Report of the Workshop on Cetacean Skin Diseases

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Participants: Bedrinana-Romano, Bertellotti, Best, Bolános, Brownell, Carlson, Castro, Flach, Flores, Gallego, Galletti, George, Kasuya, Klaich, Marcondes, Mattila, Moore, Moreno, Ott, Paniz-Mondolfi, Parsons, Pearson, Ridoux, Robbins, Rogan, Rosa, Rose, Rowles (Chair), Sanino, Siciliano, Taylor, Uhart, Van Bressem and Varisco.

1. INTRODUCTORY ITEMS

The Workshop was held in Santiago, Chile from 30-31 May 2008 at the Sheraton Santiago Hotel.

1.1 Welcoming remarks

The Conveners, Rowles and Van Bressem, welcomed the participants to the Workshop.

1.2 Introduction of participants

Rowles requested all participants to introduce themselves.

1.3 Election of Chair

Rowles was elected as Chair.

1.4 Appointment of Rapporteurs

Taylor, Paniz-Mondolfi, Moreno and Siciliano were appointed as rapporteurs.

1.5 Adoption of Agenda

The agenda was adopted as drafted and is given in Appendix A.

1.6 Available documents

The documents available for the workshop were: SC/60/DW1-19; SC/60/SH25; Gulland (2008); Paniz-Mondolfi *et al*. (2007); Reif *et al*. (2009). See Annex B for a list of documents.

2. OVERVIEW OF SKIN DISEASES IN CETACEANS

Van Bressem reviewed SC/60/DW8 which described the micro-organisms known or suspected to cause skin diseases in cetaceans. Viruses belonging to four families, i.e. *Caliciviridae*, *Herpesviridae*, *Papillomaviridae* and *Poxviridae*, have been detected in miscellaneous skin lesions sampled in odontocetes and mysticetes. Among bacteria, *Dermatophilus* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Pseudomonas* spp., *Streptococcus iniae* and *Vibrio* spp. have been isolated from ulcerative dermatitis, pyogranulomatous dermatitis and panniculitis, diamond skin disease and slow-healing ulcers and abscesses. *Aeremonas* spp., *Mycobacterium marinum*, *Pseudomonas* spp. and *Vibrio* spp. are normally present in the marine environment while *Erysipelothrix rhusiopathiae* and *Streptococcus iniae* are fish pathogens that also may infect cetaceans. Selection for antibiotic-resistant bacteria through the prophylactic use of antibiotics in aquaculture is a growing problem in South America and may account for the emergence of cutaneous conditions. At least four groups of fungi, i.e. *Candida albicans*, *Fusarium* spp., *Trichophyton* spp. and *Lacazia loboi*, have been observed to cause skin diseases. Lobomycosis or lacaziosis is distinguished by grayish, whitish to slightly pink, verrucuous lesions, often in pronounced relief that may ulcerate. Ciliated protozoans, likely *Kyaroikeus cetarius*, have caused invasive dermatitis in small cetaceans from the USA and Korea. The aquatic environment of cetaceans is a natural home to bacteria and fungi but cetacean skin has several mechanisms to impede invasion. Chemical contaminants may affect natural skin barriers and depress the immune system. Wounds and specific viral infection (poxvirus, herpesvirus) may provide routes of entry.

3. SKIN DISEASES IN SOUTH AMERICAN CETACEANS

3.1 Odontocetes

3.1.1 Review of skin diseases of known etiology

3.1.1.1 LOBOMYCOSIS

Paniz-Mandolfi discussed lobomycosis, a chronic insidious subcutaneous infection caused by *Lacazia loboi*, which is closely related to *Paracoccidiodes brasiliensis* Paniz-Modolfi *et al*. (2007). The first human case of lobomycosis along the Venezuelan coast region recently was confirmed in a fisherman reported to have had contact with lobsters that exhibited severe verrucous lesions similar to those observed on dolphins and turtles in the area. The presence of infection by *Lacazia loboi* in dolphins raises many questions: (1) Is the disease extending its geographic range, or are infected animals migrating from endemic areas in South America? (2) Are climatic changes influencing the appearance of new habitats for this agent? Could the disease be present in other marine species? (3) If so, could marine animals transmit the disease to humans? These questions add more uncertainties to many of the unknown pathobiological aspects of this agent; thus, it is necessary for biologists, veterinarians and physicians to share their thoughts and findings in order to begin deciphering the intricacies of this complex and enigmatic disease.

The Chair thanked Paniz-Mondolfi for his presentation and noted that the presence of the disease in humans and marine animals emphasises the connection between human health and wildlife health in an ecosystem context.

Siciliano presented SC/60/DW13 which reviewed cases of lobomycosis and lobomycosis-like disease (LLD) in common bottlenose dolphins (*Tursiops truncatus*), the Guiana dolphin (*Sotalia guianensis*) and a Bryde's whale (*Balaenoptera brydei*) from South American waters. LLD seems to be spreading in the tropical Atlantic and Pacific Oceans. The affected Delphinidae inhabit coastal waters that are biologically and chemically contaminated. Environmental pollutants may lower the immune response of the dolphins and favour the emergence of these diseases. Lobomycosis and LLD should be considered as neglected tropical diseases.

Siciliano clarified that the condition in the Bryde's whale was classified as lobomycosis-like and not lobomycosis as the lesions were examined by photographs only and no samples could be collected. Sanino commented on the potential effect of aquaculture farms on lobomycosis infections. Oyster and mussel farms operate in several areas of Brazil where the lobomycosis studies were conducted. As aquaculture operations increase in Brazil and Chile, there is a need to document the effects of aquaculture operations on cetacean health. It was noted that lobomycosis has not been reported in franciscana (*Ponotoporia blainvillei*) from Brazil.

SC/60/DW1 described mycotic dermatitis in common bottlenose dolphins from Southern Brazil in photo-identified and stranded dolphins from the Tramandaí and Mampituba estuaries, Rio Grande do Sul state. Lobomycosis and LLD were observed in two (20%) of the ten dolphins photo-identified in the Tramandaí estuary and in one (16.67%) of the six dolphins photo-identified in Mampituba estuary. From the period October 1991 to February 2008, a total of 65 bottlenose dolphin carcasses were recovered. Lobomycosis was confirmed by histology in one dolphin from Tramandai. Another dolphin had a mycotic disease of unknown etiology. Moreno noted that the impact of mycotic diseases on the small dolphin populations of Tramandaí and Mampituba communities should be evaluated further.

Moreno commented that since 1991 there has been no franciscana with lobomycosis or LLD lesions identified in stranded or incidentally caught animals in Tramandaí and Mampituba estuaries.

Rowles commented that in the United States bottlenose dolphins with lobomycosis have been followed over time and the infections appear to be chronic. Moreno noted that some of the cases were followed over time (3-4 years). Of these, two dolphins were presumed dead since they were not sighted for several months after the disease was first observed. In addition, one photo-identified dolphin (the confirmed case of lobomycosis) was later found stranded on the Tramandaí beach. Flores commented that in Baia Norte (Santa Catarina State) Southern Brazil, cases of lobomycosis have been followed since 1994 and showed progression of the disease. Flores also commented that a small population of Guiana dolphin which have been extensively photo-identified since 1993 have not shown any evidence of LLD. Moreno also clarified that lobomycosis was not the only cause of death in the reported cases.

Paniz-Mondolfi noted that even in human patients, lobomycosis is a difficult disease to follow. He noted that Reif *et al*. (2009) shows that bottlenose dolphins in the Indian River Lagoon of the US with lobomycosis had a significant decrease in the immunoglobin cell surface markers CD4 and CD21 which differentiate lymphocyte subpopulations. If this is true, it would mean that the animals are severely immuno-compromised, lacking T Helper lymphocyte function and B cell integrity making them prone to develop other infections which can be more life threatening. Paniz-Mondolfi noted that it is necessary for a good necropsy to be performed on these animals to look for other agents contributing to mortality.

Van Bressem (SC/60/DW7) described a case of severe LLD in an emaciated common bottlenose dolphin stranded on a beach at Margarita Island, Venezuela, in 2004. LLD may have contributed to the death of this specimen. The role of biological and chemical contamination in the emergence and severity of this disease should be explored.

Van Bressem discussed SC/60/DW6 which described skin lesions in 172 Indo-Pacific bottlenose dolphins from a year-round resident population living in the coastal waters off Amakusa-Shimoshima Island (130°07'E, 32°33'N), western Kyushu, Japan. In summer 2007, a likely mature male was observed with extensive, in relief, nodular skin lesions. The disease looked like lobomycosis caused by *Lacazia loboi* as reported in bottlenose and Guiana dolphins from the Americas, though other fungi may be involved. Small lumps that may represent the beginning of the disease were observed on this dolphin a year before. Three other dolphins had similar lumps.

Van Bressem presented SC/60/DW10 in which was reported the first case of LLD in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from the tropical lagoon of Mayotte, in the southwest Indian Ocean (12°50'S, 45°10'E). From July 2004 to June 2006, daylight boat-based surveys were conducted in the territorial waters of the island, including the 1,100km^2 closed lagoon where Indo-Pacific bottlenose dolphins are resident. Six of the 45 photo-identified dolphins had LLD. This is the first report of LLD in Indo-Pacific bottlenose dolphins from this region of the world.

The Workshop recognised the potential for impacts of skin diseases in small populations of cetaceans especially in areas in which there are high levels of environmental degradation. Given the information provided at this meeting, the Workshop agreed that special concern to the prevalence and impact of skin disease should be given to Guiana and bottlenose dolphins from southern and southeastern Brazil.

3.1.1.2 TATOO SKIN DISEASE

Sanino (SC/60/DW3) described tattoo skin disease (TSD), characterised by stippled dark or grey skin lesions and caused by poxviruses related to the orthopoxviruses. In South America, TSD has been documented in nine cetacean species including Chilean dolphins (*Cephalorhynchus eutropia*) (SE Pacific – southern Chile), Commerson's dolphins (*Cephalorhynchus commersonii*) (SW Atlantic – Southern Argentina), common dolphins (*Delphinus delphis*) (SE Pacific – Ecuador), long-beaked common dolphin (*Delphinus capensis*) (SE Pacific – central Peru), dusky dolphin (*Lagenorhynchus obscurus*) (SE Pacific – central Peru), Peale's dolphins *Lagenorhynchus australis* (SE Pacific – southern Chile), bottlenose dolphins (SE Pacific – central Peru and northern Chile), Guiana dolphin (SW Atlantic – Brazil) and Burmeister's porpoise (*Phocoena spinipinnis*) (SE Pacific – central Peru, north and central Chile). Generally, TSD is absent in neonates that are likely protected by passive immunity. A peak in prevalence has been observed in juveniles. Prevalence decreased in adults

as they developed active immunity. Adults can present with TSD, but it is usually associated with poor health and compromised immune systems. Therefore, TSD may be used as a bio-indicator of the general health of individuals, populations and environment. A free-share embedded online database to collect and share data is proposed in order to regionally assess the occurrence of TSD because it's only systemically known in Peru. It is likely endemic in several other regions of South America.

Sanino stressed the need for a standardised diagnostic protocol for TSD. TSD should be included in stranding and photo-ID studies. To better understand TSD and the role of pollution, there is a need for comparative studies in both polluted and non-polluted areas.

Rowles questioned whether infected animals would always express the disease or if the disease might manifest some time after infection. Sanino clarified that TSD is likely to be expressed after infection. Van Bressem commented that they always found pox virus when they biopsied lesions, but samples from normal skin with no lesions were not examined for pox virus. Rosa commented that normal 'control' skin of bowhead whales (*Balaena mysticetus*) had been examined and these samples were negative for pox virus. Rosa commented that a previous Geraci *et al*. (1979) publication had indicated that edema occurs in both active and resolved infections and wondered if others had seen edema associated with the lesions. Sanino has not seen edema or ulcerations. Van Bressem noted that Sanino recorded the first case of TSD in a calf. TSD should be considered a potential cause of mortality in calves considering that poxviruses (smallpox, monkey pox) can cause mortalities in children.

3.1.2 Review of skin diseases of unknown etiology

Flach presented SC/60/DW4 which reported on miscellaneous skin diseases or syndromes of unknown etiology including whitish, velvety lesions (WVL, often associated with unrelated skin injuries, scars and tooth rakes), large, rounded lesions (LRL, large to very large lesions with an orange or dark outline and a light inner colour) and vesicular skin disease (VSD, small to medium vesicles) in humpback whales (*Megaptera novaeangliae*), Commerson's dolphins, Chilean dolphins, false killer whale (*Pseudorca crassidens*), Guiana and bottlenose dolphins from marine waters of South America and the Antarctic. WVL are now commonly recorded through photo-identification studies in several coastal species and populations from South America. Mortality rates, if any, associated with these skin diseases is unknown. WVL do not seem life threatening and, at least in some individuals, may eventually heal. A Chilean dolphin calf with LRL died some weeks after being first sighted with the disease. While unknown bacteria or fungi superinfecting miscellaneous skin traumata and poxvirus tattoos are thought to cause WVL and LRL, vesiviruses are suspected as the aetiological agents of VSD. These various skin conditions may be indicative of a deteriorating coastal water environment and should be systematically monitored. Collection of biopsies or fresh samples for histopathology and microbial analysis is urgently needed.

Siciliano presented SC/60/DW16 which documented the occurrence of enteropathogens isolated from marine mammals in the coastal regions of Brazil. From 2003 to 2004, a total of 198 swabs were evaluated from the following marine mammal species: southern right whale (*Eubalaena australis*), bryde's whale, humpback whale, minke whale (*Balaenoptera acutorostrata*), true's beaked whale (*Mesoplodon mirus*), franciscana, Guiana dolphin, Atlantic spotted dolphin (*Stenella frontalis*), rough-toothed dolphin (*Steno bredanensis*), common dolphin and South American sea lion (*Otaria byronia*). The authors examined the presence of *Plesiomonas shigelloides*, *Vibrio* and *Aeromonas* species in swabs from the blowhole, anus, genital slit, mouth, nostrils, umbilicus, eyes and open wounds in marine mammals beached or accidentally captured in fishing nets in southeastern (RJ) and southern (RS) coastal regions of Brazil. The results showed 222 isolates cultured including: *Vibrio alginolyticus, V. parahaemolyticus, V. vulnificus, V. fluvialis Aeromonas veronii* biogrupo *veronii, A. caviae*, *A. hydrophila*, *Aeromonas* sp. and *Plesiomonas shigelloides*.

Van Bressem asked whether swabs from skin lesions were also examined. Siciliano clarified that this was a broad preliminary survey, and bacteria had not yet been isolated from skin lesions in franciscana. Paniz-Mondolfi noted the importance of establishing the normal flora of these animals in order to be able to identify what is abnormal. Siciliano clarified that they are looking for normal flora. Mattila inquired whether one might be able to make the assumption that the by-caught animals are normal, healthy animals compared to the stranded animals. Siciliano pointed out that the by-caught Guiana dolphin were not always in good health.

3.2 Mysticetes

SC/60/DW18 reported on miscellaneous skin diseases of unknown etiology in the following categories: tissue damage, disseminated vesicles, ulcerated lesions, small whitish rounded lesions, wrapping scars, irregular light gray patches, irregular stippled lesions, light red granulomatous tissue associated with scars, irregular reddish spots, irregular white marks, irregular whitish round marks, parallel scars, congenital malformation, abnormal growth of mass tissue, possibly tumoral and hypo-pigmentation in humpback whales from Breeding Area G in Ecuador and Colombia and Breeding Area A in Brazil. In Ecuador, between 1997 to 2004, 4,116 humpback whales were observed during 868 trips on 629 days on the water. In Columbia, 120 humpback whales were observed and analysed from 2001 to 2006. In Brazil from 2006 to 2007, 13,040 humpback whale images were obtained during cruise surveys and 186 were analysed for this study. A total of 159 individuals with lesions were sighted, described and recorded in a database. Of these, 109 cases were sighted in Abrolhos Bank in Brazil. Forty-three were sighted in Machalilla National Park in Ecuador and seven were sighted in Coqui Cove in Colombia.

This was the first attempt to analyse and compare data on skin lesions in humpback whales from two different south ocean basins. Castro and colleagues intend to standardise the methodologies and increase efforts to better understand how the lesions occur and how this affects the conservation of this species. A tremendous amount of data remains to be analysed and will be provided in future papers.

Castro clarified that the lesions are seen in the Abrolhos Bank, Machalilla National Park and Coqui Cove of South America. It has not yet been able to separate identified lesions by sex of individual whale, but in time this may be possible when the genetic analyses are completed.

SC/60/SH25 reported different types of skin lesions identified from blue whales, *Balaenoptera musculus*, photographed off the northwestern coast of Isla Grande de Chiloe, Chile in 2006 and 2007. The main lesions were: (1) cookie-cutter shark, *Isistius brasilensis,* bites, and (2)

vesicular or blister lesions. Good quality photographs from 91 individual whales (left sides) were examined for lesions. Cookie-cutter shark lesions were the most commonly observed on these whales and are similar to those reported from other species of both large and small cetaceans. Skin peeling or shedding was observed on some whales and is believed to be a normal condition. One tattoo-like skin lesion was observed on a single whale in 2007. Blister lesions were common on whales in both years. The presence of blister lesions in both years may indicate that this 'disease' will be present in the population for a long time. It is unknown if these lesions contribute to mortality of blue whales frequenting Chilean waters. Additional investigations are needed that, as a minimum, must include the histological and genetic examination of the two types of disease from live or dead whales, especially the tattoo-like skin lesions. Until this work is undertaken, it will be impossible to determine if these lesions pose a conservation risk to the blue whales off southern Chile.

Brownell noted the need to take good, clear close-up pictures of skin lesions and to obtain samples of lesions from stranded animals, especially animals with extensive skin lesions. A standardised protocol for identifying types of lesions is also necessary.

SC/60/DW14 described skin lesions in southern right whale adults and calves off the coast of Valdes Peninsula, Argentina. Kelp gulls (*Larus dominicanus*) attack the whales and take pieces of skin and blubber, causing deep wounds. The study evaluated 626 photographs and obtained biopsy samples from live whales and examinations of stranded whales. Some of the gull-pecked wounds had the aspect of a volcanic-like lesion, with epithelial hyperplasia and widespread inflammation, possibly caused by the presence of opportunistic bacterial infections. Lesions of unknown origin were also observed in adults and juveniles and were categorised as: (i) stippled, (ii) pox-like, (iii) ulcerative, (iv) regular depression and (v) rounded elevations. The skin of several calves appeared abnormally scattered and wrinkled, but without the patterns observed in adults.

Varisco noted that the kelp gulls appear to attack weak parts in the skin but there is no current explanation for this phenomenon. Best questioned whether the authors had observed long raised linear lesions in this study. Varisco clarified that linear lesions were one type of lesion that was not included because it was unclear if it was a lesion or a healed scar. Varisco noted that as kelp gulls were in contact with waste material such as dumps, these birds would therefore be carriers of pathogens. Laboratory evaluations for pox virus are in progress.

Paniz-Mondolfi noted that peripheral raised borders around an ulcer mean the presence of inflammation, basically edema and erythema, that are expressions of a healthy response to infection. For this reason, biopsies should be performed in these areas of activity (borders of lesions) in order to increase the chances of isolating an infectious agent as a possible cause of the lesion.

4. SKIN DISEASES IN CETACEANS FROM OTHER CONTINENTS

4.1 Mysticetes

SC/60/DW17 discussed a number of fungal species that have evolved as opportunistic pathogens of humans and animals. These infections, which can be systemic and/or cutaneous, mostly affect immuno-compromised individuals. In animals, most cutaneous infections are caused by fungi that have adapted to utilise keratin and can cause dermatophytosis. These fungi parasitise the keratinised layer of the epidermis, hair, feathers and horn, without affecting the deeper tissue. Records on dermaphytosis in marine mammals, especially in cetaceans, are relatively rare. The authors were therefore interested to obtain an indication of the fungal taxa associated with the skin of the southern right whale. Consequently, skin samples were collected from freshly beached whales, along the coast of South Africa, to test for the presence of pathogenic fungi associated with these (presumably) debilitated animals. The control group for this study consisted of biopsies collected under sterile conditions from free-swimming individuals in St Helena Bay, South Africa. To determine the presence of fungal dermatophytes, skin samples were cultivated on a series of isolation media using classical mycological isolation methods. The fungal isolates were identified using classical, as well as molecular identification techniques. In addition, the ability of the isolates to degrade keratin was determined using simple plate assays. It was found that a range of fungi occur on the skin of beached whales, including opportunistic pathogens belonging to the yeast genera *Candida* and *Cryptococcus*. Filamentous fungi belonging to the families Chaetomiaceae, Hypocreaceae and Trichomaceae were also found. In contrast to the host of fungi on the three beached whales so-far investigated, no fungi were recovered from five of the six animals in the control group. However, two filamentous fungal species were isolated from the sixth, an apparent healthy individual. One fungus belongs to the family Sclerotiniaceae, and the other is an undescribed black yeast. The filamentous fungi isolated during this study were all able to degrade keratin and belong to fungal groups known to harbour opportunistic fungal pathogens. As a result of the variety of cutaneous fungi found on the beached whales, and the apparent lack thereof on healthy free-swimming individuals, the authors hypothesise that the presence of these fungi on the skin of southern right whales may act as an indicator of health risk. This hypothesis should be tested in future studies.

SC/60/DW15 reported on skin abnormalities observed on the chin and blowhole regions of a juvenile bowhead whale from the Bering-Chukchi-Beaufort Sea stock. The lesions from the two affected areas had differing gross appearances: the chin lesion was a 'ring' type lesion, characterised by a circumscribed, slightly raised area of skin that projected downward into the stratum intermedium, while the blowhole lesion was non-circumscribed and proliferative in appearance. A novel poxvirus in both lesions was detected by polymerase chain reaction targeting DNA toperisomerase I genes of members of the subfamily *Chordopoxvirinae*. This virus has been provisionally called cetacean poxvirus-2 (CPV-2) and is genetically distinct from dolphin poxvirus as previously described (CPV-1). The lesions were histologically characterised by vacuolated cells present in the stratum intermedium (spinosum), along with a hyperplasia of the typically thin parakeratotic layer of cells in the stratum externum. Eosinophilic intracytoplasmic inclusion bodies were visualised by light microscopy in the epithelial cells of the raised lesions. The presence of lesions may be related to stress or degraded environments. The public health significance of these lesions is unknown, though no transmission to humans has been reported. This is the first report of a poxvirus infection in a mysticete.

Rosa acknowledged the importance of using a standardised protocol and will follow up these findings with immunohistochemistry on skin lesion samples.

Mattila presented a summary of SC/60/DW3. He stressed that the presentation and paper are based on field observations and photographs, not physical examination of the lesions described. The raised lesions observed were the primary interest of the authors, as they have not been described for this species. They appear to be a relatively new development in these animals and their cause is unknown. The depressed lesions observed were assumed to be from cookie-cutter shark bites, but were included because of their similarity in shape, size and location, and therefore possible relationship to the raised lesions. Full frame, high-quality images of the left flank of humpback whales in American Samoa, Hawaii and the Gulf of Maine were examined. Raised lesions were described as primarily ovoid-shaped, horizontal in orientation and apparently more numerous toward the ventral surface, especially around the genitals, of the whales seen. In American Samoa they were found on almost every non-calf whale, but were not seen on calves. They were not seen to erupt or 'resolve', in fact the same lesions were seen on one individual in the North Atlantic (Gulf of Maine) over a period of 29 years without apparent change. Depressed lesions were assumed to be cookie-cutter shark bites and were similar in size and shape to the raised lesions, suggesting that one possible explanation for the latter is that they are bites that have healed as raised scars. Another possible cause was a reaction to remoras, as the lesions grossly resemble their suction mechanism in size and shape, and the remoras have been anecdotally documented around the genitals of humpbacks in American Samoa. The possibility of a sexually transmitted virus was also suggested given the preponderance of lesions around the genitals. The authors then used a preliminary rating system to compare the occurrence of the two types of lesions between whales in American Samoa, Hawaii and the Gulf of Maine. There were significant differences in the prevalence of raised lesions found between all three areas, with the highest occurrence in American Samoa and the least in the Gulf of Maine, with Hawaii being intermediate in occurence. It was noted that this was not what would be expected if the raised lesions were caused by human activities, including pollutants. The Gulf of Maine humpbacks also had a significantly lower incidence of cookie-cutter shark bites than the other two areas, although Hawaii and American Samoa were not different from each other. Further refinement of a standardised rating system, analysis of biopsies and examination of the lesions during necropsies were recommended, as well as further comparison between oceanic populations.

Mattila noted that if the lesions were a result of cookie-cutter sharks and remoras it would be a newly identified response that was not previously documented to injuries that have occurred over decades. Brownell added that if the calves in American Samoa are new or born nearby then all cookie cutter lesions should be open or fairly new. Paniz-Mondolfi added that the raised lesions in adult animals could be reparative changes such as fibrosis from previous traumas. Van Bressem noted that normally even if fibrosis occurs the scars are flat and not raised, therefore it is important to understand why the lesions are raised. Mattila clarified that some of the lesions heal flat. Robbins noted that although no age is known for most animals, only one animal in American Samoa did not have a lesion so it would be reasonable to believe that the lesions are present in all age classes. Participants found the remora lesion causation to be troubling due to their highly mobile nature on the animal's body and believed they would not explain the distribution pattern observed.

5. DIAGNOSTIC TOOLS AND TECHNIQUES FOR CETACEAN SKIN DISEASES

5.1 Diagnostic tools

Rowles discussed epidemiological and diagnostic approaches to skin disease assessments in cetaceans. Epidemiology is the study of the distribution and determinants of disease and can be divided into descriptive (case series, correlational, cross-sectional) and analytical (case control and longitudinal) studies. Longitudinal studies can be used to determine the impacts of skin disease on outcomes relevant to populations. Diagnostics may include visual assessments, skin scrapings, biopsy, live capture (when feasible), and assessment of environmental factors. Visual assessments are critical to understanding field applications of skin disease outcomes and must be done with standardised techniques and criteria. Diagnostic techniques on tissues include both traditional methods (culture, histopathology, and serology) and emerging techniques (PCR and microarray technology). Finally, information management, data sharing, standardisation and information dissemination are critical for the further understanding of the factors affecting skin lesions and overall cetacean health. The development of case definitions, standardised descriptors, enhanced diagnostic capacity, and web based information tracking and dissemination will be key elements to furthering our understanding of skin diseases in cetaceans. To enhance communication and standardisation, NOAA has developed a web based 'virtual rounds' and 'virtual microscope' which allows researchers from around the world to evaluate specific cases.

Mattila cautioned field biologists to develop a way to obtain unbiased samples in order to produce accurate percentages and rates for populations. Moore suggested that the 'virtual rounds' be expanded to include water quality and coastal ecology data and include environmental scientists to support case evaluations in an ecosystem context.

SC/60/DW12 summarised the results of a preliminary study on skin lesions in Commerson's dolphin from the Northern Patagonian Sea. Data were obtained from digital pictures taken during seasonal photo-identification studies in 2006-2007 at the Chubut River estuary. Only good and very good images were used. The number of identified dolphins for 2006 and 2007 were 69 and 88, respectively. The prevalence (P) of TSD and orange marks (OMD) possibly caused by diatoms in this species varied seasonally and annually. The mean annual prevalence levels were 15.43% (TSD) and 4.53% (OMD) for 2006 and 23.81% (TSD) and 11.92% (OMD) for 2007. The P of TSD and OMD showed a significant correlation with time and temperature while the difference between annual P for TSD of 2006 and 2007 tended to be significant. The authors noted the importance of using the detection probabilities of infected and uninfected individuals in order to obtain unbiased estimates of prevalence.

6. ANALYTICAL APPROACHES

6.1 Geographic distribution of skin diseases

The geographic distribution of the various skin diseases discussed vary by effort, disease (etiology) and species. Fig. 1 (Annex C) summarises the distribution of known cases of lobomycosis or lobomycosis-like disease in cetaceans from the published literature and some papers that were discussed here. Fig. 2 (Annex C) summarises the distribution of TSD in cetaceans in South America and Fig. 3 (Annex C) summarises the distribution of the miscellaneous skin lesions discussed during this Workshop.

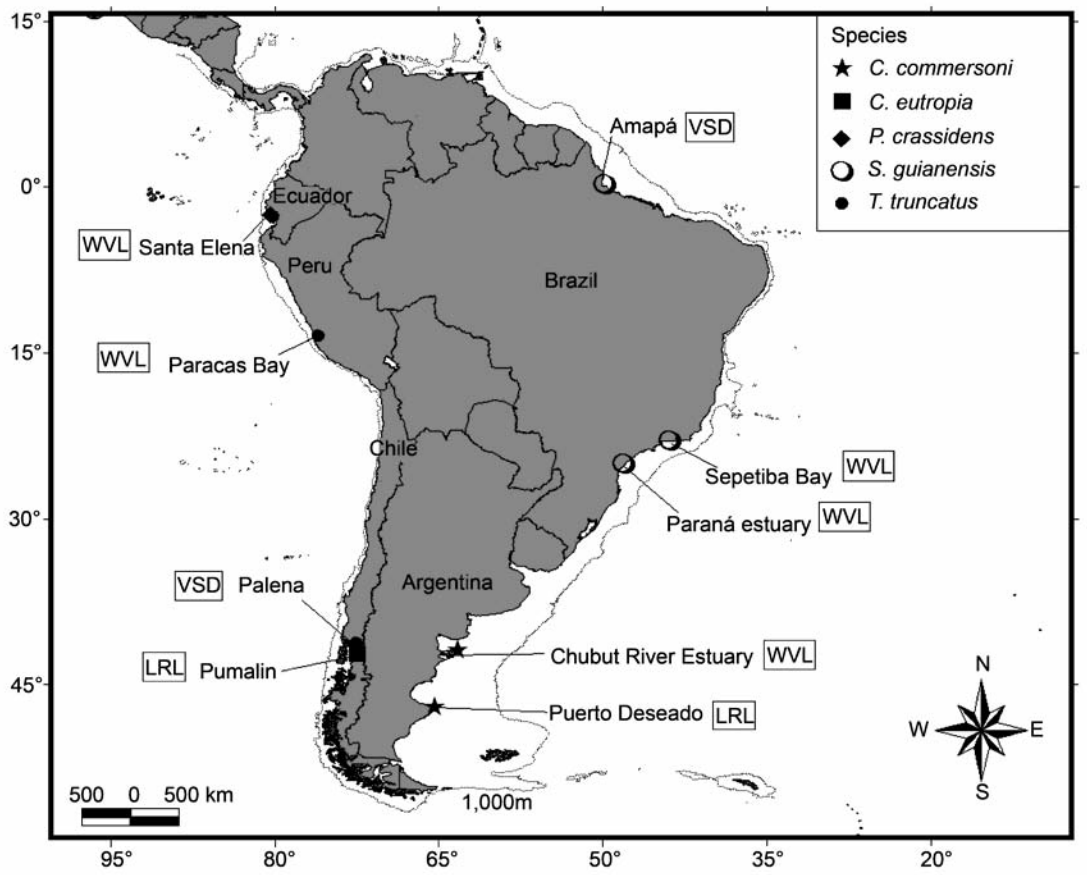

Fig. 1. Geographic distribution of Miscellaneous Skin Disease distributions in South America. WVL – white, velvety lesions; LRL – large, round cutaneous lesions; VSD – vesicular skin disease.

SC/60/DW19 compared the characteristics and prevalence of different epidermal lesions in sympatric Chilean dolphins and Peale's dolphins inhabiting the Chiloé Archipelago in southern Chile. Eight types of distinct lesions were described from long-term photographic records. Prevalence values for some infectious lesion types differed between the two species, but were not statistically significant. Chilean dolphins had higher prevalence values for tattoo-like lesions compared to Peale's dolphins. Both species seem to differ in their susceptibility and exposure to epidermal disease, which might be related to differences in habitat use, movement and residency patterns and exposure to mariculture activities which abound in the inshore waters of Chiloé. Overall prevalence values for possibly infectious diseases in both species were high in comparison with other small cetacean species.

SC/60/DW11 reported on skin lesions in 385 live-captured boto (*Inia geoffrensis*) from the Mamirauá Reserve, Brazil. Photographs of the body, head and flippers taken between 1994 and 2007 were inspected for skin pathologies. The quality of the images and the geographic position of the animals were recorded. Photographs of 120 botos did not show any apparent skin anomalies and in 116 other individuals the photographs were not of sufficient quality to allow assessment of skin condition. Skin lesions were found in 107 individuals, comprising open wounds, ulcers, abscesses, cobblestones and other pathologies. Fifty-eight percent of the botos showed large cuts and healed scars. Unidentified spots, marks or irregular skin were seen in 79% of the animals. Open wounds, ulcers and abscesses together were responsible for 87% of skin conditions, whereas 'golf-ball-disease' represented 24% of the sores, and healed scars represented 40%. Dental caries and tooth abnormalities were common in the botos, and twisted, broken and deformed mandibles were regularly encountered.

6.2 Role of environmental and individual factors in the distribution, emergence, or prevalence of skin lesions in cetaceans

SC/60/DW5 reviewed emerging and recurring infectious diseases known or suspected to have the potential to significantly impact cetacean populations, and the possible synergistic effects of environmental factors. Cetacean morbilliviruses, papillomaviruses and brucellae may affect population densities through high mortality rates (morbilliviruses) or interference with reproduction (papillomaviruses and brucellae). Evidence is available for the role of environmental factors in the emergence, recurrence, and severity of at least six infectious conditions, i.e. lobomycosis, toxoplasmosis, TSD, generalised bacterial infections, miscellaneous skin diseases and morbillivirus epizootics. Other micro-parasites of potential importance include rhabdo-, herpes- and parainfluenza-viruses as well as *Helicobacter* spp., *Streptococcus* spp., *Salmonella* spp. and *Mycobacterium marinum*. The population impact and etiology of newly emerging skin diseases in South America are unknown and represent a cause of concern.

Gulland *et al.* (2008) summarised a case study of two humpback whales (mother and female calf) that were reported on 15 May 2007 in the Port of Sacramento,

California, USA, 72 n.miles from the Golden Gate Bridge in a fresh water basin. They were first sighted by the public in Benicia in San Francisco Bay on 9 May 2007. This report represents the longest period this species has been observed in fresh water and documents their movements and skin/body condition. When first sighted, both animals showed evidence of recent linear wounds, probably ship-strikes. A variety of efforts (playbacks, oikomi pipes, fireboats, stopping traffic on bridges, stopping drilling) were undertaken to herd or lead the animals back to the ocean. During the time in fresh water, their wounds did not heal normally and overall skin condition deteriorated. The skin became progressively covered with a mat of brown/green filamentous material (possible algae and/or fungi), the skin showed a change in color and the surface became irregular. Due to concerns about infection, on 26 May 2007 antibiotics were administered to the animals (ceftiofur) through a remote dart delivery system. The next day, the animals began moving south and over the next 48 hours entered San Francisco Bay. The skin condition progressively improved as the animals moved into salt water. None of the herding efforts succeeded in moving the animals, and bridges and powerlines appeared to be obstacles to the whales' movement downriver. This is the first time that antibiotics have been delivered to a free swimming humpback whale.

7. GENERAL RECOMMENDATIONS

Several specific recommendations have been made above. The following recommendations are broad and relate to international issues.

After the presentations and discussions the Workshop recognised three categories of recommendations: (1) research needs, which include: pathogenesis, etiology, research questions addressing risk factors and exposure characterisation to environmental pollutants and other anthropogenic factors; (2) standardisation of protocols, disease and lesion characterisation, and data analyses standards; and (3) enhancing information sharing and dissemination.

7.1 Research

The utility of examining long-term datasets to determine specific outcomes for certain skin conditions, lesions or syndromes was discussed. The Workshop **agreed** that long term studies, although hard to maintain, are critically important as tools for evaluating these outcomes and **recommended** that such long term datasets be evaluated for specific epidemiological outcomes such as reproduction, survival, age distribution and disease outcome (e.g. lesions heal, progress or regress). The Workshop **strongly recommended** that whenever possible tissue samples of lesions (including the borders of the lesions) be obtained and evaluated through histopathology and molecular or other diagnostic techniques. This would build upon a close collaboration between researchers and stranding networks and by-catch observer programs to enhance the sample and data collection on normal animals and animals with lesions or syndromes. The use of the mechanisms listed below in information sharing will be critical for this collaboration. Finally it was noted that in addition to the standardised protocols, a list of identified laboratories with either diagnostic or research capabilities would be extremely useful for field teams and stranding networks. The Workshop **recommended** that a list of appropriate diagnostic laboratories be developed for each country or region.

7.2 Standardisation recommendations

There was discussion of the need for standardisation through protocols (specific to the situation or carcass condition) or field definitions in the following areas: data collection and analyses for visual assessments (e.g. photo-identification), disease characterisation (case definitions), lesion or skin condition descriptions, field collection protocols, and sample analyses protocols or standards. The Workshop recognised the need for the standardisation of a minimal suite of environmental data specific to water quality, habitat quality, prey, anthropogenic activities, and potential sources of environmental degradation or change. These standardised environmental measures should be developed and used in areas in which biological studies on skin diseases in cetaceans are ongoing. The Workshop acknowledges the critical importance of lesion documentation through photography and sample analysis and **recommended** the development of standardised protocols for the collection of the samples and the assessment of the lesions, including digital photography of the lesions.

Specific to the skin lesions themselves, the Workshop **recommended** the formation of a sub-group under CERD that will focus on producing standardised protocols for assessing lesion descriptions and characteristics, as well as disease classifications, beginning with specific skin diseases in cetaceans. The participants recognised that the number of syndromes is very high and suggested that the CERD sub-group begin by concentrating on: (i) a few major lesions on the mysticetes discussed at this Workshop, (ii) using the paper by Van Bressem *et al*. (2007) as a starting point for descriptions for small cetaceans, and (iii) developing a case definition for specific skin conditions with known etiologies. These conditions might include two diseases discussed previously and outlined above (lobomycosis and TSD) and one additional well-described disease, cutaneous herpes infections (Annex C). This sub-group would develop preliminary descriptions and would share them with a broader number of experts for peer-review and consensus. The Workshop **recommended** that the CERD Co-Chairs solicit scientists with relevant expertise to participate in this skin disease sub-group.

7.3 Data sharing and information dissemination

The Workshop **agreed** that data sharing, information dissemination and enhanced communication are key factors to future progress in determining the prevalence, incidence rates, and etiologies of skin lesions in cetaceans. This can be accomplished through descriptive and analytical approaches such as comparison between study sites or longitudinal studies. Various options and various data management schemes that might assist in this effort, including ongoing data management schemes in various countries were discussed. The Workshop **recommended** that avenues for data sharing be developed using data sharing agreements, data quality standards and definitions of fields, and using distributed databases, web portal or nested shared databases. The Workshop **recommended** the use of current and newly emerging information technology mechanisms (such as the virtual microscope) as they become available to enhance collaboration and data sharing. In addition the participants **agreed** that the use of meetings at regional, national and international levels for information dissemination,

discussion and data sharing be strongly encouraged. Given the South American focus of this meeting, the upcoming science conference in Uruguay in October 2008 would be ideal for a sub-group meeting.

8. OTHER

Participants were invited to share images of unusual skin lesions, scars and traumas of unknown origin or from areas which had not been covered by the previous papers and presentations. These included scars and lesions on bowhead whales in Alaska, bottlenose dolphins from Gibraltar and southern Brazil, as well as humpback images from Brazil and elsewhere. Some potential causes were identified, but many remained unknown. The freeform discussion of visual images was informative to the participants.

9. ADOPTION OF REPORT

The report was adopted at 16:45 on 31 May 2008.

10. REFERENCES

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Annex A

Agenda

1. Introductory items
   - 1.1 Welcoming remarks
   - 1.2 Introduction of participants
   - 1.3 Election of Chair
   - 1.4 Appointment of rapporteurs
   - 1.5 Adoption of Agenda
   - 1.6 Available documents
2. Overview of skin diseases in cetaceans
3. Skin diseases in South American cetaceans
   - 3.1 Odontocetes
     - 3.1.1 Review of skin diseases of known etiology
       - 3.1.1.1 Lobomycosis
       - 3.1.1.2 Tattoo skin disease
     - 3.1.2 Review of skin diseases of unknown etiology
   - 3.2 Mysticetes
   - 3.3 Needs, assessments and recommendations
4. Skin diseases in cetaceans from other continents
   - 4.1 Mysticetes
5. Diagnostic tools and techniques for cetacean skin diseases
   - 5.1 Diagnostic tools
6. Analytical approaches (group discussion)
   - 6.1. Geographic distribution of skin diseases
   - 6.2. Role of environmental and individual factors in the distribution, emergence, or prevalence of skin lesions in cetaceans
7. General recommendations
   - 7.1 Research
   - 7.2 Standardisation recommendations
   - 7.3 Data sharing and information dissemination
8. Other
9. Adoption of report

Annex B

List of Documents

SC/60/DW

1. MORENO, I.B., OTT, P.H., TAVARES, M., OLIVEIRA, L.R., BORBA, M.R., DRIEMEIER, D., NAKASHIMA, S.B., HEINZELMANN, L.S., SICILIANO, S. and VAN BRESSEM, M.F. Mycotic dermatitis in common bottlenose dolphins (*Tursiops truncatus*) from southern Brazil, with a confirmed record of lobomycosis disease. 11pp.
2. SANINO, G.P., VEN BRESSEM, M.F., VAN WAEREBEEK, K., FLACH, L., VIDDI, F., JEPSON, P.D., DUIGNAN, P., RAGA, J.A., DEAVILLE, R., DE OLIVEIRA SANTOS, M.C., CRESPO, E., KLAICH, J. and FÉLIX, F. Epidemiology of tattoo skin disease worldwide with a particular insight in South America [Abstract only]. 1pp.
3. MATTILA, D.K. and ROBBINS, J. Incidence of raised and depressed ovoid skin lesions on humpback whales of American Samoa. 7pp.
4. FLACH, L., VAN BRESSEM, M.F., REYES, J.C., ECHEGARAY, M., SICILIANO, S., SANTOS, M., VIDDI, F., CRESPO, E., KLAICH, J., MORENO, I.B., EMIN-LIMA, N.R., FÉLIX, F. and VAN WAEREBEEK, K. Miscellaneous skin lesions of unknown aetiology in small cetaceans from South America. 12pp.
5. VAN BRESSEM, M.F., RAGA, J.A., DI GUARDO, G., JEPSON, P.D., DUIGNAN, P., SIEBERT, U., BARRETT, T., DE OLIVEIRA SANTOS, M.C., MORENO, I.B., SICILIANO, S., AGUILAR, A. and VAN WAEREBEEK, K. Emerging and recurring diseases in cetaceans worldwide and the role of environmental stressors. 13pp.
6. SHIRAKIHARA, M., AMANO, M. and VAN BRESSEM, M.F. Skin lesions in a resident population of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from Japan. 6pp.
7. BERMÚDEZ-VILLAPOL, L.A., SAYEGH, A.J. and VAN BRESSEM, M.F. Lobomycosis-like disease in a bottlenose dolphin (*Tursiops truncatus*) from Venezuela. 3pp.
8. VAN BRESSEM, M.F., VAN WAEREBEEK, K., FLACH, L., REYES, J.C., DE OLIVEIRA SANTOS, M.C., SICILIANO, S., ECHEGARAY, M., VIDDI, F., FÉLIX, F., CRESPO, E., SANINO, G.P., AVILA, I.C., FREIJIA, N. and CASTRO, C. Skin disease in cetaceans. 11pp.
9. NO PAPER
10. KISZKA, J. and VAN BRESSEM, M.F. Lobomycosis-like disease in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from the southwest Indian Ocean. 8pp.
11. DA SILVA, V.M.F., MARTIN, A.R. and MIKESH, E. Skin disease and lesions in the boto *Inia geoffrensis* in the central Amazon. 6pp.
12. KLAICH, M.J., PEDRAZA, S.N., VAN BRESSEM, M.F. and CRESPO, E.A. Preliminary results from skin lesions study in Commerson's dolphin (*Cephalorhynchus commersonii*) from the northern Patagonian Sea. 3pp.
13. SICILIANO, S., VAN BRESSEM, M.F., MORENO, I.B., OTT, P.H., TAVARES, M., FLORES, P.A.C., FLACH, L., CESAR REYES, J., ECHEGARAY, M., DE OLIVEIRA SANTOS, M.C., VIDDI, F., CRESPO, E., KLAICH, J.M., FÉLIX, F., SANINO, G.P. and VAN WAEREBEEK, K. Review of lobomycosis and lobomycosis-like disease (LLD) in cetacea from South America. 6pp.
14. BERTELLOTTI, M., VARISCO, A., AGUADO, G. and FRANCISCO, E. Skin lesions in southern right whales (*Eubalaena australis*) off the coast of Valdés Peninsula, Argentina. 3pp.
15. ROSA, C., BRACHT, A.J., BLAKE, J.E., O'HARA, T.M., ROMERO, C.H. and SHEFFIELD, G.M. Gross and histological characterization of a newly discovered mysticete poxvirus. 8pp.
16. PEREIRA, C.S., SICILIANO, S., MORENO, I.B., OTT, P.H. and DOS PRAZERES RODRIGUES, D. Occurrence of enteropathogens isolated from marine mammals in the coastal regions of Brazil. 6pp.
17. MOUTON, M., REEB, D., THORNTON, M., BOTHA, A. and BEST, P.B. Cutaneous fungi on southern right whales (*Eubalaena australis*) from South Africa as a possible indicator of health risks. 1pp [Abstract].
18. CASTRO, C., GROCH, K., MARCONDES, M., VAN BRESSEM, M.F. and VAN WAEREBEEK, K. Miscellaneous skin lesions of unknown aetiology in humpback whales *Megaptera novaeangliae* from South America. 6pp.
19. BEDRINANA-ROMANO, L. and HEINRICH, S. Prevalence of epidermal lesions in sympatric dolphins of Isla Chiloé, southern Chile. 12pp.

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25. BROWNELL, R.L., CARLSON, C.A., VERNAZZANI, B.G. and CABRERA, E. Skin lesions on blue whales off southern Chile. 8pp.

Annex C

Summaries of specific etiologically defined skin diseases

LOBOMYCOSIS

Paniz-Mondolfi summarised information on lobomycosis.

Distribution
See Fig. 1.

Immunocompetence
Humans with lobomycosis often suffer from cell-mediated immunodeficiency but have a normal humoral immunity. Several immunological abnormalities were reported in bottlenose dolphins from the Indian river Lagoon, Florida, affected by lobomycosis. The dolphins had a severe depression of the adaptive immune response with a statistically significant lower number of the CD4+ T, CD19+ and CD21+ B Lymphocyte cells. At the present time it is unknown if this is the case in all affected specimens or whether immunocompetent dolphins may also develop the disease.

Mode of infection
The hydrophilic environment, soil and vegetation are considered to be the ecological habitats of the fungus. The agent is thought to access the skin through penetration or accidental trauma. The chronic and insidious evolution of the disease suggests an underlying defect in cell mediated immunity and the slow growth of the agent in skin phagocyte cells. Once the infection has occurred, dissemination is more likely to be lymphatic, although hematogenous and contiguous dissemination cannot be discarded.

Known risk factors
Exposure to the agent and a previous skin trauma are thought to be the main risk factors that provide a route of entry for the infection.

Once infected is always infected?
It seems likely that once the dolphins get infected and develop the disease, the disease is chronic.

Mortality
To date, only one case of visceral compromise has been described in humans and there are no reports in dolphins. The disease is essentially restricted to skin and subcutaneous tissue. Although chronic lesions predispose to the development of malignancies like squamous cell carcinoma, no fatal events have been reported in humans due to this infection. There is a lack of information in affected dolphin's necropsies that could prove lobomycosis as a direct cause of death in these animals.

Confirmed diagnosis criteria
Lobomycosis in dolphins is characterised by grayish, whitish to slightly pink, verrucuous lesions, often in pronounced relief that may ulcerate. The lesions are

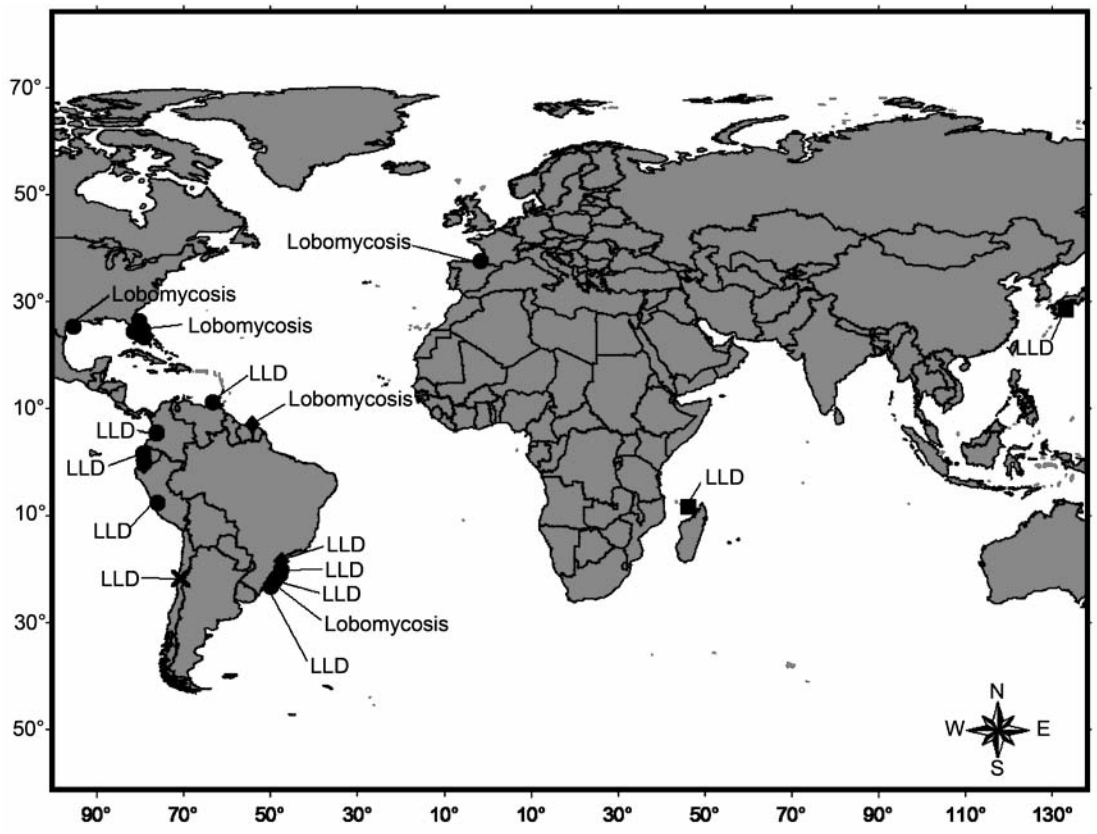

Fig. 1. Geographic distribution of lobomycosis and lobomycosis-like disease.

believed to be very typical for the infection. However, more histological studies are needed to confirm cases of lobomycosis-like diseases in dolphins from South America, the Indian Ocean and Japan.

Treatment for disease
There is no known effective treatment of lobomycosis in dolphins. Multiple antimycotic agents like ketaconazole, myconazole, trimethropin, amphotericin B and 5-fluorocytosine have not given significant benefits in humans. New generation ergosterol synthesis inhibitors azoles like voriconazole and posaconazole appear to give promising results in complex mycotic infections. Surgical excision with wide margins remains as the optimal solution at the moment.

Emergence of the disease in South America
Lobomycosis was reported for the first time in a Guiana dolphin (*S. guianensis*) from Surinam in the 1970s and later in a common bottlenose dolphin (*T. truncates*) from Southern Brazil in 1990. This disease has been rarely if at all observed in South America with regards to photo-identified, by-caught and stranded small cetaceans during the last 15 years. Thus, it is believed that lobomycosis is emerging in dolphins from this continent (both oceans).

TATTOO SKIN DISEASE

Sanino summarised information on tattoo skin diseases.

Distribution
See Fig. 2.

Immunocompetence
TSD does not necessarily prepare the path for other diseases but could be a route of entry for viral, bacterial and fungal infections as was the case for *Fusarium* spp and caliciviruses.

Mode of infection
The mode of infection is yet to be determined. Direct contact with infected animals or inhalation of virus particles are likely as in other poxvirus infections.

Known risk factors
TSD is more prevalent in small cetaceans in poor health. Polychlorinated biphenyls (PCBs) and related compounds may contribute to the severity of the disease through toxicity at the level of immune system.

Once infected is always infected?
TSD may remain in the infected individual and active lesions can re-occur, persisting in the skin cells. However, in healthy populations adult dolphins and porpoise may clear the disease.

Mortality
TSD does not cause high mortality when endemic in a population. It may be lethal in calves without maternal immunity.

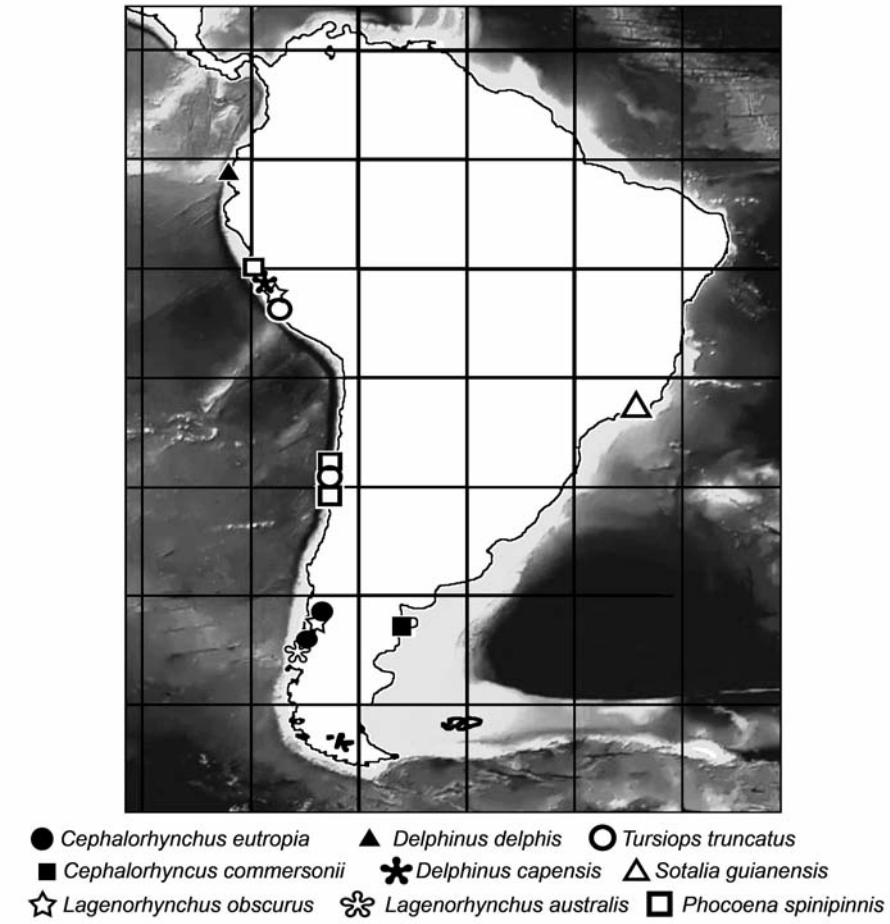

Fig. 2. Geographic distribution of Tattoo Skin Disease in South America.

Clinical disease immunocompromise
Poxviruses are not known to lower the immune response in the same way that morbilliviruses do, but this should be explored further.

Confirmed diagnosis criteria
Stippled, irregular, black, grey or yellowish skin lesions are very characteristic of TSD with poxviruses being always seen in samples taken in Peruvian small cetaceans and by PCR in dolphins and porpoises from the United Kingdom.

Treatment for disease
There is no treatment for TSD and active immunity is in general able to defeat the disease.