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Epidemiological characteristics of skin disorders in cetaceans from South American waters

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Abstract. We document the macroscopic phenotypic characteristics (relative size, location, pattern, colour, extension), prevalence and evolution of five types of skin disorders of unknown aetiology, including 'green-brown plaques' (GBP), 'orange patches' (OPA), 'cutaneous nodules' (NOD), 'pale dermatitis' (PAD) and 'expansive annular lesions' (EAL) in five odontocete species (n = 559 individuals) from the Southeast Pacific (n = 230) and Southwest Atlantic (n = 329) Oceans. GBP affected two likely-adult *Sotalia guianensis* traveling side-by-side in a freshwater area of the Cananéia Estuary in August 2009. Low salinity is suggested as predisposing factor. OPA were distinguished in three of 209 (1.4%) free-ranging *S. guianensis* in Sepetiba Bay, Brazil, during winter months of 2005-2008. Epibiont diatoms are suspected aetiological agents. NOD were chronically present in one male adult *Orcinus orca* observed off the coast of southern Brazil in 2007-2010. PAD was seen in free-ranging individuals and carcasses of *Tursiops truncatus*, *S. guianensis* and *Pseudorca crassidens* from both the Atlantic and Pacific Oceans in 1992 and in 2004-2009. Prevalence was 1% in 103 *S. guianensis* from Paranaguá Estuary (Brazil), 2.3% in 222 *S. guianensis* from Sepetiba Bay and 6.9% in 87 inshore *T. truncatus* from Paracas Bay, Peru. Although in some cases the lesions covered up to 35-40% of the visible body surface and ulcers may occur there was no evidence of mortality and, in time-series of six individuals, PAD eventually healed. In six *T. truncatus* and five *S. guianensis* acutely affected, PAD was associated with minor cutaneous injuries and scars, including tooth rakes, suggesting infection routes for opportunistic pathogens. EAL were noted in a *Cephalorhynchus eutropia* calf from Palena province, Chile, in 2003 and in a *P. crassidens* calf washed ashore dead in southern Brazil in 2009. The *C. eutropia* calf disappeared, and probably died, two weeks after first observation. Prevalence of EAL was 6.7% in 15 *C. eutropia* in 2002-2004. These data suggest that EAL are potentially lethal in calves. PAD and EAL were primarily seen in cetaceans inhabiting biologically or chemically contaminated nearshore waters. In view of their emergence and occasional severity these disorders should be the subject of systematic monitoring.

Resumo
Documentamos as características fenotípicas macroscópicas (anátomo-patologia, localização, padrão, cor e extensão), prevalência e evolução de cinco tipos de condições cutâneas de etiologias desconhecidas, incluindo 'placas marrom-esverdeadas' (PME), 'manchas alaranjadas' (MAL), 'nódulos cutâneos' (NOD), 'dermatite pálida' (DEP) e 'lesões anulares expansivas' (LAE) em cinco espécies de odontocetos (n = 559 indivíduos) do sudeste do Pacífico (n = 230) e sudoeste do Atlântico (n = 329). PME afetaram dois prováveis indivíduos adultos de *Sotalia guianensis* que deslocavam-se em paralelo em uma área de água doce do Estuário de Cananéia em agosto de 2009, sendo a baixa salinidade sugerida como um fator predisponente. MAL foram observadas em três de 209 (1,4%) *S. guianensis* na Baía de Sepetiba, Brasil, durante os meses de inverno de 2005-2008; diatomáceas epibiontes são os agentes etiológicos suspeitos. NOD estavam cronicamente presentes em um macho adulto de *Orcinus orca* observado na costa sul e sudeste do Brasil em 2007-2010. DEP foi vista em indivíduos de vida livre e carcaças de *Tursiops truncatus*, *S. guianensis* e *Pseudorca crassidens* no Atlântico e Pacífico em 1992 e em 2004-2009. A prevalência foi de 1% em 103 *S. guianensis* do Estuário de Paranaguá (Brasil), 2,3% em 222 *S. guianensis* na Baía de Sepetiba e 6,9% em 87 *T. truncatus* costeiros da Baía de Paracas, Peru. Embora em alguns casos as lesões cobrissem até 35-40% da superfície corporal visível, e úlceras possam ter ocorrido, não houveram evidências de mortalidade e, em uma série temporal de seis indivíduos, a DEP eventualmente cicatrizou. Em seis *T. truncatus* e cinco *S. guianensis* afetados de forma aguda, a DEP foi associada com injúrias cutâneas menores e cicatrizes, incluindo marcas de dentes, sugerindo rotas de infecção por patógenos oportunistas. LAE foram notadas em um filhote de *Cephalorhynchus eutropia* da Província de Palena, Chile, em 2003 e em um filhote de *P. crassidens* encalhada morta no sul do Brasil em 2009. O filhote de *C. eutropia* desapareceu, e provavelmente morreu, duas semanas após a primeira observação. A prevalência de lesões anulares expansivas foi de 6,7% em 15 *C. eutropia* em 2002-2004. Esses dados sugerem que LAE são potencialmente letais em filhotes. DEP e LAE são vistas primariamente em cetáceos que habitam águas costeiras contaminadas, biológica ou quimicamente. Em face de sua emergência e ocasional severidade, essas condições devem ser objetos de monitoramentos sistemáticos.

Introduction

About 75% of the *ca*. 88 recognized cetacean species of the world inhabit the marine and freshwater biotopes of South America. There, as in other continents, they face a number of anthropogenic threats, primarily high mortality in fisheries and severe environmental degradation. Several species, populations and communities are considered vulnerable or endangered (Culik, 2004). Especially for the latter, any source of enhanced mortality and reduction in fecundity is of concern. Increasingly documented in South American cetaceans, infectious diseases have the potential to negatively impact population abundance by elevating baseline mortality rate (*e.g.* morbillivirus disease, *Crassicauda* spp. infestation) or by negatively affecting reproduction (*e.g.* brucellosis and genital papillomavirosis) (Van Bressem *et al*., 2007; 2009*a*). Some emerging cutaneous disorders may also lead to increased mortality. Three of these conditions (tattoo skin disease-TSD, lobomycosis and lobomycosis-like disease-LLD) are well-documented in South American odontocetes[1] (de Vries and Laarman, 1973; Simões-Lopes *et al*., 1993; Van Bressem *et al*., 1993; 2007; 2009*a, b*; Daura-Jorge and Simões-Lopes, 2011). TSD is caused by poxviruses and diagnosable from irregular, grey, black or yellowish, stippled cutaneous lesions. It affects Delphinidae and Phocoenidae from Peru, Ecuador, Chile and Brazil. Prevalence levels varied between 3.5% (short-beaked common dolphin *Delphinus delphis*, Ecuador) and 62.3% (Burmeister's porpoise *Phocoena spinipinnis*, Peru) in 1992-2008 (Van Bressem *et al*., 2007; 2009*b*). Generally, juveniles had a higher probability of suffering TSD than adults, presumably because adults had acquired active immunity following infection (Van Bressem *et al*., 2009*b*). When endemic, TSD does not appear to induce a high mortality rate. However, it may kill neonates and calves without protective immunity (Van Bressem *et al*., 1999; 2003). Characterized by greyish, whitish to slightly pink, verrucous lesions, often in pronounced relief, that may ulcerate and form plaques, lobomycosis[2] and LLD naturally affect common bottlenose dolphins *Tursiops truncatus* and Guiana dolphins *Sotalia guianensis* from Brazil, Ecuador, Peru, Colombia, Venezuela and Suriname (de Vries and Laarman, 1973; Simões-Lopes *et al*., 1993; Van Bressem *et al*., 2007; Bermudez *et al*., 2009; Daura-Jorge and Simões-Lopes, 2011). Lobomycosis is caused by a dimorphic fungus resembling *Lacazia loboi* (Taborda *et al*., 1999; Hauboldt *et al*., 2000) but genetically more closely related to *Paracoccidioides brasiliensis* (order Onygenales, family Ajellomycetaceae) (Rotstein *et al*., 2009; Esperon *et al*., 2011; Ueda *et al*., 2013). LLD is

[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. (2008) Mycotic dermatitis in common bottlenose dolphins (*Tursiops truncatus*) from southern Brazil, with a confirmed record of lobomycosis disease. Paper SC/60/DW1 presented to the IWC Scientific Committee, May 2008, Santiago, Chile.
Available at http://iwc.int/index.php?cID=1790&cType=document

[2]Sometimes referred to as lacaziosis.

Table 1. Prevalence of four types of skin disorders in cetaceans from South America. Abbreviations are: N = total number of specimens examined, Prev= prevalence, FR= free-ranging, BC= by-catch, ST= stranded, GBP= green-brown plaques, OPA= orange patches, PAD=pale dermatitis, EAL= expansive annular lesions.

Country & species	Habitat	Region	Ocean province	Sampling period	Specimens	N	GBP Prev	OPA Prev	PAD Prev	EAL Prev
Brazil										
Sotalia guianensis	Inshore/estuarine	Sepetiba Bay	SW Atlantic	2005-2008	BC	10	0%	0%	20%	0%
Sotalia guianensis	Inshore/estuarine	Sepetiba Bay	SW Atlantic	2005-2008	FR	209	0%	1.4%	1.4%	0%
Sotalia guianensis	Inshore/estuarine	Sepetiba Bay	SW Atlantic	2005-2008	ST	3	0%	0%	0%	0%
Sotalia guianensis	Inshore/estuarine	Paranaguá Estuary	SW Atlantic	2006-2008	FR	103	0%	0%	1%	0%
Sotalia guianensis	Inshore/estuarine	Cananéia Estuary	SW Atlantic	2009	FR	2	100%	0%	0%	0%
Ecuador										
Pseudorca crassidens	Oceanic	Santa Elena Peninsula	SE Pacific	1992	ST	28	Unk	Unk	3.6%	Unk
Chile										
Cephalorhynchus eutropia	Inshore/estuarine	Pumalin	SE Pacific	2002-2004	FR	15	0%	0%	0%	6.7%
Peru										
Tursiops truncatus	Inshore/estuarine	central Peru	SE Pacific	1993-1994	BC	19	0%	0%	0%	0%
Tursiops truncatus	Oceanic	central Peru	SE Pacific	1985-1995	BC	65	0%	0%	0%	0%
Tursiops truncatus	Inshore/estuarine	Paracas Bay	SE Pacific	2004-2009	FR	87	0%	0%	6.9%	0%
Tursiops truncatus	Inshore/estuarine	Supay-La Mina	SE Pacific	2004-2006	FR	15	0%	0%	0%	0%

highly reminiscent of lobomycosis but its aetiology is unknown (Van Bressem *et al.*, 2007). Prevalence of the disorders varied from 1.6% (Gulf of Guayaquil, Ecuador) to 20% (Tramandaí Estuary, Brazil) in 1990-2009[1] (Van Bressem *et al.*, 2007; Daura-Jorge and Simões-Lopes, 2011). At least eight inshore *T. truncatus* with advanced lobomycosis or LLD died or disappeared in Brazil and Venezuela in 1990-2009[1] (Simões-Lopes *et al.*, 1993; Bermudez *et al.*, 2009; Daura-Jorge and Simões-Lopes, 2011). Impairment in adaptive immunity, possibly related to chronic exposure to pollutants, may play a role in the pathogenesis of lobomycosis (Reif *et al.*, 2009).

Over the past decade five other types of formerly unobserved skin disorders, here referred to as 'green-brown plaques' (GBP), 'orange patches' (OPA), 'cutaneous nodules' (NOD), 'pale dermatitis' (PAD) and 'expansive annular lesions' (EAL) have emerged in some odontocete populations on the Pacific and Atlantic coasts of South America[3] (Van Bressem *et al.*, 2007). These and similar conditions are also increasingly reported worldwide, may be severe and are sometimes associated with the death or disappearance of affected individuals[4] (Wilson *et al.*, 1997; Riggin and Maldini, 2010; Maldini *et al.*, 2010; Van Bressem *et al.*, 2007; 2013). However, general knowledge of their aetiology, epidemiology, pathogenesis and prognosis is still patchy. In this context, we decided to study their phenotypic and epidemiological characteristics, and to examine their progression and potential impact on odontocete populations from the Southwest Atlantic and Southeast Pacific Oceans.

Material and Methods

Sample composition

The prevalence and macroscopic characteristics of cutaneous conditions were documented in five odontocete species (pooled, n = 559 individuals) occurring off Pacific (n = 230) and Atlantic (n = 329) South America, mainly in free-ranging individuals (n = 432), but also in stranded cetaceans (n = 33) and in animals killed in fisheries interactions (n = 94) (Table 1). The study covered three types of habitat in tropical to cold-temperate climates: inshore/estuarine (shallow, nearshore waters on the continental shelf, semi-enclosed bays, estuaries and fjord entrances), outer neritic (non-inshore shelf waters)

[3]Flach, L., Van Bressem, M-F., Reyes, J.C., Echegaray, M., Siciliano, S., Santos, M., Viddi, F., Crespo, E., Klaich, J., Moreno, I., Tavares, M., Félix, F. and Van Waerebeek, K. (2008) Miscellaneous skin lesions of unknown aetiology in small cetaceans from South America. Paper SC/60/DW4 presented at the 60th meeting of the Scientific Committee of the International Whaling Commission, Santiago, Chile, 1-13 June 2008. http://iwc.int/index.php?cID=1790&cType=document

[4]Barry, K.P., Gorgone, A.M. and Mase, B. (2008) *Lake Pontchartrain, Louisiana Bottlenose Dolphin Survey Summary* 28 April 2008–10 May 2008. Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA Protected Resources and Biodiversity Division, PRBD Contribution: PRBD-08/09-01.

and oceanic waters (beyond the shelf edge, depth > 200m) (Table 1). Some data analysed in this study were obtained from a previously published paper on the preliminary identification of skin disorders in odontocetes from South America (Van Bressem *et al*., 2007).

Free-ranging individuals

This category comprised photographic material of four delphinid species collected for individual identification purposes (Table 1) during boat and shore-based surveys in 1991-2009[5, 6] (Reyes *et al*., 2002; Santos and Netto, 2005; Santos and Rosso, 2007). Individual dolphins were identified through natural marks (Würsig and Jefferson, 1990). Maturity status (calf, juvenile, adult) was estimated from relative body size, behavioral clues, colour pattern and from the verified duration of residence (Wells *et al*., 1980; Shane, 1990; Santos and Rosso, 2007; 2008; Izodoro and Le Pendu, 2012). A minimum prevalence of skin conditions was estimated for bottlenose dolphins from Paracas Bay, Peru, for Guiana dolphins from Sepetiba Bay and Lagamar Estuary (including the Paranaguá and Cananéia estuaries), Brazil, and for Chilean dolphins *Cephalorhynchus eutropia* in Reñihue Fjord, Palena province, Chile.

Specimens from strandings and bycatch

Carcasses of Guiana dolphins from Brazil, false killer whales *Pseudorca crassidens* from Ecuador and Brazil and bottlenose dolphins from Peru were examined macroscopically for cutaneous diseases, predominantly under field conditions, in 1992-2009 (Table 1). In females, sexual maturity was determined from the presence of at least one *corpus luteum* or *corpus albicans* in one of the ovaries, or evidence of lactation or pregnancy. In males, it was ascertained from the presence of seminal fluid in at least one epididymis. When it could not be determined directly, maturity was inferred from body size and mean standard body length at sexual maturation for the populations involved (Van Waerebeek *et al*., 1990; Félix *et al*., 1992).

Skin conditions

For the 26 individuals that scored positive for a skin disorder, macroscopic phenotypic characteristics (relative size, location, pattern, colour, extension) were evaluated from digital photographs by two scientists (MFB, KVW) who exchanged insights in the process towards a consensus view. Only high-quality images were utilized. For each free-ranging individual, the affected fraction of the visible dorsal body surface (VBS) exposed when surfacing was estimated. In dead animals, the affected fraction was evaluated for the entire body surface. Time series allowed an evaluation of relative progress in three bottlenose dolphins, three Guiana dolphins (two from Sepetiba Bay; one from Paranaguá Estuary), one Chilean dolphin and one killer whale *Orcinus orca*. Five phenotypically distinctive skin conditions of unknown aetiology were recognized as follows:

(1) *Green-brown plaques* (GBP): circular plaques, green-brown in colour;

(2) *Orange patches* (OPA): generally non-raised, orange or rusty-coloured dots, some of which coalesce to form larger blotches;

(3) *Cutaneous nodules* (NOD): circumscribed skin nodules, grey or normally pigmented;

(4) *Pale dermatitis* (PAD): a suspected primary infectious or super-infected skin condition characterized by irregular, slightly raised skin sores, pale in colour, either ulcerated or with a smooth velvety aspect;

(5) *Expansive annular lesions* (EAL): ring-like lesions consisting of a sharply circumscribed light grey outer ring and a paler inner core either of which may or may not be partially obscured by an orange tinge.

The greatest diameter of the lesions was estimated photogrammetrically on the comparative basis of either an estimated dorsal fin height of (i) 230mm for Peruvian inshore bottlenose dolphin, Paracas Bay (J. Reyes and M. Echegaray, unpub. data); (ii) 70mm in a Chilean dolphin calf, Reñihue

Figure 1. Green-brown plaques in two Guiana dolphins *Sotalia guianensis* from the Cananéia Estuary on 29 August 2009.

Figure 2. Orange marks and dots in Guiana dolphin calf *Sotalia guianensis* SEP-43 from Sepetiba Bay on 16 July 2008.

[5]Viddi, F.A., Van Bressem, M-F., Bello, M. and Lescrauwaet, A.K. (2005) First records of skin lesions in coastal dolphins off southern Chile. Page 25 *in* Abstracts, *16th Biennial Conference on the Biology of Marine Mammals,* 12-16 December 2005, San Diego, California, USA.
[6]Flach, L. (2006) Photo-identification study reveals human threats towards estuarine dolphins in southeast Brazil. Page 46 *in* Siciliano, S., Borobia, M., Barros, N.B., Marques, F., Trujillo, F. and Flores, P.A.C. (Eds) *Workshop on Research and Conservation of the Genus* Sotalia. 19-23 June 2006, Armação dos Búzios, Rio de Janeiro, Brazil.

Table 2. Characteristics of skin disorders in cetaceans from South America. Abbreviations are: Spec. = specimens, SM = sexual maturity, Assoc.= association, FR = free-ranging, ST = stranded, BC = bycatch, MAT = mature, U = unknown, PR = possible remains, NA = data not available, DF = dorsal fin.

Disease/ dolphin	Species	Region	Spec.	Sex	SM	Location	Stage of disease	Date first seen	Assoc. wounds/ scars	Number lesions	Relative size
Greenish-brown plaques											
CAN 'A'	*S. guianensis*	Cananéia Estuary, BR	FR	U	U	DF, flank, head, back	acute	27 Aug 2009	No	>10	medium to large
CAN 'B'	*S. guianensis*	Cananéia Estuary, BR	FR	U	U	Head, back, DF	acute	27 Aug 2009	No	>10	medium to large
Orange patches											
SEP 43	*S. guianensis*	Sepetiba Bay, BR	FR	U	Calf	left flank, back, head	acute	16 Jul 2008	No	>30	small to large
SEP 22	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	left flank	acute	Jun 2007	No	>5	small to large
SEP 022	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	back, DF	acute	7 Jun 2005	No	>5	small to large
Cutaneous nodules											
KW	*O. orca*	northern Parana and São Paulo, BR	FR	M	MAT	back, flank	chronic	Mar 2005-Jan 2007	No	>10	small to medium
Pale dermatitis											
FF-SN	*P. crassidens*	Sta Elena Peninsula, EC	ST	U	U	fluke	acute	20 Nov 1992	possibly	1	large
PBD015	*T. truncatus*	Paracas Bay, PE	FR	U	U	DF	acute	10 Nov 2004	Yes	1	large
PBD029	*T. truncatus*	Paracas Bay, PE	FR	U	U	DF	acute	24 Jul 2004	Yes	2	large
PBD030	*T. truncatus*	Paracas Bay, PE	FR	F	MAT	DF	PR	17 Aug 2005	Yes	2	large
PBD041	*T. truncatus*	Paracas Bay, PE	FR	F	MAT	body	acute	25 Jun 2009	Yes	>5	large
PBD055	*T. truncatus*	Paracas Bay, PE	FR	U	U	DF, back	acute	25 Jul 2006	Yes	1	medium to large
PBD060	*T. truncatus*	Paracas Bay, PE	FR	F	MAT	body	acute	25 Jul 2006	Yes	>10	small to large
PBD078	*T. truncatus*	Paracas Bay, PE	FR	M	MAT	DF	PR and acute	20 Aug 2008	Yes	>3	small to large
GUA 'S'	*S. guianensis*	Paranaguá Estuary, BR	FR	U	MAT	body	PR	Mar 2007	No	>10	small to large
SEP 3	*S. guianensis*	Sepetiba Bay, BR	BC	M	MAT	beak	acute	22 Nov 2006	Yes	4	medium to large
SEP 17	*S. guianensis*	Sepetiba Bay, BR	BC	M	MAT	beak	acute	24 Jul 2007	Yes	>3	small to large
SEP 33	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	body	acute	1 Jul 2005	Yes	>30	small to large
SEP 41	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	body	acute	28 Oct 2007	Yes	>15	small to large
SEP 23	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	DF, back	PR	14 Jun 2007	No	>20	small to large
SEP 24	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	beak	acute	14 Jun 2007	Yes	1	large
SEP 26	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	DF, back	PR	15 Jan 2007	No	7	small to large
SEP 34	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	DF, back	PR	15 Jan 2007	No	8	small to medium
SEP 27	*S. guianensis*	Sepetiba Bay, BR	FR	U	MAT	DF, back	PR	19 Jan 2007	No	>10	small to large
Expansive annular lesions											
Chilean_19	*C. eutropia*	Palena province, CL	FR	U	Calf	body	acute	11 Feb 2003	No	1 to 5	small to large
ES-Pcr	*P. crassidens*	Rio Grande do Sul, BR	ST	M	Calf	body	acute	12 Sep 2009	Yes	>30	small to large

BR = Brazil, CL = Chile, EC = Ecuador, PE = Peru

Fjord (F. Viddi, unpub. data); (iii) 1420mm for a male killer whale, southern Brazil (Durban and Parsons, 2006); or mean dorsal fin height of (iv) 155mm, n = 10 in Guiana dolphins, Sepetiba Bay (L. Flach, unpub. data), (v) 130mm, n = 30 in Guiana dolphins, Paranaguá Estuary (M. Santos, unpub. data); or (vi) 2120mm standard body length of false killer whale calf, southern Brazil[7]; or (vii) 860mm fluke span for adult false killer whale, Santa Elena (Fish, 1998). The lesions were further classified as small (< 10mm), medium-sized (11-20mm) or large (> 20mm) on the basis of their greatest relative diameter.

Results

Green-brown plaques

Medium-sized to large GBP, some coalescing, some raised, were seen dorsally in two likely-adult Guiana dolphins of unknown sex, traveling side-by-side (Fig. 1), in a turbid-water area of the Cananéia Lagoon estuary (24°54'S, 47°49'W), São Paulo State, Brazil, on 27 August 2009. Their behaviour was normal during the 50min observation period. Water temperature was 21°C, depth ranged 2.8-6m and salinity was 0ppm. The plaques did not seem associated with other pre-existing traumas and covered 20-30% of VBS. The dolphins showed no other photo-identifiable natural marks and were not sighted again, impeding evaluation of progress of the condition. Interestingly, GBP were not detected in the Cananéia population during winter (June-July) surveys of the estuary in 2010 with an average water temperature of 18°C.

Orange patches

OPA were recognized in three (1.4%, n = 209) photo-identified, free-ranging Guiana dolphins in Sepetiba Bay (23°00'S, 43°96'W), in 2005-2008. Estimated to be two-months old, calf SEP-43 showed numerous disseminated patches on the left flank (Fig. 2). Two adults had each a minimum of five blotches on the dorsum, flanks and dorsal fin. OPA generally were not associated with pre-existing skin traumas or scars (Table 2) and covered *ca*. 5-10% of VBS. Some smaller blotches coalesced to form larger ones. With none of the dolphins re-sighted, progress could not be evaluated. The three cases occurred in winter (June and July).

Cutaneous nodules

Several medium to large NOD were observed on the dorsum and flanks of a solitary adult male killer whale free-ranging in the coastal waters of northern Paraná (25°20'S, 45°05'W), southern Brazil, in March 2005. NOD were still present when this specimen was photographed off Praia de Maresias, São Paulo (23°48'S, 45°23'W) in January 2007 (Fig. 3, Lodi and Farias-Junior, 2011), southeastern Brazil. The killer whale was seen again repeatedly in 2008-2010 along the coasts of São Paulo and Rio de Janeiro states (Lodi and Farias-Júnior, 2011). Though image resolution was insufficient for skin disease assessment, the condition evidently had not been lethal in this five-year period.

Figure 3. Cutaneous nodules in a male killer whale *Orcinus orca* off Praia de Maresias, São Paulo, January 2007.

Figure 4. (a) Acute pale dermatitis in Guiana dolphin *Sotalia guianensis* SEP-41 from Sepetiba Bay on 28 October 2007; (b) Convalescent stage of pale dermatitis in SEP-41 on 4 March 2008.

Pale dermititis

Acute PAD lesions ranged in colour from off-white to light grey, and could affect as much as 35-40% of VBS, specifically on the dorsum, dorsal fin, flanks, flukes and rostrum (Fig. 4a;

[7]E. Secchi, pers. comm. to MFB, 17 November 2009

Figure 5. (a) Acute, ulcerated pale dermatitis in bottlenose dolphin *Tursiops truncatus* PBD-15 from Paracas Bay on 10 November 2004. (b) Healed pale dermatitis in PBD-15 on 18 January 2005.

Figure 6. (a) Expansive annular lesions in a Chilean dolphin calf *Cephalorhynchus eutropia* from the Northern Fjords, Chile, on 13 February 2003; (b) the lesions have expanded and coalesced by 20 February 2003.

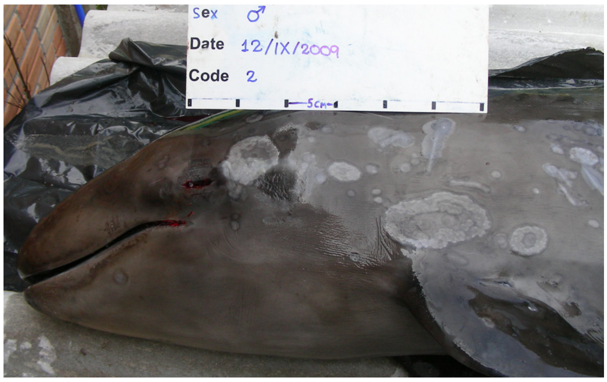

Figure 7. Expansive annular lesions in a false killer whale calf *Pseudorca crassidens* from Southern Brazil on 12 September 2009.

Table 2). When clinical features of the disease regressed, the epidermis gradually re-pigmented, the lesions lost their raised and velvety aspect, and skin ulcers resolved. Although normal pigmentation eventually returned, light-grey blotches remained visible in some animals. When present in a population, prevalence of the disorder varied from 1–6.9%.

Case study 1: Sepetiba Bay

Minimum population prevalence of acute PAD was 2.3% in 222 Guiana dolphins from Sepetiba Bay, in 2005-2008. All affected dolphins were adults. Specifically, prevalence was 1.4% in 209 free-ranging dolphins, 20% in 10 by-caught specimens and null in three stranded specimens (Table 1). In live dolphins SEP-33 and SEP-41 the lesions had a velvety aspect (Fig. 4a). In SEP-33 PAD was associated with a deep incision scar in front of the dorsal fin, possibly inflicted by fishing gear (Van Bressem *et al*., 2007), and with toothrakes on the left flank. First observed in July 2005, the lesions faded over a six-month period and light-grey blotches were all that remained in January 2006. In SEP-41, the sores were first seen in October 2007 and were mainly associated with scars. Though severe (Fig. 4a) they resolved in 4.5 months leaving only greyish blemishes (Fig. 4b). Recrudescence of acute PAD was not observed. PAD lesions on the lower jaw and gape in mature males SEP-17, SEP-24 and SEP-3 were also associated with minor injuries and scars (Table 2). In Sepetiba Guiana dolphins, pale dermatitis affected an estimated 1-40% of VBS. Healing skin lesions, possibly PAD, were present on the rostrum, dorsal fin and dorsum of four other free-ranging Guiana dolphins (Table 2).

Case study 2: Paranaguá Estuary

In the Paranaguá Estuary (25°22'S, 48°25'W), Paraná state, southern Brazil, PAD prevalence was 1% in 103 Guiana dolphins in 2006-2008. Likely-regressing PAD, not associated with skin traumas, affected some 10% of VBS in a free-ranging adult Guiana dolphin in March 2007. The dermatitis had completely healed by early August 2007.

Case study 3: Paracas Bay

Acute PAD struck 6.9% of 87 photo-identified inshore bottlenose dolphins in the Paracas Bay (Paracas National Reserve, 13°49'S, 76°16'W), south-central Peru, in 2004-2009 (Table 1). Five cases occurred during winter months (June-August) and the sixth case also recurred in July (Table 2). The reason for this apparent seasonality is unknown. In all acutely affected dolphins PAD lesions covered *ca*. 3-20% of VBS, were sometimes ulcerated and associated with toothrakes and other minor skin lacerations. The epidermis of the dorsal fin of two dolphins (PBD-15 and PBD-29) was ulcerated when first observed, exposing the dermis (Fig. 5a). The skin subsequently healed over a period of, respectively, two and six months, in 2004-2005 (Fig. 5b), leaving a white scar in PBD-15 and light-grey marks in PBD-29. A milder recrudescence was noted in PBD-15 in July 2007. The lesions had healed again by July 2008 and no further dermatitis was observed in 2009. The velvety sores detected in July 2006 in PBD-55 had partially regressed by February 2008. Light grey marks surrounded linear incisive injuries on the dorsum of dolphins PBD-41 (June 2009) and PBD-78 (August 2009). Transient in the bay, female PBD-60 had severe PAD lesions on the dorsum and flanks in July 2006, after which she was not seen again. White scars evoking healed PAD were also present on the dorsal fin of PBD-030 in August 2005. With the exception of one old female without skin condition, no inshore bottlenose dolphins were found stranded during beach surveys in the Paracas Bay area in 2004-2009. Notably, no skin conditions were observed in 1999-2004 among a group of 15 inshore bottlenose dolphins resident in Supay-Arquillo (Table 1), a less-contaminated area on the south coast of the Paracas Peninsula, shielded from anthropogenic influences. Furthermore, acute PAD was not detected in 84 closely examined, freshly dead bottlenose dolphins of both inshore and offshore populations (*sensu* Van Waerebeek *et al*., 1990) taken in fisheries off central Peru in 1985-1995 (Table 1).

Case study 4: Chanduy

An adult male false killer whale of a group of about 60 individuals that stranded at Chanduy (02°25'S, 80°40'W), Santa Elena Peninsula, Ecuador, in November 1992 had large, pale, PAD-type velvety marks affecting approximately 20% of the flukes. One of them, located on the anterior border of the left fluke, measured 450x110mm and was associated with a fresh wound. Prevalence was 3.6% for 28 examined carcasses.

Expansive annular skin lesions

Case study 1: Reñihue Fjord

An estimated one month old Chilean dolphin calf at Reñihue Fjord (42°34'S, 72°30'W), Palena Province, Chile, had large EAL on the dorsum flanks and head on 11 February 2003. The core lesion had an orange tinge and was surrounded by a light grey ring. Another lesion seemed ulcerated. By 13 February, the calf's EAL condition had progressed, *i.e*. the core lesion on its right flank had increased in diameter and became erythematous, while new blemishes were visible on both flanks, dorsum and head (Fig. 6a). Medium-sized grayish and ulcerative sores also affected the dorsal fin of the accompanying resident female CE065, presumably the mother. Over the next few days new sores kept emerging in the calf, existing ones expanded and merged, then turned light grey (Fig. 6b). The calf was last seen on 24 February with an estimated 30-40% of the VBS affected. During the 14 days of observation it struggled to breathe and swim. Female CE065 was repeatedly re-sighted alone in March and April 2003 and, presumably, the calf had died. Prevalence of the disease in 15 Chilean dolphins from Reñihue Fjord was 6.7% in 2002-2004. Though TSD was present in this population at that time (Van Bressem *et al*., 2009*b*), no typical tattoo lesions were detected in the calf or its mother.

Case study 2: Rio Grande do Sul

A male false killer whale calf washed ashore dead in Rio Grande do Sul, Brazil, in September 2009[7] with numerous, small to large, light grey EALs disseminated over its body (Fig. 7). Some lesions were associated with pre-existing linear scars and covered *ca*. 15% of the left side. It is suspected that the calf death was related to the cutaneous condition. Skin samples were collected in 10% formaldehyde and await analysis.

Discussion

With one exception, none of the observed cutaneous conditions could be biopsied or otherwise sampled and their aetiology remains unknown. However, the fact that phenotypically recognizable clinical symptoms occurred in photo-identified individuals, allowing assessment of disease progression and survival of several dolphins, deserved their documentation. Photographic examination has proven useful to classify skin disorders and study their epidemiological features in cetaceans worldwide (Wilson *et al*., 1997; 1999; Van Bressem *et al*., 2003; 2009*b, c*; 2013; Maldini *et al*., 2010; Daura-Jorge and Simões-Lopes, 2011; Bertulli *et al*., 2012). Time series of the affected VBS fraction provides a safe and practical tool for monitoring the evolution and severity of skin conditions (Van Bressem *et al*., 2003; Murdoch *et al*., 2008; Kiszka *et al*., 2009; Riggin and Maldini, 2010; Daura-Jorge and Simões-Lopes, 2011; this paper). Nevertheless, collection of samples from freshly dead animals is urgently needed to investigate the aetiologies of cutaneous disorders. Biopsy sampling with invasive techniques such as darts delivered by crossbows should however be carefully evaluated on a case-by-case base for the risk that a cutaneous pathogen may become inoculated in underlying tissues or even the bloodstream, apart from the inevitable harassment of chasing potentially weakened animals.

During this study green-brown plaques were observed only in a pair of Guiana dolphins in a freshwater area of the Cananéia Estuary on 27 August 2009. GBP were not seen in 20 Guiana dolphins swimming in a slightly more saline (4ppt) part of the estuary on the same day. However, a very similar disorder to GBP was recognized from photographs of seven bottlenose dolphins trapped in Lake Pontchartrain, southeastern Louisiana, USA, in April-May 2008, and also exposed to a hypo-saline environment (salinities, 1-3ppt; Barry *et al*., 2008). We suggest that fresh or very low salinity waters may facilitate, or even trigger, the development of GBP-like syndrome in marine odontocetes. The prognosis of GBP affected dolphins is unclear.

Present cases of orange patches are highly reminiscent of epidermal diatoms in Dall's porpoises *Phocoenoides dalli* and harbour porpoises *Phocoena phocoena* in the North Pacific (Holmes *et al*., 1993; Norman *et al*., 2004). The pathogenic significance of diatoms is limited (Norman *et al*., 2004), if not nihil. Guiana dolphins with OPA were only observed during the winter in Sepetiba Bay, when mean sea surface temperature was 23.9°C (Flach *et al*., 2008). We suggest that 'orange film' and 'orange hue', 'orange patches' described in bottlenose dolphins from, respectively, the British Isles (Wilson *et al*., 1997) and California (Maldini *et al*., 2010) are equivalent to our OPA. Diatoms have long been reported from blue *Balaenoptera musculus* and fin *B. physalus* whales in the Southern Ocean (Bennett, 1920), as well as from sperm whales *Physeter macrocephalus* off South Africa (Best, 1969).

The cutaneous nodules seen in the single killer whale persisted at least 21 months. A nodular skin disease resembling lobomycosis was observed in 2006-2007 in 14 Guiana dolphins from the Paranaguá Estuary, close to the Cananéia Estuary (Van Bressem *et al*., 2009*c*). However, clinical manifestations were different *i.e*. raised, grey, orange or reddish, sometimes ulcerated, skin lumps (Van Bressem *et al*., 2009*c*). Cutaneous nodules unrelated to lobomycosis were reported in free-ranging Indo-Pacific bottlenose dolphins *Tursiops aduncus* from Japan, in Irrawaddy dolphins *Orcaella brevirostris* from Malaysia, India and Bangladesh, and in common minke whales *Balaenoptera acutorostrata* and a white-beaked dolphin *Lagenorhynchus albirostris* from Icelandic waters (Bertulli *et al*., 2012; Van Bressem *et al*., 2013; 2014). Though likely infectious their aetiology is still unknown.

Acute pale dermatitis appears consistent with 'white lesions', 'cream lesions' and 'white fin-fringe lesions' described from bottlenose dolphins in Scotland (Wilson *et al*., 1997) and perhaps the 'white lesions' in North Atlantic right whales *Eubalaena glacialis* (Hamilton and Marx, 2005). Creamy-white lesions on dorsum and dorsal fin, most likely PAD, were also reported in 1999 from more than a third of *ca*. 120 bottlenose dolphins resident in the Gambia River estuary, West Africa. A large part of this community abandoned the estuary for several months and apparently moved towards open sea, thus impacting local dolphin-watching operations. When dolphins returned to the estuary, skin lesions had cleared[8]. In the present study PAD was encountered in three delphinid species in tropical and subtropical waters from South America. Although some cases appeared severe, prognosis was good with most affected dolphins partially or entirely clearing symptoms over a six-month period. Recrudescence occurred in at least one specimen. PAD seems to be a chronic, self-limiting infectious disorder for which there is no evidence of associated mortality, although overall fitness may be reduced to some degree. We suggest that toothrakes and other minor underlying skin traumas may offer routes of entry for superinfecting pathogens. Of 11 animals with active PAD in Peru and Brazil, eight were seen in winter (June-August) and a ninth case, first diagnosed in November 2004, regressed in summer (January 2005) but was recrudescent in winter (July 2007). Low sea surface temperature may be a predisposing factor.

Macroscopically, EAL were reminiscent of the cutaneous sores observed in a bottlenose dolphin calf from the Sado Estuary, Portugal (Van Bressem *et al*., 2003), a bottlenose dolphin calf from Monterey Bay, California (Riggin and Maldini, 2010) and a Commerson's dolphin *Cephalorhynchus commersonii* from Argentinean Patagonia (Van Bressem *et al*., 2007). In the calf from the Sado Estuary where TSD was highly prevalent, the lesions were believed to be superinfected tattoos (Van Bressem *et al*., 2003). Poxvirus-induced tattoo lesions were also thought to have facilitated the entry of fungi and bacteria in an Atlantic white-sided dolphin *Lagenorhynchus acutus* and a pygmy sperm whale *Kogia breviceps* stranded on the US Atlantic coast in 1991 and diagnosed with mycotic dermatitis (Frasca *et al*., 1996). In South America, EAL were detected only in cetacean populations in cool and temperate waters, although populations in lower latitudes were also under scrutiny (Van Bressem *et al*., 2007). Besides, a similar condition was recognized in a humpback whale *Megaptera novaeangliae* photographed in Admiralty Bay (62°10'S, 058°25'W), King George Island, in the summer of 1994[9]. EAL may constitute a potentially severe, even lethal, cetacean disease. Of two affected calves, one was found dead with disseminated EAL lesions and the other in morbid condition disappeared and almost certainly died within two weeks after the condition was first diagnosed.

With the exception of two presumably oceanic false killer whales, all PAD and EAL-affected cetaceans inhabited biologically and chemically degraded nearshore waters. The community of inshore bottlenose dolphins studied in Peru ranges in a coastal strip between Paracas Bay and Tambo de Mora which is often heavily affected by raw sewage from surrounding towns (El Chaco, San Andrés, Pisco) and vast quantities of organic material released by fish-meal factories,

[8]Van Waerebeek, K., Barnett, L., Camara, A., Cham, A., Diallo, M., Djiba, A., Jallow, A.O., Ndiaye, E., Samba Ould Bilal, A.O. and Bamy, I.L. (2003) Conservation of cetaceans in the Gambia and Senegal 1999-2001, and status of the Atlantic humpback dolphin. WAFCET-2 Report, UNEP/CMS, Bonn, Germany. 55 pp.
[9]E. Secchi, pers. comm. to MFB, 15 February 1996

resulting in severe eutrophication[10]. The factories also release effluent with caustic soda when cleaning machinery[11]. In addition, ballast water from cargo ships entering Paracas Bay to dock at the Camisea Liquefied Natural Gas (LNG) platform or San Martín harbour may introduce alien, potentially dangerous, micro-organisms[10] (Ruiz *et al*., 2000; Drake *et al*., 2007). Coastal development including new beach-front hotels and other tourist facilities along the shores of the Paracas Bay, the expansion of the LNG plant and increasing ship traffic represent accumulating challenges to the inshore bottlenose dolphin communities of Ica department. Brazil's Paranaguá Estuary also has a large port, surrounded by expanding urbanization. An illegal shrimp farm operated in 2006-2007 (M. Santos, pers. obs) and may have adversely affected the Paranaguá Estuary habitat. Home to two large ports, Sepetiba Bay suffers chemical and organic aquatic pollution and eutrophication (Copeland *et al*., 2003; Molisani *et al*., 2004). Chile's Reñihue Fjord was home to three open-net salmon farms which heavily consumed prophylactic antibiotics and released biological and chemical contaminants directly into the ocean in 2003-2004 (Cabello, 2004; 2006). The two cases of EAL and PAD in false killer whales occurred off the coasts of heavily polluted regions. Although banned in much of the Northern Hemisphere since the 1970s due to their high toxicity to humans and wildlife, several organochlorine compounds are still widely used in South America[12]. In Rio Grande do Sul, one of the most industrially developed regions of southern Brazil, organochlorine pesticides supporting high agricultural production have long entered the southwest Atlantic ecosystems (Marsili, 2000). Salinas, in Ecuador's Santa Elena Province, features a major yacht marina, a large fishing port for artisanal and industrial vessels as well as a port for petroleum tankers, leading to heavy maritime traffic. The adjacent coast boasts a string of densely-populated seaside resorts and is characterized by high levels of microbiological and chemical pollution[13]. Intense shrimp-farming and fishing plants in this area also produce large quantities of sludge and organic material[14].

The exponential increase in reports of, often severe, skin disorders in cetaceans worldwide (Van Bressem *et al*., 1993; 2007; 2009*c*; 2013; Wilson *et al*., 1999; Reif *et al*., 2006; Barry *et al*., 2008; Maldini *et al*., 2010; Riggin and Maldini, 2010; Daura-Jorge and Simões-Lopes, 2011; Bertulli *et al*., 2012; Fury and Reif, 2012) cannot alone be explained by intensified field research, and suggests a causal link with markedly deteriorating coastal environments. Climate change may also play a role in the emergence of skin diseases by increasing pathogen survival through higher water temperatures and by changing pathogen and host interaction dynamics (Burek *et al*., 2008). Mounting levels of solar ultraviolet radiation may further favour the development of cutaneous diseases by inducing DNA damage in the epidermis (Bowman *et al*., 2013).

We conclude that at least two (EAL and PAD) of the five skin conditions described here may be associated with, and indicative of, a compromised nearshore environment. Their emergence in small populations of vulnerable odontocetes is of particular concern and should be closely monitored.

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