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Assessment of epidermal condition and calf size of Fiordland bottlenose dolphin *Tursiops truncatus* populations using dorsal fin photographs and photogrammetry

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ABSTRACT: The bottlenose dolphin *Tursiops truncatus* population in Doubtful Sound, New Zealand, has declined by over 34% since 1995 and is subject to potential impacts from tourism and habitat modification via freshwater discharge from a hydroelectric power station. The bottlenose dolphin population in neighbouring Dusky Sound is exposed to much lower levels of tourism and the fiord receives only natural freshwater runoff. We used dorsal fin identification photographs from both populations to compare levels of epidermal disease and laser photogrammetry to measure the dorsal fin base length of calves (<1 yr old) to assess differences in calf size and birth seasonality between the populations. Epidermal lesions were common in both populations (affecting >95% of individuals), but lesion extent was 4 times higher in Doubtful Sound. Lesion extent was higher for female dolphins than for males in Doubtful Sound, but not in Dusky Sound. In Dusky Sound calves were larger at first observation and were born over a longer period. The short calving season in Doubtful Sound may be an adaptation to localized temperature conditions. Anthropogenic impacts may contribute to the higher levels of epidermal disease in the Doubtful Sound population. The higher extent of epidermal lesions in females and the smaller size of calves in Doubtful Sound may be a factor in the low survival of calves in the population.

KEY WORDS: Calf size · Epidermal lesions · Doubtful Sound · Dusky Sound · Laser photogrammetry · *Tursiops truncatus*

INTRODUCTION

Knowledge of wildlife health is important in conservation management because the occurrence and spread of disease can be sensitive indicators of anthropogenic impacts (Deem et al. 2001). Comparing the health status of different populations can help determine how different anthropogenic impacts affect ecosystems. For coastal cetaceans, like bottlenose dolphins *Tursiops truncatus*, typical health assessments require invasive techniques that involve either live capture of individuals or biopsy darting to collect tissue and/or blood samples (e.g. Reif et al. 2008a). Recently, photography and photogrammetry have emerged as powerful non-invasive tools that researchers can apply to evaluate the health status of individual cetaceans (Wilson et al. 1999, Pettis et al. 2004).

The bottlenose dolphin population inhabiting Doubtful Sound, New Zealand, has declined by more than 34% since 1995, with just 56 individuals (coefficient of variation [CV] = 1.3%) residing in the fiord in 2007 (Currey et al. 2007). Another bottlenose dolphin population inhabits neighbouring Dusky Sound and contains 102 (CV = 0.9%) individuals, but no information

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is available on population trends (Currey et al. 2008a). The populations in Doubtful and Dusky Sounds, combined with a population in northern Fiordland, form a regional population that meets the International Union for Conservation of Nature (IUCN) Red List criteria for being listed as critically endangered (Currey et al. 2009). The Doubtful Sound population is effectively closed with no immigration observed since 1990 (Lusseau et al. 2003, Currey et al. 2007). Less information is available for the other Fiordland populations, but surveys conducted to date in Dusky Sound have not sighted any individuals from the other populations (Currey et al. 2007, 2008a).

Bottlenose dolphins in Doubtful Sound are subject to anthropogenic impacts from tourism (e.g. Lusseau 2003) and habitat modification via freshwater discharge from a hydroelectric power station that amounts to triple the natural catchment runoff (Gibbs 2001). In contrast, the dolphin population in Dusky Sound inhabits a fiord system that receives only natural freshwater runoff and, because of its isolation, experiences much lower levels of tourism than does Doubtful Sound (Lusseau et al. 2006).

Demographic analyses show that the decline of the Doubtful Sound dolphin population is driven by poor calf survival, which declined by more than half between the periods 1994 to 2001 and 2002 to 2008 (Currey et al. 2008b). Additionally, several aspects of the behavior of bottlenose dolphins in Doubtful Sound suggest that they are potentially stressed by cumulative interactions with tour boats (Lusseau 2003). Physiological stress can result in reduced immunity to disease, and high levels of epidermal disease could indicate impaired immune systems (Harzen & Brunnick 1997, Reif et al. 2008b). Epidermal lesions are common among bottlenose dolphins, and are more severe in populations exposed to cold water and low salinity (Wilson et al. 1999). Because such epidermal lesions might be an indicator of health, we undertook a comparative study of lesion prevalence and extent in bottlenose dolphins in Doubtful Sound, which are affected by tourism and habitat modification, versus bottlenose dolphins in Dusky Sound, where such effects are minimal. We also used laser photogrammetry (Rowe & Dawson 2008) to measure the dorsal fin base length of 'newborn' calves (i.e. young-of-the-year) from both fiords to gather morphometric data on the members of the population for which survival is especially poor (Currey et al. 2008b). Poor condition was identified previously as the primary cause of calf mortality in a population of bottlenose dolphins in the Indian Ocean (Mann & Watson-Capps 2005). Hence, photogrammetry of newborn calves could reveal a link between calf size and survival.

MATERIALS AND METHODS

Data collection. Dorsal fin photographs were collected from Doubtful Sound (45° 30′ S, 167° 00′ E) and Dusky Sound (45° 45′ S, 166° 35′ E) in November to December 2007 (early summer), February to March 2008 (late summer) and June to July 2008 (winter). Systematic surveys were conducted following a pre-determined route (Schneider 1999, Currey et al. 2008a). Dorsal fin photo-identification allowed all individual dolphins (calves, sub-adult and adults) to be identified by unique patterns in naturally occurring marks that ranged from permanent fin nicks to temporary tooth rakes (see Currey et al. 2007 for details). Each photograph was compared with, and matched to, previously identified individuals in the photo-identification catalogue. Photographs were required to be well exposed, in sharp focus, with the dorsal fin orientated parallel to the photographer, and occupying a large proportion of the frame. Photographs were taken with a Nikon D70s camera equipped with an AF Nikkor lens (80–200 mm f2.8 or 70–200 mm f2.8 VR). Sex of dolphins was (1) determined from observation of their genital region, or (2) assumed to be female if they had consistent close association with a calf, or (3) assigned based on a morphometric model (Currey et al. 2008a).

The majority of dolphins from Dusky Sound were sexed via a morphometric model based on sexually dimorphic characters (Currey et al. 2008a). Young dolphins are not noticeably dimorphic (Read et al. 1993) and the model required dolphins younger than 4 yr old to be excluded. For the Dusky Sound sample, young dolphins were excluded if the base length of their dorsal fin failed to exceed a threshold measurement derived from 4 yr old individuals from Doubtful Sound (Currey et al. 2008a). Using the sexes predicted by this model allowed for comparison by sex as well as location. To ensure that we were comparing 'like with like' we also excluded dolphins younger than 4 yr old from the Doubtful Sound dataset using age data from the long-term research program.

Extent of epidermal lesions. Epidermal lesions were identified by their physical characteristics; lesions may appear as white, black or blue-grayish plaque-like patches with sharp or indistinct edges and include circular, outline and amorphous shapes (Fig. 1; see Wilson et al. 1997 for more detail). Lesions are distinct from scarred areas, which are composed of scratch marks, tooth rakes and scar tissue resulting from direct physical injury. The etiology or etiologies of the lesions observed in this study were not known, nor was it known whether the variation in appearance of lesions represented stages of the same disease or a different disorder (Wilson et al. 1999, Bearzi et al. 2009). Recent studies that used photography have

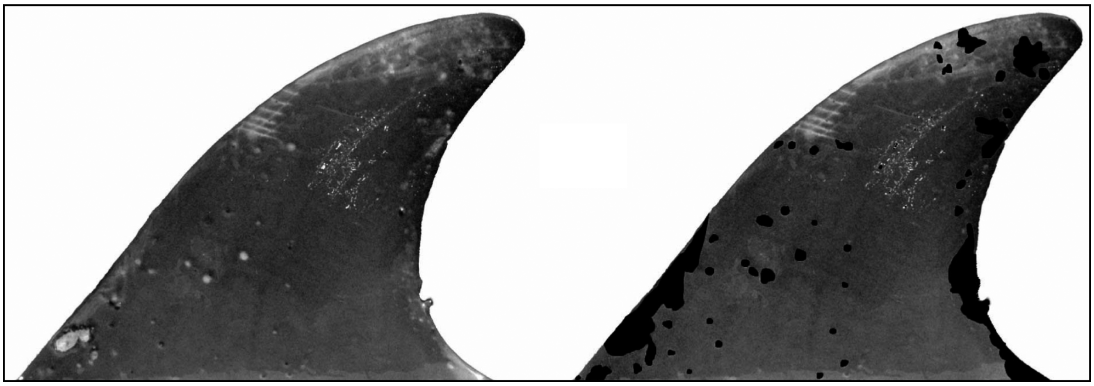

Fig. 1. *Tursiops truncatus*. Example of an extracted bottlenose dolphin dorsal fin image (left). Black marks represent the traced areas of epidermal lesions (right). This dorsal fin had epidermal lesions covering 11.4 % of its surface area

identified specific epidermal diseases (e.g. lobomycosis, Murdoch et al. 2008), but only after the disease had been confirmed to be present in the population after individuals were captured and lesions biopsied. This same approach could not be taken in Fiordland because no stranding or biopsy data are available for histological identification and validation of lesion classifications. Therefore, regardless of the type or types of lesion observed, we considered their presence and extent to be indicators of the general epidermal condition of the affected individual.

To provide a measure of lesion extent (equivalent to severity in Wilson et al. 1997, 1999, Rowe & Dawson 2009), the proportion of an individual's dorsal fin covered by all lesions was measured following methods developed by Rowe & Dawson (2009) (Fig. 1). To eliminate any effect of season on lesion extent we only analyzed photographs taken during the late summer field trips.

Laser photogrammetry. Laser photogrammetry, also known as laser metrics, uses 2 parallel lasers that project dots a known distance apart onto the object being photographed. Provided the object of interest is perpendicular to the axis of the lasers, measurements are possible at any range over which the dots are visible. Laser photogrammetry has been used to measure the dorsal fins of free-ranging killer whales *Orcinus orca* (Durban & Parsons 2006) and bottlenose dolphins (Rowe & Dawson 2008). The laser photogrammetry system was calibrated daily to reduce bias. Care in photography and stringent quality grading of photographs minimized parallax error (Rowe & Dawson 2008). Laser-metric photographs were subject to the same quality grading procedures as described above for photo-identification, with the addition that it was required that the laser dots were clearly visible in each photograph.

We used laser photogrammetry to measure the dorsal fin base length (DBL) of calves (<1 yr old). We made the assumption that the DBL would be a reasonable proxy for calf size because (1) dorsal fin morphology seems unlikely to vary over the short distance between the populations (~46.3 km [~25 nautical miles]) and (2) DBL is a straight-line distance that runs parallel to the long axis of the body. In Hector's dolphins *Cephalorhynchus hectori* DBL has a stronger relationship to total length than other characters able to be measured from the dorsal fin (Webster et al. 2009). Calves were easily identified in Doubtful Sound because all dolphins born since 2004 were of known age. In Dusky Sound calves were identified based on observations of their size relative to the mother, prominence of fetal folds and erratic surfacing behavior. Dorsal fin base length was measured following the technique of Rowe & Dawson (2008) (Fig. 2). An estimate of DBL was only calculated if there were multiple high-quality photographs of an individual calf. The measurement error (ME) associated with this technique has been quantified and was small enough (5.17 %) that replicate measurements of the same individual were averaged for further analysis (Rowe & Dawson 2008).

Statistical analysis. Measurements of lesion extent were not normally distributed so non-parametric tests were used. Wilcoxon rank sum tests of lesion extent between dolphins from Doubtful and Dusky Sounds, and between the sexes within each fiord were performed in JMP 6.0 (SAS Institute). Laser-metric photographs were taken of calves over the 3 periods of field work, but we possessed insufficient corresponding samples to enable analyses using repeated measures. Hence, we used unpaired 2-tailed *t*-tests to compare measurements of calves when first observed in both fiords, and between calves from Dusky Sound that were first observed during the first 2 field trips and those first observed during the winter field trip.

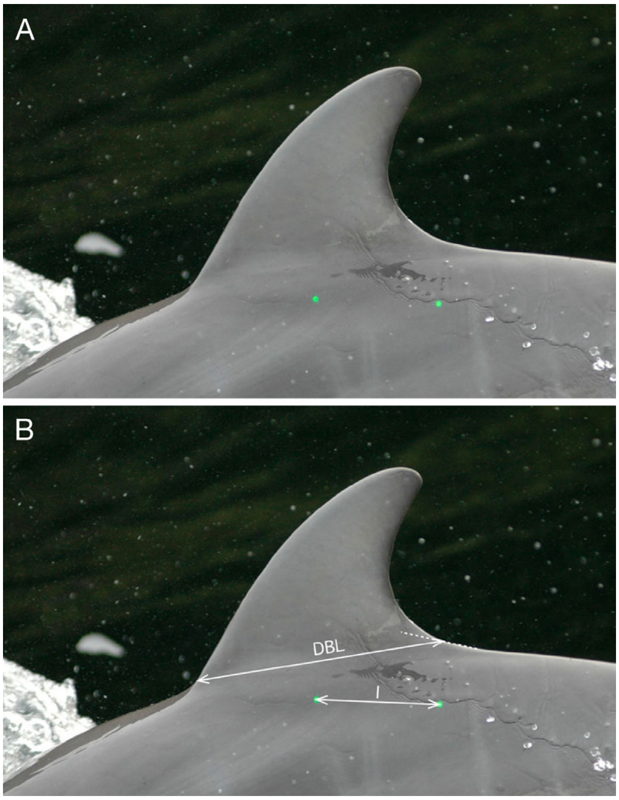

Fig. 2. *Tursiops truncatus*. (A) The 2 fixed-distance laser dots (green) projected onto the upper flank of a bottlenose dolphin calf (<1 yr old). (B) The dorsal fin base length (DBL) was measured between the anterior and posterior insertions of the dorsal fin, defined (following Rowe & Dawson 2008) to be the beginning of the dorsal fin crease on the leading edge and where the plane of the back deviated to the plane of the dorsal fin (broken white line). Measurements were calibrated to actual size based on the measured dimensions between the laser dots (l)

RESULTS

Epidermal lesions

Dorsal fin identification photographs of 121 bottlenose dolphins (42 from Doubtful Sound, 79 from Dusky Sound) were analyzed for lesion prevalence and extent. The sample represented 98% of the subadult and adult dolphins in each fiord. Epidermal lesions were prevalent across both populations (100% in Doubtful Sound, 96.2% in Dusky Sound), but dolphins from Doubtful Sound had a significantly larger proportion of their dorsal fin covered in lesions than those in Dusky Sound (Z = 6.460, p < 0.0001). The median percentage coverage of dolphin lesions in Doubtful Sound was 6.23% (inter-quartile range [IQR]: 4.34 to 10.24), 4 times the median percentage coverage of 1.48% (IQR: 0.61 to 2.62) in dolphins from Dusky Sound. Within Doubtful Sound the extent of epidermal lesions was significantly higher for females than for males (Z = –2.25, p < 0.024, Fig. 3) but no gender differences were observed in Dusky Sound (Z = –0.16, p = 0.875, Fig. 3).

Calf dorsal fin size

Over the study period 16 calves were observed, 4 in Doubtful Sound and 12 in Dusky Sound. New calves were observed during all field trips to Dusky Sound (6 calves in late summer, 3 calves in early summer and winter), but only in late summer in Doubtful Sound. Not all calves observed during a single field trip were photographed with sufficient replication to allow a measurement estimate to be calculated. Overall, however, replicate laser-metric photographs were obtained for 15 of the 16 calves. A total of 143 laser-metric photographs of calves were measured (average, 5.9 photographs per individual per field trip; range, 2 to 11). Calves from Doubtful Sound were smaller at first observation than those from Dusky Sound (unpaired *t*-test, p = 0.043), suggesting that calves from Doubtful Sound are either smaller at birth or born later in the season than those in Dusky Sound (Fig. 4).

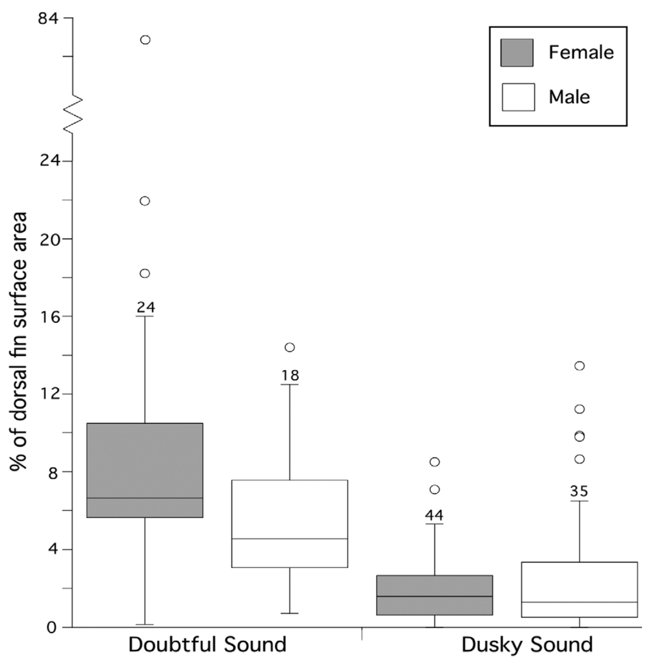

Fig. 3. *Tursiops truncatus*. Box and whisker plot showing percentage of dorsal fin surface area covered by epidermal lesions in bottlenose dolphins from Doubtful Sound and Dusky Sound. Whiskers show minimum and maximum values; boxes show lower and upper quartile; midlines show medians, and circles show outliers. Values above each box represent the number of individuals in each sample

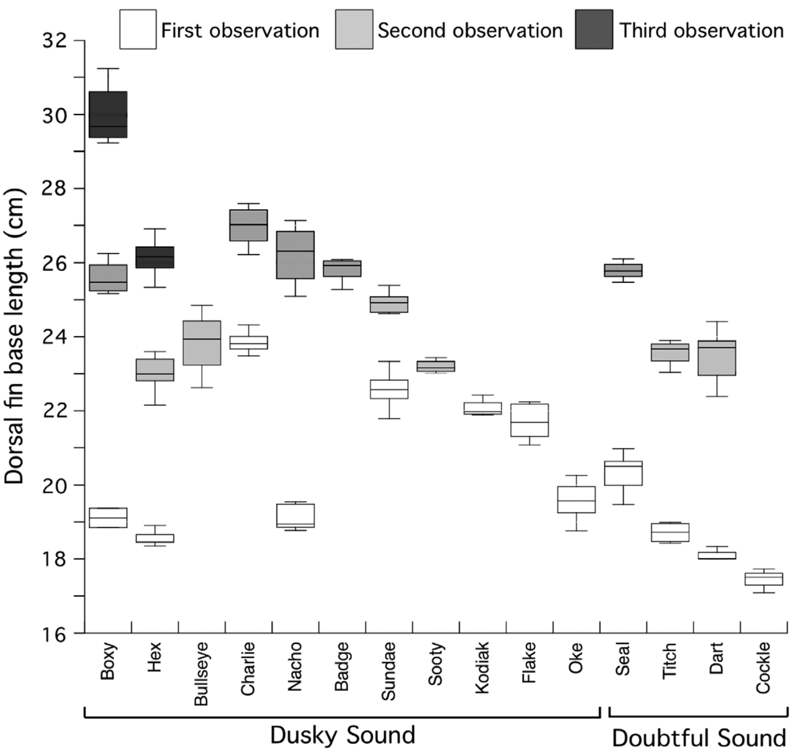

Fig. 4. *Tursiops truncatus*. Box and whisker plot showing dorsal fin base length of bottlenose dolphin calves (<1 yr old; names given on *x*-axis) from Dusky Sound and Doubtful Sound during a series of field trips between November and December 2007, February and March 2008 and June and July 2008. Whiskers show minimum and maximum values; boxes show lower and upper quartile and midlines show median

Three new calves were observed during the winter field trip to Dusky Sound. These were significantly smaller in DBL than other calves born in Dusky Sound in early and late summer (unpaired *t*-test, $p = 0.003$), suggesting that they were born later, between March and July after the late summer field trip. One of the calves first observed in July has a DBL similar to some of the calves that were less than 2 mo old during the late summer trip (Fig. 4), which suggests it was born very late in the season, perhaps in May or June. The smallest calf measured in this study was from Doubtful Sound. This calf was not sighted in winter, despite repeated sightings of its mother, and is presumed to have died.

DISCUSSION

Epidermal disease, of various descriptions and etiologies, has been identified in many coastal populations of bottlenose dolphins. Wilson et al. (1999) compared the rate of epidermal lesions among 10 bottlenose dolphin populations in the USA, Europe and New Zealand. In their study, lesions were common to all populations but resident populations in Scotland and New Zealand exhibited the greatest extent of epidermal lesions. This was hypothesized to be a result of physiological stresses associated with living at the extremes of the species' distribution. Considering the close geographic proximity of Doubtful and Dusky Sounds, the much higher extent of epidermal lesions in Doubtful Sound indicates that lesion extent is not simply a function of living at high latitude, but is a result of factors specific to a population's habitat.

The pathological causes of epidermal lesions in cetaceans are not entirely known, but fungal, bacterial, protozoan and viral etiologic agents have been identified (Taborda et al. 1999, Van Bressem et al. 2008). Studies on wild bottlenose dolphins have proposed links between environmental and anthropogenic factors and skin lesions. Potential factors include chemical pollution (Wilson et al. 1997, Van Bressem et al. 2008, Bearzi et al. 2009) and variations in salinity and water temperature (Wilson et al. 1999, Reif et al. 2006, Murdoch et al. 2008). Doubtful and Dusky Sounds are not subject to chemical pollution, but the salinity and temperature regime in Doubtful Sound is affected by the freshwater discharge from the power station (Gibbs 2001). Dolphins inhabiting an area of the Indian River Lagoon in Florida, which is characterized by freshwater intrusion and lower salinity, show a high prevalence of the skin disease lobomycosis (Reif et al. 2006, Murdoch et al. 2008). Gulland et al. (2008) proposed exposure to low salinity waters as the probable cause of the deteriorating epidermal condition of a humpback whale *Megaptera novaeangliae* and her calf when they made an 11 d foray into the Sacramento River, California. Mechanisms by which variation in salinity could cause epidermal disease include interference with osmoregulation of the epidermis, and lesions are a visible expression of systemic physiological stress caused by environmental conditions (Wilson et al. 1999).

Could the Doubtful Sound dolphin population's consistent exposure to low salinity conditions be responsible for the higher extent of lesions in the population? Both Doubtful and Dusky Sounds receive high annual rainfall that results in a well-defined low salinity layer

(LSL) on the surface of the fiords. In Dusky Sound, the LSL is temporally variable, but the additional freshwater discharge into Doubtful Sound augments the LSL and ensures it is a constant feature in the fiord (Gibbs 2001). The salinity of LSL in the inner regions of Doubtful Sound is less than 10 psu (Gibbs et al. 2000), which falls well outside the range recommended for the maintenance of dolphins in captivity (25 to 35 psu). Bottlenose dolphins in other locations make occasional forays into low salinity environments, but the Doubtful Sound dolphins are unusual in that they are exposed to a distinct low salinity layer throughout the year. Other potential causes of lesions are that dolphins in Doubtful Sound are exposed to higher levels of skin pathogens or are in poorer immunological condition than those in Dusky Sound.

The hypothesis that the freshwater discharge is a factor in the higher rates of epidermal disease in Doubtful Sound dolphins compared with those in Dusky Sound does not explain the disparity in lesion extent between the sexes. Dolphins in Doubtful Sound live in mixed-sex schools (Lusseau et al. 2003) and, therefore, encounter broadly the same temperature and salinity regimes. Females that are accompanied by calves may be exposed to the LSL more than males because their calves do not need to spend time at depth to forage, but this has not been quantified. The difference in lesion extent may also result from the different physiological constraints between the sexes. Compared with males, females have shorter dive intervals and a delayed reaction to tour boat disturbance, which suggests that females are more physiologically constrained (Lusseau 2003). Physiological stress can lead to reduced immune response in marine mammals (Fair & Becker 2000). Epidermal diseases, like lobomycosis, are thought to represent an opportunistic infection in an immune-compromised host (Reif et al. 2008b). It follows that the higher rates of lesioning in female dolphins in Doubtful Sound may be a reflection of their immunological condition.

For bottlenose dolphins with seasonal breeding, calving usually peaks between summer and autumn, coinciding with the highest water temperatures, but calving can also be associated with peak periods of food availability (Urian et al. 1996). In Doubtful Sound almost all births occur within a short summer season from December to February (Haase & Schneider 2001). Our results suggest that the distribution of births is wider in Dusky Sound, extending from early December to May or June. If this pattern is confirmed by future studies, it would be similar to that observed in bottlenose dolphins ca. 1300 km north in the Bay of Islands, where births peak in summer months but can occur between November and July (Constantine 2002).

The timing of breeding and calving can vary among conspecific, same-latitude, dolphin populations due to females in each population adapting to local conditions (Urian et al. 1996). Births in Doubtful Sound are strongly associated with the periods of highest surface water temperature (Haase & Schneider 2001). Low water temperatures may decrease core body temperature, and it is thought that dolphins of small mass, such as neonates, would be particularly vulnerable to thermal stress (Yeates & Houser 2008). Birthing during the warmest months may help the thinly insulated calves avoid thermal stress. The wider calving season in Dusky Sound suggests that the Dusky Sound dolphins may experience less thermal stress, despite being marginally farther south.

The results from this study contribute to a body of circumstantial evidence that suggests the freshwater discharge from the hydroelectric power station may be affecting the bottlenose dolphins of Doubtful Sound. The population decline is driven by a decline in calf survival that coincided with an increase in freshwater discharge from the power station (Currey et al. 2008b). The distinct seasonal distribution of the population within Doubtful Sound, previously thought to be an adaptation to life at high latitude, is not evident in Dusky Sound, and appears to be a response to seasonal patterns in surface water temperature, which is influenced by freshwater discharge (Schneider 1999, Currey et al. 2008a).

We have found the extent of epidermal disease to be significantly higher in Doubtful Sound than in Dusky Sound, which may be due to the Doubtful Sound dolphin population's exposure to an anthropogenically altered salinity regime. Although we need more data from Dusky Sound to confirm our observations of a much wider calving season, it does appear that the narrow calving season in Doubtful Sound may not be consistent across Fiordland, and could be an adaptation to localized temperature conditions within Doubtful Sound. To examine the potential influence on dolphins of the freshwater discharge into Doubtful Sound comparative studies of calving seasonality, calf survival, calf size, salinity and sea surface temperature are needed in both Dusky and Doubtful Sounds. In the meantime, the results of this study and of previous work suggest that management of the potential effects of the freshwater discharge into Doubtful Sound should be precautionary.

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