



Epidemiological investigation of tattoo-like skin lesions among bottlenose dolphins in Shark Bay, Australia

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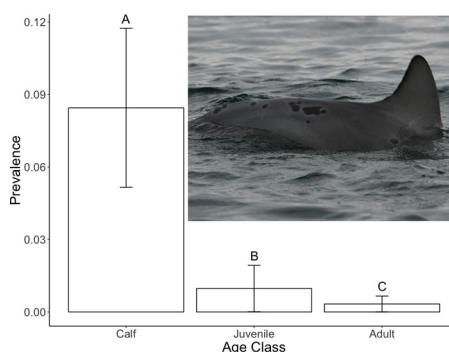
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HIGHLIGHTS

- Tattoo-like skin disease (TSD) presents as skin lesions in bottlenose dolphins.
- TSD is associated with age, with young animals having the highest prevalence.
- TSD dynamics could be a potential way to measure anthropogenic effects.
- TSD prevalence may offer insight into population health.

GRAPHICAL ABSTRACT



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ABSTRACT

Bottlenose dolphins are excellent bioindicators of ocean ecosystem health for three reasons: (a) as long-lived apex predators they accumulate biotoxins and contaminants; (b) they are visible, routinely appearing at the water's surface in coastal areas, often coming into close contact with humans; and, (c) they exhibit a range of pathogenic lesions attributable to environmental degradation. In this study, we analyzed tattoo-like skin lesions in a population of *Tursiops aduncus* studied for 30+ years in Shark Bay, Australia, a UNESCO World Heritage Site. We provide important baseline data by documenting epidemiological patterns of tattoo-like skin lesions in a healthy, free-ranging population that builds on the previous data of tattoo skin disease (TSD) derived from free ranging, stranded, and dead dolphins. Individual dolphins were classified as symptomatic with tattoo-like skin disease if at least one photograph showed a lesion similar to TSD. The average age of infection was 26.6 months (± 34.8 months) with the symptomatic period lasting 137 ± 29.8 days. Overall prevalence of tattoo-like skin disease in the population was 19.4%. Age, but not sex, was significant, with yearlings (1–2 years) exhibiting tattoo-like lesions more than younger and older calves. Tattoo-like lesions were rare among juvenile and adult dolphins ($N = 68$ calves, 4 juveniles, and 3 adults). We hypothesize that the lower prevalence in youngest calves (<1 year) is due to maternal immunity, while older individuals (>2 years) have infection-acquired immunity, as reported for other small cetaceans. The low prevalence of tattoo-like lesions in Shark Bay compared to other populations with poxvirus is consistent with reproductive and demographic viability analyses. Furthermore, by documenting the demography of the disease, we can monitor changes in the prevalence of tattoo-like lesions as a sentinel indicator of ecosystem health.

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1. Introduction

As long-lived top predators, cetaceans accumulate toxins and contaminants from both the marine environment and consumed prey. As such, they serve as important sentinels of ecosystem health (Van Bresseem et al., 2015; Wells et al., 2004). Although health assessments of wild cetaceans are difficult to administer without capture, skin lesions provide visible evidence of disease, such as lobomycosis, dermatitis caused by herpes virus, and rhomboid lesions caused by *Erysipelothrix rhusiopathiae*, a potentially lethal disease (Barr et al., 1989; Mouton and Botha, 2012; Van Bresseem et al., 2009a). Consequently, increasing prevalence of cetacean skin lesions can potentially signal weakened immunity (Van Bresseem et al., 2009a), possibly as the result of stress from environmental perturbation (Fury and Reif, 2012; Waples and Gales, 2002), habitat degradation due to increasing anthropogenic effects (Stephens et al., 2012), concurrent infection (Schulman and Lipscomb, 1999), and/or the presence of pollutants in the environment (Reif et al., 2008; Stephens et al., 2012; Wilson et al., 1999).

Bottlenose dolphins (*Tursiops aduncus* and *T. truncatus*) are some of the most common and recognizable species of cetaceans due to their coastal living and dominance in aquaria. Furthermore, humans come into contact with bottlenose dolphins, whether in controlled environments or in the wild (Samuels et al., 2003). Worldwide, there are over 20 sites where wild dolphins have regular in-water human contact, often including physical contact, including in Shark Bay (Foroughirad and Mann, 2013; Samuels et al., 2003). Therefore, the potential for zoonotic and reverse zoonotic transmission is germane to public health (Waltzek et al., 2012).

Bottlenose dolphins may contract many skin diseases of viral, fungal, ciliate, and bacterial origins (Schulman and Lipscomb, 1999; Van Bresseem et al., 2009b). Cetacean poxvirus - originally identified by Geraci and colleagues - is common and typically identified by gray or black circular pigmentation (Baker, 1992; Barnett et al., 2015; Fiorito et al., 2015; Flom and Houk, 1979; Fury and Reif, 2012; Hart et al., 2012; Geraci et al., 1979; Van Bresseem and Van Waerebeek, 1996; Van Bresseem et al., 2003; Van Bresseem et al., 2009a; Van Bresseem et al., 2015; Van Bresseem et al., 2017). The lesions resulting from poxvirus were subsequently named “tattoo skin disease” (TSD) by Van Bresseem and colleagues (Van Bresseem et al., 2003) and have been found in numerous cetacean populations (Barnett et al., 2015; Blacklaws et al., 2013; Bracht et al., 2006; Flom and Houk, 1979; Geraci et al., 1979; Van Bresseem and Van Waerebeek, 1996). Association between poxvirus and TSD has been substantiated with investigations on dead, stranded, or captive animals, although some studies have combined observations of free-ranging dolphins with deceased individuals (Van Bresseem et al., 2009a). The viral infection manifests as large, flat round lesions with dark coloration, a characteristic stippled pattern, and a darker border that fade to a lighter color before fading entirely (Geraci et al., 1979; Van Bresseem et al., 2003). Genetic analysis of cetacean poxvirus places it in the *Poxviridae* subfamily (Blacklaws et al., 2013; Bracht et al., 2006). Sequencing and phylogenetic analyses indicate that cetacean poxviruses are most closely related to *Orthopoxvirus* (Bracht et al., 2006; Blacklaws et al., 2013), and clusters into its own genus (Barnett et al., 2015). While cetacean poxviruses are common in captivity and have not been shown to be zoonotic (Van Bresseem et al., 2017), pinniped poxviruses such as sealpox (*Parapoxvirus*) have been directly transmitted to humans (Van Bresseem et al., 2009b; Waltzek et al., 2012).

In dolphin populations where TSD has been studied, juveniles (defined as >9 months but not sexually mature) are affected more than adults or nursing calves (Van Bresseem et al., 2009a). It is hypothesized that maternal antibodies passed through milk to young calves offer protective immunity, but as milk composition changes over time, such passive immunity is reduced (Langer, 2009; Van Bresseem and Van Waerebeek, 1996; Van Bresseem et al., 2003). TSD has been studied in different species and populations located off the coasts of Chile, Peru,

Argentina, Brazil, the United States, Canada, United Kingdom, Portugal, and New Zealand, among others (Baker, 1992; Barnett et al., 2015; Fiorito et al., 2015; Hart et al., 2012; Van Bresseem et al., 2003; Van Bresseem et al., 2015). To date, poxvirus-like disease has been studied in *T. aduncus* in eastern Australia, uncovering a correlation between changing salinity due to flooding and infection rates (Fury and Reif, 2012). Additionally, poxvirus was confirmed in the Swan River in western Australia and was associated with morbillivirus infection and chemical pollution (Stephens et al., 2012).

Our objectives were to characterize and report the demographics of tattoo-like skin lesions in the resident *T. aduncus* population in Shark Bay, Australia for the first time. As the population is bisexually philopatric and resident year-round (Tsai and Mann, 2013), individuals are frequently re-sighted and tracked over many years, allowing for longitudinal study of infected individuals. Similar to other populations, this disease appeared as black or white circular lesions on the skin surface. Despite the similarity in the presentation of symptoms, we refrained from referring to this disease as TSD due to the lack of virological confirmation and refer to it as tattoo-like lesions or tattoo-like skin disease henceforth. We investigated the overall prevalence, symptomatic period, and demographic (age and sex) patterns of tattoo-like lesions in Shark Bay dolphins. We also examined the effect of tattoo-like lesions on calf survival and specifically consider individuals that have high contact with humans. Based on previous studies, we did not expect to find a sex difference in tattoo-like lesions (but see Van Bresseem et al., 2017), but we did expect to find higher prevalence of tattoo-like lesions in calves (defined as nursing individuals) than in juveniles (defined as weaned, but sexually immature individuals) or adults.

Shark Bay is the oldest provisioning site in Australia where up to five adult female dolphins are fed by humans in nearshore waters up to three times a day, a tourist attraction that is controlled by the Western Australia Department of Biodiversity, Conservation and Attractions. Provisioning creates opportunities for zoonotic transmission via skin-to-skin contact between dolphins and humans (Foroughirad and Mann, 2013). Beyond the potential for zoonotic transmission, the characterization of tattoo-like lesions in Shark Bay is vital to providing a baseline for this disease in order to accurately use it as a bioindicator and assess the impacts of ecosystem changes.

2. Methods

2.1. Study site and population

The Shark Bay Dolphin Research Project (SBD RP) is a longitudinal study, running continuously since 1984 with data on >1700 individuals (Mann, 2000). Shark Bay is a UNESCO World Heritage Site with few anthropogenic impacts relative to other cetacean study sites (Mann and Karniski, 2017). Dolphins in the Shark Bay population are uniquely identified by distinctive markings on their dorsal fins, allowing for long-term identification of individuals (Karniski et al., 2015). In Shark Bay, calves remain dependent on their mothers for four years on average (Mann et al., 2000). After weaning, dolphins are considered juveniles until sexual maturity around 10 years of age (age at first pregnancy; Mann et al., 2000, J.M. unpublished data), when they are considered adults (Brook et al., 2000; Kemper et al., 2014; Wells et al., 1987). Age and sex determinations were the same methods as reported previously (Karniski et al., 2015; Mann et al., 2000).

2.2. Tattoo-like lesion identification

We used two sources of data to identify suspected cases of tattoo-like lesions: (a) photographs of identified individuals; and (b) observer descriptions of lesions, marks, scars, growths, and discolorations. Dolphins with suspected tattoo-like lesions were selected from a database of 8142 photographs, wherein all visible marks, scars, etc. in each photo are recorded and visually inspected. We searched the

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