *et al.*, 2008; Feingold & Evans, 2014). Usually, individuals tend to be seen in groups ranging in size from a few animals up to hundreds. Occasionally, they have been observed swimming alone. Compared to sheltered inshore marine areas, bottlenose dolphins appear to aggregate in large numbers in exposed coastline areas and offshore waters (Shane *et al.*, 1986). These aggregations have been related to the high level of protection they provide from predators and the species feeding strategies (Wells & Scott, 1999; Connor *et al.*, 2000; Reynolds *et al.*, 2000; Pesante *et al.*, 2008; Feingold & Evans, 2014). Variability in social structure has been documented in different bottlenose dolphin populations (Reynolds *et al.*, 2000; Pesante *et al.*, 2008; Feingold & Evans, 2014). However, the general pattern of the associations among the individuals are quite fluid and they live in fission-fusion communities where the animals form small groups of variable size and composition (Würsig & Würsig, 1977; Wells *et al.*, 1987).

The bonds between male individuals are powerful and can last 20 years, as described in Sarasota Bay (Florida) and Shark Bay (Western Australia) (Wells *et al.*, 1987; Wells, 1991; Connor, 2000; Mann *et al.*, 2000). On the other hand, the bonds among female individuals vary and are characterised being as weaker than male-male bonds (Mann *et al.*, 2000). The networks that females create are bigger but involve moderate bonds (Mann *et al.*, 2000). For female individuals, the bonds related to maternal kin are important in terms of safeguard of resources or protection from predators in the dolphin pod. Females tend not to travel together, in case the available resources are insufficient to cover the feeding needs of at least one individual (Connor, 2000).

According to Whitehead and Mann (1999), alloparenting may contribute to female bonding. It has been suggested that the distribution and abundance of resources may influence the level of association among the individuals (Whitehead & Mann, 1999). In addition, the reproductive state of the females defines the frequency of encounter and association with males. The intensity of interactions grows when females are at the peak of the menstruation cycle (Wells *et al.*, 1987).

The association between juvenile males and females begins when they leave their maternal care (Wells *et al.*, 1987). Compared to female bonds, bonds amongst males are triggered before the

weaning period finishes (Wells *et al.*, 1987; Magileviciute, 2006). Bottlenose dolphins of both sexes spend 10 years in the post-weaning period before they follow their adult life (Connor *et al.*, 2000). During this period, they learn how to develop their social and foraging skills and their sexual behaviour (Connor *et al.*, 2000).

Nevertheless, this highly social behaviour includes the risk of competition for resources and the transmission of parasites and other pathogens that may impact the general health of the population (Wey *et al.*, 2008). In order for the group to function properly, the formation of it should provide more benefits to its individuals from socialising than costs (Connor, 2000).

### **1.3 Factors affecting bottlenose dolphin populations**

In 2008, the conservation status of the bottlenose dolphin has been classified by the IUCN Red List as of “Least Concern” due to its widespread distribution (IUCN, 2008). However, coastal populations are considered vulnerable to human activities (Reeves *et al.*, 2003) and in Europe it is listed in Annex II of the Habitats Directive, as one of two cetacean species that require special protective measures (Council Directive 92/43/EEC). The health and dynamics of various local populations of bottlenose dolphins are imperilled among others by pollution, marine habitat alteration, climate change and global warming, prey depletion, by-catch and boat traffic (Wells & Scott, 1999; Reeves *et al.*, 2003).

Although climate change may have impacted local *T. truncatus* populations, it is unclear to what extent this may have happened because of the lack of data (Evans & Bjørge, 2013). Bottlenose dolphins and other marine mammals are prone to infectious diseases as a result of global warming (Geraci & Lounsbury, 2002; Harvell *et al.*, 2002; Lafferty *et al.*, 2004; Burek *et al.*, 2008; Kiszka *et al.*, 2009; Van Bresseem *et al.*, 2009a). Additionally, climate change favours the development and transmission of diseases and the susceptibility of the host (Harvell *et al.*, 2002). Also, stress levels and vulnerability of the individuals to various diseases, such as skin diseases, seems to increase with rising sea water temperatures (Haebler & Moeller, 1993; Harzen & Brunnick, 1997; Lafferty *et al.*, 2004).

Moreover, an increase in various skin diseases resulting in epidermal lesions has been reported in the Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Kiszka *et al.*, 2009). Although the cause of skin lesions remains uncertain, it appears that rapid coastal urbanization and several environmental factors contributed to their occurrence (Kiszka *et al.*, 2009). Finally, since the

prevalence of epidermal diseases appears to be related to temperature rise, it has been suggested that the evaluation of differences among populations may reveal environmental changes (Geraci *et al.*, 1979; Wilson *et al.*, 1999a; Van Bresseem *et al.*, 2007, 2009a; Bearzi *et al.*, 2009).

#### **1.4 Skin Lesions on bottlenose dolphins (*Tursiops truncatus*)**

Cetacean skin disorders have been recorded as far back as the 1950s (Simpson *et al.*, 1958; Slijper, 1962; Greenwood *et al.*, 1974; Van Bresseem *et al.*, 2008b). Among others, infectious agents such as bites from large fish and damage caused by parasitic copepods (Gill *et al.*, 2000; Visser *et al.*, 2010; Nichols *et al.*, 2011; Samarra *et al.*, 2012), ultraviolet solar radiation (Martinez-Levasseur *et al.*, 2011), and low salinity (Wilson *et al.*, 1999b; Gulland *et al.*, 2008) may all cause skin lesions and abnormalities (Bertulli *et al.*, 2012). Likewise, skin lesions are caused by viruses, fungi, bacteria, and protozoans (Kiszka *et al.*, 2009). Bite marks from conspecifics and/or other species of dolphins, cookiecutter shark (*Isistius* spp.) sea lampreys (*Lampetra tridentate*, *Petromyzon marinus*) have been reported on several cetacean species worldwide (Shetter, 1949; Pike, 1951; Baker., 1992; Moore *et al.*, 2003; Barnett *et al.*, 2009; Ólafsdóttir *et al.*, 2009; Dwyer *et al.*, 2011; Nichols *et al.*, 2011; Bertulli *et al.*, 2012).

The Lobomycosis infection in populations of bottlenose dolphins from the Northwest Atlantic was described in detail by Caldwell *et al.* (1975) (Also see § 1.5 for more details). The clinical symptoms and histopathology of the tattoo skin disease (TSD) were reported in free-ranging and captive dolphins by Geraci *et al.* (1979). In recent years, the ecology and epidemiology of epidermal diseases including TSD, lobomycosis and lobomycosis-like disease (LLD) were studied in wild dolphin populations (Reif *et al.*, 2006; Van Bresseem & Van Waerebeek, 1996; Van Bresseem *et al.*, 2003a, b, 2007, 2008a & b).

The intensity and extent of skin lesion prevalence in cetacean populations may reflect their general health status and the levels of environmental stress that they are under (Wilson *et al.*, 1999a; Pettis *et al.*, 2004; Hamilton *et al.*, 2005; Van Bresseem *et al.*, 2008a, 2009a, 2012). Anthropogenic factors such as inland water inputs and natural factors like water quality degradation appear to contribute to skin lesion development on cetaceans (Harzen and Brunnick, 1997; Wilson *et al.*, 1999a; Reif *et al.*, 2006; Van Bresseem *et al.*, 2007, 2009b; Kiszka *et al.*, 2009).

Over the last 20 years, skin lesions in different populations of bottlenose dolphins have been studied worldwide via photo-ID techniques. Climate change and anthropogenic activities seem to reinforce the appearance and development of skin lesions and diseases (Bearzi *et al.*, 2009; Hart *et al.*, 2012). In some cases the decline of certain bottlenose dolphin populations in British waters was secondary, among other reasons, attributed to skin diseases (see Thomson & Hammond, 1992). These infections are considered to be more likely to be a long-term feature of bottlenose dolphin populations and to occur regardless of age, rather than being transitory although some kinds may change over time (Wilson *et al.*, 2000).

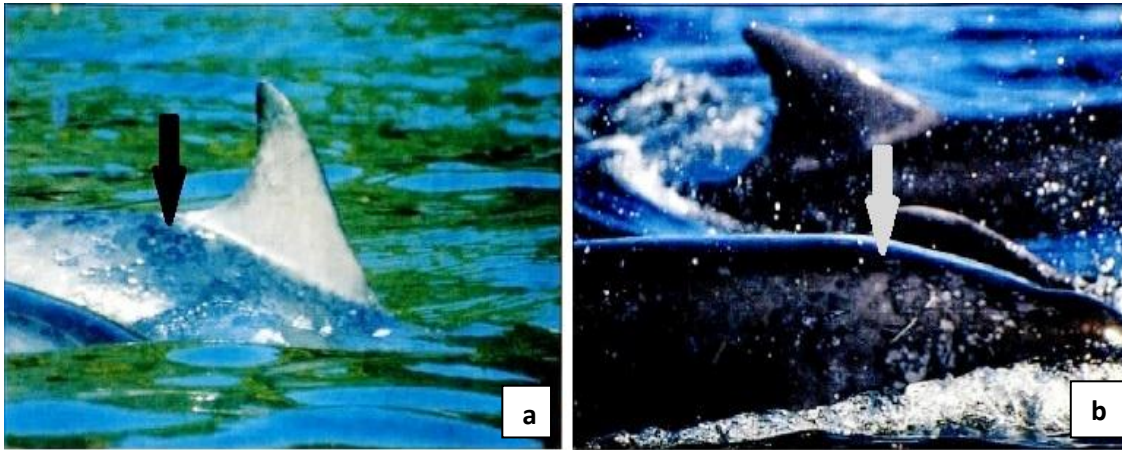
Previous studies, pointed out that the occurrence of skin lesions observed in wild bottlenose dolphin populations worldwide was between 63% and 100% (Harzen & Brunnick, 1997; Wilson *et al.*, 1999a; Bearzi *et al.*, 2009; Rowe *et al.*, 2010; Maldini *et al.*, 2010). The skin lesion categorisation used in most of the studies is mainly related to their colour and texture and has been described in Thompson and Hammond (1992) and Wilson *et al.* (1997).

### **1.5 Categories of skin lesions**

The epidermal lesions on bottlenose dolphins have been categorized into several types of abnormality, according to their colour and texture (Thompson & Hammond 1992; Wilson *et al.*, 1997; Figs. 1a & b, 2). However, over time, several studies have introduced more skin lesion categories either from photo-ID techniques (for example nodules, tattoo lesions) or by microscopic analysis of infected skin samples taken from stranded individuals (for example papillomavirus) (Rehtanz *et al.*, 2009). As a result, in some cases, wherever possible the skin lesions have been categorised by the microorganism (virus, bacteria or fungi) infection on the dolphin skin (Van Bresseem *et al.*, 2003; Hart *et al.*, 2012). The basic skin lesion categories on bottlenose dolphins are described below:

*Thompson and Hammond (1992):*

This skin lesion categorisation involved Dark lesions (Fig. 1a), Ring lesions (Fig. 1b), De-pigmentation (Fig. 2) and Injuries/Deformities.



**Fig. 1: Different individuals from the bottlenose dolphin population in the Moray Firth (Scotland) and the types of skin lesions affecting them; a) Dark lesions and b) Ring lesions (modified photos from Thompson & Hammond, 1992)**



**Fig. 2: Example of an individual from the Moray Firth population (Scotland) suffering from de-pigmentation (modified photo from Thompson & Hammond, 1992)**

*Wilson et al. (1997):*

After five years the Wilson *et al.* (1997), classified dermal diseases based on Thompson and Hammond (1992), adding more categories including ring lesions, black lesions (Fig. 3A1), dark-fringed spots (Fig. 3A2), pale lesions, abraded fin tip (Fig. 3A3), white lesions (Fig. 3A4), cream lesions (Fig. 3B), cloudy lesions (Fig.3C), white fin-fringe lesions (Fig. 3D), lunar lesions (Fig 4), orange lesions and injuries/deformities.

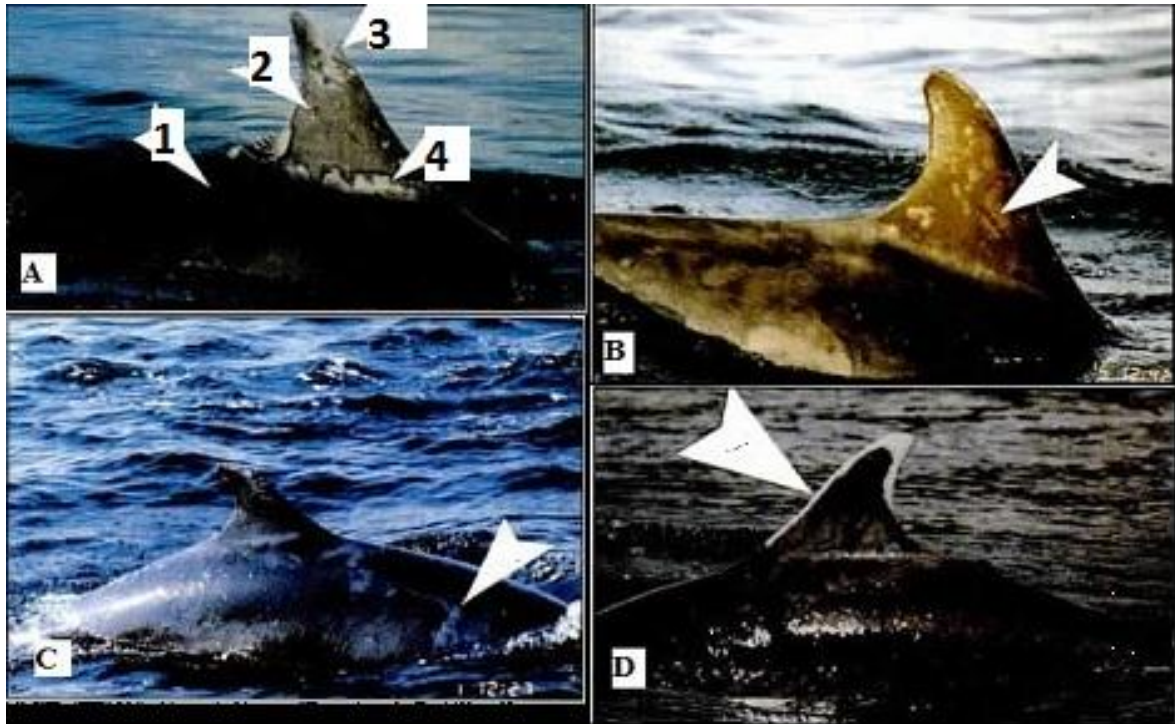


Fig. 3: A) Black lesions (1), Dark-fringed spots, (2) Abraded fin tip (3), and White lesions (4), B) Cream lesions, C) Cloudy lesions and D) White fin-fringe (modified photos from Wilson *et al.*, 1997)



Fig. 4: Juvenile with lunar lesions (modified photo from Wilson *et al.*, 1997)

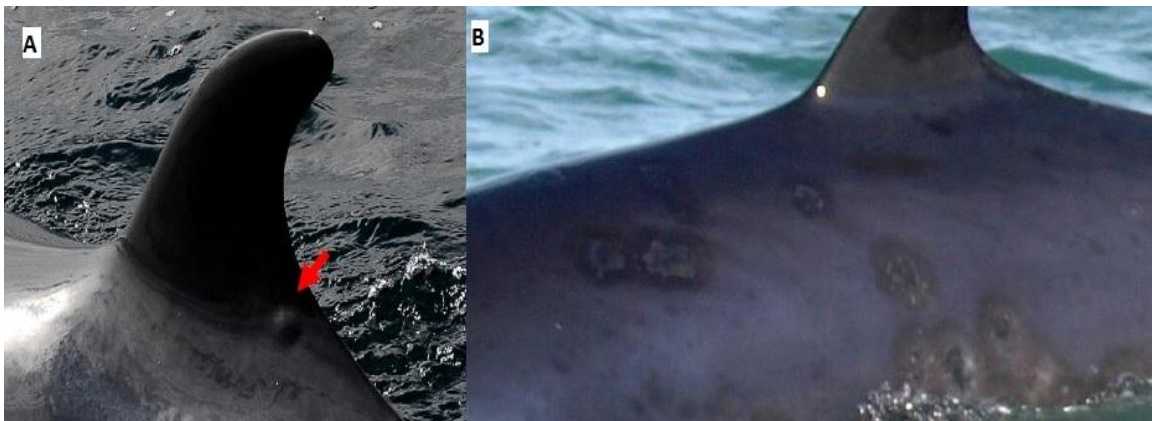
*Other skin lesion categories:*

*Nodules, cutaneous elevations and expansive annular lesions*

Other studies using photo-ID techniques have also added nodules (Fig. 5), cutaneous elevations (Fig. 6A), or expansive annular lesions (Fig. 6B) as skin lesion categories, since the cause of them (viral, fungal or bacterial) could not be confirmed (Bertulli *et al.*, 2012; Van Bresseem *et al.*, 2014).



**Fig. 5:** Nodules observed in an adult *Orcaella brevirostris* from Chilika Lagoon, India (source: Van Bresseem *et al.*, 2014)



**Fig. 6:** Cutaneous elevations (A) and expansive annular lesions (B) in common minke whales from Skjálfandi Bay (source: modified photo from Bertulli *et al.*, 2012)

## **1.6 Skin lesion categories according to the host-microorganism**

In several cases skin tissue has been obtained from stranded individuals for further microscopic analysis. The results of these studies have indicated that bottlenose dolphins may be covered by skin lesions derived from a variety of viruses, bacteria, fungi, parasites (Higgins, 2000; Birkun,



2002; Buck *et al.*, 2006; Vaughan *et al.*, 2007; Esperón *et al.*, 2008; Van Bresseem, 2008a, b, c; van Elk *et al.*, 2009; Rehtanz *et al.*, 2009; 2012; Bossart *et al.*, 2010; Cruz *et al.*, 2014). The groups of the different pathogens found on bottlenose dolphins and the causes of dermal lesions and other pathological events are described as follows:

#### **a) Viruses**

##### *Caliciviruses*

The Calicivirus of the genus *Vesivirus* was detected in tattoo skin lesions and old scars in bottlenose dolphins (Van Bresseem, 2008d). The virus caused vesicles that were rapidly eroded and resulted in shallow ulcers in one of the individuals sampled (Smith *et al.*, 1983).

##### *Herpesviruses (HVs)*

Particles of Herpes-like virus were isolated from skin lesions in bottlenose dolphins (Manire *et al.*, 2006; Smolarek Benson *et al.*, 2006). Also, according to Smolarek Benson *et al.* (2006), alpha herpesviruses may be responsible for skin or systematic infections that lead to death.

##### *Papillomaviruses (PVs)*

Six out of nine classified types of PVs have been recorded as affecting bottlenose dolphins (Rector *et al.*, 2006; Rehtanz *et al.*, 2006). Recently, it has been suggested that papillomas (Fig. 7) in the species may result in malignant transformation of benign papillomatous lesions (Bossart *et al.*, 2005). In free-ranging bottlenose dolphins, a high prevalence of papillomas has been documented, and recent studies have suggested that it may be related to high tumor prevalence (Van Bresseem *et al.*, 1996; Bossart *et al.*, 2005; Bossart, 2006, 2007). Finally, the co-existence of PVs and HVs in individuals appears to contribute to tumor development and/or tumor progression and oncogenesis (Cruz *et al.*, 2014).

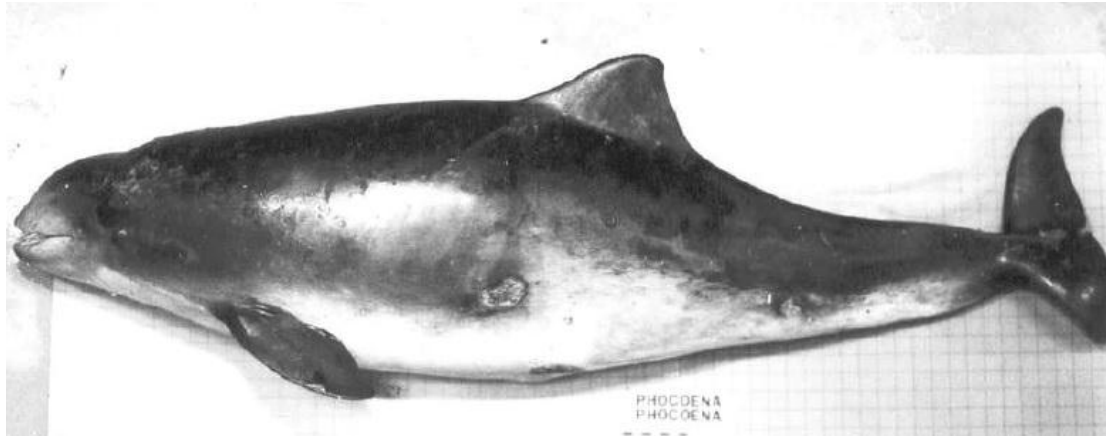


Fig. 7: A *Phocoena phocoena* infected by papillomavirus. Germany 1993 (source: Van Bresseem *et al.*, 2008d)

### *Poxviruses*

Poxviruses cause tattoo skin disease (TSD), which are characterised by very typical, irregular, grey, black or yellow, stippled lesions in bottlenose dolphins (Fig. 8) (Bracht *et al.*, 2006, Pearce *et al.*, 2008). Previous studies have suggested that tattoo skin lesions are possibly the route for other pathogens and this contributes to the severity of the lesions (Flach *et al.*, 2008; Van Bresseem *et al.*, 2008c).



Fig. 8: Very infected tattoo-like lesions on *Tursiops truncatus*. (source: Van Bresseem *et al.*, 2003)

### **b) Bacteria**

Several bacteria have been isolated from skin lesions in bottlenose dolphins (Van Bresseem, 2008d). The majority of these are opportunistic and likely result from a weakened immune defense in these individuals (Van Bresseem, 2008d).

### *Aeromonas* spp.

According to Cusick and Bullonck (1973), *Aeromonas hydrophila* may cause ulcerative dermatitis and has been recorded in bottlenose dolphins. Also, it is considered as the aetiology of skin diseases in cetaceans from South Africa (Van Bresseem, 2008d).

### *Erysipelothrix rhusiopathiae*

In bottlenose dolphins, *Erysipelothrix rhusiopathiae* may result in sub-acute to chronic dermatological disease and septicemia in captive cetaceans (Geraci *et al.*, 1966). It is characterized by greyish rectangular shaped skin lesions on the body, snout and melon. It can lead to anorexia and weakness (Higgins, 2000). This bacterium is considered to be a common infection in fish. It is suggested that bite injuries from other cetaceans or ingestion of infected fish is the access point of the disease.

### *Pseudomonas* spp.

According to Diamond *et al.* (1979), the disease caused by this pathogen was expressed extensively in one bottlenose dolphin in the form of hard, round, skin nodules with necrotic centres. *Pseudomonas* spp. may cause extensive dermatitis, skin necrosis, ulceration, bronchopneumonia, osteomyelitis and septicemia, all of which may eventually leading to death (Avalos-Téllez *et al.*, 2010).

### *Streptococcus* spp.

Different species of *Streptococcus* spp. have been found in skin abscesses (Avalos-Téllez *et al.*, 2010). Bacteria of this genus have been isolated from skin lesions and appear to be opportunistic (Buck *et al.*, 1991). They tend to infect animals under stress (Moeller *et al.*, 2003).

### *Vibrio* spp.

The species of *Vibrio damsela*, *V. vulnificus*, *V. parahaemolyticus*, *V. alginolyticus* and *V. fluvialis* have been documented in slow-healing ulcers and various wounds on bottlenose dolphins held in captivity (Schroeder *et al.*, 1985; Fujioka *et al.*, 1988; Pereira *et al.*, 2007).

### c) Fungi

#### Candidiasis

The fungus *Candida albicans* in bottlenose dolphins has been documented to cause death (Nakeeb *et al.*, 1977; Dunn *et al.*, 1982). Usually candidiasis results in widespread, granulating and occasionally ulcerated dermal lesions (Van Bressem, 2008d).

#### *Lacazia loboi*

*Lacazia loboi* is a pathogen causing Lobomycosis or Lacaziosis. The disease expresses itself in bottlenose dolphins through whitish, pinkish or grayish, verrucous lesions, often leading to ulcerations (Migaki *et al.*, 1971) (Fig. 9). Regardless of the fact that the infection develops slowly, it may lead to death (Van Bressem *et al.*, 2007; Bermúdez-Villapol *et al.*, 2008).

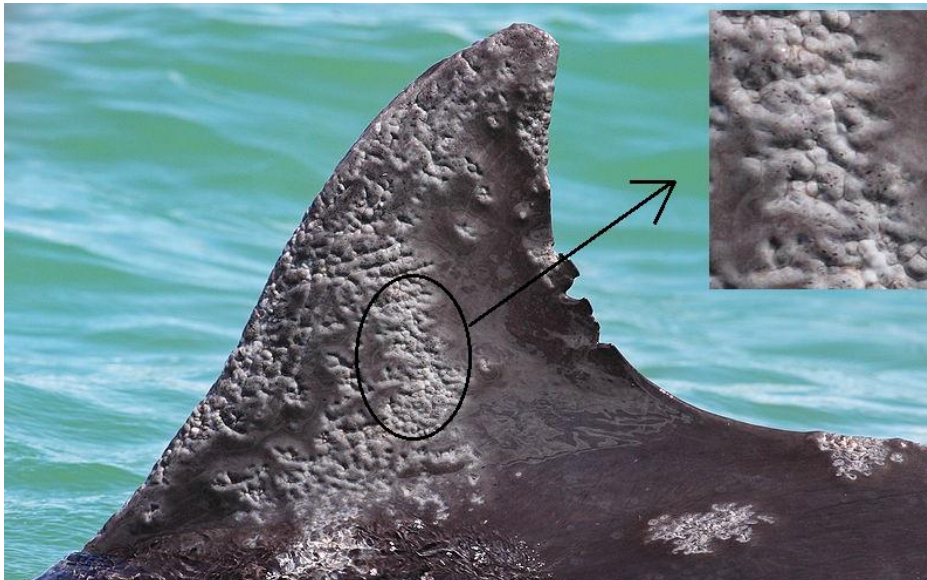


Fig. 9: Skin lesion caused by Lobomycosis infection on a bottlenose dolphin in Costa Rica (modified photo from Wikipedia, <http://en.wikipedia.org/wiki/File:Lobomycosis-fungus-lesions.jpg>)

#### *Trichophyton* spp.

The fungus *Trichophyton* spp. was isolated from superficial nodules detected on the body of a captive bottlenose dolphin in Japan (Hoshina *et al.*, 1956).

### d) Parasites

Bottlenose dolphins have been reported to exhibit dermatitis associated with invasive ciliates (Choi *et al.*, 2003). Distinct ulcers with often subcutaneous, necrosis and inflammation are the main characteristics of the infection (Schulman & Lipscomb, 1999). The ciliates observed in the

skin lesions were morphologically identical to *Kyaroikeus cetarius* (Snieszek *et al.*, 1995; Poynton *et al.*, 2001). Possibly due to their opportunistic nature, they take advantage of skin injuries in individuals (Schulman & Lipscomb, 1999). Unidentified helminthes have also been isolated, causing tumor-like tissues found on the skin of individuals (Zakharova *et al.*, 1978b; Birkun, 2002).

## **1.7 Welsh bottlenose dolphins and skin lesions**

Between two and three hundred bottlenose dolphins inhabit Cardigan Bay annually, making it the largest coastal population of the species in the British Isles (Pesante *et al.*, 2008; Feingold & Evans, 2014).

Also, the individuals in Cardigan Bay have been classified as “residents”, “visitors” and those visiting the area occasionally depended on the re-sightings number (Feingold & Evans, 2011). Residents are those who have been re-sighted over 12 occasions and constitute 42% of the individuals in the bay. Those observed between 4 and 11 times are classified as occasional visitors make up the 30% of the individuals. Transients are those individuals observed less than three occasions (Veneruso & Evans, 2012a).

Numbers are greatest during the summer months, whereas in winter the population is much more wide-ranging and it occurs mainly off North Wales and further north in the Irish Sea (Baines *et al.*, 2002; Ugarte & Evans, 2006; Pesante *et al.*, 2008; Feingold *et al.*, 2011). In recent years, there is some indication that the numbers inhabiting Cardigan Bay SAC may be declining although the overall Welsh population appears to be stable (Feingold & Evans, 2014). Although female dolphins give birth throughout the year in Cardigan Bay, 76% of all births are recorded between July and September (Feingold & Evans, 2014). The calving interval within the bay ranges from 2 to 7 years, with an average between births of 3 years (Feingold & Evans, 2014).

The abundance and density of the bottlenose dolphin in Cardigan Bay has been monitored by Sea Watch Foundation since 2001, using a combination of line transect survey and mark-recapture analysis by photo-ID. A Welsh bottlenose dolphin ID catalogue has been created and by 2013 contained the photo-ID of 378 individual dolphins, of which 248 were distinctively marked (Feingold & Evans, 2013).

Since 1989, skin lesions have been documented on the bodies of these dolphins via photo-ID methods (see Baines *et al.*, 2002; Pesante & Evans, 2008) and the epidermal lesions appear to be most intense in the dorsal fin area of the individuals. Photo-ID surveys however only became systematic and regular after 2001 (Feingold & Evans, 2014).

Over time, similar skin conditions to those described above (sections 1.4 & 1.5) have been found on individual bottlenose dolphin populations from both Special Areas of Conservation (SACs) in Cardigan Bay. Magileviciute (2006) found that one or more skin abnormalities occurred on 61% of individuals examined in the Cardigan Bay population, with the dark-fringed spots infections (DFS) being the most prevalent skin lesion among individuals. The current project updates analyses of the prevalence and development of skin lesions on Welsh bottlenose dolphins and investigates whether there is a possible relationship between the prevalence of lesions with environmental and biological variables. The study and assessment of skin lesions on bottlenose dolphins is useful since they indicate the appearance and persistence of contagious diseases amongst individuals (Rotstein *et al.*, 2009). Finally, monitoring the prevalence and intensity of skin lesions on bottlenose dolphins will help in disease surveillance and possibly will indicate any local environmental change that causes lesions (Mouton & Botha, 2012; Hart *et al.*, 2012).

## **1.8 Aims and objectives**

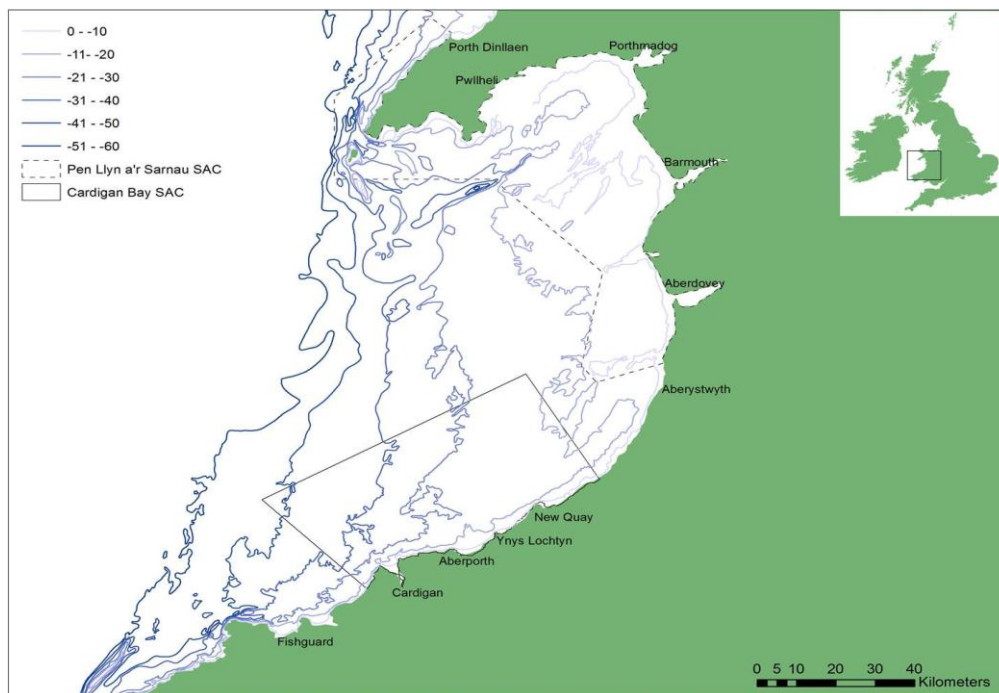
The main aim of this study was to collate and record the existing scientific information concerning the skin lesion prevalence on Welsh bottlenose dolphins. Another aim is the investigation of factors that may affect the spatio-temporal trends in skin lesion prevalence amongst the bottlenose dolphin population within Cardigan Bay. An attempt will also be made to determine whether climatic factors, such as sea surface temperature, influence the prevalence of skin lesions in dolphin populations in Cardigan Bay. To achieve this, five objectives were set:

- To review and revise the existing skin lesion categories on the Welsh bottlenose dolphins and, if possible, modify these to match more closely with the related potential causes of them
- To estimate the types, prevalence and development of skin lesions on individual bottlenose dolphins using photo-ID data collected during boat-based surveys in Cardigan Bay since 2001

- To determine whether the prevalence of specific skin lesions is related to age, sex, and home range of dolphins, using photo-ID data collected since 2001 (Sea Watch Foundation database)
- To determine if the distribution of skin lesions along the body of individuals is related to certain types of lesions over time.
- To define whether any fluctuations in sea water temperature are related to the prevalence of skin lesions over time.

## 2. Study area

Cardigan Bay is the largest bay in Britain and is located in the west coast of Wales between Pembrokeshire in the south and the Llyn Peninsula in the north (Fig. 10). Cardigan Bay covers area of approximately 5500 km<sup>2</sup> with average and water depth 40 m (Evans, 1995). The maximum water depth is 50m, becoming shallower from west to east (Evans, 1995).



**Fig. 10: Cardigan Bay, including Cardigan Bay SAC and Pen Llyn a'r Sarnau SAC (source: Feingold & Evans, 2014: NRW Monitoring Report)**

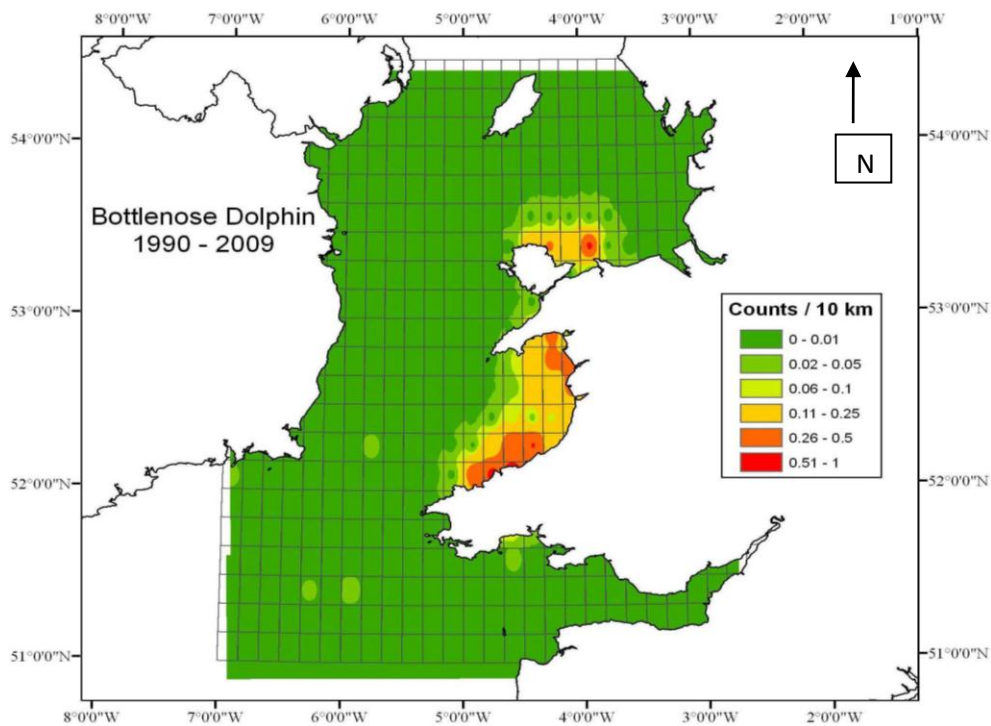
The mean annual sea water temperature range in Cardigan Bay is 11° C although temperature fluctuations occur in the shallow waters of the bay (Evans, 1995). Locally, water temperatures

and quality and salinity are affected by the input of fresh water from the rivers Dyfi, Aeron, and Teifi (CCW, 2005). Since the bay is considered shallow in general, the action of wave and wind dictate the mechanisms of physical dispersion of nutrients (CCW, 2005).

Semi-diurnal tides enter the bay by the St. Georges Channel and prevail in the area (Field Studies Research Council, 1992). In addition, the seabed sediment distribution is dependent on the speed of the tidal current, and ranges from gravel (in the high energy current) to mud (in the low energy current) with sand and rocks in between (Evans, 1995). According to Annex II of the EU Habitats and Species Directive, bottlenose dolphin requires spatial protective measures (Council Directive 92/43/EEC). In order to conserve the populations of the species in Cardigan Bay, two marine Special Areas of Conservation (SACs) have been established here.

One of these is Cardigan Bay SAC where bottlenose dolphins are the main reason for the designation, and the other is Pen Llyn a'r Sarnau SAC where the species is a qualifying feature (Feingold & Evans, 2014).

Cardigan Bay is the largest of two UK principal coastal areas used by semi-resident bottlenose dolphin populations, and with the highest abundance (Pesante *et al.*, 2008; Feingold & Evans, 2014, see also Fig. 11).



**Fig. 11: Map of Bottlenose dolphin distribution and densities of the populations in Welsh waters (source: Baines & Evans, 2012: Atlas of Marine Mammals of Wales)**



As ‘top’ predators, bottlenose dolphins are opportunistic feeders with a diet including a wide variety of benthic and pelagic, solitary and schooling, fish and cephalopods (Evans & Hintner, 2012). Documented prey of ten bottlenose dolphins from Scottish waters included gadoids (cod, saithe, whiting, haddock), salmon, sprat, sandeels, flatfish and cephalopods (Santos *et al.*, 2001). Although there have been no analyses of stomach contents of Welsh bottlenose dolphins (since very few animals strand and they generally have empty stomachs), animals have been directly observed taking a variety of prey, including sea bass, salmon, conger eel, garfish, sandeel, and small shark species (Pesante *et al.*, 2008; Evans & Hintner, 2012). There is some indication that in coastal waters of Cardigan Bay, bottlenose dolphins feed in small groups mainly on demersal and benthic prey (Feingold & Evans, 2014).

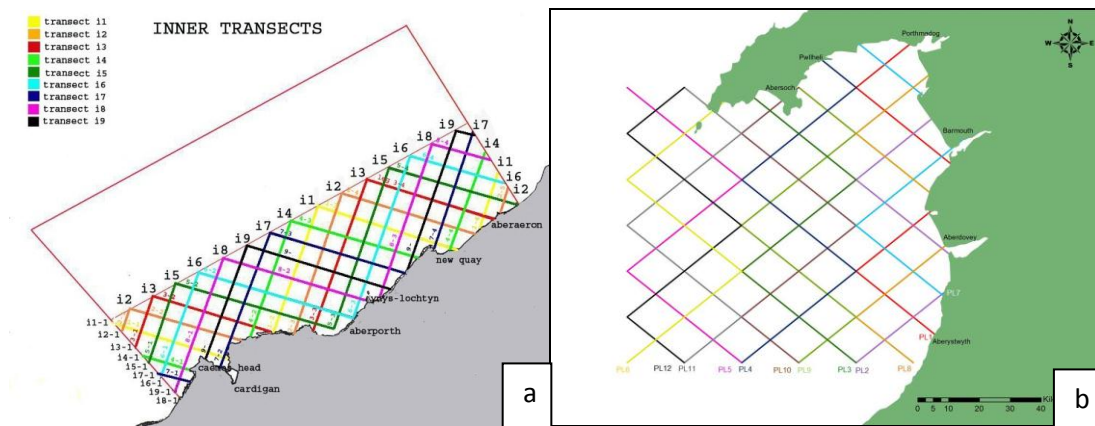
The coastal area from Aberaeron to Cardigan appears to be particularly important for the species, especially the region of New Quay and the surrounding area (Feingold & Evans, 2014). The coastal areas of Ynys Lochlyn, Mwnt and Aberporth are also important for bottlenose dolphin populations, along with the Sarns (Sarn Badrig, Sarn y Bwch, and Sarn Cynfelin) and Tremadog Bay within Pen Llyn a’r Sarnau SAC (Feingold & Evans, 2014). The species populations in Cardigan Bay include a mix of residents, transients and occasional visitors (Feingold & Evans, 2014). Most monitoring effort has been within the Cardigan Bay SAC, which covers an area of approximately 1039 km<sup>2</sup> (Baines *et al.*, 2002; Feingold & Evans, 2014), although since 2006, there has been increasing survey effort in northern Cardigan Bay.

### **3. Methods**

#### **3.1 Field data collection**

The necessary data were collected by applying photo-ID techniques through boat-based surveys that were conducted in Cardigan Bay. The samplings took place mostly within the boundaries of Cardigan Bay SAC operating out of New Quay, but with line transect surveys also in the Pen Llyn a’r Sarnau SAC out of Pwllheli harbour.. In order to estimate the abundance and the density of the populations, line transects involving “Distance” sampling and opportunistic photo-ID using mark-recapture techniques were combined during dedicated surveys (Fig. 9). Within Cardigan Bay SAC, the transect line design followed a zigzag pattern between the coast and either the median parallel or the outer boundary of the SAC (the former being sampled twice as much as the latter). However, during 2014 fieldwork period, the transect lines surveyed were

confined to those in the inner area of the SAC. In the rest of Cardigan Bay, line transects extended over the entire Bay (Fig. 12a & b).



**Fig. 12:** a) The transect lines followed in the inner area of Cardigan Bay for the 2014 study and b) the transects conducted over northern Cardigan Bay (including Pen Llyn a’r Sarnau SAC), most of these transects have been undertaken also in July 2014 (source: Feingold & Evans, 2014: NRW Monitoring Report)

Each transect was chosen at random. The vessel used in Cardigan Bay SAC was the 9.7m motor vessel “*Dunbar Castle 2*” with a 120 hp power diesel engine (Fig. 13). Two primary observers were allocated to the roof of the vessel where they had eyesight of 3.5m height above the sea level, with two separate independent observers. The overall number of observers in a survey averaged five but occasionally the number ranged from 6 to 10 observers. In northern Cardigan Bay (including Pen Llyn a’r Sarnau SAC), the vessel used was the 11m RIB “*Pedryn*” with a 150 hp diesel engine and an observer eye height of 3m. The overall number of observers ranged between 4 and 6, using the same methodology but a single independent observer. Every survey was carried out in sea states of Beaufort scale between 0 and 3, and good visibility (no fog or heavy rain). If visibility was low or the sea state over 3, the trip was cancelled or any data collected were not used in subsequent analyses.



**Fig. 13:** The photo shows “*Dunbar castle 2*” vessel and the positions the primary and independent observers occupy during the surveys

Effort and sighting data were also collected and recorded on specially designed effort and sightings sheets, following the protocol of the Sea Watch Foundation (see Appendix 1). Effort data were recorded every 15 minutes or when conditions changed, and included effort status (type of survey: casual watch, dedicated survey, linear transect line, photo-ID), time of day, position (co-ordinates), visibility conditions (precipitation presence/absence, glare), sea state, and swell height. Every sighting was documented on the sighting form, and referenced in the same way on the effort form. On the sighting form, data regarding the group size, number of adults, juveniles and calves, and behaviour (fraction of movement, reaction to the boat, and activity such as foraging, resting, social, and sexual behaviour) were recorded. When a sighting occurred, the boat approached the individuals slowly and preferably on a parallel but closing course in order to start the photo-ID photo sampling (see also Würsig & Jefferson, 1990; Würsig & Evans, 2001 for details).

All the photographed individuals found in an encounter were considered to make up a group. The size of the group was determined by the overall number of individuals heading in the same direction or being involved in similar activity at a distance of 100m or less (Wells *et al.*, 1987; Feingold & Evans, 2013). The age of the dolphins was defined according to body size, skin colour, swimming behaviour and the proximity to an adult (Bearzi *et al.*, 1997; Feingold & Evans, 2014).

Gender of individuals was determined depending on whether this could be determined. However the age classification for 2014 was female adults, male adults, unknown adults and calves. Due to the fact that calves and juveniles were not always easy to be distinct in every case, both categories were grouped as 'calves'. Potential males were considered marked individuals with a large heavy body size (Fig.). Mothers with calf/calves were recorded as adult females (Fig.). However, wherever bow-riding or aerial behaviour was expressed by the dolphins, the sex was confirmed in photos displaying the genital area (Smolker *et al.*, 1992).

The photo-ID procedure was completed when both sides of the dorsal fin and, when possible, backs of all of the individuals were properly photo sampled. A photo-ID encounter lasted a maximum of 40 minutes, as dictated by the photo-ID license issued by Natural Resources Wales (NRW). Therefore, individuals with avoidance behaviour or extended dives were not recorded in order to respect the time limitation. In order to be able to separate the different encounters during photo-sampling, spacer photos were used ("blank" photo of a non dolphin subject subsequent to the image sequence" (Würsig & Jefferson 1990)). Additionally, photo-ID notes were saved on video during the encounters. To increase the reliability of the data taken and to ensure that all individuals were photographed, at least two photographers were present.

The photographic equipment used included Canon EOS 7D and 40D DSLR cameras with 18-200 mm, 70-300 mm or 75-300 mm zoom lenses. A Canon 1000D with a 75-300 mm lens was also used occasionally. The pictures were generally exposed using the minimum diaphragm aperture at a shutter speed of 1/1000 sec. Finally, dorsal fin features (nicks & pigmentation patterns) of all individuals photographed were matched and compared with data from the Sea Watch Foundation photo-ID database for the period 2001-2013.

## **3.2 Data Analysis**

### **3.2.1 Photo-ID matching**

In order to compare how skin lesions have developed over time in the Welsh bottlenose population, photo-identification matching was essential. All photos taken from the surveys were analysed following the Sea Watch Foundation matching protocol using the ACDSee 5.0.1 digital imaging software. Only high resolution and quality photos were used in order to avoid false positive or false negative errors (Scott *et al.*, 1990; Stevick *et al.*, 2001). The individuals depicted in the photos were identified based on their nicks, scars, lesions and the shape of their

dorsal fin (Wilson *et al.*, 1999a). The age of the individuals, where possible, was defined visually in the field and by the photo-ID catalogue. Each dolphin is listed in the ID catalogue with a unique reference code-number according to the level of mark ('W' for well marked, 'S' for slightly marked, and 'U' for unmarked individuals). Also, the photos were categorised as 'Marked' where individuals had marks allowing identification, 'Right' where photos of the right side of the dolphin, displayed marks on the dorsal fin, and 'Left' where photos of the left side of the dolphin displayed marks on the dorsal fin. In order to analyse further the spatio-temporal trends of skin lesions in the dolphin, only photos from identified individuals were used.

### **3.2.2 Sea surface temperature data**

Annual sea surface temperature (SST) values for the Cardigan Bay area were retrieved in order to investigate whether skin lesion development is related to environmental factors. Data for the period 2001-2010 were obtained from the British Oceanographic Data Center (BODC). For the later period 2011-2014 SST data were obtained by NOAA High Resolution optimum interpolation (OI) following the SST analysis by Reynolds & Smith (1994) available on a daily basis at 0.25 degree resolution from 1981 onwards (<http://www.esrl.noaa.gov/psd/>).

### **3.3 Statistical analysis**

A multivariate analysis was undertaken using PRIMER v.6 (Clarke & Warwick, 2001) in order to check how the prevalence of each type of lesion cluster varied over the period 2001-14. The Spearman coefficient correlation was used to check for possible relationships between the prevalence of the different skin lesions in the overall population, but also to test for relationships between the prevalence of the different skin lesion types that were found to affect the calves and the adults (following Wilson *et al.*, 1999).

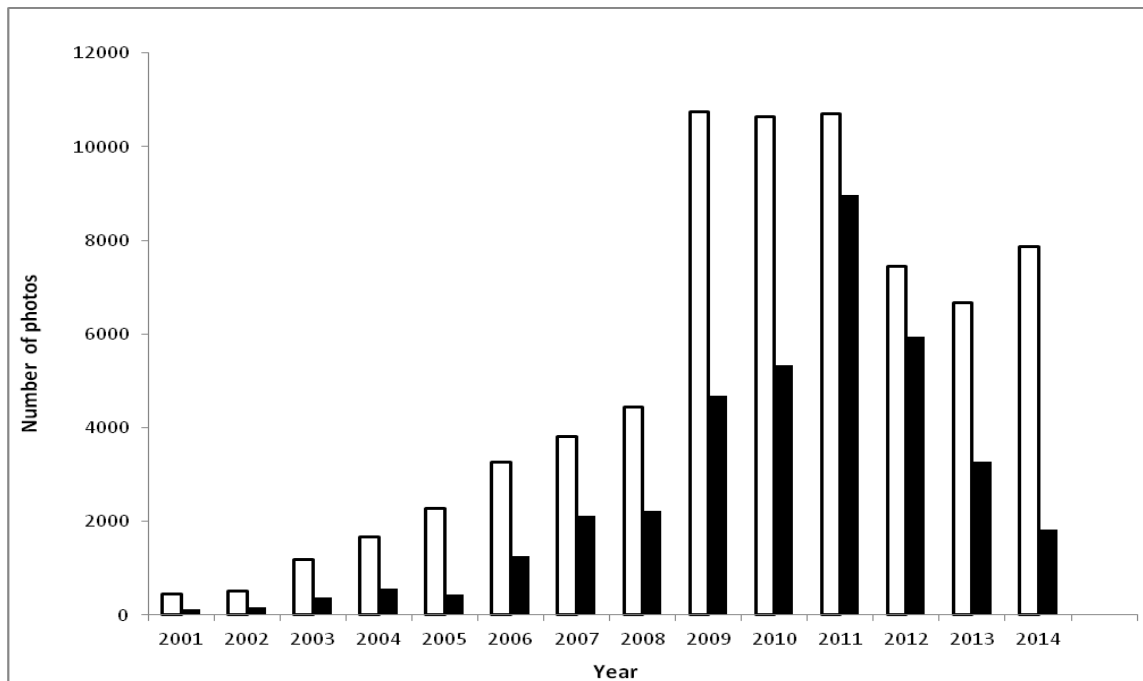
One way Anova and Kruskal Wallis were used to examine the differences in prevalence of different skin lesion types among the different age groups. Similar methodology was used to test the prevalence of the different skin lesion categories between the different body areas affected by them and between the different genders (following Wilson *et al.*, 1997). Also, Tukey *post hoc* test was used to determine variations in the prevalence of different lesion types among the different body areas, age and sex groups (following Wilson *et al.*, 1997). The same methodology was also applied in order to test whether there were significant differences in the prevalence of different skin lesions between resident, transient and visitors individuals of the population.

A  $\chi^2$  test of independence was applied in order to investigate possible relationship between the residency of individuals and skin lesion prevalence (following Hart *et al.*, 2012). In order to investigate if the prevalence of skin lesions was related to environmental factors a  $\chi^2$  test of independence was performed (following Hart *et al.*, 2012). Monthly SST values from Cardigan Bay were examined to investigate whether there was any relationship to the prevalence of dermal lesions among the dolphins.

## 4. Results

### 4.1 Photo analysis

A total of 71,610 photos were taken in the period 2001-14, of which 36,800 (51.4%) were classified as of medium to high quality, and therefore usable for skin lesion detection (Fig. 14). During the period of May to July 2014, 7,872 photos were taken. Due to the quality requirements for analysis of different types of skin lesions, of those taken in 2014, only 1,830 photos were used.



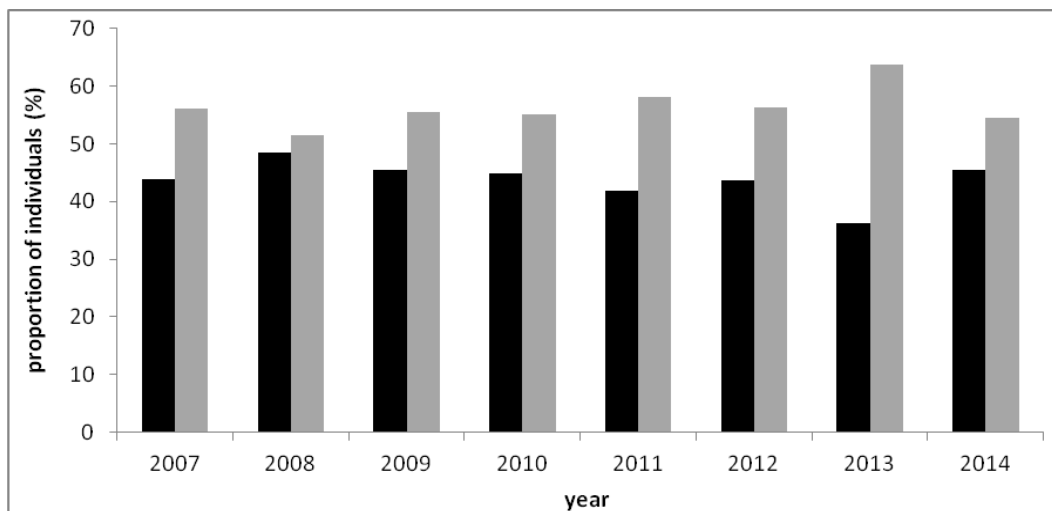
**Fig. 14: Number of photos taken (white bars) and used (black bars) for detection and analysis of skin lesion types (n=71,610)**

During the period 2001-2014 (up to July), good quality images of 260 individuals were analysed for skin lesion type categorisation and prevalence. Out of those, 214 were identified from the Sea

Watch Foundation Welsh bottlenose dolphin photo-identification catalogue. The remaining 46 were unidentified individuals due to lack of nicks and marks on their dorsal fin.

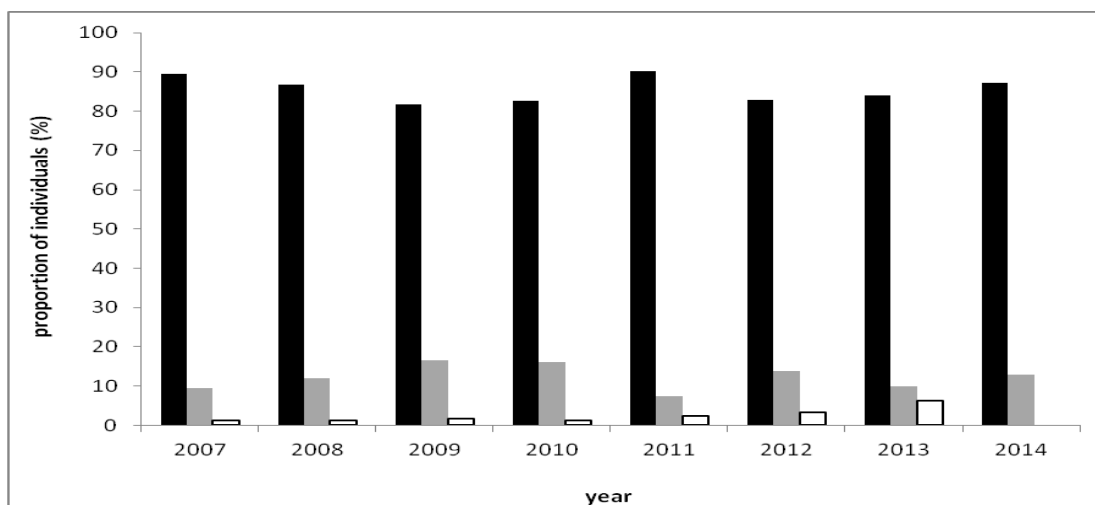
## 4.2 Population trends

In the period 2001-14, the identified females and males constituted 57.1% and 42.9% respectively (n=175). In eight out of 14 years, males were in lower proportions than females in the sample (Fig. 15). Individuals accompanied by one or more calves were determined as females



**Fig. 15: Proportions (%) of males (black) and females (grey) of individuals per year for the period 2007-14 (n=83)**

Adults comprised 76.9%, while the calves made up 17.3% and juveniles 5.3% (n=245). The same pattern was observed throughout the last eight years (Fig. 16).



**Fig. 16: Proportions (%) of adults (black), calves (grey) and juveniles (white) per year for the period 2007-14 (n=225)**

## 4.3 Skin lesions

### 4.3.1 Skin lesion categorisation

After the completion of individual matching and identification, skin lesion categorisation took place. The first attempt to categorise skin lesions in the Welsh bottlenose dolphin populations was made by Magileviciute in 2006. Since then, there has been no further information regarding the skin lesion categories on these populations. In 2014, an attempt was made for a new classification by combining the skin lesion categories used by Wilson *et al.* (1997), Magileviciute (2006), Van Bresseem *et al.* (2007, 2009b, 2014), Maldini *et al.* (2010), Bertulli *et al.* (2012) and Hart *et al.* (2012). Prevalence was defined as the proportion of photo-identified individuals showing dermal lesions (following the protocol developed by Rothman & Greenland, 1998). Since there was no chance to define the aetiology of those lesions via biopsies, the skin lesions were detected and categorised visually, based upon the dorsal and dorso-lateral views of the individual's body.

Skin lesions were grouped into the following categories:

- Dark black dot lesions (Bertulli *et al.*, 2012). This group includes small black dot lesions that are similar to those derived from herpesvirus (Fig. 17).



**Fig. 17:** Calf 008-03L covered by dark black dot lesions (DBD). The photo was taken in Cardigan Bay in 2012 (photo: © Sea Watch Foundation, 2012)

- Discolouration (Maldini *et al.*, 2010). Whitish areas that result in lighter than usual colouration of the skin are described in this group. These may be found in patches (in areas larger than a human hand) or widespread along the body of the animal (Fig. 18).





**Fig. 18: Individual 194-07w exhibiting fin discolouration . The photo was taken in Llyn Peninsula in 2014 (photo: © Elena Akritopoulou/Sea Watch Foundation, 2014)**

- Dark fringed spots (Wilson *et al.*, 1997). Usually circular areas of pale skin surrounded by a light ring. Sometimes these can be confused with white or cream lesions in animals with darker skin colouration (Fig. 19).



**Fig. 19: The individual 117-03s was observed in Cardigan Bay SAC in 2013 carrying DFS (1) and tooth rakes/scars (1) among other skin lesions on its dorsal fin (photo: © Sea Watch Foundation, 2013)**

- Orange lesions (Wilson *et al.*, 1997; Hart *et al.*, 2012). This category includes orange hues & orange patches. Orange patches are described as distinct patches of orange colouration with rounded or irregular edges. An orange hue is described, with big areas of

skin unaffected by other type of lesions, appearing to be orange. These areas are mostly on the belly and/or flanks.

- Abraded fin tip (Wilson *et al.*, 1997). This category refers to white lesions that occur on the leading edge of the fin at or near the top only, with a diffused edge (Fig.20). The surface is scraped and reminiscent of injuries that occur in stranded dolphins when they have rolled about on the beach (B. Wilson, *pers. comm.*).

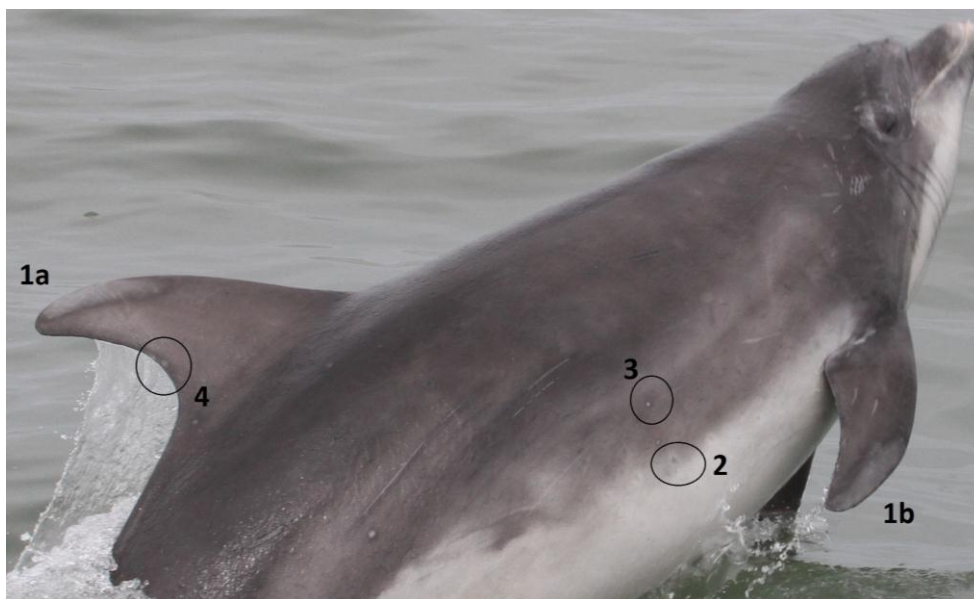


**Fig. 20: AFT on Voldermort's (023-03w) dorsal fin, as observed in 2012 in Cardigan Bay SAC (photo: © Sea Watch Foundation, 2012)**

- White fin fringe (Wilson *et al.*, 1997). White fin fringes tend to occur around entire (or much of the) margins of the dorsal fin and have distinct borders (Fig. 21). Interestingly, some of the individuals exhibiting white fin fringe lesions in other locations (for example Moray Firth population of bottlenose dolphins) had the same around their tail flukes and flippers (B. Wilson, *pers. comm.*) (Fig. 22).



**Fig. 21: Gandalf (014-01w) exhibiting WFF. The photo was taken in Cardigan Bay SAC in 2003 (photo: © Sea Watch Foundation, 2003)**



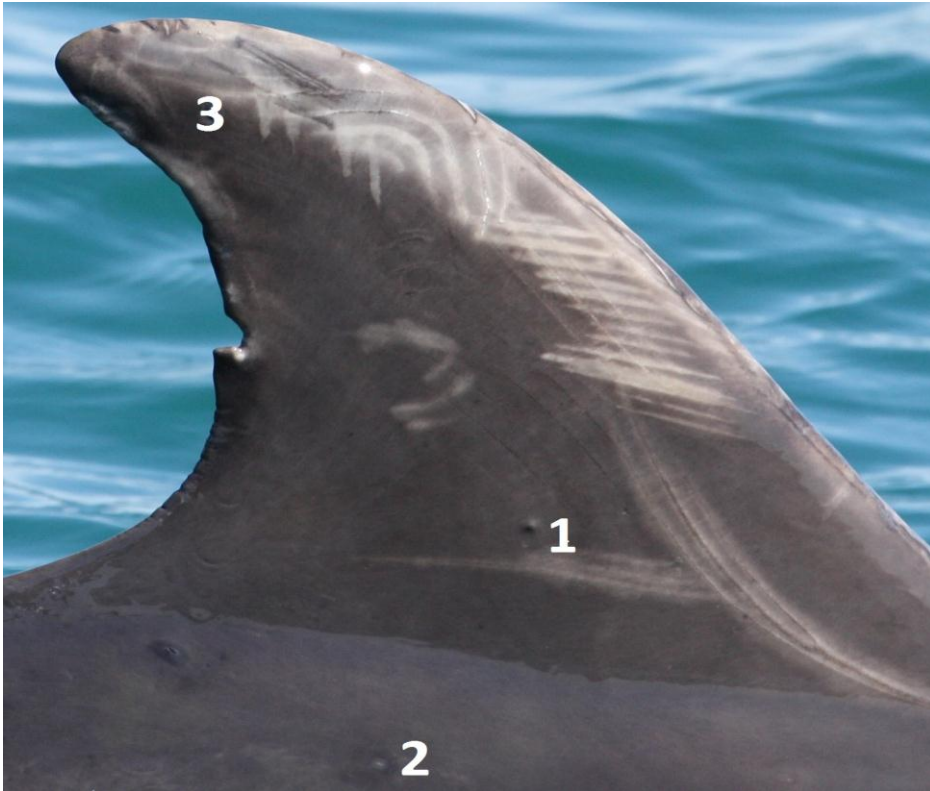
**Fig. 22: Unidentified individual in the area of Anglesey carrying WFF (1a & b), WFS (2), tooth rakes/scars (3) and DFS (4) (photo: © Sea Watch Foundation, 2010)**

- Tooth rakes and scars (Wilson *et al.*, 1997; Bertulli *et al.*, 2012). This category includes tooth rakes occurring during dolphins' social interaction, and scars caused either from anthropogenic activity (vessel collision) or unknown sources.
- White lesions (Wilson *et al.*, 1997). Circular or shapeless white patches with rounded edges. Sometimes they have a “chalky and matt” appearance (Fig. 23).



**Fig. 23: Voldermort in 2008 in Cardigan Bay SAC suffering from WL (photo: © Sea Watch Foundation, 2008)**

- Black lesions (Wilson *et al.*, 1997). Black rounded or shapeless patches with rounded edges are included in this lesion group and are larger in size than the dark black dot lesions.
- Cloudy lesions (Wilson *et al.*, 1997). These skin lesions are frequently found on the dorsal fin and back. They are blue-greyish lesions with rounded, discrete and diffused edges.
- Nodules (Van Bresseem *et al.*, 2014). This category includes nodules of unknown origin. Nodules were defined as “circumscribed elevations of the skin, generally with a normal pigmentation and unbroken integument” (Fig. 24a & b).



**Fig. 24a:** The individual 163-05s was recorded in 2013 in Cardigan Bay SAC carrying nodules (1), DFS (2) and tooth rakes/scars among other skin lesions (photo: © Sea Watch Foundation, 2013)



**Fig. 24b:** The individual 062-06w was recorded in 2014 in Cardigan Bay SAC carrying nodules on the tip of dorsal fin. Other lesions observed were tooth rakes/scars and discolouration in the margins of the dorsal fin that could be WFF (photo: © Emilia Benavente/ Sea Watch Foundation, 2014)

- Velvety lesions (Flach *et al.*, 2008). This category includes lesions described previously as lunar lesions (Wilson *et al.*, 1997; Magileviciute, 2006). The lesions are characterised as grayish to whitish, verrucous lesions that sometimes lead to ulcerations. During the healing period lesions cicatrised losing their velvety texture and become light and/or grayish. The normal skin colouration most of the times but in some individuals whitish blotches also observed. These lesions are thought to be associated with unrelated skin injuries, scars and tooth rakes (Flach *et al.*, 2008)
- Cream lesions (Wilson *et al.*, 1997). They are similar to white lesions, but their cream colour and diffused edges differentiate them.
- White fringed spots (Wilson *et al.*, 1997; Hart *et al.*, 2012). Normal or black coloured small circular areas of skin, that are surrounded by cream or white light rings
- Annular marks (Bertulli *et al.*, 2012). These lesions are characterised by hyperplastic lesions very much like wart lesions. Wart lesions were documented in harbour porpoises (*Phocoena phocoena*) in the Bay of Fundy.

#### **4.3.2 Possible other skin lesion categories observed on Welsh bottlenose dolphins**

Besides the skin lesion categories as described above, some of the photos suggested that pox and pox-like viruses lesions (Figs. 25 & 26) and tattoo lesions (Fig. 27) may apply to some of the Welsh bottlenose dolphins. However, the photos did not meet the quality requirements, and so the potential skin lesion types were omitted in the current study. Examples of those photos are found below. Additionally examples of photos meeting the requirements for skin lesion determination compared to poor quality photos taken from the Welsh waters are shown below (Fig.28). The ones taken in Welsh waters were blurry or long distanced and even if zoomed in, the lesion was not distinguishable enough to be categorised (Fig. 29).



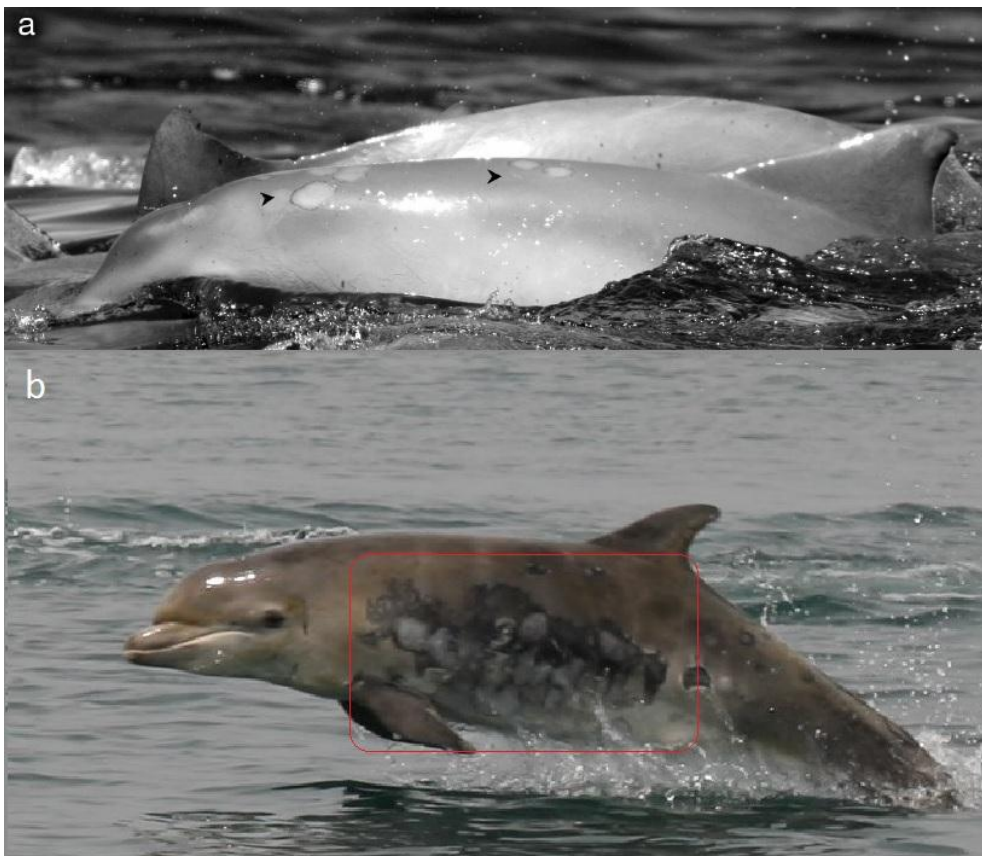
**Fig. 25:** This photo depicts the individual 115-01w with lesions similar to pox viruses. However, the photo quality is poor and the lesion category was not included in this study. The photo was taken in Cardigan Bay SAC, in 2007 (photo: © Sea Watch Foundation, 2007)



**Fig. 26:** Titania (058-04w) possibly exhibiting similar lesions to pox viruses. The photo was taken in the Llyn Peninsula in 2013 (photo: © Sea Watch Foundation, 2013)

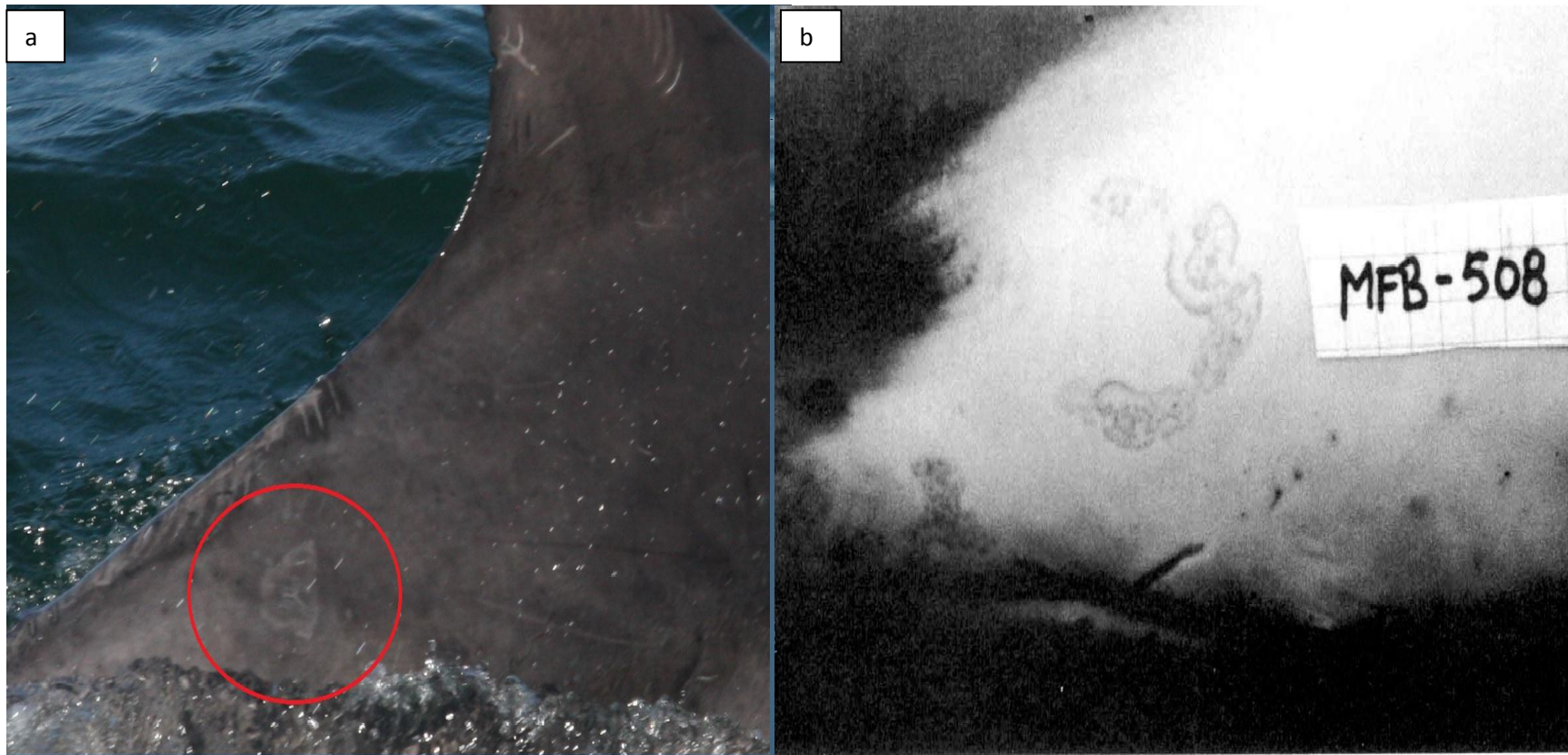


**Fig. 27:** The individual 197-07s was observed at the Llyn Peninsula in 2007 carrying a similar lesion to tattoo lesions. The quality of the photos of this encounter were characterised as poor (droplets and sun reflection on the flank did not allow the confirmation of the lesion type), and tattoo lesions were therefore not included in this study (photo: © Sea Watch Foundation, 2007)



**Fig. 28:** A *S. guianensis* suffering from regressing tattoo lesions (arrowheads). The photo was taken in Sepetiba Bay, Brazil (a) and an unidentified *T. truncatus* calf suffering also possibly from tattoo lesions on the right flank. The photo was taken in Cardigan Bay SAC in 2010. The comparison of these photos reveals the differences in photo quality regarding the requirements for skin lesion analysis (photos: a: Van Bresseem *et al.*, 2009; b: © Sea Watch Foundation, 2010)

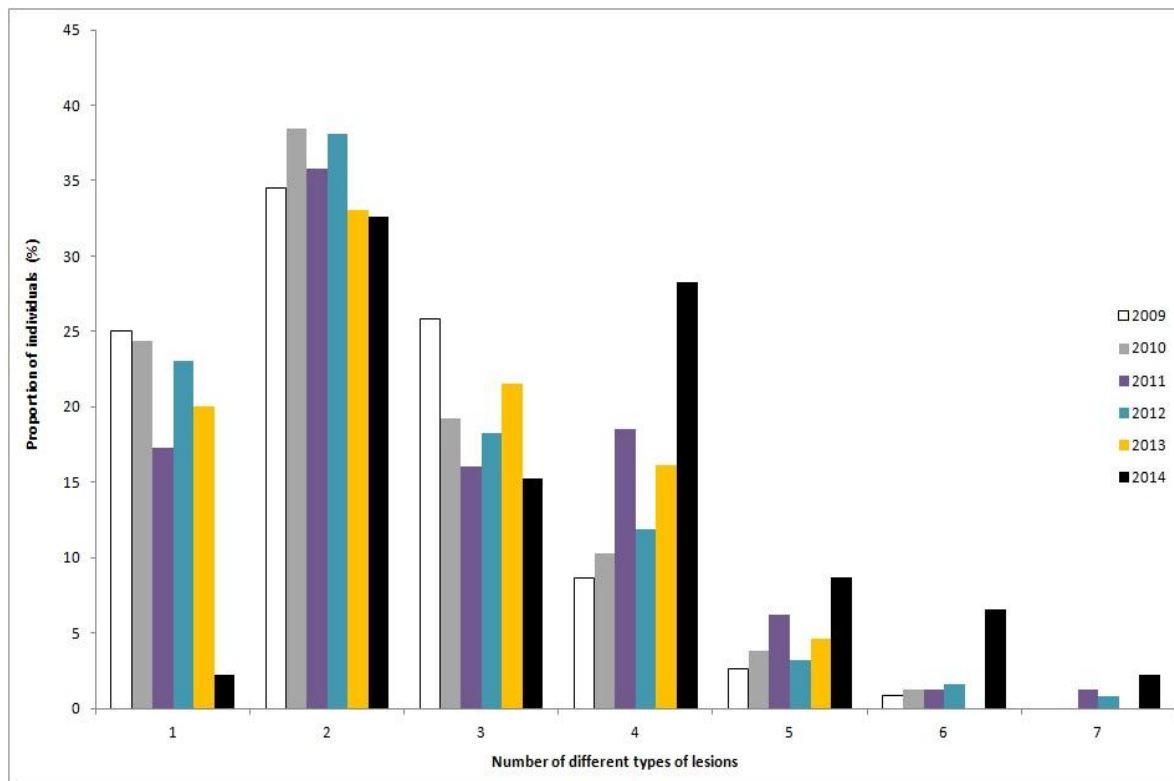




**Fig. 29: a) The transient individual 248-11s was observed in the coastal waters of northern Anglesey and possibly suffers from pox virus lesions located on the back of the body. The photo is blurry and droplets from the dolphin's swimming activity makes the classification difficult, b) a long-beaked common dolphin from Peru affected by irregular pox virus marks on the area of the belly (photos: a: © Sea Watch Foundation, 2011; b: Van Bresse *et al.*, 1996)**

#### 4.4. Skin lesion prevalence

During the period 2007-14, 72.8% (185/254) of individuals were affected by more than one type of lesion. Of those, 55.7% (103/185) were affected by two different types and 23.8% by three different types of skin lesions and 1.6% (3/185) were found to carry as many as seven different types of lesions (Fig. 30).

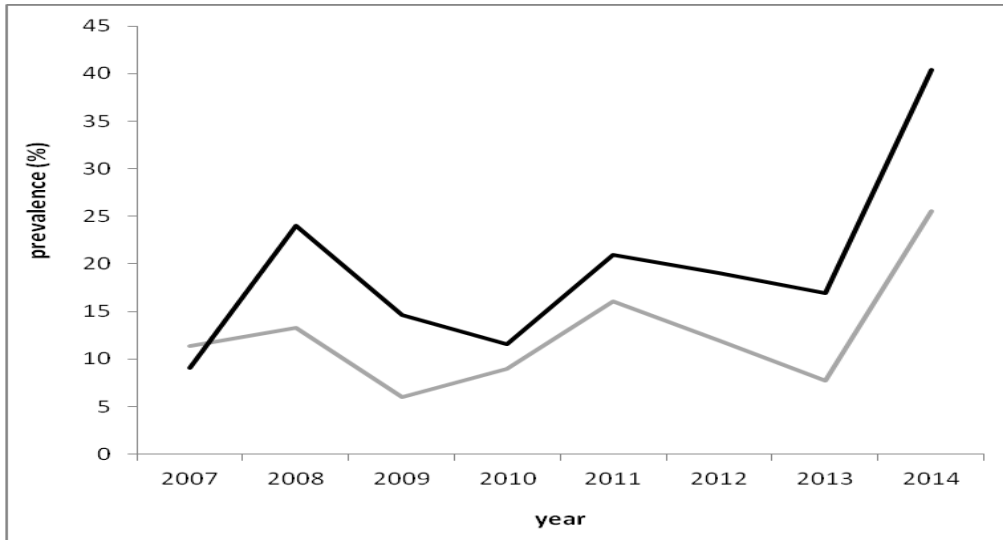


**Fig. 30: Proportions (%) of individuals affected by different numbers of skin lesion types for the period 2011-14 (black: 2014, yellow: 2013, blue: 2012, purple: 2011, grey: 2010, white: 2009)**

The more prevalent types of lesions among the dolphins over time appeared to be tooth rakes/scars(84%), white lesions (WL) (43.8%), cloudy lesions (CL) (23.4%), dark fringed spots (DFS) (20%), abraded fin tips (AFT) (15%) white fringed spots (WFS) (12.6%), dark black dots (DBD) (11.3%), white fin fringe (WFF) (9.5%) and black lesions (BL) (5.4%) (Table 2). The others (discolouration, orange lesions, nodules, velvety CR and annular marks) were all found on less than 5% of individuals and not considered further.

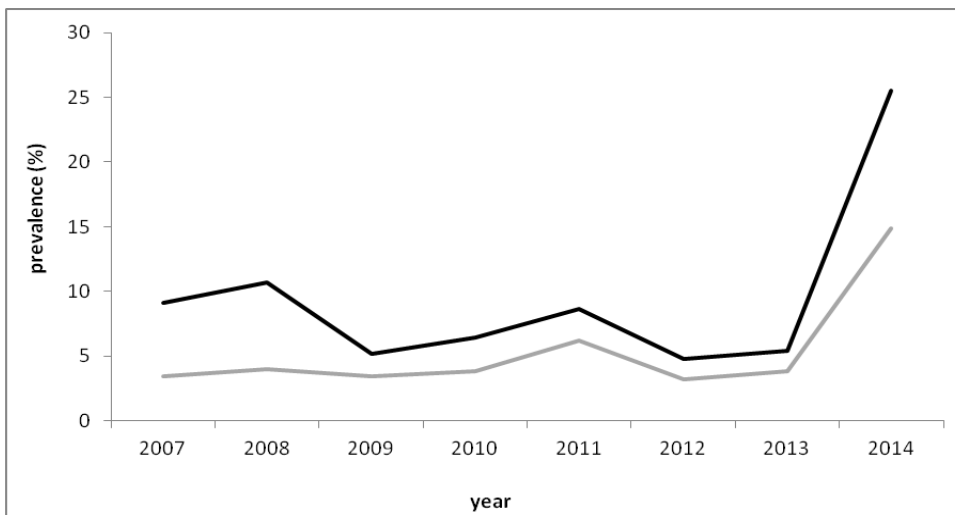
The results of Spearman coefficient correlations showed a strong positive correlation between tooth rakes/scar and the lesion types of BL ( $r_s=0.978$ ) and AFT ( $r_s=0.456$ ). The same analysis resulted in a strong positive correlation between WL and the following lesions: DFS ( $r_s=0.493$ ), WFF ( $r_s=0.610$ ), WFS ( $r_s=0.452$ ), DBD ( $r_s=0.736$ ) and BL ( $r_s=0.536$ ). Strong positive

correlations were also observed between DFS and WFF ( $r_s=0.289$ ), CL ( $r_s=0.493$ ) and WFS ( $r_s=0.767$ ). Also, a strong positive correlation was observed between WFF and WFS ( $r_s=0.667$ ), CL ( $r_s=0.476$ ), DBD ( $r_s=0.610$ ), BL ( $r_s=0.707$ ) and AFT ( $r_s=0.823$ ). The DFS (dark fringed spots) and WFS (white fringed spots) both followed a similar pattern in prevalence (Fig. 31).



**Fig. 31: Prevalence of DFS (black) and WFS (grey) (%) for the period 2007-14 (n=254)**

In addition, the WFF (white fin fringe) and BL (black lesions) followed a similar prevalence trend (Fig. 32).



**Fig. 32: Prevalence of WFF (black) and BL (grey) (%) for the period 2007-14 (n=254)**

A multivariate analysis was undertaken using PRIMER v.6 (Clarke & Warwick, 2001) in order to check how the prevalence of each type of lesion cluster varied over the period 2001-14. The

MDS configuration shows similarity regarding each skin lesion prevalence for the period 2007-14 (Figs. 33 & 34).

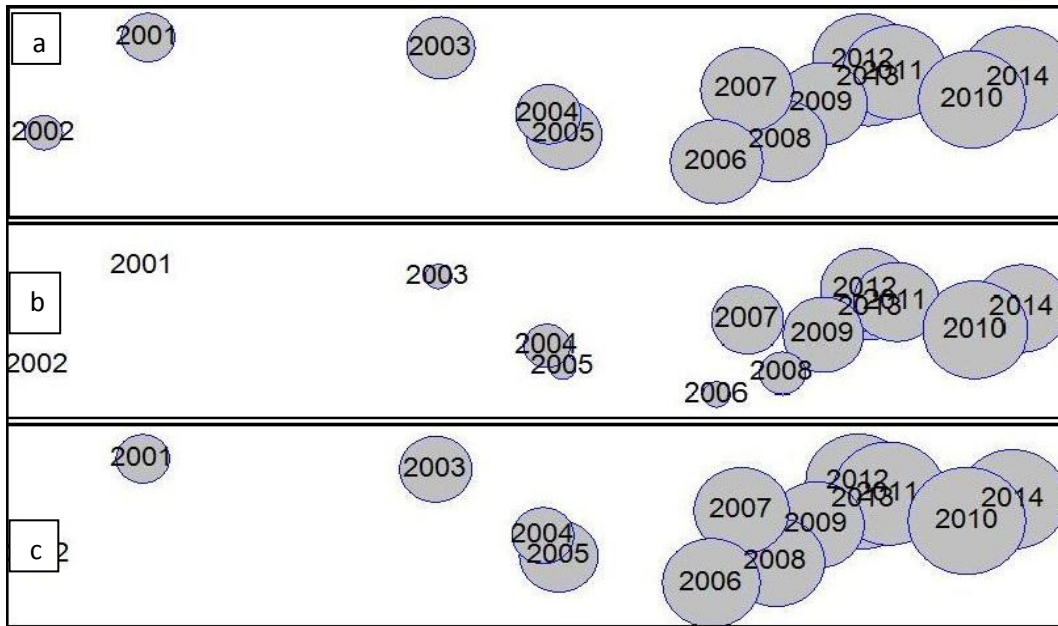


Fig. 33: MDS plot of similarity results for the main skin lesion prevalence over time. It appears that after 2007 the types of lesion are grouped better, a) for tooth rakes/scars, b) WFS, c) WL

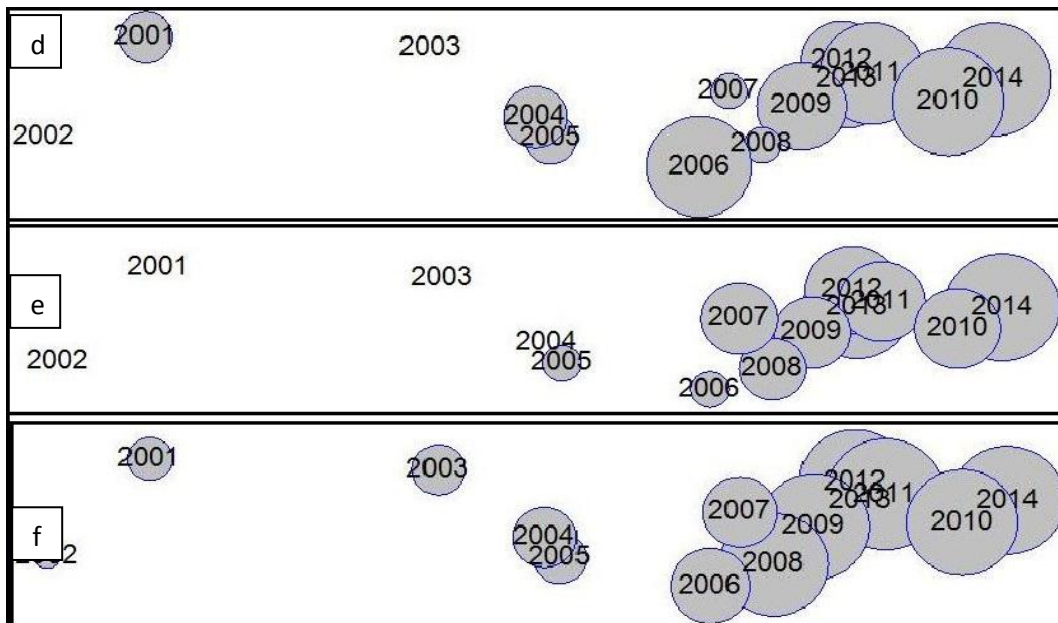


Fig. 34: MDS plot of similarity results for the main skin lesion prevalence over time. It appears that after 2007 the types of lesion are grouped better d) CL e) DBD, f) DFS

For this reason, further investigation of spatio-temporal trends of skin lesions and further statistical analysis were applied to the period 2007-14. Sample sizes ranged between 47-130 individuals. To display the trends and patterns graphically (Fig. 35), the proportions of the actual

numbers of abundance were used instead of averages. The p value of significance was taken as 0.05.

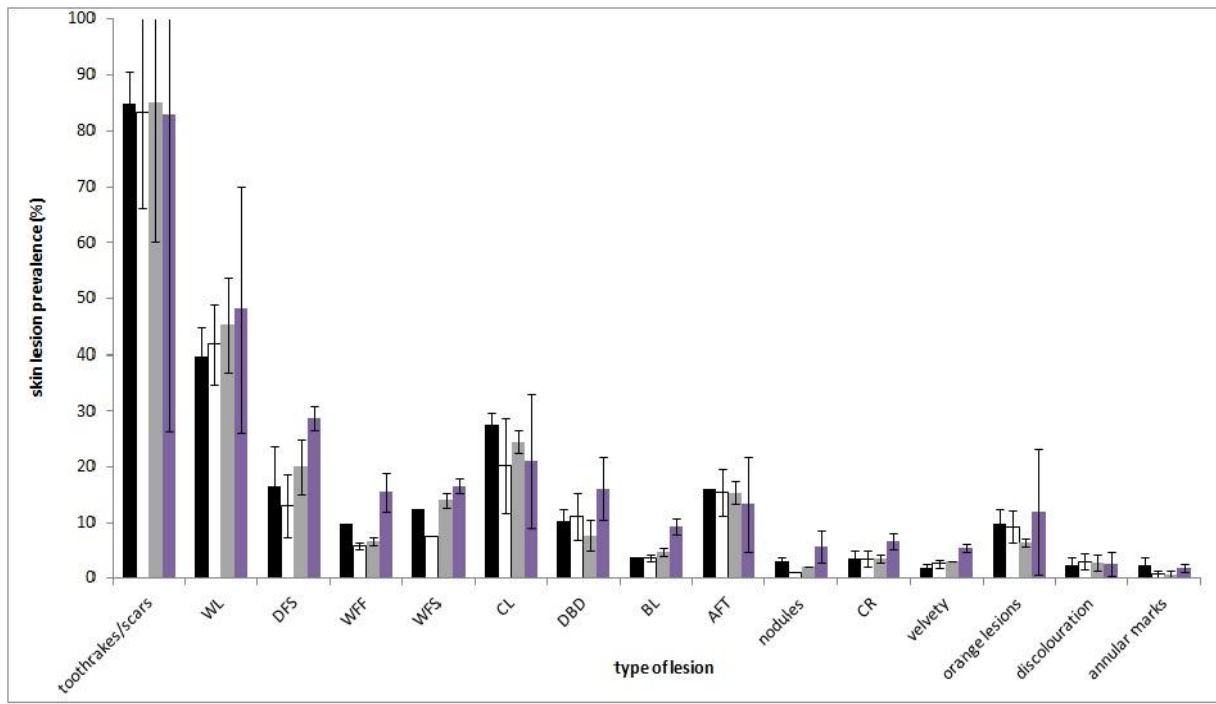
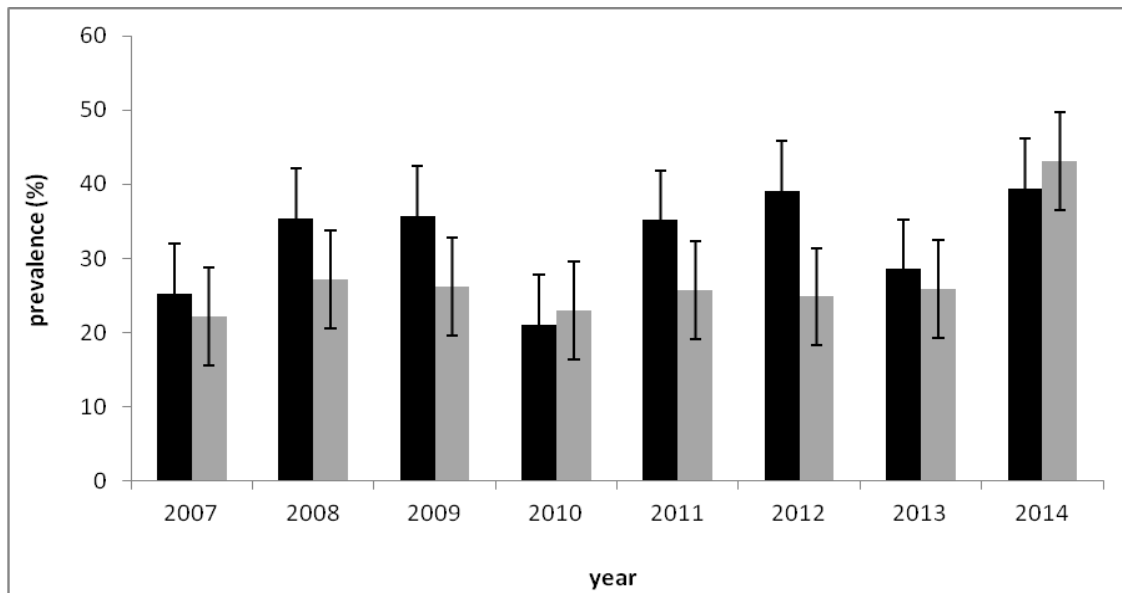


Fig. 35: The type of lesions and the averages proportion (%) of individuals affected by them for the period 2007-14. Averages were taken every two years (2007-08: purple, 2009-10: grey, 2011-12: white, 2013-14: black)

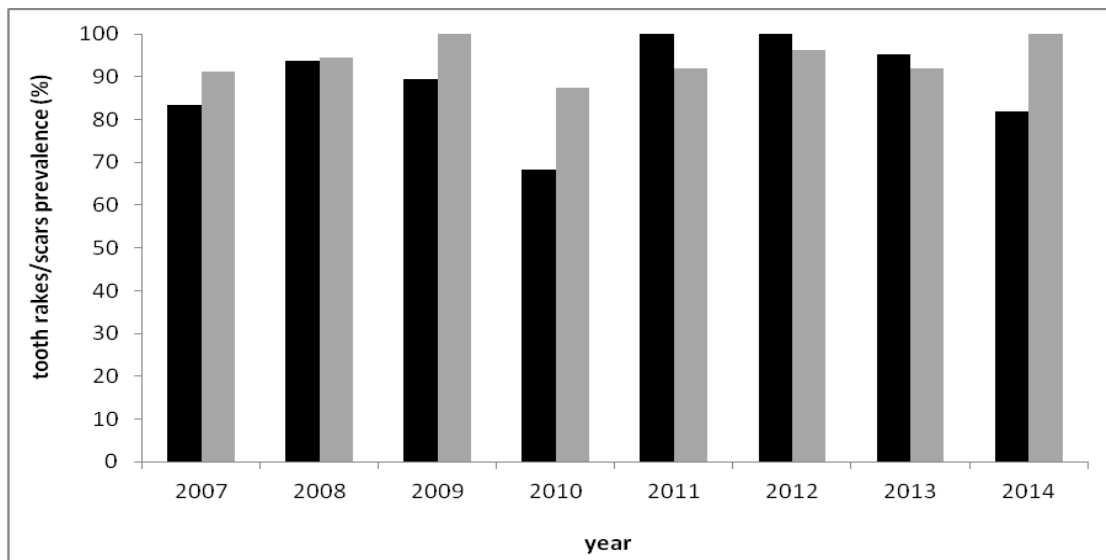
#### 4.4.1 Skin lesion prevalence in relation to gender

The average prevalence of all skin lesion types among the different sex classes did not appear to differ over time ANOVA  $F(1,14)=2.60$ ,  $p=0.129$ . (Fig. 23). In average, tooth rakes/scars (94,2%), WL (45.4%) and WFS (11.7%) were the lesion types with the higher prevalence ratios. Also, tooth rakes/scars (89%), AFT (44%) and CL (38.7%) were the lesion categories mainly observed on males. Skin lesions were more prevalent on females than males only in 2010 and 2014 of the eight years examined (Fig. 36).



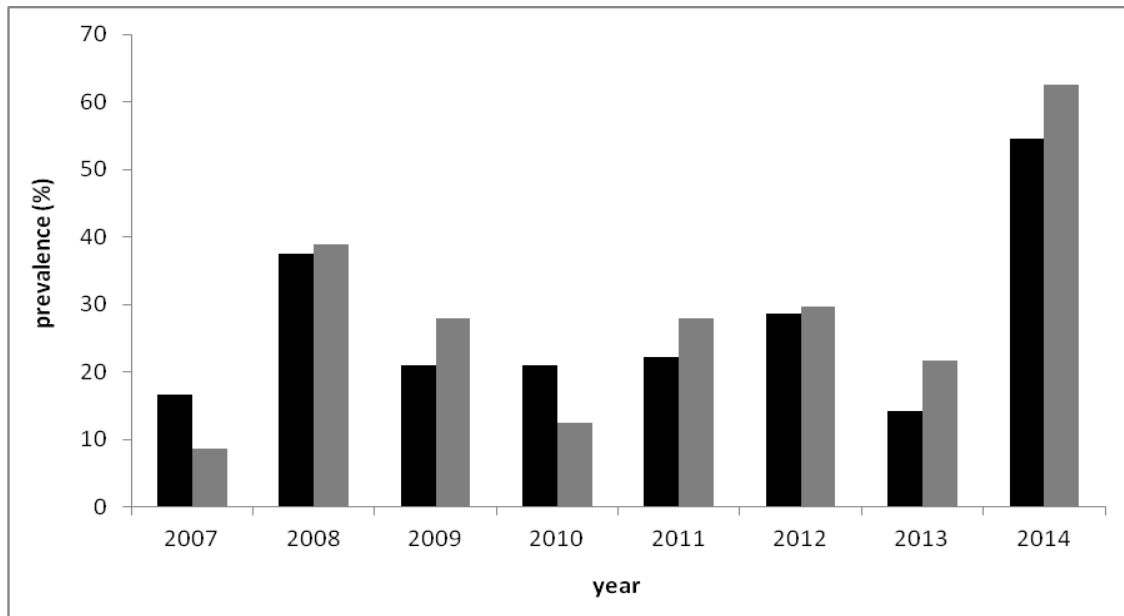
**Fig. 36: Average prevalence (%) of skin lesions (all types) over time, according to gender. The grey bars refer to females and the black bars to males (n= 84)**

Prevalence of tooth rakes/scars according to gender were 89.1% on males and 94.1% on females over the period 2007-14 (Fig. 37). However, the Kruskal-Wallis test indicated that tooth rakes/scars were not statistically different between the different sex groups  $\chi^2(1)=0.716$ ,  $p=0.397$  (Table 1). Tooth rakes/scars occurred more in males than females in three (2011 2012 and 2014) of the eight years.



**Fig. 37: Prevalence of tooth rakes/scars on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=78)**

Although DFS appeared to be the only type of skin lesion that expressed a general trend, no statistical significant differences were found in the prevalence between the two genders  $F(1,14)=0.04$ ,  $p=0.953$ . The prevalence of DFS on males and females over time appeared to decline, but after 2011 a rise was noted (Fig. 38). The rest of the skin lesion types did not exhibit any obvious trend in prevalence (see Figures in Appendix 2), but the prevalence of WL, WFS and AFT were significantly different between the different sexes (Table 1).



**Fig. 38: Prevalence of DFS on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=37)**

The average WL prevalence for the period 2007-14 was found to be higher in females (45.4%) than in males (31.7%) ( $p=0.02$ ), while the average AFT prevalence was higher in males (43.8%) than in females (3.3%) ( $p=0.001$ ). Similarly, the average prevalence of WFS in the same period was higher in males (28.9%) than in females (11.7%) ( $p=0.001$ ) (Table 1).

**Table 1: The ANOVA and Kruskal-Wallis results of important types of skin lesions found in the current study. The Tukey HSD post hoc test was used for ANOVA to detect where was the statistically significant differences and Mann Whitney U was used for the Kruskal-Wallis outcome, df=1. Transformation used: Fourth root, A value was set at 0.05, \* $\gamma$ =significant difference in prevalence among the different gender categories,  $\infty$ = no significance among groups (males; n=36, females; n=48).**

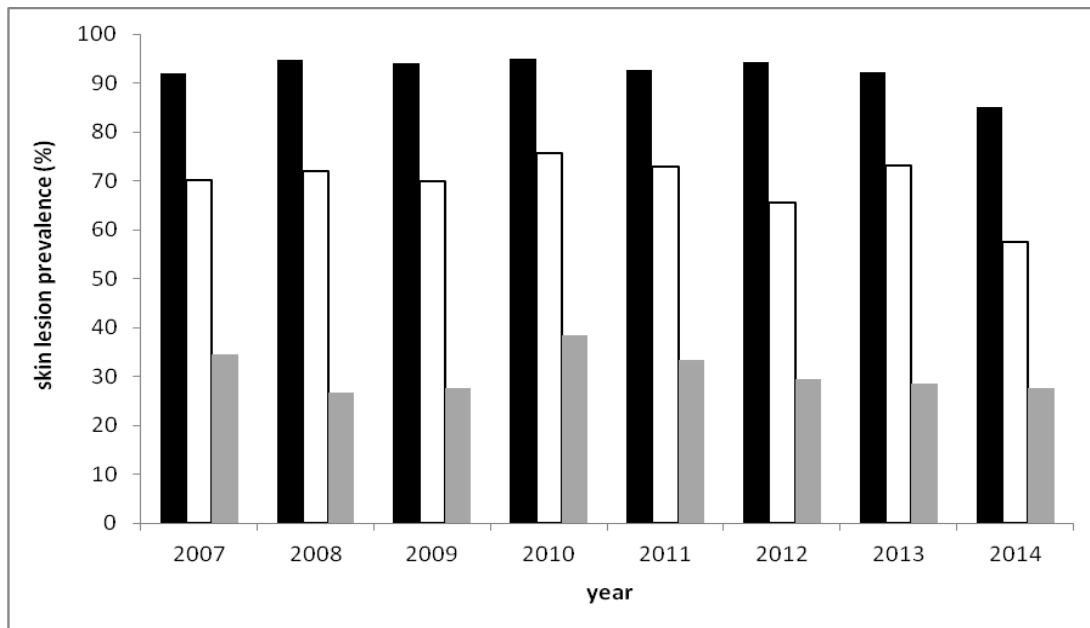
| Type of skin lesion | Males              | Females            | F/ $\chi^2$                       | P           | Pairwise Comparisons $\gamma^*$    |
|---------------------|--------------------|--------------------|-----------------------------------|-------------|------------------------------------|
|                     | Mean ( $\pm$ )SE   |                    |                                   |             |                                    |
| Tooth rakes/scars   | 0.97(0.02)         | 0.98 (0.01)        | $\chi^2= 1.66$                    | .397        | $\infty$                           |
| <b>WL</b>           | <b>0.74 (0.02)</b> | <b>0.81 (0.02)</b> | <b>F= 6.04</b>                    | <b>.028</b> | <b>higher in females (p= .002)</b> |
| DFS                 | 0.71 (0.03)        | 0.71 (0.11)        | F=.004                            | .083        | $\infty$                           |
| <b>WFS</b>          | <b>0.72 (0.02)</b> | <b>0.57 (0.02)</b> | <b>F=19.46</b>                    | <b>.001</b> | <b>higher in males (p= .001)</b>   |
| DBD                 | 0.49 (0.07)        | 0.63 (0.02)        | F= 3.25                           | .093        | $\infty$                           |
| CL                  | .76 (0.04)         | 0.07 (0.02)        | F=.506                            | .488        | $\infty$                           |
| WFF                 | 0.63 (0.03)        | 0.47 (0.08)        | F=3.49                            | .083        | $\infty$                           |
| BL                  | 0.34 (0.1)         | 0.25 (0.1)         | F= .42                            | .526        | $\infty$                           |
| <b>AFT</b>          | <b>0.8 (0.02)</b>  | <b>0.25 (0.09)</b> | <b><math>\chi^2= 33.08</math></b> | <b>.001</b> | <b>higher in males (p= .000)</b>   |

#### 4.4.2 Skin lesion prevalence in relation to body area affected

The different types of skin lesions were found to occur over three main body areas: dorsal fin, back and flanks. Some other body areas were also found to be affected by epidermal lesions. These included the head, flippers, tail, and in some cases the entire body. However, due to their low sample sizes, statistical analysis and plots of trends were applied only for the main three body areas, and the most common skin lesion categories.

The dorsal fin was the area where skin lesions were most prevalent, with 92.5% of of the individuals over time, while the back and flanks were 69.6% and 30.8% respectively (n=254). (Fig. 39).





**Fig. 39: Prevalence (%) of skin lesions on the dorsal fin (black), back (white) and flanks (grey) for the period 2001-14 (n=254)**

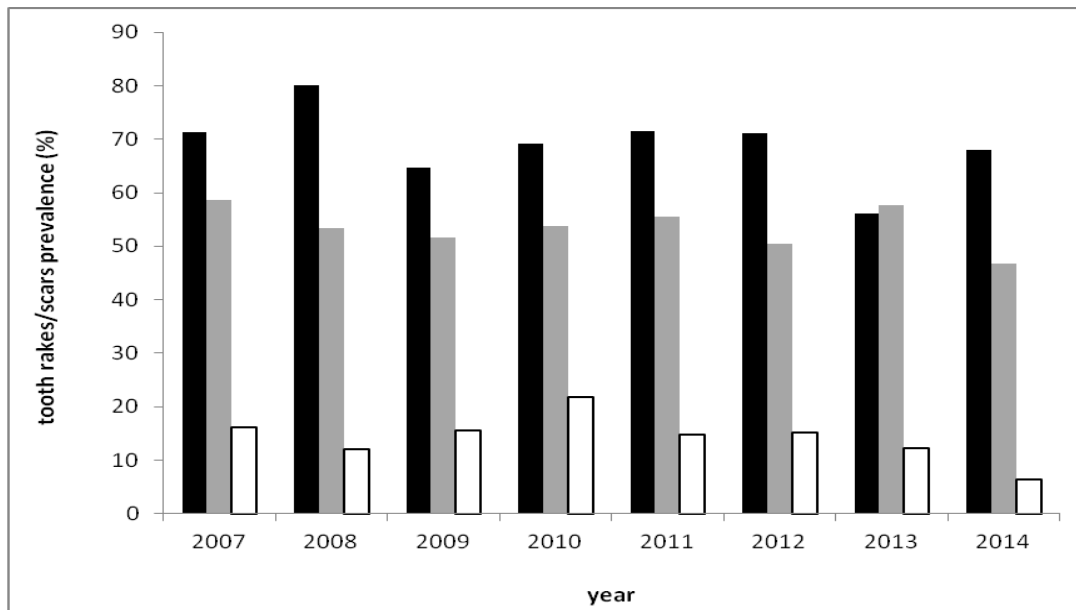
Also, in the first run of the statistical analysis, four categories of body areas were included. The categories were “dorsal fin”, “back”, “side” and “other”. The last category consisted of data referring to other body areas such as the snout, the flippers and tail of the individuals but lesions in these areas were rare, and sample sizes were insufficient for them to stand as separate categories. The result of the initial analysis showed that the data for the fourth category was also insufficient, and to avoid outliers and bias, the category was omitted and a one-way ANOVA and Kruskal-Wallis test were conducted again. Tukey post hoc analysis and Mann Whitney U tests were applied to investigate the pair wise comparisons (Table 2).

The prevalence of all skin lesion categories apart from DBD, BL and WFS, were found not to be significantly different between the different body areas (Table 2).

**Table 2: The ANOVA and Kruskal-Wallis results of important types of skin lesions found in current study. The Tukey HSD post hoc test was used for ANOVA to detect where was the statistically significant differences,  $df=2$ . Transformation used: Fourth root,  $\alpha$  value was set at 0.05,  $\gamma$ =significant difference among the different body areas categories,  $\varnothing$ = no significance among groups (n=254)**

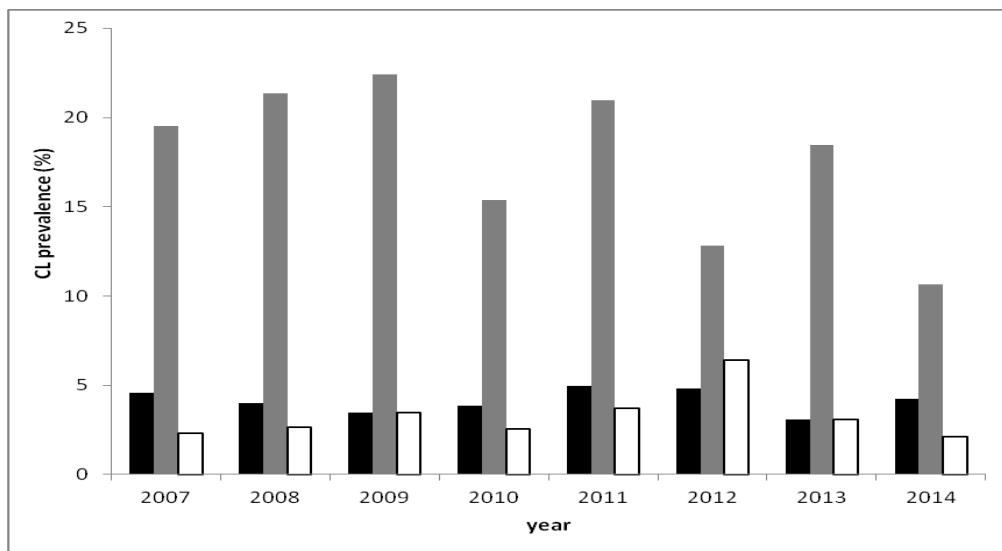
| Type of skin lesion | Dorsal Fin         | Back               | Flanks             | F              | P           | Pairwise Comparisons $\gamma$  |
|---------------------|--------------------|--------------------|--------------------|----------------|-------------|--|
|                     | Mean ( $\pm$ )SE   |                    |                    |                |             |  |
| Tooth rakes/scars   | <b>0.91 (0.08)</b> | <b>0.85 (0.05)</b> | <b>0.61 (0.02)</b> | <b>17.56</b>   | <b>.000</b> | <b>Prevalence higher on: dorsal fin than flanks (p= .000) &amp; back (p= .009), back than flanks (p= .000)</b> |
| WL                  | <b>0.77 (0.01)</b> | <b>0.56 (0.01)</b> | <b>0.46 (0.01)</b> | <b>3.33</b>    | <b>.000</b> | <b>higher on: dorsal fin than back (p= .000) &amp; flanks (p= .000), back than flanks (p= .000)</b>            |
| DFS                 | <b>0.52 (0.01)</b> | <b>0.56 (0.02)</b> | <b>0.43 (0.03)</b> | <b>7.28</b>    | <b>.004</b> | <b>Lower on flanks than dorsal fin (p= .047) &amp; back (p= .003)</b>  |
| WFS                 | 0.46 (0.02)        | 0.44 (0.04)        | 0.42 (0.06)        | 0.182          | .835        | $\varnothing$  |
| DBD                 | 0.37 (0.05)        | 0.43 (0.03)        | 0.45 (0.02)        | 1.55           | 0.335       | $\varnothing$  |
| CL                  | <b>0.85 (0.20)</b> | <b>2.03 (0.23)</b> | <b>0.48 (0.24)</b> | <b>F=13.26</b> | <b>.000</b> | <b>Higher on the back than-dorsal fin (p= .000) &amp; side (p= .000)</b>                                       |
| BL                  | 0.36 (0.05)        | 0.33 (0.05)        | 0.17 (0.06)        | 3.06           | .068        | $\varnothing$  |

Over time, the prevalence of tooth rakes/scars differed significantly between the different body areas tested  $F(2,21)=8.72$ ,  $p=0.001$  (Table 2), being more prevalent on the dorsal fin (73%) followed by the back (53.5%) and flanks (14.2%) (Fig. 40).



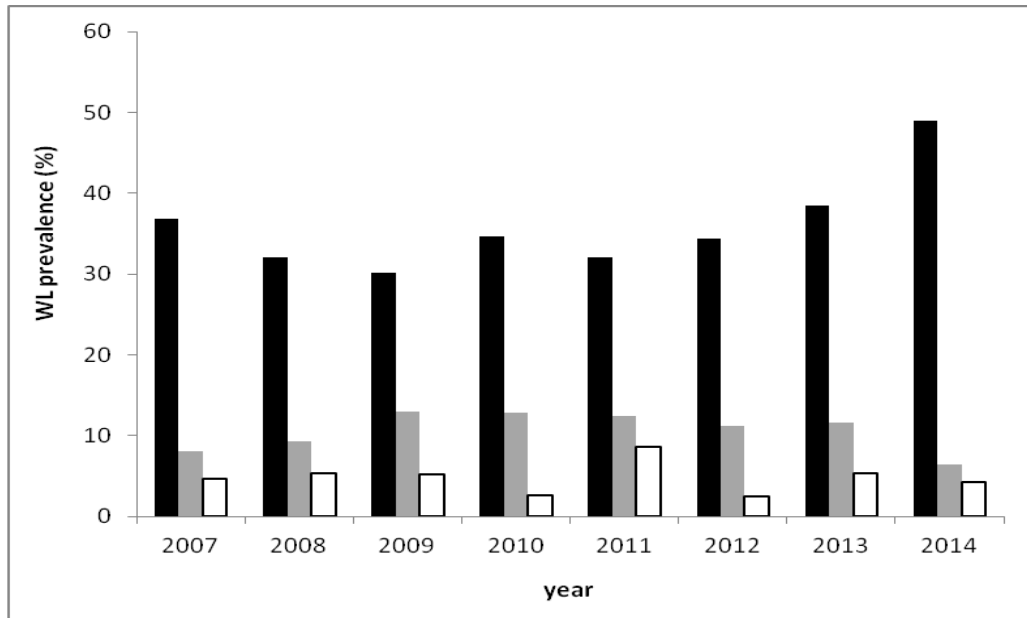
**Fig. 40: Prevalence (%) of tooth rakes & scars over time on the dorsal fin (black), back (grey) and flanks (white) for the period 2007-14 (n=213)**

The prevalence of CL was also statistically significantly different  $F(2,21)=13.26$ ,  $p=0.001$  (Table 2). The dorsal fin was affected on 4.1% of the individuals, while the back and the flanks was affected on 17.7% and 3.3% of individuals respectively. The prevalence on the back was also higher than on the rest of the body in previous years (Fig. 41). The CL lesions were mostly detected on the back, followed by the dorsal fin (Table 2).



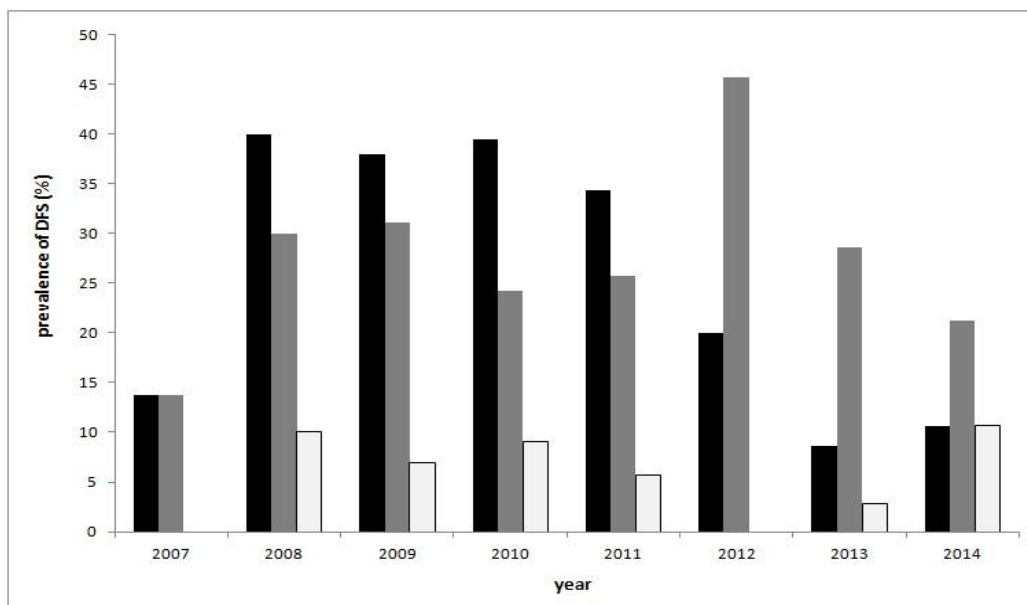
**Fig. 41: Prevalence (%) of CL on the dorsal fin (black), back (grey) and flanks (white) for the period 2007-14 (n=52)**

In all eight years, WL lesions were significantly different between the three main body areas tested  $F(2,21)$ ,  $p=0.001$  (Table 2) and more common on the dorsal fin than any other body area (Fig. 42, Table 2).



**Fig. 42: Prevalence (%) of WL on the dorsal fin (black), back (grey) and flanks (white) for the period 2007-14 (n=91)**

The DFS lesions were also found mainly on the back and dorsal fin (Fig. 43), and their prevalence was significantly different between three body areas,  $F(2,21)=7.28$ ,  $p=0.004$  (Table 2).



**Fig. 43: Prevalence (%) of DFS on the dorsal fin (black), back (grey) and flanks (white) for the period 2007-14 (n=41)**

### 4.4.3 Skin lesion prevalence in relation to age

The majority of the individuals with skin lesions were adults (n=173), in all eight years, although sample sizes for calves and juveniles were generally lower than adults and especially for juveniles. For this reason, they were considered as one group (n=51) (Fig. 44). WFF and AFT lesions were in fact absent from calves in all years examined. For this reason, they were omitted from the statistical analysis.

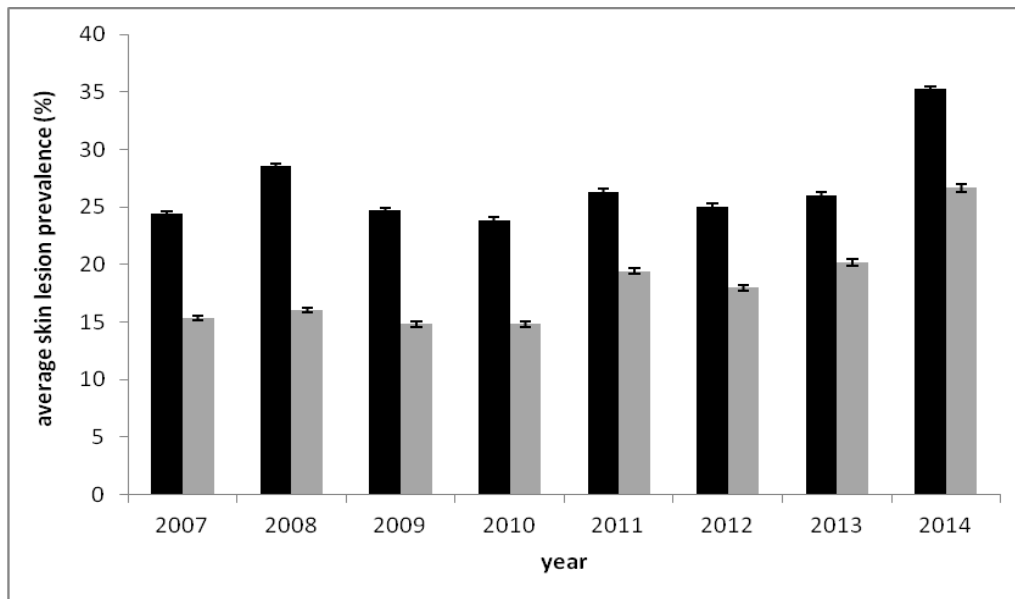
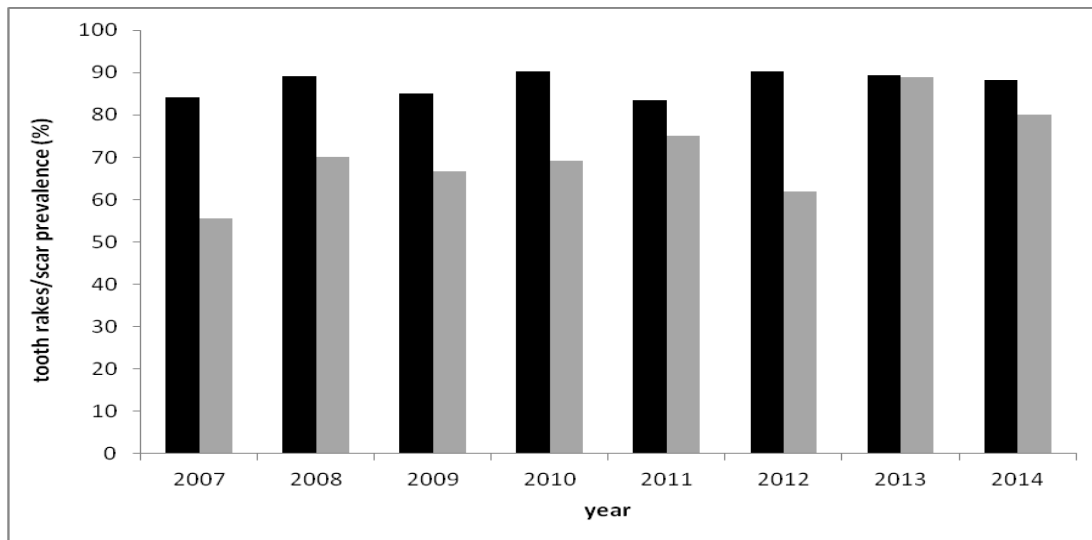


Fig. 44: Average prevalence of all skin lesion types on adults (black) and calves (grey) for the period 2007-14 (n=225)

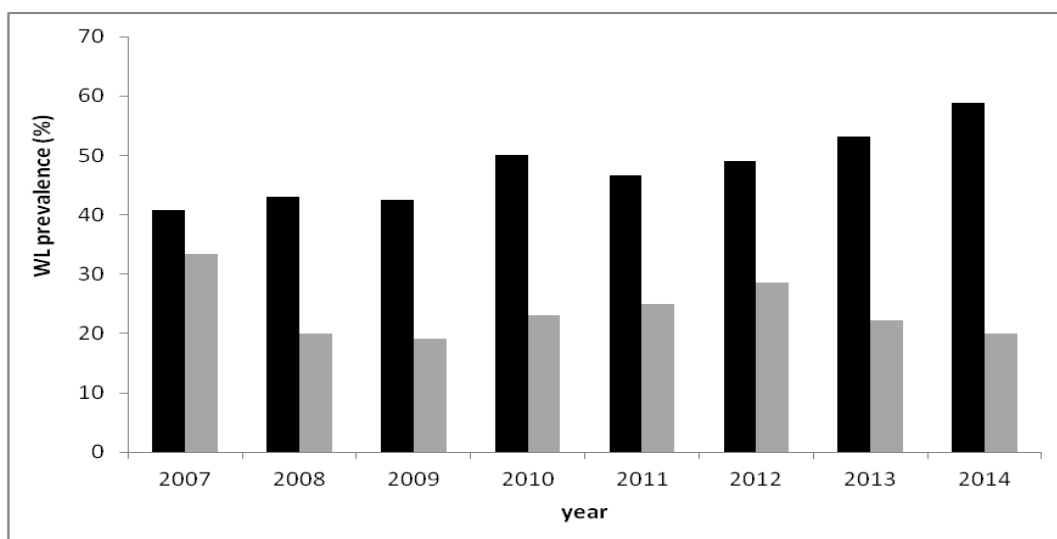
The prevalence of the average skin lesions was found to significantly differ between the two main age groups (ANOVA  $F(1,14)=22.63$ ,  $p=0.001$ ). The tooth rakes/scars did not show any particular trend over time apart from being constantly at a high ratio each year in both age groups (Fig. 45). However, the prevalence of them was significantly different between the different age classes ( $\chi^2(1)=8.67$ ,  $p=0.003$ ; Table 3).



**Fig. 45: Prevalence (%) of tooth rakes/scars on adults (black) and calves (grey) (n=193)**

The Spearman coefficient correlation showed a strong positive correlation between tooth rakes/scars and WL ( $r_s=0.671$ ), WFF ( $r_s=0.599$ ), WFS ( $r_s=0.606$ ), BL ( $r_s=0.510$ ), AFT ( $r_s=0.663$ ) and between WFS and WFF ( $r_s=0.648$ ), AFT ( $r_s=0.772$ ), BL ( $r_s=0.592$ ). Also strong positive correlation was observed between AFT and WFF ( $r_s=0.661$ ) and BL ( $r_s=0.507$ ).

In general, WL prevalence appeared to increase on adults over the period 2007-14 but decline on calves (Fig. 46). The one way ANOVA indicated that WL prevalence was significantly different between the two age groups  $F(1,14)=78.1$ ,  $p=0.001$  (Table 3)



**Fig. 46: Prevalence (%)WL on adults (black) and calves (grey) for the period 2007-14 (n=80)**

Although prevalence of DFS, WFS and BL were significantly different between the age classes (Table 3), they were also observed to be absent on calves for three, five and six years

respectively (see Figures in Appendix 3). The WFS were more prevalent on adults than calves). WFS were present on calves only in 2010, 2012 and 2013 (Fig. 47). The other main skin lesion types did not exhibit any other obvious pattern in prevalence dependent on age (see Appendix 3).

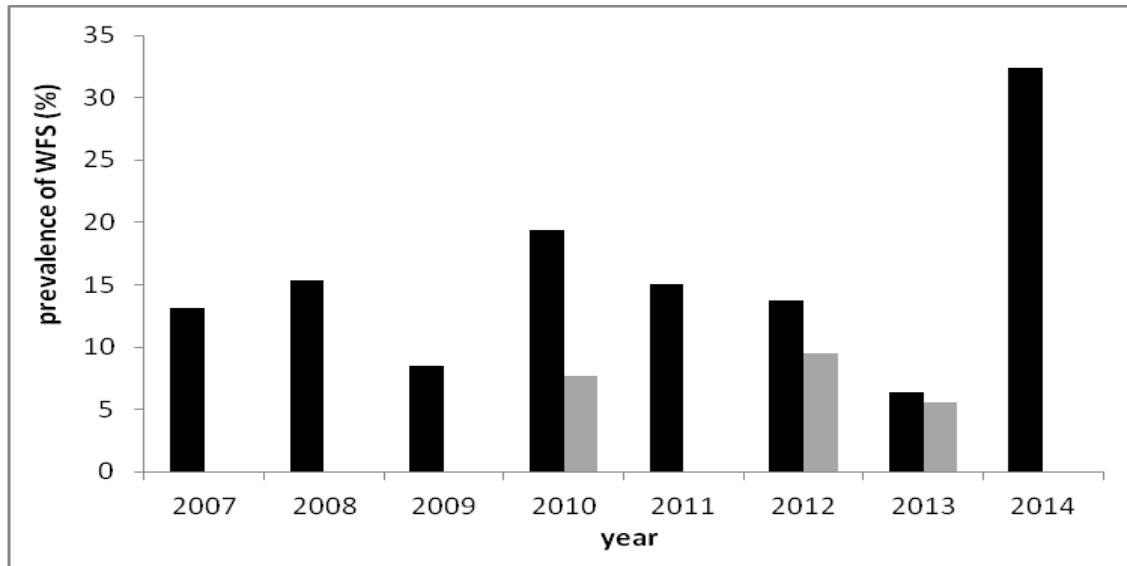


Fig. 47: Prevalence (%) of WFS on adults (black) and calves (grey) for the period 2007-14 (n=25)

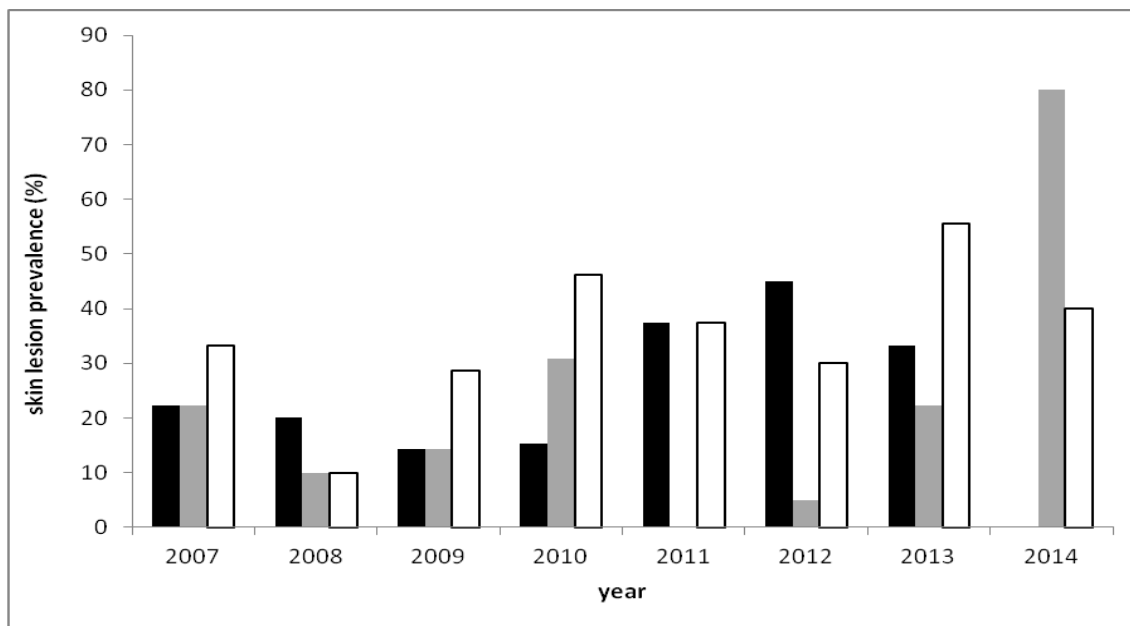
Table 3: The ANOVA results of important types of skin lesions found in current study. Adults; n=173 and calves; n=51. *df*=1. Transformation used: Fourth root, A value was set at 0.05

| Type of skin lesion | Adults           | Calves      | F/ $\chi^2$    | P     |
|---------------------|------------------|-------------|----------------|-------|
|                     | Mean ( $\pm$ )SE |             |                |       |
| Tooth rakes/scars   | 0.96 (0.03)      | 0.91 (0.01) | $\chi^2= 8.67$ | .003. |
| WL                  | 0.83 (0.04)      | 0.69 (0.01) | F= 78.1        | .000  |
| DFS                 | 0.67 (0.02)      | 0.39 (0.12) | $\chi^2= 4.97$ | .043  |
| DBD                 | 0.59 (0.01)      | 0.59 (0.09) | F= 0.29        | .594  |
| CL                  | 0.68 (0.24)      | 0.62 (0.09) | F= 0.42        | .529  |
| WFS                 | 0.61 (0.02)      | 0.19 (0.09) | $\chi^2=17.73$ | .001  |
| BL                  | 0.43 (0.06)      | 0.11 (0.22) | F= 9.48-       | .000  |

Additionally, focusing only upon skin lesion types that affected calves over time, it was observed that different types, such as the OL (orange lesions) were affecting them more compared to adults (Fig. 48). In conclusion, the main types of skin lesions observed in high prevalence on calves were the tooth rakes/scars (62.7%), OL (35.1%), CL (23.5%), DBD (23.1%), and WL (22.4%) (Figs. 49-52).

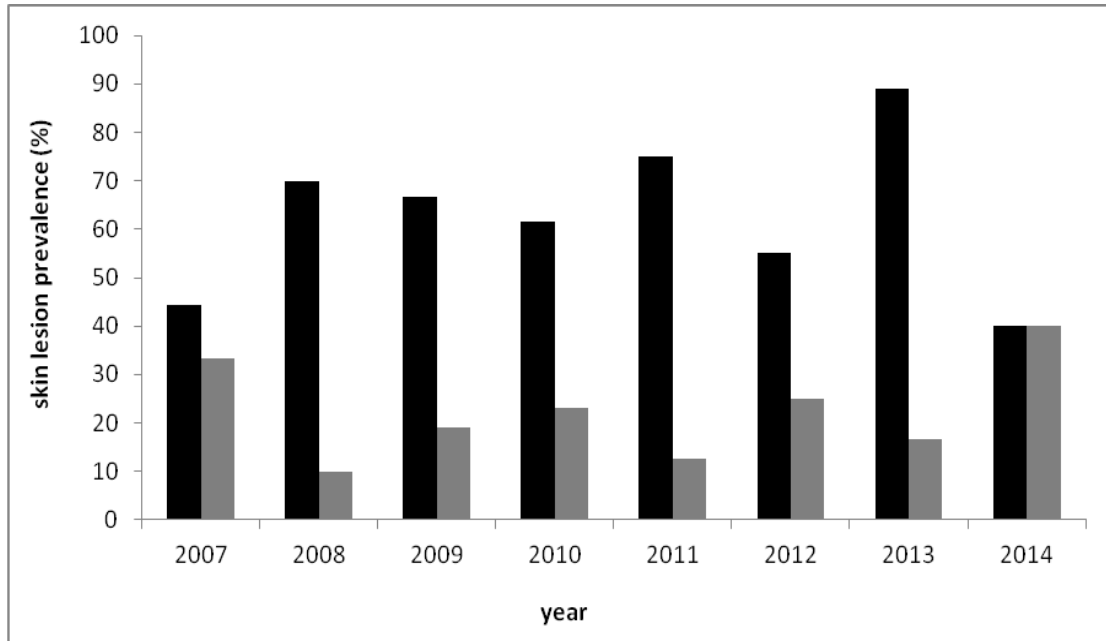


**Fig. 48:** The calf 091-08s suffering from OL. Cardigan Bay SAC, 2013. The extent of OL covered almost all the body (photo: © Sea Watch Foundation, 2013)



**Fig. 49:** Prevalence (%) of CL (black), DBD (grey) and OL (white) on calves for the period 2007-14 (n=51)





**Fig. 50: Prevalence (%) of tooth rakes/scars (black) and WL (grey) on calves for the period 2007-14 (n=51)**

The Spearman coefficient correlation showed a strong positive correlation between tooth rakes/scars and WL ( $r_s=0.905$ ), and between CL and DBD ( $r_s=0.707$ ) (Fig 51).



**Fig. 51 : The calf of 133-03S covered in DBD, and partially OL. Photo taken in Cardigan Bay SAC in July 2009 (photo: © Sea Watch Foundation, 2009)**



**Fig. 52: The calf of the individual 069-01s that was observed in Cardigan Bay SAC in 2009 was covered in CL (photo: ©Sea Watch Foundation, 2009)**

#### **4.5 Residency of individuals in relation to skin lesion prevalence**

In order to test whether the prevalence of skin lesions depended upon residency to a particular region, individuals that had been observed only in Anglesey, in Cardigan Bay SAC, and Pen Llyn a'r Sarnau SAC were investigated for skin lesions. For the period 2007-14, 23 individuals were observed to use only Cardigan Bay SAC as their home range, 17 individuals only Anglesey and only five individuals were found to use solely the Pen Llyn a'r Sarnau SAC. Due to the fact that the sample size of the dolphins using only the region of Pn llyn a'r Sarnau SAC was smaller than for the other two regions, five individuals from each region were chosen randomly for statistical analysis.

A  $\chi^2$  test of independence was applied to examine the relationship between the average skin lesion prevalence of all types of skin lesions and the different home ranges. The result showed that the relationship between these variables was not significant ( $\chi^2(4, N=15)=6, p=0.188$ ). The same test was applied for individuals recorded only in the Anglesey area and only in Cardigan Bay SAC, using a larger sample size ( $n=34$ ). The individuals again were chosen randomly. Similarly, the  $\chi^2$  test of independence found no significant association between the average prevalence of skin lesions and individuals from the different regions ( $\chi^2(1, N=34)=2, p=0.317$ ).

## **4.6 Skin lesion prevalence in relation to resident, visitors and transient individuals**

The prevalence of the different types of skin lesions was also determined in relation to whether the individuals were considered to be residents, transients, or occasional visitors. Residents were defined as individuals seen over 12 times, occasional visitors as individuals seen 4 to 11 times, and transients as those encountered less than four times (Feingold & Evans, 2012). In this study, all three different types of individuals were found. The residents comprised 61.7% (129/209), visitors 30.1% (63/209), and transients 6.7% (14/209).

For sample size homogeneity and in order to check statistically for significant differences in skin lesion prevalence between the different groups of individuals, sub-samples were randomly taken from each group. The Kruskal Wallis H test indicated that the average prevalence of skin lesions between resident, visitors and transient individuals were not significantly different ( $\chi^2(2)=2$ ,  $p=0.368$ ).

## **4.7 Sea Surface Temperature**

Sea surface temperature (SST) data for the study area was obtained from the British Oceanographic Data Centre (BODC) and NOAA. Due to the seasonality of occurrence of the species in Cardigan Bay, the sampling period was confined to April to September in the years 2007-14 (Fig. 36). Average SST values for those months were tested separately for correlations with the average prevalence of all skin lesions of each year. The maximum value of SST was 15.8°C in August 2008 and the minimum value was 8.0°C in April 2008.

A  $\chi^2$  test of independence was performed to examine the relationship between the average skin lesion prevalence of all skin lesion types and the monthly average SST values over time. The results showed that the relationship between these variables was not significant for any month except June ( $\chi^2(1, N=8)=56.0$ ,  $df=49$ ,  $p=0.026$ ).

## **4.8 Skin lesion extent**

In some cases, the pattern of skin disorders was noticeable when analyzing photos taken in subsequent years. A first attempt was made to examine qualitatively the extent of skin lesions, and a small number of individuals are depicted in a sequence of photos below, showing the

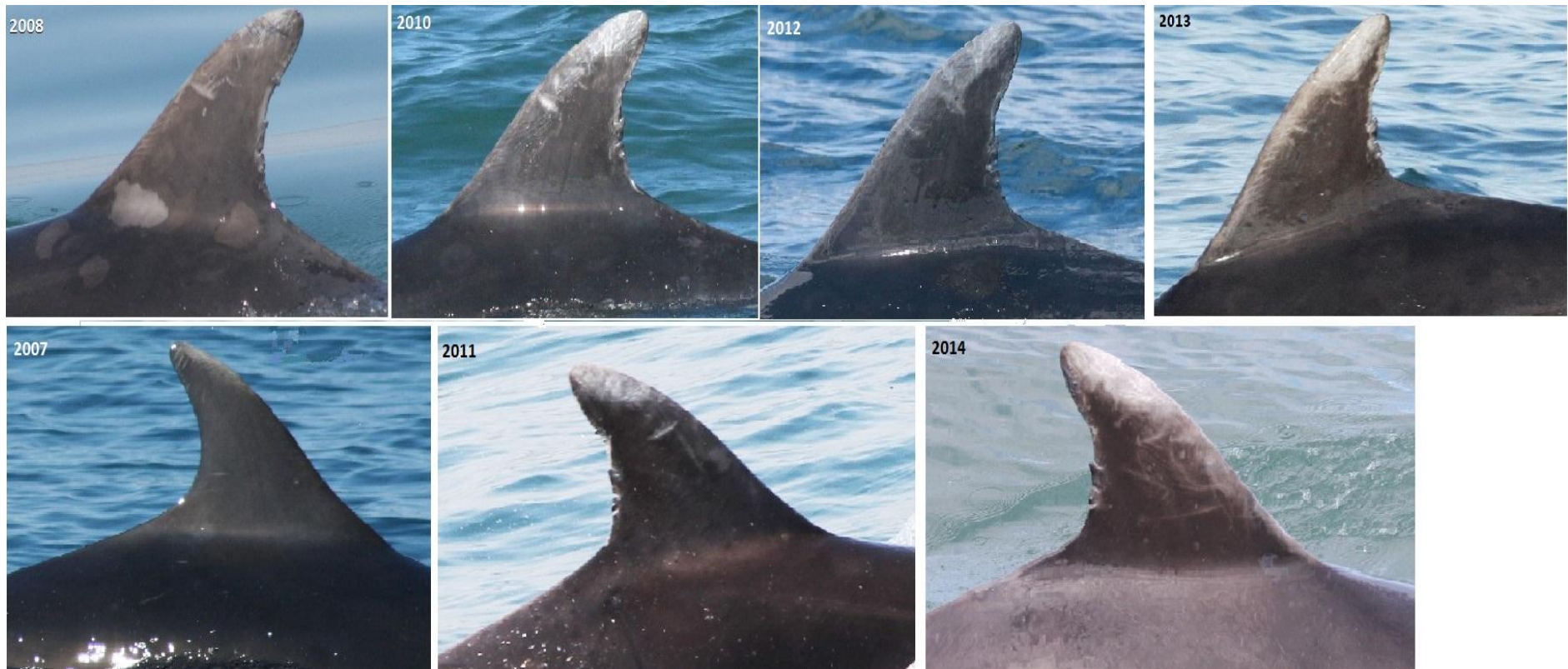
development of their lesions over time. The animals chosen, had a wide home range (including Anglesey, the Llyn Peninsula, and Cardigan Bay SAC) rather than any particular area. Those dolphins were:

- 194-07w. This individual, exhibited fin discoloration. Over time, the intensity of the discoloration and extent did not show a particular pattern apart from swapping over the years (Fig. 53).
- Voldermort (023-03w). Voldermort, exhibited mostly WL and AFT. However, DFS, DBD and WFS were also observed over time on the dorsal fin and flanks of both sides. In contrast to the WL no certain trend of presence/absence of DFS, DBD and WFS was observed over time (Fig. 54)
- 003-01 (Checkmate). The main skin lesions observed on the dorsal fin of Checkmate were the tooth rakes/scars. Additionally, OL in 2008 were observed but over time appeared to subside. The photo makes it easy to see that the extent and intensity of the tooth rakes increased with time, and possibly led to the de-pigmentation on the tip of the dorsal fin (Fig. 55)
- Smoothy (017-03w). Finally, Smoothy was identified in the photos to suffer from WFF and tooth rakes/scars on the dorsal fin. However, it seemed that there were no noticeable changes on the intensity or extent of WFF over time. (Fig. 56)



o

**Fig. 53: Display of photos taken in sequential years, showing the development and pattern of the skin discoloration located on the dorsal fin of the resident individual with the code name 194-07w. (Source: © Sea Watch Foundation 2008-2012, © Elena Akritopoulou/Sea Watch Foundation 2014)**



**Fig. 54: Display of Voldermort (023-03w) in photos taken in sequential years showing the development and pattern of the WL located on the left side of the dorsal fin and flank. Also the development of the AFT over time is obvious on both sides. Note that in 2007 only tooth rakes and de-pigmentation are shown on the tip of the fin (Source: © Sea Watch Foundation 2008-2013, © Elena Akritopoulou/Sea Watch Foundation 2014)**



**Fig. 55: Display of Checkmate (003-07R) in photos taken in sequential years showing the development and pattern of the tooth rakes located on the tip of the dorsal fin. Also in 2008 among others, the development of the OL and WFS is noted. Over time, the type of skin lesions which affected Checkmate seemed to either swap in other types or become more intense (Source: © Sea Watch Foundation, 2008-2013)**



**Fig. 56: Smoothy (017-03w) developed WFF over time. However, the extent or intensity of it seemed unchanged each year. Also, the tooth rakes shown on the dorsal fin in 2006 seemed to be in the same state over time (Source: © Sea Watch Foundation, 2006-2013)**



## 5. Discussion

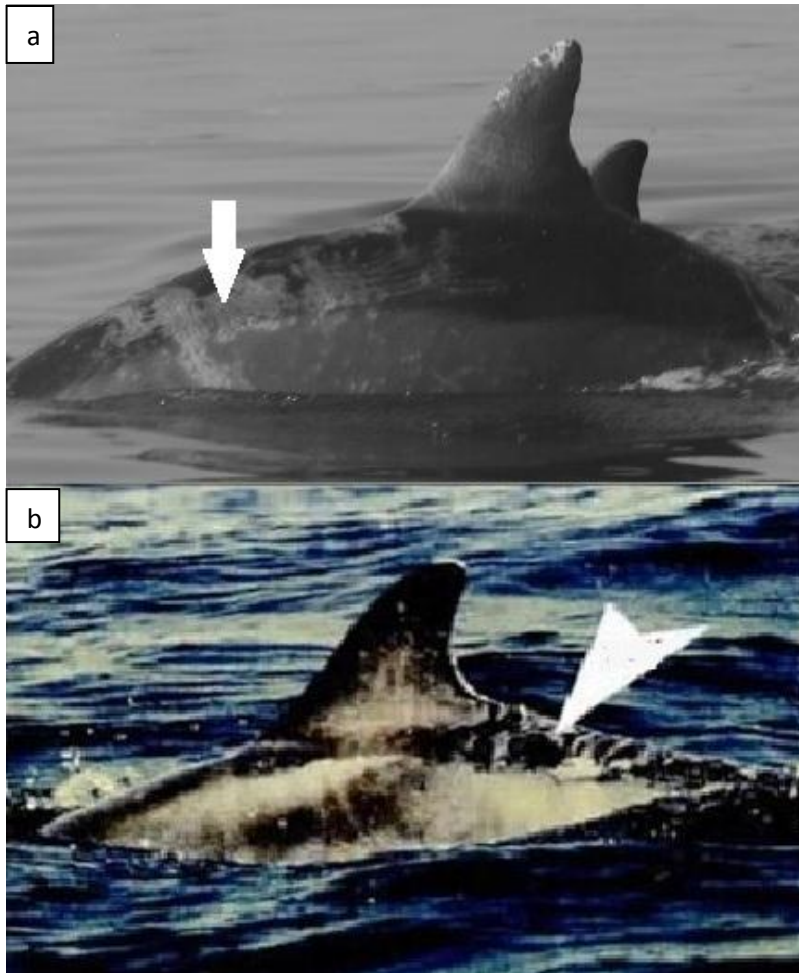
Previous studies indicated that prevalence of skin lesions observed in wild bottlenose dolphin populations worldwide is relatively high between 63% and 100% (Harzen & Brunnick, 1997; Wilson *et al.*, 1999a; Bearzi *et al.*, 2009; Rowe *et al.*, 2010; Maldini *et al.*, 2010). In the UK, the first to describe skin lesion types in bottlenose dolphins were Thompson and Hammond (1992) in the Moray Firth (Scotland). Wilson *et al.* (1997) further studied this population, classifying skin lesions and creating a baseline for a number of other studies related to skin lesions. Magileviciute (2006) examined the social networks of Welsh bottlenose dolphins and used Wilson *et al.*'s. (1997) skin lesion classification to conduct a preliminary study on skin lesions. She found that 61% of the population was affected by lesions. The present study has focused upon spatio-temporal patterns of skin lesions in the Welsh bottlenose population over the period 2001-14, using photo-ID techniques.

Photographic equipment (underwater and digital SLR autofocus cameras, and associated software packages) and methodologies (photo-ID and skin sampling when possible) have improved over time, providing a greater availability of more detailed photographic information (Thompson & Hammond 1992; Harzen & Brunnick 1997; Wilson *et al.*, 1997; Van Bresseem *et al.*, 2003; Magileviciute, 2006; Kiszka *et al.*, 2009; Maldini *et al.*, 2010; Hart *et al.*, 2012). In Cardigan Bay, digital photography started to be used as a tool for photo-ID techniques from 2006 onwards, the earlier images being available for analysis only as scans. This may explain why in this current study, relatively few images were used from earlier years and why the data were better clustered after 2006-07

In order to classify the skin lesions, a review of the existing literature was first made along with personal communication with some of the authors (for example Marie Françoise Van Bresseem, Ben Wilson). The lesion categories were defined by a combination of observations made in the field and photo analysis. However, as shown in §3.3.2, there is a possibility that the skin lesion categories exhibited in this study may be fewer than the actual number affecting the Welsh dolphins. Additionally, there is great variability in skin lesion categories among different studies. Based upon visual observations, the results of the current study followed this pattern.

Wilson *et al.* (1992), describes lunar lesions as a black, grayish and white skin lesions where their “surface are extensive, uneven, raised and pitted and gave the skin an appearance of

corroded aluminum”, while Flach *et al.* (2008), describes the regressing form of white velvety lesions as “ulcerated and cicatrized lesions, which while healing lost their velvety aspect and turned progressively light and dark gray”. Due to the fact that different authors described similarly those different skin lesions and the photos taken in different cases appeared also similar (Fig.57), the velvety lesions reflected lunar lesions in this study.



**Fig. 57: a) velvety lesions and b) lunar lesions (modified photos from: a: from Van Bressema *et al.*, 2008d; b: Wilson *et al.*, 1997)**

Previous studies considered tooth rakes/scars as a skin lesion category (Thompson & Hammond, 1992; Harzen & Brunnick, 1997; Wilson *et al.*, 1999a; Bearzi *et al.*, 2009; Rowe *et al.*, 2010; Maldini *et al.*, 2010). They were also included in this study due to the fact that they can provide access into the skin for a number of pathogens (such as *Vibrio* spp., viruses or parasites) allowing them to invade (Van Bressema *et al.*, 2008d), and to create lesions, but it would also explain how different types of lesions could be found at different places on the dorsal fin rather than all over the body like a rash (B. Wilson *pers. comm.*).

The prevalence of tooth rakes/scars in the current study was the highest recorded for the Welsh individuals over time. The reason for this could be attributed to the highly social interaction and behaviour of the species, but may also explain why they were spotted mostly on the dorsal fin and back (Schulman & Lipscomb, 1999, Moore & Newman, 2000). However, in many cases tooth rakes and AFT co-existed on the dorsal fin at the same point and they also showed a statistically significant association. As an example, Voldermort (023-03w) was observed over time to develop AFT on the tip of the dorsal fin, where biting frequency tends to be high. This might explain the correlation found between AFT and tooth rakes/scars

The assessment of the etiology of skin lesion prevalence is not easily accomplished without tissue sampling, but even then, to detect the pathogens causing them is rarely achievable. For example, Wilson *et al.* (1997; 1999; 2000), working on the Moray Firth bottlenose dolphin population attempted several times to obtain tissue samples from stranded individuals. However, by the time the lesions were visible, the pathogen was not detectable. The only exception to that were lesions derived by pox-viruses and Lobomycosis infections (B. Wilson *pers. comm.*). Hart *et al* (2012) isolated pox-viruses from DFS lesions and delphinid herpesvirus 3 from WFS lesions in bottlenose dolphins from the Northwest Atlantic. Both types of lesions were also detected in 2014 in the Welsh bottlenose dolphin population, and were found among the main skin lesion types that were significantly associated with one another. Although the reason for this association is uncertain, combined with the above findings, it could raise concern for the health status of the Welsh population of bottlenose dolphins. The fact that the etiology of none of the lesion types of this study is known can only lead to assumptions or comparisons with results derived from testing the different variables.

Around one-third of individuals were covered by more than three different types of skin lesions. Consequently, the need for more systematic and skin lesion centered research is needed, in order to assess sufficiently the population dynamics of the species and contribute to determining its sustainability. This applies especially in the light of possible declines in the numbers of individuals using Cardigan Bay SAC (Feingold & Evans, 2014).

## **5.1 Skin lesion prevalence in relation to gender**

In previous studies, females and calves were significantly more prevalent to skin lesions than adult males (Wilson *et al.*, 1997). Females and calves accompany each other and share the same

home range, while socialising with the same individuals in the pod (Natoli *et al.*, 2005). However, in the current study there was no clear difference between the sexes for most lesion types. In some years, prevalence was greater in males and for others in females.

Apart from DFS, no obvious skin lesion patterns were noticed over time. It may be that the time frame of eight years is insufficient for skin lesions to exhibit trends or that if trends exist, other important variables may not have been considered. The DFS may have shown a trend by chance due to the relatively small sample size (n=37). In this instance, female individuals were more prevalent to the skin lesion than males although that could be due to the high proportion of females in the sample, as females are easier to sex than males when they are accompanied by calves. Nonetheless, the higher prevalence of tooth rakes/scars and AFT on males could be explained by their intense social behaviour and the male-male bond (Wells *et al.*, 1987; Wells, 1991).

## **5.2 Skin lesion prevalence in relation to body area affected**

The skin lesions were observed to be more prevalent on the dorsal fin of individuals. This may happen due to the fact that the dorsal fin is more exposed than other areas of the body. Only CLs were more exposed on the back, and as observed numerically, in different body areas. However, investigating the calves specifically, it was found that CL and DBD lesions affected mainly the head. The prevalence of those two lesions was also found to be associated. The reason for that is uncertain since the etiology of those lesions is unknown.

## **5.3 Skin lesion prevalence in relation to age**

Regarding the classification by age and its relationship with skin lesion prevalence over time, it appeared that all skin lesion types were distributed differently between adults and calves, with adults generally covered more by skin lesions than calves. This result differs from other studies where calves have higher prevalence and this is thought to be due to loss of their passive immunity against the infectious agents (Wilson *et al.*, 1997; Van Bressen *et al.*, 2003). The fact that a separate analysis conducted only on calves showed that other lesions were also more prevalent than in adults, supports this. Other possible explanations for the generally lower lesion prevalence on calves are that calves are still under their mother's protection, and their social and

foraging skills have not yet fully developed (Connor *et al.*, 2000), or it may simply be that sample sizes of calves and juveniles in the Welsh population are too small to detect such trends.

Adults exhibited WFF, WL, tooth rakes/scars and AFT lesions. These lesions were mostly observed on the dorsal fin and in several cases were associated with one another. WFF and AFT were absent from calves. It may be that AFT and WFF lesions are gained from frequent biting, or injury while socializing (B. Wilson *pers. comm.*). Given the fact that the social behaviour of bottlenose dolphin individuals begins after they leave maternal care, this could explain the observed trends between adults and calves. For certain types of lesions, some trends appeared to develop in relation to age over time. Different types of lesions affected individuals of different age and to a different extent. Calves were prone to fewer lesion types but also in OL, while adults were not. The reasons for these events are unclear but may be due to the lower immune defence system of calves leading in and the extreme cases to calves being completely covered by lesions such as DBD, CL or OL (Figs. 15b, 35, 38, & 39). The more developed immune response system towards infectious agents in adults may explain their lower skin lesion extent (Van Bressen *et al.*, 2003).

#### **5.4 Residency of individuals in relation to skin lesion prevalence**

No association was found between the prevalence of skin lesions and the residency to a particular region. Since the great majority of the individuals of the Welsh population range over all of Cardigan Bay and into North Wales (Feingold & Evans, 2013), the sample sizes may have been too small (particularly around Anglesey) to detect differences between residents occupying particular regions. It was only after 2007 in fact that survey coverage was extended to north and east Anglesey (Feingold & Evans, 2013).

Overall, almost 40% of the population has been observed to use as home range Cardigan Bay SAC, Pen Llŷn a'r Sarnau SAC and the Isle of Anglesey (Feingold & Evans, 2013). However, only 7% of the individuals were observed to use solely Cardigan Bay SAC, 8% solely Anglesey, and 3% only Pen Llŷn a'r Sarnau SAC as their home range. The low proportions of individuals exhibiting local residency and small home ranges therefore probably account for the lack of difference in lesion prevalence between the areas, compounded by variation in home ranges among different individuals (Veneruso & Evans, 2012b; Feingold & Evans, 2013).

## **5.5 Skin lesion prevalence in relation to resident, visitors and transient individuals**

When an individual is observed in Cardigan Bay more than 6 years and in more than 12 occasions, is classified as resident individual of the bay. However, it is possible it would be migrating annually in winter time off the Isle of Anglesey and beyond. In these terms of lesion prevalence, residency is defined differently than the previous section. Individuals resident to the Cardigan Bay make up between 52 and 63% of the overall population (Feingold & Evans, 2013). In order to test whether infectious burdens might be introduced into the local population by visitors or transients, comparisons of the prevalence of skin lesions between residents, visitors and transient individuals within the Welsh population were made. Similar to the local residency results, no association was detected in skin lesion prevalence between the three groupings - residents, visitors and transients. However, the fact that the sample size of the transients was smaller than the other groups defined the overall sample size of this analysis and possibly the outcome.

## **5.6 Sea Surface Temperature (SST) and skin lesion prevalence**

In general, it appears that SST has no effect upon skin lesion prevalence. There was a relationship between SST values in June and skin lesion prevalence over time. However, this may be due to chance when applying multiple different comparisons in which eventually significance may appear (Czaja *et al.*, 1986).

## **5.7 Extent**

A preliminary investigation was made to determine the extent of skin lesions on the Welsh population of bottlenose dolphins. The approach was made only qualitatively from visual observations on a small sub-sample of the population. However, differences in the extent of some lesion types were noted on individuals over time. Environmental factors such as salinity and temperature and/or pollution may contribute to that (Geraci *et al.*, 1979; Wells & Scott, 1999; Wilson *et al.*, 1999a; Reeves *et al.*, 2003; Bearzi *et al.*, 2009; Kiszka *et al.*, 2009; Van Bresseem *et al.*, 2007, 2009a).

## 6. Conclusions

The Welsh population of bottlenose dolphins is larger than the populations from the Moray Firth and Shannon Estuary. Magileviciute (2006) studied the social networks in the Welsh bottlenose dolphin population and used Wilson *et al's* (1997) skin lesion classification as her tool to classify the skin lesion types observed on individuals. The current study focused upon spatio-temporal patterns of skin lesions on the Welsh bottlenose population for the period 2001-14 using photo-ID techniques. However, due to the scarcity of data for the period 2001-06, the investigation was limited to the last eight years.

An attempt was made to classify the skin lesions according to the related potential causes of them, based on the literature and with the assistance of Marie Van-Bresseem and Ben Wilson. A total of 15 lesion categories were defined, of which nine were mainly found to have affected the Welsh bottlenose dolphins. These were tooth rakes/scars (84%), white lesions (WL) (43.8%), cloudy lesions (CL) (23.4%), dark fringed spots (DFS) (20%), abraded fin tips (AFT) (15%), white fringed spots (WFS) (12.6%), dark black dots (DBD) (11.3%), white fin fringe (WFF) (9.5%), and black lesions (BL) (5.4%). Tooth rakes/scars were included as a lesion type in this study due to the fact that they provide pathogens such as *Vibrio* spp. with a potential route into the skin to invade and create lesions (Van Bresseem *et al.*, 2008d).

It appeared that 73% of individuals were affected by at least one type of lesion, whilst 56% of the population carried more than two different types of lesion over time. Comparisons of skin lesion prevalence were made between the two genders and between age groups. During the period 2010-14, females, as in other studies, were found to have a greater prevalence of skin lesions, particularly tooth rakes/scars (94%), WL (45%) and WFS (12%), while males had a prevalence of tooth rakes/scars (89%), AFT (44%) and CL (39%). In contrast to previous studies, the overall skin lesion prevalence was observed in higher proportions in adults than in calves. A separate analysis was made solely on the calves, which showed a prevalence of tooth rakes/scars (63%), OL (35%), CL (23.5%) WL (23%) and DBD (23%). Additionally, the extent of lesions was investigated qualitatively. Calves were found to be extensively covered by skin lesions compared to adults. In general, the extent was studied by visual observations *in situ* and qualitative analysis of photos on a subsample of individuals. Obvious changes were noted on the development of skin lesions over time.

Residency and home range of the individuals were also examined in relation to skin lesion prevalence. The analysis included individuals that use solely particular areas as their home range. The results indicated that there was no significant association in skin lesion prevalence in individuals between the different areas. Furthermore, the investigation whether there is a relationship in prevalence of skin lesions between resident, visitors and transient individuals found no difference, suggesting that neither transients nor visitors were introducing infections into the resident population, although sample sizes of the latter two groups may have been too low to test this fully.

The possible relationship between skin lesion prevalence and environmental factors such as monthly sea temperature was explored using SST values for Cardigan Bay and applying multiple comparisons. In general, the result showed no relationship between those variables, except for June. This could be a multiplicity statistical error since variation in SST values within Cardigan Bay is not significant.

The prevalence of skin lesions on the bottlenose dolphin populations has been used among others as a health indicator (Wilson *et al.*, 1999a; Pettis *et al.*, 2004; Hamilton *et al.*, 2005; Van Bresseem *et al.*, 2008a, 2009a, 2012). The list of pathogens that have been isolated by skin lesions has proved to lead either to poor health and/or death of the individuals (Smith *et al.*, 1983; Bossart *et al.*, 2005; Rector *et al.*, 2006; Rehtanz *et al.*, 2006; Smolarek Benson *et al.*, 2006). Since, pox-viruses and herpesviruses have been isolated by DFS and WFS respectively in other studies (Hart *et al.*, 2012), the strong presence of these in the Welsh bottlenose dolphins raises questions regarding the health of the population. Photos of low quality depicting individuals with the possibility of carrying other categories of lesions such as pox, pox-like lesions and tattoo lesions are also shown in this study. The pathological nature of these lesions suggests the need for further investigation on skin lesion categories that may affect the Welsh population of bottlenose dolphins. A further systematic and quantitative study of the prevalence and extent of skin lesions is needed in order to assess and better understand the patterns of skin lesions on this population. Accurate evaluation would lead to better management towards the sustainability of this important population.



## 7. Limitations and Future Recommendations

The limitations of this study involved among others the quantity and quality of data for the period examined. The initial aim was to investigate the trends of skin lesions over the period 2001-14. However, the time frame of investigating the data had to be reduced to eight years (2007-14) since the scanned images of individuals covering the period 2001-06 (before digital photography was used routinely) were not of the same resolution for skin lesion detection/classification. Also, when it comes to body area affected by skin lesions, the numbers of backs and flanks investigated in this study may be reduced since the photos were taken according to photo-ID protocols targeting the dorsal fin. Consequently, it is important that photos taken during field work is not only directed towards photo-ID, but is also appropriate for skin lesion detection and classification of other body areas besides the dorsal fin

Compared to Magileviciute (2006), the results of this study are not comparable since the skin lesion categorisation was different and more lesion types were included in this study. Therefore, the prevalence of skin lesions was differently estimated. More research is needed regarding the determination of the skin categories covering the Welsh dolphins. The more accurate determination of skin lesion types will lead to more accurate estimation of lesion prevalence and to better evaluation of the health of the dolphins and their habitats (Pettis *et al.*, 2004; Hamilton *et al.*, 2005; Van Bresseem *et al.*, 2008a, 2009a, 2012).

The Sea Watch Foundation holds a large database of identified animals that is updated every year, including information on gender and age of the individuals. Annually, new information is collected regarding the individuals. However, for the separate analyses of age and sex with regards to skin lesion prevalence, only individuals of known sex and gender were included for the proper investigation of those factors. Consequently this suggests that in order to investigate the skin lesion patterns in relation to those two factors accurately, more years of study is needed. The annual Cardigan Bay monitoring project run by Sea Watch constantly provides new scientific information regarding the dynamics of the Welsh population of bottlenose dolphins. In the future, this information will lead to the determination of the sex and gender of more animals. In these terms, the following years of monitoring of skin lesion patterns upon the dolphins should provide bigger samples of useful data.

Another limitation was the time frame of this project, allowing the test of only one environmental variable, but also to investigate the extent of skin lesions on the individuals only qualitatively. Previous studies have pointed out that the epidermal disorders may be affected also by salinity and pollution, and with an increase in stress levels amongst individuals (Wells & Scott, 1999; Wilson *et al.*, 1999a; Reeves *et al.*, 2003; Bearzi *et al.*, 2009). For example, the natural defence of the skin may be affected by several chemical contaminants which can weaken the immune-defence system of individuals (Van Bressem *et al.*, 2008d). In the future, the investigation of more environmental variables such as salinity, pH, nutrients and pollution in relation to skin lesion prevalence and extent might reveal a positive relationship between them. Consequently, the systematic investigation of patterns of skin lesion extent quantitatively or qualitatively is important not only for conservation research of the population overall but also for studies of age, sex and home range of sub-groups. Nemoto *et al.* (1977), for example, studied the diatoms on the skin of Franciscana dolphins and reported that orange hues could be film caused by the attachment of diatoms. Since the extent of OL on calves was notable in this study, the investigation of a potential relationship between nutrients and extent would be interesting. Moreover, the field season of 2014 was not completed and therefore the data set of this year lacks some information. In the future, the opportunity for full coverage of the sampling period would assess any possible temporal bias.

The Welsh population of bottlenose dolphins is considered relatively small (albeit the largest coastal population of the species in UK) and the resident individuals make up 52 to 63% of the overall population (Feingold & Evans, 2013). In this study, the residents affected by skin lesions constituted 62% of the overall data set. However, the small number of individuals tested was a limitation in itself. It is possible that the investigation for skin lesions on those different groupings of individuals may need a bigger time frame in order for a good quantity of data to be collected. Additionally, in this study in order to test skin lesion trends and home range/connectivity comparisons were made only for the individuals solely using specific areas. However, the numbers of individuals used were small, as shown above. In the future, more comparisons between individuals that use larger home ranges may reveal important information since more individuals could be included.

In order to fully assess the health levels of the Welsh bottlenose dolphins for skin lesions, and their contribution to the status of the UK populations, a systematic comparative qualitative and quantitative study that includes also the Scottish populations would be useful. The creation of a

database that includes information on skin lesions for each individual maintained annually for Cardigan Bay would help to safeguard and monitor the dynamics of the population. It would also be useful to develop software packages similar to those used in matching procedures, which would help measure and calculate the prevalence and extent of different skin lesions on individuals.

Several studies have previously pointed out that by taking tissue samples from stranded animals, pathogens could be isolated from skin lesions via biopsies and other biological techniques (for example PCR and Gram staining procedures). The information that could be collected by the results of these techniques would be invaluable. Although the procedure needs special circumstances in order to be successful (such more and fresh samples), collaboration with other institutes in the UK that could provide the facilities and the equipment for that would be extremely helpful in revealing new scientific information.

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## 9. Appendices

### Appendix 1. Sea Watch Foundation effort (a) and sighting (b) forms

a

Page ..... of .....



## 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<br>GMT/BST | LATITUDE<br>(degrees, decimal minutes) | LONGITUDE<br>(degrees, decimal minutes) | BOAT<br>COURSE | SPEED<br>(knots) | EFFORT<br>TYPE | SEA<br>STATE | SWELL<br>HEIGHT | VISIBILITY | BOAT<br>ACTIVITY | SIGHT.<br>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.



# VESSEL-BASED SIGHTINGS RECORDING FORM

Date (dd/mm/yyyy) ..... 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/GM | 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. Figures of skin lesion prevalence in relation to gender

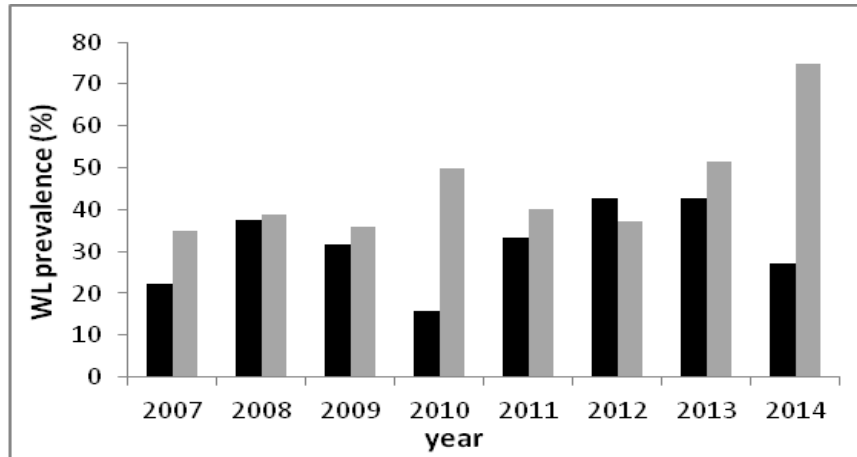


Fig. 58: Prevalence of WL on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=35)

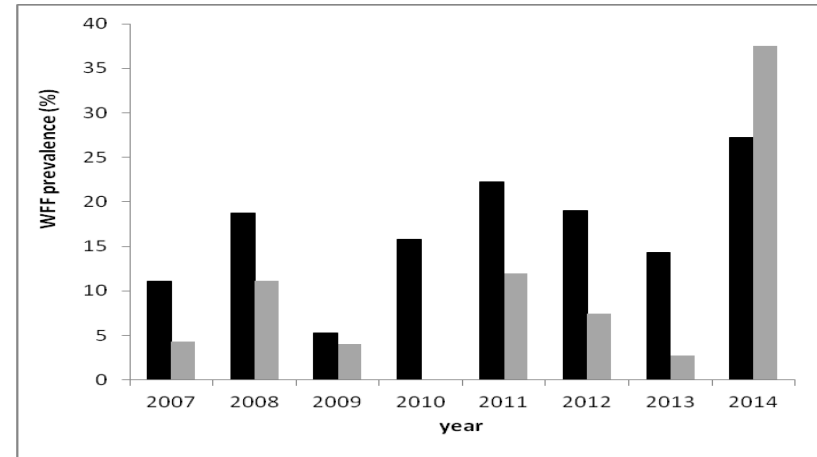


Fig. 59: Prevalence of WFF on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=17)

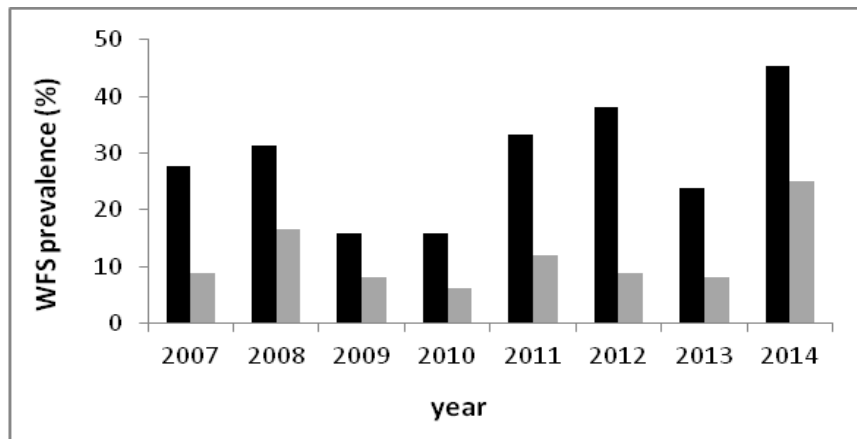


Fig. 60: Prevalence of WFS on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=29)

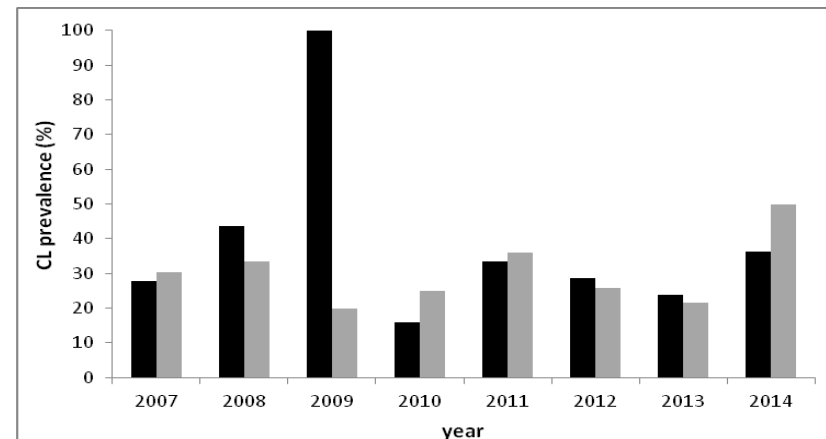
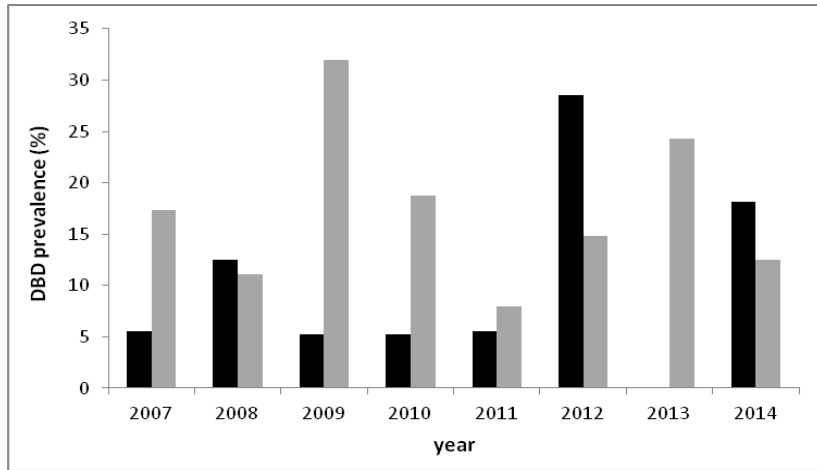
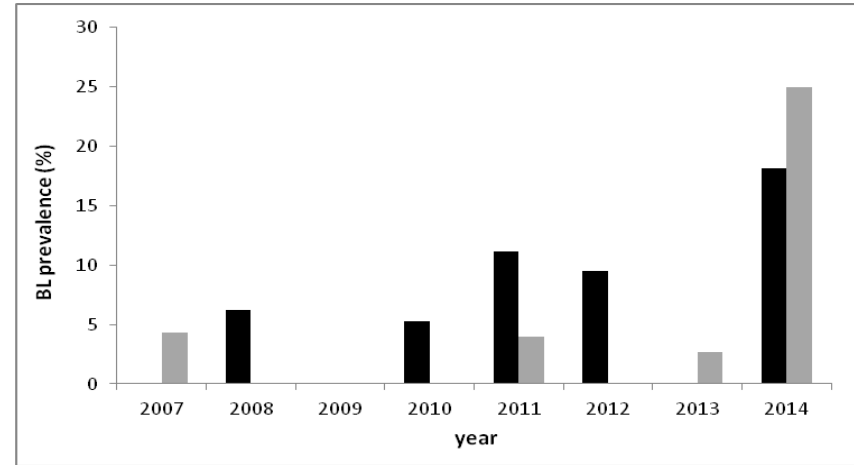


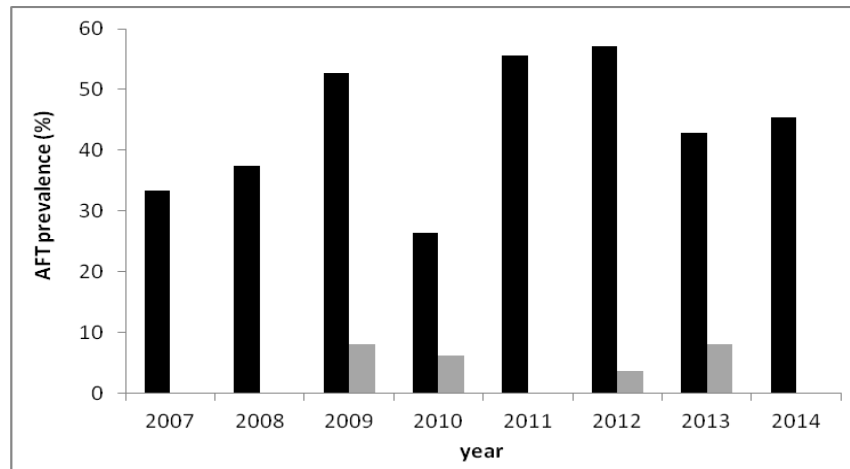
Fig. 61: Prevalence of CL on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=45)



**Fig. 62:** Prevalence of DBD on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=15)

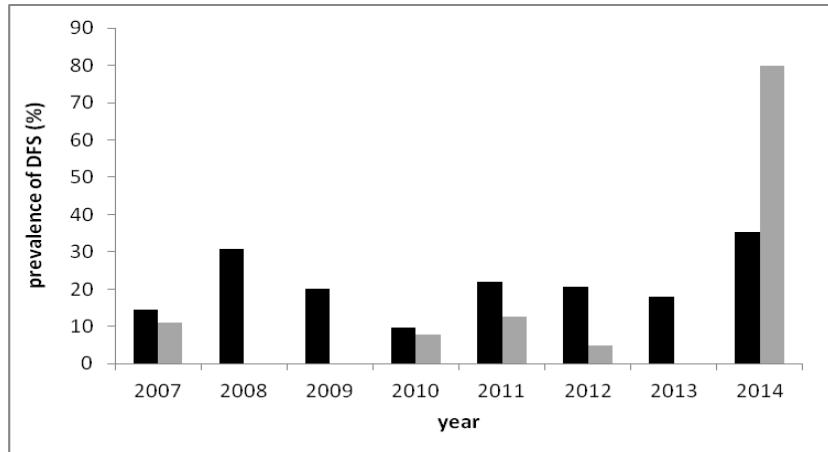


**Fig. 63:** Prevalence of BL on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=16)

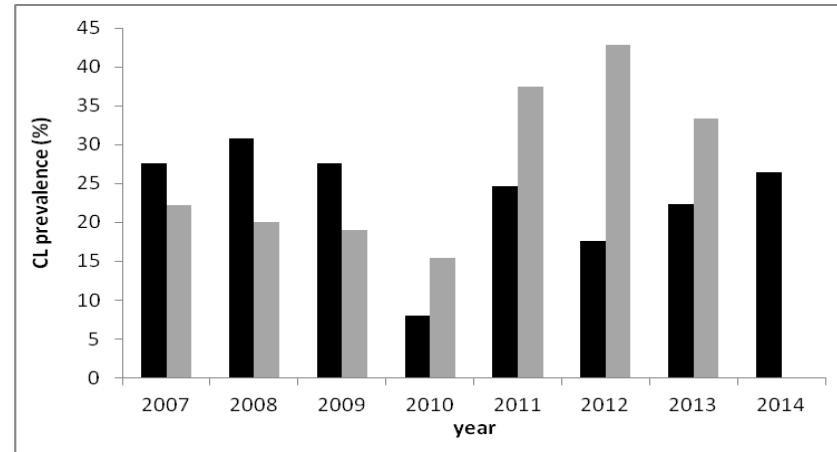


**Fig. 64:** Prevalence of AFT on females (grey) and males (black) Welsh bottlenose dolphins for the period 2007-14 (n=25)

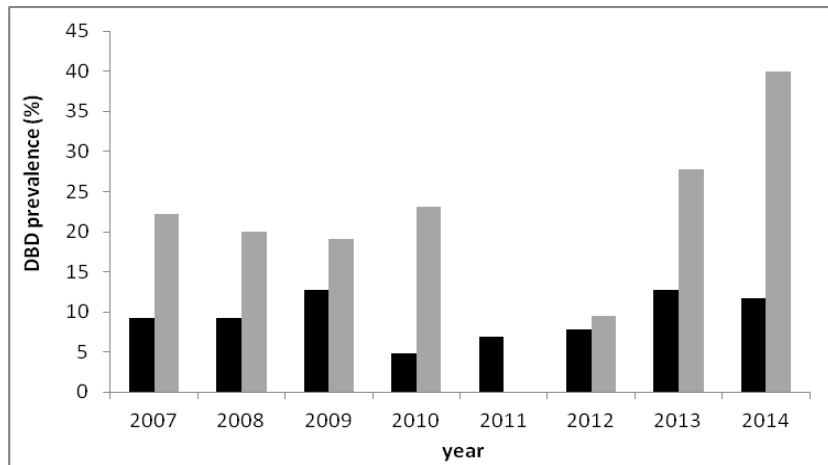
### Appendix 3. Figures of skin lesion prevalence in relation to age



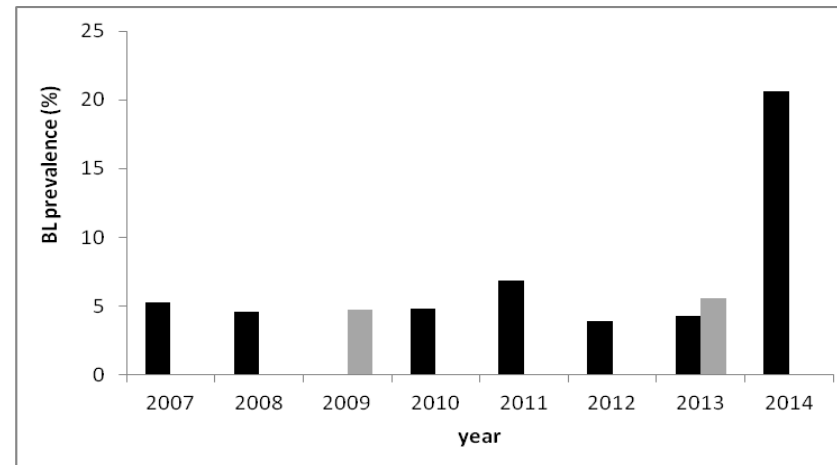
**Fig. 65: Prevalence of DFS on adults (black) and calves (grey) for the period 2007-14 (n=112)**



**Fig. 66: Prevalence of CL on adults (black) and calves (grey) for the period 2007-14 (n=54)**



**Fig. 67: Prevalence of DBD on adults (black) and calves (grey) for the period 2007-14 (n=42)**



**Fig. 68: Prevalence of BL on adults (black) and calves (grey) for the period 2007-14 (n=34)**