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## Lacaziosis in Bottlenose Dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida, USA

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**ABSTRACT:** The objective of this study was to document the presence of the fungal granulomatous skin disease lacaziosis in stranded Indian River Lagoon (IRL) bottlenose dolphins (*Tursiops truncatus*). From 1 January 2007 through 31 December 2007, stranded dolphins from the northern part of the IRL were thoroughly examined, and appropriate tissue samples were collected. The intralesional fungal agent (*Lacazia loboi*) was identified histologically in three bottlenose dolphins. Histologically, lacaziosis has been previously documented in IRL dolphins inhabiting the southern portion of the lagoon. Our findings suggest that the disease occurs throughout the lagoon. Enhanced monitoring of the prevalence of lacaziosis in dolphins throughout the IRL is needed to assess changes in population health.

**Key words:** Bottlenose dolphin, Indian River Lagoon, lacaziosis, *Lacazia loboi*, *Tursiops truncatus*.

Lacaziosis is a chronic granulomatous skin disease that is caused by the yeast-like organism, *Lacazia loboi* (formerly: *Loboa loboi*) that is yet to be cultured. The pathogen seems to be the phylogenetic sister taxon to the dimorphic onygenal pathogen *Paracoccidioides brasiliensis* (Herr et al., 2001). Although little is known about the epidemiology of the disease, it is suspected that the causal agent is a saprobic, hydrophilic pathogen (Ajello, 1998; Pradinaud, 1998). The disease has two known hosts, humans and dolphins. Positive identification of the organism is based on the unique morphology exhibited in lesions in both species. The use of additional diagnostics, including Western blot analysis and polymerase chain reaction can be useful in identifying and classifying *L. loboi* (Haubold et al., 1998; Herr et al., 2001;

Mendoza et al., 2008); however, these advanced methodologies support the usefulness of characteristic morphology of *L. loboi* to effectively identify the pathogen. Lacaziosis is considered zoonotic, with one documented case of an aquarium worker contracting the disease from an infected dolphin (Symmers, 1983). In humans, the disease causes nodular parakeleoidal lesions that typically occur on the ears and extremities of the individual. The disease is endemic to the Latin American countries, although there have been a few anomalous cases in North America in patients that had traveled to endemic areas (Burns et al., 2000; Elsayed et al., 2004). Transmission in both humans and dolphins is thought to be facilitated by trauma, injuries, or abrasions to the skin serving as portals of entry for the organism and may be correlated with immunodeficiency (Bossart, 1984; Pecher and Fuchs, 1988; Funchs et al., 1990; Reif et al., 2006, 2008; Xavier et al., 2006). In dolphins, the disease is found to occur on the head, flippers, flukes, dorsal fin, and peduncle (Caldwell et al., 1975; Dudok Van Heel, 1977) and has been documented along the coasts of the Gulf of Mexico, South America, and the coasts of Florida (Migaki et al., 1971; De Vries and Laarman, 1973; Caldwell et al., 1975; Bossart, 1984; Cowan, 1993; Simões-Lopes and Paula, 1993). The lesions are generally limited to the skin and present as a chronic proliferative and ulcerated dermatitis and cellulitis.

The Indian River Lagoon (IRL) is a shallow estuary system that extends approximately 220 km along the east coast of

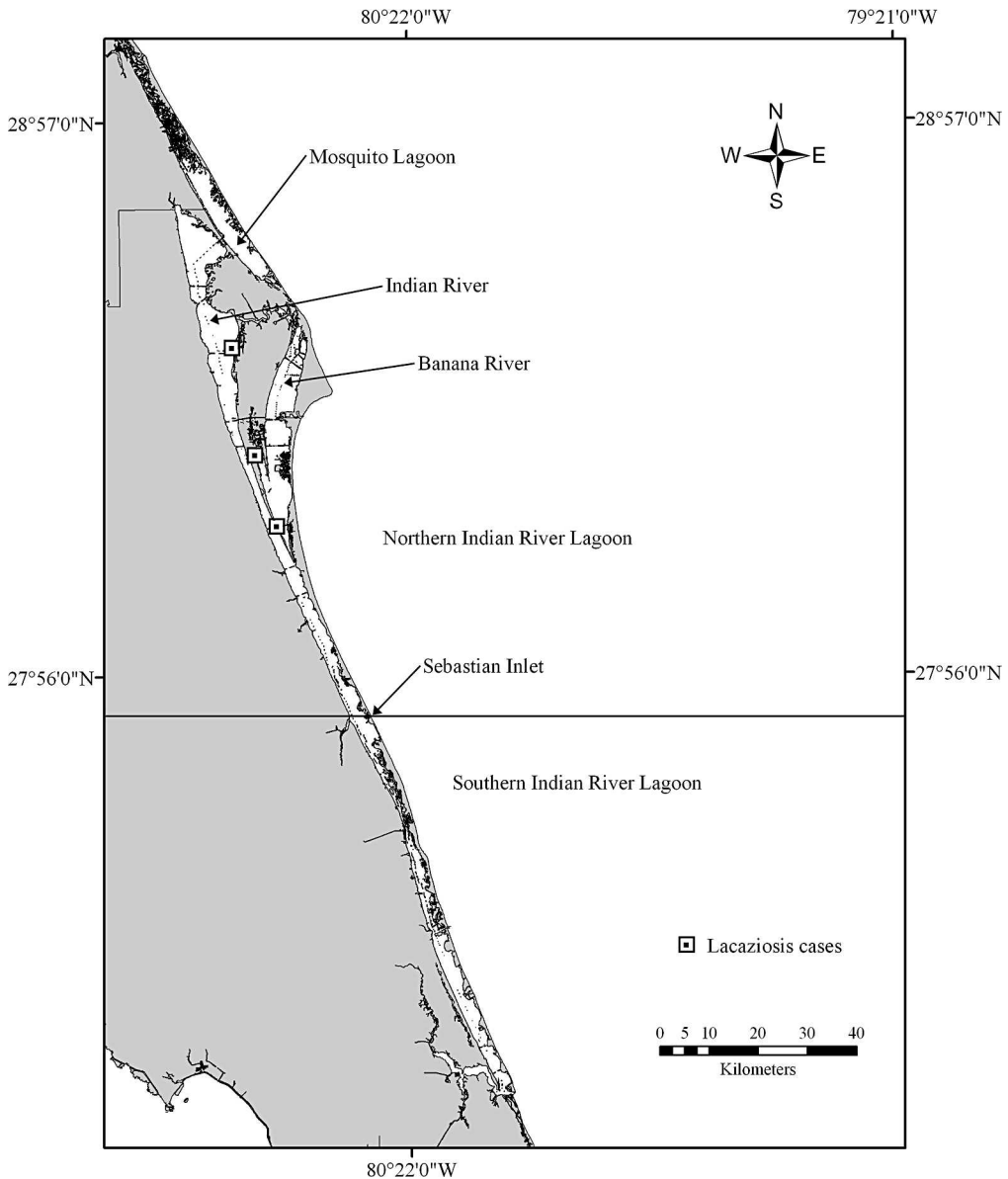


FIGURE 1. Map of the Indian River Lagoon, Florida, USA, and stranding locations for lacaziosis cases in the present study. Northern and southern lagoon is defined as north and south of Sebastian Inlet (arrow).

central Florida, USA (Mulligan and Snelson, 1983; Fig. 1). Although the estuary is open to the Atlantic Ocean at five inlets, the term “river” is a misnomer because there is no consistent flow pattern or gradient and the majority of water fluctuation is caused by wind currents (Mulligan and Snelson, 1983; Smith, 2001). The closed nature of the lagoon makes it

susceptible to an influx of terrestrial pollutants (Woodward-Clyde, 1994). In fact, it is suspected that diminishing seagrass coverage and loss of species diversity are related to contaminant accumulation and declining water quality (Virnstein et al., 1983; Sigua et al., 2000). In recent years, increased IRL dolphin mortality (Stolen et al., 2007) as well as

reports suggesting that the population is immune compromised (Bossart et al., 2003) have caused a growing concern for the health of Indian River Lagoon dolphins. This has resulted in efforts to assess the overall health of the population (Goldstein et al., 2006). The objective of this study was to document the presence of lacaziosis in IRL dolphins inhabiting the northern reaches of the lagoon.

From 1 January 2007 through 31 December 2007, all live, fresh dead, or moderately decomposed (Geraci and Lounsbury, 2005) bottlenose dolphins that were recovered stranded within the northern Indian River Lagoon (Fig. 1) were examined. Three bottlenose dolphins that exhibited advanced decomposition (Geraci and Lounsbury, 2005) were not included in the study. Total body length was measured as straight line length from the tip of the rostrum to the fluke notch (Norris, 1961). Sex was determined by external examination and internal examination of gonads. Teeth were extracted for age estimation, decalcified, sectioned using a freezing microtome, and stained with hematoxylin using standard methods (Myrick et al., 1983). Ages were estimated by counting the growth layer groups in the teeth (Hohn et al., 1989). All thickened, ulcerated, or irregular skin lesions were sampled via full thickness biopsy and preserved in 10% neutral-buffered formalin. Skin samples were paraffin embedded, sectioned at 3–5  $\mu\text{m}$ , and stained with hematoxylin and eosin and/or periodic acid Schiff and examined. Because the organisms have a characteristic morphology but have yet to be cultured, diagnosis was based on identifying a granulomatous dermatitis or cellulitis containing the *L. loboi* organisms. Thorough necropsies were conducted on dead animals, and tissue samples for histologic evaluation were collected, preserved, and processed using standardized procedures (Geraci and Lounsbury, 2005). Attempts were made to determine a cause of death for all animals.

In total, 33 IRL dolphins were examined during the study period. Three (9%) of the animals examined had raised, nodular lesions on the pectoral flippers, head, and lateral trunk. The first case stranded on 11 February 2007. This adult male (250-cm) bottlenose dolphin (Hubbs-0717-Tt) was stranded alive in Merritt Island, Florida, along the eastern shore of the Indian River (28°12.526'N, 80°38.442'W) and died on-site shortly afterward. The animal was emaciated and had multifocal pale areas of skin discoloration covering much of its body. Grossly the animal had chronic mandibular misalignment due to a healed fracture in the right ramus. The dolphin had extremely worn teeth and was estimated to be 22 yr old. An area of granulomatous thickening was present at the cranial and ventral aspect of the mandible. Histologically, this was an area of chronic fibrosis and inflammation without intralesional organisms. The left shoulder joint and atlanto-occipital joints demonstrated chronic and active arthritis with a marked, irregular and multinodular thickening of the joint capsule and erosion of the articular surfaces. The animal had unilateral otitis media in the left ear and had several trematodes (presumptively *Nasitrema* sp.) in necrotic, hemorrhagic tissue around the left ear. There was a 6-cm  $\times$  12-cm area of granulomatous thickening that was opaque, nodular, and ulcerated along the leading edge of the right pectoral flipper (Fig. 2A). In addition, multiple chronic conditions were present including hepatic periportal fibrosis, mild verminous pneumonia, and mild and multifocal fibrosing cardiomyopathy. The cause of death was determined to be emaciation and debilitation from the multiple chronic conditions.

On 6 June 2007, a free-swimming bottlenose dolphin (Hubbs-0738-Tt) was captured and relocated from a small retention pond in Merritt Island, Florida (28°20.37'N, 80°41.14'W) where the animal had been trapped for more than a week. The adult female (234 cm) had

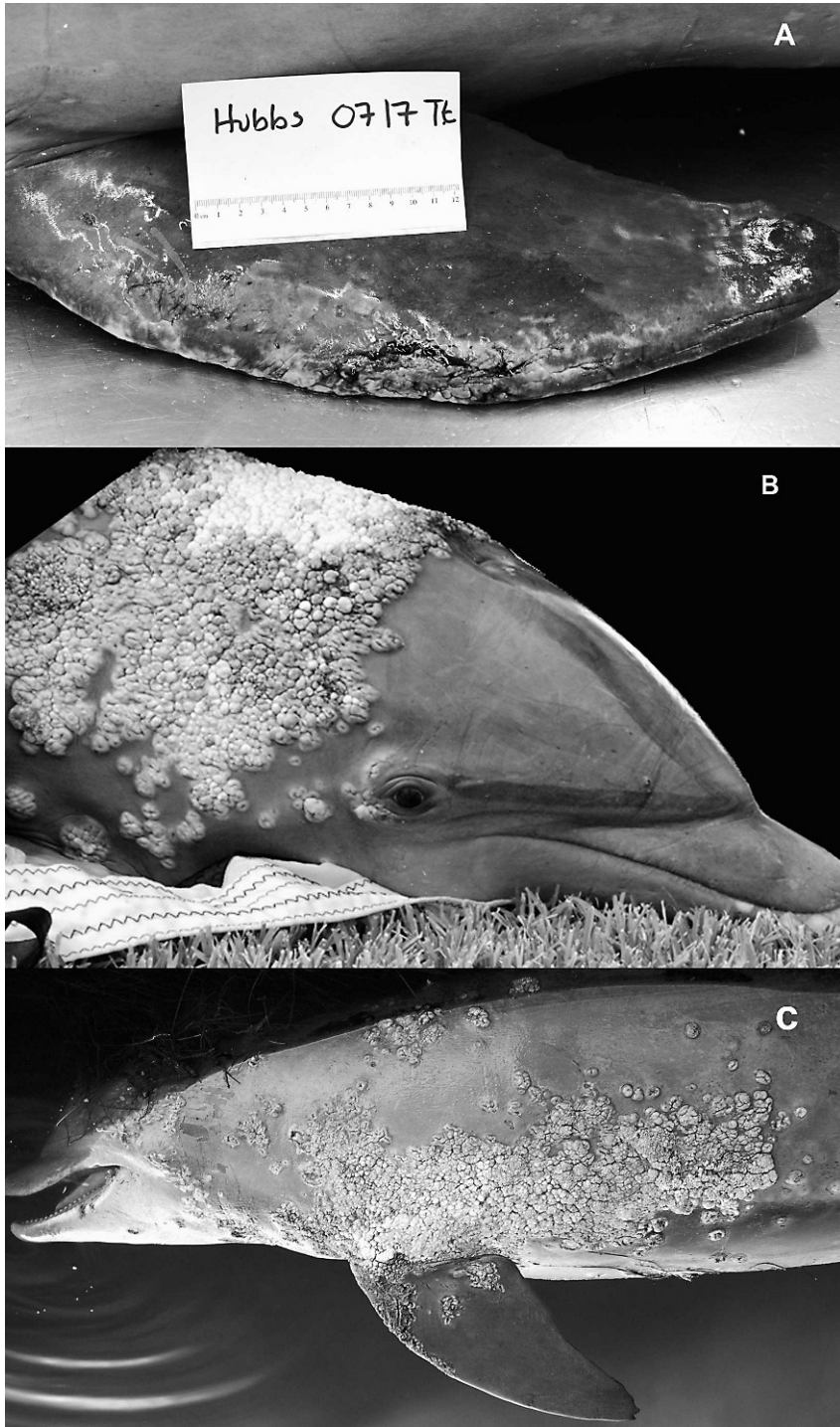


FIGURE 2. A. Case 1: Lacaziosis along leading edge of the right pectoral flipper of a live stranded bottlenose dolphin (Hubbs-0717-Tt). B. Case 2: A live stranded Indian River Lagoon, Florida, USA, with extensive lacaziosis circumscribing the head (Hubbs-0738-Tt). C. Case 3: Extensive lacaziosis covering the left lateral aspect of a dead stranded Indian River Lagoon dolphin (Hubbs-0744-Tt).



extensive cutaneous lesions on the left and right lateral and dorsal aspects of the cervical region, caudal to the eyes and blowhole, surrounding the head laterally and extending caudally 28 cm (Fig. 2B). Isolated lesions were also present on the dorsal fin. Lesions were opaque, nodular and sometimes ulcerated granulomatous thickenings. Before release, multiple skin biopsies were collected from the left lateral cervical region and preserved in 10% neutral-buffered formalin. Hematologic evaluation included a complete blood count and serum chemistry evaluation, and all values were within the normal range. On 2 July 2007, the dolphin was recovered dead approximately 5.4 km from the release site. Although autolysis precluded histologic evaluation of the tissues, the presumed cause of death was a large fish lodged in the esophagus. This animal was estimated to be 32 yr old.

On 3 July 2007, a dead adult (270-cm) male bottlenose dolphin (Hubbs-0744-Tt) stranded in Titusville, Florida (28°32.18'N, 80°43.96'W). Although the animal was moderately decomposed, the granulomatous thickening and extensive verrucose, nodular skin lesions were apparent caudal to blowhole on the head, along the left lateral aspect of the cervical region and along the thorax extending from the insertion of the left pectoral flipper to the base of the dorsal fin and on the left pectoral flipper (Fig. 2C). This animal was determined to be 27 yr old. Due to the state of decomposition and the lack of overt gross findings, the specific cause of death was not determined for this animal.

Histologic examination of skin from the proliferative regions in all three cases revealed multifocal, well circumscribed, patchy areas of histiocytic granulomatous dermatitis. Granulomas contained a mix of fibrous connective tissue and lymphocytes, plasma cells, macrophages, and multinucleate giant cells. Contained within these areas were thick-walled, globose cells that were approximately 10  $\mu$ m in diameter and were connected by narrow necks

(Fig. 3). Histologic findings are consistent with the morphologic diagnosis of multifocal granulomatous dermatitis with the intralesional fungal agent *L. loboi*.

Lacaziosis in bottlenose dolphins is endemic to the southeastern US waters. Many of the published accounts have come from the IRL and east coast dolphins (Caldwell et al., 1975; Bossart, 1984; Bossart et al., 2003; Reif et al., 2006). Anecdotal reports have documented the disease throughout the lagoon for decades (Caldwell et al., 1975; Odell, pers. comm.). More recently, Reif et al. (2006) examined 75 free-swimming IRL dolphins for lacaziosis. The study concluded that lacaziosis exhibited geographic variation with no cases seen in the northern portions (Fig. 1) compared with 30% of the animals in the southern portion exhibiting the disease. Based on photographic analysis, another study estimated disease prevalence at 6.8% throughout the lagoon and 4.9% in our study area, the northern part of the IRL (Murdoch et al., 2008). Based on histologic examination, our study documented lacaziosis prevalence to be nearly twice the photographic estimate for the northern portion of the lagoon (3/33 or 9%). Live animal resighting data for two of our cases confirmed that these animals were resident to the northern portion of the IRL (Mazzoil, pers. comm.).

Lacaziosis has been found to occur in older and possibly immune-compromised animals (Bossart, 1984; Reif et al., 2006, 2008); therefore, it is not surprising that animals in this study were relatively old (22–32 yr) with multiple chronic conditions present in some cases. Bottlenose dolphins from the Indian River Lagoon have been found to live up to 35 yr (Stolen et al., 2002). Studies on IRL dolphin health have found high concentrations of mercury (Durden et al., 2007), a toxic element that can compromise immune health (Moszczynski, 1997), and lingual and genital papillomas that may be related to immune dysfunction (Bossart, 2007).

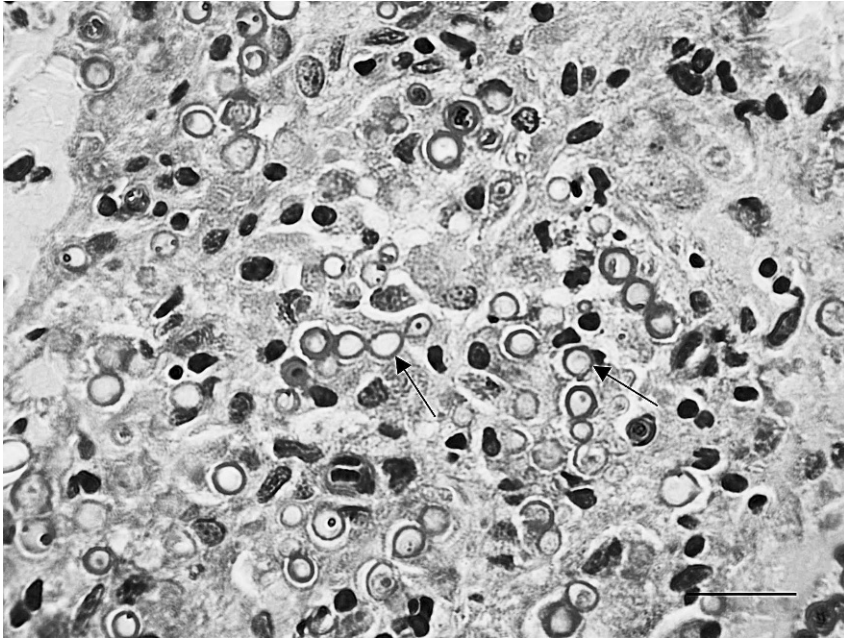


FIGURE 3. Photomicrograph of skin tissue from a bottlenose dolphin (Hubbs-0717-Tt) with lacaziosis. Note the globose cells (9–12  $\mu\text{m}$  in diameter) (arrows) that are connected by narrow necks. PAS stain. 60 $\times$ . Scale=30  $\mu\text{m}$ .

The apparent diminished health of these animals coupled with the stagnant nature of the ecosystem may contribute to the presence of this apparently endemic disease. Future studies are warranted to determine the prevalence of the disease over time and its relation to the age, overall health status, and immunologic status of the population.

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