

# Epidemiological pattern of tattoo skin disease: a potential general health indicator for cetaceans

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**ABSTRACT:** The presence of tattoo skin disease (TSD) was examined in 1392 free-ranging and dead odontocetes comprising 17 species from the Americas, Europe, South Africa, New Zealand and Greenland. We investigated whether TSD prevalence varied with sex, age and health status. TSD was encountered in cetaceans from the Pacific and Atlantic Oceans as well as in those from the North, Mediterranean and Tasman Seas. No clear patterns related to geography and host phylogeny were detected, except that prevalence of TSD in juveniles and, in 2 species (dusky dolphin *Lagenorhynchus obscurus* and Burmeister's porpoise *Phocoena spinipinnis*), in adults was remarkably high in samples from Peru. Environmental factors and virus properties may be responsible for this finding. Sex did not significantly influence TSD prevalence except in the case of Peruvian *P. spinipinnis*. Generally, there was a pattern of TSD increase in juveniles compared to calves, attributed to the loss of maternal immunity. Also, in most samples, juveniles seemed to have a higher probability of suffering TSD than adults, presumably because more adults had acquired active immunity following infection. This holo-endemic pattern was inverted in poor health short-beaked common dolphins *Delphinus delphis* and harbour porpoises *Phocoena phocoena* from the British Isles, and in Chilean dolphins *Cephalorhynchus eutropia* from Patagonia, where adults showed a higher TSD prevalence than juveniles. Very large tattoos were seen in some adult odontocetes from the SE Pacific, NE Atlantic and Portugal's Sado Estuary, which suggest impaired immune response. The epidemiological pattern of TSD may be an indicator of cetacean population health.

**KEY WORDS:** Tattoo skin disease · Poxviruses · Cetaceans · Epidemiology · Health status

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## INTRODUCTION

Tattoo skin disease (TSD) in cetaceans is characterised by irregular, grey, black or yellowish, stippled skin lesions that may occur on any part of the body but show a preferential distribution depending on the species (Van Bresseem & Van Waerebeek 1996). With some experience, tattoo lesions (or 'tattoos') are readily distinguished macroscopically from other types of integument blemishes and scars. Individual tattoos may persist for months, or even years, and recur. They eventually heal and convert into light grey marks that may or may not have a darker outline and a darker centre (Van Bresseem et al. 2003). TSD has been observed in several species of free-ranging odontocetes in the North Atlantic and eastern Pacific Oceans and in the Mediterranean Sea, as well as in captive common bottlenose dolphins *Tursiops truncatus* (see Van Bresseem et al. 1999). It was also recently reported in an Alaskan bowhead whale *Balaena mysticetus*, though no images of the lesions were provided (Bracht et al. 2006). TSD is caused by poxviruses (Flom & Houk 1979, Geraci et al. 1979, Van Bresseem et al. 1993) that belong to a new genus of the subfamily *Chordopoxvirinae* (family *Chordopoxviridae*), but have a common, most immediate ancestor with terrestrial poxviruses of the genus *Orthopoxvirus* (Bracht et al. 2006). Poxviruses affecting Delphinidae and Phocoenidae belong to different species (Pearce et al. 2008). They are thought to induce humoral immunity that protects neonates and young calves from the disease (Smith et al. 1983, Van Bresseem & Van Waerebeek 1996, Van Bresseem et al. 2006a). Published and unpublished observations of tattoo occurrence linked to age–growth data suggest that passive immunity in Peruvian dusky dolphins *Lagenorhynchus obscurus* and Burmeister's porpoises *Phocoena spinipinnis* as well as in *T. truncatus* from the Sado Estuary, Portugal, lasts at least until 6 to 9 mo of age (Van Bresseem & Van Waerebeek 1996, Chávez-Lisambart 1998, Van Bresseem et al. 2003).

Though clinical and epidemiological data do not indicate that poxvirus infection induces a high mortality rate when endemic, it may kill neonates and calves without protective immunity and may affect host population dynamics (Van Bresseem et al. 1999). TSD, for instance, may have contributed to the decline of *Tursiops truncatus* from the Sado Estuary by possibly affecting juvenile survival. Besides, the presence of very large tattoo lesions and their persistence (over 3 years) in adults were suggestive of immune deficiencies (Van Bresseem et al. 2003). In search of a general epidemiological pattern of TSD and of the potential relation between health status and TSD epidemiology, we studied the occurrence of tattoo lesions in cetaceans from several ocean provinces, the results of which we present here.

## MATERIALS AND METHODS

The presence of TSD was examined in 1392 individuals of 17 cetacean species from the Pacific, the Atlantic and the southwestern Indian Oceans, as well as from the North, Baltic, Mediterranean and Tasman Seas. Animals were free-ranging ( $n = 468$ ), had stranded ( $n = 182$ ), had died traumatic deaths ( $n = 741$ ) in fisheries interactions (most of them) and from inter-specific aggression (a few) or died from an unknown cause ( $n = 1$ ) (Tables 1 & 2).

The cetaceans studied occupied all types of habitat, ranging from inshore/estuarine (waters of the shallower parts of the continental shelf, including those semi-enclosed by land, near river estuaries and the entrance of fjords), neritic (continental shelf waters up to about 200 m depth) and oceanic (waters beyond the shelf with a depth greater than 200 m) (Tables 1 & 2). Populations that straddled 2 habitats were assigned to the habitat in which they spend most time. Raw data that led to previously published papers on the epidemiology of TSD in Peruvian small cetaceans (Van Bresseem & Van Waerebeek 1996) and *Tursiops truncatus* from the Sado Estuary (Van Bresseem et al. 2003) were re-analysed.

**Dead specimens.** The majority of specimens examined died entangled in nets or stranded in the period 1984 to 2008 (Tables 1 & 2). Condition varied from fresh to early decomposition and most had intact skin. The entire body surface was examined for tattoos. Several specimens were frozen before examination. Sexual maturity was determined directly from a macroscopic and/or histological examination of the genital tract and mammary glands or was inferred from standard body length and life history parameters for these populations (Collet & Saint Girons 1984, Slooten 1991, Van Waerebeek 1992, Calzada 1995, Lockyer 1995, Reyes & Van Waerebeek 1995, Peddemors 1999, Duignan et al. 2003). The age of some animals was determined by counting growth layer groups in teeth (Perrin & Myrick 1980, Hohn et al. 1989, Slooten 1991, Duignan et al. 2003, P. J. Duignan & C. Lockyer unpubl. data).

**Free-ranging dolphins.** Skin lesions in free-ranging *Tursiops truncatus* from Slovenia, Portugal and Peru, Guiana dolphins *Sotalia guianensis* from Brazil and Chilean dolphins *Cephalorhynchus eutropia* from northern Patagonia, Chile, were detected from photos taken during small-boat surveys (Reyes et al. 2002, Van Bresseem et al. 2003, Viddi et al. 2005, Flach et al. 2008, Genov et al. 2008). Considering that in these animals generally only upper body parts were visible, the reported prevalences represent minimum values. Dolphins were individually identified from natural marks (Würsig & Jefferson 1990). Maturity status (calf, juvenile, adult) was estimated from relative body size and

Table 1. Characteristics of 30 samples of odontocetes (N = 1322) examined for presence of tattoo skin disease

Code	Species	Ocean province (country, locality)	Habitat	Sampling period	Sample size
<b>Free-ranging</b>					
1f	<i>Tursiops truncatus</i>	Mediterranean Sea (Slovenia, northern Adriatic Sea)	Inshore	2002–2006	80
2f	<i>Tursiops truncatus</i>	NE Atlantic (Portugal, Sado Estuary)	Inshore/estuarine	1994–1997	35
3f	<i>Tursiops truncatus</i>	SE Pacific (Peru, Paracas Bay)	Inshore	2004–2008	79
4f	<i>Sotalia guianensis</i>	SW Atlantic (Brazil, Sepetiba Bay)	Inshore/estuarine	2005–2008	206
5f	<i>Cephalorhynchus eutropia</i>	SE Pacific (Chile, Northern Patagonia)	Inshore/fjords	2002–2004	13
<b>Bycatch, <i>T. truncatus</i> attack<sup>a</sup> or other traumas of unknown origin</b>					
1b	<i>Delphinus capensis</i>	SW Indian (South Africa, KwaZulu-Natal)	Neritic	2000	25
2b	<i>Tursiops aduncus</i>	SW Indian (South Africa, KwaZulu-Natal)	Inshore/estuarine	2000	19
3b	<i>Phocoena phocoena</i>	North Sea (British Isles)	Neritic	2004–2006	10
4b	<i>Phocoena phocoena</i>	NE Atlantic (British Isles)	Neritic	2004–2006	26
5b	<i>Phocoena phocoena</i>	Baltic Sea (Germany)	Neritic	1991–1995	8
6b	<i>Phocoena phocoena</i>	Greenland (Nuuk & Paamint)	Neritic	1995	14
7b	<i>Delphinus delphis</i>	NE Atlantic (British Isles)	Oceanic	2004–2006	18
8b	<i>Lagenorhynchus obscurus</i>	SE Pacific (Peru, central coast)	Neritic	1993–1994	196
9b	<i>Phocoena spinipinnis</i>	SE Pacific (Peru, central coast)	Neritic	1993–1994	77
10b	<i>Delphinus capensis</i>	SE Pacific (Peru, central coast)	Neritic	1993–1994	54
11b	<i>Tursiops truncatus</i>	SE Pacific (Peru, central coast)	Oceanic	1993–1994	12
12b	<i>Delphinus delphis</i>	SE Pacific (Ecuador, central coast)	Oceanic	1993	28
13b	<i>Sotalia guianensis</i>	SW Atlantic (Brazil, Northern Rio de Janeiro)	Inshore/estuarine	1988–2004	91
14b	<i>Pontoporia blainvillei</i>	SW Atlantic (Brazil, Northern Rio de Janeiro)	Neritic	1989–2005	104
15b	<i>Stenella frontalis</i>	SW Atlantic (Brazil, Northern Rio de Janeiro)	Neritic	1992 & 1996–1999	8
16b	<i>Cephalorhynchus hectori hectori</i>	SW Pacific & Tasman Sea (New Zealand, South Island)	Inshore	1997–2005	37
17b	<i>Sotalia guianensis</i>	SW Atlantic (Brazil, Sepetiba Bay)	Inshore/estuarine	2005–2008	8
<b>Stranded</b>					
1s	<i>Phocoena phocoena</i>	North Sea (British Isles)	Neritic	2004–2006	29
2s	<i>Phocoena phocoena</i>	NE Atlantic (British Isles)	Neritic	2004–2006	46
3s	<i>Phocoena phocoena</i>	North Sea (Germany)	Neritic	1991–1995	12
4s	<i>Delphinus delphis</i>	NE Atlantic (British Isles)	Oceanic	2004–2006	9
5s	<i>Stenella coeruleoalba</i>	Mediterranean Sea (Spain, Valencian Community)	Oceanic	2000–2007	40
6s	<i>Tursiops truncatus</i>	Mediterranean Sea (Spain, Valencian Community)	Likely inshore	2000–2006	8
7s	<i>Cephalorhynchus hectori hectori</i>	SW Pacific (New Zealand, South Island)	Inshore	1997–2005	27
8s	<i>Sotalia guianensis</i>	SW Atlantic (Brazil, Sepetiba Bay)	Inshore/estuarine	2005–2008	3

<sup>a</sup>Only detected in sample 4b, where at least 13 specimens died as a result of these attacks

Table 2. Other records of tattoo skin disease in cetaceans. S: stranded; B: bycatch; F: free-ranging; N:P indicates negative and positive cases in the sample analyzed

Species	Ocean province (country)	Habitat	Sampling period	Sample type	N:P
<i>Hyperoodon ampullatus</i>	North Sea (British Isles)	Oceanic	2006	S	0:1
<i>Phocoena phocoena</i>	NW Atlantic (Bay of Fundy, Canada)	Neritic	1984	B	0:1
<i>Phocoena phocoena</i>	NE Pacific (US)	Neritic	2007	Unknown	0:1
<i>Phocoena spinipinnis</i>	SE Pacific (central Chile)	Neritic	1998–2002	S & B	1:3
<i>Cephalorhynchus commersonii</i>	SW Atlantic (Patagonia, Argentina)	Neritic	2005	F	0:1
<i>Cephalorhynchus eutropia</i>	SE Pacific (Guaitecas Archipelago, Northern Patagonia, Chile)	Inshore/estuarine	2007	F	19:4 <sup>a</sup>
<i>Cephalorhynchus hectori maui</i>	Tasman Sea (North Island, New Zealand)	Inshore	1997–2003	S & B	2:1
<i>Delphinus delphis</i>	NE Atlantic (Cascais, Portugal)	Oceanic	1990	B	0:1
<i>Stenella coeruleoalba</i>	NE Atlantic and North Sea (British Isles)	Oceanic	2004–2006	S & B	3:1
<i>Sotalia guianensis</i>	SW Atlantic (Guaraquecaba, Brazil)	Inshore/estuarine	2007	F	0:1
<i>Lagenorhynchus australis</i>	SE Pacific (Guaitecas Archipelago, Northern Patagonia, Chile)	Inshore/estuarine	2007	F	21:8 <sup>a</sup>
<i>Tursiops truncatus</i>	SE Pacific (Choros Islands, Chile)	Inshore	1998	F	0:1

<sup>a</sup>Not all individuals of this community have yet been examined; hence prevalence estimates and statistical analysis would be premature

behavioural clues (Wells et al. 1980, Goodall et al. 1988, Shane 1990).

**Tattoo lesions.** Tattoos were identified on the basis of their typical appearance, i.e. irregular, dark gray, black or yellowish marks with a stippled pattern

(Fig. 1). The corporal topography of these marks as well as their number and relative size (small, medium, large and very large) were noted, though not systematically. Light gray, irregular marks surrounded by a black line were considered regressing tattoos

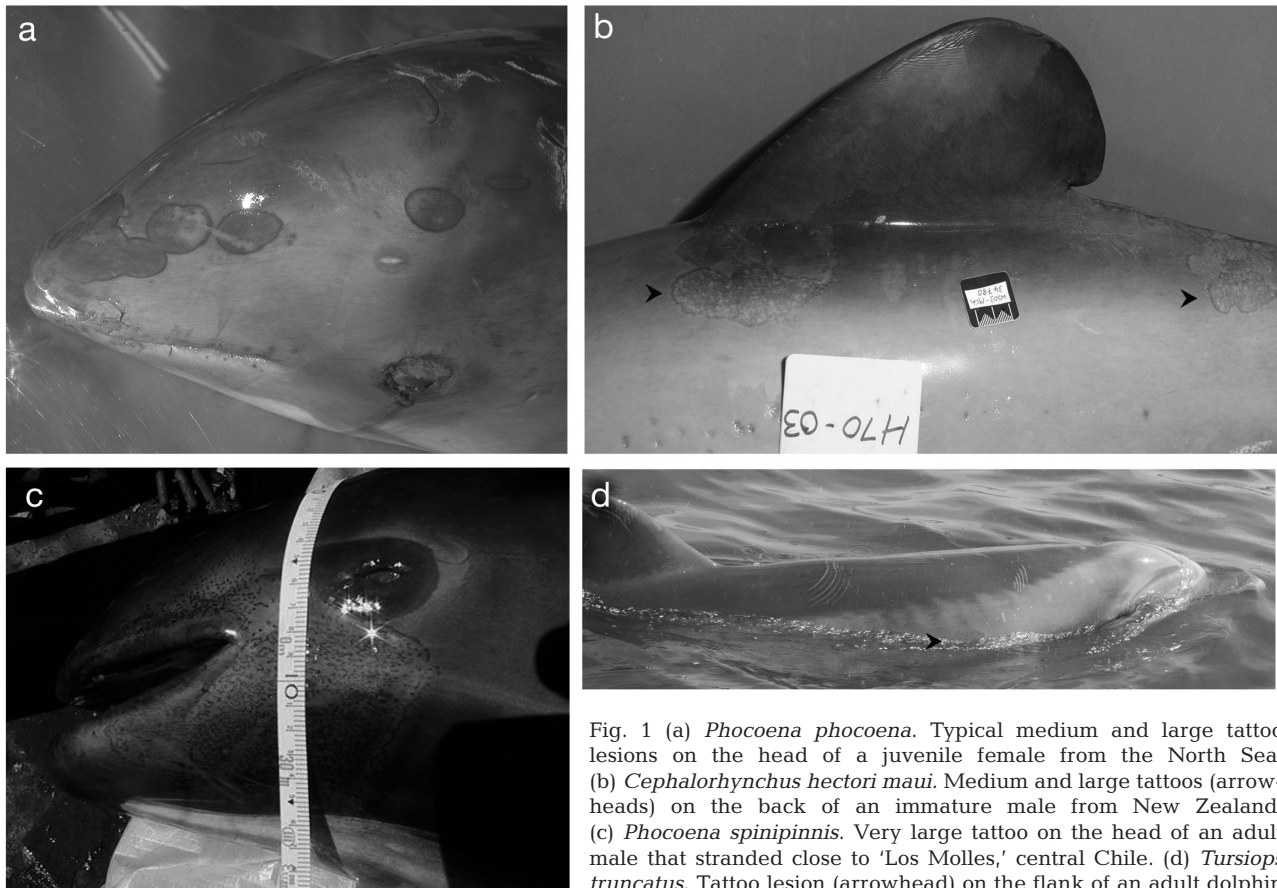


Fig. 1 (a) *Phocoena phocoena*. Typical medium and large tattoo lesions on the head of a juvenile female from the North Sea. (b) *Cephalorhynchus hectori maui*. Medium and large tattoos (arrowheads) on the back of an immature male from New Zealand. (c) *Phocoena spinipinnis*. Very large tattoo on the head of an adult male that stranded close to 'Los Molles,' central Chile. (d) *Tursiops truncatus*. Tattoo lesion (arrowhead) on the flank of an adult dolphin from Paracas Bay, Peru



Fig. 2. (a) *Sotalia guianensis*. Regressing tattoos (arrowheads) on the dorsum of a dolphin from Sepetiba Bay, Brazil. (b) *Cephalorhynchus eutropia*. Regressing tattoos (arrowheads) on dorsum and dorsal fin of an adult dolphin from Reñihue Fjord, northern Patagonia, Chile

(Fig. 2). Light gray, mostly rounded marks without a dark outline were regarded as healed lesions. To avoid bias, only animals observed by the authors were included. For most images the first author confirmed tattoo lesions. Only active tattoos, including regressing but not healed tattoos, were considered for the statistical analysis. TSD aetiology was confirmed by electron microscopy in Peruvian small cetaceans (Van Bresseem et al. 1993, Van Bresseem & Van Waerebeek 1996) and by PCR in harbour porpoises *Phocoena phocoena* and striped dolphins *Stenella coeruleoalba* from the British Isles (Pearce et al. 2008). Though investigations on the aetiology of TSD in cetaceans from other ocean provinces could not be carried out, it is likely that it was also caused by poxviruses. Indeed, poxviruses are the only infectious agents consistently observed by electron microscopy or detected by PCR in tattoos from several species of odontocetes (Flom & Houk 1979, Geraci et al. 1979, Van Bresseem et al. 1993, 1999, Bracht et al. 2006).

**Geographic distribution.** On the basis of the results of this study, published records (Geraci et al. 1979, Bossart et al. 2003, Bracht et al. 2006, Van Bresseem et al. 1993, 1999, 2006b, Bearzi et al. 2009) and unpub-

lished data archived at CEPEC, we mapped the known distribution of TSD.

**Statistical analyses.** With the exception of Peale's dolphin *Lagenorhynchus australis* and *Cephalorhynchus eutropia* communities from Guaitecas Archipelago, northern Patagonia, Chile, that are still under study, samples with  $n \geq 7$  animals, grouped by species, geographical region and sampling type (free-ranging, stranded and traumatic death) were considered as observational units for the statistical analysis (Table 1). Free-ranging and stranded odontocetes as well as cetaceans that had suffered a traumatic death were treated separately, as they differed in the screening effort for TSD and population representation. *Phocoena phocoena* from the British Isles was split into a North Sea population and a NE Atlantic population, including specimens from the English Channel and the Irish and Celtic Seas (see Donovan & Bjørge 1995). Short-beaked common dolphins *Delphinus delphis* from the British Isles were assumed to belong to a single population (Murphy et al. 2006) (Table 1). As the exact age of specimens was rarely available, 3 age classes were inferred from the correlation between maturity status and standard body length in each sam-

ple and defined as follows: (1) neonates and young calves until 6 to 9 mo<sup>1</sup> (hereafter referred to as calves), which are likely protected by maternal immunity in populations where the virus is endemic; (2) older calves, juveniles and subadults (hereafter referred to as juveniles), which are likely not protected anymore by passive immunity, have not yet acquired active immunity and thus are susceptible to TSD; and (3) sexually mature animals (adults) that may or may not have active immunity against the virus. Geographical variability in infection patterns of TSD was described based on a scatter plot of prevalences in juveniles and adults for each sample (note that calves were not

infected except in 1 case, see Table 3). Sterne's method was used to determine 95% CIs for prevalence values (Reiczigel 2003).

We investigated the effects of host sex and age class upon the epidemiological patterns of TSD separately. Sampling type may influence TSD detection, arguably the lowest in free-ranging animals, regardless of sex or age. Geography (e.g. spatial autocorrelation), phylogeny (e.g. similarity in host susceptibility) and virus strain/species (e.g. differences in virulence and infectiousness) may have contrasting effects with regard to sexual or age-related differences in cetacean poxvirus infections. We applied Zelen's test to pinpoint samples that deviated from the common pattern and made attempts to account for such deviations (see below). We further examined whether odontocetes that had died in poor health (PH, i.e. starvation, infectious and parasitic diseases) exhibited a higher prevalence of

<sup>1</sup>*Phocoena phocoena* <105.5 cm, Mediterranean *Stenella coeruleoalba* <120 cm, *Cephalorhynchus hectori* ssp. <90 cm, South American *Delphinus delphis* <150 cm, *Lagenorhynchus obscurus* <140 cm and *P. spinipinnis* <130 cm

Table 3. Occurrence of tattoo skin disease in cetaceans, grouped according to age and sex classes. Numbers separated by colons indicate specimens without:with tattoos. Unk.: animals for which the sex and/or age class were unknown. Population sample codes are provided in Table 1

Sample code	Calf			Juvenile			Mature			Unk.
	Male	Female	Unk.	Male	Female	Unk.	Male	Female	Unk.	
<b>Free-ranging</b>										
1f						1:0			79:0	
2f						5:5			23:2	
3f			4:0			0:2			14:2	57:0
4f									195:11	
5f						3:0			5:5	
<b>Bycatch, <i>T. truncatus</i> attack or other traumas of unknown origin</b>										
1b	1:0			4:0	3:0		11:0	6:0		
2b		1:0		8:0	3:0			7:0		
3b		1:0		2:0	1:1		4:0	1:0		
4b	1:0	3:0		7:0	7:0		2:0	6:0		
5b				4:0			1:0	3:0		
6b				1:0	3:0		5:0	5:0		
7b	1:0			5:0	1:1		5:0	5:0		
8b	7:0	9:0		16:20	18:15		34:16	38:23		
9b	2:0	4:0		4:18	4:5		4:20	11:5		
10b				9:27	2:4		9:1			1:1
11b				0:3	1:2		4:0	2:0		
12b	11:0	11:0		2:1	1:0		1:0			1:0
13b				24:0	13:0		31:0	21:0		2:0
14b				41:0	29:0		14:0	18:0		2:0
15b				3:0	4:0			1:0		
16b	2:0	1:0		3:4	4:1		12:2	5:1	1:0	0:1
17b				1:0			7:0			
<b>Stranded</b>										
1s	5:0	2:0		6:1	3:0		7:2	2:1		
2s	6:0	2:0		13:1	14:2		2:1	4:1		
3s				5:0	2:0		1:0	2:0		2:0
4s	1:0			1:0	1:0		0:1	4:1		
5s	2:0	4:1		8:1	6:1		8:0	9:0		
6s					3:1		1:0	2:0		1:0
7s	1:0	6:0		5:1	1:1		5:1	6:0		
8s	2:0			1:0						

TSD than those that had died a traumatic death (TD). This was only possible in *Phocoena phocoena* and *Delphinus delphis* from the British Isles (codes 3b, 4b, 7b, 1s, 2s, 4s, see Table 1), where thorough necropsies had been carried out and the cause of death determined unequivocally. Sparse and unbalanced data precluded the use of a single model including both factors (Table 3). For the same reason multivariate models further controlling for (at least) host phylogeny and spatial (geographical) autocorrelation (see Peres-Neto 2006 and references therein) would have generated highly biased results (see Agresti & Hartzel 2000). The potential influence of confounding factors, potential interactions between factors, or statistical dependency among observations upon the results obtained are further clarified in the discussion section. We sought general conclusions about the effect of host sex and age upon the likelihood of suffering TSD by combining results from a number of samples. Within this sort of 'meta-analytical' approach, we found it useful to estimate the central tendency and variability in effect sizes across samples rather than simply testing departures from a null hypothesis (Nakagawa & Cuthill 2007, Thompson 2007, Levine et al. 2008). Accordingly, statistical tests were accompanied by the estimation of the magnitude of effect sizes, and the precision of these estimates.

We selected 2 statistics for effect size, i.e. difference of prevalence (DP) and odds ratio. DP was chosen because (1) it is easily interpretable and (2) it is possible to set confidence limits for DP even when neither of the 2 samples to be compared was infected with TSD (see Agresti & Min 2001 for details). DPs between sexes were obtained as males minus females. DPs between age classes were obtained as juveniles minus calves, and juveniles minus adults; a positive DP was expected in both comparisons according to Van Bresseem & Van Waerebeek (1996). Exact 95% CI for DP was set in each sample by inverting a 2-sided unconditional test for difference of proportions (see Agresti & Min 2001 for details). The odds ratio measures the increase (or decrease) in odds of suffering TSD between selected groups (e.g. males vs. females). It was selected because it is one of the most important comparative risk measurements in epidemiology (Kahn & Sempos 1989). More significantly the odds ratio forms the basis of Mantel-Haenszel's test, which allows examining significant departures from a ratio of 1 using samples as a stratifying variable. The use of a single test allows a more precise estimate of a general sex or age class effect, and greatly enhances statistical power particularly when sample sizes are small (Agresti & Hartzel 2000). However, Mantel-Haenszel's test is meaningful only when odds ratios do not differ among samples. We used Zelen's exact test for homogeneity of odds ratios as it performs well for unbalanced designs with sparse

data (Reis et al. 1999). Assessment of homogeneity also allowed identifying those samples that departed from the common odds ratio pattern. Therefore, the test could be used indirectly to explore confounding effects of other factors that were not modeled (e.g. geography, phylogeny or sample type). For the group of samples for which homogeneity held, we calculated the 95% CI for the common odds ratio and carried out exact Mantel-Haenszel's tests for departure from 1 (Agresti & Hartzel 2000).

The software Quantitative Parasitology v. 3.0 (Reiczigel & Rószka 2005) was used for the calculation of confidence intervals for prevalence, and Statxact v.8 for the remaining statistical analyses. Exact tests (permutational p-values) were always preferred because sample sizes were small, sparse and unbalanced. However, when calculations were very time-consuming, a Monte Carlo estimate of p-values based on 100 000 random samples from the reference set was used.

## RESULTS

### Characteristics of the disease

Tattoos were typical in all affected species (Fig. 1). In dolphins and porpoises for which tattoo size was recorded, it ranged from  $10 \times 7$  to  $245 \times 245$  mm. Very large lesions ( $>150$  mm in at least 1 dimension) were seen in adults of samples 2f, 8b, 9b (Table 1), a *Phocoena phocoena* from the NE Atlantic (not included in statistics) and a *P. spinipinnis* from central Chile (Fig. 1c). Tattoo number per animal ranged from 1 to more than 50. They were distributed on the flanks, back, belly, throat, tailstock, head, dorsal fin and flippers. A preferential distribution was observed in *Lagenorhynchus obscurus*, *P. spinipinnis* and long-beaked common dolphins *Delphinus capensis* from Peru (Van Bresseem & Van Waerebeek 1996). In *P. spinipinnis* from Peru and Chile tattoos were more frequently seen on the head. Similarly, in *P. phocoena* these lesions were often seen on the head (55.5%) and flanks (55.5%) ( $n = 9$ ). Regressing tattoos and tattoo remains were observed in most species examined in this study (Fig. 2).

### Geographical pattern

Compiled TSD records from the Americas, Europe and New Zealand are presented in Fig. 3. The disease is widespread and affects various cetacean species from different habitats. The most southerly and northerly TSD records were detected in free-ranging *Lagenorhynchus australis* and *Cephalorhynchus eutropia* from the Guaitacas Archipelago ( $43^{\circ} 52' S$ ,

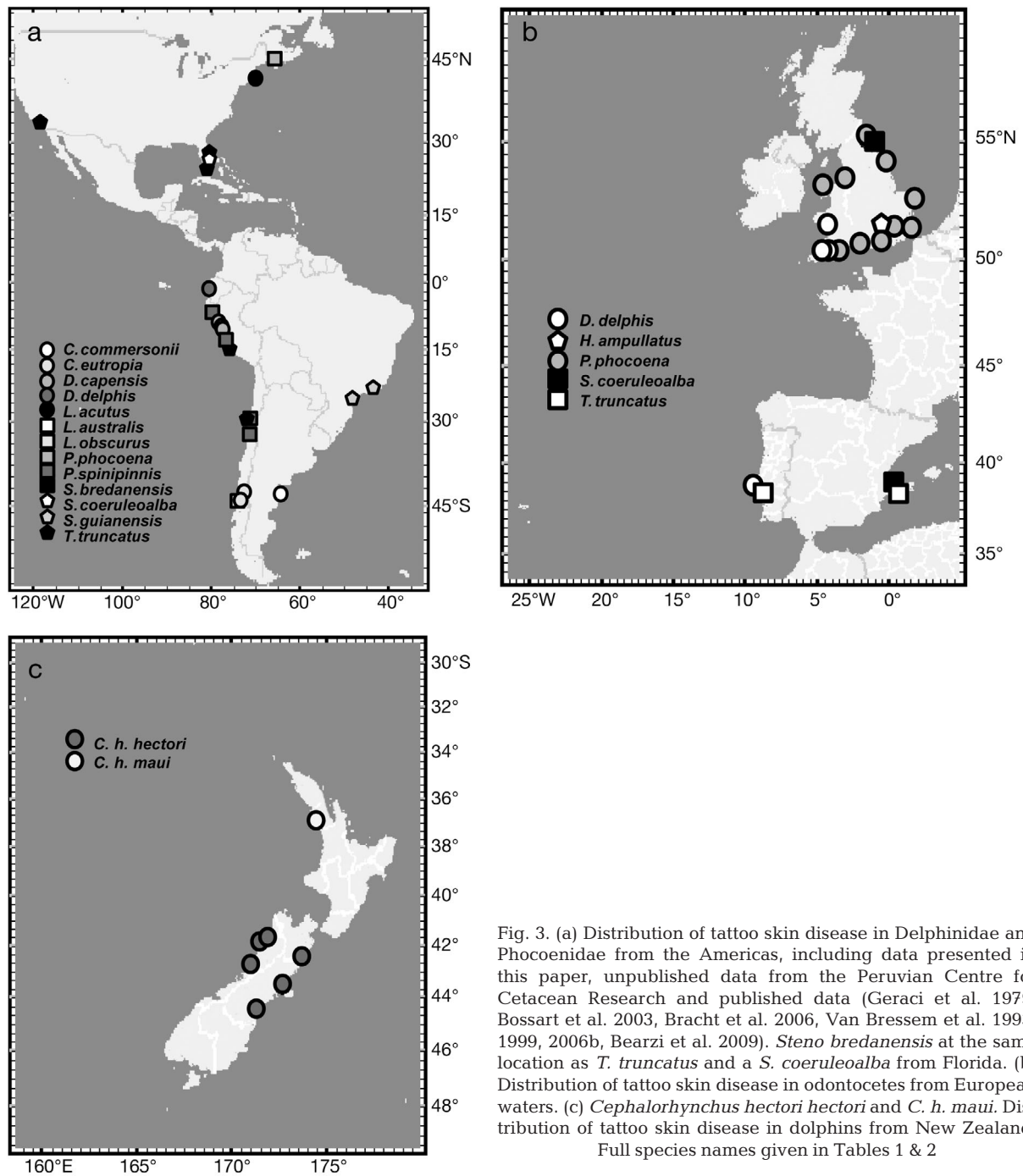


Fig. 3. (a) Distribution of tattoo skin disease in Delphinidae and Phocoenidae from the Americas, including data presented in this paper, unpublished data from the Peruvian Centre for Cetacean Research and published data (Geraci et al. 1979, Bossart et al. 2003, Bracht et al. 2006, Van Bressem et al. 1993, 1999, 2006b, Bearzi et al. 2009). *Steno bredanensis* at the same location as *T. truncatus* and a *S. coeruleoalba* from Florida. (b) Distribution of tattoo skin disease in odontocetes from European waters. (c) *Cephalorhynchus hectori hectori* and *C. h. maui*. Distribution of tattoo skin disease in dolphins from New Zealand. Full species names given in Tables 1 & 2

73° 45' W), Chile (Fig. 3a), and in a *Phocoena phocoena* stranded on the coast of Northumberland (55° 07' N 1° 30' W), UK (Fig. 3b), respectively. During this study TSD was not observed in the following samples: free-ranging *Tursiops truncatus* from the northern Adriatic (1f), bycaught *Delphinus capensis* (1b) and Indo-Pacific bottlenose dolphins *T. aduncus* (2b) from South Africa; bycaught *Sotalia guianensis* (13b), franciscana *Pontoporia blainvillei* (14b) and Atlantic spotted dol-

phin *Stenella frontalis* (15b) from northern Rio de Janeiro, Brazil; stranded and bycaught *P. phocoena* from the German North (3s) and Baltic Seas (5b) and Greenland (6b) (Table 1). However it was present in free-ranging *S. guianensis* from southern Rio de Janeiro (4f) and in *P. phocoena* from the British part of the North Sea (1s, 3b). In both cases, sample sizes seemed large enough to rule out that inconsistencies were caused by false negatives. In the case of *S. guia-*



*nensis* these observations may be related to the origin of the samples. Indeed, the *S. guianensis* from northern and southern Rio de Janeiro likely belong to 2 distinct communities which may not intermingle (A. P. di Benedetto & L. Flach pers. obs.) and thus may not share the same micro-organisms. The origin of the differences in prevalence between North Sea *P. phocoena* collected along the German and British coasts remains unknown. In many samples, the 95% CIs for prevalence were wide due to small sample size. No clear patterns related to geography and host phylogeny were observed, except that TSD prevalence in all juveniles as well as in adult *L. obscurus* and *Phocoena spinipinnis* tended to be higher in samples from Peru (Fig. 4).

**Sex**

Values of DP between males and females of each sample were scattered around 0 and the 95% CI included 0, except in male *Phocoena spinipinnis* from Peru (sample 9b), which exhibited a significantly higher prevalence than females (Fig. 5, Table 3). Note that the precision of DP estimates was generally low because of small sample sizes (Fig. 5).

The hypothesis of a homogeneous odds ratio for all samples could not be rejected (Zelen's exact test,  $n = 14$ ,  $p = 0.076$ ), nor could the hypothesis that the common odds ratio did not differ from 1 (exact  $p = 0.095$ ) with a point estimate of 1.44 (95% CI: 0.96 to 2.16). However, when sample 9b was removed, evidence that the null hypothesis of homogeneity should not be rejected was stronger (exact  $p = 0.963$ ), and the common odds ratio was far closer to 1 with a point estimate of 1.027 (95% CI: 0.665 to 1.60). We conclude that there is no evidence that sex influences TSD prevalence, except in the case of *P. spinipinnis* from Peru (see sample 9b in Fig. 5).

**Age class**

With the exception of a single specimen of *Stenella coeruleoalba* from the Mediterranean Sea, TSD was not detected in calves from any species for which this age class was represented (Table 3). Accordingly, a positive value of DP between juveniles and calves was generally observed (Fig. 6a). The hypothesis of homogeneous odds ratio for all samples could not be rejected (Zelen test = 0.019,  $n = 11$ , exact  $p = 0.066$ ) and the common odds ratio was 48.74 (95% CI: 8.78 to 1039.0), departing very significantly from 1 (Mantel-Haenszel test, exact 1-tailed  $p < 0.0001$ ). In summary, there is a general pattern of increase of TSD infections in juveniles compared to calves.

A positive trend was also observed in DP values of juveniles vs. adults (Figs. 4 & 6b), although in the 3 samples of stranded cetaceans from the British Isles (1s, 2s and 4s) and the sample of free-ranging *Cephalorhynchus eutropia* from southern Chile (5f),

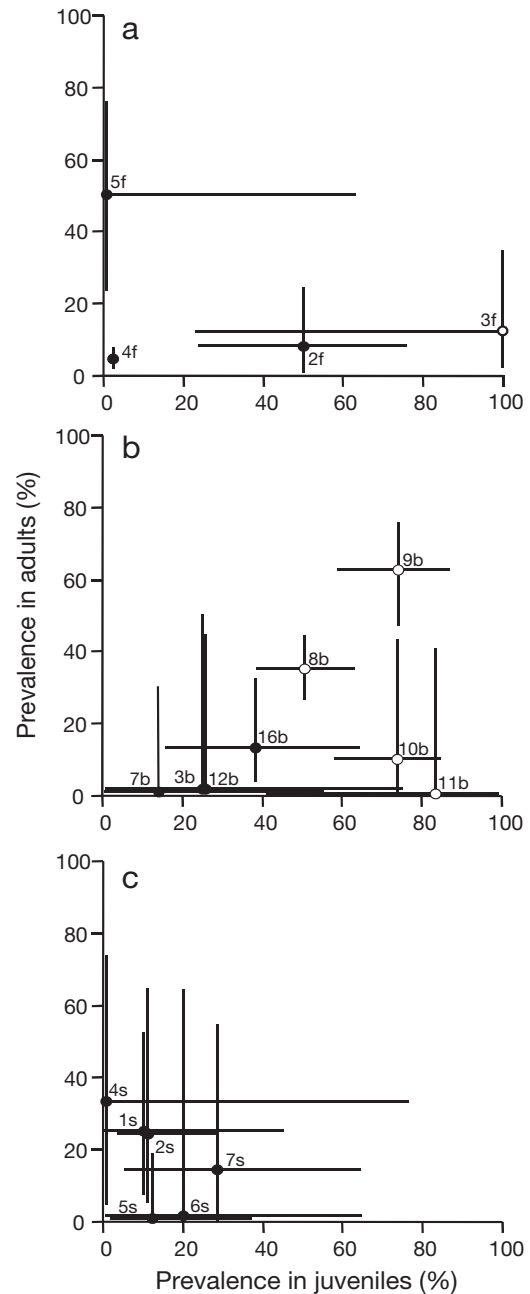


Fig. 4. Scatter-plot of prevalence (in percentage) of tattoo skin disease (TSD) in juvenile and adult cetaceans in samples from different geographical regions with at least 1 positive case. (a) Free-ranging dolphins. Note that the x coordinate for sample 4f is arbitrary as juveniles were not present in this sample (see Table 3). (b) Cetaceans that died a traumatic death. (c) Stranded odontocetes. Bars represent 95% CIs. Sample codes as in Table 1

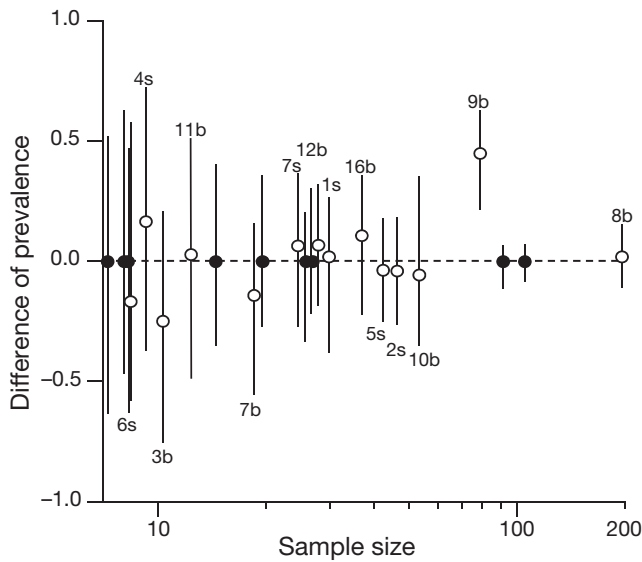


Fig. 5. Values of difference of prevalence (males minus females) of tattoo skin disease (TSD) in samples of cetaceans from different geographical regions. O: samples with at least a positive case for TSD; ●: samples in which TSD was not detected. Bars represent the 95% CIs. Sample codes as in Table 1

the prevalence of adults exceeded that of juveniles (Table 3; Fig. 6b). Odds ratios were not homogeneous across samples (Zelen statistic = 0.430,  $n = 17$ , Monte Carlo  $p = 0.0014$ ). When these 4 samples were removed, rejection of the null hypothesis of homogeneity was not accepted (Zelen statistic < 0.001,  $n = 13$ , Monte Carlo  $p = 0.061$ ). In this case the common odds ratio was 3.41 (95% CI: 2.21 to 5.29), with juveniles having significantly higher odds of suffering TSD (exact Mantel-Haenszel test, exact 1-tailed  $p < 0.0001$ ). Thus, there is a tendency for juveniles to have greater probability of TSD than adults, but this tendency can be inverted in some cetacean populations.

### Health status

We examined whether the health status could influence the probability of suffering from TSD by comparing animals from waters off the British Isles that died PH vs. TD. Samples comprised of 23 *Delphinus delphis* (negative:positive for TSD: PH 3:2; TD 17:1); 68 *Phocoena phocoena* from the NE Atlantic (PH 37:5; TD 26:0), 36 *P. phocoena* from the North Sea (PH 22:4; TD 9:1) and 2 *Stenella coeruleoalba* (PH 0:1 and TD 1:0) that were thoroughly necropsied. The difference in prevalence of TSD in animals that died PH vs. TD was always positive, ranging from 0.064 to 1. Odds ratios were homogeneous across samples (Zelen's statistic: 0.193,  $n = 4$ ,  $p =$

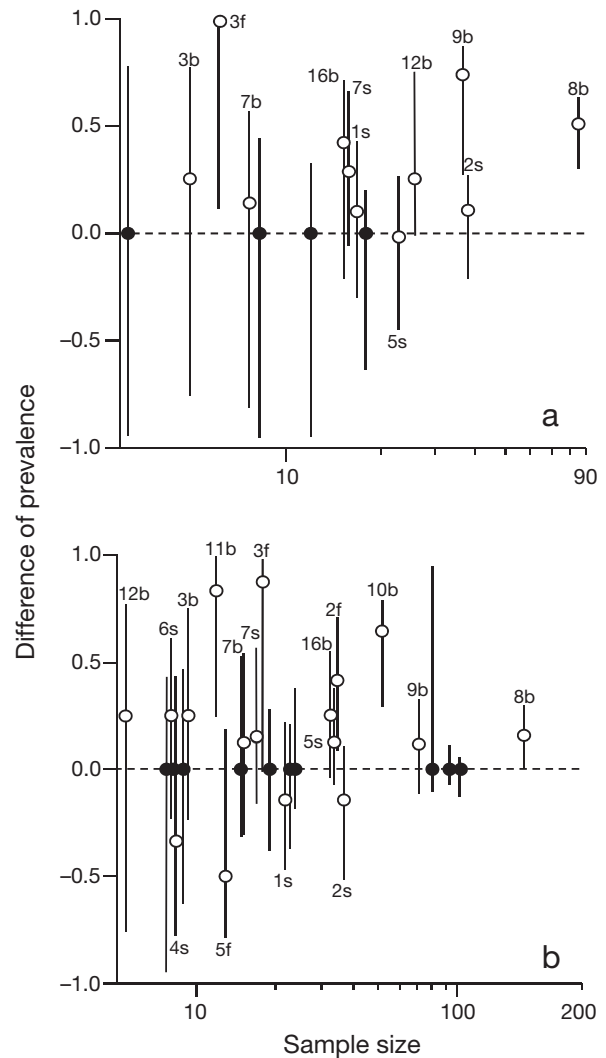


Fig. 6. Values of difference in prevalence of tattoo skin disease (TSD) in samples of cetaceans from different geographical regions. (a) Juveniles minus calves. (b) Juveniles minus adults. O: samples with at least 1 positive case for TSD; ●: samples in which TSD was not detected. Bars represent the 95% CIs. Sample codes as in Table 1

0.365) and the common odds ratio (7.16, 95% CI: 1.46 to 57.5) departed significantly from 1 (exact 1-tailed  $p = 0.011$ ). Interestingly, the odds ratio of TSD infections between juveniles and adults differed significantly (Zelen's test,  $p < 0.01$ ) in TD vs. PH samples (TD: juvenile 24:2; adult 23:0; PH: juvenile 31:5; adult 18:7). We conclude that cetaceans in the PH group exhibited a greater prevalence of TSD, and that prevalence remained high in adults as compared with cetaceans that died a traumatic death. Four of the 5 positive adult *P. phocoena* also had a high number of tattoos and 1 of the 2 positive adult *D. delphis* showed a large (110 mm) tattoo.

## DISCUSSION

During this study we examined the prevalence of TSD in several cetacean species and populations (Tables 1 & 2, Fig. 3). The disease was encountered in 13 species and in 2 subspecies from 5 water bodies (Tables 2 & 3): the Atlantic and Pacific Oceans as well as the North, Mediterranean and Tasman Seas. Our data only confidently suggest that in some ocean provinces TSD is frequent and affects several sympatric, odontocete species (e.g. off Peru and around the British Isles) whereas in other regions TSD infections occur less frequently or remain undetected in most species examined (e.g. SW Atlantic). Peruvian coastal waters appear to represent a high-risk area for TSD. Prevalence was remarkably high in both delphinids and phocoenids, especially in juveniles. Although no abundance estimates exist, frequent sightings of large to very large groups (high 100s to 1000s of individuals per group, K. Van Waerebeek pers. obs.) and apparently sustained annual bycatches of several thousand specimens of each species over many years (e.g. Van Waerebeek & Reyes 1994, Alfaro-Shigueto et al. 2008) hint that Peruvian populations of *Lagenorhynchus obscurus*, *Delphinus capensis*, offshore *Tursiops truncatus* and *Phocoena spinipinnis* are very large. High densities of individuals are thought to facilitate virus maintenance and continued infection of juveniles. The poxviruses circulating in Peruvian populations may also be more infectious than those present in other ocean provinces. However, they have not yet been characterized, preventing further discussion. Environmental stressors such as fishery interactions and pollution may also play a role in lowering the immune response (Clark et al. 2006, Hall et al. 2006). There was no obvious relationship between epidemiological values and geography and host phylogeny. However, as TSD screening effort and host sample size were strongly uneven among samples, this should be further studied before any definitive conclusion can be drawn. The occurrence of TSD in several ocean provinces and its holoendemic pattern in many populations further suggest that cetacean poxviruses have been infecting cetaceans for a long time (Bracht 2005, Pearce et al. 2008).

We investigated the influence of host sex and age upon the prevalence of TSD in several species and ocean provinces after verifying that the general pattern was not significantly confounded by sample type, geography, phylogeny or interaction between host sex and age. With the exception of Peruvian *Phocoena spinipinnis*, prevalence of the disease was similar in both sexes in all species examined. We discerned a general pattern of an increase of TSD in juveniles compared to calves, likely because juveniles had lost

maternal humoral immunity and become fully receptive to the virus, as previously suggested (Van Bresseem & Van Waerebeek 1996). Juveniles revealed a significantly higher probability of having TSD than adults in most samples, with the exception of cetaceans in poor health from the British Isles and *Cephalorhynchus eutropia* from southern Chile. A high percentage of adults likely had developed active immunity following infection and were protected against re-infection. This typical holoendemic pattern appeared to be inverted in poor health odontocetes as demonstrated in *Delphinus delphis* and *P. phocoena* from the British Isles, where prevalence was significantly higher in adults than in juveniles. Geraci et al. (1979) also reported that in captive dolphins the development of TSD was linked to general poor health. High exposure to polychlorinated biphenyls (PCBs) was previously shown to increase the risk of mortality from infectious diseases in *P. phocoena* from the British Isles, presumably because of their immunosuppressive effects (Jepson et al. 1999, 2005, Hall et al. 2006). It is possible that a high prevalence of TSD in adults reflects a depressed immune system, but this should be further studied. The presence of very large tattoos in some adult small cetaceans from the SE Pacific, NE Atlantic and Sado Estuary evokes 'progressive vaccinia', a life-threatening complication of smallpox vaccination in humans with immunological deficiencies, and similarly suggests an impaired immune response (Van Bresseem & Van Waerebeek 1996, Van Bresseem et al. 2003). An adult *P. spinipinnis* from central Chile (Fig. 1c) presenting many tattoos, one of them very large, was also diagnosed with pneumonia.

In summary, this is the first time that a clear, general age-related epidemiological pattern is reported for TSD. Departure from this pattern, i.e. TSD prevalence remaining high in adults, occurred in some cetacean populations and, at least in 1 region, was associated with individuals that had died in poor health. It is thus possible that the epidemiological pattern of TSD is an indicator of cetacean population health. Future research should seek to correlate the presence, number and size of tattoo lesions with quantitative data on contaminant loads, including PCB congeners amongst others. Inshore and neritic cetaceans living in a contaminated environment, stressed by fisheries interactions and disturbance from dense shipping may be physiologically challenged to mount an adequate immune response against infectious agents.

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