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

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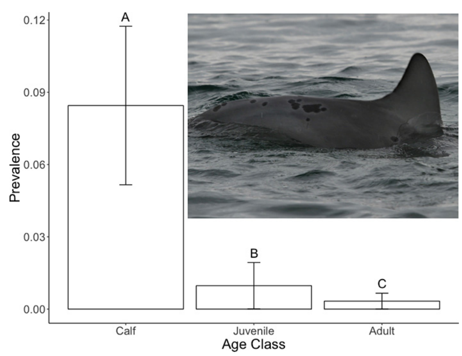
HIGHLIGHTS

- 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 Bressem 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 Bressem et al., 2009a). Consequently, increasing prevalence of cetacean skin lesions can potentially signal weakened immunity (Van Bressem 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 Bressem et al., 2009b). Cetacean poxvirus - originally idenitfied by Geraci and colleageus - 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 Bressem and Van Waerebeek, 1996; Van Bressem et al., 2003; Van Bressem et al., 2009a; Van Bressem et al., 2015; Van Bressem et al., 2017). The lesions resulting from poxvirus were subsequently named “tattoo skin disease” (TSD) by Van Bressem and colleagues (Van Bressem 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 Bressem 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 Bressem 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 Bressem 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 Bressem et al., 2017), pinniped poxviruses such as sealpox (*Parapoxvirus*) have been directly transmitted to humans (Van Bressem 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 Bressem 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 Bressem and Van Waerebeek, 1996; Van Bressem 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 Bressem et al., 2003; Van Bressem 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 Bressem 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 (SBDRP) 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 database for terms such as "black spot disease," "black circle disease," "lesions," "fungus," "growths," or "odd skin coloration." Compiled photos of individuals were examined systematically by the same observer (S.P.) to assess whether tattoo-like lesions were present or not. If skin lesions were mentioned but did not have a corresponding photograph (one case), it was excluded from our analyses. In each case, we prioritized photographic evidence over observer description. A total of 418 photos were found with identifiable tattoo-like lesions, with each dolphin having an average of 4.93 (SD = 3.75) relevant photos showing a) tattoo-like lesions, b) affected body parts prior to infection, and/or c) affected body parts post-infection.

Once all individuals with tattoo-like lesions were identified, we analyzed photos of those individuals to determine the earliest and latest date in which the afflicted body part showed symptoms of the disease. We visually assessed lesion progression to characterize disease stages, following the disease progression set forth by Van Bressem and colleagues (Van Bressem et al., 2003).

Once the population of individuals with tattoo-like lesions was determined, we re-examined each photograph to categorize four stages of disease progression. Although we examined the frequency of tattoo-like lesions by body part, this analysis was limited by the fact that the dorsal area routinely breaks the water's surface and is photographed frequently (e.g., dorsal fin, lateral and dorsal side) while the tail flukes, head and ventral sides are seldom photographed. Individuals with a relatively well-documented infectious bout (i.e. photographs spanning three or more disease categories) were included in the analysis to determine the symptomatic period.

2.3. Characterizing the demographic distribution of tattoo-like lesions

To calculate prevalence, we defined our population sample as all individuals that survived to age three and had a minimum of five sightings within the first three years of life. A cutoff of five or more sightings was used to enhance our confidence of observing tattoo-like lesions. Of this sample, individuals with tattoo-like lesions were identified as discussed above, and the remaining individuals in the sample were considered unaffected. We used individuals seen between 2002 and 2015 as those years were heavily sampled. We calculated a population prevalence using the sample of diseased and unaffected individuals and created a 95% confidence interval by bootstrap resampling with 1000 replicates (package "boot" in R version 3.4.1) (R Core Team, 2017). Using this same population, we took the subset of individuals that are provisioned animals and calculated prevalence with a 95% confidence interval by bootstrap resampling with 1000 replicates (package "boot" in R version 3.4.1) (R Core Team, 2017).

To calculate age-specific prevalence of tattoo-like lesions, we binned ages for the population by year. For this count, animals were only included if they had been sighted at least 15 times over their lifetime (offspring were included if their mothers were seen 15 times). The 15-sighting minimum was chosen to obtain a more accurate estimate of the total population size from year to year in our study area and reduce the inclusion of infrequently sighted individuals that live in neighboring communities. The annual disease-afflicted and disease-free populations were averaged over the years 2002–2015, and ages within each age class were summed. We calculated average age-specific prevalence for each age class (calf, juvenile, and adult) and created a 95% confidence interval by bootstrap resampling with 1000 replicates (package "boot" in R version 3.4.1) (R Core Team, 2017). We then used the bootstrapped samples in an ANOVA to test for an age effect on the prevalence of tattoo-like lesions and ran pairwise t-tests to determine differences among age classes. The prevalence of tattoo-like lesions prevalence was also compared within the calf age group by comparing calves aged 0–1 years, 1–2 years, and 2–3 years using pairwise t-tests.

To investigate association between the presence of tattoo-like lesions and calf survival, we defined our population sample as all individuals who had been seen at least five times within the first three years of life and had a known calf mortality status (binomial survival to age 3, Y or N). Calf mortality is determined by repeated sightings of the mother without her calf and the calf never being sighted again. Since there is no emigration (Tsai and Mann, 2013), this method is reliable for determining calf mortality (Mann et al., 2000). Individuals with tattoo-like lesions were identified as described above. We used a Fisher's Exact test to test for sex bias among infected individuals and disease associations with survival.

All statistical analyses were conducted in R, version 3.4.1 (R Core Team, 2017).

2.4. Mortality

Calves younger than age 3 were considered dead if their mothers were sighted without them and the calf was never seen again. Juveniles and adults were considered dead if they were not sighted for 3 years or more, despite being sighted every year prior. If a dolphin was not sighted every year, death dates were assigned as the maximum gap between sighting years after three times that gap was passed. For example, if a dolphin was sighted annually but there were occasional one-year gaps, 6 years would have to pass of no sightings before a death date was assigned as 2 years after the last sighting. Strandings are rare in Shark Bay and dead dolphins are rarely found due to inaccessible coastline and a plethora of sharks (Heithaus and Dill, 2002).

3. Results

Tattoo-like lesions are detectable when dark black or gray circular or oval marks appear. The lesions range in size and have not been directly measured but seem to average ~5 cm in diameter. The lesions begin to change color over time, with the inside gradually lightening until the spot has become light inside with a darker border. The lesions will continue to fade until they are light gray/white throughout, before eventually fading entirely, leaving no trace behind (Table 1, Figs. 1 and 2). While the photographic evidence of tattoo-like lesions is biased towards areas that frequently break the water's surface, such as the dorsal side and dorsal fin, tattoo-like lesions have been photographed across the entire body, including near the genital slit, on the beak, and on the tail flukes. Individuals that were photographed with a lesion from any of the four categories were included in the analyses.

A total of 79 dolphins, photographed between 1989 and 2015, showed evidence of tattoo-like lesions ($N = 68$ calves, 4 juveniles, 3 adults, and 4 young animals of unknown age class). Of these 79 dolphins, 26 were male, 23 were female, 30 of unknown sex, and 10 individuals died within one year of disease onset. We identified the mean symptomatic period of tattoo-like lesions in this population to be 137 days (SD = 29.8 days) with a median of 137 days ($N = 10$, Fig. 3). Determination of the symptomatic period for the disease was complicated by the fact that some dolphins appeared to have recurrent tattoo-like lesions. For example, a male calf was photographed with category 4 tattoo-like lesions in November 2009 and then was photographed with category 2 tattoo-like lesions in April 2010. Two other male calves showed a similar pattern of tattoo-like lesions

Table 1
The categorizing scheme that was followed in order to classify the disease present and thereby the stage of the disease with which a dolphin is currently afflicted.

Category	Description
1	Brand new disease occurrence where the lesions are very dark in coloration
2	Lesions have exhibited a color change and non-uniform throughout
3	Lesions are light, although not necessarily uniform in color, on the inside with a darker border
4	Lesions are light throughout potentially having a dark border; Lesions are generally light gray in this stage and beginning to substantially fade

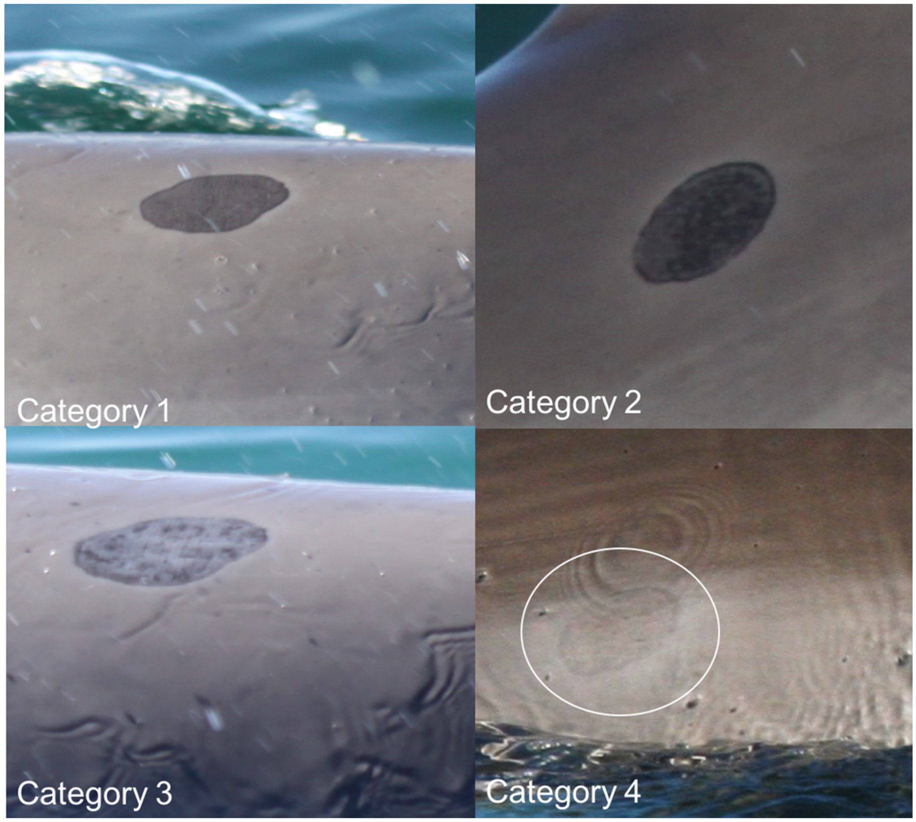

Fig. 1. Representative lesions showing the four stages of tattoo-like lesions as they progress from a newly acquired infection to fully healed. Category 1 is the earliest stage of the disease and Category 4 is the latest stage of the disease. The lesions pictured for Category 1 and 3 are the same lesion as is the lesion pictured for Category 2 and 4. Both dolphins pictured were nursing calves when these photographs were taken. The contrast has been increased for the Category 4 image to better show the lesion.

recurrence. However, most dolphins progressed from Category 1 to 4 of the disease and have not been seen with tattoo-like lesions since.

The prevalence of tattoo-like lesions in the Shark Bay population was 19.4% ($N_{disease} = 48$, $N_{total} = 199$, 95% CI = 14.6%–24.3%). The prevalence of tattoo-like lesions within the subset of provisioned dolphins was similar at 15% ($N_{disease} = 3$, $N_{total} = 17$, 95% CI = 0.00%–30.6%). The prevalence of tattoo-like lesions among calves (defined as 0–3 years old) was 8.45% ($N_{disease} = 6$, $N_{total} = 66$, 95% CI = 1.97%–14.7%). The prevalence of tattoo-like lesions among juveniles and adults (defined as 3–10 years old and >10 years old, respectively) was much lower: juvenile = 0.971% ($N_{disease} = 1$, $N_{total} = 103$, 95% CI = 0.00%–2.86%), adult = 0.330% ($N_{disease} = 1$, $N_{total} = 303$, 95% CI = 0.00%–0.98%).

We found the average age of an individual with tattoo-like lesions was 26.6 months (ranging from 1 to 212 months, or 0–18 years) and that tattoo-like skin lesions were associated with age class (calf, juvenile, adult) ($Z = -1.8899$, $df_{treatment, error} = 17, 66$, $P = 0.019$) (Fig. 4). Specifically, we found that calves were more affected than juveniles ($t = 155.23$, $P < 0.00001$) and adults ($t = 174.44$, $P < 0.00001$). The prevalence of tattoo-like lesions in juveniles was significantly higher than in adults ($t = 44.013$, $P < 0.00001$). Calves <12 months and >24 months had tattoo-like lesions significantly less than calves between 1 and 2 years old ($t_{<12mos.} = -3.82$, $P < 0.0008$; $t_{>24mos.} = 4.6714$, $P < 0.0002$) (Fig. 5).

For our analyses of the association between tattoo-like lesions and survival, we used a subset of 53 disease individuals ($N_{survive\ to\ 3} = 48$, $N_{did\ not\ survive\ to\ 3} = 5$) and 246 unaffected individuals ($N_{survive\ to\ 3} = 199$, $N_{did\ not\ survive\ to\ 3} = 47$). Tattoo-like lesions were not significantly associated with survival to age 3 ($P = 0.1$, 95% CI = 0.838–7.68, odds ratio = 2.262). There was no significant sex bias in tattoo-like lesion presentation ($P > 0.9$, odds ratio = 0.966, 95% CI = 0.488–1.919).

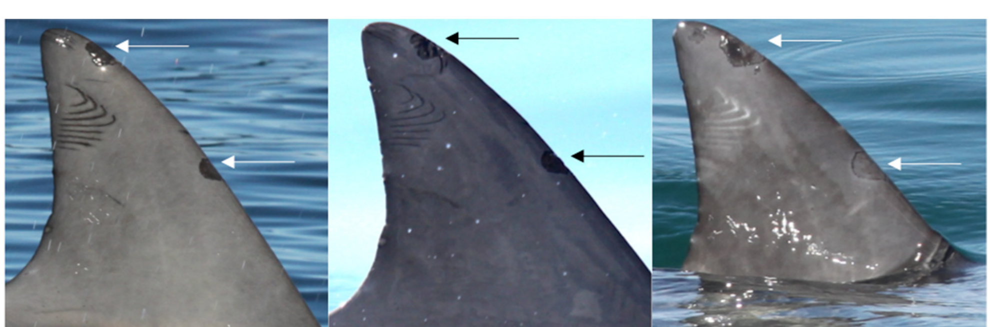

Fig. 2. A representative progression from Category 1 to Category 3 of tattoo-like lesions in the same individual. The dolphin was 22 months old at the onset of symptoms. The brightness and contrast in the middle photograph have been increased to better show the lesion.

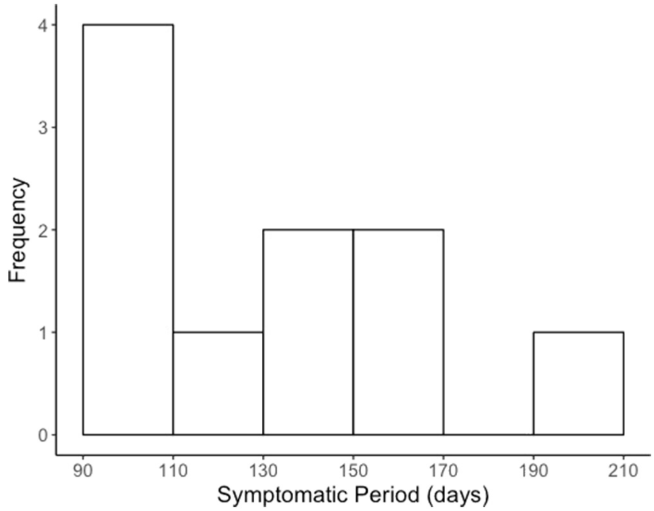

Fig. 3. Histogram showing the average symptomatic period of tattoo-like lesions. The symptomatic period was calculated using 10 individuals that had been photographed throughout at least 3 categories of their disease progression.

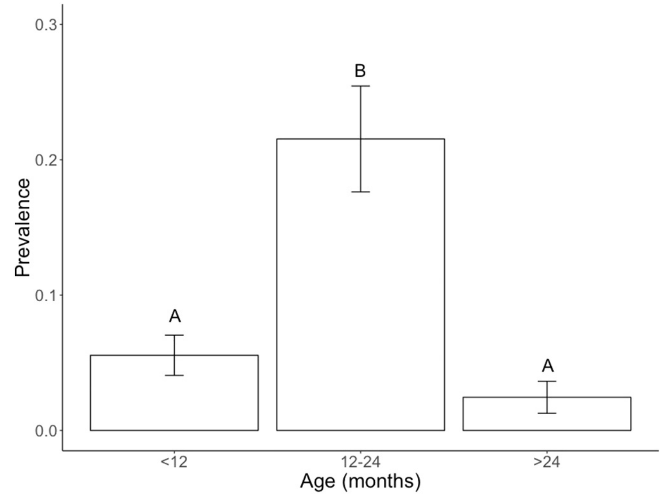

Fig. 5. Prevalence among 12 to 24-month-old individuals is significantly higher than prevalence in both <12 month olds and >24 month olds (P < 0.001 indicated by lettering). Error bars indicate one standard error above and below the mean. Only individuals aged 0–3 years old were included in this analysis.

4. Discussion

Our primary objectives were to (a) characterize tattoo-like lesions in Shark Bay dolphins for the first time, and (b) document age- and sex-specific patterns to better understand the epidemiology of tattoo-like skin disease. Although the overall prevalence of tattoo-like lesions was 19.4%, we observed precise and strong age effects with calves aged 1–2 years showing infection at a higher rate than any other age group. This pattern could assist as an early warning system regarding anthropogenic or other threats to dolphin populations and the marine environment. Given that other studies show higher prevalence of TSD, an increase in the prevalence of tattoo-like skin disease in the Shark Bay population might indicate compromised immune function, which may be linked to environmental degradation (Maldini et al., 2010; Stephens et al., 2012; Van Bressem and Van Waerebeek, 1996; Wilson et al., 1997; Wilson et al., 1999).

We found the symptomatic period for tattoo-like lesions to last approximately 130 days, but the sample size was small and the symptomatic duration may be confounded by other variables, such as age and overall health. For comparison, a study of *T. truncatus* in the Sado estuary in Portugal, found that TSD persisted for variable periods ranging from 3 to 45.5 months (Van Bressem et al., 2003). Our finding of the long symptomatic period combined with little evidence for tattoo-like lesion-caused mortality supports the hypothesized tradeoff between virulence and infectiousness duration (Anderson and May, 1982). Although tattoo-like lesions were not significantly associated with mortality, the presence of tattoo-like lesions could still be indicative of a weakened immune system and, along with concurrent infection or stresses, could be a factor in calf mortality. To reduce false negatives, we only included individuals in the non-diseased population if they were sighted five times or more before age three, increasing the chances that non-diseased individuals would be in the surviving population. Further investigation is needed on this topic.

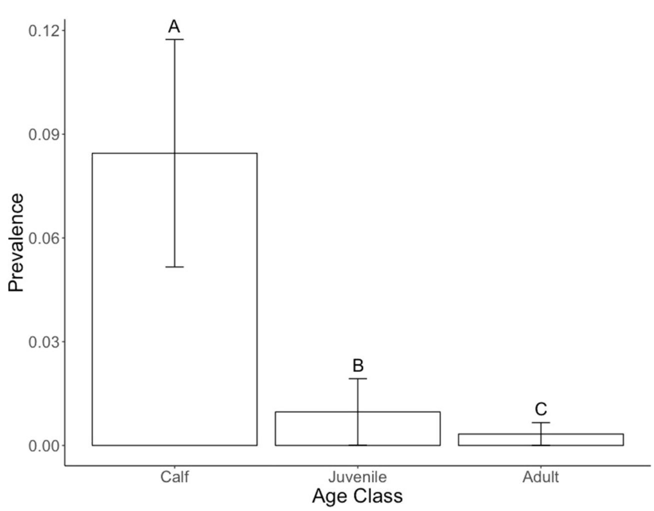

Fig. 4. Tattoo-like lesion prevalence distribution by age class. Error bars indicate one standard error above and below the mean. The lettering above each bar indicates a significant difference (P < 0.00001) between each age class.

Other delphinid populations appear to have a higher prevalence of poxvirus diseases than in Shark Bay. In Peruvian populations of dusky dolphins (*Lagenorhynchus obscurus*) and long-beaked common dolphins (*Delphinus capensis*), the prevalence of TSD was reported as 34.7% for the dusky dolphin and 61.1% for the long-beaked common dolphin (Van Bressem and Van Waerebeek, 1996). Additionally, poxvirus-like lesions affected up to 80% of coastal bottlenose dolphins in California (Maldini et al., 2010). Such unusually high prevalence could be due to pollution or other stressors such as fishery interactions or differences in population density between study sites (Mouton and Botha, 2012; Van Bressem et al., 2009a).

The age profile of tattoo-like lesions in Shark Bay dolphins is comparable to other populations with TSD despite different methods for age classification and population size. Direct comparisons are difficult to make because birth year and weaning age are frequently inferred by size at other sites, rather than being based on longitudinal data and a known birth and weaning date (Fury and Reif, 2012; Van Bressem and Van Waerebeek, 1996; Van Bressem et al., 2009a), and because the categorization into calf, juvenile, and adult differed between studies. Nevertheless, both tattoo-like lesions and TSD primarily affect younger individuals and the suggestion that TSD was more common in juveniles in free-ranging populations (Van Bressem et al., 2009a) is probably because all of Shark Bay 2-year olds were still nursing (calves), but were classified as juveniles based on available biological data at other sites. In Portuguese waters, statistical comparisons were between immature and mature dolphins, with the immature group having significantly higher prevalence (Van Bressem et al., 2003). In Van Bressem et al. (2009a), the free ranging species included were *T. truncatus*, *Sotalia guianensis*, *Cephalorhynchus eutropia* and calves were defined as individuals between ages 6–9 months based on body size and behavior. However, *T. truncatus* weaning ages are almost invariably >18 months, meaning that some nursing calves in this study would be classified as juveniles in another (Möller, 2011). Regardless, according to these studies, animals older than 9 months were more likely to have the disease than younger animals, which is in alignment with our results (Van Bressem et al., 2009a). Fury and Reif (2012) distinguished between young calves, older calves and juveniles that could tolerate separations from their mothers, and adults; they found that younger animals were more likely to have the disease and that the incidence rate was 100% in calves compared to 25% in juveniles.

In Shark Bay, we found that calves aged 0–1 years only rarely had tattoo-like lesions, and calves aged 1–2 years had tattoo-like lesions significantly more than calves aged 2–3 years. Whether this age pattern during the calf period is attributed to disease exposure or immune system development is unclear. There were two cases where individuals presented with lesions twice, but it was never confirmed that these individuals were ever fully without tattoo-like lesions between the two observed symptomatic periods. Because we found that only few adults develop lesions, perhaps only immune-sensitive individuals, such as calves, and those who do not gain immunity from their mother, presuming that the mother did not contract tattoo-like lesions, generally develop lesions. A caveat to this point is that among one mother-calf pair, both had tattoo-like lesions; the mother had tattoo-like lesions in 2004 as a juvenile approximately 10 years prior to her calf acquiring the disease. For the most part, we cannot determine which mothers had tattoo-like lesions as calves since many were juveniles or adults at the onset of our study. As such, it is hard to assess if calves would have received protective immunity through nursing because we cannot determine maternal exposure.

In general, TSD in wild bottlenose dolphins does not show a sex bias (Maldini et al., 2010), although some studies did not include sex in their analyses (Fury and Reif, 2012; Hart et al., 2012; Van Bressem et al., 2003). Captive male bottlenose dolphin had a higher incidence of TSD than females in one study (Van Bressem et al., 2017). The only reported sex difference in TSD in a free-ranging population is among Burmeister's porpoises (*Phocoena spinipinnis*) off the coast of Peru, where males were infected at twice the rate of females (Van Bressem and Van Waerebeek, 1996). To date, sex differences in TSD have not been reported in free-ranging bottlenose dolphins (Van Bressem et al., 2009a).

Although current evidence does not support the conclusion that lesions are zoonotic, the potential for zoonosis, and reverse zoonosis, should be considered, especially in locations where close human-dolphin contact regularly occurs. In Shark Bay, there is a small provisioning program that includes 5 adult females and their offspring that visit the shores as a tourist attraction (Foroughirad and Mann, 2013). Although humans are not allowed to initiate physical contact with dolphins, the animals in the provisioning program do make physical contact with humans, often rubbing on their legs or making occasional contact with swimmers. This contact may create a potential pathway for transmission from dolphins to humans. Recruitment of new individuals to provisioning is limited to two existing matrilines (i.e., only daughters of females who were provisioned will be introduced), so their disease histories would be known. Poxviruses with zoonotic transmission from marine mammals to humans include sealpox (Waltzek et al., 2012). Additionally, a virus from the same family, monkeypox, is zoonotic and is associated with childhood mortality in unvaccinated individuals; the virus was originally zoonotic from the monkey family *Cercopithecidae* although transmission is now endemic in humans (Damon, 2011; Lewis-Jones, 2004).

Other than the provisioning program, Shark Bay has very low anthropogenic impact. Given the high reproductive rate of the population (Manlik et al., 2016), and the low prevalence of tattoo-like lesions (this study), we suggest that tattoo-like lesions may be a good indicator of population health (Van Bressem et al., 2009a). Shark Bay waters are relatively pristine but not exempt from environmental threats. For example, extreme heat and flooding in Shark Bay in 2010–2011 caused a massive seagrass die off (Fraser et al., 2014; Thomson et al., 2015). Cascading impacts of ecosystem disturbance are a serious stressor for top predators. Careful documentation of the prevalence and age-specificity of tattoo-like lesions and other skin lesions in wild dolphins can be useful bioindicators when environmental conditions deteriorate. Given that photo-ID catalogs are available for most long-term studies of dolphins (Mann and Karniski, 2017), similar analyses or meta-analyses across populations would be useful as it could be mapped to environmental events. Further research is required to understand the social, environmental, abiotic, and anthropogenic drivers of tattoo-like skin disease and characterize the consequences on dolphin health.

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Conflict of interest

The authors have no conflicts of interest to report.

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