

Lacaziosis-like disease among bottlenose dolphins *Tursiops truncatus* photographed in Golfo Dulce, Costa Rica

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ABSTRACT: Lacaziosis (also known as lobomycosis) is a chronic dermal disease caused by the fungal agent *Lacazia loboi*, which affects both humans and dolphins. Photographic data have been used to identify lacaziosis-like disease (LLD) among dolphins in the waters of North and South America, and here we report LLD in bottlenose dolphins *Tursiops truncatus* off the coast of Costa Rica, the first reporting in Central American waters. During the periods of 1991 to 1992 and 2010 to 2011, 3 research teams conducted separate dolphin surveys in the Pacific tropical fiord Golfo Dulce, and each documented skin lesions in the resident population of bottlenose dolphins. Photo-ID records were used to identify LLD-affected bottlenose dolphins and to assess their lesions. Findings showed between 13.2 and 16.1% of the identified dolphins exhibited lesions grossly resembling lacaziosis. By combining efforts and cross-referencing photographic data, the teams explored the presence of LLD in Golfo Dulce over a time gap of approximately 20 yr. Our findings expand the geographical range of the disease and offer insight into its longevity within a given population of dolphins.

KEY WORDS: Skin lesions · Cetaceans · *Lacazia loboi* · LLD · Eastern Pacific · Central America

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INTRODUCTION

Lacaziosis (also called lobomycosis) is a chronic mycotic disease of the skin caused by the dimorphic organism *Lacazia loboi*, which naturally affects only humans and dolphins (Taborda et al. 1999). While the

disease process is similar in both hosts, fungal cells from infected human tissues appear twice as large as those found in dolphins (Haubold et al. 2000) and only one suspected case of zoonotic transmission has been documented (Symmers 1983). In dolphins, lacaziosis manifests as raised, sometimes ulcerated or

plaque-like, epidermal lesions of grayish, whitish, or slightly pinkish coloration, which may spread to disfigure large areas of the dorsal, dorsolateral, and peduncular regions of the body. Lacaziosis-like disease (LLD; originally lobomycosis-like disease, Van Bresse et al. 2007) refers to lesions that appear to be lacaziosis but for which the etiologic agent is unconfirmed. LLD is typically classified through photographic analysis, and there is some validation of presumptive diagnosis. For instance, in a study of 16 bottlenose dolphins classified with LLD via photos, sampled biopsies confirmed lacaziosis in 75% ($n = 12$) of the cases (Murdoch et al. 2008).

Lacaziosis and LLD have previously been described among 3 species of Delphinidae inhabiting the waters of at least 9 countries or territories: Guiana dolphins *Sotalia guianensis* in Brazilian estuaries (de Vries & Laarman 1973, Van Bresse et al. 2009), Indo-Pacific bottlenose dolphins *Tursiops aduncus* in Mayotte (Kiszka et al. 2009), and in bottlenose dolphins *T. truncatus* off the coasts of the US (Caldwell et al. 1975, Reif et al. 2006, Murdoch et al. 2008, Rotstein et al. 2009, Burdett Hart et al. 2011), France (Symmers 1983), Venezuela (Bermúdez et al. 2009), Brazil (Simões-Lopes et al. 1993, Daura-Jorge & Simões-Lopes 2011), Colombia, Ecuador, and Peru (Van Bresse et al. 2007). LLD is considered an emerging disease with increasing reports in a growing number of countries (Van Bresse et al. 2007, Bermúdez et al. 2009, Kiszka et al. 2009, Rotstein et al. 2009, Burdett Hart et al. 2010); hence, there is a need to better understand the dynamics of this infectious condition and define the potential limits of its range.

Although the yeast-like pathogen has not yet been cultured *in vitro* and little is known about its growth and route of transmission, *Lacazia loboi* is likely to be hydrophilic in nature, penetrating traumatized skin (Paniz-Mondolfi et al. 2012). Infected dolphins exhibiting dermal dissemination have also revealed significant compromise of adaptive immunity. Among other health-related abnormalities, Reif et al. (2009) showed a decline of absolute T cells and B cells in 8 Atlantic bottlenose dolphins with lacaziosis; lymphocyte proliferation was impaired, and antibody titers to certain common marine microorganisms were also reduced. Data suggest that epidermal lesions may be associated with lower salinity (Wilson et al. 1999, Daura-Jorge & Simões-Lopes 2011) and may also reflect problems in the marine environment, including anthropogenic contamination (Van Bresse et al. 2009, Daura-Jorge & Simões-Lopes 2011).

Golfo Dulce, Costa Rica, has been recognized as a distinctive aquatic habitat with fiord-like characteristics rare to the tropics. The deep inner basin is of tectonic origin and periodically anoxic (Hebbeln & Cortés 2001), with notable hydrological fluctuations in salinity and temperature (Svendsen et al. 2006). The inlet supports an abundance of marine life (Morales-Ramírez 2011). While other species of delphinids have been reported inside the embayment (Acevedo-Gutiérrez et al. 1997), only bottlenose dolphins and pantropical spotted dolphins *Stenella attenuata* are considered resident, living sympatrically (Acevedo-Gutiérrez & Burkhart 1998, Cubero-Pardo 2007, Oviedo 2007). Bottlenose dolphins are generally seen in all parts of Golfo Dulce with an overall preference for shallow waters close to shore and in the midwaters between the 2 largest coastal human communities, Puerto Jiménez and Golfito (Acevedo-Gutiérrez & Burkhart 1998, Cubero-Pardo 2007, Oviedo 2007). During 1991 to 1992 and 2010 to 2011, 3 research teams conducted separate, unrelated on-water surveys to collect data on dolphins in Golfo Dulce. Initially unaware of any dermal diseases in the resident dolphins, each research team photographed inshore bottlenose dolphins exhibiting raised skin lesions consistent with lacaziosis. Data were later combined and cross-referenced with the objectives of (1) confirming the presence of LLD among the population of bottlenose dolphins in Golfo Dulce and (2) exploring disease persistence and progression among individuals seen during both study periods. Here, we collectively report our findings, the first documented cases of LLD in Central America.

MATERIALS AND METHODS

Study area

Golfo Dulce, centered around 08° 33' N, 083° 14' W, is a tropical fiord-like embayment located on the South Pacific coast of Costa Rica (Fig. 1). This curved inlet is approximately 50 km in length and 10 to 15 km wide with a surface area of 750 km² (Wolff et al. 1996). Bathymetrics describe a steeply sloped inner basin reaching a maximum depth of 215 m separated from a shallow outer basin by a 60 m sill. The top of the water column is characterized by lower salinity maintained in part by freshwater dispensed from 4 major rivers, the Rincón, Esquinas, Tigre, and Coto Colorado, plus several smaller tributaries (Svendsen et al. 2006). Dense mangroves are associated with the riparian areas and the remainder of the

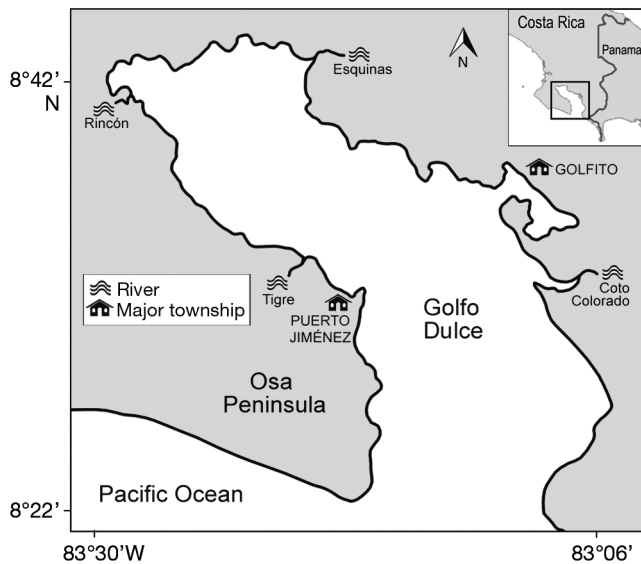


Fig 1. Study site in Golfo Dulce, Costa Rica, in Eastern Pacific waters of Central America

shoreline topography varies from steep forested slopes to sandy beaches (Wolff et al. 1996).

Research teams

All 3 research teams surveyed the full area of Golfo Dulce with at least 2 experienced observers per boat at all times. However, each team used a distinct methodology. A. Acevedo-Gutiérrez (AA) conducted cetacean surveys in the embayment from September 1991 through December 1992 (Acevedo & Smultea 1995, Acevedo-Gutiérrez et al. 1997, Acevedo-Gutiérrez & Burkhart 1998), spanning 3 prescribed periods: January to April, May to August, and September to December. The team split up, using two 5 m inflatable boats with 25 hp engines and nonrandom surveys canvassed the entire Gulf approximately every 5 survey days. The total effort by AA's team was 2719.7 survey hours. B. L. Bessesen (BB) undertook 2 multi-species surveys of Golfo Dulce (Bessesen 2012, Bessesen & Saborío-R. 2012): one in January to February 2010 using a 6 m vessel with a 50 hp engine, and the other in July to August 2011 using a 3 m vessel with a 15 hp engine. The Gulf was divided into 4 geographical areas and the team traversed 1 area per day, generally canvassing the full Gulf every 4 survey days. The effort by BB totaled 423.7 survey hours. L. Oviedo (LO) has been studying cetaceans around the Osa region since 2005 (Oviedo 2007, Oviedo & Solís 2008, Oviedo Correa et al. 2009). The

team's 2010 and 2011 Golfo Dulce dolphin surveys covered 2 study periods each year: June to October and November to May. Sightings were recorded from a 9 m boat with a 115 hp engine, and the search effort alternated between sectors (inner basin, outer area, transitional-oceanic) to ensure representative coverage of the study area. The LO team logged a total of 778 survey hours during 2010 to 2011.

Photo-identification and LLD assessment

All 3 research teams collected photographic data during bottlenose dolphin observations and cataloged individuals based on unique dorsal fin profiles (Würsig & Würsig 1977, Würsig & Jefferson 1990). Photos of identified dolphins were then screened, and images showing epidermal lesions were separated for disease analysis. Individuals were classified as LLD if photo quality was good enough, e.g. high resolution, in focus, to confirm that the lesion characteristics resembled lacaziosis in gross appearance, i.e. raised contours and gray/white/pink in coloration (Migaki et al. 1971, Reif et al. 2006, Van Bressemer et al. 2007; Figs. 2 & 3). Cases that appeared likely to be LLD but did not meet the criteria for confirmation were classified as 'possible LLD' (p-LLD; Burdett Hart et al. 2011). These cases included animals with photographic evidence suggestive of early stages of LLD based on longitudinal observations from lesion progression modeling (Burdett Hart et al. 2010), animals with photographic evidence suggesting lacaziosis-like lesions but lacking follow-up images to confirm that the disease was chronic, and animals with lesions suggestive of LLD despite poorer image resolution (Fig. 4). Photos of dermal disruptions that were too distant, or suggestive of other causal factors, e.g. trauma, were not included in analyses (Burdett Hart et al. 2011).

Statistical calculations

Due to certain constraints on data, BB and LO did not combine photo-ID records of bottlenose dolphins, and data from each team were separately analyzed. A reliable population estimate for 2010 to 2011 could not be rendered, so a disease prevalence statistic (proportion of lesioned dolphins within the population) could not be calculated. The disease percentages provided are based on the number of diseased individuals relative to the number of dolphins identified by each team.

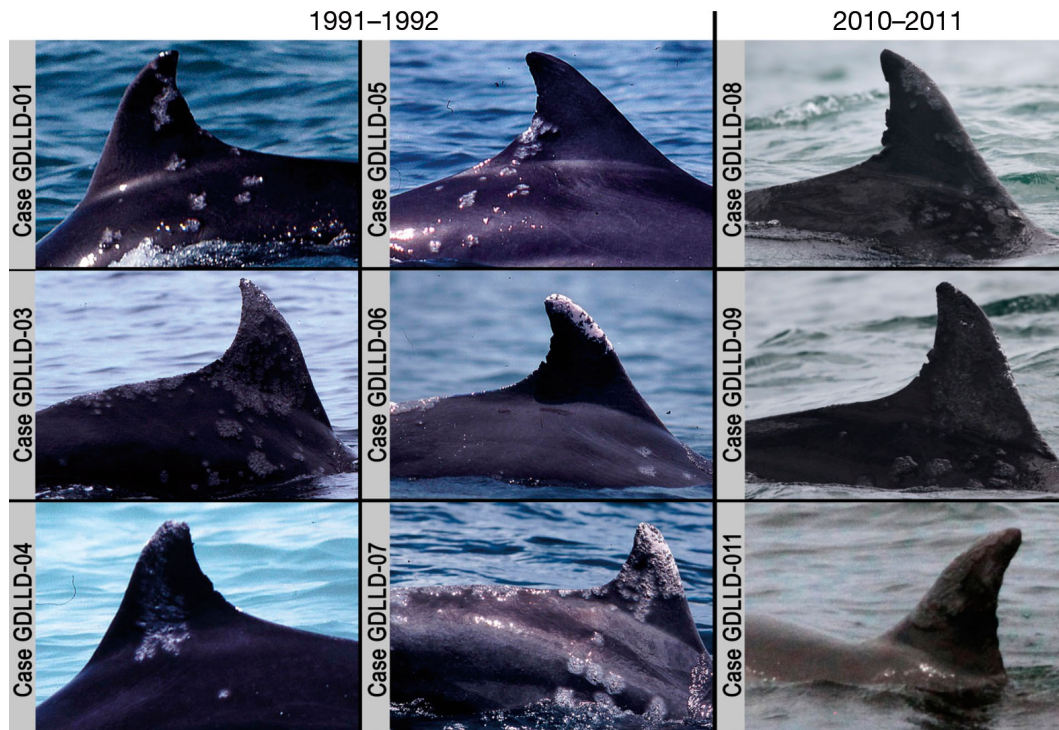


Fig. 2. *Tursiops truncatus*. Cases of lacaziosis-like disease (LLD) divided by study period. For Cases GDLLD-02 and GDLLD-10, refer to Fig. 3

RESULTS

1991–1992. AA identified 56 individual bottlenose dolphins through photographs collected during his surveys, all of which were screened for LLD lesions. Nine bottlenose dolphins were classified as having LLD ($n = 7$) or p-LLD ($n = 2$), representing 16.1% of the identified dolphins (Figs. 2–4, Table 1). If p-LLD cases were excluded, the percentage of LLD was 12.5% of the identified dolphins.

2010–2011. Between BB and LO, a total of 5 LLD cases (Figs. 2 & 3) and 5 p-LLD cases were classified among distinct bottlenose dolphins photographed during the 2010 to 2011 time period. Most of the LLD cases ($n = 4$) but none of the p-LLD cases overlapped. The data for each team are as follows: BB identified 68 individual bottlenose dolphins, including 9 with lesions characteristic of lacaziosis, thereby representing 13.2% of the identified dolphins. The BB cases were classified as 5 LLD (7.4% of the identified dolphins) and 4 p-LLD. During the same years, the LO team identified 38 individual bottlenose dolphins and photographed 5 with lacaziosis-like lesions, also representing 13.2% of the identified dolphins. The LO cases were later classified as 4 LLD (10.5% of the identified dolphins) and 1 p-LLD (Fig. 4, Table 1).

Cross-referenced data. Two of the study dolphins photographed by AA were also photographed during the 2010 to 2011 surveys (Table 1). In 1991, Case GDLLD-02 showed limited dissemination of bilateral dermal lesions on the lower trailing edge of the dorsal fin, extending slightly onto the dorsum. When resighted by BB in 2011, those lesions appeared more extensive, yet localized to the original area with much of the cranial dorsum still unaffected (Fig. 3a). The second dolphin, Case GDLLD-10, did not exhibit lesions in photographs taken by AA in 1991, but 20 yr later, GDLLD-10 was photographed by LO and BB with extensive LLD lesions (Fig. 3b).

DISCUSSION

To date, several studies have reported the presence of lacaziosis or LLD among bottlenose dolphin populations in South America (Simões-Lopes et al. 1993, Van Bressemer et al. 2007, 2009, Bermúdez et al. 2009, Daura-Jorge & Simões-Lopes 2011) and in the US (Caldwell et al. 1975, Reif et al. 2006, Murdoch et al. 2008, Rotstein et al. 2009, Burdett Hart et al. 2011). By presenting documented cases of LLD in dolphins in Central America, we expand the spatial range of

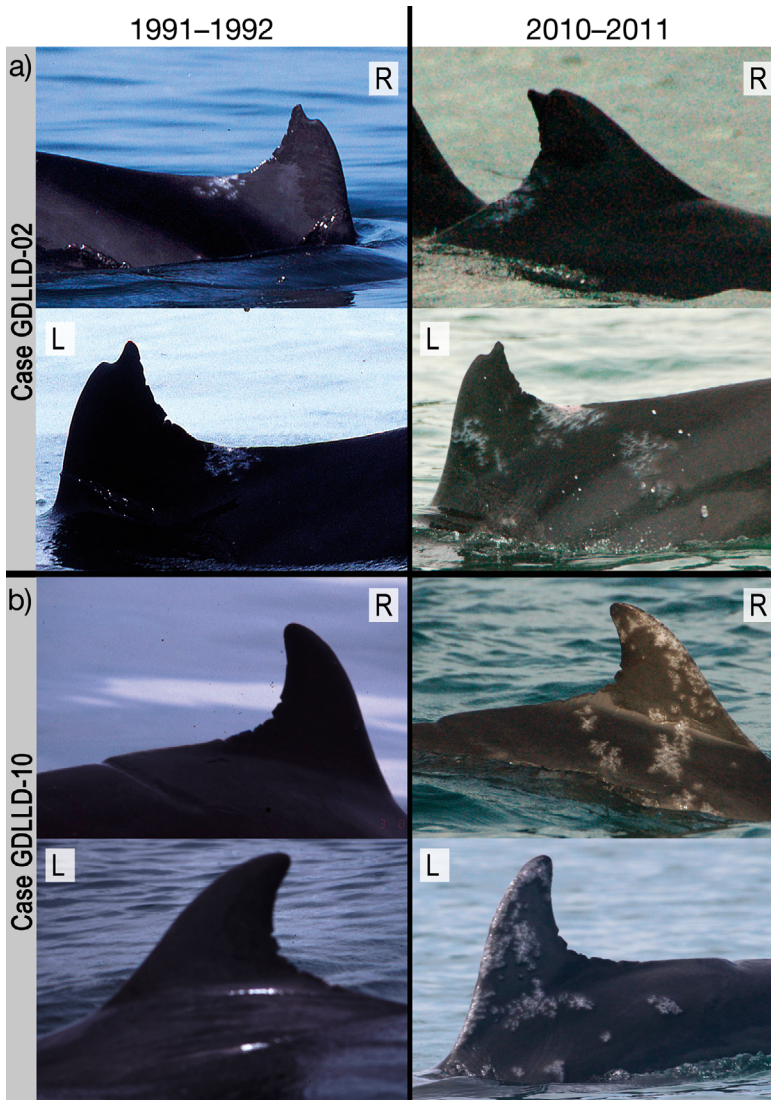


Fig. 3. *Tursiops truncatus*. Bilateral dorsal images (R: right, L: left) show disease progression for the 2 cases of lacaziosis-like disease (LLD) recorded between study periods: (a) GDLLD-02; (b) GDLLD-10, appeared lesion-free in 1991–1992

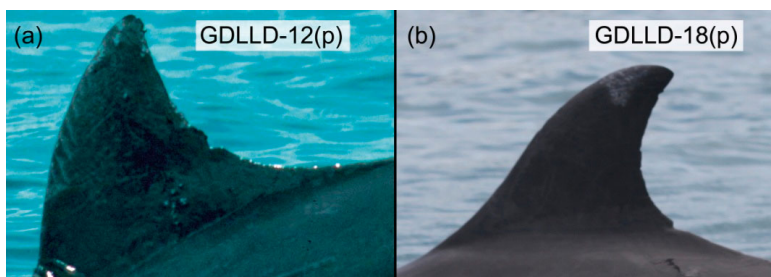


Fig. 4. *Tursiops truncatus*. Examples of 'possible lacaziosis-like disease' (p-LLD). (a) Small raised nodules suggestive of early stages of lacaziosis based on longitudinal observations from lesion progression modeling (Burdett Hart et al. 2010); (b) snowflake pattern suggests LLD, but we were unable to confirm that the lesion was raised and lack follow-up images to confirm that the disease was chronic

the disease and bridge the gap between North and South America. By including 2 study periods between 1991 and 2011, we also provide some insight on the progression of the disease among and within individuals. Worldwide, only a couple of studies of lacaziosis or LLD in defined dolphin populations have shown data spanning at least 20 yr, both in Florida, USA: Indian River Lagoon (Murdoch et al. 2008) and Sarasota Bay (Burdett Hart et al. 2011, Hart et al. 2012). While it is clear that LLD has been present in Golfo Dulce bottlenose dolphins for over 2 decades, only 1 case was sighted during both study periods (GDLLD-02). One new case was also discovered between the 1991–1992 and 2010–2011 data sets (GDLLD-10), presuming that animal did not have undetected lesions during the first study period. Case GDLLD-02 exhibited a slower dissemination of lesions compared to Case GDLLD-10. Other studies have shown similar variation in disease progression between individual dolphins (Kiszka et al. 2009, Burdett Hart et al. 2010), although the reasons for these differences in lesion growth remain unknown.

For all 11 LLD cases presented, bilateral photographs were obtained and LLD lesions were confirmed on both sides of the dorsum. Such evidence suggests that when LLD lesions are present on one side, they are likely to be present on the other side as well. It is important to acknowledge limitations of using dorsal photographs to determine the scope of disease, as some individuals may present lesions on areas of the body not readily observed or photographed above the water surface, which would also bias the overall count of infected dolphins. Although population-based disease prevalence could not be established in Golfo Dulce, calculating the percentage of identified bottlenose dolphins with lesions characteristic of lacaziosis (LLD and p-LLD combined) showed some consistency between study periods at 16.1% in 1991–1992 and 13.2% in 2010–2011.

Table 1. *Tursiops truncatus*. All cases of lacaziosis-like disease (LLD) and possible LLD for bottlenose dolphins, recorded inside Golfo Dulce, Costa Rica, during 3 separate surveys in 1991 to 1992 and 2010 to 2011 (AA: A. Acevedo-Gutiérrez; BB: B. Bessesen; LO: L. Oviedo). 'Photos' refers to the side(s) of the dolphin photographed, and 'Lesions' refers to the side(s) of the dolphin with visible lesions (R: right; L: left; B: both). Cases highlighted in **bold** (GDLLD-02 and GDLLD-10) are the only cases sighted during both study periods

Dolphin case number	No. of sightings			Photos	Lesions
	1991–92 AA	2010–11 BB LO			
LLD					
GDLLD-01	40			B	B
GDLLD-02	53	2		B	B
GDLLD-03	12			B	B
GDLLD-04	8			B	B
GDLLD-05	10			B	B
GDLLD-06	18			B	B
GDLLD-07	25			B	B
GDLLD-08		4	3	B	B
GDLLD-09		5	8	B	B
GDLLD-10	39	4	6	B	B
GDLLD-11		1	2	B	B
Possible LLD					
GDLLD-12(p)	37			B	B
GDLLD-13(p)	22			R	R
GDLLD-14(p)		2		L	L
GDLLD-15(p)		1		B	R
GDLLD-16(p)		1		L	L
GDLLD-17(p)		1		B	B
GDLLD-18(p)			2	L	L

Reported disease prevalence estimates for lacaziosis or LLD among dolphin populations in North America range from 1.9% (Charlotte Harbor, Florida; Burdett Hart et al. 2011) to 12% (Indian River Lagoon, Florida; Reif et al. 2006), and estimates for South America range from 1.6% (Gulf of Guayaquil, Ecuador) to as high as 33% (Mampituba Estuary in Southern Brazil; Van Bressemer et al. 2007). The closest known population of LLD dolphins to Golfo Dulce is in Columbia's Bahía Málaga and surroundings, where prevalence has been calculated at 5.4% (Van Bressemer et al. 2007). By comparison, the moderately high percentage that we have detected raises the possibility that LLD affects the long-term health of the Golfo Dulce bottlenose dolphin population. Pan-tropical spotted dolphins also use the study area, yet to date no lacaziosis-like lesions have been documented on spotted dolphins in the embayment. The lack of LLD among that species raises additional questions about disease transmission and susceptibility. Lacaziosis in humans was reported in Costa

Rica as early as the 1950s (Trejos & Romero 1953); however, to our knowledge, no stranding cases have provided biopsy confirmation of lacaziosis among any dolphin species in the region. Future opportunities to collect lesion samples from stranded bottlenose dolphins should be pursued.

Based on the findings of Wilson et al. (1999), Murdoch et al. (2008), and Burdett Hart et al. (2011), it is possible that fluctuations in salinity could influence the occurrence and severity of dermal lesions on bottlenose dolphins. As a fiord-like habitat, salinity fluctuations are significant in Golfo Dulce and may play a role in our findings. While any connection between habitat degradation and LLD may still be speculative, it is worth noting that Golfo Dulce is facing escalating anthropogenic pressures (Wolff et al. 1996, MINAET-SETENA 2011, 2012), which are increasing chemical contamination of the marine environment (Spongberg 2004). Further studies are needed to explore the role of environmental factors on lesion development in Golfo Dulce. More broadly, we hope that our findings will provide insight for researchers in other regions affected by LLD, who are attempting to piece together a better understanding of this infectious disease through dynamic means.

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