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M-F. van Bressem, Marcos César de Oliveira Santos, Júlia Emi de Faria Oshima. Skin diseases in Guiana dolphins () from the Paraná estuary, Brazil: A possible indicator of a compromised marine environment. *Marine Environmental Research*, 2009, 67 (2), pp.63. 10.1016/j.marenvres.2008.11.002 . hal-00563063

HAL Id: hal-00563063

<https://hal.science/hal-00563063>

Submitted on 4 Feb 2011

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Accepted Manuscript

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PII: S0141-1136(08)00235-3

DOI: [10.1016/j.marenvres.2008.11.002](https://doi.org/10.1016/j.marenvres.2008.11.002)

Reference: MERE 3299

To appear in: *Marine Environmental Research*

Received Date: 26 March 2008

Revised Date: 30 October 2008

Accepted Date: 1 November 2008



Please cite this article as: Van Bressem, M-F., de Oliveira Santos, M.C., Emi de Faria Oshima, J., Skin diseases in Guiana dolphins (*Sotalia guianensis*) from the Paraná estuary, Brazil: A possible indicator of a compromised marine environment, *Marine Environmental Research* (2008), doi: [10.1016/j.marenvres.2008.11.002](https://doi.org/10.1016/j.marenvres.2008.11.002)

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**Skin diseases in Guiana dolphins (*Sotalia guianensis*) from the Paraná estuary,
Brazil: a possible indicator of a compromised marine environment**

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ABSTRACT

We report on the presence of lobomycosis-like disease (LLD) and nodular skin disease (NSD) in a community of Guiana dolphins (*Sotalia guianensis*) inhabiting the biologically and chemically contaminated Paran  estuary (Brazil) and on their absence in the community living in the cleaner Canan ia estuary. Prevalence rates of LLD and NSD were 3.9% and 12.6%, respectively, in 103 photo-identified (PI) dolphins from the Paran  estuary in the period 2006-2007. Adults and calves were affected. Lobomycosis-like lesions may be extensive and form large plaques. Skin nodules were sometimes ulcerated and associated with cutaneous traumas suggesting that traumatic injuries may play a role in the pathogenesis of this condition. In two adult dolphins, NSD evoked the beginning of LLD. In 1996-2007 none of the 200 PI Canan ia *S. guianensis* had LLD or NSD, a highly significant difference. Interestingly, these dolphins were reported to harbour relatively low concentrations of organochlorines. LLD and NSD are possibly indicators of environmental changes.

Keywords: diseases, lobomycosis-like disease, nodules, skin, pollution, *Sotalia guianensis*, Lagamar estuary, coastal waters, Brazil.

1. INTRODUCTION

Caused by the fungal pathogen known as *Lacazia loboi* (Taborda et al., 1999) (syn. *Loboa loboi*; Caldwell et al., 1975), lobomycosis (or lacaziosis) naturally affects humans, bottlenose dolphins (*Tursiops truncatus*) and Guiana dolphins (*Sotalia guianensis*) (de Vries and Laarman, 1973; Caldwell et al., 1975; Simões-Lopes et al., 1993; Reif et al., 2006; Van Bressem et al., 2007). The natural reservoir of *L. loboi* is unknown but soil and vegetation seem to be likely sources of infection for humans (Honda et al., 2007). Lobomycosis in dolphins is characterized by grayish, whitish to slightly pink, verrucous lesions, often in pronounced relief that may ulcerate (Migaki et al., 1971). *T. truncatus* from the Indian River Lagoon (IRL), Florida, affected by cutaneous lobomycosis, were found to have significant impairment in adaptive immunity possibly related to chronic exposure to environmental stressors (Reif et al., 2008). Variation in salinity and water temperature may also play a role in the infection (Reif et al., 2006). Lobomycosis and lobomycosis-like disease (LLD), a disease very similar to lobomycosis but for which a histological diagnostic is missing, have been reported in inshore dolphins from several South American countries with the first case reported in a *S. guianensis* caught in the estuary of the Surinam River in 1971 (de Vries and Laarman, 1973; Simões-Lopes et al., 1993; Van Bressem et al., 2007). Photo-identification was shown to be a useful tool to detect and monitor these diseases (Van Bressem et al., 2007; Murdoch et al., 2008).

Two communities of *S. guianensis* inhabit the Lagamar estuary (Figure 1), Brazil. One dwells in the coastal waters surrounding the island of Cananéia (25°03'S; 47°55'W) and the other lives along the northern coast of the Paraná state (25°22'S; 48°25'W). The first one has been studied since 1996 (e.g. Santos and Rosso, 2007; 2008; Santos et al., 2000; 2001; 2002; 2003) while photo-identification surveys of the second one started in April 2006. In the Cananéia and Paraná areas 200 and 103 dolphins were photo-identified, respectively. Approximately 180 km long, the Lagamar estuary is

located between the states of São Paulo and Paraná and is in close contact with the Southwest Atlantic Ocean. It is surrounded by large mangrove forests and was designated a National Protected Area in 1984 (Schaeffer-Novelli et al., 1990). It is, however, severely impacted by anthropogenic factors. A channel dug at the end of the 1880s to connect its northern limit to the Ribeira do Iguape River has caused dramatic changes in its fauna composition and structure (Tommasi, 1985). Though prohibited by law in Brazil since 1985, chlorinated hydrocarbons were vastly used till the end of the 1990s and still contaminate the estuary (Ferreira et al., 1980; Mattos, 2002). In the southern portion of the estuary, Paranaguá harbour, Brazil's second most important port, was built in 1935. Oil spills are common in this area as well as shipwrecks inside and outside local estuarine waters (Wiczorek, 2006). As top predators, small cetaceans are exposed to, and affected by, persistent organic pollutants (POP) including polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds (Ross et al., 1996; Ross 2002).

Here we report on the presence of LLD and possibly related nodular skin disease (NSD) in *S. guianensis* from the Paraná estuary and on their absence in the community inhabiting the Cananéia area.

2. MATERIAL AND METHODS

2. 1. Image and data collection

Photo-identification boat-based (15 and 30hp motorized vessels) surveys were conducted from May 1996 to August 2007 in the Cananéia estuary and from April 2006 to September 2007 in the northern portion of the Paraná state. The survey path followed a zig-zag pattern to maximize chances of encountering dolphins. Surveys were conducted in good sea conditions (Beaufort Scale = 0-2). A 35mm reflex camera using printed films and with a 300mm zoom lens was used till May 2004 and

subsequently replaced by digital cameras with 300 and 400mm lenses. Photographs were taken at distances ranging from two to 10 m with shutter speeds ranging between 1/500s to 1/2000s.

2.2. Dolphins

All dolphins were individually identified from natural marks present on the dorsal fin and body (Würsig and Würsig, 1977; Würsig and Jefferson, 1990). About 100,000 and 26,000 pictures of dorsal fins were examined for the Cananéia and Paraná dolphins, respectively. On most occasions, all individuals from a group including calves were photographed. Females were identified on the basis of an accompanying calf. Calves are defined as individuals whose body length is one-third or less than that of other dolphins in the area and that usually remain close to their mothers in the infant position (Mann and Smuts, 1999; Mann et al., 2000). Adults are large, robust dolphins.

2.3. Nodular skin disease and lobomycosis-like disease

Nodular skin disease is characterized by circumscribed, raised, grey, orange or reddish, sometimes ulcerated, skin lumps. Lobomycosis-like disease consists in raised, gray-white to slightly pink, verrucous, at times ulcerated, proliferating lesions that may form large plaques, and grossly most closely resemble those caused by *L. loboi* in *T. truncatus*.

The lesions were counted and their broadest size estimated on the basis of a mean dorsal fin base length of 23 cm in adults (Santos et al., unpublished data). They were further classified as small (≤ 10 mm), medium (>10 mm and < 30 mm) and large (≥ 30 mm). Severity of the disease was scored as follows: 1= one small to medium-sized lesion, 2= two to seven, small to medium-sized, localized nodules, 3= five or more, small to large lesions affecting more than one body areas, 4= more than 10 lesions, aggregating, affecting several body areas and covering approximately 10% or more of the

visible body surface.

3. RESULTS

In the Paraná estuary we observed LLD and NSD in 18 (17.4%) of the 103 dolphins photo-identified in 2006-2007. Four (3.9%) dolphins including a calf, an adult female and two adults of unknown sex had LLD with severity scores ranging from 2 to 4 (Table 1). In two adults, including the female, the lesions were extensive and proliferating and affected the back, flanks and tailstock (Figure 2a). Individual lesions ranged from an approximate 5 to 50 mm in their broadest dimension (Table 1). When congregating the sores formed large plaques in adult female 'J' (approximately 355 x 25 mm, 283 x 16 mm and 119 x 08 mm) and in adult 'L' of unknown sex (about 180 x 80 mm-the sizes of the two other visible plaques could not be measured). In the calf the disease was apparently restricted to the back and dorsal fin (Figure 2b). The elevated white lumps measured around 8 till 20 mm in their broadest dimension. All dolphins were seen in Guaraqueçaba bay (25S 16' 14.2", 48W 21' 06.1", Figure 1) and surrounding areas in February-August 2007. On the only picture available, the calf's mother had a small white and round mark on her right side that was not considered to be LLD or NSD but may represent a very early stage of these diseases (Figure 2b).

NSD was seen in 14 (12.6%) dolphins including 13 adults and a large calf with severity scores ranging from 1 to 3 (Table 1). The nodules were seen on the flanks, back and tailstock. They ranged from an estimated 5 to 45 mm in their broadest dimension, were occasionally ulcerated (Figure 3a, Table 1) and were sometimes associated with skin traumas (Figure 3b) suggesting that wounds may play a role in the pathogenesis of this disease. In two adult dolphins of unknown sex, the lumps evoked the beginning of LLD: though still circumscribed they were grouped on a small area behind the dorsal fin and had a tendency to form plaques (Figure 4). However, these lesions were only

sighted once and their evolution is unknown. Nine of the 13 adults with lumps were seen in Guaraqueçaba bay and surroundings. The mother of the calf with the reddish lump did not have visible skin lesions but only one picture was available. None of the 200 Cananéia dolphins had LLD or NSD in the period 1996-2007, a highly significant ($\chi^2 = 37.32$, $df = 1$, $P = 1^{-09}$) difference.

4. DISCUSSION

This photo-identification study reports for the first time on the presence of LLD and NSD in a community of *S. guianensis* inhabiting the Paraná estuary, Brazil. It is also the first time that LLD is observed in a calf. The epidemiological status of LLD and NSD in *S. guianensis* from the Lagamar estuary is unknown. The presence of extensive lobomycosis-like lesions in two adults, and minor lesions in a third, suggests that the disease has been present for at least some months in this community. Prevalence of LLD was 3.9% in 103 dolphins photo-identified in 2006-2007. In inshore *T. truncatus* from South America, prevalence levels of this syndrome varied from 1.6% (Gulf of Guayaquil, Ecuador) to 20% (Tramandaí estuary, Brazil) in the period 1990-2007 (Van Bressem et al., 2007). The role of *L. loboi* as the aetiological agent of LLD was confirmed by histology in a dead *T. truncatus* from the Tramandaí estuary, Brazil, visually diagnosed as positive when still alive (Van Bressem et al., 2007; Moreno et al., 2008). Further studies will examine whether this fungus is also the causal agent of LLD in *S. guianensis* from the Paraná estuary.

Though the 'nodular skin disease' observed in the Paraná dolphins suggests an early form of LLD, it may represent another disorder. Besides, *L. loboi* at least four other pathogens are known to cause skin nodules in small cetaceans. *Streptococcus iniae*, primarily a bacteria of fishes, triggered 'golf ball disease', a condition characterised by multiple subcutaneous abscesses in botoes (*Inia geoffrensis*) kept in captivity in the US (Bonar and Wagner, 2003). Lumps resembling golf ball

disease were seen in *I. geoffrensis* from the Mamirauá Sustainable Development Reserve, Brazil, but the aetiological agent of these lesions is unknown (da Silva et al., 2008). There is no other report of this syndrome in free-ranging river dolphins, and the organism has not been reported in fishes from Brazil (Agnew and Barnes, 2007). An uncharacterised papillomavirus (PV) caused cutaneous warts in a harbour porpoise (*Phocoena phocoena*) stranded in Germany in December 1993 (Van Bressem et al., 1999). Nevertheless, the warts had a different aspect from the lumps observed during this study, the PV disease did not seem to be very contagious and has not been reported in South American cetaceans. *Fusarium* spp. hyphae caused raised, firm, erythematous, 2 to 5 mm cutaneous nodules that were most prominent on the head, trunk, and the caudal portion of an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) and a pygmy sperm whale (*Kogia breviceps*) stranded along the northeast coast of the USA in 1991 (Frasca et al., 1996). *Fusarium* spp. are widespread in South America causing diseases in plants and animals (Ortoneda et al., 2004; Guilhermetti et al., 2007). A fungus identified as *Trichophyton* spp. was seen in conical, ulcerated, 16-36mm nodules on the trunk of an Atlantic *T. truncatus* kept in captivity in Japan (Hoshina and Sugiura 1956). The role of these pathogens in the aetiology of the cutaneous nodules in *S. guianensis* should be further explored. If NSD represents indeed an early form of LLD, prevalence of this syndrome would reach 17.4% in *S. guianensis* from the Parana estuary.

Although more than 100,000 photographs of *S. guianensis* inhabiting the Cananéia estuary were analysed over a period of 10 years, LLD and NSD were never seen in this community. Interestingly, these dolphins were reported to harbour relatively low concentrations of organochlorines (Yogui et al., 2003). On the other hand, prevalence of LLD and possibly related NSD was relatively high (17.4%) in the individuals from the Paraná estuary. A large port, small villages with up to 2,000 inhabitants and an illegal shrimp farm that operated for a short time in 2006-2007 (Santos, pers.

observations) likely biologically and chemically contaminated this area. Aquaculture, especially shrimp and salmon farms, in South America is known to heavily use prophylactic antibiotics that may alter the normal skin fauna and create an appropriate environment for fungal invasion (Frasca et al., 1996; Cabello, 2004, 2006). Water ballast from the numerous ships transporting grain could bring alien micro-organisms (Ruiz et al., 2000; Drake et al., 2007) including *L. loboi* into the Paraná estuary. Interestingly all inshore *T. truncatus* populations from South America affected by LLD inhabited heavily polluted waters surrounding large ports and cities and, in the case of Guayaquil, also harbouring intense shrimp farming activities (Van Bressem et al., 2007). Environmental changes may favour the dissemination of the *L. loboi* and promote its introduction in naïve populations and communities.

5. CONCLUSIONS

The high prevalence of LLD and NSD in the *S. guianensis* from the Paraná estuary and their absence in the well-studied community of the Cananéia estuary is striking. These diseases may be indicators of environmental changes. Their aetiology, pathogenesis, epidemiology, evolution and impact on survival of coastal dolphins should be further explored.

6. ACKNOWLEDGEMENTS

Long-term photo-identification studies in the Lagamar estuary have been sponsored by the Cetacean Society International (CSI), the Whale & Dolphin Conservation Society (WDCS), The Humane Society of the United States (HSUS), The Society for Marine Mammalogy (SMM), the Earthwatch Institute (EWI), and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, process numbers 01/05128-8, 05/59439-5, 05/54149-9). M. Santos received a fellowship from FAPESP and J. de Faria obtained a fellowship from Agência Nacional do Petróleo (ANP), Financiadora de

209 Estudos e Projetos (FINEP), Ministério da Ciência e Tecnologia (MCT), Programa de Recursos
210 Humanos da ANP para o setor de petróleo e gás – PRH-ANP/MCT. Logistical support was provided
211 by the Instituto Oceanográfico da Universidade de São Paulo (Cananéia research station) and the
212 Núcleo Pró-Ação de Guaraqueçaba da Pontifícia Universidade Católica do Paraná. Ednilson da Silva
213 and Eduardo Pacífico helped in fieldwork surveys. The work of M-F Van Bresseum was funded by
214 the WDCS and CSI. We kindly thank two anonymous reviewers for their constructive comments on
215 the manuscript.

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329 8. FIGURE CAPTIONS

- 330 Figure 1. Map of the Lagamar estuary, Brazil, including the Cananéia and Paran  estuaries
- 331 Figure 2a. Extensive lobomycosis-like disease in adult female *S guianensis* ‘J’
- 332 Figure 2b. Localised lobomycosis-like disease in calf *S. guianensis* ‘G’.

333 Figure 3a. Ulcerated nodules on the back of adult *S. guianensis* 'C'.

334 Figure 3b. Reddish nodule on tailstock and lesions associated with cutaneous traumas on the back of
335 adult *S. guianensis* 'M'.

336 Figure 4. Nodules evoking the beginning of LLD on the back of adult *S. guianensis* 'O'.

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338 **9. TABLE 1**

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341 **10. FIGURES**

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Table 1. Details of Guiana dolphins (*Sotalia guianensis*) from Guaraqueçaba and adjacent areas affected by lobomycosis-like disease (LLD) and nodular skin disease (NSD)

Dolphin	Sex	Age class	Date	NSD	LLD	Nber	Severity	Description
J	female	adult	8 Aug 07	No	Yes	several	4	dark gray and brownish, at times ulcerated, small to large nodules forming plaques on flanks and back.
L	indet	adult	8 Feb 07	No	Yes	several	4	extensive, small to large, nodular lesions forming plaques on dorsal fin, back and tailstock.
G	indet	calf	8 Jul 06	No	Yes	3	2	two whitish, small to medium, verrucous lesions on back behind dorsal and one on dorsal fin.
V	indet	adult	2 Aug 07	No	Yes	2	2	small to medium lesions on the back, apparently associated with a skin trauma.
A	indet	adult	30 Jul 06	Yes	No	5	3	in relief small to large lesions on dorsal fin and left flank.
B	female	adult	30 Jul 06	Yes	No	2	2	small to medium nodules on back and left flank.
C	indet	adult	8 Jul 06	Yes	No	3	2	ulcerated, medium-sized nodules on the back below dorsal.
D	indet	adult	8 Jul 06	Yes	No	3	2	small to large, grouped nodules on left flank.
E	indet	adult	8 Jul 06	Yes	No	2	2	a small nodule on the back and a medium on tailstock.
F	female	adult	8 Jul 06	Yes	No	1	1	medium-size, ulcerated nodule on left flank.
H	indet	calf	8 Aug 07	Yes	No	1	1	heart shaped, medium, reddish nodule on the back before dorsal fin.
M	indet	adult	8 Feb 07	Yes	No	1-3	2	one medium-size reddish nodule on tail stock, two in relief lesions associated with skin traumas on left flank.
N	indet	adult	8 Jan 07	Yes	No	~ 7	2	small to medium nodules associated with scars and evoking the beginning of LLD on the back behind dorsal fin
O	indet	adult	31 Jul 06	Yes	No	5	2	medium, whitish nodules evoking the beginning of LLD on the back behind dorsal fin.
P	indet	adult	8 Aug 07	Yes	No	1	1	reddish, medium-size nodule on right flank.
Q	indet	adult	8 Aug 07	Yes	No	5	2	medium-size, whitish nodules on the left flank and back behind dorsal fin.
R	female	adult	8 Aug 07	Yes	No	1	1	medium-size nodule on the back behind head.
S	indet	adult	2 Aug 07	Yes	No	1	1	small nodule on the back behind dorsal.

Specimens are ordered by disease and code. Abbreviations are: indet= indeterminate, nber= number.











