Life history of the long-finned pilot whale (*Globicephala melas edwardii*); insights from strandings on the New Zealand coast

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Abstract

Extensive research has been conducted on long-finned pilot whale (*Globicephala melas*) populations in the North Atlantic, based predominantly on samples collected from drive fisheries. However, the species remains poorly understood in the Southern Hemisphere. Prior to this study, almost nothing was known of the biology or ecology of the unique long-finned pilot whale subspecies of the temperate south (*G. m. edwardii*). Despite recognition as data-poor by the International Union for the Conservation of Nature, *G. m. edwardii* has been classified as "Not Threatened" by the New Zealand Threat Classification System. Although pilot whales are not necessarily under threat, it can be argued that cetacean populations whose abundance, distribution, habitat use and life history remain unknown are most at risk, since population declines are likely to go unnoticed. *G. m. edwardii* mass strands frequently on the New Zealand coast and data collected from stranding events are the primary data for this population. This thesis contributes new understanding of the biology and ecology of this data-poor subspecies and identifies important relationships between mass stranding events and life history characteristics that have significant implications for the conservation of long-finned pilot whales in New Zealand waters. Specifically, this research presents novel information regarding: (1) growth rates, growth patterns/allometry, sexual dimorphism, (2) age structure, survival and mortality (3) male sexual maturation, (4) female reproductive parameters, and (5) spatiotemporal stranding patterns of *G. m. edwardii* on the New Zealand coast. Estimated length-at-birth, maximum size and age, survivorship, and average length and age at the attainment of sexual maturity are all reported to be lower in *G. m. edwardii* than in the North Atlantic subspecies (*G. m. melas*), indicating that geographic variation in life history occurs in this species, likely reflecting population-specific adaptation to local habitats. This study makes a significant contribution to the current scientific understanding of the poorly understood southern subspecies of the long-finned pilot whale, by providing the first life history data for *G. m. edwardii* in New Zealand waters.

Table of Contents

List of figures

List of tables

List of acronyms

AIC	Akaike Information Criterion
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APR	Annual pregnancy rate
ASM	Average age at attainment of sexual maturity
CA	*Corpus albicans*
CaI	Calving interval
CI	Confidence interval
CL	*Corpus luteum*
CLO	*Corpus luteum* of ovulation
CLP	*Corpus luteum* of pregnancy
CrI	Credible interval
df	Degrees of freedom
DIP	Demographically independent population
DOC	Department of Conservation (New Zealand)
DT	Seminiferous tubule diameter
ESU	Evolutionary significant unit
F	Female
GLG	Growth layer group
HPDI	Highest Posterior Density Interval
IUCN	International Union for Conservation of Nature
IWC	International Whaling Commission
LFPW	Long-finned pilot whale
LOOIC	Leave-One-Out Information Criterion
LSM	Average length at attainment of sexual maturity
M	Male
MCA	Medium *corpus albicans*
MMAP	Marine Mammal Action Plan
MMPA	Marine Mammal Protection Act (New Zealand)
MSE	Mass stranding event
NAMMCO	North Atlantic Marine Mammal Commission
NZ	New Zealand
NZCeTA	New Zealand Cetacean Tissue Archive
NZPWP	New Zealand Pilot Whale Project
NZPWD	New Zealand Pilot Whale Database
NZPWTA	New Zealand Pilot Whale Tissue Archive
NZTCS	New Zealand Threat Classification System

NZWSDB	New Zealand Whale Stranding Database
OCA	Old *corpus albicans*
PrR	Post-reproductive representation
PSIS	Pareto-smoothed importance sampling
SD	Standard deviation
SE	Standard error
SFPW	Short-finned pilot whale
SSD	Sexual size dimorphism
TBL	Total body length
YCA	Young *corpus albicans*

Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institute of higher learning.

Emma Louise Betty

Date: 06 March 2019

Statement of publication and co-authorship

Chapters 5 and 7 of this thesis represent manuscripts that have been submitted to peer-reviewed journals and are currently in review in for consideration for publication. Chapters 3, 4, and 6 are in preparation for submission to peer-reviewed journals.

The following people and institutions contributed to the work undertaken as part of this thesis:

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Mark Orams, Auckland University of Technology & University of the Sunshine Coast = Author 4
Bethany Hinton, Massey University = Author 5
Adam Smith, Massey University = Author 6
Mike Ogle, Department of Conservation = Author 7
Hannah Hendriks, Department of Conservation = Author 8

The candidate was the lead author for each manuscript and responsible for the research design, sample collection, laboratory work/sample preparation, data analyses and interpretation, and preparation of manuscripts. The co-authors contributed to the collection of samples/data, laboratory work/sample preparation, some data analyses, interpretation of results, supervision of the candidate, and revisions of manuscripts for publication. All co-authors have approved the inclusion of the joint work in this doctoral thesis. Contributions of co-authors are outlined below:

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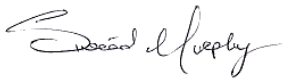
Author 1 (Sinéad Murphy):  Date: 03 March 2019

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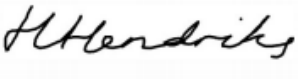
Author 8 (Hannah Hendriks):  Date: 04 March 2019

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Chapter 1

Introduction

Long-finned pilot whales offshore, northern New Zealand, January 2018.

1.1 Background

Pilot whales, *Globicephala* spp., are a widely distributed species complex of large dolphins (over 7m for adult males) belonging to the Order Cetacea, Suborder Odontoceti, and Family Delphinidae. The two currently recognised species of the genus *Globicephala* are: *G. melas*, Traill 1809 (the long-finned pilot whale; herein LFPW) and *G. macrorhynchus*, Gray 1846 (the short-finned pilot whale; herein SFPW). The name 'pilot whale' originated with an early theory that a pod is piloted by a leader. Other common names for these species include pothead whale and blackfish.

Externally, the LFPW closely resembles its short-finned relative; they both have a robust body, thick tail stock, bulbous and exaggerated melon with only a slightly discernible rostrum, and a broad, falcate dorsal fin (Jefferson et al. 2008; Figure 1.1). Usually, pilot whales are almost entirely black to dark greyish brown, with a white to light grey anchor-shaped patch on the chest, extending back to the urogenital area (Jefferson et al. 2008). Although the two species are readily distinguishable using osteological characters such as tooth count, flipper length and skull morphology (Sergeant 1962b, Van Bree 1971, Olson 2018) or by genetic analyses (Oremus et al. 2009), they are generally not distinguishable at sea by physical characteristics alone (Jefferson et al. 2008, Olson 2018). The two species range widely; however, there seems to be only limited distributional overlap. The LFPW has an anti-tropical distribution and is found in the cold-temperate waters of the North Atlantic and Southern Hemisphere, whereas the SFPW has a circum-global distribution and is found mainly in the tropics and subtropics (Olson 2018; Figure 1.2). Regions of overlap occur in the South Pacific; North Atlantic; off southern Africa; and off southern Brazil, Uruguay, and northern Argentina (Jefferson et al. 2008).

Based on some differences in body markings, the geographically separated LFPW populations in the Northern and Southern Hemispheres were described as different species by (Rayner 1939); however, Davies (1960) later reduced this distinction to subspecies level: *Globicephala melas melas* in the North Atlantic and *Globicephala melas edwardii* in the Southern Hemisphere. Although LFPWs are now absent in the North Pacific, fossil skulls identified as a potential third (un-named) subspecies have been found at several archaeological sites in Japan (Kasuya 1975) and Alaska (Frey et al. 2005), suggesting extinction in this ocean basin since the 8th to 12th century. More recently, Oremus et al. (2009) found that although LFPWs show strong mitochondrial DNA differentiation between ocean basins, the presence of shared haplotypes between North Atlantic and Southern Hemisphere forms suggests that current subspecies designations may require revision.

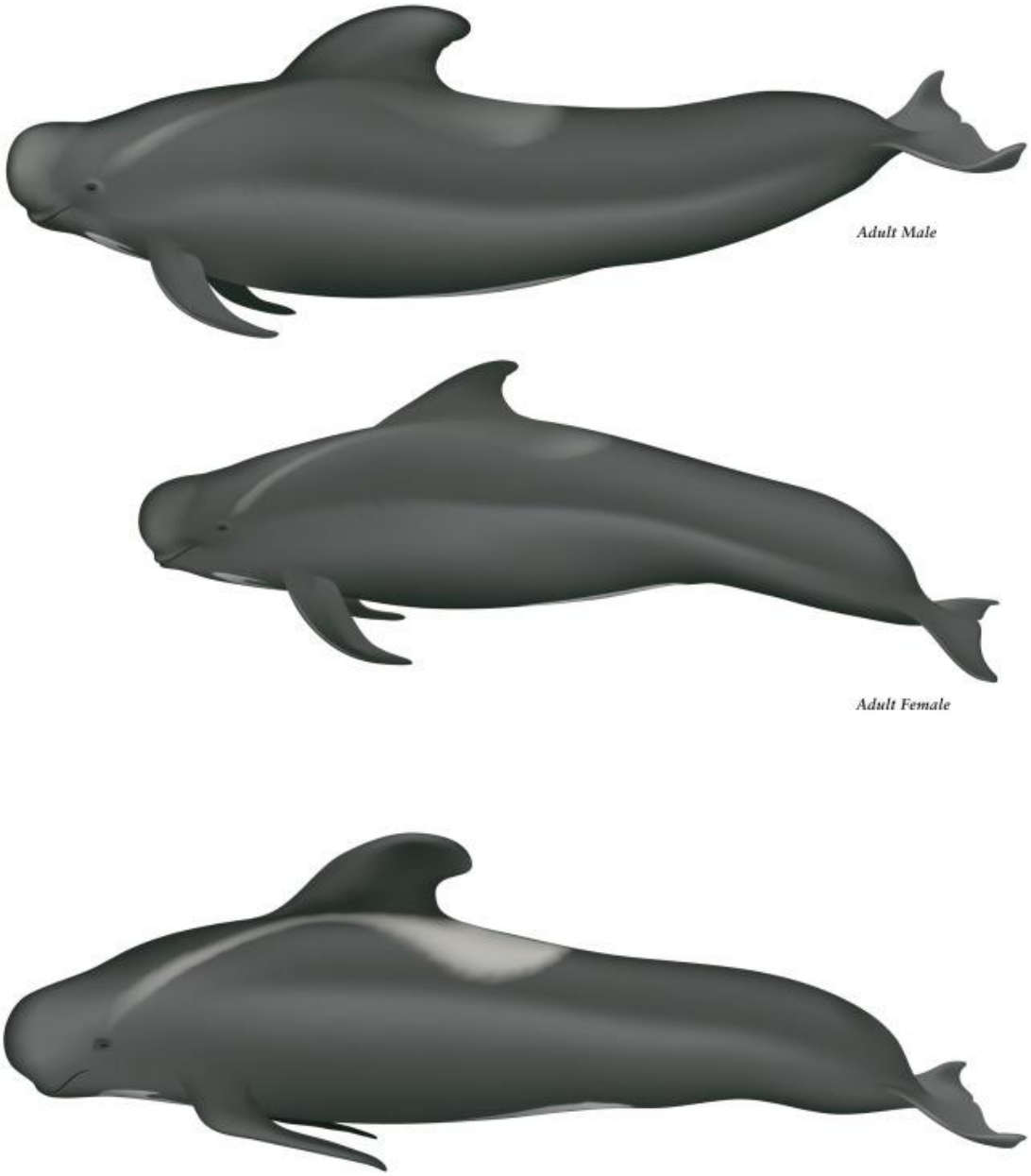


Figure 1.1. Full body view of the long-finned pilot whale *Globicephala melas* (lower illustration) and the short-finned pilot whale *Globicephala macrorhynchus* (upper two illustrations, showing marked sexual dimorphism).

Source: Olson (2018). Illustrations by Uko Gorter.

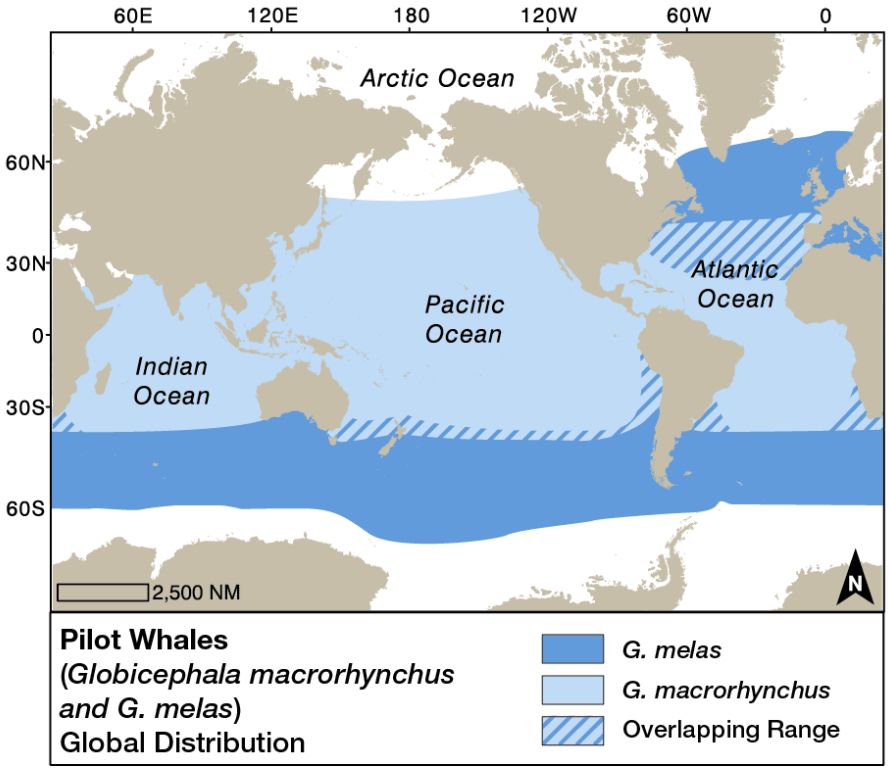


Figure 1.2. Worldwide distribution of pilot whales (*Globicephala* spp.).

Source: Adapted by Nina Lisowski from Olson (2018).

Two geographical forms of the SFPW have been described from Japanese waters, and are known as the 'Naisa'and the 'Shiho' forms (Kasuya et al. 1988a). Kasuya et al. (1988a) listed the visual characteristics separating the two forms as the shape and brightness of the saddle mark and the contour of the adult male melon when seen from above. They also described the segregation between the two forms in the western North Pacific, and suggest that the SFPWs in the eastern North Pacific may exhibit similar segregation. Recent genomic work suggests three SFPW types within the species: (1) an Atlantic Ocean type, (2) a western/central Pacific and Indian Ocean (Naisa) type, and (3) an eastern Pacific Ocean and northern Japan (Shiho) type (Van Cise et al. 2019). These three types appear to represent two subspecies, separated by the East Pacific Barrier: (1) the Shiho SFPW in the eastern Pacific Ocean and northern Japan, and (2) the Naisa SFPW throughout the remainder of the species' range (Van Cise et al. 2019).

Both species of pilot whales generally prefer deep, open ocean waters (Canadas and Sagarminaga 2000, Canadas et al. 2002, Aguilar de Soto et al. 2008), and are found in highest densities over the outer continental shelf or continental slope (Bloch et al. 2003b, Minton et al. 2018). In the North Atlantic, LFPWs are reported to follow their prey (squid and mackerel) inshore and into continental shelf waters during the summer and autumn (Reeves et al. 2003). Very little is known of pilot whale movements in the Southern Hemisphere. Often referred to

as one of the most gregarious cetaceans, pilot whales are often found in groups of up to several hundred individuals, although the average group size is generally around 20 whales (Jefferson et al. 2008). Their social system is assumed to be matrilineal, with groups consisting of several generations of maternally-related individuals (Kasuya and Marsh 1984, Amos et al. 1993b, Heimlich-Boran 1993). They are well-known for their highly cohesive behaviour which is considered to be a contributing factor towards their propensity to mass strand (Perrin and Geraci 2002).

Long and short-finned pilot whale records frequent the New Zealand Whale Stranding Database (NZWSDB); however, LFPWs are the most frequent mass stranding cetacean on the New Zealand coast (NZWSDB, extracted on 20/07/2018). The International Union for Conservation of Nature (IUCN) threat classification listing for both pilot whale species has recently been updated from 'Data Deficient' (Taylor et al. 2008) to 'Least Concern' (Minton et al. 2018). However, there are few empirical data on some populations of these species, especially in the Southern Hemisphere (Minton et al. 2018). Extensive research has been conducted on both LFPW and SFPW populations in the Northern Hemisphere, based mostly on samples collected from drive fisheries (e.g. Sergeant 1962a, Kasuya and Marsh 1984, Donovan et al. 1993). These studies have revealed significant morphological and biological differences between populations, and identified the presence of distinct subpopulations within the same geographic area, for example, Naisa and Shiho forms of the SFPW in Japanese waters (Kasuya and Tai 1993). Surprisingly, almost nothing is known of the biology or ecology of either species in the Southern Hemisphere (including the southern subspecies of LFPW; *G. m. edwardii*). Despite this lack of empirical data on the species in the broader area, and the long history of mass stranding events (MSEs) on the New Zealand coast, the official New Zealand Threat Classification System (NZTCS) categorises SFPWs as 'Migrant', and LFPWs as 'Not Threatened' (Baker et al. 2016). These categorisations are based on general assumptions about the species in New Zealand and, as a consequence, improved understanding of population ecology, key habitat requirements, threats, and strandings are recognised as high priorities for effective conservation management of pilot whales in New Zealand waters (Suisted and Neale 2004).

Effective conservation management is dependent on an accurate understanding of the species being managed. It is widely accepted that knowledge of the demography, growth, reproduction, diet, and mortality of wildlife populations, is essential for their conservation and management (Stockin and Orams 2009, Jefferson et al. 2012). This thesis seeks to contribute to this need and provides the first substantive biological data on the poorly understood southern subspecies of the LFPW (*G. m. edwardii*). Consequently, the research presented in this thesis

provides an important contribution to our scientific understanding of LFPWs in New Zealand waters and serves as a base of fundamental biological data from which further work on this species can be undertaken.

1.2 Mass stranding events as a research opportunity

Animal life history studies are designed to collect data on age, body size, physical maturity, sexual maturity, and feeding ecology from many individuals to estimate parameters that characterise how species' allocate resources to growth, reproduction, and survival (Chivers 2002, Chivers 2018). Estimated parameters can include age-specific growth and pregnancy rates, the average age at attainment of sexual maturity, calving interval, and longevity. Age is the primary independent variable for all studies because age explicitly demonstrates the trade-off in resource allocation to growth vs. reproduction during an animals' life. Life history data may be collected by observing individual animals directly via photo-identification, tagging, marking or telemetry studies, or by post-mortem sampling to obtain such things as teeth for aging, body length measurements for quantifying growth rates, gonads for determining reproductive condition, and stomach contents for describing diet (Chivers 2018).

Although they are opportunistic and have inherent biases, analyses of data collected from stranded cetaceans can provide useful insights into the biology of the stranded cetacean species (Perrin and Geraci 2002). Many cetacean species, such as many of the beaked whales (Ziphiidae) are known only from strandings (Thompson et al. 2013). Even a decomposing carcass on a beach can yield invaluable biological information on anatomy, genetics, life history, feeding ecology, predators, parasites, disease, and contaminant load (Perrin and Geraci 2002). MSEs offer a particularly valuable population sample (albeit potentially biased), and as such provide opportunities to obtain data such as sex ratio, age structure, pregnancy rate, lactation period and relatedness within a group (Perrin and Geraci 2002). Although a common observation in cetacean stranding records is an over-representation of very young and much older individuals, it has been reported by Sergeant (1982) that groups of pilot whales stranded on the Newfoundland coast do resemble groups driven ashore by fisheries in terms of age and sex composition. Sergeant (1982) also reports no obvious difference between the state of cetaceans deliberately driven ashore by humans and those that stranded from natural or unknown causes, thus the value of data collected from MSEs to science is unquestionable (Perrin and Donovan 1984).

Although LFPWs frequently strand in high numbers on the New Zealand coast (Brabyn 1991), very little is known about the biology and ecology of the species here, and elsewhere in the Southern Hemisphere. Exceptions are an examination of a pod stranded in Patagonia (Crespo et al. 1985), genetic investigations on DNA diversity and kinship (Oremus et al. 2009, Oremus et al. 2013), skull morphometry of specimens stranded in Argentina (Marina et al. 2019), female reproductive parameters estimated from a single MSE in Argentina (Soto et al. 2017), an unpublished report on life history and contaminant levels in New Zealand waters (Schroder and Castle 1998), and a few published papers on the diet and foraging ecology of LFPWs in New Zealand (Beatson et al. 2007a, Beatson et al. 2007b, Beatson and O'Shea 2009), Tasmanian (Beasley et al. 2019) and Kerguelen waters (Fontaine et al. 2015). This thesis presents novel data on the life history of *G. m. edwardii* using samples and data obtained from post-mortem examinations of individuals stranded on the New Zealand coast, under marine mammal research permits issued by the Department of Conservation (Per/HO/2008/02, AK-31924-MAR RNW/NO/2011/03 and 39635-MAR).

1.3 Thesis aim and objectives

This thesis aims to improve our understanding of the life history of LFPWs in New Zealand waters and to identify any relationships between pilot whale MSEs and life history characteristics that may have implications for conservation. To achieve this aim, there are five key research objectives:

Objective 1: Describe the growth rates, allometric relationships and sexual dimorphism of LFPWs stranded on the New Zealand coast.

Objective 2: Examine the age structure and construct age and sex-specific life tables, survivorship curves, and mortality schedules for *G. m. edwardii* in New Zealand waters, using age-at-death data from stranded individuals.

Objective 3: Classify the stages of sexual maturation in male *G. m edwardii*, and define indicators of sexual maturity.

Objective 4: Estimate the reproductive parameters of female *G. m edwardii*, and investigate evidence of reproductive senescence and seasonality.

Objective 5: Identify spatiotemporal trends in the New Zealand LFPW stranding record.

These five objectives are investigated and presented in Chapters 3 to 7, respectively.

1.4 Thesis structure

This thesis is organised into eight chapters (Table 1.1), comprising five research chapters (Chapters 3 – 7) that have been written in publication format and represent manuscripts that are either in review or in preparation for imminent submission. This format has resulted in some unavoidable repetition, particularly in relation to methods sections. However, every effort has been made to limit duplication where appropriate.

In Chapter 1, this chapter, the thesis is introduced and the thesis aims and objectives are outlined. In Chapter 2, the research context is established through a review of relevant literature and critical gaps in biological knowledge of the southern subspecies of the LFPW in New Zealand waters are identified.

In Chapter 3, an investigation of sex-specific growth rates, allometry and sexual dimorphism of *G. m. edwardii* is presented, and in Chapter 4, data on the age structure, survival, and mortality of LFPWs in New Zealand waters are provided.

In Chapter 5, the reproductive biology of male LFPWs stranded on the New Zealand coast is examined. Classification of male *G. m. edwardii* according to their stage of sexual development, determination of the estimated average age and total body length at the attainment of sexual maturity, and examination of predictors of sexual maturation provide new insights on the mating system and strategies used by *G. m. edwardii*.

In Chapter 6, a description of the life history of female LFPWs in New Zealand waters is presented. Parameters such as the average age and length at sexual maturity, ovulation rate, gestation period and foetal growth rate, average date of conception, average length at birth, lengths of lactation and resting periods, annual pregnancy rate, and calving interval are estimated. Ovarian symmetry, senescence, and indicators of seasonality are also assessed.

In Chapter 7, spatial and temporal patterns of LFPW strandings on the New Zealand coast are examined using all LFPW data held in the NZWSDB (1874 – 2017) but with emphasis placed on strandings between 1978 and 2017. Following the establishment of the New Zealand Marine Mammal Protection Act in 1978, cetacean strandings were more actively recorded from this point on.

In the final chapter (Chapter 8), the key results of this thesis are discussed, the conservation and management implications for New Zealand LFPWs are identified, and recommendations are made for future research.

Table 1.1. Thesis structure by chapter, outlining purpose and methods used.

Chapter	Purpose	Methods
1 Introduction	• Describe the research problem • Outline thesis aim and objectives • Describe thesis structure	• Literature review
2 Literature Review	• Establish the context of the research • Introduce relevant literature • Highlight current knowledge gaps	• Literature review
3 Age & Growth	• Study 1 to achieve Objective 1 • Estimate length-at- birth • Establish growth curves to estimate growth rates and predict length and age at physical maturity • Describe growth patterns (allometry) and sexual dimorphism	• Literature review • Age estimation • Bayesian logistic regression model • Growth modelling • Allometric analysis • Discriminant analysis
4 Survivorship	• Study 2 to achieve Objective 2 • Define the age structure of NZ LFPWs • Predict survivorship • Predict mortality	• Literature review • Construct life tables • Model survivorship and mortality
5 Male Reproduction	• Study 3 to achieve Objective 3 • Classify the stages of male sexual maturation • Estimate male ASM, LSM and other indicators of attainment of sexual maturity	• Literature review • Histological analysis • Bayesian logistic regression models
6 Female Reproduction	• Study 4 to achieve Objective 4 • Determine female reproductive status • Estimate female ASM and LSM • Estimate reproductive parameters (i.e. length of gestation, lactation and resting periods, APR, CaI) • Investigate evidence of senescence and reproductive seasonality	• Literature review • Gross examination • Histological analysis • Bayesian logistic regression models • Calculation of reproductive parameters
7 Strandings	• Study 5 to achieve Objective 5 • Examine spatiotemporal trends in the NZ LFPW stranding record	• Literature review • Spatiotemporal analyses
8 Conclusions	• Summarise key findings and discuss how they relate to the thesis aim • Demonstrate original contributions • Describe implications of this research • Describe remaining knowledge gaps • Identify future research priorities	• Literature review • Critique of work • Self-reflection

Chapter 2

Literature review

Long-finned pilot whales offshore, northern New Zealand, January 2018. Note presence of calf with foetal folds still visible.

This chapter reviews relevant literature with a focus on research that has been previously published on pilot whales. Particular attention is given to scientific knowledge on age and growth, male reproduction, female reproduction, strandings, and implications for conservation and management. Gaps in knowledge are identified, from which the aim and specific objectives for this thesis are derived. Finally, I summarise the key points of my literature review and discuss how they relate to my thesis aim and objectives.

2.1 Age and growth

Age estimation

Determining age structure, natural longevity (maximum age), and age-at-death of individuals removed by anthropogenic activities, or during stranding events, is crucial not only for understanding the dynamics of a population but also for determining if particular age groups are more or less at risk. Several methods have been used to attempt to determine the age of whales, as reviewed by the International Whaling Commission (IWC; Perrin and Myrick 1980). The present standard for toothed whales is to count growth layer groups (GLGs; Perrin and Myrick 1980) in the dentine and cementum of the teeth, as initially identified by Sergeant (1959). Counting GLGs in teeth is a reliable way to estimate age in many mammalian species because they indicate chronological age, similar to calculating the age of a tree by counting the numbers of growth rings in its trunk (Laws 1952, Scheffer and Myrick 1980). Age determination of marine mammals, based on tooth GLGs has become a standard procedure in population assessment and management, with age-specific estimates for fecundity or mortality used to project population growth (Hohn 2009).

GLGs in teeth are incremental layers that start accumulating after birth. Odontocetes have monophyodont dentition, meaning they do not possess deciduous teeth (milk teeth) and so their teeth contain a complete growth record of the individual. They are also homodont, which means that all teeth are the same shape (e.g. conical or peg-shaped) and contain the same layering, except anterior and posterior underdeveloped teeth. Therefore, any normally developed tooth should provide a good estimation of age (Hohn 2009). Teeth are composed of three types of hard tissue; enamel, dentine, and cementum (Figure 2.1). Newly erupted teeth are composed of a thin-walled dentine cap already covered with enamel, encapsulating a papilla of soft tissue known as the dental pulp, located in the pulp cavity. The pulp contains living connective tissue and cells called odontoblasts, which give rise to new dentine (Hohn 2009). Dentine is a calcified connective tissue that accumulates in layers on the internal surface of the tooth adjacent to the pulp cavity in such a way that the layers formed most recently are closer to the pulp cavity (Hohn 2009). Cementum, also a mineralised connective tissue, deposits in concentric layers on the external surface of the root (Hohn 2009).

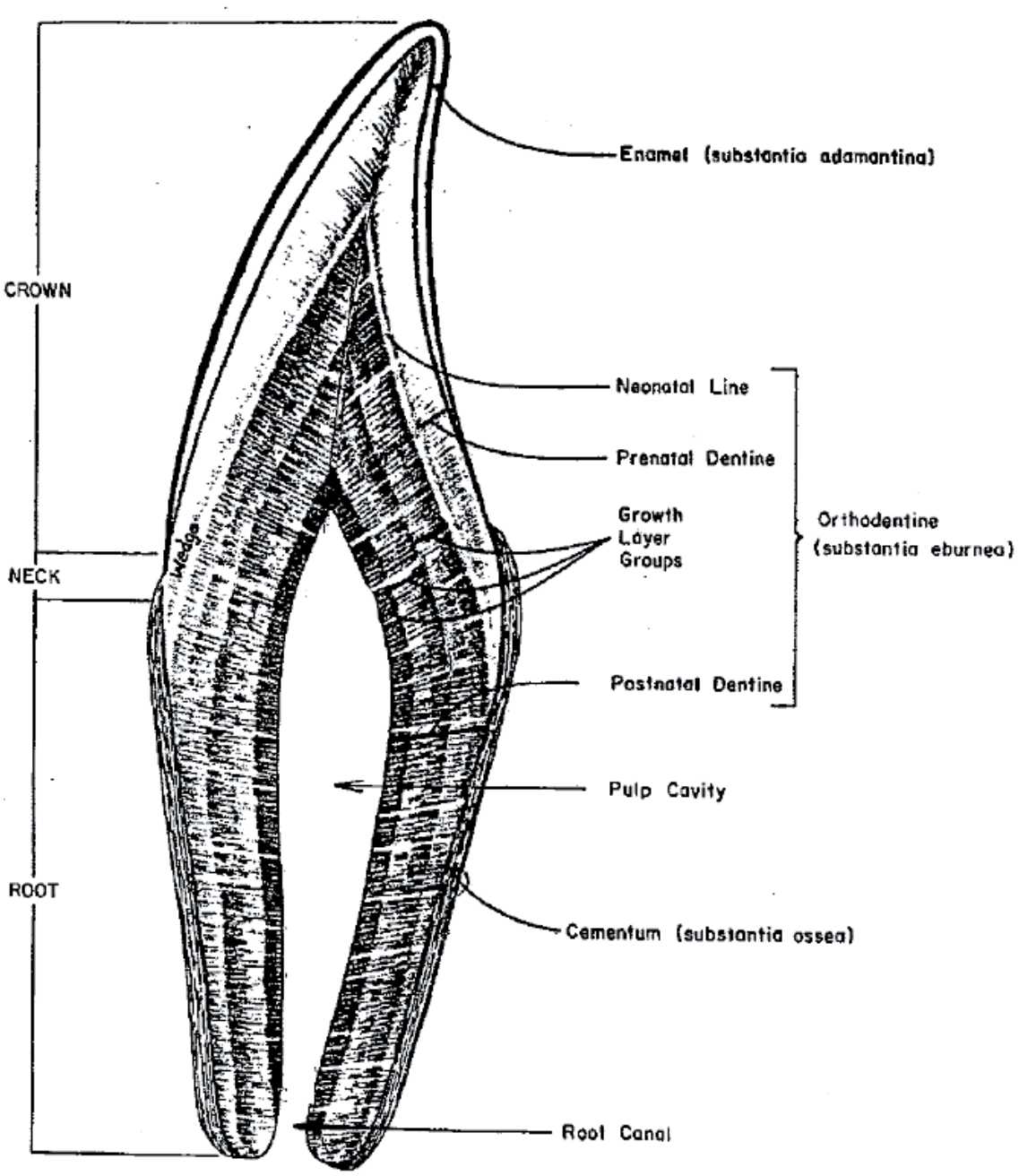


Figure 2.1. A generalised illustration of a longitudinal section of a dolphin tooth.

Source: Perrin and Myrick (1980).

Accurate age estimation from counts of dentinal GLGs first requires the identification of the neonatal line, a hypo-calcified band in the dentine, which is deposited at birth and therefore represents age zero. The neonatal line delineates the pre- and post-natal dentine tissue, after which the first incremental layer begins to be deposited. GLGs are countable units within dental hard tissue and can be recognised from their cyclic repetition, which must involve at least one change, i.e. between translucent and opaque, dark and light, ridge and groove, more stained and less stained (Klevezal 1980, Perrin and Myrick 1980). These alterations are caused by variation in the content and distribution of the mineral component of the tooth, e.g. hydroxyapatite, which results in differences in density and stainability (Klevezal 1980, Luque et al. 2009a). Incremental deposition rates have been calibrated for many marine mammal species in captivity via the use of tetracycline, an antibiotic used as a fluorescent marker in teeth, which is incorporated permanently into the mineralising tissue and can be observed under ultraviolet light (Gurevich et al. 1980, Myrick et al. 1984). Examination of GLGs in dental tissue of known-age odontocetes can also be used to validate incremental deposition rates; for most species, GLGs are deposited in the dental tissue annually (Murphy et al. 2012). Calibration of age estimates is essential for reliable age estimation. Many population parameters are sensitive to age estimation deviations, and lack of adequate calibration could lead to incorrect interpretations (Hohn et al. 1989).

Estimation of age in pilot whales was originally based on counting GLGs in thin untreated transverse sections of the dentine only (Sergeant 1959, 1962a). However, significant progress was subsequently made in odontocete age determination techniques (Perrin and Myrick 1980), with Kasuya and Matsui (1984) establishing an age determination method for the SFPW using annual GLGs in dentine and cementum as observed in decalcified and haematoxylin stained longitudinal tooth sections. It is now accepted that readings of decalcified and stained tooth sections give a more accurate estimation of age, especially for older individuals, and have since been used successfully in life history studies of both SFPW (e.g. Kasuya and Tai 1993) and LFPW populations (e.g. Kasuya et al. 1988b, Bloch et al. 1993a, Lockyer 1993a).

Lockyer (1993a) examined patterns of dentine and cementum deposition in the northeast Pacific SFPW and, using teeth from known-age individuals, demonstrated that age could be determined from any undamaged tooth in the jaw. Both decalcified stained sections and thin untreated sections of teeth from northeast Pacific SFPWs that had been receiving tetracycline treatments, in captivity, for periods of up to seven years were examined (Lockyer 1993a). Deposition rates of dentinal and cemental GLGs were validated as one GLG per year,

confirming the suitability of tooth GLGs for pilot whale age determination (Lockyer 1993a). It is assumed that this layering pattern is similar in both pilot whale species (Bloch et al. 1993a).

The most extensive study of pilot whale biology to date was conducted from July 1986 to July 1988, based on drive fishery catches of LFPWs from the Faroe Islands (Donovan et al. 1993). Teeth examined during this international research programme estimated the oldest male LFPW at 46 years and the oldest female at 59 years (Bloch et al. 1993a, Lockyer 1993a). These ages are considerably older than previously found for LFPWs in the North Atlantic (Sergeant 1962a, Martin et al. 1987, Kasuya et al. 1988b, Sigurjonsson et al. 1993), or recorded from the Southern Hemisphere to date (Crespo et al. 1985, Schroder and Castle 1998, Soto et al. 2017; see Table 2.1). Before the current study, maximum recorded ages of LFPWs in the Southern Hemisphere were 31 and 35 years for males and females, respectively (Schroder and Castle 1998, Soto et al. 2017). Estimated longevity of LFPWs in the Faroe Islands is, however, very similar to that of the SFPW analysed from the Japanese fishery where the oldest male was 45 years and the oldest female 62 years (Kasuya and Matsui 1984, Kasuya and Tai 1993). The lower estimates of LFPW longevity from elsewhere in the North Atlantic (Sergeant 1962a, Martin et al. 1987, Sigurjonsson et al. 1993), and Southern Hemisphere (Crespo et al. 1985, Schroder and Castle 1998, Soto et al. 2017) probably reflects that those studies were based on small sample sizes and/or used less reliable age estimation techniques.

Growth

Knowledge of growth patterns is needed to understand a species' life history strategy as it describes how individuals allocate resources to growth, reproduction, and survival (Chivers 2018). Growth models are important, and permit detailed comparisons with other populations of a species and monitoring of changes within a population (Stolen et al. 2002). They also allow estimations of age and body length at physical maturity to be calculated. Physical maturity is defined when skeletal growth stops, following the fusion of the vertebral epiphyses with the centrum (Chivers 2018). Single von Bertalanffy and Gompertz growth models have been used to model growth in a number of cetacean species, including pilot whales (Bloch et al. 1993a), however, two-phase models have been found to provide a better fit for several delphinid species (e.g. Perrin et al. 1976, Perrin et al. 1977, Danil and Chivers 2007, Murphy et al. 2009).

Pilot whales have been found to share several features of life history traits with other large odontocetes; long life span, delayed maturity, differential rates of maturation in males and females, sexual dimorphism, seasonal mating, and extended calving intervals (Olson 2018). Although maximum body lengths probably vary geographically, males appear to have a faster

growth rate and attain a larger body size than females (see Table 2.1). The pattern of growth for both species is similar, except for the first year, when the LFPW shows a slower growth rate than the SFPW (Kasuya and Matsui 1984, Bloch et al. 1993a). Rapid neonatal growth is followed by a less rapid but continual growth phase during the juvenile years. Growth slows even further after the attainment of sexual maturity, at about eight years in females and 16 years in males, and ceases some years later (Kasuya and Matsui 1984, Bloch et al. 1993a). Physical maturity of LFPWs off the Faroe Islands was reached at a length of *c.* 570 cm and between 25 and 30 years in males, and *c.* 425 cm and *c.* 30 years in females (Bloch et al. 1993a).

Although most mammals have determinate growth, and the same was assumed for cetaceans, studies on the growth of LFPWs taken off the Faroe Islands (Bloch et al. 1993a), Iceland (Sigurjonsson et al. 1993), and Britain (Martin et al. 1987) indicate that male LFPWs may continue growing past the estimate previously taken as the asymptotic length. Female pilot whales off the Faroe Islands were found to grow longer than those off Newfoundland (Sergeant 1962a, Kasuya et al. 1988b) and Iceland (Sigurjonsson et al. 1993) but with similar body lengths to those stranded on the British coast (Martin et a. 1987; see Table 2.1). The longer females and protracted growth pattern observed in the eastern North Atlantic may be explained by the favourable feeding possibilities in the area or may point to the existence of more than one population in the North Atlantic (Bloch et al. 1993a).

Morphometric studies allow animal body size and shape to be studied. Studies of morphometry include not only the morphological diversity that can occur between the sexes, such as sexual differences in growth patterns and growth rates (Weckerly 1998) but also intraspecific geographical variation in morphology (Bloch and Lastein 1993, Mazák 2010, Marina et al. 2019). The presence or lack of sexual dimorphism can also convey information about the life of the animal and its behaviour within social groups (Shine 1989, Isaac 2005, Mesnick and Ralls 2018). Aside from the pronounced sexual dimorphism in pilot whale total body length and weight, there is also some evidence of sexually dimorphic characteristics of both flippers and flukes, with male LFPWs reported to have longer pectoral flippers and longer and wider flukes than females of similar body lengths (Bloch et al. 1993b). Immature LFPWs tend to have significantly smaller flippers and flukes in proportion to body length compared to mature whales of both sexes (Bloch et al. 1993b). The proportion of the length of the pectoral flipper to body length is also one of the external morphological characteristics used to distinguish LFPWs from SFPWs (Leatherwood et al. 1976). Pectoral flipper length as a percentage of body length is generally 18 to 30% for *G. melas* and 14 to 19% for *G.*

macrorhynchus (Van Bree 1971, Yonekura et al. 1980, Nores and Perez 1988, Bloch et al. 1993b), although overlap does exist between the two species (Bloch et al. 1993b).

Geographic variation in intraspecific morphology has also been recorded for both SFPWs and LFPWs (Kasuya et al. 1988a, Bloch and Lastein 1993, Kasuya and Tai 1993, Marina et al. 2019). The SFPW off Japan is divided into two morphologically differing forms latitudinally by east-west running currents. The northern form lives in colder, more seasonally marked waters than the southern form, and attains a much larger size (Kasuya et al. 1988a, Kasuya and Tai 1993). Quite the opposite is the case for LFPWs in the North Atlantic where the currents run from southwest to northeast, dividing the waters longitudinally in a western and eastern gyre by the mid-Atlantic Ridge (Bloch and Lastein 1993). LFPWs occur under the same temperate conditions but on either side of the front. The differences in LFPWs between the two localities are not as distinct as found for SFPWs off Japan (Kasuya et al. 1988a, Kasuya and Tai 1993), presumably as a result of similar environmental conditions on both eastern and western sides of the North Atlantic (Bloch and Lastein 1993). It is, however, still possible to separate North Atlantic LFPWs into two forms based on morphometric proportions of the body; a likely artefact of reproductive isolation (Bloch and Lastein 1993). There have been comparatively few studies of age, growth, and morphometry of pilot whales in the Southern Hemisphere, resulting in a general lack of knowledge on the demographic parameters of LFPW throughout most of its southern range, including New Zealand.

2.2 Male reproduction

Research on the reproductive biology of cetaceans requires detailed studies of both sexes (e.g. Kasuya and Marsh 1984, Slooten 1991, Hohn et al. 1996, Murphy 2004). However, historically, most studies on cetacean reproduction have focused on females, while research that exclusively examines male reproduction remains rare (Plön and Bernard 2007). It is also important to acknowledge that most recent studies on the reproduction of cetaceans use samples from stranded or by-caught animals, thus utilising tissue which may not have been collected immediately after death. In addition, these tissues may not be adequately preserved for histological examination. Consequently, results should be interpreted cautiously. Nonetheless, even with slightly autolysed reproductive tissue, it is possible to identify the stages of maturity (Plön and Bernard 2007).

Table 2.1. TBL and age data available for the SFPW (*Globicephala macrorhynchus*) and LFPW (*G. melas*) from various geographical areas.

		G. macrorhynchus		***G. melas melas***				***G. melas edwardii***	
Location		Japan		Britain[4]	Faroe Islands[5,6]	Iceland[7]	Newfoundland[8,9]	Argentina[10, 11]	New Zealand[12]
		"Southern form"[1,2]	"Northern form"[3]						
Source		Drive fishery	Drive fishery	Stranding	Drive fishery	Stranding	Drive fishery	Stranding	Stranding
Sampling period		1983 – 1988	1982 – 1988	1982 – 1985	1986 – 1992	1982 – 1986	1951 – 1959	1982, 2009	1992 – 1996
Average length-at-birth (cm)		140[a] (*n* = 47)	185[b]		177[a] (*n* = 143)		M: 178[c] (*n* = 59) F: 174[c] (*n* = 49)		
Asymptotic length (cm)	M	474[d] (*n* = 35)	650[e] (*n* = 11)	550 – 600[f] (*n* = 21)	580[g] (*n* = 965)		557[h] (*n* = 5)		
	F	364[d] (*n* = 181)	467[e] (*n* = 58)	400 – 450[f] (*n* = 31)	445[g] (*n* = 1,478)		489[h] (*n* = 53)	441[i] (*n* = 31)	
Age at asymptotic length (yrs)	M	27[d] (*n* = 35)	25 – 30 (*n* = 111)	> 20[h] (*n* = 21)	> 46 (*n* = 965)		21 – 25[i] (*n* = 152)		
	F	22[d] (*n* = 181)	25 – 30 (*n* = 173)	> 20[h] (*n* = 31)	32 (*n* = 1,478)		21 – 25[i] (*n* = 275)		
Maximum length (cm)	M	525 (*n* = 200)	720	630	625 (*n* = 1,190)	595 (*n* = 55)	617 (*n* > 1,275)	538 (*n* = 7)	584 (*n* = 24)
	F	405 (*n* = 449)	510	546	512 (*n* = 1,635)	475 (*n* = 119)	511 (*n* > 1,951)	483 (*n* = 62)	470 (*n* = 37)
Maximum age (yrs)	M	45.5 (*n* = 170)	44.5 (*n* = 111)	20[j] (*n* = 21)	46 (*n* = 967)	34 (*n* = 38)	35.5[k] (*n* = 153)	16 (*n* = 5)	31 (*n* = 16)
	F	62.5 (*n* = 373)	61.5 (*n* = 173)	25[j] (*n* = 31)	59 (*n* = 1,482)	34 (*n* = 92)	56.5[k] (*n* = 284)	35 (*n* = 40)	35 (*n* = 19)

[a] length-at-birth estimated by logistic regression; [b] length-at-birth estimated from relationship of mean neonatal length and mean TBL of females at sexual maturation; [c] length-at-birth estimated as mean of overlapping foetus and neonate TBL; [d] asymptotic length and age estimated from growth curve "drawn by eye"; [e] asymptotic length estimated as mean TBL of individuals > 30 years; [f] asymptotic length estimated from length frequency distribution; [g] asymptotic length estimated using a single Gompertz growth model; [h] asymptotic length estimated as mean TBL of individuals > 25 years; [i] asymptotic length estimated using a single von Bertalanffy growth model; [j] age estimated using less reliable method: acid etching; [k] age estimated using less reliable method: transverse tooth sections.

Sources: [1]Kasuya and Matsui (1984), [2]Kasuya and Marsh (1984), [3]Kasuya and Tai (1993), [4]Martin et al. (1987), [5]Bloch et al. (1993a), [6]Lockyer (1993a), [7]Sigurjonsson et al. (1993), [8]Sergeant (1962a), [9]Kasuya et al. (1988b), [10]Crespo et al. (1985), [11] Soto et al. (2017), [12]Schroder and Castle (1998).

Sexual development and attainment of sexual maturity

The definition of sexual maturity in male cetaceans is complex (Perrin and Donovan 1984) as there is no single criterion for the attainment of sexual maturity (Perrin and Henderson 1984). Testis size, testis histology (i.e. stage of spermatogenesis and diameter of the seminiferous tubules), sperm abundance, the presence of sperm in the epididymis, and serum testosterone levels have all been used to estimate the attainment of sexual maturity (Plön and Bernard 2007). Testosterone is the primary androgenic hormone in male mammals and influences the maturation of sex organs, including initiating and maintaining spermatogenesis (Atkinson and Yoshioka 2007). In all species examined, testosterone concentrations increase around the onset of sexual maturity, making it useful as a diagnostic tool (Desportes et al. 1994b, Kita et al. 1999, Kjeld et al. 2004, Kjeld et al. 2006, Robeck and Monfort 2006, Atkinson and Yoshioka 2007). However, considerable individual and seasonal variation in testosterone levels have been observed and results must, therefore, be interpreted with caution (Desportes et al. 1994b). Spermatogenesis is the primary indicator of sexual development in male mammals, both with respect to the attainment of maturity and initiation of breeding in seasonally reproducing species (Plön and Bernard 2007). Therefore, histological examination of the testes remains the most accurate way to determine attainment of sexual maturity in male cetaceans (Murphy et al. 2005).

There is some inconsistency as to the number of different maturity stages that can be defined in cetaceans. Some studies distinguish between three stages: immature, pubertal (also called prepubescent or maturing), and mature animals (e.g. Best 1969, Hohn et al. 1985, Sorensen and Kinze 1994). Others define four stages of maturity as immature, pubertal, young mature, and mature (e.g. Collett and Saint-Girons 1984, Murphy et al. 2005), or immature, early maturing, late maturing, and mature (e.g. Kasuya and Marsh 1984, Desportes et al. 1993b, Kasuya and Tai 1993). Typically, immature testes are characterised by tightly packed, narrow seminiferous tubules with no lumen, embedded in abundant interstitial tissue, and lined by an epithelium comprising two types of cells, the supportive nurse (Sertoli) cells and spermatogonia (Desportes et al. 1993b, Murphy et al. 2005). Testes classified as mature are characterised by large seminiferous tubules with an open lumen, very little interstitial tissue and all stages of maturation of germinal cells are present, including the final germinal cells, spermatozoa (Desportes et al. 1993b, Murphy et al. 2005; see Figure 2.2). Full testicular activity is characterised by the presence of spermatozoa in the lumen of the seminiferous tubules and epididymis (Murphy et al. 2005). Unfortunately, for comparative purposes, the definitions of pubertal or maturing stages vary greatly between species and studies. However,

most authors report that pubertal and maturing testes are characterised by proportions of interstitial tissue and tubule diameters intermediary to those observed in immature and mature testes (e.g. Kasuya and Marsh 1984, Hohn et al. 1985, Desportes et al. 1993b, Plön 2004, Murphy et al. 2005).

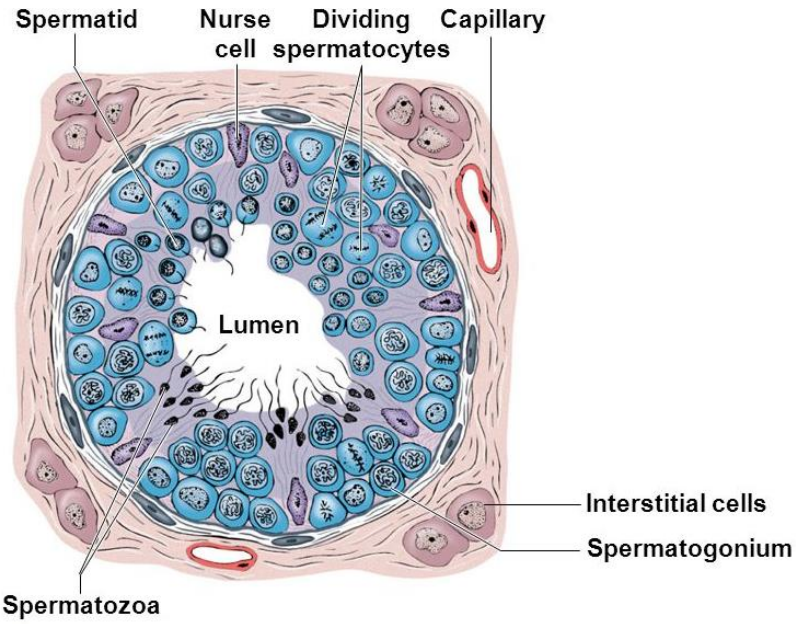


Figure 2.2. Illustration of a mature seminiferous tubule cross-section, showing all cell types involved in spermatogenesis.

In most mammalian species, both testes mature at the same rate (e.g. Collett and Saint-Girons 1984, Miyazaki 1984, Desportes 1994, Van Waerebeek and Read 1994, Plön 2004) and therefore usually only one testis is examined (Plön and Bernard 2007). In some cetacean species, for example, the sperm whale (*Physeter macrocephalus*; Best 1969), bowhead whale *(Balaena mysticetus*; O'Hara et al. 2002), sei whale (*Balaenoptera borealis*; Masaki 1976), and the LFPW (Desportes 1994), zonal maturation of the testes has been observed. The testes of sperm whales, bowhead whales, and sei whales all appear to mature from the centre outwards (Best 1969, Masaki 1976, O'Hara et al. 2002) and this has been used as a guide for gonadal sampling (Plön and Bernard 2007). However, the pattern of maturation in pilot whale testes does not seem as clear. One study identified that in LFPW, the core of the testis matures the latest, thus suggesting the testis core is the most appropriate place for sampling (Desportes 1994), i.e. all regions of the testis would then be fully mature. However, no significant zonal or bilateral differences have been detected in maturity and seminiferous tubule diameter in the testes of SFPWs (Kasuya and Marsh 1984).

Several previous studies have described the reproductive biology of male pilot whales, with varying levels of detail. Many of the earlier attempts to estimate age (ASM) and length (LSM) at the attainment of sexual maturity for North Atlantic LFPWs were based on small sample sizes (e.g. Morton 1963, Cowan 1966, Martin et al. 1987, Bloch 1992, Sigurjonsson et al. 1993). Sergeant (1962a) carried out a more thorough investigation of LFPWs taken in the fishery off Newfoundland, but limitations of the data and the narrow seasonal spread of sampling only allowed him to give approximate estimates of ASM and LSM. Moreover, problems in age estimation (as described above) rendered Sergeant's (1962a) estimated life history parameters inaccurate (Kasuya et al. 1988b). Extensive, and comparable, reproductive studies have since been conducted on both short and long-finned pilot whales in the Northern Hemisphere, based on large numbers of samples collected from the SFPW drive fishery in Japan (Kasuya and Marsh 1984, Kasuya and Tai 1993), and the LFPW drive fishery in the Faroe Islands (Desportes et al. 1993b, Desportes 1994, Desportes et al. 1994b). There appear to be many similarities in the reproductive biology of the two pilot whale species, with ASM at *c.* 17 years, and the cessation of testis growth at *c.* 25yrs (Kasuya and Marsh 1984, Desportes et al. 1993b, Kasuya and Tai 1993).

Desportes et al. (1993b) report a lower sperm density in male LFPWs over 34 years and suggest that this may reflect a decline in potential reproductive success. No evidence for such a decline was observed in SFPWs off Japan (Kasuya and Marsh 1984, Kasuya and Tai 1993). However, as the oldest male LFPWs off the Faroe Islands were still undergoing spermatogenesis, and testis weight did not appear to decline with age, the decline in sperm density may not necessarily mean that older males could no longer successfully reproduce. In their review of reproductive ageing, Harman and Talbert (1985) highlight that although there is considerable evidence that males in many species do show an overall reduced reproductive capacity with age, there is a high degree of individual variation and some males retain their full reproductive function into old age.

Reproductive seasonality

Although reproductive seasonality is well known in terrestrial mammals (e.g. Delgadillo et al. 1999, Pereira et al. 2005), the factors influencing seasonality in cetaceans are not well understood (Plön and Bernard 2007). This can be, at least in part, attributed to the many complexities of the marine environment, including largely unpredictable spatial and temporal variation in both biological and environmental factors (Sorensen and Kinze 1994). Reproductive seasonality has been widely researched in fish and marine invertebrates, but it is considerably more challenging to identify seasonal cues in cetaceans (Plön and Bernard 2007).

As males must time their reproductive cycle around the conditions that are most advantageous for the females, reproductive seasonality has mostly been examined relative to the timing of the estrus period (Plön and Bernard 2007).

The male seasonal reproductive cycle is complex and different parameters can peak at different times of the year (Desportes et al. 1993b). Seasonal reproduction requires that males have a sufficient quantity of viable sperm when females are receptive. Therefore, in seasonally reproducing species, testicular activity would be expected to peak shortly before, or coinciding with, the time of female estrus (Atkinson and Yoshioka 2007). However, there are many different degrees of male reproductive seasonality (Perrin and Henderson 1984), and the study of reproductive seasonality in male cetaceans is difficult as it requires samples to be collected throughout the year. However, because most samples are opportunistically collected from stranded or by-caught animals, a seasonally unbiased sample is not always possible (Plön and Bernard 2007). Although some of the following studies have relied on seasonal sampling and thus may be biased, indicators of male seasonality have included changes in testis weight (e.g. Neimanis et al. 2000, Murphy et al. 2005), testis length (e.g. Desportes et al. 1993b, Neimanis et al. 2000), testis volume (e.g. Gaskin et al. 1984), seminiferous tubule diameters (e.g. Neimanis et al. 2000, Murphy et al. 2005), sperm abundance (e.g. Hohn et al. 1985, Desportes et al. 1993b), spermatogenetic activity (e.g. Sergeant 1962a, Desportes et al. 1993b, Murphy et al. 2005), and serum testosterone levels (e.g. Wells 1984, Desportes et al. 1994b, Kjeld et al. 2006).

Seasonal cycles of testicular growth and increased spermatogenic activity, followed by partial involution with associated regression of spermatogenic activity, have been reported in many cetacean species, including the vaquita (*Phocoena sinus*; Hohn et al. 1996), short-beaked common dolphin (*Delphinus delphis*; Murphy et al. 2005), dusky dolphin (*Lagenorhynchus obscurus*; Van Waerebeek and Read 1994), spotted dolphin (*Stenella attenuata*; Hohn et al. 1985), and LFPW (Desportes et al. 1993b). Complete cessation of spermatogenesis (aspermatogenesis), outside the mating period, has been reported in harbour porpoises (*Phocoena phocoena*) in the North-east Atlantic (Neimanis et al. 2000). In contrast, species such as sperm whales (Best 1969, Mitchell and Kozicki 1984), dwarf sperm whales (*Kogia sima*; Plön 2004), and common bottlenose dolphins (*Tursiops truncatus*; Cockcroft and Ross 1990), show evidence of continuous spermatogenesis throughout the year, with minimal variation in levels of production. Studies of captive bottlenose and spinner dolphins (Harrison and Ridgway 1971, Wells 1984), and wild-caught mysticetes (Fukui et al. 1996, Mogoe et al. 2000, Kjeld et al. 2004, Kjeld et al. 2006), indicate that serum testosterone levels change seasonally,

with highest concentrations observed during the breeding season. In North Atlantic minke whales (*Balaenoptera acutorostrata*) and fin whales (*Balaenoptera physalus*), combined studies of serum hormone levels and morphological gonadal characteristics have indicated that measurements of testosterone levels not only correlate to anatomical/histological data (Fukui et al. 1996, Mogoe et al. 2000) but may also surpass them in sensitivity of detecting cyclical changes in the male reproduction system (Kjeld et al. 2004, Kjeld et al. 2006).

Testicular activity in LFPWs around the Faroe Islands is diffusely seasonal, with an overall 1.5-fold increase in testis weight, and 2.5-fold increase in testosterone concentrations between March and September (Desportes et al. 1993b). However, elevated testis weights, testosterone concentrations and high densities of spermatozoa were still recorded in some individuals between October and February (i.e. outside the proposed mating period), suggesting that testicular activity does not completely cease (Desportes et al. 1993b). The reproductive patterns of male and female LFPWs off the Faroe Islands appear to synchronise well, with conceptions and births estimated to occur mainly from spring to early autumn, with a few births still recorded into the winter months (Desportes et al. 1993b, Martin and Rothery 1993). As (early pregnancy) foetal mortality appears to be common in LFPWs (Martin and Rothery 1993, Desportes et al. 1994a), the protracted seven-month period of elevated testicular activity may be an adaptive feature enabling females that abort a foetus early in pregnancy to conceive again within the same mating season (Desportes et al. 1993b). Although similar signs of seasonality in sperm production of SFPWs off Japan have been detected, the seasonally-limited sampling period has prevented a complete investigation (Kasuya and Marsh 1984, Kasuya and Tai 1993).

Mating strategy

Mammalian reproductive strategies are diverse and variable, and marine mammals are no exception. Cetacean mating systems are not considered monogamous (each individual with a single partner) but can be either polygynous (some males with two or more partners) or polygynandrous (males and females both with multiple partners; Murphy et al. 2005, Mesnick and Ralls 2009). Both polygynous and polygynandrous male mating strategies and associated competition among male cetaceans have led to various reproductive adaptations such as relatively large testis size, sexual dimorphism, and secondary sexual characteristics (Murphy et al. 2005, Mesnick and Ralls 2009). Relationships between sexual dimorphism, testis size, and mating systems are so strong that both testis size and sexual dimorphism may be useful as indicators of the mating system of a species (Gomenido et al. 1998, MacLeod 2010, Dines et al. 2015).

The primary male mating strategy in mammals is dispersing from the natal group, searching for receptive females, and spending as little time as possible with them other than to mate, or to defend access to those females (Greenwood 1980, Murphy et al. 2005, Mesnick and Ralls 2009). In odontocetes, male dispersal has been documented in sperm whales (Best et al. 1984, Whitehead and Weilgart 2000), and several delphinid species (Connor et al. 2000). The mating strategy of male pilot whales is still unknown, as more information is needed from genetic studies and detailed analysis of group structure, together with behavioural studies (Desportes et al. 1993b). Dispersal of maturing SFPWs has been suspected on the basis of an uneven distribution of maturing males among captured schools, and the lack of males between 10 and 20 years of age (Kasuya and Marsh 1984). There is also some evidence from the North Atlantic, including the existence of male-only groups (Sergeant 1962a, Bloch 1992, Desportes et al. 1993a), that maturing and young mature male LFPWs may, at least temporarily, move away from their natal groups to aggregate in other groups and/or form non-breeding groups (Desportes et al. 1993b). Genetic evidence indicates that, while male LFPWs remain in their natal pod, they do not father calves in that pod, and therefore mating must occur when two or more pods meet (Amos et al. 1993a). Although *G. m. edwardii* has a wide distribution in the Southern Hemisphere, studies of gonadal development have not been conducted, nor have any definite conclusions been drawn regarding their mating seasonality and strategies.

2.3 Female reproduction

Knowledge of female reproductive parameters is particularly important in populations where there is a lack of baseline information on the population size before the introduction of anthropogenic pressures (Murphy et al. 2009). Even in the absence of direct exploitation, knowledge of the reproductive parameters of cetacean populations is essential to build a complete understanding of their life history. Such information would enable an assessment of possible current and future impacts on a population such as adverse anthropogenic activities that may result in marine pollution and habitat degradation (Plön 2004).

Reproductive anatomy

The reproductive tract of cetaceans is typically mammalian. The uterus is bicornate (twin-horned), and the foetus usually develops in one of the horns (Boyd et al. 1999). The proliferation of the endometrial lining of the uterus is stimulated as part of the seasonal reproductive cycle, but there is no menstrual blood loss as in some other mammalian species (Boyd et al. 1999). The mammary glands are elongate in shape, positioned along the main body axis, and produce thick creamy milk that has a fat content ranging from 14 to 53%

(Lockyer 1984, 1993b, Boyd et al. 1999). The ovaries are both equally functional in mysticetes, however, some odontocete species tend to ovulate only from the left ovary initially, with right ovarian activity occurring later in life (Boyd et al. 1999). Once ripe, an egg is released from an ovarian follicle, triggering the development of a *corpus luteum* (CL), which shrinks if fertilisation does not take place, but continues to function if pregnancy occurs (Boyd et al. 1999; see Figure 2.3). Regardless, the CL eventually degenerates into a *corpus albicans* (CA), which continues to regress (Murphy et al. 2010).

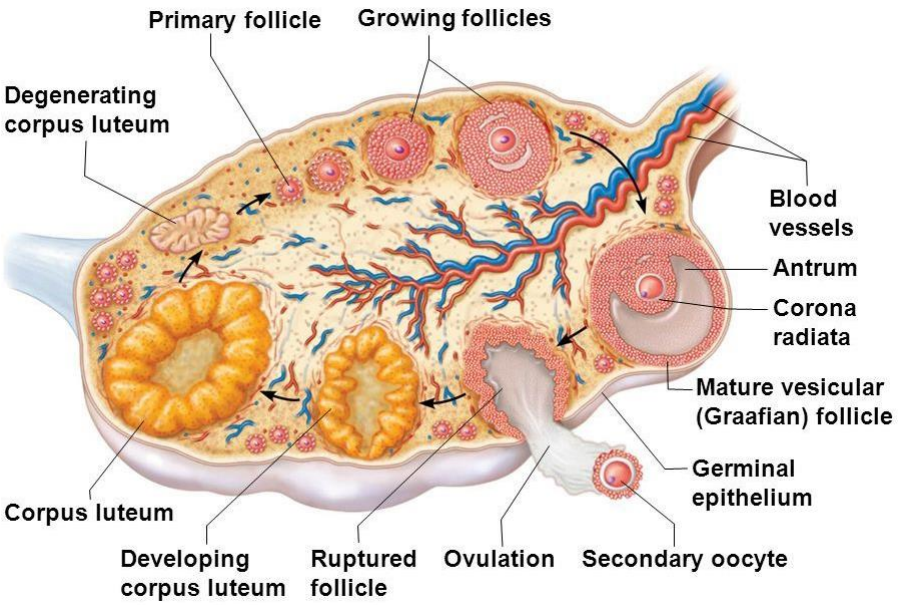


Figure 2.3. Sagittal view of a mammalian ovary showing the developmental stages of an ovarian follicle.

Attainment of sexual maturity

Unlike the gradual sexual maturation process in male cetaceans, female maturation is a rapid process, with the attainment of sexual maturity defined as the age at which a female has ovulated at least once (evidenced by at least one *corpus* on her ovaries; Perrin and Donovan 1984). The *corpora*, where they reflect the reproductive history of a species (Perrin and Donovan 1984), can be related to the age of the female to determine the age at first ovulation, the reproductive lifespan of the individual, and can indicate the reproductive ability of the population (Plön 2004). In addition to estimating the age at first ovulation, the age at first conception and first lactation can also be determined (Perrin and Donovan 1984). In cases where a foetus has not been observed, the development of stretch marks along the uterine body, a marked increase in the diameter of one of the uterine horns, and an increase in vascular supply to the uterus are indications that a female has previously borne a large foetus

(Benirschke et al. 1980). These uterine changes indicate that the female has carried a substantial foetus, but will occur regardless of whether the foetus is aborted or carried until term (Benirschke et al. 1980). Irreversible changes that occur in the mammary glands at the time of first lactation indicate whether a female has borne a calf until at least full term, but not whether she has successfully maintained it until weaning (Benirschke et al. 1980). The biases and degrees of precision resulting from different approaches to estimating the age at sexual maturity have been reviewed by (Perrin and Reilly 1984) and in more detail by (DeMaster 1984) who reported the mean age of first time ovulators provided the best estimate. Therefore, ASM will be used synonymously with the average age at first ovulation throughout the remainder of this thesis.

In female LFPWs, ASM is one of the few parameters in which there appears to be a substantial difference between the Faroese population sampled in the late 1980s (Martin and Rothery 1993) and the Newfoundland population sampled in the 1950s (Sergeant 1962a; Table 2.2). Although only a small Newfoundland sample was examined (*n* = 16 mature females), Sergeant (1962a), later supported by Kasuya et al. (1988b), reported a mean age at sexual maturity of six to seven years. Female LFPWs examined from the Faroese drive fishery were reported to ovulate for the first time at a mean age of approximately eight years (Martin and Rothery 1993). These ASM values were found to be significantly different, but in the absence of accurate estimates of population size before and after exploitation, and comparable measures of ASM before exploitation, the reasons for Newfoundland LFPWs reaching maturity almost two years earlier than the Faroese individuals was not elucidated (Martin and Rothery 1993). It is interesting to note that the attainment of sexual maturity in Faroese LFPWs seems to be triggered by the attainment of a critical body length (median 375 cm, range *c*. 350 – 418 cm) rather than either age or body mass (Martin and Rothery 1993), as also noted in other cetaceans (e.g. Lockyer 1984).

Persistence of corpora and ovulation rate

In most mammalian species, CAs are reabsorbed and eventually disappear (Boyd et al. 1999). However, there is evidence that CA scars remain on the ovaries of some cetaceans for the life of the individual recording, in theory, the total number of ovulations, most of which represent pregnancies (Ivashin 1984, Marsh and Kasuya 1984, Boyd et al. 1999). However, questions been raised concerning the validity of this assertion (Brook et al. 2002, Takahashi et al. 2006, Dabin et al. 2008) and there is still no agreed resolution on the matter (Murphy et al. 2010). In pilot whales, CAs are believed to be retained indefinitely and are therefore an indication of the ovulation rate (Marsh and Kasuya 1984, Martin and Rothery 1993), but not necessarily the

Table 2.2. Comparison of reproductive parameters and characteristics of SFPWs (*Globicephala macrorhynchus*), and LFPWs (*G. melas*) from various locations.

		G. macrorhynchus		***G. melas melas***		***G. melas edwardii***
Location		Japan		Faroe Islands[4, 5]	Newfoundland[6, 7]	Argentina[8]
		"Southern form"[1, 2]	"Northern form"[3]			
Source		Drive fishery	Drive fishery	Drive fishery	Drive fishery	Stranding
Sampling period		1983 – 1988	1982 – 1988	1986 – 1992	1951 – 1959	2009
Sexual maturity						
Mean length at sexual maturity (cm)	M	422	560	494	430 – 490	
	F	316	390 – 400	375	356[a]	366
Mean age at sexual maturity (yrs)	M	17	17	17	12	
	F	9	8 – 9	8.3	6 – 7[a]	8
Age dependence in reproduction						
Age of the oldest pregnancy (yrs)		35.5	36.5	55	39.5	
Age at the oldest ovulation (yrs)		39.5	40.5	55	39.5	
Age at the oldest lactation (yrs)		50.5	43.5		39.5	
Post-reproductive females (% of mature)		25		< 5[b]	≤ 5	0
Reproductive cycle						
Mean ovulation rate (yr^{-1})		0.70 – 0.14 (7 – 39yrs)		0.25	0.3 – 0.5	0.41
Annual pregnancy rate (APR %)		32.2	28.3	28.6[c]	24.6	37[d]
Gestation period (mo.)		14.9	*c.*15	12	15.5 – 16	
Mean body length at birth (cm)		140	185	176.8	174 – 178	
Mean lactation length (yrs)		2.23	2 – 2.78	3.4	1.75 – 1.83	
Mean resting period (yrs)		1.75	1.20 – 1.67	< 0.75		
Mean calving interval, all ages (yrs)		7.78	5.1 – 7.1	5.1	3.3	2.4[e]
Lifetime reproductive output (calves)		4 – 5		3 – 4	9	
Reproductive Seasonality						
Seasonality of breeding		Diffuse, unimodal	?More distinct, unimodal	Diffuse, ?bimodal	Diffuse	Diffuse
Peak month(s) of conception		May	October – November	April – June, September	April – May	
Peak month(s) of birth		July – August	December – January	April – June, September	August	

[1]Kasuya and Marsh (1984), [2]Marsh and Kasuya (1984), [3]Kasuya and Tai (1993), [4]Martin and Rothery (1993), [5]Desportes et al. (1993), [6]Sergeant (1962), [7]Kasuya et al. (1988), [8]Soto et al. (2017).
[a]Estimated from evidence of first ovulation. [b]Ovulation continued through life in many females, but inter-pregnancy interval increases with age and pregnancy is rare after 40 yrs. [c]Pregnancy rate was considered to be positively biased in this sample. [d]Estimated as proportion of mature females pregnant. [e]Estimated directly from ovulation rate.

pregnancy rate, as ovulations have been reported to occur without a subsequent pregnancy in a number of delphinid species (e.g. Benirschke and Marsh 1984, Perrin and Reilly 1984, Cockcroft and Ross 1990, Murphy et al. 2010). Although some investigators claim to be able to distinguish between the scars of infertile ovulations vs. ovulations that resulted in pregnancy (e.g. Harrison 1969, Harrison and Brownell Jr 1971, Ivashin 1984, Takahashi et al. 2006), the majority have not been able to reliably differentiate between the two (e.g. Perrin et al. 1976, Benirschke et al. 1980, Lockyer 1984, Marsh and Kasuya 1984, Perrin and Donovan 1984, Slooten 1991). In bottlenose dolphins and *Stenella* spp., CAs are only reported to be retained when they have originated from a CL of pregnancy (Harrison 1969, Perrin and Reilly 1984, Brook et al. 2002).

The number of ovulations per female per annum is termed the annual ovulation rate and is usually obtained from the slope of the regression of age on total number of *corpora* scars in mature females (e.g. Marsh and Kasuya 1984, Myrick et al. 1986, Cockcroft and Ross 1990, Martin and Rothery 1993). Large variations have been reported in odontocete ovulation rates (Sergeant 1962a, Perrin and Donovan 1984, Myrick et al. 1986), including inter-population differences (Van Waerebeek and Read 1994). Age-specific ovulation rates can be calculated when the sample is large enough to be subdivided into age classes (e.g. Marsh and Kasuya 1984, Perrin and Donovan 1984, Marsh and Kasuya 1986). However, there is also usually high population-level variability in the number of ovarian *corpora* scars between individual females of the same age class (e.g. Perrin et al. 1976, Marsh and Kasuya 1984, Cockcroft and Ross 1990). Factors contributing to this variation include unreliability of age estimates, individual variation in the ASM, changes in ovulation rate during the reproductive life span, individual variation in ovulation rate, number of pregnancies, and female position in the social organisation (Perrin et al. 1976, Perrin et al. 1977, Marsh and Kasuya 1984, Murphy et al. 2010). Given the relationship between estimated age and the number of corpora is not usually linear, some researchers have fitted a curve to these data (Perrin et al. 1976, Marsh and Kasuya 1984). Previous studies have suggested that the ovulation rate decreases continuously throughout life in both SFPWs (Marsh and Kasuya 1984) and LFPWs (Martin and Rothery 1993).

Gestation, foetal growth and birth size

The gestation period is one of the least variable reproductive parameters within species of delphinids, and mammals in general (Kiltie 1982). Estimation of the gestation period is essential for population management because it comprises one portion of the calving interval (Perrin and Reilly 1984). Foetal growth rates are important for determining the age of foetuses

and estimating the length of gestation (Perrin and Reilly 1984). Estimates of average birth size are used to estimate the length of gestation and timing of births. Length-at-birth is also the starting point for growth curves and ultimately contributes to estimates of the reproductive capacity of a population (Perrin and Reilly 1984).

Mammalian foetal growth is generally considered to have two stages: (1) non-linear growth phase — the early weeks of development, after conception, when the embryo/foetus accumulates mass slowly, and its length increases non-linearly; and (2) linear-growth phase — when foetal body length begins to increase at an approximately constant rate (Huggett and Widdas 1951). The second linear phase is thought to continue throughout most or all of the remaining gestation period (Huggett and Widdas 1951). There appears to be a relationship between the length of gestation, foetal growth rate and birth size, such that larger delphinids have longer gestation periods than the smaller species (Perrin and Reilly 1984). However, as a foetus can only be measured after the death of the mother (or if an aborted foetus is recovered), the rate of growth has to be inferred (Martin and Rothery 1993). Most small odontocete species have a gestation period of 12 months or less (Boyd et al. 1999), but in larger species such as killer whales (Perrin and Reilly 1984) this ranges up to 16 months and has been recorded lasting up to 19 months in the sperm whale (Best et al. 1984).

Although unable to give certain and precise values for the rate of foetal growth and gestation period, Martin and Rothery's (1993) estimates for Faroese LFPWs differ greatly from those calculated by Sergeant (1962a) for the LFPWs off Newfoundland or by Kasuya and Marsh (1984) for SFPWs off Japan (see Table 2.2). As acknowledged in these studies, the considerable differences in the estimates of these important parameters can at least be partially explained by the inherent bias of the analytical methods and data sets used, with the growth rate estimates of Sergeant (1962a) and Kasuya and Marsh (1984) likely to be underestimates (Martin and Rothery 1993). As such, *G. m. melas* is now believed to have a gestation period closer to 12 months, rather than the 15 to 16 months previously suggested (Martin and Rothery 1993). Usually, one calf is produced from each pregnancy (Boyd et al. 1999), and average lengths-at-birth are reported to be approximately 177 cm for *G. m. melas* (Sergeant 1962a, Martin and Rothery 1993) and 140 and 185 cm for the southern and northern forms of the SFPW, respectively (Kasuya and Matsui 1984, Kasuya and Tai 1993; Table 2.2).

Lactation, weaning, and resting period

The length of lactation can be highly variable in odontocetes, with the lactation period lasting between 10 and 20 months in the majority of species, but continuing for several years after

parturition in species such as sperm and pilot whales (Sergeant 1962a, Best et al. 1984, Marsh and Kasuya 1984, Cockcroft and Ross 1990, Boyd et al. 1999). Kasuya and Marsh (1984) studied the age composition of entire SFPW pods taken in the Japanese drive fishery and suggested that suckling might sometimes last up to eight years in female calves and 13 to 15 years in male calves. However, given the mean lactation periods for both pilot whale species (2 – 3 yrs for SFPWs, 3.4 yrs for LFPWs; Table 2.2) are considerably less than these extreme values, and some solid food is consumed from six months onwards, the function is likely to be social rather than nutritional in the later stages of lactation (Kasuya and Marsh 1984, Martin and Rothery 1993, Boyd et al. 1999). Extended calving intervals are not necessarily associated with seasonal feeding habits or accumulation of body fat reserves and may be useful as a learning phase for the young in methods of cooperative feeding and foraging strategies where echolocation may be an important function (Brodie 1969). The age at weaning is usually estimated from the oldest suckling calf in the sample, but this may be biased by individual variation in growth rate (Plön 2004); a different method determines the presence of lactose in the stomachs of calves (Best et al. 1984). Although the age at weaning can be estimated using either method, these estimates may be biased in species such as sperm and pilot whales that continue 'social suckling' for long periods (Plön 2004). To overcome this bias, alternative methods to estimate age and length at weaning are to: (1) identify the smallest calves found with solid food in the stomach (e.g. Kasuya and Marsh 1984, Learmonth et al. 2014), and (2) use published formulae for the relationship between estimated female asymptotic length and length at weaning (Huang et al. 2009).

In both pilot whale species, the calving interval and duration of lactation increase with maternal age, which may mean: (1) higher calf survival, (2) milk is provided to calves other than the mother's own, and (3) increased investment in calves with advancing age of the mother (Marsh and Kasuya 1984, Martin and Rothery 1993). Post-reproductive lactating female SFPWs have been reported feeding not only their own offspring but also other calves and juveniles in the pod and this may be related to the tight social organisation of pilot whales (Kasuya and Marsh 1984). The matriarchal pod structure of pilot whales, where there is considerable support from other females, may favour the survival of the young more than for other less social odontocetes, or for the baleen whales where the mother is alone with her calf (Boyd et al. 1999).

Simultaneous lactation and pregnancy has been reported for a number of cetacean species, including both pilot whale species (Best 1968, Perrin et al. 1976, Perrin et al. 1977, Marsh and Kasuya 1984, Miyazaki 1984, Kasuya and Tai 1993, Martin and Rothery 1993), though is usually

only observed in a very low percentage of the total sample of mature females. Detailed investigations of this phenomenon in pilot whales have shown females either conceive towards the end of the lactation period and/or once lactating females become pregnant, they cease lactation shortly afterwards (Kasuya and Tai 1993, Martin and Rothery 1993). Mature females that are not pregnant or lactating are usually classified as resting; the resting period is a stable part of the reproductive cycle of many cetaceans (Best 1968, Lockyer 1984, Perrin and Reilly 1984). The resting period is thought to aid in refilling the energy reserves of females exhausted by the high costs of lactation and can range between two and 15 months but on average lasts four to five months in odontocetes (Perrin and Reilly 1984).

Annual pregnancy rate and calving interval

The annual pregnancy rate (APR) and mean length of the calving interval (CaI) are usually estimated from the proportion of mature females that are pregnant (including those that are simultaneously pregnant and lactating; Perrin and Reilly 1984). In *G. m. melas*, Martin and Rothery (1993) estimated that the length of the reproductive cycle, including ovulation, conception, pregnancy, lactation and 'resting', lasts about five years on average (Table 2.2), but suggested the interval between births is shorter in young mature individuals and increases through life as the female matures. Older females spend more time 'resting' between weaning one calf and conceiving the next (Martin and Rothery 1993). The duration of lactation also increases with age, thus extending the CaI even more in older females (Martin and Rothery 1993). As discussed below, pilot whales (a small minority of LFPWs, though the majority of SFPWs) are reported to cease ovulations at some stage in their lives, due to either ovarian follicle exhaustion or ovarian dysfunction (Marsh and Kasuya 1984, Martin and Rothery 1993).

Senescence and post-reproductive lifespan

In general, fertility and reproductive success are low in newly mature female cetaceans, reaching a peak in young mature animals, following which fertility maintains a plateau until it (often) declines with age (Best et al. 1984, Martin and Rothery 1993, Boyd et al. 1999). Females are defined as reproductively senescent, or post-reproductive, if conceiving or sustaining a successful pregnancy is no longer possible because of age-related changes to the reproductive system (Marsh and Kasuya 1986). The occurrence of reproductive senescence has been observed in females of several odontocete species including sperm whales (Best 1980), killer whales (Foster et al. 2012), false killer whales (Photopoulou et al. 2017), beluga (*Delphinapterus leucas*; Suydam 2009), narwhal (*Monodon monoceros*; Garde et al. 2015), and both SFPWs (Marsh and Kasuya 1984) and LFPWs (*G. m. melas*; Sergeant 1962a, Martin and

Rothery 1993). Senescent females have not been reported in other odontocete species, for example, common bottlenose dolphins (Cockcroft and Ross 1990) or short-beaked common dolphins (Murphy et al. 2009). However, not all criteria used to indicate senescence are reliable, as previously reviewed by Marsh and Kasuya (1986). The primary factors influencing the age at senescence are the depletion of oocytes and age-related degenerative changes of the uterus (Marsh and Kasuya 1986). In a few mammalian species, including humans (*Homo sapien*), SFPWs, killer whales, beluga, and narwhal, females can spend a significant proportion of their adult lifespan post-reproductive, which is contradictory to classical life history theory (Ellis et al. 2018b). It has also been suggested that false killer whales have a substantial post-reproductive lifespan (Photopoulou et al. 2017), though more data are needed to establish the extent and frequency of post-reproductive life in false killer whales (Ellis et al. 2018b).

Marsh and Kasuya (1984) conducted a detailed examination of ovarian aging in the SFPW and reported an age-specific decline in the pregnancy rate, paralleled by a decline in the ovulation rate, and a high incidence of infertile ovulations (atresia) in older females. Approximately 25% of mature female SFPWs (n = 298) examined by Marsh and Kasuya (1984) had senescent ovaries, which were severely depleted of oocytes (presumably because of the exceptionally high atresia rate), and they concluded that SFPWs appear to cease ovulating before 40 years of age. Females less than 40 years of age were classified as senescent only if their ovaries did not contain a CL, and/or a young or medium-sized CA, or macroscopic follicles that were not obviously atretic (Marsh and Kasuya 1984). Female SFPWs are reported to have a post-reproductive life expectancy of 14 years, on average (Marsh and Kasuya 1984). Curiously, senescent females are observed much less frequently in LFPWs (< 5% of mature female *G. m. melas*; Martin and Rothery 1993), and *G. m. melas* does not appear to have a significant post-reproductive lifespan (Ellis et al. 2018b).

Recent work suggests that the demographic consequences of certain life history characteristics are important in the evolution of post-reproductive lifespans (Johnstone and Cant 2010, Croft et al. 2015, Ellis et al. 2018b). SFPWs share several life history characteristics with resident killer whales, beluga and narwhal; the only other cetacean species for which a substantial post-reproductive lifespan has been identified (Ellis et al. 2018b). Limited information available on SFPWs, resident killer whales, beluga, and narwhal suggest they are all sexually dimorphic, highly social, have low lifetime productivity, and are known or believed to exist in stable matrilineal groups of closely related females, with strong mother-offspring associations and a long period of dependency (Bigg 1982, Kasuya and Marsh 1984, Heimlich-Boran 1993, Palsbøll et al. 1997, Whitehead and Mann 2000, Marcoux et al. 2009, Colbeck et al. 2013, O'Corry-

Crowe et al. 2018). However, it is important to note that such characteristics do not presuppose the evolution of post-reproductive lifespans (Ellis et al. 2018b). Available evidence suggests that LFPWs exhibit similar life history characteristics and social structure to SFPWs (Amos et al. 1993b, Martin and Rothery 1993), yet LFPWs do not appear to have a significant post-reproductive lifespan (Martin and Rothery 1993, Ellis et al. 2018b).

It has been identified that there remains a considerable amount still to be discovered regarding the occurrence and evolution of post-reproductive lifespan in toothed whales (Ellis et al. 2018b), including pilot whales. For example, the occurrence of reproductive senescence has not been investigated in female *G. m. edwardii*. However, data needed to examine reproductive senescence are often difficult to gather for long-lived species such as cetaceans, as samples need to be unbiased and sample sizes need to be large to obtain a meaningful number of old females. Also, unless all the females in a population cease breeding at approximately the same age, identification of post-reproductive females will be extremely difficult, even with age-specific fecundity data (Marsh and Kasuya 1986). Data from drive fisheries and MSEs are suitable for investigating the existence of post-reproductive females in cetacean species, as demonstrated for SFPWs (Kasuya and Marsh 1984, Marsh and Kasuya 1984), *G. m. melas* (Martin and Rothery 1993), and false killer whales (Photopoulou et al. 2017). Therefore, the frequent MSEs on the New Zealand coast (see Chapter 7), provide a valuable opportunity to conduct the first investigation of reproductive senescence in female *G. m. edwardii*.

Reproductive seasonality

Many mammalian species show synchrony and seasonality of reproduction, and most data describing seasonal reproduction in marine mammals are inferred from the calving season (Plön 2004, Plön and Bernard 2007). Some odontocetes appear to have a definite calving season, which is often protracted over a few months (e.g. Best 1968, Sergeant 1973, Van Waerebeek and Read 1994), while others show births year-round with a calving peak over part of the year; a phenomenon termed ‘diffusely seasonal’ (e.g. Sergeant 1962a, Harrison and Ridgway 1971, Cockcroft and Ross 1990, Martin and Rothery 1993). At high latitudes, births occur in a well-defined peak and are timed to maximise the availability of food during periods of high energy demands. Seasonal reproduction is pronounced in many odontocetes such as sperm whales (Best et al. 1984, Whitehead and Weilgart 2000), harbour porpoises (Read 1990, Börjesson and Read 2003), and many delphinid species (Perrin and Reilly 1984, Robeck et al. 2009). In contrast, tropical odontocetes such as *Stenella* spp., reproduce year-round (Kasuya

1972, Miyazaki 1977, Barlow 1984, Whitehead and Mann 2000). However, variation in calving season is not merely a consequence of latitude.

Reproductive timing may also vary among populations. For example, common bottlenose dolphin populations show variation in the timing of parturition, with no apparent correlation with latitude (Urian et al. 1996, Thayer et al. 2003, McFee et al. 2014). Further, differences in seasonality at the population level have been reported in striped dolphins (Miyazaki 1984, Meynier et al. 2010), harbour porpoises (Read 1990, Sorensen and Kinze 1994, Börjesson and Read 2003), and SFPWs (Kasuya and Marsh 1984, Kasuya and Tai 1993). These were attributed to differences in climate (Sorensen and Kinze 1994), food availability (Barlow 1984, Kasuya and Tai 1993, Meynier et al. 2010), food quality (Read 1990, Sorensen and Kinze 1994), variation in water temperature (Kasuya and Tai 1993, Meynier et al. 2010) and degree of human exploitation (Barlow 1984).

In *G. m. melas*, a diffusely seasonal, bimodal breeding pattern has been proposed, with a primary peak in boreal spring/early summer and a secondary peak in autumn (Martin and Rothery 1993). The seasonal frequency of ovulations in *G. m. melas* suggest that ovulations probably occur throughout the year, and thus the observed mating (or conception) season, i.e. early spring through until autumn, may be due to the reported seasonal trend in male reproductive activity (Sergeant 1962a, Desportes et al. 1993b, Martin and Rothery 1993). Knowledge of the reproductive biology and seasonality of female pilot whales in the Southern Hemisphere is lacking.

2.4 Strandings

> *"It is not known for what reason they run themselves aground on dry land; at all events, it is said they do so at times and for no obvious reason."*
> *—Aristotle, 350 BCE (Historia Animalia, Book IX, Ch.48).*

Strandings of cetaceans have occurred along the shores of most coastal nations for centuries. When stranding records are examined, four main types of strandings can be identified: (1) single strandings, (2) mass strandings (MSEs), (3) mass mortalities and Unusual Mortality Events (UMEs), and (4) out of habitat situations (Moore et al. 2018). The majority of these events are single strandings, involving individuals that have either become ill or died before coming ashore, but occasionally large groups of apparently healthy cetaceans strand live. MSEs almost always involve odontocetes rather than mysticetes and more often pelagic than inshore species (Moore et al. 2018). Curiously, most of the whales that die during an MSE do not show

any pathology (Perrin and Geraci 2002). It is not understood why apparently healthy cetaceans strand *en masse*, although there are a variety of both natural and anthropogenic hypotheses including coastal topography and oceanography (Brabyn and McLean 1992, Walker et al. 2005, Hamilton 2018); meteorological and geomagnetic conditions (Evans et al. 2005, Bradshaw et al. 2006, Mazzariol et al. 2011); seismic activity and sonar noise (Fernandez et al. 2005, Southall et al. 2006, Southall et al. 2013). It is likely that a number of factors contribute to any one stranding event, and these vary on a case-by-case basis.

Most cetaceans involved in an MSE often die *in situ* or a short distance away from the original stranding site (Martin et al. 1987). There is usually no reason to suspect that mass stranded groups are unrepresentative of the population as a whole, these events, therefore, provide a valuable opportunity to study the biology of the species involved (Martin et al. 1987). MSEs should not be confused with mass mortality events or UMEs where animals strand dead (or dying) over an extended period of time (Moore et al. 2018). UMEs have been associated with infectious diseases such as morbillivirus epidemics (e.g. Fernández et al. 2008, Van Bressem et al. 2014), biotoxins (e.g. Torres de la Riva et al. 2009, Bengtson Nash et al. 2017), and oil spills (e.g. Venn-Watson et al. 2015).

Pilot whales are among the species most often involved in MSEs throughout their range (Minton et al. 2018), but it is not known whether anthropogenic activity plays a role in these events (Hayes et al. 2017). In most cases, the cause(s) of MSEs could not be identified (e.g. Bogomolni et al. 2010, Dolman et al. 2010). Pilot whales tend to strand in large numbers of mixed ages and sexes on gently sloping beaches, and strandings often recur in a specific geographic area; for example, Cape Cod, Massachusetts, U.S. (McFee 1990, Wiley et al. 2001, Sweeney et al. 2005), Tasmania, Australia (Evans et al. 2005, Kemper et al. 2005, Gales et al. 2012, Beasley et al. 2019), and Golden Bay, New Zealand (Gaskin 1968, Baker 1981, Brabyn 1991). Although the reasons behind these strandings remain unknown, the strong social cohesion of pilot whales is thought to be a factor for groups to strand in large numbers, and to re-strand after being re-floated by human-initiated efforts, whatever the underlying reasons may be (Sergeant 1982, Perrin and Geraci 2002). However, many re-stranding events may simply be explained by disorientation and weakness of the whales resulting from the initial stranding event (Geraci and Lounsbury 2005).

Sporadic data on cetacean strandings (including both beach-cast and live stranded) in New Zealand date back to 1840, although it was not until the introduction of the New Zealand Marine Mammal Protection Act (MMPA) in 1978 that it became government policy to

document cetacean strandings. Following the establishment of the MMPA in 1978, the New Zealand Whale Stranding Database (NZWSDB) was created in 1988 and compiled existing records within the Department of Conservation (DOC) and those previously held by the National Museum of New Zealand (Te Papa Tongarewa), Ministry of Agriculture and Fisheries, published and private records of Frank Robson (1984), Bill Gaskin (1968), International Whaling Commission reports (Cawthorn 1978, 1979, 1982) and newspaper archives. A list of the categories used in the NZWSDB and an analysis of records to April 1989 are presented by Brabyn (1991). The NZWSDB is administered by DOC and includes spatial and temporal information on each reported stranding, with a subset of stranded individuals examined by specialist cetacean biologists or veterinary pathologists.

Both LFPW and SFPW records frequent the NZWSDB, with LFPWs known to be the most common mass stranded cetacean species. Brabyn (1991) reported that pilot whale strandings occur year-round on the New Zealand coast, but more often over the summer period. Whangarei Harbour, Mahia Peninsula, Golden Bay, and the Chatham Islands appear to be particularly prone to LFPW strandings and have been referred to as stranding 'hot-spots' (Brabyn 1991). DOC has taken an active interest in the management of cetacean strandings on the New Zealand coast in recent years, but decisions as to the planning and prediction of effort have been hampered by a lack of scientific data on which to base management decisions. There has been no analysis of the New Zealand pilot whale stranding record post-1989.

2.5 Conservation status

Knowledge of species identity, population structure, demographics, reproductive biology and habitat use is required for effective conservation management of any population (Stockin 2008). Although the LFPW frequently mass strands on New Zealand (Brabyn 1991; see Chapter 7 for further detail) and Australian (Evans et al. 2005, Kemper et al. 2005, Beasley et al. 2019) coasts, there is a severe lack of empirical data on LFPW biology and ecology throughout the Southern Hemisphere and no current information available on population status in this region (Minton et al. 2018). Despite the lack of research undertaken on the LFPW in the Southern Hemisphere, the International Union for Conservation of Nature (IUCN) Red List of Threatened Species has recently updated the global threat classification for LFPWs from 'Data Deficient' (Taylor et al. 2008) to 'Least Concern' (Minton et al. 2018). However, it is recognised that Southern Hemisphere populations are especially 'data-poor', indicating that more research is needed to adequately determine their conservation status (Minton et al. 2018). LFPWs are also currently treated by the IUCN as a single species, even though there is evidence that they may

comprise a complex of two or more species. If so designated, the IUCN classification may change, and some potential new species may warrant listing under a higher category of risk but, as an immediate priority, consideration should be given to the separate assessment of the two subspecies; *G. m. melas* in the North Atlantic and *G. m. edwardii* in the South Hemisphere (Minton et al. 2018).

The North Atlantic subspecies (*G. m. melas*) continues to be harvested for food in the Faroe Islands and Greenland, but these localised drive fisheries do not appear to have resulted in any detectable declines in abundance (NAMMCO 2018b, Pike et al. 2019). However, this will need to be reassessed if LFPWs are found to represent a species complex (Taylor et al. 2008, Taylor et al. 2011). It is also possible that large culls (average 850 individuals per annum) in the Faroe Islands, which have occurred over many centuries, may have had a significant but undetected impact on this highly social and wide-ranging species (Minton et al. 2018). The North Atlantic Marine Mammal Commission (NAMMCO) is planning a thorough assessment of North Atlantic *G. m. melas* in 2019 (NAMMCO 2018b).

Other threats that could cause widespread declines include high levels of anthropogenic noise (e.g. military sonar and seismic surveys; Parsons et al. 2008, Sivle et al. 2012, Parsons 2017), incidental mortality from fisheries (Leeney et al. 2008, Verborgh et al. 2016, Olson 2018), and contaminants. High contaminant levels have been documented in LFPWs off the Faroe Islands (Dam and Bloch 2000, Nielsen et al. 2000, Sonne et al. 2010) and Tasmania (Weijs et al. 2013), with their meat considered a potential health hazard for human consumption in the Faroe Islands (Simmonds et al. 1994, Weihe et al. 1996, Weihe and Debes Joensen 2012). In addition, predicted impacts of global climate change on the marine environment might induce changes in LFPW range, prey distribution, abundance and/or migration patterns (MacLeod 2009, Simmonds and Eliott 2009, Fielding 2010). Such threats may be causing undetected declines in abundance and/or changes in distribution in regions for which little or no empirical data are available (Minton et al. 2018), for example, the Southern Hemisphere and New Zealand in particular.

In New Zealand waters, LFPWs are listed as 'Not Threatened' according to the official New Zealand Threat Classification System (NZTCS; Baker et al. 2016). Remarkably, this status appears to have been assigned in the absence of any abundance, density or life history data. Although LFPWs may not be under threat globally, it can be argued that cetacean populations whose abundance, distribution, habitat use and reproductive biology remain unknown are most at risk, since population declines are likely to go unnoticed (Stockin and Orams 2009).

Failure to monitor and recognise local population declines can threaten the national (and eventually the international) status of once-common species, for example, the bottlenose dolphin (Vermeulen and Bräger 2015). Lack of empirical data is indeed a risk for the New Zealand LFPW population which, until the current study, has been the focus of only a few preliminary studies related to diet (Beatson et al. 2007a, Beatson 2008, Beatson and O'Shea 2009) and contaminants (Schroder and Castle 1998).

2.6 Summary

Most previous research on pilot whales has been based on populations inhabiting the North Atlantic, and North Pacific, where drive fishery catches, together with MSEs, have provided extensive information on the biology and ecology of these whales (e.g. Sergeant 1962a, Kasuya and Marsh 1984, Kasuya and Matsui 1984, Martin et al. 1987, Donovan et al. 1993, Kasuya and Tai 1993). Despite the identification of a unique subspecies of LFPW (*G. m. edwardii*) in the temperate south (Davies 1960), and the occurrence of frequent MSEs on New Zealand (Brabyn 1991; see Chapter 7 for further detail) and Australian (Evans et al. 2005, Kemper et al. 2005, Beasley et al. 2019) coasts, very little is known of the biology and ecology of LFPWs in the Southern Hemisphere. With the exception of female reproductive parameters estimated from a single MSE in Argentina (Soto et al. 2017), and an unpublished report on life history and contaminant levels in New Zealand waters (Schroder and Castle 1998), very little information is available on the life history of *G. m. edwardii*. Current conservation status categorisations as 'Least Concern' (The IUCN Red List of Threatened Species; Minton et al. 2018) and 'Not Threatened' (NZTCS; Baker et al. 2016) are based on general assumptions about the species and, as a consequence, improved understanding of the species is recognised as a high priority for effective conservation management of LFPWs in New Zealand waters (Suisted and Neale 2004).

This thesis addresses critical gaps in the biological knowledge of *G. m. edwardii* by defining key life history parameters for the subspecies using samples and data obtained from post-mortem examinations of individuals stranded on the New Zealand coast. The overall goal of this thesis is to improve our understanding of the life history of LFPWs in New Zealand waters and to identify any relationships between MSEs and life history characteristics that may have implications for conservation. Specifically, key objectives were to: (1) describe growth rates, allometric relationships, and sexual dimorphism; (2) model survivorship and mortality; (3) classify the stages of male sexual maturation, and define indicators of male sexual maturity; (4) estimate female reproductive parameters and investigate evidence of reproductive

senescence and seasonality; and (5) identify spatiotemporal trends of LFPW strandings on the New Zealand coast. These five objectives are investigated and presented in Chapters 3 to 7, respectively. As such, this thesis provides guidance to researchers and managers on baseline life history parameters that can be used to assist with monitoring and conservation management of a species for which there are few published data in the Southern Hemisphere.

Chapter 3

Age, growth, and sexual dimorphism of the long-finned pilot whale (*Globicephala melas edwardii*) in New Zealand waters

A pod of long-finned pilot whales offshore, northern New Zealand, January 2018.

In this chapter, an investigation of sex-specific growth rates, allometric relationships and sexual dimorphism of long-finned pilot whales (*Globicephala melas edwardii*) is presented to achieve the first research objective:

Objective 1: Describe the growth rates, allometric relationships and sexual dimorphism of long-finned pilot whales stranded on the New Zealand coast

This chapter is a reformatted version of the following manuscript:

Betty EL, et al. (in prep). Age, growth, and sexual dimorphism of the long-finned pilot whale (*Globicephala melas edwardii*) in New Zealand waters; insight from strandings. Marine Mammal Science.

3.1 Abstract

There is a lack of population-level information available on the biological parameters of long-finned pilot whales (LFPWs; *Globicephala melas edwardii*) in the Southern Hemisphere, despite frequent mass strandings on New Zealand and Australian coasts. In this study, age, growth, allometric relationships, and sexual dimorphism are described using teeth and 14 external morphometric measurements obtained from 515 male, 776 female, and 229 unsexed individuals that stranded along the New Zealand coastline between 1948 and 2017. Ages or age ranges were estimated for 405 whales (including 239 females and 163 males) by examination of growth layer groups (GLGs) in the dentine of decalcified thin sections of teeth. Maximum ages of 31 and 38 years were estimated for males and females, respectively. Females ranged in length from 160 to 500 cm (modal size class 400 – 449 cm) and males from 165 to 622 cm (modal size class 500 – 549 cm). Length at birth was estimated at 163 cm. Gompertz and von Bertalanffy growth models indicated a preliminary early, rapid growth phase, followed by a second phase of slower growth, in both sexes. For males, a two-phase growth model also indicated a moderate growth spurt later in life (*c.* 12 – 13 years). Asymptotic lengths were calculated as 570 and 438 cm for males and females, respectively, attained at an estimated 40 years in males and 30 years in females. This study demonstrates age-related changes in growth rates between male and female LFPWs and strong evidence of sexual size dimorphism, with males being significantly larger than females for 13 out 14 characters measured. Sexual shape dimorphism was also evident in the external pectoral flipper length, fluke width, and dorsal fin height, with males having comparatively longer flippers, wider flukes, and taller dorsal fins than females. Estimated length-at-birth, maximum ages, and sexual shape dimorphism for *G. m. edwardii* differ to that previously reported for the North Atlantic subspecies (*G. m. melas*), which may indicate subspecies or population-level differences in morphology, longevity, and sociality.

3.2 Introduction

Growth models and morphometric analyses have wide applicability in answering questions related to species biology, ecology, and conservation (Fortune et al. 2012). For example, growth models can be used to assess temporal changes in body size, allowing inferences to be made about changes in population status (e.g. Calkins et al. 1998, Fearnbach et al. 2011). In addition, morphometric and allometric studies allow various aspects of body shape and size to be studied, including not only the morphological diversity that can occur between the sexes of a species (Weckerly 1998), but also geographical variation in morphology within a species, i.e. subspecies, populations (Bloch and Lastein 1993, Mazák 2010). The presence or lack of sexual dimorphism can also impart information about the life of the animal and its behaviour within social groups (Shine 1989, Isaac 2005, Mesnick and Ralls 2018). Historically, data used to describe external morphology and size-at-age of cetaceans were collected from whaling ships, whaling stations, and drive fisheries, primarily to assist in the management of exploited stocks (e.g. Laws 1959, Best 1970, Bloch et al. 1993a). More recently, cetacean growth has been examined using data from fisheries bycatch and stranding events (e.g. Read and Tolley 1997, Murphy and Rogan 2006, Denuncio et al. 2017). Therefore, growth models and morphometric data exist for many commercially exploited and bycaught species but are mostly unavailable for other species, subspecies, and populations, for example, the Southern Hemisphere subspecies of the long-finned pilot whale (LFPW; *Globicephala melas edwardii*).

Most research on the LFPW to date has focused on the North Atlantic subspecies (*G. m. melas*), where drive fishery catches, together with MSEs, have provided extensive information on the biology of the subspecies (e.g. Sergeant 1962a, Donovan et al. 1993). There have been comparatively few studies of *G. m. edwardii*, resulting in a general lack of knowledge on the biological parameters of LFPW throughout most of its southern range, including New Zealand. Although maximum body lengths vary geographically, male LFPWs appear to have a faster growth rate and attain a larger body size than females (Martin et al. 1987, Bloch et al. 1993a, Sigurjonsson et al. 1993). The maximum lengths and ages recorded for both male (630 cm and 46 yrs) and female (546 cm and 59 yrs) *G. m. melas* in the North Atlantic (Sergeant 1962a, Martin et al. 1987, Kasuya et al. 1988b, Bloch et al. 1993a) exceed those recorded to date for male (584cm and 31 years) and female (483 cm and 35 years) *G. m. edwardii* from the Southern Hemisphere (Crespo et al. 1985, Schroder and Castle 1998, Soto et al. 2017). Such differences in maximum length and age indicate demographic parameters likely vary between the two subspecies.

Investigations of parameters such as age structure, length-at-birth, growth, maximum total body length, sexual dimorphism, and natural longevity (maximum age) are required to allow detailed comparisons among subspecies and populations of LFPWs, and assess temporal changes within populations (Stolen et al. 2002). Monitoring these parameters can provide an objective means of assessing the resilience of a population to increasing environmental pressures (Caughley 1977, Evans and Hindell 2004). Such assessments are particularly important for effective conservation management of protected species such as marine mammals (Moore and Read 2008).

G. m. edwardii occurs within New Zealand waters year-round, and frequently mass strands in high numbers on the New Zealand coast (Brabyn 1991, Berkenbusch et al. 2013; see Chapter 7). Schroder and Castle (1998) conducted a preliminary investigation of growth and reproduction in *G. m. edwardii*, based on specimens stranded on the New Zealand coast between 1992 and 1996. Preliminary growth curves were presented, though the sample sizes of male (n = 16) and female (n = 19) LFPWs were not adequate to evaluate possible ontogenetic variation in growth rates or estimate asymptotic lengths. Using data collected from MSEs on the New Zealand coast between 1948 and 2017, this study presents some of the first empirical estimates of many demographic parameters for *G. m. edwardii*, specifically: 1) length-at-birth, 2) sex-specific growth curves, 3) allometric relationships, and 4) sexual dimorphism.

3.3 Materials and methods

Data collection

The dataset analysed in this study includes LFPW morphometric data recorded in the New Zealand Whale Stranding Database (NZWSDB; administered by the New Zealand Department of Conservation [DOC]) up to December 2017. The cleaned dataset of 1520 LFPWs was composed of 776 females, 515 males, 229 individuals of unknown sex, and spanned 70 years from 1948 to 2017, though most data were obtained from carcasses stranded between 1978 and 2017. Fifteen standard external body measurements outlined in Norris (1961), as well as the sex (determined by gross examination of external genital opening) of stranded cetaceans, are routinely recorded by DOC rangers or cetacean researchers. Fourteen of these measurements are relevant to LFPWs, comprising nine length measurements, an axillary girth measurement, pectoral fin length and width measurements, dorsal fin height, and tail fluke width (Figure 3.1, Table 3.1). Depending on the stage of decomposition and severity of scavenger damage, not all external measurements could always be taken. In the case of large

MSEs, often only the sex, total body length (TBL), and axillary girth, or sometimes only sex and TBL, were recorded. Sex (determined by gross examination of external genitalia) and TBL were also recorded for 31 foetuses recovered during post-mortem examinations.

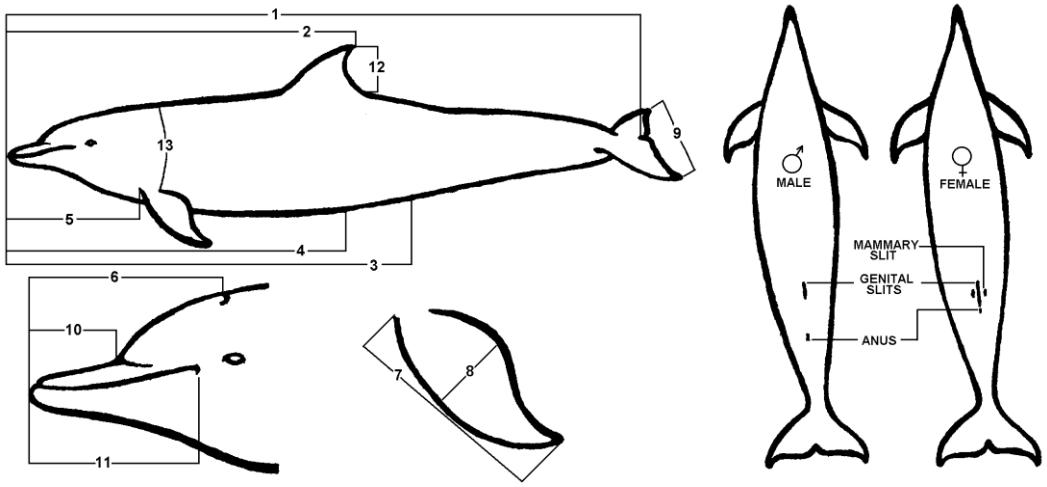


Figure 3.1. Fourteen external morphometric characters (1 – 13, plus length of genital slit) measured from LFPWs stranded on the New Zealand coast.

Source: New Zealand Department of Conservation Whale & Dolphin Stranding/Accident/Death Report, see Appendix 3A).

Table 3.1 External measurements/characters taken of the New Zealand LFPW.

No.	Character	Abbreviation
1	Total length (from tip of upper jaw to deepest part of fluke notch)	1_Total_length (TBL)
2	Tip of upper jaw to tip of dorsal fin	2_Ujaw_dorsal
3	Tip of upper jaw to anus	3_Ujaw_anus
4	Tip of upper jaw to genital slit	4_Ujaw_genital
5	Tip of upper jaw to front (forward insertion) of flipper	5_Ujaw_flipper
6	Tip of upper jaw to blowhole	6_Ujaw_blowhole
7	Length of flipper (external)	7_Flipper_length
8	Greatest width of flipper	8_Flipper_width
9	Greatest width of tail flukes	9_Fluke_width
10	Length of rostrum	10_Snout_length
11	Length of gape (tip lower jaw to corner of mouth)	11_Ujaw_gape
12	Height of dorsal fin	12_Height_dorsal
13	Axillary girth (immediately behind flipper, around body)	13_Axill_girth
14	Length of genital slit	14_Genital_slit

To check measurements for transcription errors, inter-observer error (since measurements were taken by a number of different people), and outliers, regression analysis was carried out on each character by plotting it against TBL, separately for males and females (after Murphy and Rogan 2006) using the SPSS statistical software package, version 24 (IBM 2016). Any correctly transcribed data points found to be more than three standard deviations from the fitted line were omitted from the dataset.

Age estimation

Of the 1520 LFPWs examined in this study, teeth from 405 whales (including 239 females and 163 males) involved in MSEs between 2006 and 2017 were collected for age estimation purposes. Age estimation was performed by counting annual growth layer groups (GLGs) in decalcified and stained longitudinal sections of teeth (Perrin and Myrick 1980; Figure 3.2). Tooth preparation methods for this study were adapted from Lockyer (1993a); for a detailed explanation of methods see Appendix 3B. Between three and ten teeth from each whale were collected from the middle of the upper or lower jaw, and either stored in 70% ethanol or frozen. Prior to processing for age determination, all teeth were catalogued, measured and photographed with identification labels for archival reference. At least one of the least worn/damaged/curved teeth from each whale was selected, rehydrated if stored in ethanol or defrosted if frozen, and cleaned using a scalpel blade or tooth extractor. Teeth were mounted longitudinally in the centre of a slide with mounting media Crystalbond™, and ground down on both sides, using a GEMMASTA GF4 faceting machine equipped with a 600 grit wheel, in order to obtain a 3 to 5 mm longitudinal section through the centre of the tooth, including the crown and the root. After removal of the mounting media, the teeth were decalcified with hydrochloric acid (RDO, Apex Engineering Products Corporation, Aurora, Illinois) until they were slightly pliable. Decalcification times ranged from four hours for teeth of neonates to *c.* 24 to 36 hours for adult teeth. Decalcified teeth were sectioned at approximately 25 µm on a carbon dioxide freezing stage of sledge microtome, using Tissue-Tek® as a mounting medium. Sections were then stained with Erlich's haematoxylin and 'blued' in a weak ammonia solution. The best sections (i.e. those cut through the centre of the pulp cavity) were mounted permanently on glass slides using DPX mounting medium.

Sections were examined under a binocular microscope for GLGs in the dentine (10 – 40×) and cementum (100 – 400×). The GLGs in the postnatal dentine were found to be considerably more distinct than the cemental GLGs. As the pulp cavity was not found to be completely occluded in any specimen examined (see Appendix 3C), GLGs in the postnatal dentine were used to assess age in this study. All sections were read by two or three individuals, including at

least one expert reader (ELB or SM). Readers evaluated the tooth sections three times independently, without prior knowledge of body length or sex, and then compared assessments to assign the best age estimate or an age range for each animal according to Hohn and Fernandez (1999). If readers disagreed on the age, the sections were examined again. If the difference was higher than one GLG, all readers re-read the tooth, and if no agreement was reached another tooth was sectioned and read by all readers. If the increments were still difficult to count on the second tooth, all readers discussed the interpretation and either reached an agreed age or judged the tooth to be unreadable. Individuals for which age could not be estimated reliably were excluded from further analysis. Calves that did not possess a neonatal line in the tooth, or had a neonatal line forming, with no additional postnatal dentine, were classified as newborns.

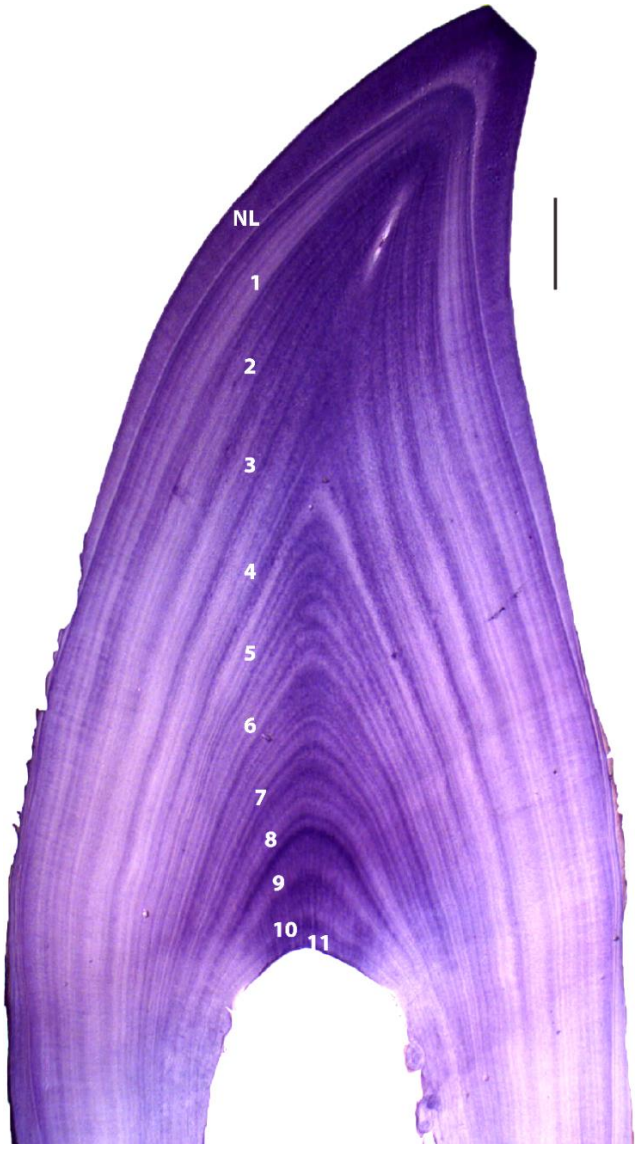


Figure 3.2. Growth layer groups (GLGs) in a male LFPW (GM46) aged at 11 yrs.

NL = neonatal line. Scale bar = 1mm. Note open pulp cavity.

Length-at-birth

The probability of birth (p) as a function of TBL was modelled using Bayesian logistic regression, as follows:

$$y_i \sim \text{Bernoulli}(p_i)$$

$$\log\left(\frac{p_i}{1-p_i}\right) = \alpha + \beta x_i$$

where i indexes individuals, y_i is either 0 (unborn) or 1 (born), x_i gives the lengths of individuals, and α and β are the intercept and slope parameters to be estimated. All Bayesian logistic regression models were fitted using the 'brms' package (Bürkner 2017) for modelling in R (R Development Core Team 2018). Weakly informative prior distributions (Student's $t(3, 0, 10)$) were assumed for α and β. The quantity $x_{50} = -\alpha/\beta$ was defined as the median length-at-birth; i.e., the length at which the probability of birth is 50%. The posterior distribution of this quantity provided a set of plausible values that could then be summarised. This model was fitted to a dataset of (*n* = 338) all foetuses and postnatal whales ≤ 350 cm for which TBL measurements were available, to obtain an estimate of x_{50} pooled across the sexes.

To assess any difference in median length-at-birth (x_{50}) between male and females, three other Bayesian logistic regression models were fitted. For these models, a reduced, balanced dataset was used, which included all foetuses and the 100 smallest post-birth cases for each sex (excluding all individuals of unknown sex). The longer individuals were excluded because they were much larger than the plausible length-at-birth and including them substantially slowed the fitting of the models. In model 1, the sexes had the same intercepts and the same slopes; in model 2, the sexes had different intercepts but the same slopes; in model 3, the sexes had different intercepts and different slopes. The Leave-One-Out Information Criterion (LOOIC; with Pareto-smoothed importance sampling and refitting models for observations with Pareto $k > 0.7$; see the 'loo' package for R; Vehtari et al. 2017, 2018) was used to compare the relative accuracy of out-of-sample predictions of birth status, and compute 'stacking' model weights based on their predictive accuracy (Yao et al. 2018). Model stacking was then used to calculate weighted estimates of x_{50} for males and females, and the difference in x_{50} between the two sexes. This model-stacking approach is an alternative to choosing a single 'best' model, allowing estimation of the difference in birth lengths for males *vs* females while taking into account the uncertainty regarding the utility of the candidate models (Yao et al. 2018).

Posterior distributions for quantities of interest were summarised with means and 95% Highest Posterior Density Intervals (HPDI).

Two additional statistics were calculated to enable comparisons with previously published length-at-birth estimates for the northern subspecies of LFPW (*G. m. melas*):

1. Mean-overlap – the mean of overlapping foetal and calf lengths by including the value of the largest non-overlapping foetus and the smallest non-overlapping calf (Bloch et al. 1993a, Börjesson and Read 2003). The difference in the mean-overlap statistic between males and females was tested using a Student's *t*-test.

2. Mean neonatal length – the mean length of calves that did not possess a neonatal line in the tooth or had a neonatal line forming, with no additional postnatal dentine, i.e. classified as a newborn (Kasuya and Marsh 1984, Bloch et al. 1993a, Murphy et al. 2009).

Growth models

Several growth curves were considered with the primary focus on sex-specific von Bertalanffy and Gompertz models fitted to the age-length data. The von Bertalanffy and Gompertz growth models have been used to model growth in many cetacean species, including pilot whales (Bloch et al. 1993a).

The von Bertalanffy model (von Bertalanffy 1938, Bloch et al. 1993a) is as follows:

$$L_t = A[1 - bexp(-kt)]$$

and the equation for the Gompertz growth model (Laird 1966, Fitzhugh 1976, Bloch et al. 1993a) is:

$$L_t = A\{\exp[-bexp(-kt)]\}$$

where L_t is total body length (TBL) at age (t), A is the asymptotic value, b is the constant of integration, and k is the growth rate constant.

Both these models limit growth to a monotonically decreasing function and cannot represent multiple phases of growth. Using the equations above, two-phase von Bertalanffy and Gompertz growth models (Perrin et al. 1976), were also used to simultaneously fit separate equations to the age-at-length data, using an iterative least-squares method. The two-phase

model was used to account for the secondary growth spurt observed in many delphinids (e.g. Perrin et al. 1976, Murphy et al. 2009, McFee et al. 2010, Jefferson et al. 2012). The intersection point of the two models was estimated as the age at which the total sum of squares for the fit of both models is the smallest (Perrin et al. 1976, Danil and Chivers 2007). Growth curve parameters for the models were estimated using SPSS, version 24 (IBM 2016). The most appropriate model was selected using the Akaike Information Criterion (AIC).

Allometry

To analyse growth patterns, and compare them between the sexes, allometric growth equations for 13 body components were created in the form:

$$y = ax^b$$

where y is the character (dependent variable), x is the TBL (independent variable), b is the growth coefficient, and a is the intercept (Schmidt-Nielsen 1993). Negative allometry is indicated when the growth coefficient is significantly < 1, positive allometry is indicated when the growth coefficient is significantly > 1, and isometric allometry is indicated when the coefficient is not significantly different from 1 (Read and Tolley 1997). To test the null hypothesis H_0: $b = 1$, the test statistic (t_s) was calculated as:

$$t_s = (b - 1)/\mathrm{SE}_b$$

where $b =$ slope, $SE_b =$ standard error of slope, $\mathrm{df} = n - 2$ and $\alpha = 0.05$, using Student's t-test tables. Comparing slope analysis was performed to compare growth coefficient values between male and female pilot whales, $\mathrm{df} = n - 4$ and $\alpha = 0.05$, using Student's t-test tables. Data were used from all physically immature and mature pilot whales and analysed using SPSS, version 24 (IBM 2016). No post-hoc adjustments were made to *p*-values.

Sexual dimorphism

Sexual dimorphism was investigated only in physically mature individuals (defined as TBL ≥ 0.9 × asymptotic length to account for the lack of clear asymptote in the male growth data). Following Murphy and Rogan (2006), dimorphism was measured in two ways: sexual size dimorphism without correcting for body size; and sexual shape dimorphism, to account for variations in body length. Sexual size dimorphism investigates overall variations in body size and differences in shape. Sexual shape dimorphism investigates the differences in shape only, i.e. the relative size of a body part. The relationships between sex and morphometric measurements were explored using charts and Spearman's rank correlation coefficients. All

physically mature male and female morphometric data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) before analysis.

Univariate analysis

Each morphometric measurement was analysed separately for males and females by carrying out Welch's univariate analysis of variance (ANOVA) and univariate analysis of covariance (ANCOVA). Welch's ANOVA was used to investigate size and shape variation between the sexes. Using TBL as the covariate, ANCOVA removed the effect of body size on individual characters and investigated sexual variations of body shape only. All morphometric data were transformed to a logarithmic scale [$\log_{10}(x)$] prior to ANCOVA analysis. The tip of the upper jaw to genital slit measurement (4_Ujaw_genital) was excluded from ANCOVA analysis due to the differing position of the genital slit between the sexes. The length of rostrum measurement (10_Snout_length) was also excluded from ANCOVA as it was not found to have a linear relationship with TBL. No post-hoc adjustments were made to *p*-values.

Multivariate analysis

Sexual dimorphism was also investigated using multivariate analysis. Multivariate analysis accounts for any correlation between dependent variables and is, therefore, more powerful than univariate analysis. However, multivariate analysis is sensitive to missing values. In this study, if individuals were missing measurements for more than five characters, they were eliminated from the dataset. The remaining missing variables were calculated using multiple imputation (linear regression method). The resulting dataset was used to carry out linear discriminant function analysis to investigate sexual dimorphism in body size and shape. Insufficient sample sizes (see Table 3.5 for *n* values) were available for the following characters, and they were omitted from the analysis: length of rostrum (10_Snout_length) and length of genital slit (14_Genital_slit). The tip of upper jaw to genital slit measurement (4_Ujaw_genital) was also excluded from multivariate analysis due to the differing position of the genital slit between the sexes.

3.4 Results

The sample

Total body length (TBL) for the entire data set ranged from 160 to 622 cm (*n* = 1520), with a modal size class of 400 to 449 cm (median 423; Figure 3.3). Where sex was reported, females and males ranged in TBL from 160 to 500 cm (*n* = 776), and from 165 to 622 cm (*n* = 515), respectively. Age was estimated for 384 LFPWs measuring from 176 to 485 cm for females (*n* =

227) and 180 to 622 cm for males (*n* = 154) (Appendix 3D; see Chapter 4 for further information on age structure). Age ranges or a minimum age were obtained from a further 22 whales due to difficulties in counting growth layer groups in their dentine and cementum. Females ranged from 0 to 38 years, and males from 0 to 31 years, with 99% of the sample sexed (Appendix 3D). Of the individuals for which age was estimated, 21 (5%) were younger than 1-year-old; these individuals ranged in TBL from 176 to 222 cm in females and 180 to 250 cm in males, thus highlighting rapid growth in the first year.

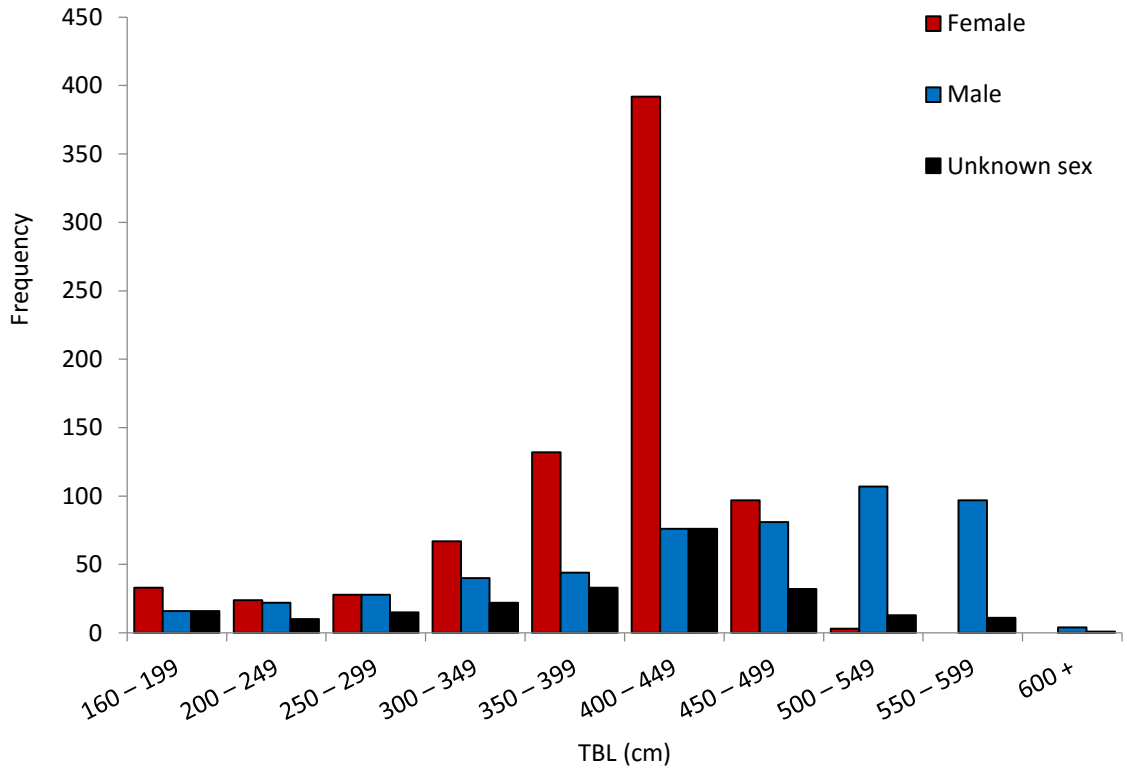


Figure 3.3. Length-frequency distributions for female (*n* = 781), male (*n* = 523) and unknown sex (*n* = 230) LFPWs stranded on the New Zealand coast between 1948 and 2017.

Length-at-birth

A total of 31 foetuses were recorded during post-mortem examinations measuring between 5 and 176 cm TBL. The smallest male and female calves (confirmed live-born via field observations) measured 165 and 160 cm TBL, respectively, and the largest foetuses of both sexes measured 176 cm. Overall, there were seven foetuses and 15 neonates measuring between 160 and 176 cm TBL. The results for each of the statistics used to compare estimated length-at-birth are summarised in Table 3.2.

No difference in the median length-at-birth between males and females was detected. Model 1, which contained no parameters for sex, had the lowest LOOIC score and greatest LOOIC

weight (0.794); models 2 (different intercepts for the sexes) and 3 (different slopes and intercepts for the sexes) had LOOIC weights of 0.216 and 0.000, respectively. Given the lack of sexual dimorphism, the overall median length-at-birth is estimated to be 163.1 cm (95% HPDI = 154.2, 170.5), based on a model that disregarded sex and a dataset that contained all males, females, and sex-unknown cases with length ≤ 350cm (*n* = 338; Figure 3.4).

The additional two statistics: (1) mean-overlap, and (2) mean neonatal length, were calculated for comparison with the northern subspecies (*G. m. melas*) only. Using the mean-overlap statistic, no significant difference was found between the estimated length-at-birth for males and females (*t* = 1.39; *p* = 0.19). The pooled estimate for males and females combined was 171 cm (95% CI = 169 – 173 cm, *n* = 22). Because it is based on naïvely taking the average length from all postnatal and prenatal calves, this method is expected to be very sensitive to having an unbalanced sample. In the current dataset, there were many more postnatal than prenatal measurements available, so the average of these lengths is unsurprisingly much higher than the estimated median length-at-birth obtained from the logistic regression. For the mean neonatal length statistic, the dataset comprised five newborn females and one newborn male with no neonatal line. Given such small sample sizes, only the pooled estimate of average length-at-birth of 182 cm (95% CI = 175 – 188 cm, *n* = 6) was calculated. This method is also expected to overestimate birth length since it includes only postnatal animals. Given the expected upward bias of the additional methods, the Bayesian logistic regression method is preferred for estimating median length-at-birth. This method is able to include both prenatal and postnatal data, and also has the advantage of being able to define any quantity of interest (e.g., x_{50} and the difference in x_{50} between sexes) and summarise the plausible values of such quantities using a posterior distribution.

Growth

Significant postnatal sexual size dimorphism was evident, and therefore separate growth curves were created for males and females, with unsexed individuals omitted from the models. Gompertz and von Bertalanffy equations were used to describe growth in both male and female LFPWs, with the von Bertalanffy model providing a better fit for both males and females (Table 3.3). The points of intersection at the *y*-axis were determined by selecting the curves that best fitted the dataset.

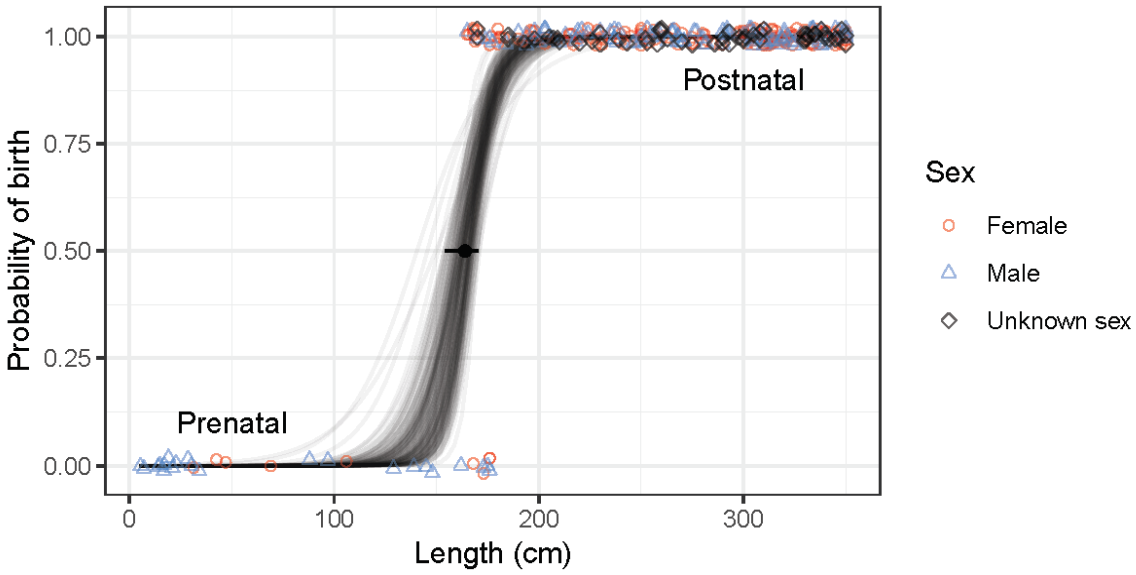


Figure 3.4. The lengths of prenatal and postnatal LFPWs (points), with a posterior sample of 200 logistic curves for the mean probability of birth as a function of length (thin grey lines) using a model that disregarded sex, fitted to *n* = 338 cases.

A small amount of transparency and vertical 'jitter' was added to help visualise overlapping points. The central black point and thin horizontal line show the mean and 95% highest posterior density interval for the estimated median length-at-birth (i.e., the length at which the probability of birth is 50%).

Table 3.2. Length-at-birth (cm) of LFPWs, calculated using three different methods.

	All			**Female**			**Male**		
Method	*n*	$\bar{x}$	95% interval	*n*	$\bar{x}$	95% interval	*n*	$\bar{x}$	95% interval
Logistic regression	**338**	**163**	**154 – 171**	156	164	154 – 171	127	165	154 – 174
Mean overlap	22	171	169 – 173	13	170	168 – 172	7	173	170 – 176
No neonatal line	6	182	175 – 188						

The logistic regression estimate for the full dataset (males, females, unknown sex) is considered the best estimate of median length-at-birth for NZ LFPWs. 95% intervals from the Bayesian logistic regression models were highest posterior density intervals; for the other methods, they are standard 95% confidence intervals.

In the first five years of growth, the TBL of both males and females increases rapidly (Figure 3.5, Figure 3.6). Using the single von Bertalanffy model, females continue grow rapidly until they reach a TBL of 420 cm at approximately 14 years of age, after which the rate of growth slows to less than 3 cm per year and less than 1 cm per year by 22 years and TBL of approximately 434 cm (Figure 3.5, Figure 3.7). The asymptotic value obtained for female TBL was 438 cm, attained at approximately 30 years of age (Table 3.3, Figure 3.5). In this study, physical maturity is considered to be attained once a TBL of 0.9 × the asymptotic length is reached; for females, this is at 394 cm and 10 years.

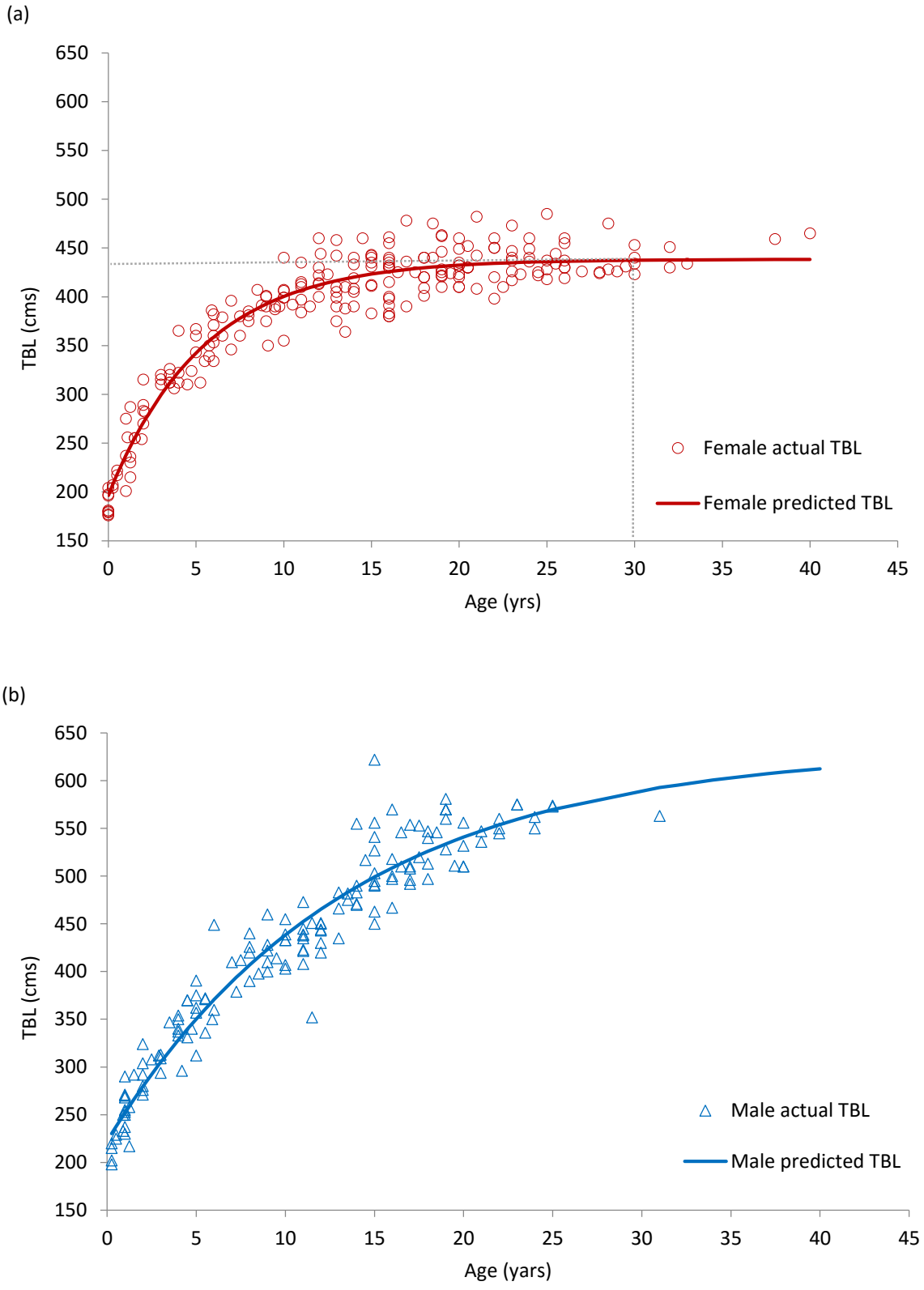


Figure 3.5. Single von Bertalanffy growth curves superimposed on length-at-age data for (a) female (*n* = 227) and (b) male (*n* = 154) LFPWs. Dotted lines indicate estimated TBL and age at attainment of asymptotic length.

Note: Males do not appear to reach a clear asymptote when growth is modelled using a single growth curve.

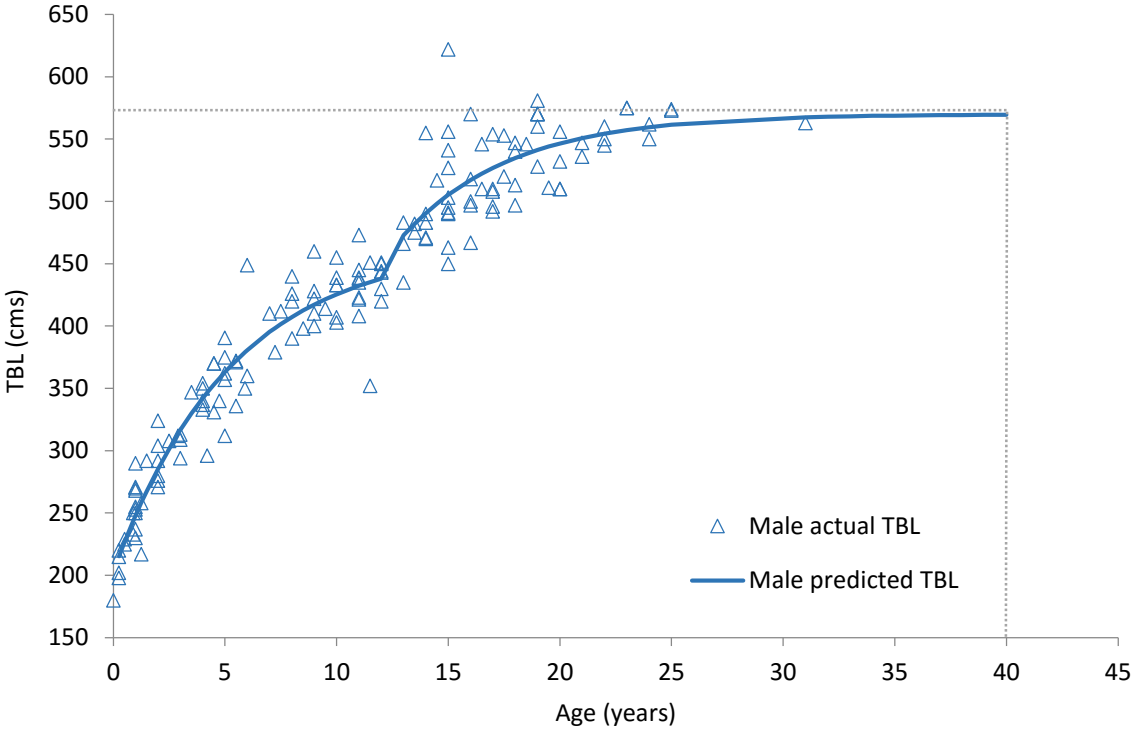


Figure 3.6. Two-phase von Bertalanffy growth curve superimposed on length-at-age data for (b) male LFPWs (*n* = 154). Dotted lines indicate estimated TBL and age at attainment of asymptotic length.

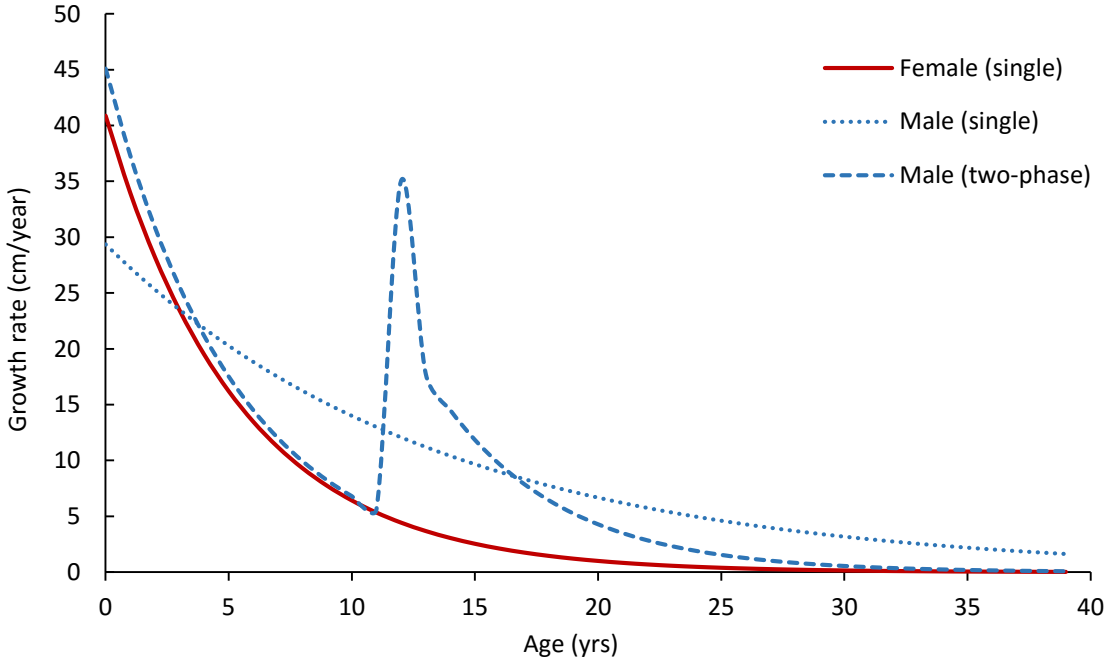


Figure 3.7. Estimated growth rates (cm/year) for male (dashed line, *n* = 154) and female (solid line, *n* = 227) LFPWs as estimated from single (male and female) and two-phase (male) von Bertalanffy growth models.

Note: Secondary growth spurt in males, observed in the two-phase model, estimated to occur at *c*. 13 yrs of age.

Table 3.3. Estimated growth parameters, 95% confidence intervals (95% CI) and Akaike Information Criterion scores (AIC) for the Gompertz and von Bertalanffy growth curves derived from male (M) and female (F) LFPW data.

	A **(95% CI)**		***B*** **(95% CI)**		***k*** **(95% CI)**		**AIC score**	
Model	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Single Gompertz	435.5 (430.9 – 440.2)	602.0 (573.5 – 630.5)	0.76 (0.72 – 0.81)	0.96 (0.91 – 1.01)	0.23 (0.21 – 0.25)	0.11 (0.09 – 0.13)	1759.49	1795.01
Two-phase Gompertz (< 13 yrs)		453.5 (434.5 – 472.5)		0.78 (0.72 – 0.83)		0.25 (0.20 – 0.30)		1770.96
Two-phase Gompertz (> 13 yrs)		569.2 (531.6 – 606.8)		3.05 (-4.53 – 10.63)		0.22 (0.02 – 0.42)		1770.96
Single von Bertalanffy	**438.4 (433.3 – 443.5)**	**633.9 (592.1 – 674.9)**	**0.55 (0.53 – 0.57)**	**0.65 (0.63 – 0.67)**	**0.19 (0.17 – 0.20)**	**0.07 (0.06 – 0.09)**	**1753.69**	**1790.10**
Two-phase von Bertalanffy (< 13 yrs)		**465.2 (440.1 – 490.2)**		**0.56 (0.54 – 0.59)**		**0.19 (0.14 – 0.24)**		**1770.11**
Two-phase von Bertalanffy (> 13 yrs)		**570.0 (530.8 – 609.1)**		**2.38 (-3.41 – 8.17)**		**0.20 (0.01 – 0.40)**		**1770.11**

The single (female) and two-phase (male) von Bertalanffy models are considered to provide the best estimates of asymptotic length for NZ LFPWs.

Following the initial growth spurt, male LFPWs appear to undergo a second growth spurt at around 12 to 13 years and continue to grow for longer than females. For males, a two-phase von Bertalanffy model was used to account for the apparent growth spurt around the age of sexual maturity (13.5 yrs; see Chapter 5). The two-phase model provided a significantly better fit than the single von Bertalanffy model for the male data, though the two-phase Gompertz model also provided a good fit (Table 3.3, Figure 3.6). Using the two-phase von Bertalanffy model, the estimated change point (i.e. growth spurt) for male LFPWs occurred at approximately 13 years and 465 cm TBL. After this point, rapid growth continues until they reach a length of approximately 554 cm at around 22 years of age, followed by slower growth (less than 3 cm/year) until the estimated asymptotic length of 570 cm is reached at approximately 40 years of age (Figure 3.6, Figure 3.7). In this study, male growth did not reach a clear asymptote but continued to increase slowly with age; physical maturity (TBL of 0.9 × asymptotic length) is considered to be attained at 513 cm and 16 years.

Allometry

Significant allometry was observed for 10 out of 13 and 11 out of 13 morphometric measurements in females and males, respectively, which is more than expected to be significant by chance (5%). Nearly all linear body measurements (Ujaw_dorsal, Ujaw_anus, Ujaw_genital, Ujaw_flipper, Ujaw_blowhole, Snout_length, Ujaw_gape, Genital_slit; see Table 3.1) were negatively allometric in both male and female LFPWs, except genital slit length which exhibited isometric growth in females (Table 3.4). The growth coefficients for the length of the genital slit (t = 1.923, df = 110, p = 0.044) provided evidence that females have a higher growth rate for this character than males. The auxiliary girth measurement exhibited isometric growth in males and was negatively allometric in females, although there was no evidence for variation in growth rate between males and females (t = 1.726, df = 341, p = 0.085). Pectoral flipper width was negatively allometric in growth in both sexes. However, pectoral flipper length and fluke width were isometric in females but positively allometric in males. In females, the height of the dorsal fin exhibited negative allometric growth, whereas in males it was isometric in growth. Significant variation was evident between the sexes for five out of 13 body measurements, which is more than expected to be significant by chance (5%). Specifically, sexual variation was evident in the allometry of appendage measurements, with males having a higher growth rate than females (Table 3.4), i.e. pectoral flipper length (t =

2.428, df = 478, p = 0.016), pectoral flipper width (t = 2.272, df = 274, p = 0.024), fluke width (t = 4.248, df = 385, p < 0.001), height of dorsal fin (t = 2.028, df = 279, p = 0.044).

Sexual dimorphism

Table 3.5 shows the mean ($\bar{x}$), standard error (SE) and range for morphometric characters in physically mature (i.e. TBL ≥ 0.9 × asymptotic length) LFPWs. The mean lengths obtained for physically mature males and females were 550 and 432 cm, respectively, giving a sexual size dimorphism (SSD) ratio of 1.27. As observed in Figure 3.8 and Figure 3.9, considerable sexual dimorphism is evident, with males delineated from females by TBL and external flipper length in particular.

Univariate analysis

Sexual size dimorphism was also evident in physically mature LFPWs when tested using Welch's ANOVA. TBL and 12 out of 13 other external measurements exhibited sexual size dimorphism (variation in body size and/or body shape), more than expected to be significant by chance (5%). Male LFPWs were significantly larger in all measurements taken except the length of the genital slit (14_Genital_slit; Table 3.5). However, only three out of 11 measurements (still more than the 5% expected by chance) were found to be sexually shape dimorphic using ANCOVA, with males having considerably longer flippers, wider flukes, and taller dorsal fins than females, irrespective of TBL (Table 3.5).

Multivariate analysis

Linear discriminant analysis was used to examine differences between males and females with respect to a linear combination of 11 morphometric measurements. A single discriminant function accounted for 100% of the sexual dimorphism observed in New Zealand LFPWs (using pooled multiple imputation data: Wilk's λ = 0.110, χ^2 = 371.457, df = 11, canonical correlation = 0.943, p < 0.001). Table 3.5 presents the standardised canonical discriminant function coefficients for the 11 morphometric measurements. Functions at the group centroids were -1.804 for females and 4.421 for males, using pooled multiple imputation data. Reclassification of cases based on the new canonical function was highly successful: 100% of the cases were correctly reclassified into their correct sex category.

Table 3.4. Allometric growth relationships for 13 body measurements regressed against TBL for both female (F) and male (M) LFPWs.

	Female	SE (*b*)	*n*	r^2	*b*	Male	SE (*b*)	*n*	r^2	*b*	F *vs* M
2_Ujaw_dorsal	$y = 1.104x^{0.874}$	0.010	289	0.961	< 1	$y = 1.243x^{0.854}$	0.010	202	0.975	< 1	ns
3_Ujaw_anus	$y = 0.775x^{0.964}$	0.010	198	0.980	< 1	$y = 0.822x^{0.955}$	0.012	128	0.980	< 1	ns
4_Ujaw_genital	$y = 0.782x^{0.946}$	0.012	296	0.951	< 1	$y = 0.781x^{0.933}$	0.015	207	0.951	< 1	ns
5_Ujaw_flipper	$y = 0.971x^{0.703}$	0.014	344	0.884	< 1	$y = 1.031x^{0.692}$	0.015	245	0.901	< 1	ns
6_Ujaw_blowhole	$y = 0.760x^{0.657}$	0.025	281	0.712	< 1	$y = 0.922x^{0.630}$	0.024	211	0.768	< 1	ns
7_Flipper_length	$y = 0.187x^{1.006}$	0.018	291	0.917	ns	$y = 0.130x^{1.071}$	0.020	191	0.937	> 1	F < M
8_Flipper_width	$y = 0.116x^{0.872}$	0.019	160	0.930	< 1	$y = 0.080x^{0.938}$	0.022	118	0.939	< 1	F < M
9_Fluke_width	$y = 0.189x^{1.020}$	0.017	234	0.936	ns	$y = 0.092x^{1.144}$	0.023	155	0.940	> 1	F < M
10_Snout_length	$y = 0.272x^{0.410}$	0.136	139	0.063	< 1	$y = 0.213x^{0.412}$	0.124	75	0.131	< 1	ns
11_Ujaw_gape	$y = 0.474x^{0.691}$	0.041	191	0.599	< 1	$y = 1.133x^{0.546}$	0.035	131	0.657	< 1	ns
12_Height_dorsal	$y = 0.172x^{0.823}$	0.041	168	0.707	< 1	$y = 0.094x^{0.933}$	0.036	115	0.858	ns	F < M
13_Axill_girth	$y = 0.964x^{0.903}$	0.028	207	0.839	< 1	$y = 0.620x^{0.974}$	0.030	138	0.884	ns	ns
14_Genital_slit	$y = 0.099x^{0.977}$	0.103	71	0.566	ns	$y = 0.936x^{0.614}$	0.158	43	0.269	< 1	F > M

Growth patterns have been determined in the form of $y = ax^b$, where x = TBL (cm); y = character (cm); b = growth coefficient; a = intercept. SE = standard error for growth coefficient; n = sample size; r^2 = correlation coefficient; F vs M, comparison of slopes between sexes with TBL as the independent variable; ns = no significant evidence ($p > 0.05$) that $b \neq 1$, or F ≠ M. For explanation of character codes see Table 3.1 and Figure 3.1.

Table 3.5. Mean ($\bar{x}$), standard error (SE), range and sample size (*n*) of 14 morphometric measurements, with results of Welch's ANOVAs, ANCOVAs, and multivariate linear discriminant analysis comparing physically mature male and female LFPW data.

	Female				**Male**						
	$\bar{x}$ **(cm)**	**±SE**	**Range (cm)**	***n***	$\bar{x}$ **(cm)**	**±SE**	**Range (cm)**	***n***	**ANOVA *p***	**ANCOVA *p***	**SCDFC**
1_Total_length (TBL)	431.9	0.9	394 – 500	519	550.0	1.5	513 – 622	188	***	na	0.801
2_Ujaw_dorsal	220.2	0.9	186 – 250	184	270.9	1.7	242 – 300	71	***		-0.178
3_Ujaw_anus	273.4	1.1	246 – 300	119	333.2	2.8	296 – 370	43	***		0.056
4_Ujaw_genital	240.2	1.0	203 – 285	186	272.8	2.1	246 – 330	64	***	na	na
5_Ujaw_flipper	68.1	0.3	55 – 83	216	78.5	0.7	66 – 99	80	***		-0.026
6_Ujaw_blowhole	40.2	0.3	31 – 54	176	47.2	0.6	36 – 60	68	***		0.109
7_Flipper_length	85.5	0.5	70 – 107	183	113.7	1.1	85 – 132	67	***	***	0.275
8_Flipper_width	23.1	0.2	19 – 28	87	30.1	0.5	25 – 36	38	***		-0.041
9_Fluke_width	92.2	0.6	70 – 108	139	125	1.5	94 – 150	53	***	*	0.161
10_Snout_length	3.9	0.2	1 – 10	80	3.1	0.3	1 – 6	26	**	na	na
11_Ujaw_gape	30.4	0.4	19 – 38	113	34.7	0.7	26 – 42	48	***		-0.040
12_Height_dorsal	25.4	0.4	16 – 33	91	34.6	0.8	22 – 47	38	***	**	0.127
13_Axill_girth	230.3	2.6	188 – 320	125	285.2	5.5	216 – 360	44	***		-0.032
14_Genital_slit	37.4	1.6	19 - 62.5	37	42.1	4.4	22 – 75	14			na

SCDFC = standardised canonical discriminant function coefficients, na = not analysed, *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. M > F for all sexually dimorphic characters. For explanation of character codes see Table 3.1 and Figure 3.1.

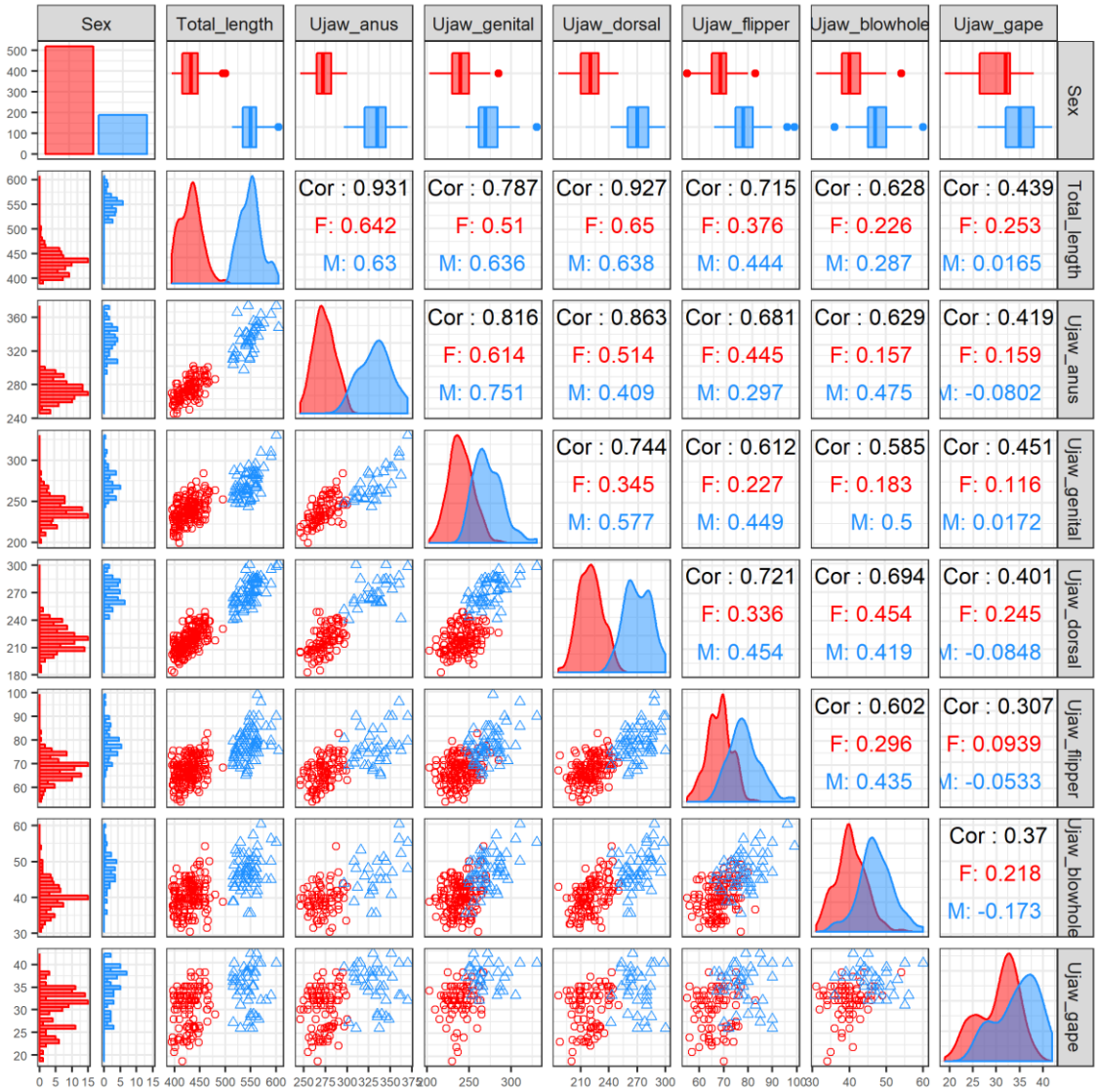


Figure 3.8. Sex versus seven linear body measurements of New Zealand LFPWs.

TBL (male: *n* = 188, female *n* = 519), Ujaw_anus (male: *n* = 43, female *n* = 119), Ujaw_genital (male: *n* = 64, female *n* = 186), Ujaw_dorsal (male: *n* = 71, female *n* = 184), Ujaw_flipper (male: *n* = 80, female *n* = 216), Ujaw_blowhole (male: *n* = 68, female *n* = 176), and Ujaw_gape (male: *n* = 48, female *n* = 113). Colours represent the individuals' sex: female = red, male = blue. Cor = Spearman's rank correlation coefficients. All measurements in cm.

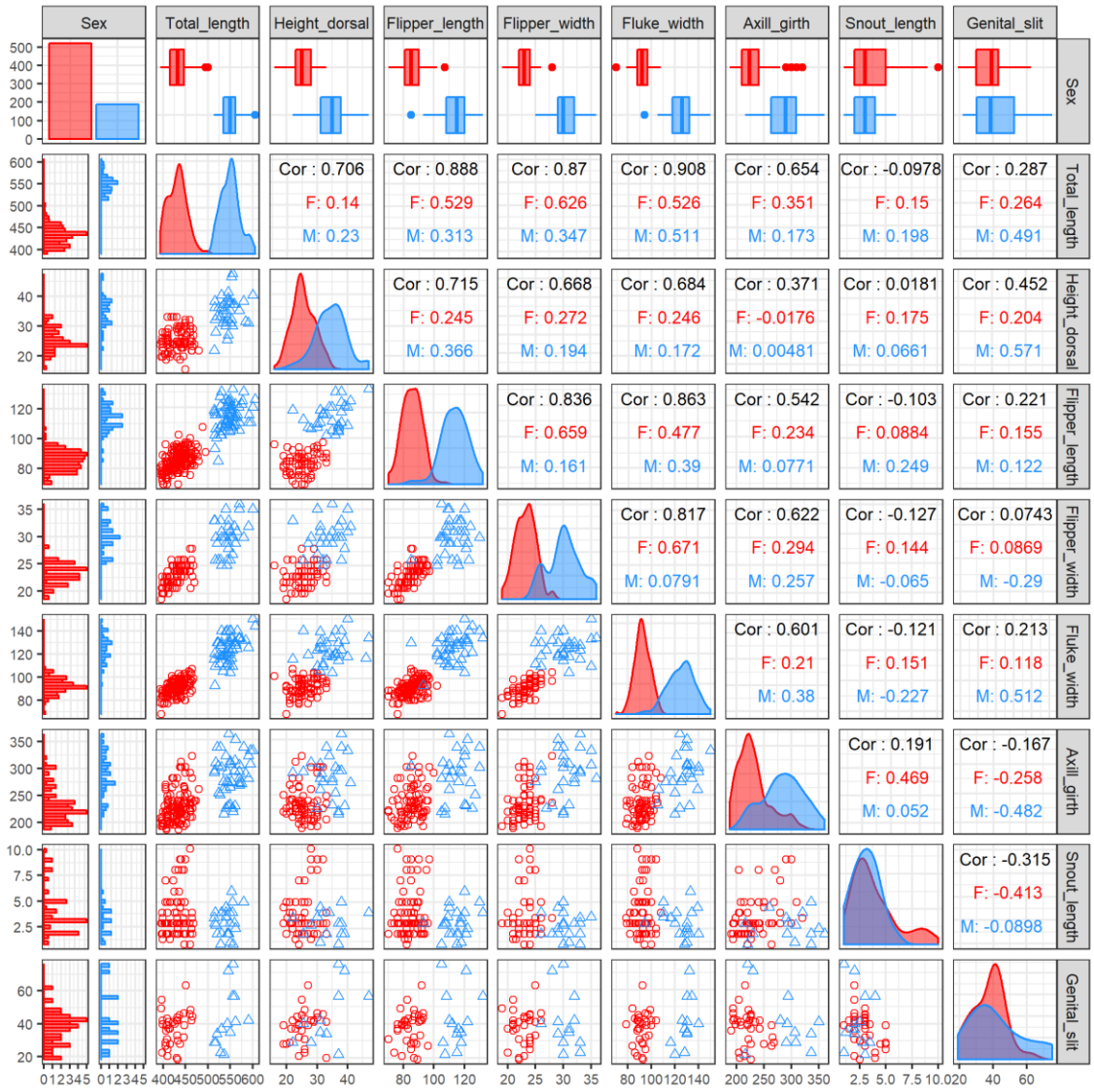


Figure 3.9. Sex versus TBL, four appendage measurements, axillary girth, snout and genital slit length of New Zealand LFPWs.

TBL (male: *n* = 188, female *n* = 519), Height_dorsal (male: *n* = 31, female *n* = 91), Flipper_length (male: *n* = 67, female *n* = 183), Flipper_width (male: *n* = 38, female *n* = 87), Fluke_width (male: *n* = 53, female *n* = 139), Axill_girth (male: *n* = 44, female *n* = 125), Snout_length (male: *n* = 26, female *n* = 80), and Genital_slit (male: *n* = 14, female *n* = 37) Colours represent the individuals' sex: female = red, male = blue. Cor = Spearman's rank correlation coefficients. All measurements in cm.

3.5 Discussion

Age estimation

This study presents the maximum recorded ages for *G. m. edwardii* in New Zealand waters as 31 years for males and 38 years for females. These maximum ages are considerably lower than those recorded for *G. m. melas*, sampled from drive fisheries in both Newfoundland (male: 35 yrs, female: 56 yrs; Sergeant 1962a, Kasuya et al. 1988b) and the Faroe Islands (male: 46 yrs, female: 59 yrs; Bloch et al. 1993a; Table 3.6), and also those reported from SFPWs captured in the Japanese fishery (male: 46 yrs, female: 64.5 yrs; Kasuya and Marsh 1984, Kasuya and Matsui 1984, Kasuya and Tai 1993). However, maximum ages reported in studies of stranded *G. m. melas* in the North Atlantic (male and female: 34 yrs; Martin et al. 1987, Sigurjonsson et al. 1993) are more in line with estimates for the stranded *G. m. edwardii* reported in the current study. These differences in maximum ages between the present and past studies could be explained in any one or more of three ways: (1) older animals are present in the Southern Hemisphere subspecies under study here, but they are less likely to strand *en masse,* or their carcasses were not recovered or aged, (2) errors in age estimation resulting in an underestimate of the true age of the stranded animals, or (3) species, subspecies and population-level differences in pilot whale longevity.

Firstly, lower estimates of LFPW longevity from stranding-based studies (in both the North Atlantic, and the Southern Hemisphere; Table 3.6) could reflect the fact that they were based on smaller sample sizes than drive fishery-based studies, and may only represent sub-groups rather than the entire pod, resulting in older individuals being missed by chance. It is also possible that older individuals are less likely to strand *en masse*, and/or more likely to survive stranding events so were therefore not sampled. In this study, age and TBL were not always determined for all individuals in large MSEs. Therefore, it is possible that some older animals were missed in the sampling process. While this factor cannot be ignored, this study is based on a large, minimally biased sample (i.e. particular ontogenetic groups were not favoured, except two MSEs where adult males were targeted for gonadal sampling; see Chapter 5) that likely reflects the true age distribution of the New Zealand population.

Secondly, the lower maximum ages reported in this study (and other stranding-based studies, e.g. Martin et al. 1987, Sigurjonsson et al. 1993), compared to those reported in LFPWs sampled in Newfoundland (Kasuya et al. 1988b) and the Faroe Islands (Bloch et al. 1993a), may reflect the fact that animals were not aged using cemental layers in the current study. Lockyer

et al. (1987) and Kasuya et al. (1988b) found a strong correlation in dentinal and cemental GLGs from the teeth of LFPWs up to around 14 years of age in individuals with open or closing pulp cavities. Beyond this age, the correlation was substantially weakened, with the number of cemental layers often greater than those observed in the dentine when the pulp cavity was closed/occluded (see Figure 1 in Kasuya et al. 1988b). In the current study, only growth layer counts from the dentine were used for age estimation as the readability of the cemental layers was considered inferior, and this was also reported for SFPWs (Kasuya 2017). Although the pulp cavities were undoubtedly still open in the oldest animals sampled in the current study (see Appendix 3C), it was not possible to prove or disprove that readable dentine is still being deposited in the oldest animals in the New Zealand sample. The close similarity between the growth curves derived by Kasuya et al. (1988b) and Bloch et al. (1993a), based on dentinal and cemental layers, and those based only on dentinal counts in this study (Figure 3.5) suggests that the latter age estimates are reliable. Nevertheless, given the degree of uncertainty of age estimates for teeth where the pulp cavity is almost occluded, it is perhaps sensible to follow Martin et al. (1987) in considering age estimates for older animals (> 20 yrs) as minima.

Finally, while acknowledging potential caveats associated with sampling from strandings vs. drive fisheries, and age estimation using dentinal vs. cemental growth layers, ages of the oldest *G. m. edwardii* sampled to date were younger than those of *G. m. melas* by 21 years in females or by 15 years in males (see Table 3.6). The potential difference in longevity between the two subspecies is supported by other estimates of life history parameters, where *G. m. edwardii* attains sexual maturity at a younger age and smaller body size (see Chapters 5 and 6) and could exhibit a higher natural mortality rate (see Chapter 4).

Length-at-birth

Methods previously used to estimate length-at-birth were applied in this study to allow comparisons with previous studies of *G. m. melas* in the North Atlantic. Using a comparable method to our preferred (logistic regression) method, Bloch et al. (1993a) reported a considerably larger length-at-birth (177 cm) for *G. m. melas* off the Faroe Islands than estimated for LFPWs off New Zealand (*G. m. edwardii*; 163 cm) in this study. The mean-overlap statistic was also applied to *G. m. melas* by Bloch et al. (1993a) off the Faroe Islands, and Sergeant (1962a) off Newfoundland, with both studies again estimating larger length-at-birth for the northern subspecies than those reported for the southern subspecies in this study (Newfoundland: 178 cm males [59 foetuses: 31 calves] and 174 cm females [49 foetuses: 43 calves], Faroe Islands: 177 cm males and females combined [49 foetuses: 39 calves], New Zealand: 171 males and females combined [7 foetuses: 15 calves]).

Table 3.6. TBL and age data available for LFPWs from various geographical areas.

		Globicephala melas melas				*Globicephala melas edwardii*	
Location		Britain[1]	Faroe Islands[2,3]	Iceland[4]	Newfoundland[5,6]	Argentina[7, 8]	New Zealand[9]
Source		stranding	drive fishery	stranding	drive fishery	stranding	stranding
Sampling period		1982 – 1985	1986 – 1992	1982 – 1986	1951 – 1959	1982, 2009	1948 – 2017
Average length-at-birth (cm)			177[a] (*n* = 143)		M: 178[b] (*n* = 59) F: 174[b] (*n* = 49)		163[a] (*n* = 338)
Asymptotic length (cm)	M	550 – 600[c] (*n* = 21)	580[d] (*n* = 965)		557[e] (*n* = 5)		570[g] (*n* = 154)
	F	400 – 450[c] (*n* = 31)	445[d] (*n* = 1,478)		489[e] (*n* = 53)	441[f] (*n* = 31)	438[f] (*n* = 227)
Age at asymptotic length (yrs)	M	> 20[h] (*n* = 21)	> 46 (*n* = 965)		21 – 25[i] (*n* = 152)		40 (*n* = 154)
	F	> 20[h] (*n* = 31)	32 (*n* = 1,478)		21 – 25[i] (*n* = 275)		30 (*n* = 227)
Maximum length (cm)	M	630	625 (*n* = 1,190)	595 (*n* = 55)	617 (*n* > 1,275)	538 (*n* = 7)	622 (*n* = 515)
	F	546	512 (*n* = 1,635)	475 (*n* = 119)	511 (*n* > 1,951)	483 (*n* = 62)	500 (*n* = 776)
Maximum age (yrs)	M	20[h] (*n* = 21)	46 (*n* = 967)	34 (*n* = 38)	35.5[i] (*n* = 153)	16 (*n* = 5)	31 (*n* = 154)
	F	25[h] (*n* = 31)	59 (*n* = 1,482)	34 (*n* = 92)	56.5[i] (*n* = 284)	35 (*n* = 40)	38 (*n* = 227)

[a] length-at-birth estimated by logistic regression; [b] length-at-birth estimated as mean of overlapping foetus and neonate TBL; [c] asymptotic length estimated from length frequency distribution; [d] asymptotic length estimated using a single Gompertz growth model; [e] asymptotic length estimated as mean TBL of individuals > 25 yrs; [f] asymptotic length estimated using a single von Bertalanffy growth model; [g] asymptotic length estimated using a two-phase von Bertalanffy growth model; [h] age estimated using less reliable method: acid etching; [i] age estimated using less reliable method: transverse tooth sections. Sources: [1]Martin et al. (1987), [2]Bloch et al. (1993a), [3]Lockyer (1993a), [4]Sigurjonsson et al. (1993), [5]Sergeant (1962a), [6]Kasuya et al. (1988b), [7]Crespo et al. (1985), [8] Soto et al. (2017), [9]this study.

The overlap in lengths between the largest foetus (176 cm) and the smallest newborn calf (160 cm) in the current study is less than that found for *G. m. melas* off Newfoundland (190 and 165 cm; Sergeant 1962) and the Faroe Islands (191 and 163 cm; Bloch 1993a). No foetuses measured over 176 cm in the New Zealand data, which in turn resulted in a lower estimated length-at-birth using both the logistic regression and the mean-overlap statistic. The smaller length-at-birth obtained in this study may be due to a lack of sampling of larger sized foetuses, though it seems unlikely near-term foetuses would be missed given the estimated peak calving period (i.e. early austral summer, see Chapter 6) coincides with the peak stranding season (i.e. late austral spring through austral summer, see Chapter 7). Therefore, the smaller estimated length-at-birth for *G. m. edwardii* may represent true morphological variations between the northern and southern subspecies of LFPW.

The mean length of calves that do not possess a neonatal line in the tooth, or have a neonatal line forming (mean neonatal length), should not be considered an estimate of length-at–birth; it is unavoidably upwardly biased since only postnatal calves are considered. The fact that this method returned the highest estimates in this study (182 cm) and in the Faroe Islands study (200 cm) indicates that the neonatal line is not formed exactly at birth, but several weeks or perhaps months later, as previously suggested for *G. m. melas* by Bloch et al. (1993a) and also for SFPWs by (Kasuya and Matsui 1984).

Growth

Although the von Bertalanffy growth model was preferred in this study, due to slightly lower AIC scores for both males and females, the Gompertz growth model is also considered adequate for describing growth in this species (and is the preferred growth model for *G. m. melas*; Bloch et al. 1993a). Growth models for *G. m. edwardii* indicated an early period of rapid growth, followed by a decrease in growth velocity and a period of sustained but slower growth in both sexes. In males, there also appears to be a secondary growth spurt at around the age of sexual maturity (ASM = 13.5 yrs; see Chapter 5), which is followed by a period of slower growth but with no clear asymptote reached by the model (Figure 3.6) due to the low number of older males sampled. Table 3.6 presents a review of published data on geographical variations in the predicted asymptotic TBL value, estimated age at attainment of asymptotic length, and maximum TBLs recorded for LFPWs. Estimates of asymptotic and maximum lengths of LFPWs in the current study are similar to those previously reported for both *G. m. edwardii* and *G. m. melas* elsewhere; with the exception of a longer estimated asymptotic length for female *G. m. melas* off Newfoundland (which was not estimated using a comparable modelling

approach) and a much longer maximum female body length recorded for LFPWs off the British coast (see Table 3.6).

Although most mammals have determinate growth, and the same was assumed for marine mammals, LFPWs off New Zealand appear to continue to grow, albeit at a much-reduced rate, until well into old age (*c.* 30 yrs for females and ≥ 40 yrs for males). The lack of clear plateaus produced by the models, particularly in males, may be an artefact of the small numbers of males older than 20 years of age (*n* = 12) and females older than 30 years (*n* = 4) in the New Zealand sample. Previous growth curves constructed from studies of LFPWs taken in drive fisheries off the Faroe Islands (Bloch et al. 1993a) and stranded on the coasts of Iceland (Sigurjonsson et al. 1993) and Britain (Martin et al. 1987) also indicate that these animals, and males in particular, may continue growing beyond the predicted asymptotic lengths. Physical maturity in *G. m. melas* off the Faroe Islands, determined from vertebral epiphyseal fusion, was reported to be reached at the age of 25 to 30 years for males and *c.* 30 years for females (Bloch et al. 1993a). However, while some *G. m. melas* were found to be physically mature at 25 to 30 years; others were still growing, resulting in the apparent protracted growth pattern (Bloch et al. 1993a) that was also observed in *G. m. edwardii* in the current study (particularly pronounced for males).

In this study, male *G. m. edwardii* attained greater asymptotic TBL than females as a result of a secondary growth spurt and a more prolonged period of growth. The presence of a such a growth spurt confined to males has been reported in other delphinid species including common bottlenose dolphins (*Tursiops truncatus*; Cheal and Gales 1992) and estuarine dolphins (*Sotalia guianensis*; Rosas et al. 2003) and was previously suggested for *G. m. melas* by Kasuya et al. (1988b). Size differences between males and females in the current study, and an extended period of male growth, may be a result of several factors including differences in: (1) reproductive strategies (Read et al. 1993), (2) foraging ecology (Cockcroft and Ross 1990), and (3) resource partitioning (Bernard and Hohn 1989). Female LFPWs have been documented to attain sexual maturity much earlier than males (see Chapters 5 and 6, Desportes et al. 1993b, Martin and Rothery 1993), and it has been suggested that as females reach sexual maturity (ASM = 6.7 yrs for *G. m. edwardii* off New Zealand; see Chapter 6), considerable energy is diverted from growth in size to reproduction (i.e. gestation and lactation; Reynolds et al. 2000). In contrast, male growth velocity spikes around the age of sexual maturation (ASM = 13.5 yrs for *G. m. edwardii* off New Zealand; see Chapter 5), and continues to surpass females after attaining sexual maturity, possibly to increase body size for investment in greater social activity (e.g. physical competition; Bryden 1986).

The body growth equations presented in this study are important for two reasons. Firstly, they document detailed growth curves for *G. m. edwardii* for the first time, and secondly, they can provide population parameters by which temporal changes in the population of LFPWs in the New Zealand region can be assessed. In other marine mammal populations, increases in individual growth rates have been attributed to greater prey availability per capita associated with declines in population sizes (e.g. Lockyer 1978, Hanks 1981, Lockyer 1981, Kasuya 1991, Trites and Bigg 1992). Therefore, if the demographic parameters of this species change, the results of the study can be used as a baseline for future assessments of the condition of the population, as slower body growth rates may reflect poorer condition of individuals (Hanks 1981).

Allometry and sexual dimorphism

Sexual size dimorphism was evident in the current study, with male *G. m. edwardii* significantly larger than females in TBL, and in 12 of the additional 13 characters measured. Genital slit length was the only measurement for which female *G. m. edwardii* exhibited a higher growth rate than males. However, this measurement is not comparable between the sexes due to the sexual variation in the position of the genitals. The mean TBLs obtained for physically mature (i.e. TBL above 0.9 × estimated asymptotic length) males and females were 550 and 432 cm, respectively, giving an SSD ratio of 1.27. A comparable SSD ratio of 1.27 can be calculated from published lengths at attainment of physically maturity (as assessed via vertebral fusion) in male (570 cm) and female (450 cm) *G. m. melas* (Bloch et al. 1993a). The extent of sexual size dimorphism varies widely among the odontocetes, with males larger than females in many species, but with some of the most pronounced sexual size dimorphism found in sperm whales, narwhal (*Monodon monoceros*), beluga (*Delphinapterus leucas*), killer whales, and pilot whales (reviewed by Dines et al. 2015). The degree of sexual dimorphism could be related to several biological factors including behaviour, social structure, mating system, the sex ratio of the breeding population, and/or environmental factors such as habitat, distribution, diet and prey abundance (Murphy and Rogan 2006).

Aside from the pronounced sexual dimorphism in TBL, evidence of sexual shape dimorphism in the length of the pectoral flippers, the width of the tail flukes, and height of the dorsal fin were also found in *G. m. edwardii*, with males having longer flippers, wider flukes and taller dorsal fins than females of similar body lengths. Males also exhibited higher growth rates than females in all appendage characters measured (i.e. pectoral flipper length and width, fluke width and dorsal fin height); an increase which could have occurred during the growth spurt around the onset of sexual maturity in males (see above). Among odontocetes, there is

considerable diversity in sexual shape dimorphism, including differences in the size and shape of the dorsal fin, flukes, flippers, and other external structures such as postanal humps (Mesnick and Ralls 2018). Adult males have longer and broader flukes and flippers in some species, which may function to give more propulsion (Mesnick and Ralls 2018), and dorsal fins of adult males are particularly exaggerated in some species, e.g. killer whales (Clark and Odell 1999) and SFPWs (Kasuya 2017). The significance of sexual differences in dorsal fin shape and size is not well understood, but they may serve a thermoregulatory function and/or a visual signal in mating interactions (Mesnick and Ralls 2018). Among the many forms of killer whales, there are differences in the relative degree of both sexual size dimorphism and sexual shape dimorphism of the dorsal fin (e.g. Durban et al. 2017). Similar differences appear to occur between the two LFPW subspecies, with sexual shape dimorphism of the flippers and flukes evident in both subspecies (Bloch et al. 1993b), but dorsal fin height only apparent in *G. m. edwardii*. Such differences are likely due to variation in ecology and sociality among subspecies, populations, or forms (Mesnick and Ralls 2018), though this has not been tested.

3.6 Conclusions

The current study has provided the first detailed descriptions of growth, allometry and sexual dimorphism of *G. m. edwardii*, using data collected from MSEs on the New Zealand coast. Age-related changes in growth rates between male and female LFPWs and strong evidence of sexual size dimorphism are demonstrated, with males attaining a larger body size than females. Sexual shape dimorphism was also evident in pectoral flipper length, fluke width, and dorsal fin height, with males having considerably longer flippers, wider flukes, and taller dorsal fins than females. Estimated length-at-birth and maximum ages for *G. m. edwardii* are considerably lower than previously reported for *G. m. melas*, which may indicate subspecies or population-level differences in morphology, longevity, and sociality. Through providing new insights into the life history of LFPWs, the results of this study may help inform future conservation management of the species in the Southern Hemisphere.

Chapter 4

Age structure, survival and mortality rates of long-finned pilot whales (*Globicephala melas edwardii*) stranded on the New Zealand coast

Long-finned pilot whales mass stranded on West Ruggedy Beach, Stewart Island, New Zealand, February 2010.

Photograph credit: New Zealand Department of Conservation.

In this chapter, data on the age structure and models of survivorship and mortality of long-finned pilot whales (*Globicephala melas edwardii*) stranded on the New Zealand coast are provided, to achieve the second research objective:

Objective 2: Examine the age structure and construct age and sex-specific life tables, survivorship curves, and mortality schedules for *G. m. edwardii* in New Zealand waters, using age-at-death data from stranded animals.

This chapter is a reformatted version of the following manuscript:

Betty EL, et al. (in prep). Age structure, survival and mortality rates of long-finned pilot whales (*Globicephala melas edwardii*) estimated from New Zealand mass stranding data. Marine Mammal Science.

4.1 Abstract

Life tables, and the survivorship and mortality curves derived from them, have proven to be powerful tools for conservation. In this study, data gathered from 227 female and 154 male long-finned pilot whales (LFPWs; *Globicephala melas edwardii*) that stranded on the New Zealand coast were used to construct the first age and sex-specific life tables for *G. m. edwardii*. Survivorship curves were fitted to the data using (1) a traditional maximum likelihood approach, and (2) Siler's competing-risk model. Life table construction and subsequent survival and mortality curves revealed distinct differences in the age and sex-specific survival and mortality rates, with females outliving males. Both sexes revealed slightly elevated rates of mortality among the youngest age classes (< 2 yrs) with mortality rates decreasing and remaining relatively low until the average life expectancy is reached; 11.3 years for males and 14.7 years for females. Overall (total) mortality is estimated to be 8.8% and 6.8% per annum for males and females, respectively. The mortality curve resembles that of other large mammals, with high calf mortality and an exponentially increasing risk of senescent mortality. An accelerated mortality rate was observed in mature female LFPWs, which is in contrast to the closely related short-finned pilot whale (SFPW; *G. macrorhynchus*) that selects for an extension to the post-reproductive life span. The reason for the observed differences in the mortality rate acceleration between the two pilot whale species has not been established and warrants further investigation. Life table data, when collected in a standardised and comprehensive manner, offer an ability to assess changes in population parameters over time, providing essential information for conservation management.

4.2 Introduction

Models of population viability are fundamentally based on age structure (Caughley 1977, Barlow and Boveng 1991). Determining the age structure of a population is, therefore, the essential first step when studying population dynamics (Evans and Hindell 2004). Further, the parameters that have been interpreted to reflect significant changes to population abundance or resource availability are those specific to age, e.g. age at sexual maturation, age-specific fecundity rates, and growth parameters of individuals in the population (Caughley 1977, Evans and Hindell 2004). Additionally, determining age-at-death of individuals removed by anthropogenic activities (e.g. drive fisheries or fisheries bycatch), or during mass stranding events (MSEs), is crucial not only for understanding the dynamics of a population, but also for determining if particular age groups are more or less at risk. In cetaceans, changes at the population level can occur due to threats such as by-catch, directed fisheries, competition with fisheries, disease, habitat modification, mass mortality events (e.g. MSEs) and predation, and populations can vary in their ability to recover (Bearzi et al. 2003, Murphy and Rogan 2006, Currey et al. 2009).

Life tables and survivorship curves have proven to be powerful conservation tools when combined with models that predict the susceptibility of certain age classes to anthropogenic impacts (e.g. Crouse et al. 1987, Moore and Read 2008). Survival and mortality rates can be inferred directly by following one or more cohorts through time, or indirectly from analysis of age distribution of live (Caughley 1966, Barlow and Boveng 1991) or dead (Caughley 1966, Stolen and Barlow 2003) individuals. All methods involve assumptions that are unlikely to be fully satisfied but are often estimated well enough for practical purposes (Stolen and Barlow 2003). Life tables based on age-at-death data have been presented for several species of large terrestrial mammals (e.g. Caughley 1966, Laws 1968, Spinage 1972); however, published examples of age-structured life tables for marine mammals are rare (Barlow and Boveng 1991, Stolen and Barlow 2003).

The most accessible source of information about the population structure of most cetaceans is in the stranding record (Saavedra 2018). The long-finned pilot whale (LFPW; *Globicephala melas edwardii*) is regularly observed within New Zealand waters, and also frequently mass strands in high numbers on New Zealand (Brabyn 1991; see Chapter 7) and Australian coasts (Evans et al. 2005, Gales et al. 2012, Beasley et al. 2019). The majority of animals involved in MSEs often die *in situ* or very close to the original stranding site. Given that the mass stranded pods of LFPWs have been found to reflect the age and sex composition of entire pods driven

ashore by fisheries (Sergeant 1962a), and there is usually no reason to suspect that mass stranded pods are unrepresentative of the population (Martin et al. 1987), MSEs provide a valuable opportunity to study the demography of the species in New Zealand waters. The current study investigated the age structure of *G. m. edwardii* stranded on the New Zealand coast between 2006 and 2017. Specifically, this study presents age and sex-specific (1) life tables, (2) survivorship curves, and (3) mortality schedules for *G. m. edwardii* in New Zealand waters, using age-at-death data from stranded animals.

4.3 Materials and methods

Age estimation

Teeth from 405 whales (239 females, 163 males and three of unknown sex) stranded on the New Zealand coast between 2006 and 2017 were collected for age estimation purposes. Age estimation was performed by counting annual growth layer groups (GLGs) in decalcified and stained longitudinal sections of teeth, as described by Perrin and Myrick (1980). Tooth preparation methods for this study were adapted from Lockyer (1993a), and all sections were read by at least two individuals; for further explanation see Chapter 3. Individuals for which age could not be estimated reliably were excluded from further analysis. Calves that did not possess a neonatal line in the tooth, or had a neonatal line forming, with no additional postnatal dentine, were classified as newborns. Individuals were considered immature if they were aged younger than the estimated age at attainment of sexual maturity (ASM), or mature if they were older than or equal to the estimated ASM, i.e. 13.5 and 6.7 years for males and females respectively (see Chapters 5 and 6).

Life tables, survivorship, and mortality rates

Life tables, including age-specific survivorship and mortality rates for both male and female LFPWs, were constructed using two approaches: (1) following the traditional approach as described by Caughley (1966) and Krebs (1989) and (2) fitting the Siler competing-risk model (Siler 1979, Barlow and Boveng 1991, Bloch et al. 1993a, Stolen and Barlow 2003, Moore and Read 2008) to smooth the age-at-death data. Traditional life tables have previously been applied to stranded cetaceans (e.g. Stolen and Barlow 2003, Evans and Hindell 2004, Murphy et al. 2007) where theoretical populations are constructed with corresponding abundance by age. Vectors of age-specific survival and mortality are then estimated from this population structure where $n_x =$ the number of individuals alive at age x; $d_x =$ the number of individuals dying during the age interval x to $x + 1$; $l_x =$ the proportion of the animals surviving to the start of age x; $q_x =$ the proportion of animals alive at age x that die before

age $x+1$ (i.e. mortality rate); $e_x =$ average (remaining) life expectancy for individuals at age x; $\sum d_x / \sum l_x =$ overall (total) annual average mortality rate.

Mortality and survival rates directly derived from observational age-at-death data are generally imprecise and may be biased (e.g. underrepresentation of young ages) and therefore model-based estimates are preferred. In this study, the Siler model was used to smooth the age-at-death data, and avoid violating the requirements of a vertical life table (i.e. that the frequency of each age class x is equal to or greater than age class $x+1$), because it adequately fits expected mortality patterns for a wide range of long-lived species such as marine mammals (Siler 1979, Barlow and Boveng 1991, Stolen and Barlow 2003). In the Siler model, survivorship at a given age $l(x)$ is expressed as the product of three competing risks:

$$l(x) = l_j(x).\, l_c(x).\, l_s(x)$$

where $l_j(x) = \exp\{(a_1/b_1)[1-\exp(-b_1 x)]\}$ is an exponentially decreasing risk due to juvenile risk factors, $l_c(x) = \exp\{-a_2.x\}$ represents a constant risk experienced by all age classes, $l_s(x) = \exp\{(a_3/b_3)[1-\exp(-b_3 x)]\}$ is the exponentially increasing risk due to senescence, x is a given age and a_n and b_n are the Siler parameters. The total mortality at a given age $\mu(x)$ is the sum of the juvenile mortality $\mu_j(x)$, the constant mortality affecting all age classes $\mu_c(x)$, and the senescent mortality $\mu_s(x)$:

$$\mu(x) = \mu_j(x) + \mu_c(x) + \mu_s(x)$$

Total mortality can be calculated using the Siler parameters (a_1, b_1, a_2, a_3, b_3) as follows:

$$\mu(x) = a_1 \exp(-b_1 x) + a_2 + a_3 \exp(b_3 x)$$

The above equation describes the general shape of the mortality curve using five parameters that account for initially increasing (and subsequently decreasing) risk of an individual dying at the beginning of life, a constant risk through life, and increased risk due to senescence. The competing-risk Siler model was fitted to the LFPW age-at-death data using the Nelder and Mead (1965) optimisation method implemented in the "strandCet" package (Saavedra 2018) in R (R Development Core Team 2018). The life table calculations for both the Siler and traditional methods were constructed using estimated ages and are based on a hypothetical cohort of 1000 LFPWs. In order to construct these life tables, it was assumed that (1) MSEs of LFPWs on the New Zealand coast representative of the population, (2) carcass recovery and tooth collection from MSEs were independent of the age and sex, and (3) the population has a

stable age distribution and a zero growth rate (referred to as a stationary age distribution; Caughley 1966).

4.4 Results

Age structure

Age was estimated for 227 females and 154 male LFPWs (Figure 4.1) stranded in 14 independent events (Figure 4.2). Age ranges or a minimum age were obtained from a further 22 whales due to difficulties in counting GLGs in their dentine and cementum; these individuals were not included in the subsequent life table construction. Females ranged from 0 to 38 years and males from 0 to 31 years (Figure 4.1). The aged male sample was composed of younger individuals than the female sample, with 28% of males younger than five years and 90% younger than 20 years, compared with 18% of females younger than five years and 71% younger than 20 years. Overall, there was a lack of males older than 20 years in the dataset. For further assessment of the age-sex class composition of LFPWs stranded on the New Zealand coast, see Chapter 7.

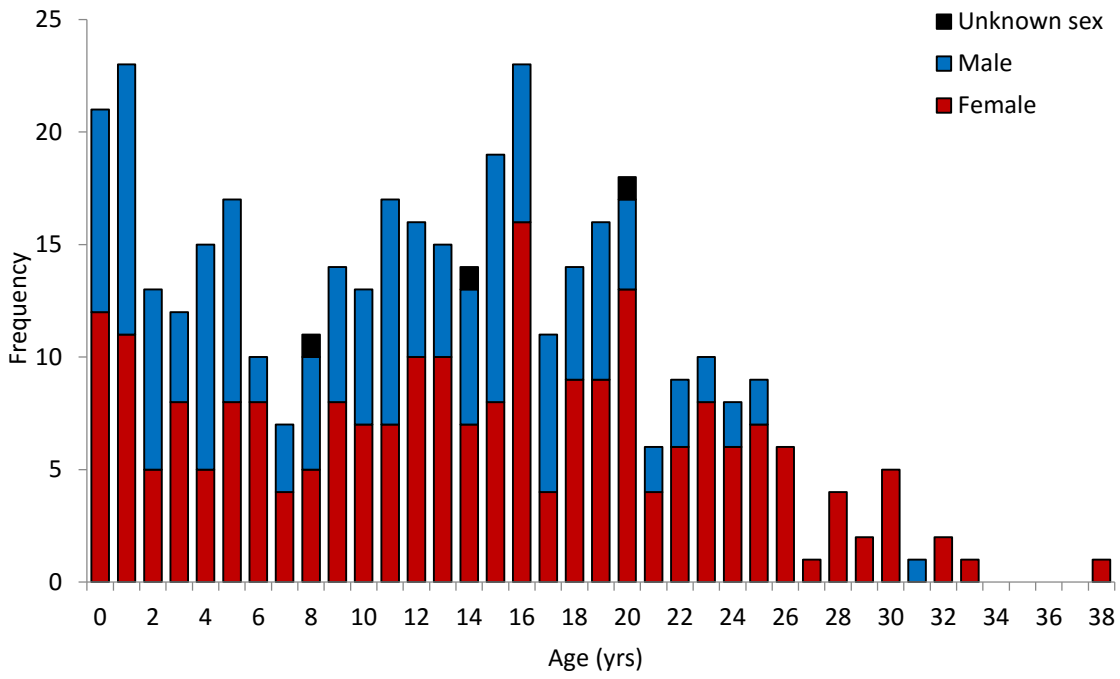


Figure 4.1. Age distribution of the female (*n* = 227), male (*n* = 154) and unknown sex (*n* = 3) stranded LFPWs used for survivorship and mortality schedules.

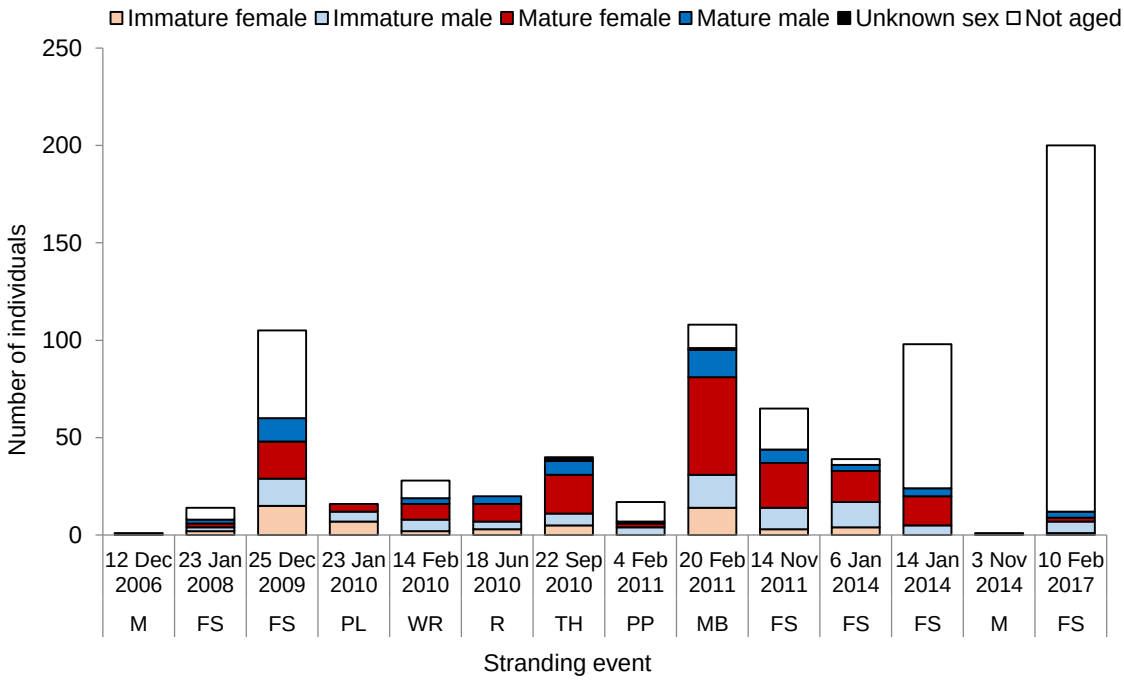


Figure 4.2. Sex and maturity composition of aged LFPWs, by stranding event.

Categories: immature female (< estimated ASM of 6.7 years, *n* = 57; see methods), immature male (< estimated ASM of 13.5 years, *n* = 93; see methods), mature female (≥ ASM, *n* = 170), mature male (≥ ASM, *n* = 61), unknown sex (*n* = 3), not aged (*n* = 368). Stranding locations: M = Muriwai, Auckand; FS = Farewell Spit, Golden Bay; PL = Port Levy, Banks Peninsula; WR = West Ruggedy Beach, Stewart Island; R = Ruapuke, Waikato; TH = Te Horo Beach, Far North; PP = Port Puponga, Golden Bay; MB = Mason Bay, Stewart Island.

Life tables, survivorship, and mortality rates

Life table construction (Table 4.1, Table 4.2, Table 4.3) and subsequent survival (Figure 4.3) and mortality curves (Figure 4.4) showed distinct differences in the age and sex-specific survival and mortality rates for New Zealand LFPWs. Both sexes showed slightly elevated rates of mortality among the youngest age classes (< 2 yrs) with mortality rates decreasing and remaining fairly low until 11 years of age for males and 15 years of age for females (Table 4.2, Table 4.3, Figure 4.4). Using data from the traditional life table (Table 4.1), the overall (total) mortality for the population of LFPWs in New Zealand waters is estimated to be approximately 7.3% per annum, with an average life expectancy at birth estimated to be 13.6 years. Using data from the sex-specific model life tables (Table 4.2, Table 4.3), the overall (total) mortality is estimated to be 8.8% and 6.8% per annum for males and females, respectively. Average life expectancy at birth is 11.3 years for males and 14.7 years for females.

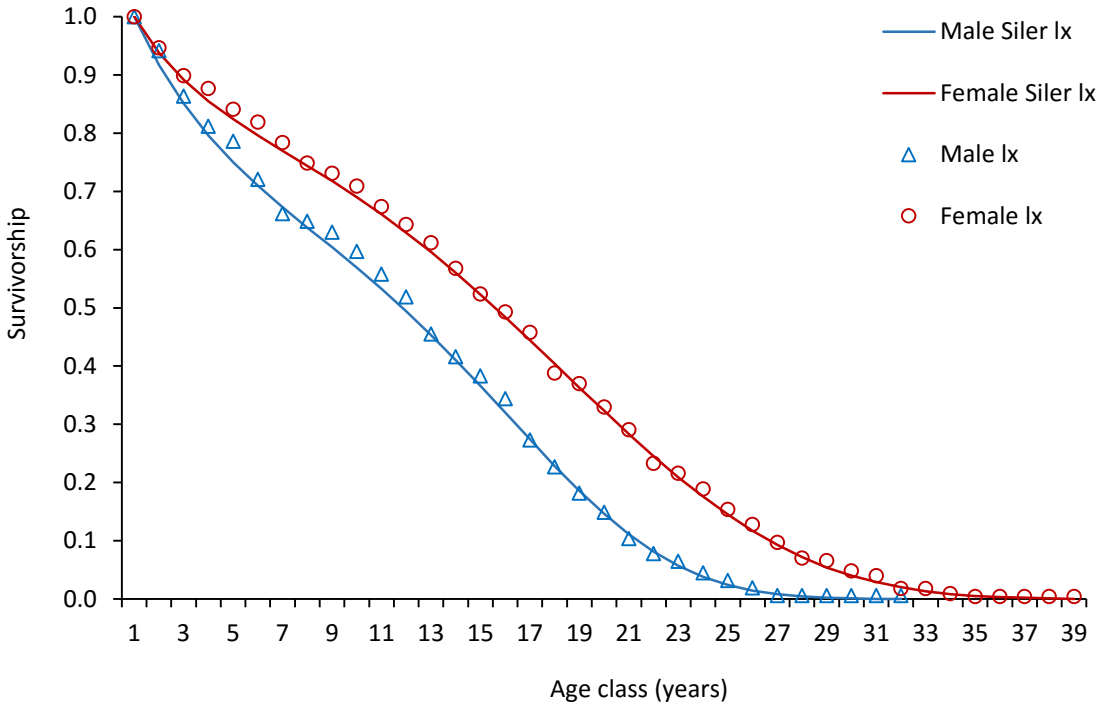


Figure 4.3. Age-specific survivorships (l_x) for male and female LFPWs.

Points are based on traditional life table calculations (l_x) and smoothed curves were fitted using the Siler model (Siler l_x). Age class = age x to $x + 1$.

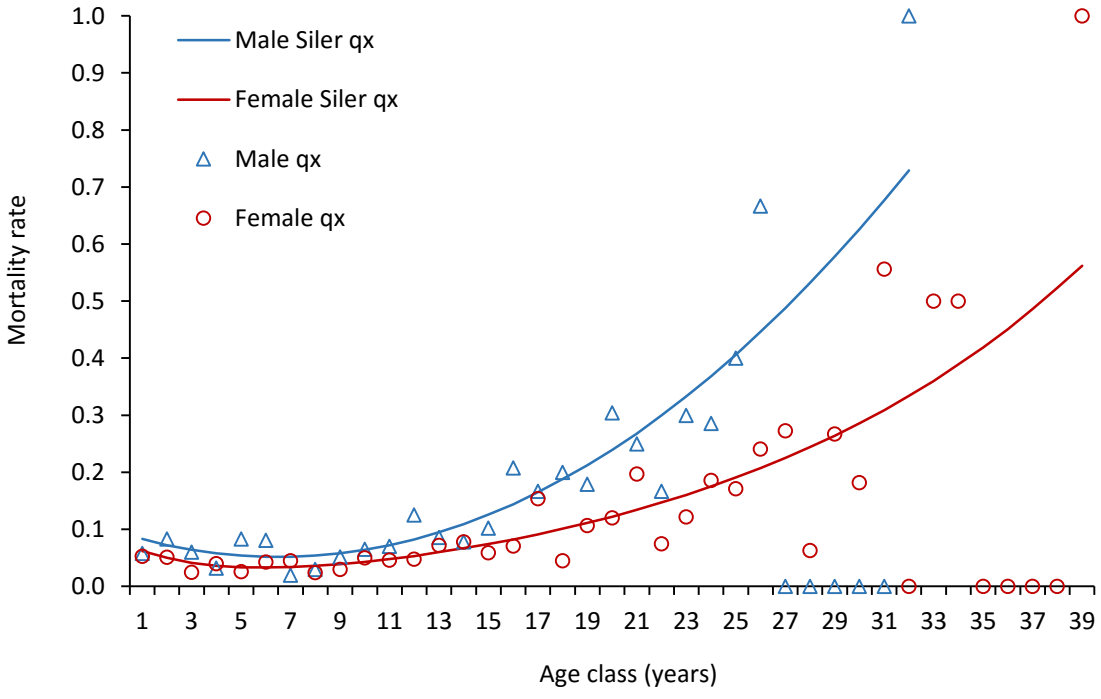


Figure 4.4. Age-specific mortality rates (q_x) for male and female LFPWs.

Points are based on traditional life table calculations (q_x) and smoothed curves were fitted using the Siler model (Siler q_x). Age class = age x to $x + 1$.

Table 4.1. Life table for both sexes (including unknown sex) of LFPW based on individuals mass stranded on the New Zealand coast between 2006 and 2017 (*n* = 384).

Age (x)	No. individuals	% of total	n_x	d_x	l_x	q_x	e_x
0	21	5.5	1000	55	1.000	0.055	13.628
1	23	6.0	945	60	0.945	0.063	13.358
2	13	3.4	885	34	0.885	0.038	13.194
3	12	3.1	852	31	0.852	0.037	12.679
4	15	3.9	820	39	0.820	0.048	12.124
5	17	4.4	781	44	0.781	0.057	11.680
6	10	2.6	737	26	0.737	0.035	11.322
7	7	1.8	711	18	0.711	0.026	10.700
8	11	2.9	693	29	0.693	0.041	9.955
9	14	3.6	664	36	0.664	0.055	9.341
10	13	3.4	628	34	0.628	0.054	8.826
11	17	4.4	594	44	0.594	0.075	8.272
12	16	4.2	549	42	0.549	0.076	7.858
13	15	3.9	508	39	0.508	0.077	7.421
14	14	3.6	469	36	0.469	0.078	6.956
15	19	4.9	432	49	0.432	0.114	6.458
16	23	6.0	383	60	0.383	0.156	6.163
17	11	2.9	323	29	0.323	0.089	6.121
18	14	3.6	294	36	0.294	0.124	5.619
19	16	4.2	258	42	0.258	0.162	5.273
20	18	4.7	216	47	0.216	0.217	5.096
21	6	1.6	169	16	0.169	0.092	5.231
22	9	2.3	154	23	0.154	0.153	4.661
23	10	2.6	130	26	0.130	0.200	4.320
24	8	2.1	104	21	0.104	0.200	4.150
25	9	2.3	83	23	0.083	0.281	3.938
26	6	1.6	60	16	0.060	0.261	4.087
27	1	0.3	44	3	0.044	0.059	4.176
28	4	1.0	42	10	0.042	0.250	3.375
29	2	0.5	31	5	0.031	0.167	3.167
30	5	1.3	26	13	0.026	0.500	2.600
31	1	0.3	13	3	0.013	0.200	3.200
32	2	0.5	10	5	0.010	0.500	2.750
33	1	0.3	5	3	0.005	0.500	3.500
34	0	0.0	3	0	0.003	0.000	5.000
35	0	0.0	3	0	0.003	0.000	4.000
36	0	0.0	3	0	0.003	0.000	3.000
37	0	0.0	3	0	0.003	0.000	2.000
38	1	0.3	3	3	0.003	1.000	1.000

Life table parameters are calculated using the traditional method (Krebs 1989), where $n_x =$ the number of individuals alive at age x; $d_x =$ the number of individuals dying during the age interval x to $x + 1$; $l_x =$ the proportion of the animals surviving to the start of age x; $q_x =$ the proportion of animals alive at age x that die before age $x + 1$ (i.e. mortality rate); $e_x =$ average (remaining) life expectancy for individuals at age x.

Table 4.2. Life table for male LFPWs based on individuals mass stranded on the New Zealand coast between 2006 and 2017 (*n* = 154).

Age (x)	No. males	n_x	Siler n_x	d_x	Siler d_x	l_x	Siler l_x	q_x	Siler q_x	e_x	Siler e_x
0	9	1000	1000	58	83	1.000	1.000	0.058	0.083	11.552	11.259
1	12	942	917	78	66	0.942	0.917	0.083	0.072	11.207	11.183
2	8	864	851	52	55	0.864	0.851	0.060	0.064	11.128	10.977
3	4	812	796	26	46	0.812	0.796	0.032	0.058	10.776	10.661
4	10	786	750	65	40	0.786	0.750	0.083	0.054	10.099	10.256
5	9	721	710	58	37	0.721	0.710	0.081	0.052	9.919	9.783
6	2	662	673	13	35	0.662	0.673	0.020	0.052	9.706	9.262
7	3	649	638	19	34	0.649	0.638	0.030	0.054	8.880	8.714
8	5	630	604	32	35	0.630	0.604	0.052	0.058	8.124	8.152
9	6	597	569	39	36	0.597	0.569	0.065	0.064	7.511	7.591
10	6	558	533	39	38	0.558	0.533	0.070	0.072	6.965	7.042
11	10	519	494	65	41	0.519	0.494	0.125	0.082	6.413	6.512
12	6	455	453	39	43	0.455	0.453	0.086	0.095	6.186	6.007
13	5	416	410	32	45	0.416	0.410	0.078	0.109	5.672	5.531
14	6	383	366	39	46	0.383	0.366	0.102	0.126	5.068	5.087
15	11	344	320	71	46	0.344	0.320	0.208	0.144	4.528	4.674
16	7	273	274	45	45	0.273	0.274	0.167	0.165	4.452	4.293
17	7	227	228	45	43	0.227	0.228	0.200	0.188	4.143	3.944
18	5	182	186	32	39	0.182	0.186	0.179	0.212	3.929	3.623
19	7	149	146	45	35	0.149	0.146	0.304	0.239	3.565	3.331
20	4	104	111	26	30	0.104	0.111	0.250	0.268	3.688	3.065
21	2	78	81	13	24	0.078	0.081	0.167	0.300	3.583	2.823
22	3	65	57	19	19	0.065	0.057	0.300	0.333	3.100	2.603
23	2	45	38	13	14	0.045	0.038	0.286	0.368	3.000	2.403
24	2	32	24	13	10	0.032	0.024	0.400	0.406	2.800	2.221
25	2	19	14	13	6	0.019	0.014	0.667	0.446	3.000	2.055
26	0	6	8	0	4	0.006	0.008	0.000	0.488	6.000	1.904
27	0	6	4	0	2	0.006	0.004	0.000	0.532	5.000	1.764
28	0	6	2	0	1	0.006	0.002	0.000	0.578	4.000	1.631
29	0	6	1	0	1	0.006	0.001	0.000	0.626	3.000	1.495
30	0	6	0	0	0	0.006	0.000	0.000	0.677	2.000	1.323
31	1	6	0	6	0	0.006	0.000	1.000	0.729	1.000	1.000

n_x = the number of individuals alive at age x; d_x = the number of individuals dying during the age interval x to $x+1$; l_x = the proportion of the animals surviving to the start of age x; q_x = the proportion of animals alive at age x that die before age $x+1$ (i.e. mortality rate); e_x = average (remaining) life expectancy for individuals at age x calculated using traditional life table methods (Krebs 1989). Siler (smoothed) parameters calculated using the Siler model (Siler 1979, Saavedra 2018).

Table 4.3. Life table for female LFPWs based on individuals mass stranded on the New Zealand coast between 2006 and 2017 (*n* = 227).

Age (x)	No. females	n_x	Siler n_x	d_x	Siler d_x	l_x	Siler l_x	q_x	Siler q_x	e_x	Siler e_x
0	12	1000	1000	53	62	1.000	1.000	0.053	0.062	15.018	14.729
1	11	947	938	48	46	0.947	0.938	0.051	0.050	14.800	14.634
2	5	899	892	22	37	0.899	0.892	0.025	0.041	14.544	14.345
3	8	877	855	35	31	0.877	0.855	0.040	0.036	13.884	13.920
4	5	841	824	22	28	0.841	0.824	0.026	0.033	13.424	13.405
5	8	819	796	35	26	0.819	0.796	0.043	0.033	12.758	12.834
6	8	784	770	35	26	0.784	0.770	0.045	0.034	12.287	12.235
7	4	749	744	18	27	0.749	0.744	0.024	0.036	11.818	11.625
8	5	731	718	22	28	0.731	0.718	0.030	0.039	11.078	11.017
9	8	709	690	35	30	0.709	0.690	0.050	0.043	10.391	10.421
10	7	674	661	31	31	0.674	0.661	0.046	0.048	9.882	9.843
11	7	643	629	31	34	0.643	0.629	0.048	0.053	9.308	9.285
12	10	612	596	44	35	0.612	0.596	0.072	0.060	8.727	8.752
13	10	568	560	44	37	0.568	0.560	0.078	0.067	8.326	8.242
14	7	524	523	31	39	0.524	0.523	0.059	0.074	7.941	7.758
15	8	493	484	35	40	0.493	0.484	0.071	0.082	7.375	7.299
16	16	458	444	70	41	0.458	0.444	0.154	0.091	6.865	6.865
17	4	388	404	18	41	0.388	0.404	0.045	0.101	6.932	6.454
18	9	370	363	40	40	0.370	0.363	0.107	0.111	6.214	6.065
19	9	330	323	40	39	0.330	0.323	0.120	0.122	5.840	5.699
20	13	291	283	57	38	0.291	0.283	0.197	0.134	5.500	5.354
21	4	233	245	18	36	0.233	0.245	0.075	0.147	5.604	5.028
22	6	216	209	26	34	0.216	0.209	0.122	0.160	4.980	4.721
23	8	189	176	35	31	0.189	0.176	0.186	0.175	4.535	4.432
24	6	154	145	26	28	0.154	0.145	0.171	0.191	4.343	4.160
25	7	128	117	31	24	0.128	0.117	0.241	0.207	4.034	3.904
26	6	97	93	26	21	0.097	0.093	0.273	0.225	4.000	3.663
27	1	70	72	4	18	0.070	0.072	0.063	0.244	4.125	3.436
28	4	66	54	18	14	0.066	0.054	0.267	0.264	3.333	3.223
29	2	48	40	9	11	0.048	0.040	0.182	0.286	3.182	3.021
30	5	40	29	22	9	0.040	0.029	0.556	0.309	2.667	2.830
31	0	18	20	0	7	0.018	0.020	0.000	0.334	3.750	2.649
32	2	18	13	9	5	0.018	0.013	0.500	0.360	2.750	2.477
33	1	9	8	4	3	0.009	0.008	0.500	0.389	3.500	2.309
34	0	4	5	0	2	0.004	0.005	0.000	0.419	5.000	2.142
35	0	4	3	0	1	0.004	0.003	0.000	0.451	4.000	1.965
36	0	4	2	0	1	0.004	0.002	0.000	0.486	3.000	1.759
37	0	4	1	0	0	0.004	0.001	0.000	0.523	2.000	1.477
38	1	4	0	4	0	0.004	0.000	1.000	0.562	1.000	1.000

n_x = the number of individuals alive at age x; d_x = the number of individuals dying during the age interval x to $x+1$; l_x = the proportion of the animals surviving to the start of age x; q_x = the proportion of animals alive at age x that die before age $x+1$ (i.e. mortality rate); e_x = average (remaining) life expectancy for individuals at age x calculated using traditional life table methods (Krebs 1989). Siler (smoothed) parameters calculated using the Siler model (Siler 1979, Saavedra 2018).

4.5 Discussion

Age structure

The aged dataset of LFPWs stranded on the New Zealand coast comprised immature and mature individuals of both sexes, though among the matures there were many more females than males (Figure 4.2). The sample of stranded LFPWs aged in this study (and therefore the 'population' from which survival was determined) resulted in different age structures for males and females, with very few (*n* = 12) males older than 20 years of age. The age structure of any population of animals at a given point in time is a factor of mortality and recruitment into that population. The lack of males greater than 20 years may be the result of a number of factors such as: (1) dispersal of older (mature) males from natal groups into male-only groups or temporary associations with other matrilineal groups for breeding (see Chapter 5), (2) older males being less likely to strand (or having a higher refloat success), possibly as a consequence of the social bonds with their group members not having been established or reinforced by long-term inclusion within in the group (Evans and Hindell 2004), and/or (3) higher natural mortality in males relative to females for all or part of their lifespan, resulting in females outnumbering males of a similar age (especially in adulthood), as previously described for North Atlantic *G. m. melas* (Martin et al. 1987, Bloch et al. 1993a).

Genetic data has suggested that male pilot whales mostly remain within natal groups (Amos et al. 1993a) though dispersal may occur at different points during their lifetime, with the occasional observation of male-only groups in the North Atlantic (Sergeant 1962a, Bloch 1992, Desportes et al. 1993a). Whether the cause of the age structure observed in this study is (1) the result of the dispersal of older males, or (2) a lack of older males stranding when in groups other than their natal group (due to social bonds not being established), is difficult to determine due to the lack of observations and information on these hypotheses. Male dispersal and the occurrence of 'bachelor groups' in another extreme *K*-selected odontocete species, the sperm whale (*Physeter macrocephalus*), have been well documented (Best 1979, Jaquet et al. 2000, Lettevall et al. 2002). However, the extent of male dispersal in male LFPWs is largely unknown. Limited genetic evidence suggests that male LFPWs do not father calves in their natal group, suggesting at least some temporary male dispersal for mating purposes (Amos et al. 1993a). Further molecular studies on several populations are required to establish the extent of this dispersal. Male dispersal could potentially explain some of the prevalence of females in groups of both captured (North Atlantic) and mass stranded (this study; see Chapter 7) LFPWs, but it is considered more likely a result of (3) higher male mortality in both the *G. m.*

melas (Sergeant 1962a, Martin et al. 1987, Bloch et al. 1993a) and *G. m. edwardii* (this study) populations studied.

Life tables, survivorship, and mortality rates

Methods of estimating mortality rates from age-at-death data assume that age-specific fecundity and mortality are stable and that the exponential rate of increase has been, and is currently, zero (Caughley 1966, Caughley and Sinclair 1994, Evans and Hindell 2004). This assumption is difficult to validate; the age structures of real populations are often not stable, resulting in differing amounts of variation around life-table parameters. To account for this variation, Caughley (1977) proposed a minimum sample size of 150 for accurately estimating survival. It is often difficult to obtain such large sample sizes for cetaceans but was possible in this study due to the high frequency of LFPW MSEs on the New Zealand coast (see Chapter 7). Also, long-lived species such as delphinids are buffered from perturbations from stable age distribution due to their long reproductive period and high rates of survival (Stolen and Barlow 2003). The age-at-death data reported here are based on an 11-year time series that also acts to average out the deviations that might be present in any given year.

In populations that are not stationary, vertical estimates of survivorship (i.e. based on the age structure of a population at a specific point in time) have been found to overestimate mortality rates when compared to those derived from a horizontal perspective (i.e. based on the fate of a cohort followed through time; Olesiuk et al. 1990, Evans and Hindell 2004). Apparent changes in the mortality of older groups are influenced not only by the deaths of older individuals but also by changes in the initial sizes of cohorts through time. The extent of this bias is a function of the true mortality rate and the population growth rate (Evans and Hindell 2004). As a result, it is recognised that the survival rates presented here are tentative; nevertheless, they still provide a basis for relative comparisons between species and populations.

The overall survival curve for *G. m. edwardii* in New Zealand waters (Figure 4.3) is typical of mammals and other long-lived species (Spinage 1972, Stolen and Barlow 2003). The sex-specific survival curves show that females have higher survival relative to males at all ages, with the difference being particularly marked around the age of 20 years. Mortality curves created from these life table data (Figure 4.4) approximate the typical U-shaped curves of other large mammals (Caughley 1966, Spinage 1972) but with lower than expected mortality in animals younger than two years old. Again, there are differences between the sexes with males displaying higher mortality at all age classes.

Large differences in mortality with age and sex occur in many mammals. Higher mortality among the very young has been observed in both terrestrial (Caughley 1966) and marine mammals. For example, high neonate mortality has been reported for New Zealand sea lions (neonatal mortality rate = 0.14; Castinel et al. 2007) and cetaceans such as Atlantic spotted dolphins (*Stenella frontalis*, yearling mortality rate = 0.24; Herzing 1997) and common bottlenose dolphins (*Tursiops truncatus*, yearling mortality rate = male: 0.11 and female: 0.08; Stolen and Barlow 2003). Causes of high calf mortality are difficult to identify, though it is likely that factors such as nutrition, social interactions, and predation pressures have compounding effects (Stolen and Barlow 2003). High neonate and calf mortality has been reported for LFPWs (*G. m. melas*) captured in drive fisheries in the Faroe Islands (yearling mortality rate = female: 0.15; Bloch et al. 1993a), but is not as pronounced for SFPWs in Japan (yearling mortality rate = male: 0.10 and female 0.07; Kasuya and Marsh 1984), or stranded LFPWs in the current study (first year mortality rate = male: 0.08 and 0.06: female). It would usually be expected that the actual proportion of calves dying in the population may be underestimated by the number of stranded carcasses recorded due to the more rapid decomposition, greater vulnerability to predation, and the lower detection probability of small-sized animals (Stolen and Barlow 2003). However, the age data in this study were predominantly obtained from live MSEs, which are more likely to be representative of the population than single beach cast events.

Higher rates of male mortality have been reported in the common bottlenose dolphin (Fernandez and Hohn 1998, Stolen and Barlow 2003), SFPW (Kasuya and Marsh 1984), and LFPW (*G. m. melas*) populations (Bloch et al. 1993a), and there are probably several factors that contribute to this. It has been suggested that social structure and differences in ranging patterns may be involved (Stolen and Barlow 2003). As noted earlier, for LFPWs, there is some evidence from the North Atlantic, including the occasional observation of male-only groups (Sergeant 1962a, Bloch 1992, Desportes et al. 1993a), that male LFPWs may, at least temporarily, move away from their natal groups for breeding purposes (Desportes et al. 1993b). If males do indeed move between groups, males that travel alone or in small groups may be more vulnerable to predation (Stolen and Barlow 2003). If LFPW males emigrate from their natal groups and join other groups, male-male competition could result in direct or indirect mortality, as reported in other mammalian species (Ralls et al. 1980).

Overall (total) average annual mortality rates for *G. m. edwardii* (males 8.8% and females 6.8%) are comparable to those previously estimated for *G. m. melas* (males ~8% and females ~7%; Bloch et al. 1993). However, SFPWs appear to have considerably lower overall female mortality rates than LFPWs (total average annual mortality rates: males 8.3% and females

4.5%; Kasuya and Marsh 1984). The survival curve constructed for female SFPWs caught in Japanese drive fisheries (Kasuya and Marsh 1984, Ellis et al. 2018b) shows high juvenile mortality, followed by a period of lower mortality during prime reproductive years, and then a higher mortality rate in the post-reproductive or senescent years. This is characteristic of females in long-lived mammalian species that invest heavily in each offspring (Barlow and Boveng 1991). In contrast, the results of this study suggest that female *G. m. edwardii* show a pattern of mortality more similar to *G. m. melas*. Both LFPW subspecies do not exhibit a pronounced period of relative stability and low mortality during mid-life; rather, they show a relatively stable period of low mortality until the teens followed by a steady decline with age (in a similar manner to the males; Bloch et al. 1993a, Ellis et al. 2018b).

The greatest decrease in survival of mature female *G. m. edwardii* occurred between the ages of 20 and 30 years, compared to between 30 and 40 years for mature female *G. m. melas* (Bloch et al. 1993a). The age at which the escalated decrease in survival occurs is likely to be related to the relative female longevity of each subspecies (i.e. *G. m. edwardii*: 38 yrs vs. *G. m. melas*: 59 yrs; see Chapter 3). Other mammalian species with similar longevity to *G. m. melas* demonstrate decreases in survival at a similar age, or older; for example, female SFPWs (longevity 64.5 yrs) and resident killer whales (longevity 80 yrs) show a decrease in survival between the ages of 30 and 45 years from a previously relatively stable rate of survival during the adulthood (Kasuya and Marsh 1984, Olesiuk et al. 1990). Both SFPWs and resident killer whales appear to have selected for an extension of the post-reproductive lifespan (proportion of the adult lifespan that is post-reproductive [post-reproductive representation; PrR] for resident killer whales: 0.31 and SFPWs: 0.26), whereas no significant post-reproductive lifespan (PrR 0.002), but an acceleration in the mortality rate, is observed in *G. m. melas* (Kasuya and Marsh 1984, Bloch et al. 1993a, Foote 2008, Ellis et al. 2018a, Ellis et al. 2018b). The observed variation in life history strategies between the two pilot whale species may in part be due to the social organisation within stable social groups and the benefits of cooperative foraging and multigenerational transfer of information (Marsh and Kasuya 1984, Whitehead 2015, Ellis et al. 2018b; see Chapter 6). The social structure of pilot whale pods is similar to that of killer whales (Olson 2018) but the reason for the observed differences in the mortality rate acceleration between the two pilot whale species has not been established (Foote 2008). Other species with longevity closer to *G. m. edwardii* (e.g. common bottlenose dolphins from the Indian River Lagoon system, longevity 35 yrs; Stolen and Barlow 2003) also do not show a stable period of low mortality during mid-life, but instead a gradual decline and greatest decrease in survival between 20 and 30 years. Increases in birth-related mortality,

susceptibility to predation due to the care and protection of young and greater energetic demands on females associated with gestation and lactation may affect the survival of these age groups (Evans and Hindell 2004).

Pilot whales are among the cetacean species most frequently involved in MSEs in which the entire group dies. Although the cause of these MSEs is often not determined, there is no doubt that strong social bonds exist within pilot whale groups (Olson 2018). The cultural implications of stranding-related mortality in a highly social species are not well understood, but pilot whales may be particularly vulnerable to removal of certain individuals from social groups (Wade et al. 2012). For example, one hypothesis is that large MSEs in which the entire group (or aggregation) dies, result in less social disruption to the remaining whales than the death of a small percentage of individuals from many different social groups (Wade et al. 2012). Alternatively, matriline-based knowledge or foraging specialisations may become lost in MSEs where a whole matriline dies (Williams and Lusseau 2006). In another matrilineal species, the sperm whale, differential foraging success of matrilines was identified during El Niño events (Whitehead and Rendell 2004). That different sperm whale groups seem differentially affected by altered climate conditions has implications for the impacts of global warming on sperm whales (Whitehead and Rendell 2004), and other species with similar social structure, e.g. killer whales and pilot whales. There is some genetic evidence that MSEs of LFPWs on New Zealand and Tasmanian coasts can contain multiple matrilines in a single event (Oremus et al. 2013). Thus, it has been suggested that *G. m. edwardii* off New Zealand and Tasmania form associations comprising multiple matrilineal groups (Oremus et al. 2013), as also suggested by a behavioural study of *G. m. melas* in the north-west Atlantic (Ottensmeyer and Whitehead 2003). Further investigation using samples collected from complete mass stranded groups (including live whales) is required to enable a thorough assessment of the social structure of *G. m. edwardii*.

4.6 Conclusions

Survival and mortality curves for LFPWs stranded on the New Zealand coast revealed distinct differences in the age and sex-specific survival and mortality rates. Slightly elevated rates of mortality were observed among the youngest age classes (< 2 yrs), and females outlived males. Average life expectancy at birth is 11.3 years for males and 14.7 years for females. Overall (total) average annual mortality is estimated to be approximately 8.8% for male and 6.8% for female *G. m. edwardii* in New Zealand waters. An accelerated mortality rate was observed in mature female LFPWs when compared to the closely related SFPW, a species which selects for

an extension to the post-reproductive lifespan. The reason for the observed differences in mortality rate acceleration between the two pilot whale species and social implications of stranding-related mortality have not been established and warrant further investigation.

Despite known biases with data derived from stranded individuals, the frequent MSEs of LFPWs on the New Zealand coast (in large groups of mixed ages and sexes; see Figure 4.2 and Chapter 7) provides valuable opportunities to collect data on the demography of this species in New Zealand waters. This 11-year study is the first comprehensive population assessment of New Zealand LFPWs and contributes valuable baseline data on the mortality rates of LFPWs in New Zealand waters. These data, when collected in a standardised and comprehensive manner, offer an ability to assess changes in population parameters over time, providing essential information for conservation management of LFPWs in New Zealand waters. The approach used in this study is also broadly applicable to data gathered by stranding networks in other areas. With dedicated collection of life history samples, similar age-structured models could be developed for other populations of marine mammals.

Chapter 5

Sexual maturation in male long-finned pilot whales (*Globicephala melas edwardii*) off New Zealand; defining indicators of sexual maturity

Long-finned pilot whales offshore, northern New Zealand, January 2018. Note large male attempting to mate.

In this chapter, the reproductive biology of male long-finned pilot whales (*Globicephala melas edwardii*) stranded on the New Zealand coast is examined. Males are classified according to their stage of sexual development, average age and total body length at the attainment of sexual maturity are estimated, and predictors of sexual maturation are examined to provide new insights on the mating system and strategies used by *G. m. edwardii.* This chapter addresses the third research objective of this thesis:

Objective 3: Classify the stages of sexual maturation in male *G. m edwardii*, and define indicators of sexual maturity.

This chapter is a reformatted version of the following manuscript:

Betty EL, et al. (2019). Sexual maturation in male long-finned pilot whales (*Globicephala melas edwardii*): defining indicators of sexual maturity. Journal of Mammalogy: gyz086.

5.1 Abstract

Male reproductive biology is described for the first time in the Southern Hemisphere long-finned pilot whale (*Globicephala melas edwardii*; herein LFPW), a subspecies that regularly mass strands along the New Zealand coastline. Ten mass stranding events sampled over a seven-year period enabled assessments of key life history parameters, information that is important for the conservation management of this cetacean subspecies. Sexual maturation in immature, maturing, and mature males was assessed using morphological data and histological examination of testicular tissue. Variation was observed in the age (11 – 15 yrs) and length (450 – 490 cm) at which individuals attained sexual maturity. Using Bayesian cumulative logit regression models, the average age and length at the attainment of sexual maturity were estimated to be 13.5 years and 472 cm, respectively. Combined testes weight, combined testes length, an index of testicular development (combined testes weight/combined testes length), and mean seminiferous tubule diameter were all good indicators of sexual maturity status. Combined testes length was the best non-histological indicator, and all testicular measures were found to be better indicators of sexual maturation for *G. m. edwardii* than age or total body length. Sexual maturity was attained before physical maturity (> 40 yrs and 570 cm), and at a younger age and smaller body length than previously reported for *G. m. melas* in the North Atlantic. Given the ease of collection, minimal processing, and applicability to suboptimal material collected from stranding events, it is recommended that future studies apply further effort to assessing the value of testicular size as an indicator of sexual maturity in pilot whales and other cetacean species. Estimates of the average age and length at sexual maturity for *G. m. edwardii* provided in this study may be used to inform population models required for future conservation management of the subspecies, which is subject to high levels of stranding mortality.

5.2 Introduction

Knowledge of life history is the foundation for understanding population dynamics, the vulnerability of a species or population, and its capacity to recover from large-scale mortality (Kemper et al. 2014). Understanding the reproductive biology of free-ranging cetacean populations also allows for the interpretation of data from behavioural and genetic studies, thus playing a vital role in conservation management (Plön and Bernard 2007). However, lack of data limits our understanding of the reproductive biology of many cetacean populations, including the Southern Hemisphere LFPW (*Globicephala melas edwardii*). Further, most research on cetacean reproduction to date has focused on females, primarily because female reproductive parameters are required for population modelling. Information on male reproduction can improve population models and associated conservation management strategies, while also providing insights on the health of the population (O'Hara et al. 2002, Plön and Bernard 2007, Kemper et al. 2014). Cases of reproductive disease and abnormalities have been documented in male cetaceans (Dagleish et al. 2008, Kemper et al. 2014), as have adverse effects of endocrine-disrupting chemicals, such as PCBs and DDT (Subramanian et al. 1987, Diamanti-Kandarakis et al. 2009, Murphy et al. 2018). Toxin load is a particular concern for male cetaceans because they are unable to offload their lipophilic pollutant burden via gestation and lactation (Murphy et al. 2018). Research on the reproductive biology of both sexes is important (Plön and Bernard 2007), particularly where (individual) reproductive potential has been compromised.

Spermatogenesis (production of haploid spermatozoa) is the primary indicator of sexual development in male cetaceans (Plön and Bernard 2007). Plasma and blubber testosterone levels have been used to estimate the attainment of sexual maturity in delphinids by delineating sexually immature from mature individuals (e.g. Kellar et al. 2009). However, Desportes et al. (1994b) reported that in the North Atlantic LFPW subspecies (*G. m. melas*), highest plasma testosterone concentrations were observed in pubertal males. Furthermore, testosterone concentrations were not correlated with age, body length, or body mass in *G. m. melas*, instead the large-scale individual variation observed in mature individuals was attributed to reproductive seasonality (Desportes et al. 1994b). Other studies have used smears from testicular and epididymal tissues to indicate sperm presence and relative sperm density (e.g. Kasuya and Marsh 1984, Desportes et al. 1993b). When associated data on testis size (e.g. length, weight) are collected from a large number of animals, it may be possible to predict whether animals are sexually mature based on testis size alone (O'Hara et al. 2002). However, histological examination (i.e. stage of spermatogenesis and seminiferous tubule

diameter) of the testicular tissue is the most accurate way to determine sexual maturity in male cetaceans (e.g. Murphy et al. 2005, Plön and Bernard 2007, Kemper et al. 2014).

In most cetacean species, both testes mature at the same rate (e.g. Miyazaki 1984, Desportes 1994, Van Waerebeek and Read 1994, Plön 2004) and therefore examination of a single testis is usually sufficient to determine maturity status (Plön and Bernard 2007). In some species, zonal maturation occurs within the testis, maturing from the centre outwards in the sperm (*Physeter macrocephalus*; Best 1969), bowhead (*Balaena mysticetus*; O'Hara et al. 2002), and sei (*Balaenoptera borealis*; Masaki 1976) whales. However, Desportes (1994) identified that in LFPWs the core of the testis matures last and, therefore, is the most appropriate place to sample for assessment of sexual maturity status.

Estimates of the average age at attainment of sexual maturity (ASM) and knowledge of reproductive senescence are necessary to determine the length of reproductive life at individual, population and species levels (Kemper et al. 2014), and to allow inter- and intraspecific population comparisons (Hohn et al. 1985). Compared with most terrestrial mammals, cetaceans have a *K*-selected life history strategy and are therefore more vulnerable to anthropogenic impacts and mass mortality events (Merrick et al. 2009). ASM is thought to vary with resource availability and the level of mortality in cetaceans (Fowler 1984) and therefore, may be useful as an index of the condition of the population or its relative carrying capacity (Fowler 1984, Hohn 1989). If the ASM is increasing (i.e. individuals attain sexual maturity at an older age), it is inferred that the availability of resources is decreasing and that density-dependent mechanisms are operating (DeMaster 1984).

Previous studies have described the reproductive biology of male pilot whales (*Globicephala* spp.) with varying degrees of detail. Many of the earlier attempts to estimate ASM in *G. m. melas* were based on small sample sizes (e.g. Martin et al. 1987, Sigurjonsson et al. 1993). Sergeant (1962a) carried out a more thorough investigation of *G. m. melas* taken in the drive fishery off Newfoundland, but limitations of the dataset only allowed for an approximate estimate of ASM to be reported. Also, problems with the ageing methodology employed rendered Sergeant's earlier (1962a) estimates of life history parameters to be inaccurate (Kasuya et al. 1988b). Extensive, and comparable, reproductive studies were later conducted on both LFPWs and short-finned pilot whales (*G. macrorhynchus*; herein SFPW) in the Northern Hemisphere, based on large numbers of samples collected from the *G. m. melas* drive fishery in the Faroe Islands (Desportes et al. 1993b, Desportes 1994, Desportes et al. 1994b) and the SFPW drive fishery in Japan (Kasuya and Marsh 1984, Kasuya and Tai 1993). There are many

similarities in the reproductive biology of the two pilot whale species. These include a correlation of age and body length with maturity stage, attainment of sexual maturity at *c*. 17 years, and the cessation of testis growth at *c*. 25 years with larger mature males having heavier testes than smaller ones (Kasuya and Marsh 1984, Desportes et al. 1993b, Kasuya and Tai 1993). Pilot whales also follow the general delphinid pattern of bimaturism, with males attaining sexual maturity several years after females (Perrin and Reilly 1984).

The Southern Hemisphere subspecies of the LFPW frequently mass strands alive on the coast of New Zealand (Brabyn 1991; see Chapter 7). The majority of animals involved in mass stranding events (MSEs) often die in situ or very close to the original stranding site, and there is usually no reason to suspect that mass stranded pods of pilot whales are unrepresentative of the population (Martin et al. 1987). Therefore, MSEs provide a valuable opportunity to study the biology of LFPWs. Here, tissues opportunistically collected from carcasses of *G. m. edwardii* mass stranded on the New Zealand coast were used to provide first insights on male sexual maturation in this subspecies. Morphological and histological features of the testes relevant to the stage of sexual maturation are described, and growth in testicular size is documented. More specifically, using histological examination and a set of testicular measures (combined testes length, combined testes weight, an index of testicular development, and mean seminiferous tubule diameter), the following were examined: (1) how testis growth changes with age and body length, and with the onset of sexual maturity, (2) the average age and body length at the attainment of sexual maturity, (3) potential indicators of sexual maturity, and (4) evidence of reproductive seasonality in mature males. Estimates of average age and length at the attainment of sexual maturity are compared with previous estimates for the North Atlantic subspecies, and mating strategies used by male LFPWs are considered.

5.3 Materials and methods

Sample collection

Sexual maturation was assessed for 98 male *G. m. edwardii* involved in 10 MSEs on the New Zealand coast between January 2010 and February 2017 (Figure 5.1). Teeth, gonadal samples (testes and associated epididymides), total body length (TBL), and an assessment of decomposition were obtained through standard post-mortem examination procedures (Geraci and Lounsbury 2005). Between three and 10 teeth from each whale were collected from the middle of the upper or lower jaw, and either stored in 70% ethanol or frozen for age estimation purposes. Whenever possible, both left and right testes were collected and individually weighed (with and without associated epididymides) to the nearest gram, and the

length, width, and depth were measured to the nearest millimetre. Where feasible, testes and epididymides were fixed in a 1:10 ratio of tissue sample to 10% neutral buffered formalin immediately upon removal, within 24 hours' post-mortem. Where testes were large, three × 1cm³ blocks were dissected from the centre of a mid-length cross-section from each testis prior to fixation, along with a section of the epididymis. As the majority of post-mortem examinations were carried out in remote locations, many of the samples were not able to be fixed immediately, with some frozen before fixation. Testes weights and measurements were obtained where possible but are not available for all individuals. In two MSEs, particular males were targeted for sampling to obtain a sufficient sample for assessing gonadal development in the subspecies, i.e. larger males in the 2010 Spirit's Bay MSE and males with TBLs around the average length at the attainment of sexual maturity (LSM) in the 2017 Farewell Spit MSE.

Age estimation

Up to four of the straightest teeth from each whale were selected and rehydrated if stored in ethanol or defrosted if frozen. Age estimation was performed by counting annual growth layer groups (GLGs) in decalcified and stained longitudinal sections of teeth, as defined by Perrin and Myrick (1980). Tooth preparation methods for this study were adapted from Lockyer (1993a), and all sections were read by at least two experienced readers; for further explanation see Chapter 3. Individuals for which a definitive age could not be estimated reliably were excluded from the statistical analysis. Calves that did not possess a neonatal line in the tooth, or had a neonatal line forming, with no additional postnatal dentine, were classified as newborns.

Histological assessment of reproductive organs

Stages of sexual maturity were determined from histological examination of testicular tissue following Kasuya and Marsh (1984) and Desportes et al. (1993b). The tissues were prepared using standard histological techniques, i.e. embedded in paraffin wax, sectioned at 5 µm, stained with haematoxylin and eosin (H&E) stain and mounted in DPX, a permanent medium. Histological slides were examined microscopically (100 – 500× magnification), and the stage of sexual maturity was determined by reviewing all seminiferous tubules in an approximately 1 cm² testicular section (> 100 tubules per section).

Following Murphy et al. (2005), the slides were examined for the relative quantity of interstitial tissue, size of the lumen, mean diameter of the seminiferous tubules, the relative proportion (low, medium, high) of interstitial tissue, Sertoli cells and germinal cells (e.g. spermatogonia, spermatocytes, spermatids, and spermatozoa), activity of the epididymis, and

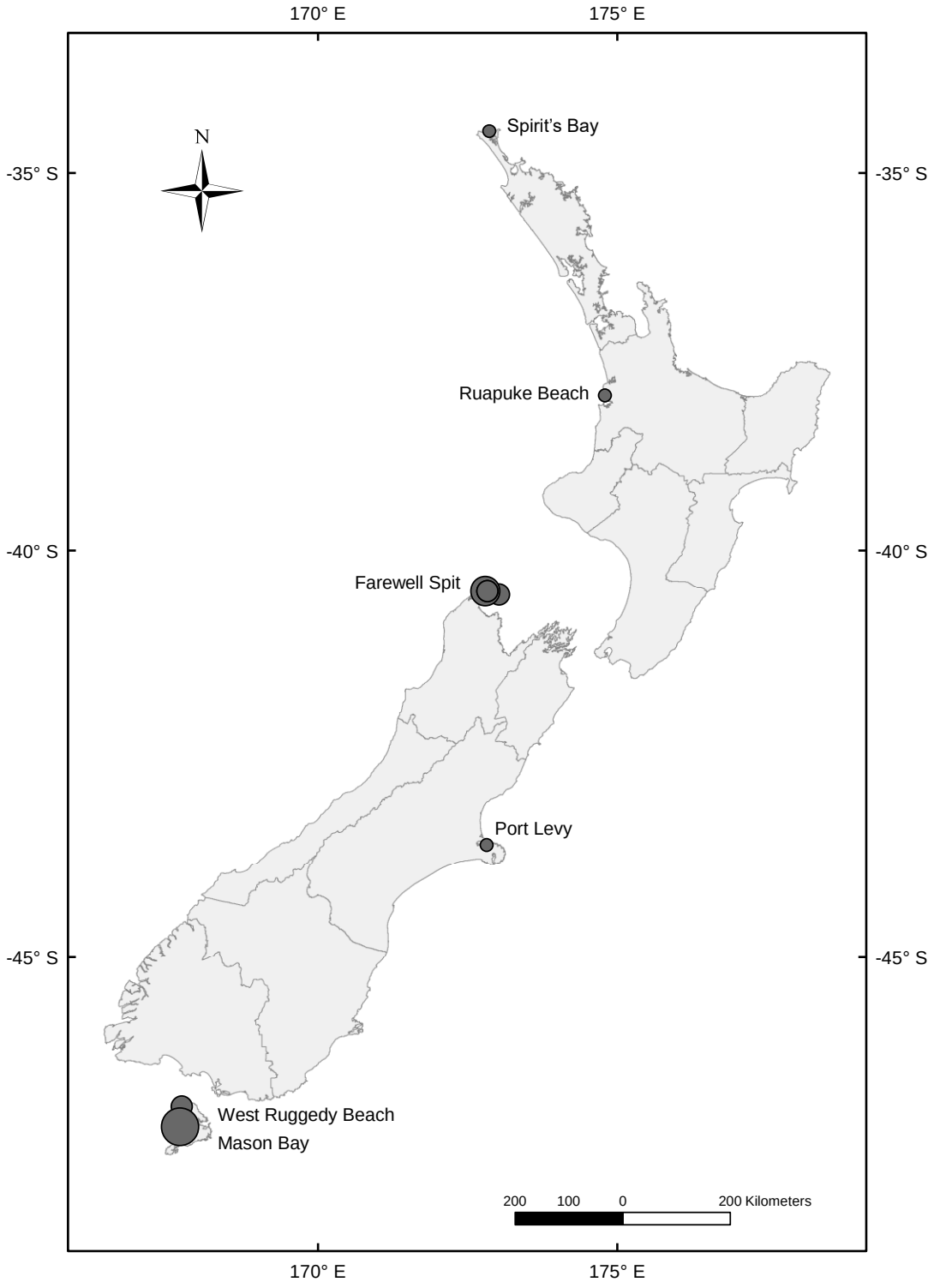


Figure 5.1. Location of LFPW MSEs on the New Zealand coast from which male reproductive samples were collected for this study.

Locations: Spirit's Bay, Far North, 22 Sep 2010 *n* = 5; Ruapuke Beach, Waikato, 18 June 2010 *n* = 7; Farewell Spit, Golden Bay, 4 Feb 2011 *n* = 5, 14 Nov 2011 *n* = 12, 6 Jan 2014 *n* = 18, 14 Jan 2014 *n* = 8, 10 Feb 2017 *n* = 7; Port Levy, Banks Peninsula, 23 Jan 2010 *n* = 4; West Ruggedy Beach, Stewart Island, 14 Feb 2010 *n* = 9; Mason Bay, Stewart Island, 20 Feb 2011 *n* = 27). The size of the location icon is representative of the number of individual males sampled at that location per MSE.

the presence and relative proportion of spermatozoa within the epididymis. In slightly autolysed tissues, some areas of the basement membrane detached from the germinal epithelium. Neimanis (1996) reported the diameter of the tubules in slightly autolysed harbour porpoise (*Phocoena phocoena*) testes did not differ significantly from fresh testes. Therefore, the mean diameter of the seminiferous tubules was based on measurements taken from the basement membranes of 10 tubule cross sections for each testis and measured on digital micrograph images using the 'Fiji' plugin (Schindelin et al. 2012) for image processing software, ImageJ2 (Rueden et al. 2017). Only 'round' tubules were measured to ensure true cross sections of the longitudinal axis (Desportes et al. 1993b, O'Hara et al. 2002), and tubules were not measured in cases of moderate or advanced autolysis (where the basement membrane could not be distinguished). Following (Desportes et al. 1993b), when both mature and immature tubules were present, at least 10 tubules of each category were measured. Each resulting mean was weighted by the corresponding proportion of mature and immature tubules, thus taking into account the varying distribution of mature and immature tubules. The overall mean diameter of the seminiferous tubules was taken as the sum of these two weighted values.

Seminiferous tubules that contained spermatozoa or spermatids were classified as mature, whereas tubules containing spermatogonia and spermatocytes only were considered immature. Individuals were categorised into maturity stages based on the earlier studies of Kasuya and Marsh (1984), Desportes et al. (1993b), and Kasuya and Tai (1993) and the proportion of seminiferous tubules that were mature, these are: immature = 0% of the tubules mature; maturing = both immature and mature tubules found, < 100% of tubules mature; mature = 100% of tubules mature. Some of the tubules examined in maturing and mature individuals lacked one or two of the cell types spermatocytes, spermatids, and spermatozoa. However, for an individual to be classified as maturing or mature, spermatozoa were present in some tubules.

Statistical analysis

An index of testicular development, normalising testis weight by testis length, thereby removing variability in testis weight due to differences in body length, has been shown to provide a useful tool for comparison of sexual maturity between populations or species (Hohn et al. 1985). In the current study, an index of testicular development was calculated as the combined testes weight in grams (excluding the epididymides) divided by the combined testes length in millimetres (Hohn et al. 1985, Desportes et al. 1993b). This index variable was then

included in a dataset of six individual-level continuous variables compiled for the 98 males, including two demographic variables (age and TBL) and four testicular variables (combined testes length, combined testes weight, index of testicular development, and mean seminiferous tubule diameter). Not all variables were recorded for all 98 individuals. The relationships among these six variables were explored, both across and within maturity stages (immature, maturing and mature), using charts and Spearman's rank correlation coefficients.

Progression through the three maturity stages was modelled using Bayesian cumulative logit models fitted with the 'brms' package (Bürkner 2017) for R (R Development Core Team 2018). To compare the utility of each of the individual-level variables for predicting maturity stage, each was used as the single predictor variable (x) in turn. Maturity stage (Y) was treated as an ordinal variable with categories $k = \{1, 2, 3\}$, representing stages immature, maturing, and mature, respectively. The probability of a male being in stage k or below ($\pi_k = P(Y \leq k)$) was modelled as:

$$\log\left(\frac{\pi_k}{1-\pi_k}\right) = \alpha_k - \beta x$$

for $k = 1, 2; \pi_3 = 1 - \pi_2$. Weakly informative prior distributions ($\text{Student's } t(3, 0, 10)$) were assumed for the three parameters (α_1, α_2, and β). The posterior distributions of $c_1 = \alpha_1/\beta$ and $c_2 = \alpha_2/\beta$ were used to estimate the values of x at which 50% of males were classified as not immature (i.e. maturing or mature), and fully mature, respectively. These values were summarised *via* the mean and 95 percentile credible intervals.

The relative utility of the demographic and testicular variables as indicators of maturity in the logistic regression models was compared using the Leave-One-Out Information Criterion (LOOIC; using the 'loo' package for R, Vehtari et al. 2017, 2018). LOOIC is a criterion for comparing the accuracy of candidate models for predicting out-of-sample data. LOO scores were estimated based on Pareto-smoothed importance sampling. Models were refitted for 'problematic' observations (i.e. with Pareto $k > 0.7$), as recommended by Vehtari et al. (2017). Only 55 complete cases were available for comparing all the fitted models with LOOIC due to the variables having different missing values. Two further comparisons of interest were made for two pairs of models using larger datasets; namely age vs TBL ($n = 90$) and mean diameter of the seminiferous tubules vs combined testes length ($n = 72$). The four testicular variables (combined testes weight, combined testes length, index of testicular development, mean diameter of the seminiferous tubules) had skewed distributions so, for each, two models were fitted: one using the raw values (x), and one using the log-transformed ($\log x$) values. Model

comparisons based on the LOOIC indicated that the log-transformed variables resulted in better estimated out-of-sample predictive accuracy. Thus, the models presented herein for all four testicular variables are based on log-transformed values. For mature males, the difference in mean diameter of the seminiferous tubules among months of the year was also tested using univariate analysis of variance (ANOVA).

5.4 Results

Stages of sexual maturation

Of the 98 male gonadal tissues examined, 33 were classified as reproductive (i.e. maturing or mature) and 65 as non-reproductive individuals (i.e. immature; Table 5.1). Two of the individuals identified as reproductive (based on the presence of spermatozoa) were too autolysed to permit further classification, i.e. not all cell types in the germinal epithelium could be distinguished. The remaining 96 males were further classified into three maturity stages (immature, maturing, mature). Descriptions of the histological appearance of the testis and epididymis at each reproductive stage (shown in Figure 5.2) are given below.

Table 5.1. Mean (±SE), range and number of samples obtained for each variable (TBL, age, combined testes weight, combined testes length, an index of testicular development [index], and seminiferous tubule diameter) for each stage of male sexual maturation.

Stages	***n***	**TBL (cm)**	**Age (yrs)**	**Combined testes weight (g)**	**Combined testes length (mm)**	**Index (g/mm)**	**Seminiferous tubule diameter (µm)**
Immature	65	337 (±10)	4.9 (±0.5)	117 (±18)	259 (±13)	0.42 (±0.04)	42.1 (±1.3)
		184–482	0–13.5	15– 515	129–570	0.09–1.26	29.2–75.3
		(*n*=65)	(*n*=62)	(*n*=48)	(*n*=60)	(*n*=48)	(*n*=59)
Maturing	5	456 (±13)	12.8 (±0.6)	1530 (±686)	595 (±83)	2.63 (±0.98)	102.1 (±12)
		420–490	12–15	790–2900	419–900	1.54–4.58	66.2–137.8
		(*n*=5)	(*n*=5)	(*n*=3)	(*n*=5)	(*n*=3)	(*n*=5)
Mature	26	518 (±7)	17.0 (±0.7)	7606 (±1297)	963 (±32)	7.94 (±1.12)	179.5 (±7.6)
		450–573	11–25	3000–13020	645–1340	4.09–12.50	126.4–236.4
		(*n*=26)	(*n*=23)	(*n*=8)	(*n*=21)	(*n*=8)	(*n*=15)
Total	98	393 (±11)	8.5 (±0.7)	1204 (±376)	450 (±35)	1.55 (±0.37)	72.0 (±6.4)
		184–573	(0–25)	15–13020	129–1340	0.09–12.50	29.2–236.4
		(*n*=98)	(*n*=92)	(*n*=59)	(*n*=86)	(*n*=59)	(*n*=79)

Note: Two males were identified as reproductive (based on the presence of spermatozoa) but were too autolysed to permit further classification as maturing or mature; parameters for these two individuals are included in totals only.

Immature testes (Figure 5.2a, b; *n* = 65) had seminiferous tubules that were narrow ($\bar{x}$ = 42.1 µm, range 29.2 – 75.3), tightly packed together, with no apparent (i.e. open) lumen, and embedded in abundant interstitial tissue. Enclosed by the basement membrane were one or two layers of two types of cells, the supportive Sertoli cells, and spermatogonia (germinal cells). The epididymis was undeveloped and empty, with a resting epithelium. No spermatocytes, spermatids, or spermatozoa were observed in the testis (or epididymis).

Maturing testes (Figure 5.2c, d; *n* = 5) contained both immature and mature tubules, with areas of considerably smaller immature tubules observed near areas of fully mature tubules producing spermatozoa. The appearances of the immature and mature areas were similar to those observed in immature and mature testes, but the size of the tubules was transitional. In maturing testes, the mean seminiferous tubule diameter was 78.6 µm (range 38.1 – 170.4) for immature tubules and 136.1 µm (range 74.2 – 201.8) for mature tubules, compared to 42.1 µm in immature testes and 179.5 µm in fully mature testes. Given the clear zonation of mature and immature seminiferous tubules, the mean seminiferous tubule diameter of maturing testes was calculated as the sum of the weighted mean immature and mature tubule diameters (as described earlier) and ranged from 66.2 to 137.8 µm ($\bar{x}$ = 102.1).

In mature testes (Figure 5.2e, f; *n* = 26), all cell types involved in spermatogenesis were observed in the tubules. Full testicular activity is characterised by the presence of spermatozoa in the lumen of the seminiferous tubules and epididymis. The relative proportion of interstitial tissue, Sertoli cells, and spermatogonia was low, and a high relative proportion of spermatocytes, spermatids and spermatozoa was observed. The germinal epithelium often contained greater than five cell layers. The epididymis was enlarged, with an actively secreting epithelium and contained spermatozoa. Large seminiferous tubules were observed, with a mean seminiferous tubule diameter of 179.5 µm (range 126.4 – 236.4).

Comparison with demographic variables

Total body length (TBL) of the entire sample examined in this study ranged from 184 to 573 cm (median 409 cm; Figure 5.3a). Although males ranged in age from 0 to 25 years, 21% of the aged sample comprised individuals younger than two years old, and 54% younger than six years old (Figure 5.3b). The high proportion of young males in the sample reflects the composition of the stranded groups, as the male component of most mass stranded groups was predominantly sexually immature (Figure 5.4a). Age and TBL for each of the maturity stages described above are summarized in Table 5.1. Age and TBL increased with maturity stage, though there was some overlap in values between stages (Table 5.1; Figure 5.5).

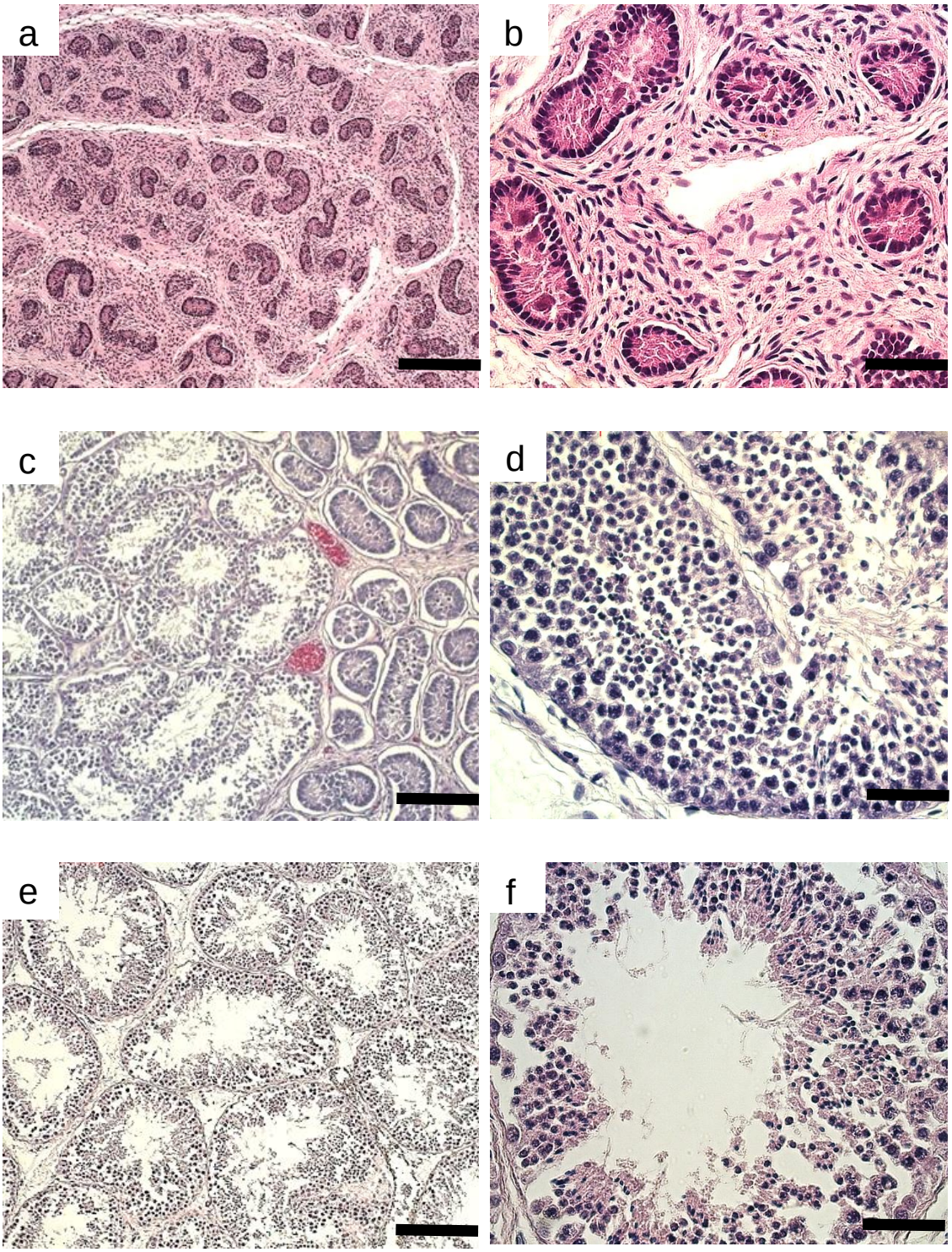


Figure 5.2. Histological appearance of immature, maturing and mature LFPW testes.

(a), (b) Immature male (GM241; neonate, TBL 213cm; combined testes weight 17.7g, mean seminiferous tubule diameter 32.6mm) showing abundant interstitial tissue, Sertoli cells lining the tubules, and spermatogonia the only germinal cells present. (c) Early-maturing male (GM530; 12yrs, TBL 420cm, combined testes weight 900g, mean seminiferous tubule diameter 90.2µm [27% tubules mature]) showing adjacent immature and mature zones and (d) Early-maturing male (GM530) mature zone showing a multi-layered seminiferous epithelium with all cell types involved in spermatogenesis, including spermatozoa. (e), (f) Mature and very active male (GM475; 15 yrs, TBL 527cm, combined testes weight 13,020g, mean seminiferous tubule diameter 236.4µm) showing little interstitial tissue and multi-layered seminiferous epithelium with all cell types involved in spermatogenesis, including spermatozoa, and a well-developed lumen. Scale bar is 200µm in (a), (c), (e) and 50µm in (b), (d), (f).

Between 11 and 13.5 years of age and length classes 450 to 480 cm, both non-reproductive (immature) and reproductive (maturing and mature) male pilot whales were observed. The estimated average age and length of the onset of maturity (i.e. the transition from immature to maturing) was 12.2 years and 454 cm, respectively (Table 5.2). The estimated average age and length at the attainment of full sexual maturity for males was 13.5 years, and 472 cm, respectively (Table 5.2, Figure 5.6). Model comparison on the basis of the LOOIC suggested that age was a better indicator of sexual maturity stage than TBL, but this result had little statistical support as the difference in LOOIC scores (6.1) was less than the standard error (SE = 9.6; *n* = 90).

Comparison with testicular variables

Variables related to testicular size (i.e. combined testes weight, combined testes length, index of testicular development, and mean seminiferous tubule diameter) for each maturity stage are summarised in Table 5.1. All demographic and testicular variables were highly positively correlated (Figure 5.5). The testicular variables followed the general pattern of increasing size with maturity stage, although, as with the demographic variables, there was some overlap between maturity stages (Table 5.1, Figure 5.5). A sharp increase in combined testes length was observed at approximately 13 years of age and 470 cm TBL, though a considerable overlap in combined testes length was found among individuals between 10 and 15 years of age and 400 to 500 cm in TBL (Figure 5.5). At approximately 11 years of age and 450 cm TBL, a rapid increase in combined testes weight was observed (see Figure 5.5). As observed in Figure 5.5, sexually immature and early-maturing males are clearly delineated from late-maturing and mature males based on combined testes weight, index of testicular development and mean seminiferous tubule diameter values. Large-scale individual variation was observed in the mature sample, with combined testes weights and lengths ranging from 3,000 to 13,020 g and from 645 to 1,340 mm. It was estimated that 50% of males have reached sexual maturity at 2,685 g in combined testes weight, 676 mm in combined testes length, 3.83 g/mm in the index of testicular development, and 125.3 µm in mean seminiferous tubule diameter (Table 5.2, Figure 5.6). Of all the modelled variables, comparison on the basis of the LOOIC indicated that the best indicators of sexual maturity were mean seminiferous tubule diameter and combined testes length (Table 5.2). It is important to note that all testicular variables were better indicators of maturity stage than age or TBL (Table 5.2).

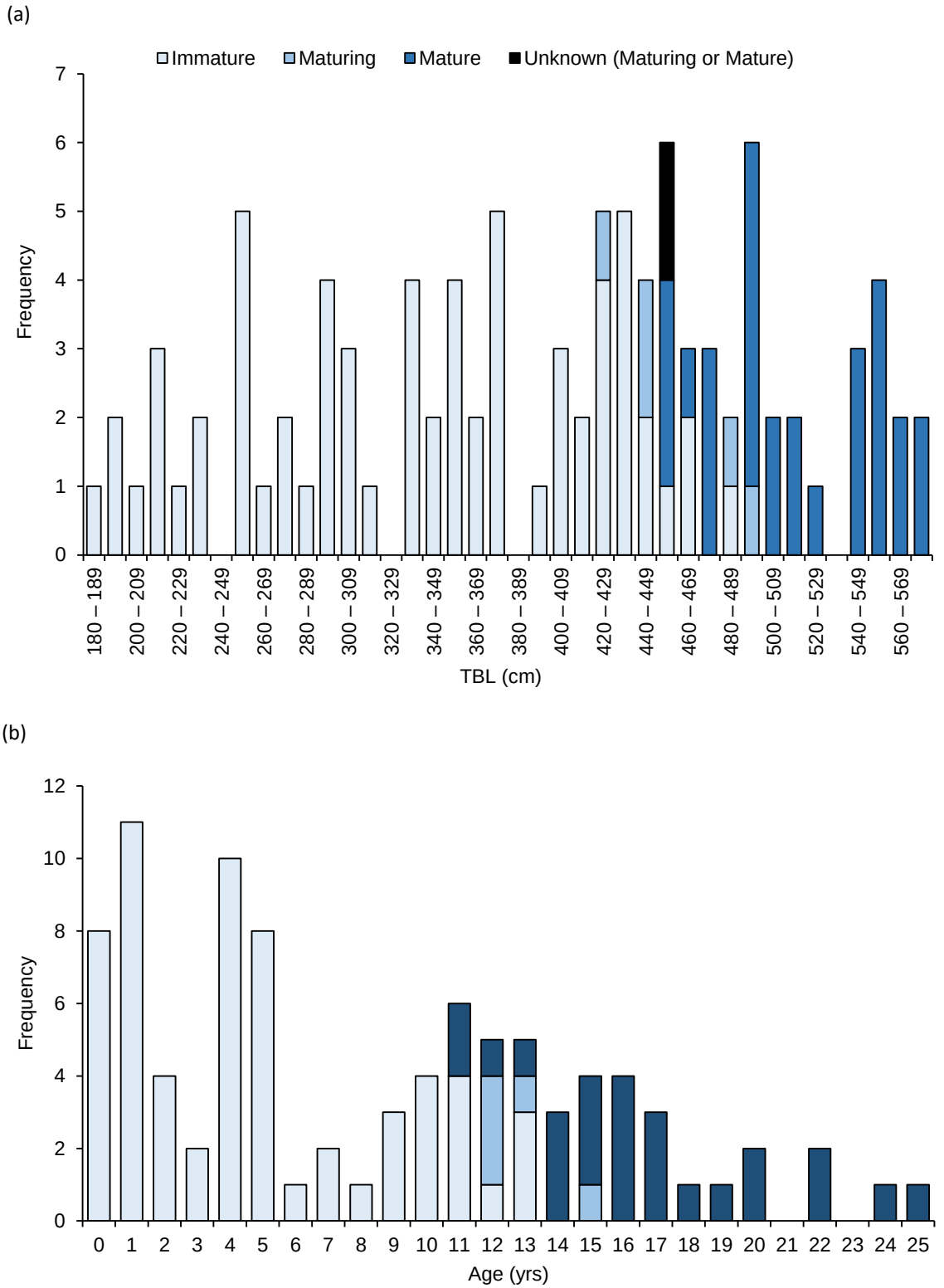


Figure 5.3 (a) Total body length (TBL; *n* = 98) and (b) age (*n* = 92) frequency distributions of immature, maturing, and mature male LFPWs.

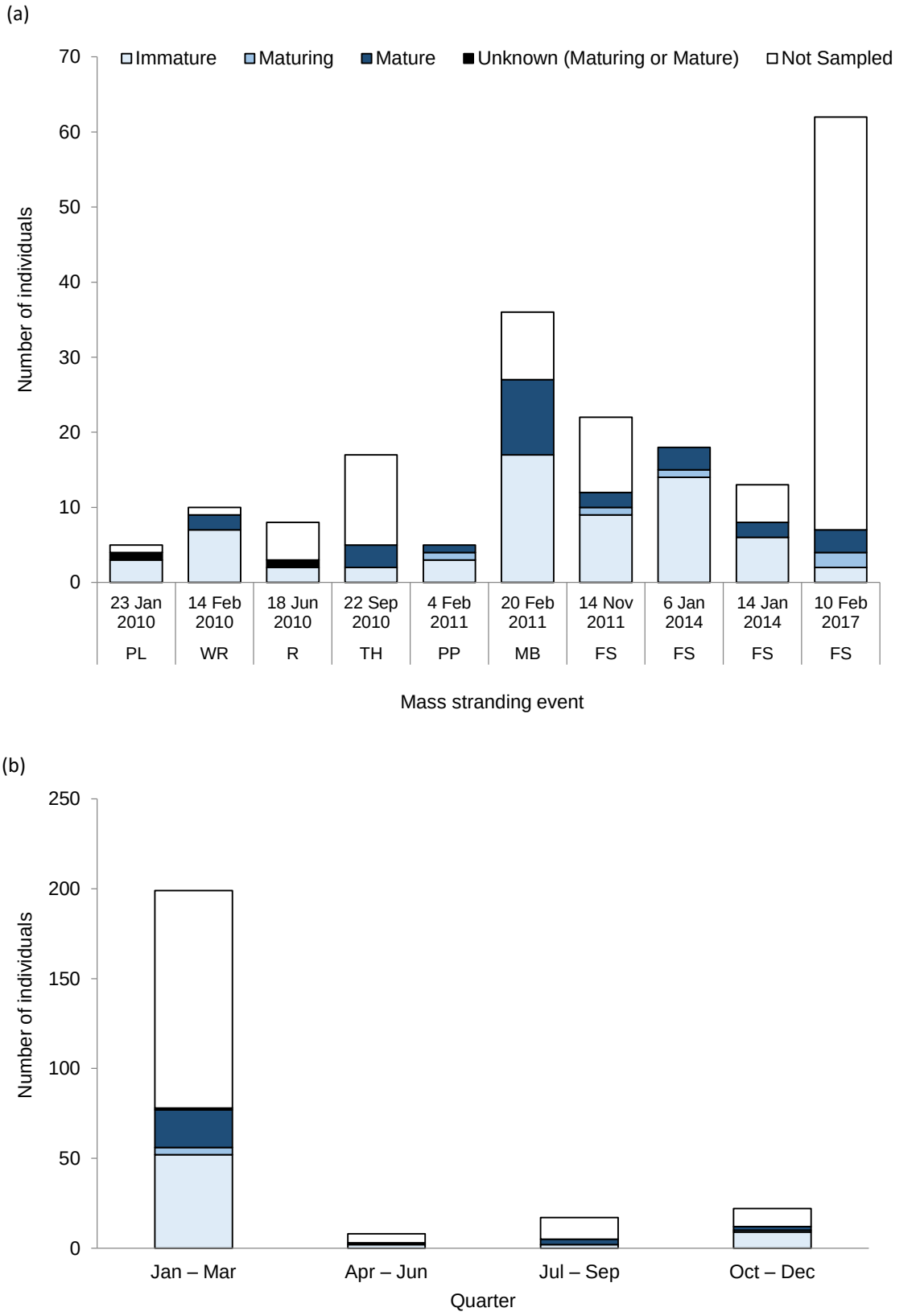


Figure 5.4. Number of male LFPW gonads collected from MSEs on the New Zealand coast by (a) MSE and (b) quarter (*n* = 98).

Stranding locations: FS = Farewell Spit, Golden Bay; PL = Port Levy, Banks Peninsula; WR = West Ruggedy Beach, Stewart Island; R = Ruapuke, Waikato; TH = Te Horo Beach, Spirit's Bay, Far North; PP = Port Puponga, Golden Bay; MB = Mason Bay, Stewart Island.

Table 5.2. Estimates of the age, total body length (TBL), combined testes weight, combined testes length, index of testicular development (index) and mean ($\bar{x}$) diameter of seminiferous tubules (mean DT) at which 50% of male LFPWs attain the two stages of sexual maturity (maturing and mature).

Indicator	Maturing $\bar{x}$ (95% CrI)	Mature $\bar{x}$ (95% CrI)	LOOIC (SE)
Age (yrs)	12.2 (11.4 – 13.0)	13.5 (12.7 –14.4)	18.73 (10.34)
TBL (cm)	454.0 (441.4 – 466.6)	471.5 (458.8 – 485.0)	22.76 (8.17)
Combined testes weight (g)	662 (465 – 1012)	2,685 (1,516 – 3,839)	10.59 (5.64)
Combined testes length (mm)	508 (447 – 579)	676 (587 – 775)	8.51 (4.45)
Index (g/mm)	1.46 (1.17 – 2.02)	3.83 (2.48 – 5.04)	11.93 (6.14)
Mean DT (μm)	76.59 (66.48 – 90.07)	125.26 (108.23 – 142.52)	7.47 (3.75)

Posterior distributions obtained using Bayesian cumulative logit regression and summarised with means and credible intervals. CrI = credible interval; LOOIC = Leave-One-Out Information Criterion (all variables together; *n* = 55); SE = standard error.

Reproductive seasonality

Most MSEs sampled in this study occurred during the first quarter (austral summer period; see Table 5.2), which is the peak stranding season for the species in New Zealand (Brabyn 1991; see Chapter 7). No mature males were sampled in the austral autumn or winter seasons (March – August). The largest male sampled during this period (TBL 450 cm, sampled in June, austral winter period) was identified as reproductive (based on the presence of spermatozoa) but was too autolysed to permit further classification into maturing or mature categories thus was not included in the statistical analysis. Spermatogenesis was observed in all mature individuals sampled in September, November, January, and February (austral spring and summer periods). Individuals with a high relative abundance of spermatozoa were also recorded in all months sampled, and there was no significant difference in the mean seminiferous tubule diameters of mature males among months (ANOVA $F_{3,11} = 0.817$, $p = 0.511$; Figure 5.7).

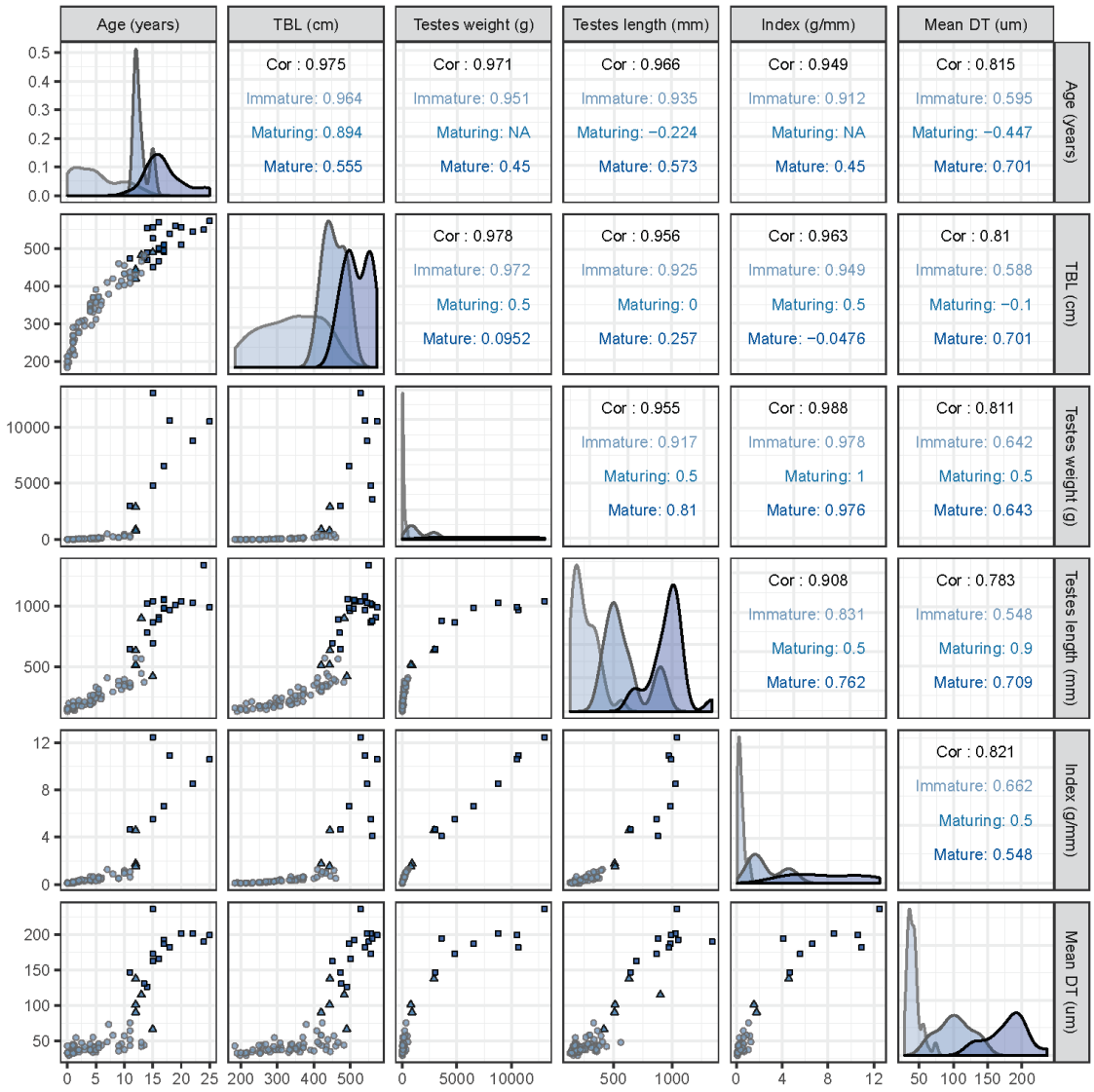


Figure 5.5. Demographic variables (age and TBL) versus testicular variables (combined testes length [age: *n* = 80, TBL: *n* = 86], combined testes weight [age: *n* = 57, TBL; *n* = 59], index of testicular development [age: *n* = 57, TBL: *n* = 59] and mean diameter of seminiferous tubules [mean DT; age: *n* = 76, TBL: *n* = 79]) for male LFPWs.

Note: All demographic and testicular variables were highly positively correlated (Cor = Spearman's rank correlation coefficients). Point types and colours represent the individuals' sexual maturity stage: immature = light blue circle, maturing = medium blue triangle, mature = dark blue square.

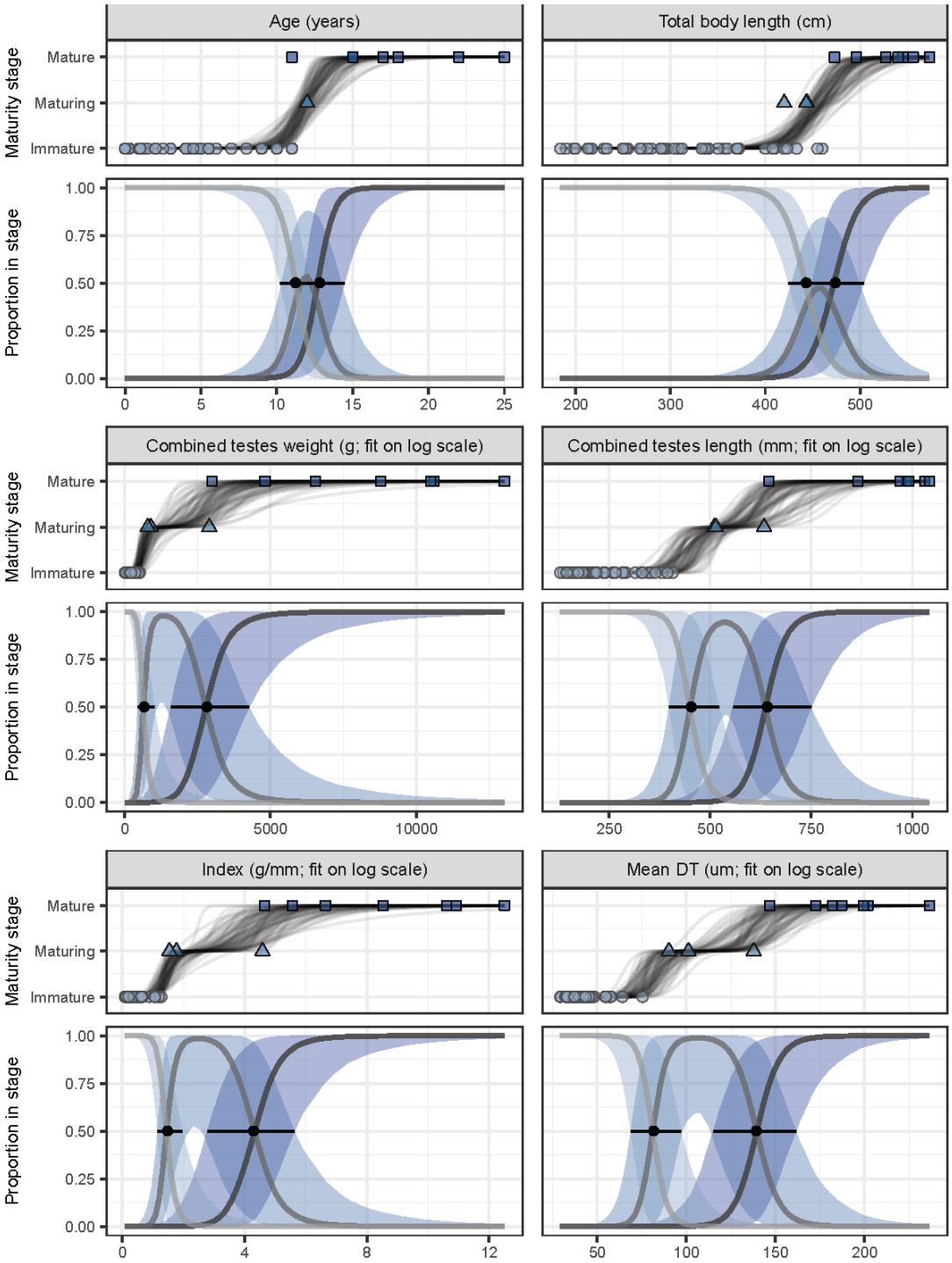


Figure 5.6. Bayesian cumulative logit regression of the sexual maturation of male LFPWs through three stages (immature, maturing, and mature) modelled as a function of one of six individual measures: age, total body length, combined testes weight, combined testes length, an index of testicular development (combined testes weight / combined testes length), and mean seminiferous tubule diameter (mean DT).

Two plots are shown for each measure. The upper plot shows the data values for the measure within each stage, with lines representing posterior predictions of the mean stage. On the lower plot, the thick lines show the estimated mean and 95% credible intervals for the probability of being in each of the three stages (increasing in maturity shown by lighter to darker lines and intervals, going left to right). The black points and thin horizontal lines show mean and 95% credible intervals of the estimated values of x at which 50% of males were classified as maturing or mature (i.e. not immature; left point and line), and fully mature (right point and line). Measures on the x-axis are shown on the raw scale, but models were fitted to the log-transformed testicular variables, as indicated.

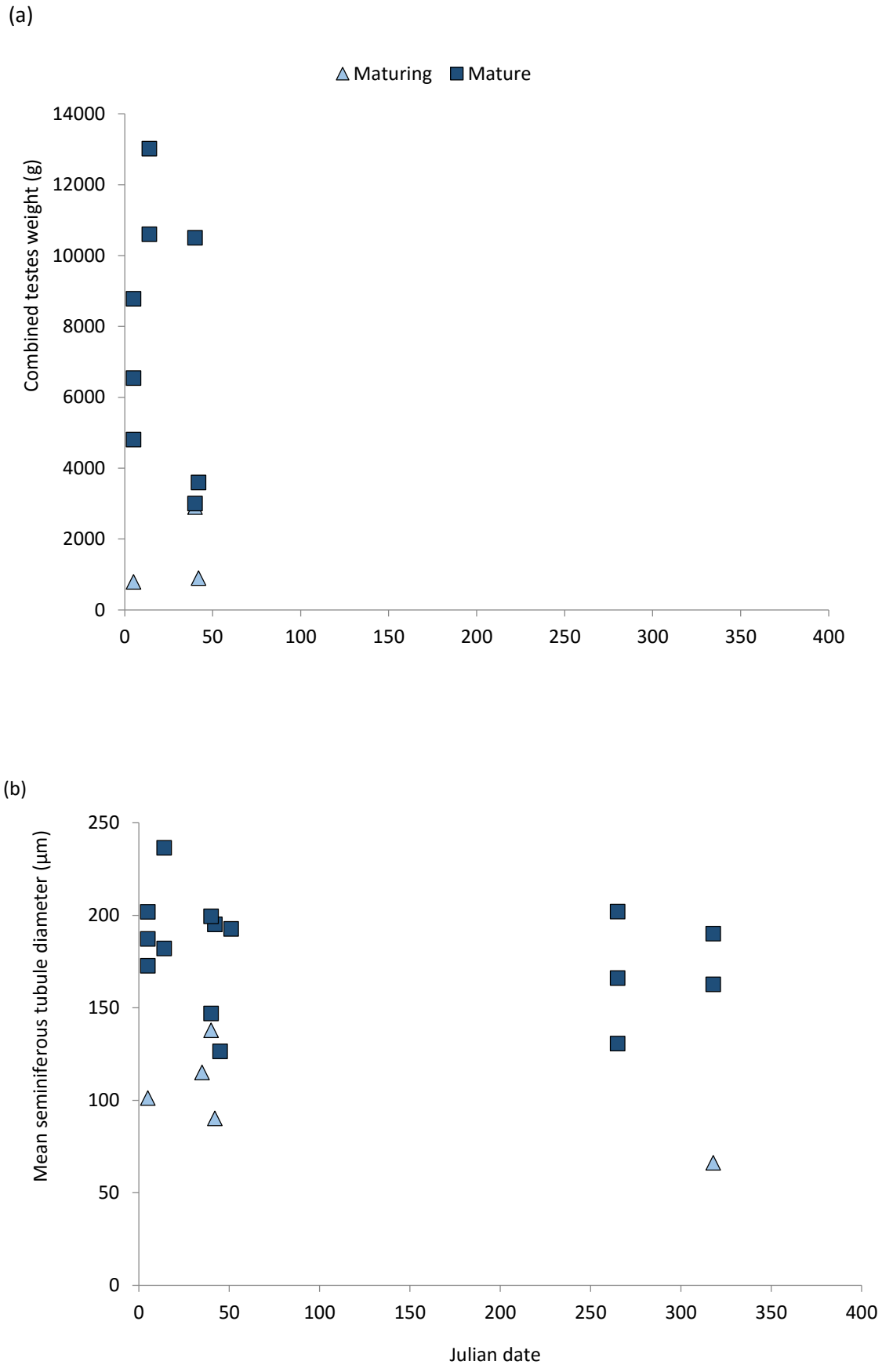


Figure 5.7. (a) Combined testes weights vs. Julian date (*n* = 11), and (b) mean seminiferous tubule diameter vs. Julian date (*n* = 20) of maturing and mature male LFPWs.

5.5 Discussion

Stages of sexual maturation

Morphological and histological analysis of male gonadal tissue from *G. m. edwardii* revealed that individuals could be readily classified into three maturity stages—namely 'immature', 'maturing', and 'mature'—as previously described for pilot whales by Kasuya and Marsh (1984) and Desportes et al. (1993b). Other studies on small delphinids defined alternative maturity stages such as 'pubertal' and 'young mature' (e.g. Miyazaki 1977, Kasuya et al. 1997, Murphy et al. 2005, Kemper et al. 2014). However, these stages are not comparable to the maturing stage reported in the current study. Maturing males are characterised by the presence of typically mature and immature seminiferous tubules in separate but adjacent areas of a testis histological section. Maturing males have been previously reported in several odontocete species, including the sperm whale (*Physeter macrocephalus*; Best 1969), melon-headed whale (*Peponocephala electra*; Amano et al. 2014), Risso's dolphin (*Grampus griseus*; Amano and Miyazaki 2004), and both the SFPW (Kasuya and Marsh 1984) and LFPW (*G. m. melas*; Desportes et al. 1993b). It is unknown whether the lack of observed 'maturing' individuals in small delphinid species reflects true differences in the sexual maturation process between small delphinids and the larger species listed above. Though, if the maturing stage is very short in duration in small delphinid species, it is possible that 'maturing' individuals may have been missed by chance.

Only five male *G. m edwardii* were classified as 'maturing' in this study, however, there were some indications of early-maturing (< 50% of tubules examined mature, n = 3) and late-maturing (between 50% and 100% of tubules examined mature, n = 2) individuals, as described for *G. m. melas* by Desportes et al. (1993b) and SFPWs by Kasuya and Marsh (1984). A rapid increase in testicular size (e.g. length, weight) was observed between the early and late-maturing individuals of both pilot whale species, suggesting that a rapid increase in testis growth is a feature of the maturing stage (Kasuya and Marsh 1984, Desportes et al. 1993, this study; see Figure 5.5). Desportes et al. (1993b) reported an apparent extended male maturing stage in *G. m. melas*, with individuals ranging in age from 11 to 22 years. Compared with *G. m. melas*, this period seems to be shorter in male *G. m. edwardii* (11 – 15 yrs); though this may be an artefact of the small sample size, and requires further investigation. The low proportion of maturing males examined suggests that sexual maturation occurs rapidly, or maturing animals are under-represented in the stranded groups examined, either due to sampling bias or age-sex segregation. However, it is unlikely that maturing animals are under-represented based on the observed age (11 – 15 yrs) and length class (420 – 490 cm) ranges (see Figure 5.3). Most

mammalian species progress through puberty at a rapid rate, and not in synchrony with their peers (Sinclair 1973). This is likely to be the case in LFPWs (Desportes et al. 1993b), and it has also been suggested for SFPWs (Goebel-Diaz 1986). As a consequence, the overall group information will likely underestimate the rate of sexual maturation and overestimate the length of the maturing stage for any one individual (Desportes et al. 1993b). Future sampling efforts should aim to target male *G. m. edwardii* around the length that sexual maturity is expected to be attained so that the duration of the maturing stage, as well as estimates of the ASM and LSM, can be further refined.

Indicators of sexual maturity

Histological examination of testicular tissue for staging spermatogenesis is required for a full assessment of sexual maturation and, within the current study, mean seminiferous tubule diameter was the best morphometric indicator of sexual maturation in *G. m. edwardii*. Mean seminiferous tubule diameter was positively correlated with combined testes weight and length in *G. m. edwardii*, as has been described in *G. m. melas* (Desportes et al. 1993b) and other delphinids (e.g. Miyazaki 1977, Kemper et al. 2014). As odontocete testes are particularly sensitive to post-mortem autolysis, especially when individuals die as a result of a live stranding event during periods of high summer temperatures, collection of fresh samples (< 24 hrs post-mortem) for histological examination is challenging (Laws 1961, Kemper et al. 2014). Thus, there is a need for the development of non-histological indicators of sexual maturation. Although body length and age data are generally considered when assessing maturity stage in other species (e.g. Murphy et al. 2005, Kemper et al. 2014), there was considerable overlap in these variables among maturity stages, even immature and fully mature males, in this and other pilot whales studies (e.g. Desportes et al. 1993b). Age was a better indicator of sexual maturation in *G. m. edwardii* than TBL, but this was not supported statistically. Previous research has suggested that testicular size data can be used to predict attainment of sexual maturity in pilot whales (Kasuya and Marsh 1984, Desportes et al. 1993b) and other small odontocete species (e.g. Perrin et al. 1977, Neimanis et al. 2000, Murphy et al. 2005, Kemper et al. 2014). The range of values for combined testes length in maturing *G. m. edwardii*, a variable that was previously suggested by Desportes et al. (1993b) to be the best (non-histological) indicator of sexual maturity in *G. m. melas*, overlapped with both the immature and mature categories. However, when a single late maturing male was included in the mature category, combined testes weight, combined testes length, and an index of testicular development (combined testes weight/combined testes length) were all considered good non-histological indicators of sexual maturation in *G. m. edwardii*. Given the ease of collection and

applicability to suboptimal material collected from stranding events, further effort should be applied to assessing the value of predicting sexual maturity on the basis of testicular size in male *G. m. edwardii.*

Attainment of sexual maturity

Using equivalent maturity stages, *G. m. edwardii* off New Zealand attained an average age at the onset of both the maturing and mature stages considerably earlier than *G. m. melas* off the Faroe Islands (Desportes et al. 1993b). The maturing stage was attained, on average, approximately two years earlier (at an ASM of 12.2 vs. 13.9 yrs and LSM of 454 vs. 486 cm), while the mature stage was attained approximately three years earlier (at an ASM 13.5 vs. 16.8 yrs and LSM of 472 vs 516 cm) (Desportes et al. 1993b, this study). Comparison with other studies of North Atlantic (e.g. Sergeant 1962a, Martin et al. 1987, Sigurjonsson et al. 1993) or Southern Hemisphere (Schroder and Castle 1998) LFPW populations is difficult since they are based on insufficient sample sizes, or did not attempt to estimate ASM or LSM using comparable methods. Comparison is possible, however, with studies of the southern and northern forms of the related SFPW (Kasuya and Marsh 1984, Kasuya and Tai 1993) since sample sizes were large and similar assessment methods and criteria were used. Many results were similar between both pilot whale species, including the correlation of age and body length with sexual maturation. The ASM occurred at approximately 17 years in both forms of the SFPW, with the LSM estimated at 422 cm for the southern form and 560 cm for the northern form (Kasuya and Marsh 1984, Kasuya and Tai 1993).

Attainment of sexual maturity is influenced by the general health of the animal, although factors such as hierarchical position, mate availability, genetics, prey availability, and consumption of prey high in contaminant levels (especially endocrine-disrupting chemicals) may also have potentially confounding effects (Murphy et al. 2005). Although individual variation in the ASM is common among mammals, population-level declines in ASM have been associated with relative reductions in population abundance. For example, relative declines in the ASM have been correlated with changes in population size due to exploitation in baleen whales (e.g. Lockyer 1984, Boyd et al. 1999) and both incidental capture (e.g. Perrin et al. 1976, Perrin et al. 1977) and exploitation (e.g. Kasuya 1985) in small delphinids. LFPWs around the Faroe Islands have been hunted since at least the ninth century (Sanderson 1992). Catches of LFPWs in the Faroese drive fishery have remained consistent for at least the last 300 years, with an average annual catch of 850 whales (range 0 – 4,480; NAMMCO 2018). The abundance of the *G. m. melas* population in the eastern North Atlantic is estimated to be approximately

778,000 (CV = 0.295) whales, based on the most extensive survey in the region conducted in 1989 (Buckland et al. 1993). Evidence that multiple subpopulations (or at least whales from multiple regions of the North Atlantic) are taken in the fishery suggests that the removal probably represents less than 1% of the local population subject to harvesting (Wade et al. 2012), and is considered sustainable by the North Atlantic Marine Mammal Commission (NAMMCO 2018a). The abundance of LFPWs in the Southern Hemisphere is not well understood, but *G. m. edwardii* does not have a history of significant exploitation, except possible isolated incidents in the 19th century (during the peak whaling period) (Reeves et al. 2003).

Possible explanations for (non-exploited) LFPWs off New Zealand having a lower ASM, and LSM, than (exploited) LFPWs off the Faroe Islands, are: (1) frequent, large MSEs may have a significant impact on the local population of *G. m. edwardii*; (2) favourable conditions in temperate Southern Hemisphere waters could potentially support further population growth (i.e. the carrying capacity has not been reached); (3) real population-level differences between the two subspecies, such as smaller mean body size and shorter lifespan in *G. m. edwardii* (see Chapter 3); (4) smaller sample size for *G. m. edwardii* leading to an underestimation of ASM and LSM; and (5) any combination of the above. Exposure to pollutants has been reported to cause a decline in spermatogenesis and fertility, and alter or delay sexual maturation in male mammals (Diamanti-Kandarakis et al. 2009). LFPWs inhabiting waters in the Faroe Islands have relatively high levels of organochlorines (DDT and PCB), cadmium, and mercury (Borrell and Aguilar 1993, Caurant et al. 1993, Dam and Bloch 2000, Sonne et al. 2010), with consumption of pilot whale meat deemed to be hazardous to the health of consumers (Simmonds et al. 1994, Weihe et al. 1996, Weihe and Debes Joensen 2012). Exposure to pollutants could potentially have caused delayed attainment of sexual maturity in some individuals, which may explain the protracted maturing stage (11 – 22 yrs) reported in *G. m. melas* outlined earlier, though this requires assessment. Alternatively, the Faroese sample may have been composed of individuals from multiple subpopulations, exhibiting different reproductive traits. Pollutant burdens of LFPWs in New Zealand waters are not well understood, but a preliminary study reported PCB concentrations to be two to three orders of magnitude lower than in North Atlantic LFPWs (Schroder and Castle 1998).

Reproductive seasonality

Large individual variation was observed in combined testes weight (3,000 – 13,020 g) in sexually mature *G. m. edwardii*, though this was not related to reproductive seasonality due to

the seasonally limited sampling of MSEs (austral spring and summer; see Figure 5.7), which occurred during the proposed peak mating period (see Chapter 6). Similar individual variation in testis weight was observed in sexually mature *G. m. melas* off the Faroe Islands (single testis weight 807 – 6,150 g, *n* = 241; Desportes et al. 1993). Testicular activity in *G. m. melas* off the Faroe Islands is diffusely seasonal, with an overall 1.5-fold increase in testis weight during the proposed mating period between March and September, i.e. boreal spring, summer and early autumn (Desportes et al. 1993b). Though, large testis weights and high spermatozoa densities were still observed in some individuals between October and February, suggesting that testicular activity does not entirely cease outside the proposed mating period (Desportes et al. 1993b).

Mating strategy

Understanding how individual males allocate their resources to reproduction is challenging. Game theory specifies that males have a fixed budget for reproduction, and there can be a trade-off between investment in precopulatory traits (e.g. body size, armaments, ornaments) and postcopulatory traits (e.g. testis size, spermatogenic efficiency; Dines et al. 2015). If males can monopolise access to females, they will place greater investment in precopulatory traits, which are used for defence and/or aggressive interactions (Parker et al. 2013, Lüpold et al. 2014, Dines et al. 2015). Thus, it has been suggested that selection for pre- or postcopulatory sexual traits can indicate the mating system of a species for which observations of mating behaviour are rare (MacLeod 2010, Dines et al. 2015). LFPWs live in matrilineal social groups, and although males remain within their natal group, limited genetic evidence suggests that they do not tend to mate within it; mating must occur when two or more groups meet, or when adult males visit other groups (Amos et al. 1993a). Prominent precopulatory traits documented in male pilot whales include sexual size dimorphism (with males on average 30% larger than females), larger and more bulbous heads, larger and thicker dorsal fins, and deeper tail stocks (Jefferson et al. 2008; Chapter 3). Most authors assume that LFPWs are polygynous, drawing attention to the sexual dimorphism observed in the species (e.g. Sergeant 1962a, Evans 1987). However, there is limited evidence for male combat in pilot whales; “their dimorphic characters appear to function as ornaments, rather than armaments” (Dines et al. 2015: page 1568). After taking into account body mass, the LFPW has the fourth largest residual testes mass in cetaceans (MacLeod 2010). The lack of a trade-off with testis size suggests that male pilot whales are not able to monopolise access to females to the same extent as those with combat, and therefore also need to invest in postcopulatory traits such as

testis size (Dines et al. 2015), suggesting that sperm competition is of importance within its mating strategy (MacLeod 2010).

In some cetacean species, including pilot whales, killer whales (*Orcinus orca*), and sperm whales, males often do not reach sexual maturity until 1.5 to 2 times the age of females, allowing them additional time for growth in size (Robeck and O'Brien 2017). This was also observed in *G. m. edwardii* off New Zealand where females attained sexual maturity 6.8 years, on average, earlier than males (see Chapter 6). Physical maturity is not obtained until well after sexual maturity in *G. m. edwardii* (asymptotic TBL 570 cm and age > 40 years; Chapter 3) and a pattern of protracted growth is suggested, similar to that reported for *G. m. melas* off the Faroe Islands (Bloch et al. 1993a). Attainment of social maturity and social dominance are recurring themes in studies of male reproduction in many mammalian taxa (Ellis 1995). Although a young male may be physiologically capable of reproducing, he is rarely able to copulate successfully with a female or compete with dominant males until he is older (Evans and Raga 2001). For example, male North Atlantic right whales (*Eubalaena glacialis*) do not successfully reproduce until at least 15 years of age, almost twice the age of first reproduction in females (Frasier et al. 2007). In Atlantic spotted dolphins (*Stenella frontalis*), reproductive success appears to be based on social standing or attainment of larger body size as the youngest known male to successfully reproduce was 18 years of age, several years after attainment of sexual maturity in the species (Green et al. 2011).

As noted earlier, combined testes weight in sexually mature males varied considerably on an individual basis. However, male *G. m. edwardii* with the largest testes (> 8 kg) were large individuals (527 – 573 cm TBL). Testicular size (combined testes length and weight, and an index of testes development) continued to increase (an apparent almost linear increase between combined testes weight with TBL in mature individuals; see Figure 5.5) after sexual maturity was attained. Although it is not known if there is an age-related increase in male reproductive success in the subspecies, the larger an individual was, the larger the investment in reproduction, as previously reported for *G. m. melas* by Desportes et al. (1993b) and SFPWs by Kasuya and Marsh (1984). This is in contrast to what has been suggested for some mammal species, where sexually mature males that are not large enough to compete directly with larger males may have relatively large testes as a consequence of greater investment in sperm competition (Stockley and Purvis 1993, Connor et al. 2000). As the sample of male pilot whales examined in this study did not contain any males over 25 years of age, no inference can be made about the potential cessation of testis growth reported in *G. m. melas* and SFPWs Kasuya and Marsh (1984).

Although *G. m. edwardii* attain sexual maturity at a smaller size and younger age than *G. m. melas* off the Faroe Islands, there are indications that testicular size is larger in the former subspecies – and amongst the largest published to date for the species. The largest combined testes weight of 13 kg and maximum single testis length of 53 cm were recorded in a 527 cm male that stranded in month of January (austral summer); the (heaviest) single testis weight of 6.7 kg (for the right testis) in this individual was heavier than the maximum single testis weight of 6.2 kg recorded in *G. m. melas* (Desportes et al. 1993b). Although associated weights were not available, several males in the current study had single testis lengths exceeding 53 cm, thus suggesting that maximum combined testes weights for *G. m. edwardii* in New Zealand waters likely exceed 13 kg. The longest single testis measured in this study was 69 cm in a 550 cm male that stranded in November (austral spring), which is considerably longer than the maximum testis length of 50 cm recorded in a *c*. 560 cm male *G. m. melas* (Desportes et al. 1993b).

Theory predicts that healthier individuals are more likely to have large testes, and be more capable of producing large volumes of protein-rich sperm (Forsyth 2001, Murphy et al. 2005), whereas relatively unfit individuals are less able to maintain large testes size and high rates of spermatogenesis (Olsson et al. 1997, Schulte-Hostedde et al. 2005). Despite smaller mean total body lengths (mature mean TBL = 518 cm in the Southern Hemisphere subspecies vs. mature mean TBL = 561 cm in the North Atlantic subspecies), attainment of a larger testicular size *G. m. edwardii*, compared with *G. m. melas* may be an indication that: (1) there are real differences in mating strategy between the two subspecies, with *G. m. edwardii* investing more in sperm competition, (2) *G. m. edwardii* is not resource-limited, (3) suffers less from disease, and/or (4) has lower exposure to endocrine disrupting chemicals, and thus can invest more heavily in reproduction.

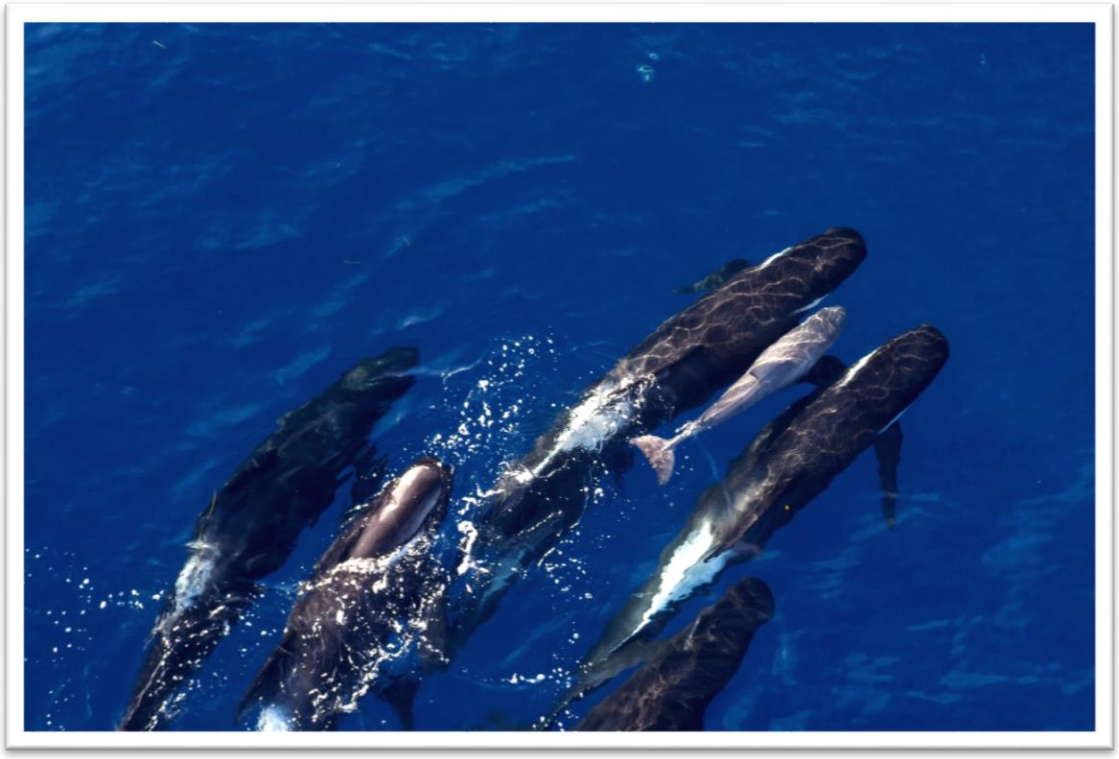
5.6 Conclusions

In summary, this study has contributed to our understanding of the reproductive biology of the Southern Hemisphere subspecies of LFPW, with first descriptions of male sexual maturation and estimates of ASM and LSM provided. The attainment of sexual maturity at a smaller body length and a younger age in *G. m. edwardii* off New Zealand compared to *G. m. melas* in the eastern North Atlantic is notable. Further sampling of maturing male *G. m. edwardii* is recommended to refine estimates of ASM, LSM, duration of the maturing stage, and indicators of sexual maturity based on testicular size. Additionally, temporal monitoring of ASM and LSM, alongside assessments of environmental and anthropogenic stressors that may impact those

parameters would provide some insight into the condition of the population and its relative carrying capacity. An examination of reproductive seasonality in addition to further information from genetic studies, detailed analysis of group structure (in MSEs and at sea), and behavioural observations of individuals are needed to confirm mating strategies, and when social maturity is attained in *G. m. edwardii* off New Zealand.

Chapter 6

Reproductive parameters of female long-finned pilot whales (*Globicephala melas edwardii*) in New Zealand waters



A long-finned pilot whale gives birth to a calf on the edge of the continental shelf. Then, with another adult, the mother lifted her newborn to the surface for its very first breath. Offshore, northern New Zealand, January 2017.

In this chapter, a description of the reproductive biology of female long-finned pilot whales (*Globicephala melas edwardii*) in New Zealand waters is presented. Parameters such as the average age and length at sexual maturity, ovulation rate, gestation period and foetal growth rate, average date of conception, average length-at-birth, lengths of lactation and resting periods, annual pregnancy rate, and calving interval are estimated. Ovarian symmetry, senescence, and indicators of seasonality are also assessed to achieve the fourth research objective:

Objective 4: Estimate the reproductive parameters of female *G. m edwardii*, and investigate evidence of reproductive senescence and seasonality

This chapter is a reformatted version of the following manuscripts:

Betty EL, et al. (in prep). Reproductive parameters of female long-finned pilot whales (*Globicephala melas edwardii).* Marine Biology.

Betty EL, et al. (in prep). Lack of evidence for a post-reproductive phase in female long-finned pilot whales (*Globicephala melas edwardii*). PLOS ONE.

6.1 Abstract

Mass stranding events (MSEs) provide a valuable source of biological information, especially for relatively inaccessible oceanic delphinids, such as long-finned pilot whales (*Globicephala melas*). However, despite the frequent occurrence of MSEs, knowledge of the life history of the southern subspecies of LFPW (*G. m. edwardii*) is limited, with no estimates of reproductive parameters available for the population utilising New Zealand waters. Reproductive samples, teeth, and morphometric data were collected from 166 females, following 14 stranding events on the New Zealand coast between 2008 and 2017. Specimens were examined for evidence of pregnancy and lactation, ovaries were weighed and the number of *corpora* determined grossly. Age was estimated from growth layer groups in the teeth. Attainment of sexual maturity was estimated to occur at an average age of 6.7 years, and body length of 356 cm. The oldest female in the sample assessed for reproductive status was 33 years, and the maximum TBL was 485 cm. In mature individuals, the total number of *corpora* increased with age and appeared to persist throughout life. Significantly more *corpora* were present on the left ovary, with a maximum of 19 *corpora* recorded in any one individual. Mean ovulation rate for mature females was 0.4 year^{-1}. Estimated annual pregnancy rate was 19%. Calving occurs throughout the year, with females estimated to produce a calf every 5.3 years; after an average gestation period of 13.6 months, an average lactation period of 19.3 months, and an average resting period of approximately two years. No evidence of senescence was found. Estimated length-at-birth, average age and length at the attainment of sexual maturity, maximum size, and maximum age are lower than best estimates reported for the North Atlantic subspecies (*G. m. melas*), indicating that large-scale geographic variation in life history occurs for this species, likely reflecting population-specific adaptations to local habitats.

6.2 Introduction

Studies of life history and reproduction are essential for cetacean conservation because they provide the information required to assess species, populations, and social dynamics (Jefferson et al. 2012, Learmonth et al. 2014, Kemper et al. 2019). Age structure and fecundity rate (i.e. annual proportion of adult females giving birth; Arso Civil et al. 2017) are the primary determining factors of recruitment to a population, yet for many cetacean species and populations, these remain unknown (Kemper et al. 2019). Age at sexual maturation and maximum age (or reproductive senescence) delimit female reproductive lifespan, and along with fecundity and inter-calving/inter-birth interval, determine an individual's lifetime reproductive output (Wade 2018, Kemper et al. 2019). For cetaceans, data from stranded and bycaught animals, despite known biases and limitations, remain the primary (and often only) source of life history information for many populations (Learmonth et al. 2014). In these circumstances, mortality data are often essential for modelling the viability of populations and assessing the effects of extrinsic influences on population dynamics (Stolen and Barlow 2003), as well as allowing inter- and intraspecific population comparisons (Hohn et al. 1985).

In a few mammalian species, including humans (*Homo sapien*), short-finned pilot whales (SFPWs; *Globicephala macrorhynchus*), and killer whales (*Orcinus orca*), females can spend a significant proportion of their adult lifespan post-reproductive, which is contradictory to classical life history theory (Photopoulou et al. 2017). Pilot whales and killer whales both form long-term stable matrilineal groups composed of closely related females (with an increase in local relatedness with age), as well as strong-mother offspring associations with long periods of dependency (Marsh and Kasuya 1990, Brent et al. 2015, Whitehead 2015, Croft et al. 2017). Neither sex disperses from the group, though males mate outside the matriline and daughters raise their offspring within the group (Amos et al. 1993b, Johnstone and Cant 2010). This type of social structure where adult males stay with their female kin and mate elsewhere is unusual among mammals (Olson 2018). Explanations for the adaptive prolonged post-reproductive lifespans include the 'mother hypothesis' and 'grandmother hypothesis', where older non-reproductive mothers maximise their inclusive fitness by aiding and enhancing the survival of others in their group (e.g. Johnstone and Cant 2010, Foster et al. 2012, Brent et al. 2015, Whitehead 2015, Croft et al. 2017).

Comprehensive studies on female reproduction have been completed for populations of long-finned pilot whales (LFPWs; *G. m. melas*) in the North Atlantic, where drive fishery catches in Newfoundland (Sergeant 1962a, Kasuya et al. 1988b) and the Faroe Islands (Donovan et al.

1993) provided the opportunity to gather biological information. These studies have shown that *G. m. melas* shares several features of life history with other large odontocetes, e.g. long life span, delayed maturity, females attaining sexual maturity before males, seasonal mating, and the production of a single calf in multiyear intervals (Sergeant 1962a, Kasuya et al. 1988b, Martin and Rothery 1993, Olson 2018). Females live past 50 years; while males reach 35 to 45 years of age (Sergeant 1962a, Kasuya et al. 1988b, Bloch et al. 1993a). Attainment of sexual maturity differs between *G. m. melas* in Newfoundland and the Faroe Islands; females off Newfoundland mature at an average of 6 to 7 years (males 12 yrs; Sergeant 1962a, Kasuya et al. 1988b), while females off the Faroe Islands mature, on average, at 8.3 years (males 17 yrs; Desportes et al. 1993b, Martin and Rothery 1993). Conceptions and births are diffusely seasonal, with peaks in summer and autumn off the Faroe Islands (Martin and Rothery 1993). The mean calving interval was estimated to be 3.3 years off Newfoundland (Sergeant 1962a) and 5.1 years off the Faroe Islands (Martin and Rothery 1993). Available evidence suggests that *G. m. melas* exhibits similar life history characteristics and social structure to SFPWs (Amos et al. 1993b, Martin and Rothery 1993) yet, curiously, *G. m. melas* does not appear to have a significant post-reproductive lifespan (Martin and Rothery 1993, Ellis et al. 2018b). Given that population variability in life history parameters exists for this species (see Chapters 2 – 5), it is important to characterise reproductive parameters for *G. m. edwardii*, specifically.

The population status and reproductive parameters of *G. m. edwardii* in New Zealand, and throughout the Southern Hemisphere are poorly understood (Minton et al. 2018). Within this region, and New Zealand in particular, large MSEs of *G. m. edwardii* are frequent, and have high *in situ* mortality rates (see Chapter 7). However, baseline population levels and changes in abundance as a result of this stranding-related mortality remains unknown (see Chapter 4). Here, reproductive parameters are estimated from post-mortem data to describe the dynamics of the *G. m. edwardii* population utilising New Zealand waters. The current study examined reproductive organs opportunistically collected from female *G. m. edwardii* stranded on the New Zealand coast and presents the first detailed descriptions of many female reproductive parameters for LFPWs in the Southern Hemisphere. Specifically, the following are investigated: (1) average age (ASM) and body length (LSM) at the attainment of sexual maturity, (2) ovarian symmetry, (3) persistence of ovarian *corpora* scars and their utility to infer ovulation rate, (4) fecundity and calving interval, (5) trends in reproductive parameters with age (i.e. evidence of senescence), and (6) indicators of seasonal breeding. Where possible, comparisons are made with estimated reproductive parameters for other *G. m. edwardii* populations in the Southern Hemisphere and *G. m. melas* populations in the North Atlantic.

6.3 Materials and methods

Sample collection

During the 10-year sampling period (2008 – 2017), reproductive data were collected from 166 female *G. m. edwardii,* following 14 independent stranding events on the New Zealand coast (Figure 6.1). Total body length (TBL) was measured to the nearest centimetre and decomposition state was assessed following Geraci and Lounsbury (2005). Where possible, teeth were collected for age determination, and reproductive organs (ovaries and uteri) were removed *in situ* via standard post-mortem procedures (after Geraci and Lounsbury 2005). Both ovaries were examined from 148 females, a single ovary from seven females, and none from 11 females. The 11 females for which no ovaries were collected were composed of five pregnant, two lactating, three resting mature, and one indeterminate mature.

Ovaries were examined grossly for the presence of *corpora albicantia* (CAs) and *corpora lutea* (CLs), and then carefully dissected from the reproductive tract. Depending on the resources available in the field, the ovaries were either fixed in 10% neutral buffered formalin immediately upon collection or, if not possible, placed in separate labelled bags and frozen before fixation in the laboratory. The maximum diameter of each uterine horn was measured, and pregnancy was established by the presence of a foetus in the uterus. If a foetus was present, it was photographed, weighed, measured, and sexed if possible (see Appendix 6A). If pregnancy was indicated by the condition of the uterus and/or a CL was present, but no foetus was found, special care was taken to look for either a tiny foetus or evidence of a recent birth (e.g. condition of the mammary glands).

Evidence of lactation was examined via external pressure applied around the mammary slit and noting any fluids emitting from the nipples, and internally by sectioning through the mammary glands and recording the presence of milk. Unfortunately, due to time and logistical constraints, not all information was collected from each carcass, so sample sizes vary between parameters and are reported throughout.

Age estimation

Age was estimated by examining decalcified and stained tooth sections using a binocular microscope (10 – 40× magnification) and counting annual growth layer groups (GLGs) in the dentine, as defined by Perrin and Myrick (1980). Tooth preparation methods were adapted from the protocol described by Lockyer (1993a) and are outlined in more detail in Chapter 3. All age estimates were initially made ‘blind’ (with no biological information on the animal),

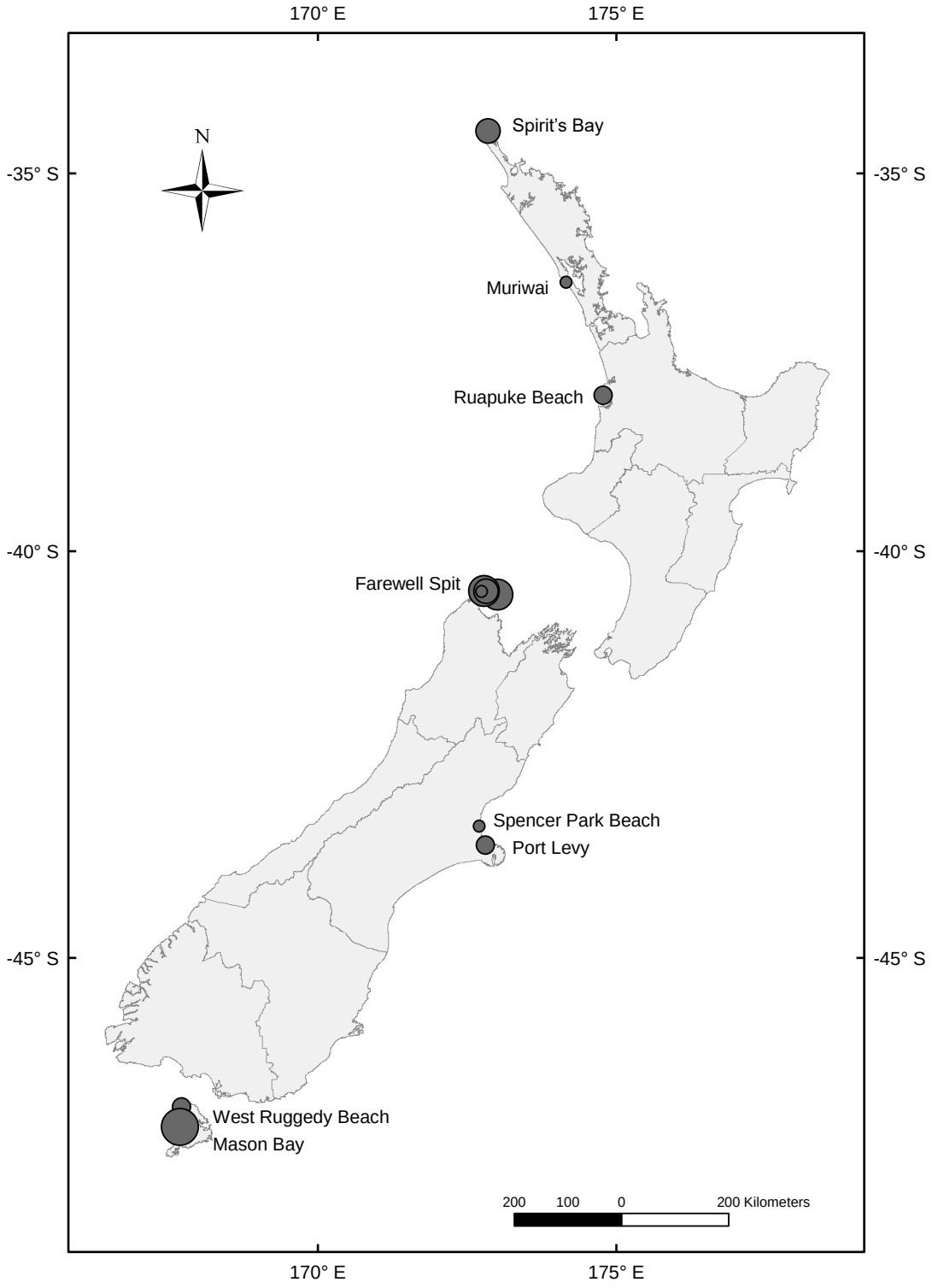


Figure 6.1. Location of LFPW stranding events on the New Zealand coast, from which female reproductive samples were collected for this study.

Locations: Spirit's Bay, Far North, 22 Sep 2010 *n* = 16; Muriwai, Auckland 03 Nov 2014 *n* = 1; Ruapuke Beach, Waikato, 18 June 2010 *n* = 7; Farewell Spit, Golden Bay 23 Jan 2008 *n* = 1, 25 Dec 2009 *n* = 2, 4 Feb 2011 *n* = 2, 14 Nov 2011 *n* = 29, 5 Jan 2014 *n* = 21, 14 Jan 2014 *n* = 13, 9 Feb 2017 *n* = 3; Spencer Park Beach, Christchurch 11 Feb 2009 *n* = 1; Port Levy, Banks Peninsula, 24 Jan 2010 *n* = 9; West Ruggedy Beach, Stewart Island, 14 Feb 2010 *n* = 10; Mason Bay, Stewart Island, 20 Feb 2011 *n* = 51). The size of the location icon is representative of the number of individual females sampled at that location per stranding event.

with replicate counts made by at least two experienced readers (see Chapter 3). Individuals for which age could not be estimated reliably were excluded from further analysis. Calves that did not possess a neonatal line in the tooth, or had a neonatal line forming, with no additional postnatal dentine, were classified as newborns.

Reproductive status

Assessment of female reproductive status was determined through ovarian, uterine and mammary gland examination, following the procedures and terminology recommended by the International Whaling Commission (IWC; Perrin and Donovan 1984) and adapted for pilot whales by Kasuya and Marsh (1984) and Martin and Rothery (1993). Females were considered sexually mature if at least one CL or CA was present on their ovaries and/or they were pregnant or lactating. Mature females were further classified into three reproductive states following the criteria of Perrin and Donovan (1984): (1) pregnant (visible presence of a foetus and a CL of pregnancy [CLP] but not lactating, based on the absence of milk in the mammary glands), (2) lactating (active mammary glands producing milk but not visibly pregnant), (3) resting (presence of a least one CL or CA but no signs of pregnancy or lactation). The resting class includes true "resting" mature females, as well as ovulating females (based on the presence of a CL and large follicles but no signs of pregnancy, i.e. CL of ovulation [CLO]). There is a possibility that some females in the very early stages of pregnancy may have been erroneously classified as "resting", with the CLP wrongly classified as a CLO. It is not known when pregnancy first becomes macroscopically detectable in pilot whales (Kasuya and Marsh 1984).

Average age and body length at the attainment of sexual maturity

Average female age and length at the attainment of sexual maturity were modelled with Bayesian cumulative logit models fitted with the 'brms' package (Bürkner 2017) for R (R Development Core Team 2018). To compare the utility of age (*n* = 150) and TBL (*n* = 161) for predicting maturity status, each was used as the single predictor variable (x) in turn. Maturity status (Y) was treated as a binary variable (0 for immature; 1 for mature), and the probability of a female being mature ($\pi = P(Y = 1)$) was modelled as:

$$\log\left(\frac{\pi}{1-\pi}\right) = \alpha + \beta x$$

Weakly informative prior distributions ($\text{Student's } t(0, 10)$) were assumed for the two parameters (intercept α and slope β). The posterior distribution of $c = \alpha/-\beta$ was used to

estimate the values of x at which 50% of females were classified as sexually mature. These values were summarised *via* the mean and 95 percentile credible intervals (CrIs).

The relative utility of age and TBL as predictors of maturity in the logistic regression models was compared using the Pareto-smoothed importance sampling (PSIS) approximation of the Leave-One-Out Information Criterion (LOOIC; using the 'loo' package for R, Vehtari et al. 2017, 2018). LOOIC compares the accuracy of candidate models for predicting out-of-sample data; thus, it is used here to compare how well maturity status would be predicted by TBL *vs* age for females not included in the dataset. The two models were refitted for the purposes of comparison, using the cases that were complete for both TBL and age (n = 146). The models were also refitted during the PSIS procedure for any 'problematic' observations (i.e., with Pareto k > 0.7), as recommended by Vehtari et al. (2017).

Ovarian examination

Before examination, formalin-fixed ovaries were rinsed in water for 24 to 48 hours, depending on their size, and then transferred to 70% ethanol. For each ovary, maximum length, width and depth were recorded to the nearest 0.1 mm using Vernier callipers, and the weight recorded to the nearest 0.1 g. Ovarian volume was calculated as the product of length, width and depth measurements. Combined weight was calculated for those females from which two ovaries were collected. *Corpora* scars present on the ovaries were described according to a classification adapted from Marsh and Kasuya (1984). *Corpora lutea* (CL) and three stages of *corpora albicantia* (CA; scars left from regressing CLs), are described as follows: CL, an endocrine gland easily recognisable as a protuberance from the ovary surface, often larger than the ovary, trabeculae and periphery obvious, usually yellow or orange in colour; young CA (YCA), moderately protruding from the surface of the ovary, appearing as round, smooth knobs with a distinct stigma, trabeculae and periphery obvious, pale orange to white internal colour; medium CA (MCA), usually slightly protruding from the ovary surface, may be round or flattened with a smooth surface, stigma and trabeculae less obvious than YCA, periphery easily identified, usually white colour internally but may have orange or brown pigment near the centre; old CA (OCA), flat or embedded into the ovary, only the stigma protruding from the ovary surface and often appearing as white "puckered plaque", trabeculae not visible and periphery traced with difficulty, white colour internally but may retain traces of brown pigment (see Appendix 6B and 6C). Large, hollow structures protruding from the ovary surface were assumed to be follicles (see Appendix 6B).

Sections of all types of ovarian scars or scars that could not be identified on gross examination were taken for histopathology. Tissues were dehydrated using graded ethanol solutions and cleared in xylene before embedding in paraffin wax, sectioning at 5 μm, staining with haematoxylin and eosin (H&E) and permanently mounting on glass slides using DPX. Ovaries from severely autolysed, or previously frozen, specimens were not included in the histological examination.

The medulla and cortex of all ovaries were sliced at approximately 2 mm intervals with the hilar region left intact to hold the slices together. *Corpora* counts for each ovary were summed for each female, using a 5× magnifying lamp to count the total number of *corpora* present. When only one ovary was available, the count was a minimum for that female. Accessory *corpora lutea* and *corpora atretica* are not included in the total *corpora* counts in this study. The diameters of all CL, CA, and the largest follicle (> 1 mm) observed in each ovary were measured to the nearest 0.1 mm on three planes using Vernier callipers, and the mean diameter of each structure was calculated as the cube root of the product of the three values, following Marsh and Kasuya (1984). The activity of both ovaries was recorded to assess symmetry.

All *corpora* data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) before statistical analysis. To test the null hypothesis of ovarian symmetry, a paired *t*-test was performed to compare the total *corpora* count on left vs. right ovaries (all mature females pooled). The difference in mean diameter between (assumed) CLOs and CLPs was tested using an independent *t*-test, and the relationship between mean CLP diameter and foetal TBL was investigated using linear regression.

Persistence of corpora and ovulation rate

For each individual, the total number of *corpora* (CL + CAs) in the ovaries was used to provide an index of the number of ovulation events, and to determine the persistence of ovarian scars. To test the hypothesis that the number of *corpora* increases with size or age, total corpora count was regressed against both age and TBL. To further investigate the persistence of *corpora* scars, the size frequency distribution of all CAs was plotted. The ovulation rate was then estimated by regressing mean *corpora* count on age, assuming CAs persist indefinitely and the slope of the regression corresponds to the rate at which the *corpora* are formed (Perrin and Donovan 1984, Martin and Rothery 1993).

Gestation period and foetal growth

Foetal growth has an initial nonlinear phase, followed by a linear phase. The gestation period was estimated using the conventional Huggett and Widdas (1951) nonlinear growth phase method:

$$\text{Total gestation period } (t_g) \ = t_0 \ + (\ t_g - t_0)$$

where t_0 is the nonlinear phase of growth, and $(\ t_g - t_0)$ is the linear phase of growth.

Following examination of a plot of TBL of foetuses against the day of year of collection, i.e. Julian date, it was apparent that in January and February (Julian dates 0 – 60) foetuses ranged from barely macroscopic to near-term (i.e. 5 – 176 cm), with many of intermediate size. The considerable variation in foetal length in the first 60 days of the year indicates that conceptions and births are not well synchronised within the population and/or the gestation period is longer than 12 months (365 days). Following Martin and Rothery (1993), the temporal spread of conceptions within an annual 'cohort' and its subsequent growth was visualised by plotting all foetal and newborn calf (i.e. neonatal line forming or just formed with no additional postnatal dentine and/or presence of visible foetal folds) lengths against Julian date, then concatenating three copies of the pattern to mimic three consecutive 'years'. It is possible to identify diagonally orientated concentrations of points from the smallest to largest foetuses, separated by regions with few points, thus providing a basis for allocating foetal specimens to a 'cohort' and suggesting that conceptions and births have a seasonal component.

The linear phase of growth $\left(\ t_g - t_0\right)$ was calculated using two approaches. First, the 'standard' method of fitting a least squares linear regression of foetus/newborn calf TBL on sampling date for a nominal 'cohort' was applied (e.g. Sergeant 1962a, Kasuya and Marsh 1984, Perrin and Reilly 1984), using 163 cm as the best estimate of the median length-at-birth (see Chapter 3). However, this method will always underestimate the rate of growth in species with low breeding synchrony (e.g. pilot whales) and result in an overestimate of gestation time (Martin and Rothery 1993). In an attempt to address this bias, a second method for estimating $\left(\ t_g - t_0\right)$ was applied by regressing sampling date on foetus/newborn calf TBL (following Martin and Rothery 1993), again using 163 cm as the best estimate of length-at-birth.

It was not possible to accurately describe either the shape or duration of early foetal growth (t_0) from data collected in this study, and it cannot be ruled out that some tiny embryos were

missed. An estimate of the nonlinear growth phase in this study is therefore dependent on published estimates of the relationship of t_0 to t_g, or $(t_g - t_0)$, but there is no universally accepted formula to adopt. Strict adherence to the guidelines of Huggett and Widdas (1951), would give $t_0 = 0.2(t_g)$, i.e. $t_0 = 0.25(t_g - t_0)$. However, Laws (1959) suggested a correction for length rather than weight data to give $t_0 = 0.22(t_g - t_0)$, therefore, in the current study, the initial nonlinear growth phase is approximated by $0.22(t_g - t_0)$ (following Martin and Rothery 1993).

Lactation, weaning, and resting period

The lactation period was calculated as the proportion of lactating females divided by the proportion pregnant in the sample, multiplied by the gestation period expressed in years:

$$\text{Lactation period } (t_l) = t_g \times l/p$$

where t_g is the length of gestation, l is the proportion of the sample lactating, p is the proportion of the sample pregnant (including both pregnant and lactating; Perrin and Reilly 1984).

Following Kasuya and Marsh (1984) and Learmonth et al. (2014), the stomach contents of neonates and calves were also examined to provide information on the timing of when solid food is taken. Data is presented on the occurrence of solid food in stomachs of animals up to 271 cm for females and up to 285 cm long for males (cut-off points identified retrospectively as the approximate size of 2-yr-olds according to fitted growth curves; see Chapter 3). Length at weaning (l_w) was estimated based on the size of the smallest calves found with solid food in the stomach, as well as by applying the (Huang et al. 2009) equation:

$$\text{Length at weaning } (l_w) = 1.239 {l_x}^{0.877}$$

where l_x is the estimated female asymptotic length (cm).

The resting period was calculated as the proportion of resting divided by the proportion pregnant in the sample, multiplied by the gestation period expressed in years:

$$\text{Resting period } (t_r) = t_g \times r/p$$

where t_r is the length of the resting period, t_g is the length of gestation, r is the proportion of the sample resting, and p is the proportion of the sample pregnant (including both pregnant and lactating; Perrin and Reilly 1984).

Annual pregnancy rate and calving interval

The annual pregnancy rate (APR) was estimated by dividing the proportion of pregnant females in the sexually mature sample by the length of gestation, expressed in years (Kasuya and Matsui 1984, Perrin and Reilly 1984, Murphy et al. 2009):

$$\text{Annual pregnancy rate } (APR) = p/t_g$$

where t_g is the length of gestation, and *p* is the proportion of sample pregnant (including individuals that were simultaneously pregnant and lactating). However, several assumptions are implicit in this model: (1) no sampling bias caused by selectivity, i.e. the distribution of reproductive conditions in the sample is the same as in the population sampled, (2) no seasonal bias exists in the sample collection or reproductive status, and (3) all pregnancies are detected (Perrin and Reilly 1984). To avoid missing the presence of early embryos, it is usually recommended to exclude samples collected during the mating period (Murphy et al. 2009). However, due to the highly seasonal occurrence of LFPW strandings on the New Zealand coast, sample sizes outside the mating period were small, and therefore all sexually mature females were included in the analysis for estimating pregnancy rates in this study. The summation method (gestation + lactation + resting periods) and the inverse of the APR were used to calculate two estimates of calving interval (Kasuya and Marsh 1984, Perrin and Reilly 1984). The estimate of the length of the cycle calculated by the summation method must be adjusted downward to take into account any overlap of lactation and pregnancy, by a factor equal to the proportion of females simultaneously pregnant and lactating (Perrin et al. 1977).

Reproductive senescence

To investigate evidence of reproductive senescence, i.e. the presence of post-reproductive females in the population, ovaries were examined (for absence of a CL, young or medium CAs, or macroscopic follicles) following the criteria outlined by Marsh and Kasuya (1984). The proportions of pregnant, lactating and resting mature females in six different age classes (5 – 10, 11 – 15, 16 – 20, 21 – 25, 26 – 30, 31 – 35 years) were also estimated to identify any changes in reproductive status with increasing age. Following Marsh and Kasuya (1984) and Photopoulou et al. (2017), the relative frequencies of CLPs and CLOs were compared in two age groups (< 15 yrs and > 15 yrs) using a Pearson chi-square test to assess whether ovulation was less likely to be followed by pregnancy in older animals.

Reproductive seasonality

In addition to noting the stranding dates of newborn calves, near-term foetuses, presence of large follicles and ovulating females in the sample, the timings of conceptions and parturition were assessed for (1) each foetus observed in the current study, and (2) each yearling calf recorded in the New Zealand Whale Stranding Database (NZWSDB; assigned to age class 0 or 1 through examination of dentinal GLGs or estimation from TBL, see below). Including calves of one year of age or less should avoid biases resulting from non-uniform sampling across the year (Martin and Rothery 1993).

Conception and birth dates were calculated for each foetus and yearling calf, based on the estimated age of individual foetuses (Börjesson and Read 2003) and calves:

$$\text{Foetal } (t) \;=\; (L_t/u) \;\times\; 30.5 \;+\; t_0$$

$$\text{Yearling calf } (t) \;=\; (L_t - L_b/(u/\,30.5))$$

where t is the foetal/calf age in days, L_t is the actual length of the foetus/calf (cm), u is the appropriate foetal, male calf, or female calf growth rate (cm mo^{-1}), 30.5 is the average number of days in a month, t_0 is the nonlinear foetal growth rate, and L_b is the estimated length-at-birth. Calf age was estimated using 163 cm as the average length-at-birth, and the length at one year of age as 237 cm for females and 248 cm for males (determined from sex-specific growth curves; see Chapter 3). Following Martin and Rothery (1993), it is assumed that the rate of growth is constant over this time so that: year 1 growth rate (my^{-1}) = length at 1 year – length-at-birth. For foetal specimens, individual conception dates were calculated by subtracting the estimated foetal age (t in days) from the date stranded (Julian date), and birth dates were projected by adding the estimated length of gestation (i.e. 414 days) to the estimated conception date. For calves, birth dates were back-calculated by subtracting the estimated calf age (t in days) from the date stranded (Julian date), and conception dates by subtracting the estimated length of the gestation period from the estimated birth date.

6.4 Results

The sample

The distribution of the stranding locations for female LFPWs included in this study is shown in Figure 6.1. The sample collected from most MSEs reflected the composition of the stranded (dead) group and was dominated by mature females (Figure 6.2a). The exception to this was the Port Levy (24 January 2010) MSE, in which there was high mortality of calves and juveniles. Most MSEs occurred during the first quarter (January – March; Figure 6.2b), which is the austral summer period and the peak stranding season for the species in New Zealand (see Chapter 7). Female TBLs in the dataset ranged from 160 to 485 cm (*n* = 161), with a modal size class of 430 to 439 cm (Table 6.1, Figure 6.3a). Age was determined for 150 female LFPWs, with age ranges for an additional eight individuals identified. Female LFPWs ranged from 0 to 33 years (Table 6.1, Figure 6.3b). The age distribution was bimodal with peaks in the age classes of yearling calves (i.e. < 1-yr-old) and 16-year-old sexually mature adults.

Reproductive status

In the sample of 166 female LFPWs for which maturity status was determined, 21.7% were classified as immature and 78.3% as sexually mature. Of the mature sample, reproductive status was determined for 90%, while 10% could only be classified as far as “indeterminate” mature (see Table 6.1). The largest proportion of the mature sample (for which reproductive status was determined) was composed of resting mature females (43.6%), followed by those that were lactating (32.5%) and pregnant (23.9%). No individuals were identified as simultaneously pregnant and lactating. However, not all animals identified as pregnant were assessed for evidence of lactation (and *vice versa*), so there is a chance that some simultaneously pregnant and lactating females may have been present in the sample.

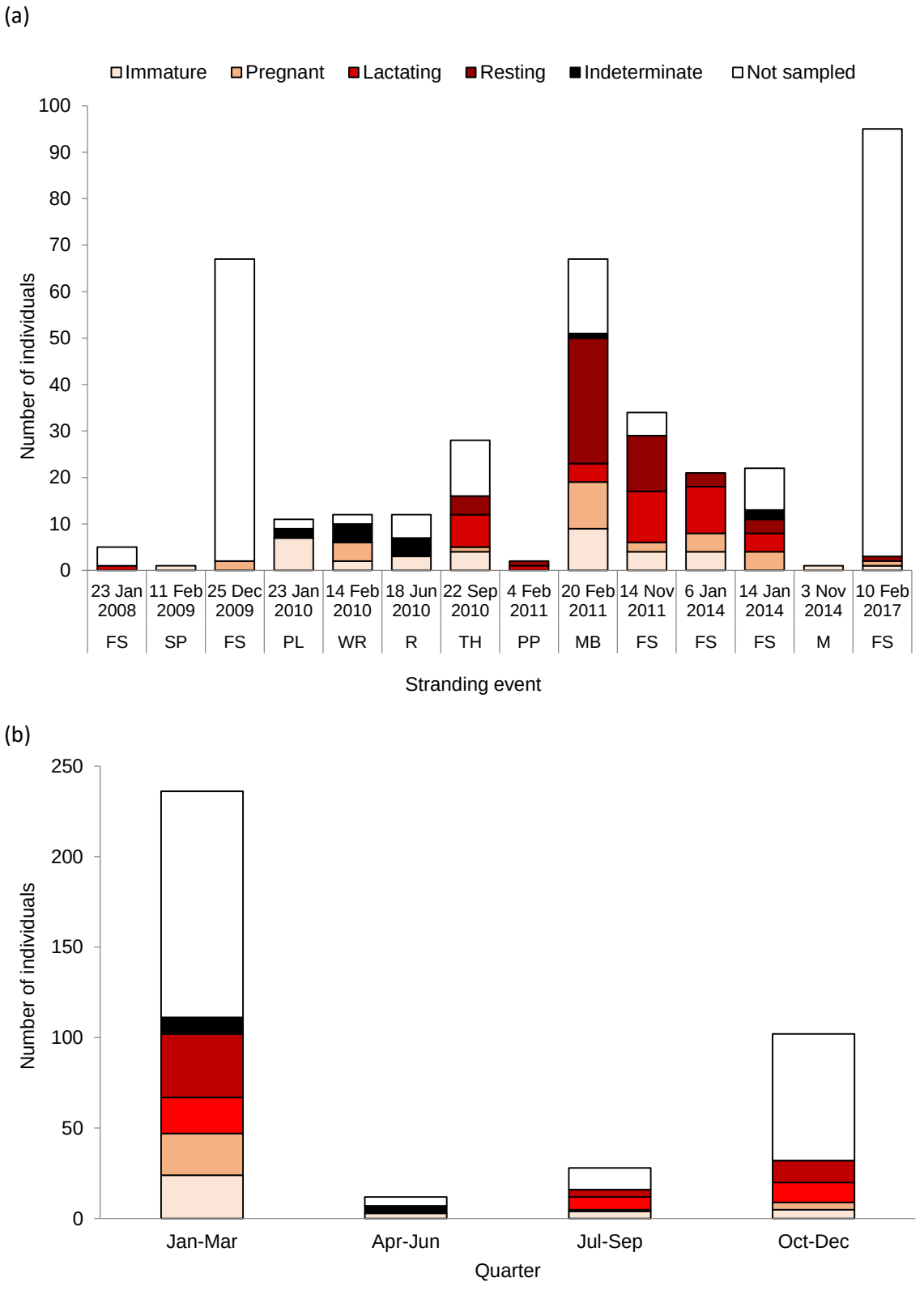


Figure 6.2. Number of female LFPWs with reproductive data collected from stranding events on the New Zealand coast by (a) stranding event and (b) quarter (*n* = 166).

Reproductive categories: immature; pregnant (foetus and CLP present); lactating; resting mature (not pregnant or lactating); indeterminate mature. Not sampled = individuals identified as female but no reproductive data collected (*n* = 212). Stranding locations: FS = Farewell Spit, Golden Bay; SP = Spencer Park Beach, Christchurch; PL = Port Levy, Banks Peninsula; WR = West Ruggedy Beach, Stewart Island; R = Ruapuke, Waikato; TH = Te Horo Beach, Spirit's Bay, Far North; PP = Port Puponga, Golden Bay; MB = Mason Bay, Stewart Island; M = Muriwai, Auckland.

Table 6.1. Mean (±SE) TBL, age and ovarian characteristics for all reproductive groups in the sample of female LFPWs examined.

Categories: immature, pregnant (foetus and CLP present); lactating; resting mature (not pregnant or lactating); indeterminate mature.

Stages	*n*	TBL (cms)	Age (yrs)	Total ovarian weight (g)	Total ovarian volume (mm^3)	Corpora count (CL +CAs)		CL present	
						L	R	L	R
Immature	36	283 (±11) 160–375 (*n*=35)	2.6 (±0.4) 0–8.5 (*n*=35)	5.9 (±1.0) 0.5–22.3 (*n*=31)	4.8 (±0.9) 0.4– 22.7 (*n*=31)	0	0	0	0
Pregnant	28	409 (±7) 346–473 (*n*=28)	16.0 (±1.4) 5–33 (*n*=26)	69.5 (±3.7) 39.1–101.3 (*n*=20)	67.8 (±4.2) 41.7–110.6 (*n*=20)	3 (±0.5) 0–8 (*n*=20)	1.7 (±0.3) 0–4 (*n*=20)	10	9
Lactating	40	416 (±3) 380–465 (*n*=37)	17.2 (±1.0) 7–30 (*n*=34)	48.7 (±3.5) 15.7–98.6 (*n*=35)	42.3 (±3.6) 13.5–100.8 (*n*=35)	4 (±0.4) 0–9 (*n*=36)	1.9 (±0.3) 0–5 (*n*=36)	5	3
Resting mature	51	428 (±3) 380–468 (*n*=48)	18.3 (±0.9) 8–30 (*n*=42)	46.7 (±2.4) 17.7–95.7 (*n*=47)	45.5 (±2.9) 16.2–105.8 (*n*=47)	5 (±0.4) 0–12 (*n*=47)	4 (±0.3) 0–9 (*n*=47)	5	7
Indeterminate mature	13	428 (±8) 380–485 (*n*=13)	20 (±1.9) 8–32 (*n*=13)	35.0 (±4.2) 14.4–63.8 (*n*=11)	38.9 (±5.3) 16.8–74.3 (*n*=11)	3 (±0.4) 1–7 (*n*=11)	3.2 (±0.5) 0-6 (*n*=11)	0	0
All mature individuals	130	420 (±2) 346-485 (*n*=126)	17.7 (±0.6) 5–33 (*n*=115)	50.2 (±1.9) 14.4–101.3 (*n*=113)	47.8 (±2.1) 13.5–110.6 (*n*=113)	4 (±0.2) 0–12 (*n*=114)	2.6 (±0.6) 0–9 (*n*=114)	20	19
Total	166	387 (±7) 160–485 (*n*=161)	14.2 (±0.7) 0–33 (*n*=150)	40.6 (±2.2) 0.5–101.3 (*n*=144)	38.6 (±2.2) 0.4–110.6 (*n*=144)	4 (±0.2) 0–12 (*n*=114)	2.6 (±0.6) 0–9 (*n*=114)	20	19

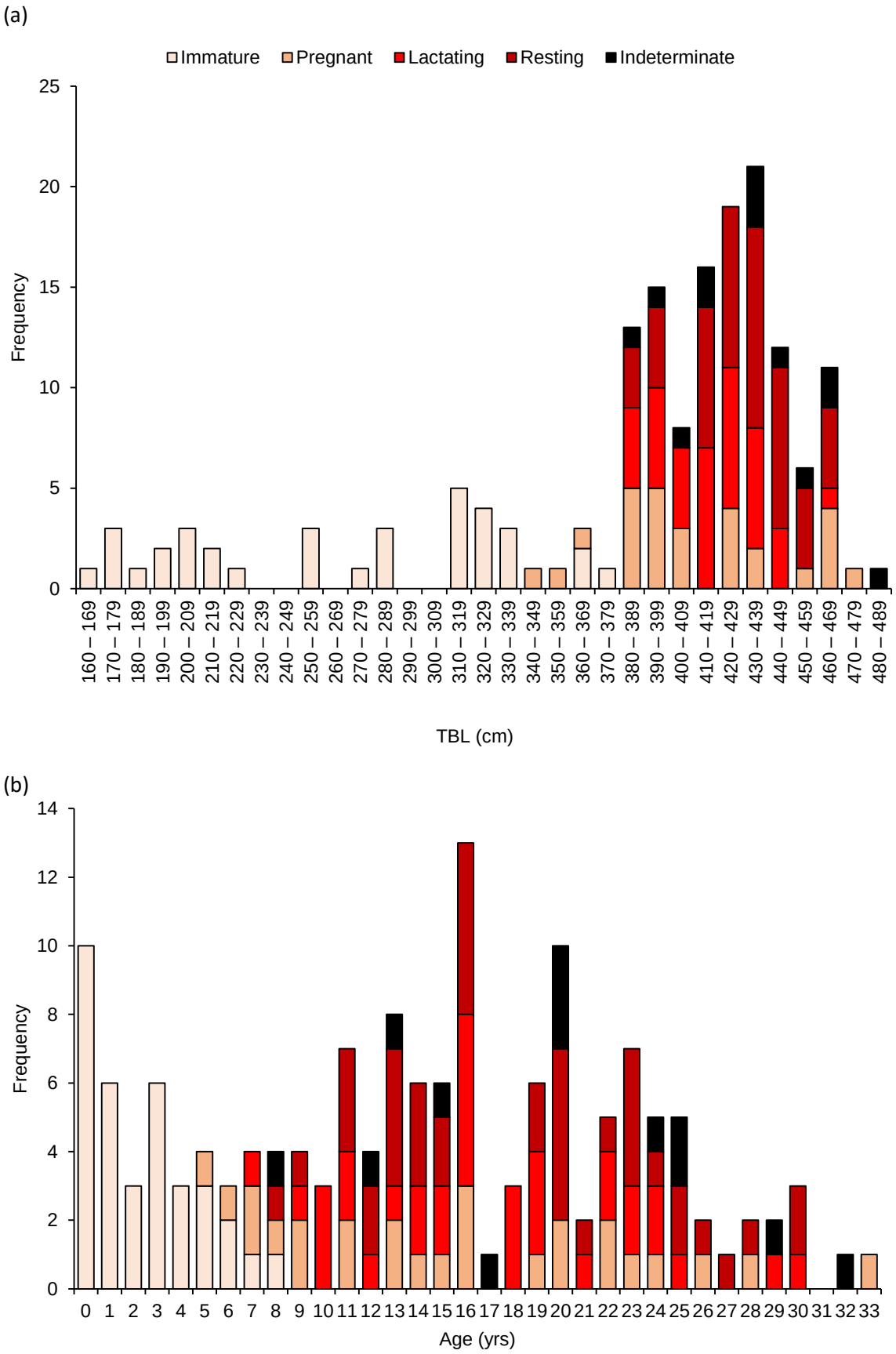


Figure 6.3 (a) Total body length (TBL; *n* = 161) and (b) age (*n* = 150) frequency distributions of female LFPWs in the sample.

Reproductive categories: immature; pregnant (foetus and CLP present); lactating; resting mature (not pregnant or lactating); indeterminate mature.

Average age and body length at the attainment of sexual maturity

Table 6.1 outlines the biological and ovarian parameters for each reproductive group. There was some overlap in the age and TBL between immature and mature females, with immature females ranging from 0 to 8.5 years in age and 160 to 375 cm TBL, and mature females ranging from 5 to 33 years and 346 to 485 cm. Average age at attainment of sexual maturity (ASM) was estimated to be 6.7 years (95% CrI = 5.8 – 7.5 yrs, *n* = 150; Figure 6.4). There was evidence that the youngest sexually mature female (GM252, 5 yrs) had recently matured as it had a single *corpora lutea* of pregnancy (CLP; 43 cm mean diameter, orange, small central cavity), no other *corpora* scars, was not lactating, and was in the early stages of her first pregnancy (14.8 cm foetus). Average TBL attained at sexual maturity (LSM) was estimated to be 356 cm (95% CI = 344 – 367 cm, *n* = 161; Figure 6.4). Again, there was evidence that the smallest sexually mature female (GM242, 346 cm) had only recently matured as it had a single CLP (41 cm mean diameter, orange, no central cavity), no other *corpora* scars, was not lactating, and was in the early stages of her first pregnancy (16.7 cm foetus). There was no evidence for either age or TBL being the better predictor of sexual maturity status; the LOOIC scores were very similar (22.1 and 21.8, respectively), with an estimated difference of 0.3 being much less than the standard error for the difference (SE = 5.4).

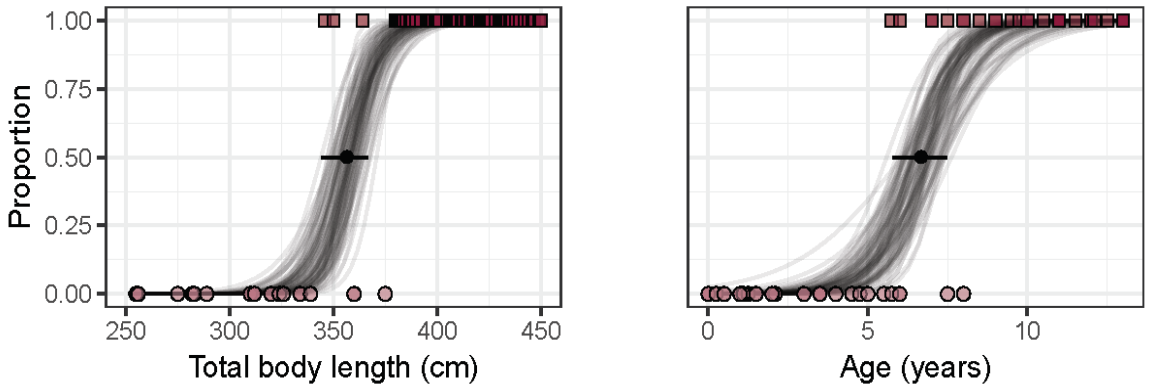


Figure 6.4. Bayesian cumulative logit regression of the maturation of female LFPWs modelled as a function of TBL and age.

The plots show the TBL and age values for immature (circles) and mature (squares) individuals, with lines representing posterior predictions of the mean. The black points and thin horizontal lines show mean and 95% credible intervals of the estimated values of x at which 50% of females were classified as mature.

Ovarian characteristics

Combined ovary weight increased from birth to about the time of sexual maturity (Figure 6.5), but there was considerable variation in combined ovary weight among mature females. The total number of *corpora* scars (CL + CAs) recorded in mature individuals ranged from 1 to 19, with a mean of 6.7 scars ± 0.4 SE (*n* = 114). *Corpora* scars were observed on both ovaries in sexually mature females (Table 6.1), but there were significantly more *corpora* present on the left ($\bar{x}$ = 4.0 ± 0.2 SE), compared to the right ($\bar{x}$ = 2.6 ± 0.2 SE) ovary when results were pooled (paired t = 5.574, df = 113, $p < 0.001$). Of a total of 730 CAs measured, 107 (14.7%) were classified as young and had a range of mean diameters from 9.8 to 29.6 mm ($\bar{x}$ = 16.9 ± 0.4 SE; Figure 6.6). The mean diameters of 318 MCAs (43.6% of total CAs) ranged from 5.6 to 22.3 mm ($\bar{x}$ = 11.3 ± 0.1 SE), and the 305 OCAs (41.8% of total CAs) from 2.0 to 14.2 mm ($\bar{x}$ = 7.9 ± 0.1 SE; Figure 6.6). There was no evidence that CAs regress below 2.0 mm.

CLs were found almost equally on the left (*n* = 20) and right (*n* = 19) ovaries. When a foetus was present, the mean diameter of the CLP was 35.6 mm ± 2.5 SE (range 25.4 – 49.2, *n* = 22). Without the presence of a foetus, the mean diameter of the (assumed) CLO was 30.2 mm ± 0.9 SE (range 17.4 – 42.9, *n* = 18), which was not significantly different than the mean CLP diameter (independent t = 3.381, df = 38, p = 0.002). The largest CL (mean diameter 49.2 mm) was found in a pregnant female that was full-term (foetus length 173 cm). However, simple linear regression revealed no significant relationship between mean CL diameter and foetal TBL observed in the sample (r^2 = 0.004, p = 0.726, n = 22).

Persistence of corpora and ovulation rate

To test the hypothesis that the number of *corpora* increases with size or age, the relationship between these parameters was examined. In our sample, positive linear relationships between total number of *corpora* scars (CL + CAs) and both TBL (r^2 = 0.284, $p < 0.001$, n = 114) and age (r^2 = 0.550, $p < 0.001$, n = 107) were significant, despite considerable variation around the regression lines (Figure 6.7, Figure 6.8a). Although significant, the linear relationship between *corpora* count and TBL was only weakly positive (Figure 6.7). The maximum number of *corpora* scars (*n* = 19) was recorded in the ovaries of two resting mature females, one aged 26 years (TBL 430 cm), and the other was estimated to be older than 30 years (TBL 468 cm) using fitted growth curves for LFPWs in New Zealand waters (Chapter 3). However, a number of females that were between 24 and 29 years of age had only three to seven *corpora* scars; including a pregnant female aged 26 years that presented with only three corpora scars (including the CLP).

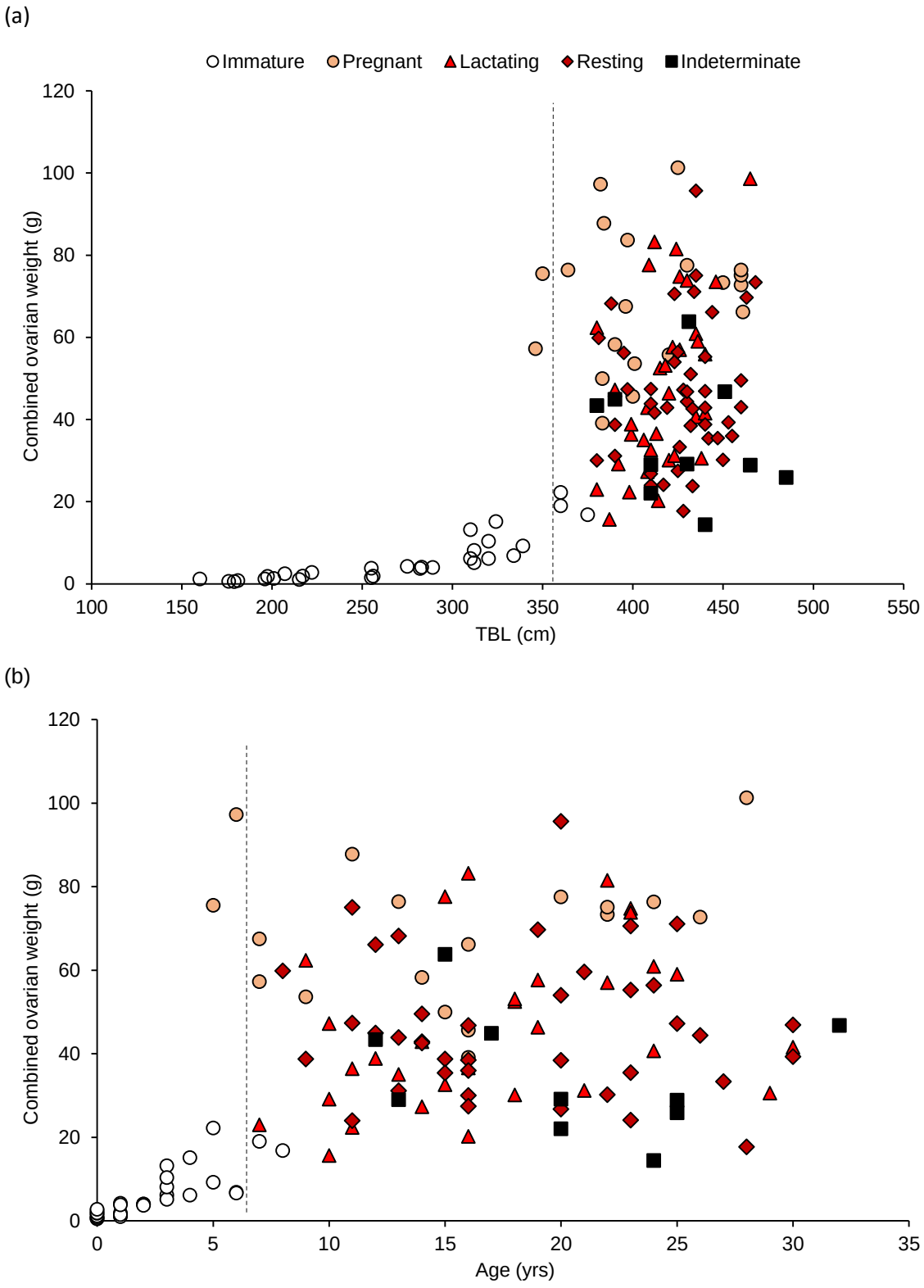


Figure 6.5. Combined ovarian weight vs (a) TBL (*n* = 139) and (b) age (*n* = 128) in the female LFPW.

Each point represents an individual whale's maturity status. Dashed lines indicate best estimates of average length and age at attainment of sexual maturity (i.e. 356 cm and 6.7 yrs. Categories: immature; pregnant (foetus and CLP present); lactating; resting mature (not pregnant or lactating); indeterminate mature.

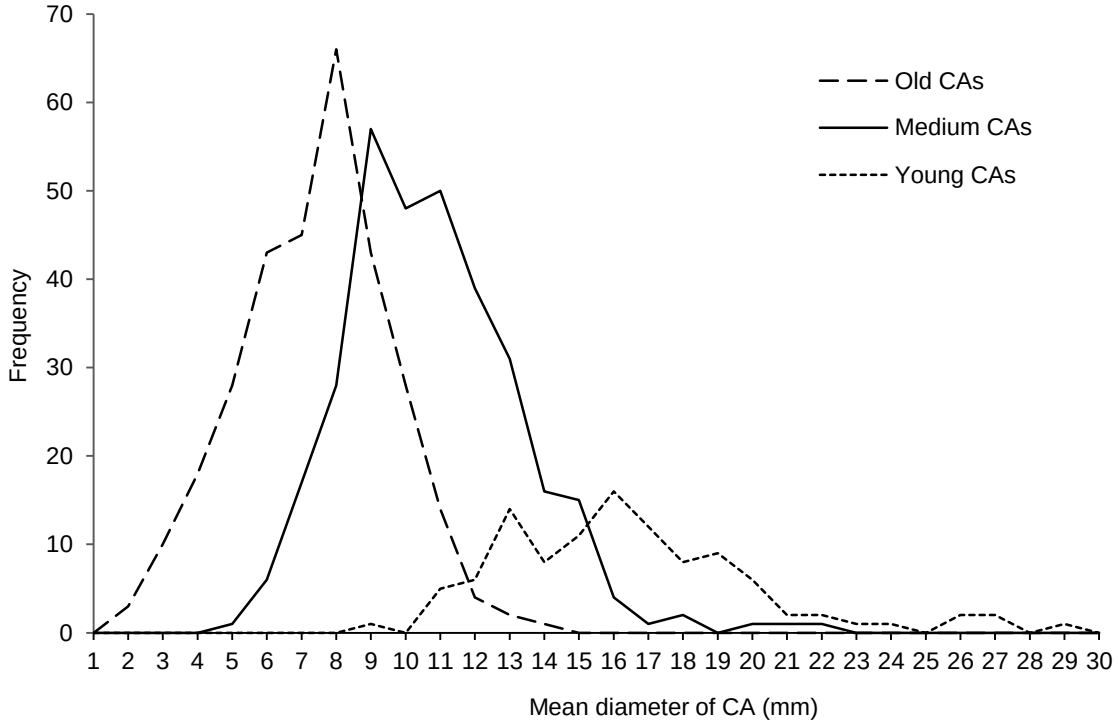


Figure 6.6. Size-frequency distributions of young, medium and old *corpora albicantia* (CAs) on the ovaries of female LFPWs (*n* = 730 CAs from 114 individuals).

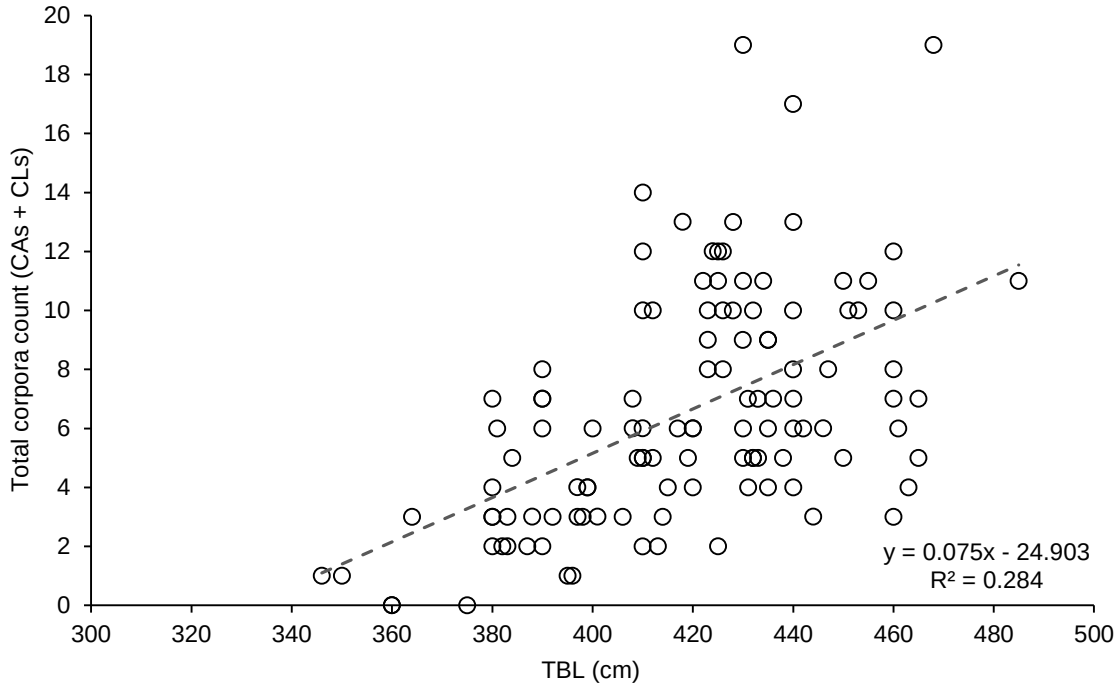


Figure 6.7. Relationship between the ovarian activity (i.e. number of ovarian *corpora*) and TBL for female LFPW ≥ 346 cm TBL (*n* = 114).

The dashed line corresponds to the linear regression.

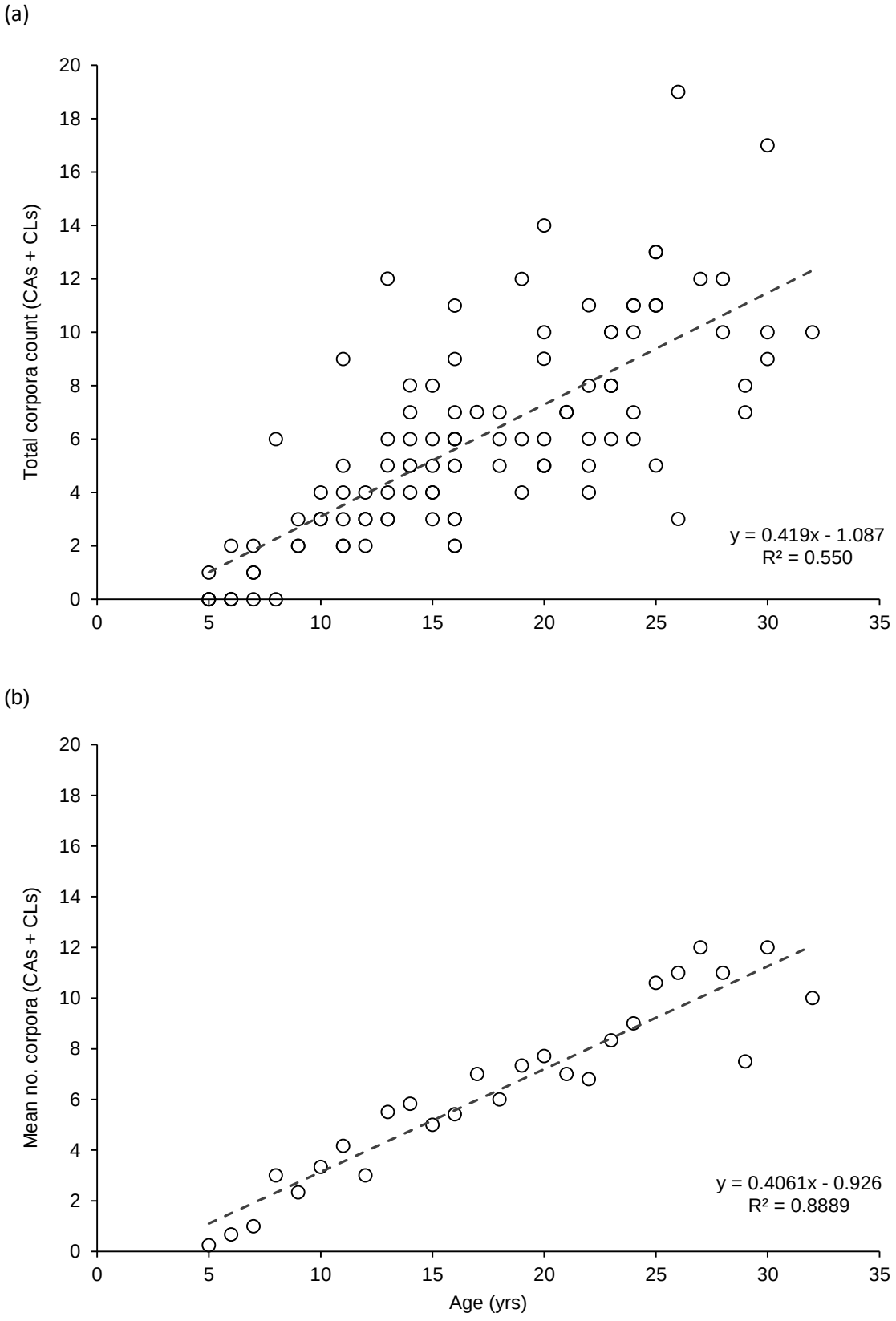


Figure 6.8. (a) Total number of *corpora* on both ovaries by age and (b) mean number of *corpora* on both ovaries by age class for female LFPWs aged ≥ 5 yrs (*n* = 107).

Dashed lines correspond to the linear regression.

To further investigate the persistence of *corpora* scars over time, the size-frequency distribution of all CAs (*n* = 730) was plotted (Figure 6.9). The highest frequency occurred in the smaller range of diameters with a peak at size class 9 mm. The observed CA sizes formed a typical bell-shaped distribution curve that is indicative of a complete and normal sample (Figure 6.9). The slight skewing to the left is due to the inclusion of several very young CAs in the plot. However, very young CAs of a large diameter are few, which suggests an initial period of rapid decline in *corpora* size. The near-normal distribution and lack of CAs less than 2 mm suggests corpora persistence in the sample of New Zealand LFPWs since the size distribution would be negatively skewed if corpora were resorbed. As it has been established that *corpora* persistence can be assumed for New Zealand LFPWs, and the linear regression fitted to the mean number of *corpora* scars on age was significant (r^2 = 0.889, p < 0.001, n = 107; Figure 6.8b), the slope, i.e. 0.406 *corpora* per year, is considered to represent the ovulation rate of the population.

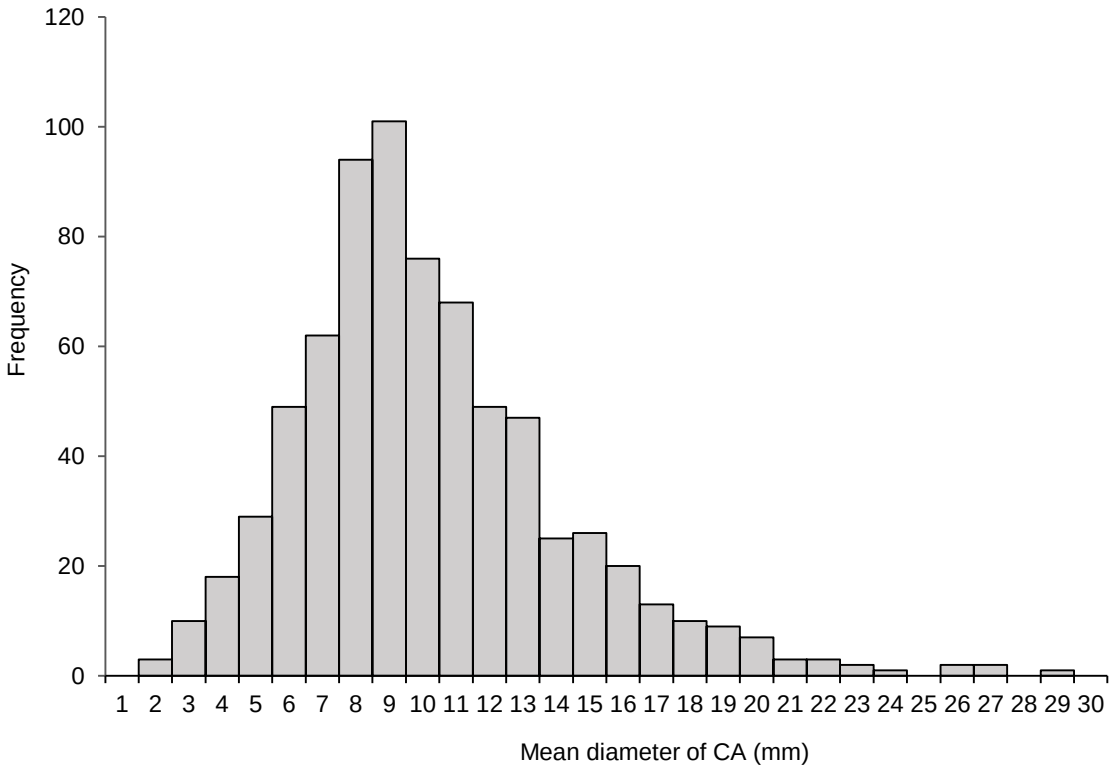


Figure 6.9. Size-frequency distribution of *corpora albicantia* scars (CAs) on the ovaries of female LFPWs (*n* = 730 CAs from 114 individuals).

Gestation period, foetal growth, and dates of conception and birth

Using the 'standard' method for estimating the linear foetal growth phase of the gestation period by regressing foetus and newborn calf TBL on sampling date for a nominal 'cohort', ($t_g - t_0$) was estimated to be 366 days or approximately 12 months (y = 0.413x + 12.072, r^2 = 0.789, p < 0.001, n = 40; Figure 6.10), and the length of the nonlinear growth phase was estimated at 80 days (t_0 = 0.22 × 366 days). Summing the linear and nonlinear gestation periods together produced a total gestation period of 446 days or approximately 14.6 months (t_g = 366 + 80 days), and a foetal growth rate of 12.6 cm per month.

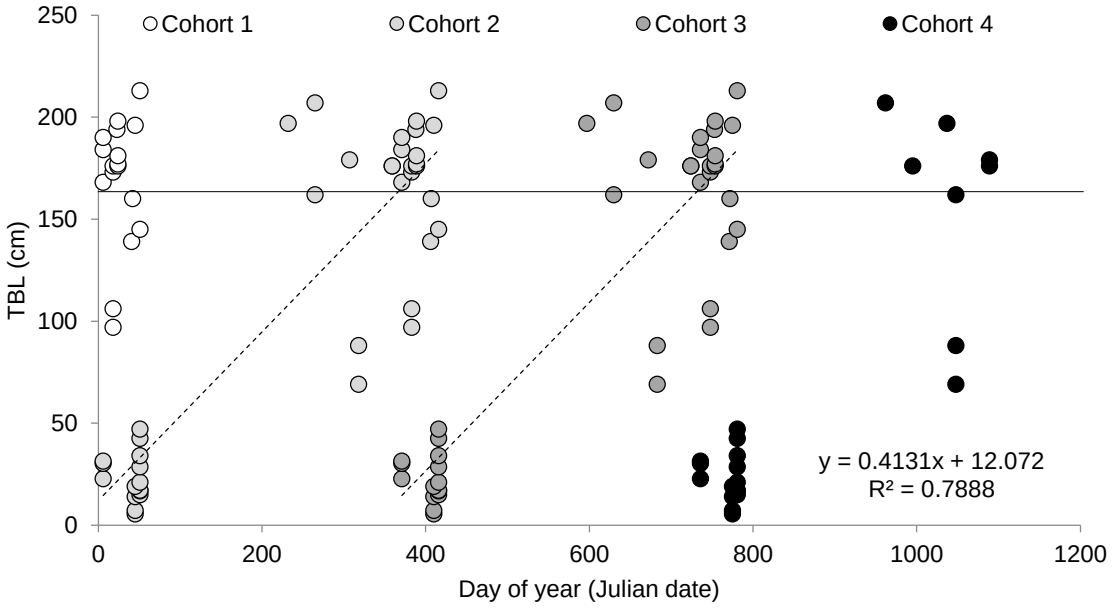


Figure 6.10. Three concatenated copies of a plot of foetal and neonatal TBL against day of year sampled (Julian date) (n = 40).

The horizontal line indicates the estimated mean length at birth (163 cm; see Chapter 3). The diagonal dotted lines indicate the growth trajectory for a nominal 'cohort' as fitted by linear regression.

Using the alternative method of estimating the linear foetal growth phase by regressing sampling date on foetus/newborn TBL (following Martin and Rothery 1993), ($t_g - t_0$) was estimated to be 339 days or approximately 11 months (y = 1.910x + 28.033, r^2 = 0.789, p < 0.001, n = 40), and the length of the nonlinear growth phase was estimated at 75 days (t_0 = 0.22 × 339 days). Summing the linear and nonlinear gestation periods together produced a total gestation period of 414 days or approximately 13.6 months (t_g = 339 + 75 days), and a foetal growth rate of 16 cm per month. The estimates calculated using the regression of sampling date on foetus/newborn TBL to estimate ($t_g - t_0$) are used in all calculations of reproductive parameters hereafeter to ensure the results of the current study are comparable to those previously reported for *G. m. melas* (Martin and Rothery 1993).

Lactation, weaning, and resting period

Using Perrin and Reilly's (1984) equation, the lactation period was calculated to last approximately 19.3 months (or 1.6 yrs). Remains of solid food were found in the stomachs of nine females less than 271 cm long, and seven males less than 285 cm long (all estimated to be < 2 yrs old) recorded between November and February. It is tentatively suggested that weaning occurs mainly during austral summer. Prey items recovered from the stomachs of the smallest weaned animals (*n* = 16) were composed of almost exclusively cephalopod beaks (94%), the majority of which were identified as arrow squid, *Nototodarus* spp. The smallest individuals with remains of solid food in the stomach were a male (1-yr-old) and female (< 1-yr-old), both measuring 215 cm TBL.

Using the Huang et al. (2009) equation, length at weaning was estimated to be 257 cm (95% CI = 255 – 260 cm) based on the estimated asymptotic female body length of 438 cm (95% CI = 433 – 444 cm). According to fitted growth curves for the species in New Zealand waters (see Chapter 3), the estimated total body length at weaning of 257 cm is expected to occur at approximately 19 months of age for females, and 21 months for males. Thus, the estimated length and age at weaning is consistent with the duration of the lactation period, i.e. 19 to 20 months, estimated using Perrin and Reilly's (1984) equation. Also using Perrin and Reilly's (1984) equation, the resting period was calculated to be 24.6 months or approximately two years.

Annual pregnancy rate and calving interval

The sample for estimating the pregnancy rate was collected between 2010 and 2017 and was composed of 130 sexually mature females, of which 28 (21.5%) were pregnant. Taking the gestation period of 1.13 years (13.6 mo.), the annual pregnancy rate (APR) in the sample was estimated at 19% (21.5/1.13). Calculated as the inverse of the APR, the overall calving interval was estimated at 5.3 years (or 63 mo.) for the New Zealand LFPW population. The summation of the gestation (13.6 mo.), lactation (19.3 mo.), and resting (24.6 mo.) periods estimated a calving interval of 4.8 years (or 57.5 mo.).

Reproductive senescence

The criteria of Marsh and Kasuya (1984) to indicate reproductive senescence (i.e. no macroscopic follicles, no CL and all CAs classified as OCAs) were applied to the ovaries in our samples; none of the 114 mature females for which both ovaries were examined showed evidence of being post-reproductive, i.e. would not ovulate again. Where both age and full

reproductive status were available for mature individuals (*n* = 102), the proportion of pregnant, lactating and resting mature individuals was determined for six age groups (5 – 10, 11 – 15, 16 – 20, 21 – 25, 26 – 30, 31 – 35 yrs; Figure 6.11). For the age class 5 to 10 years, a very small proportion of mature dolphins were resting (14%) and the majority of individuals were either pregnant (50%) or lactating (36%). After this, a decrease in the proportion pregnant and an increase in the proportion resting was noted in older age classes, except the oldest female in the sample (GM280) which was aged at 33 years and pregnant with 23.5 cm foetus. The relative frequencies of CLOs and CLPs were available for 40 known-age females. These data were arbitrarily split into two groups of approximately equal samples sizes: animals up to 15 years old and animals older than 15 years. The frequency of CLPs relative to CLOs was greater in females up to 15 years of age (10 CLPs, 8 CLOs) compared to older females (11 CLPs, 11 CLOs). However, there was insufficient evidence to support a significant difference between the groups, i.e. that ovulation was less likely to be followed by pregnancy in older animals (χ^2 = 0.123, df = 1, *p* = 0.726).

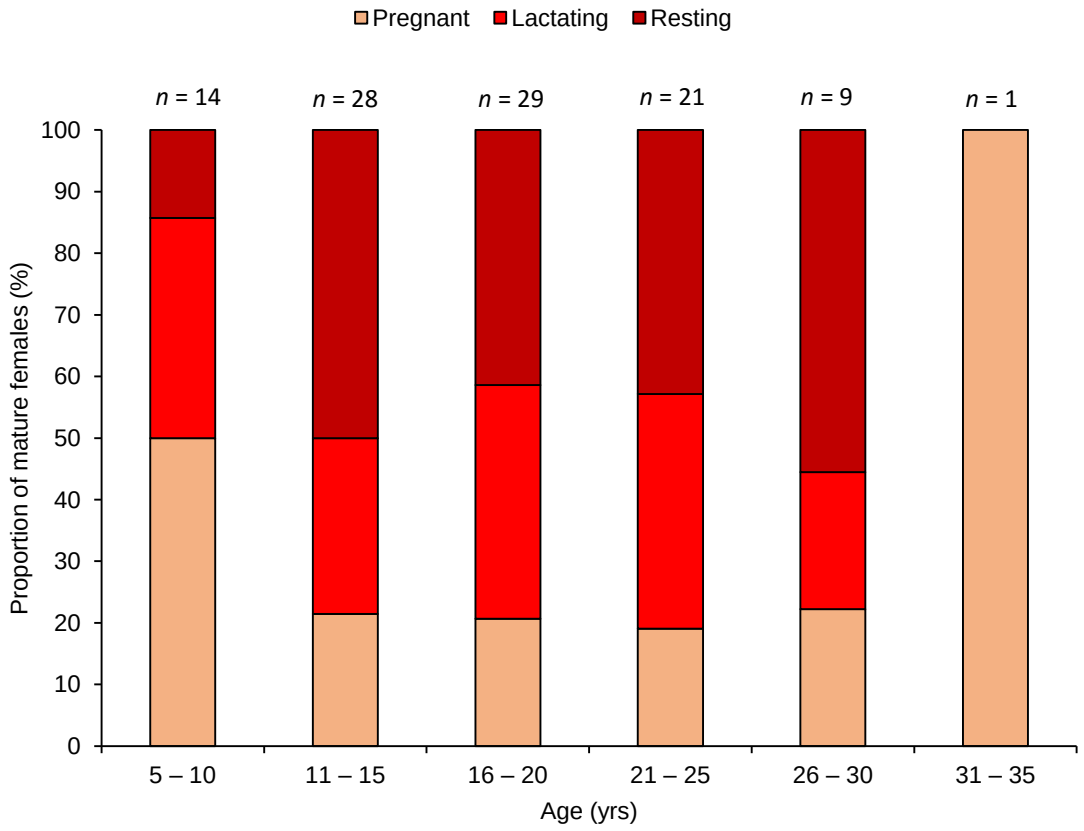


Figure 6.11. Proportion of pregnant, lactating, and resting mature female LFPWs against age (*n* = 102).

Reproductive seasonality

A total of 19 females were considered to have been examined at ovulation based on the presence of a CL but no observed foetus. Expressed as a percentage of the total number of mature females examined in each month sampled, ovulation shows a peak season in austral spring (September and November; Figure 6.12). Follicle development, as indicated by non-pregnant mature females that had a large (≥ 10mm) follicle on either ovary, was greatest between September and February, peaking in January and February (Figure 6.13).

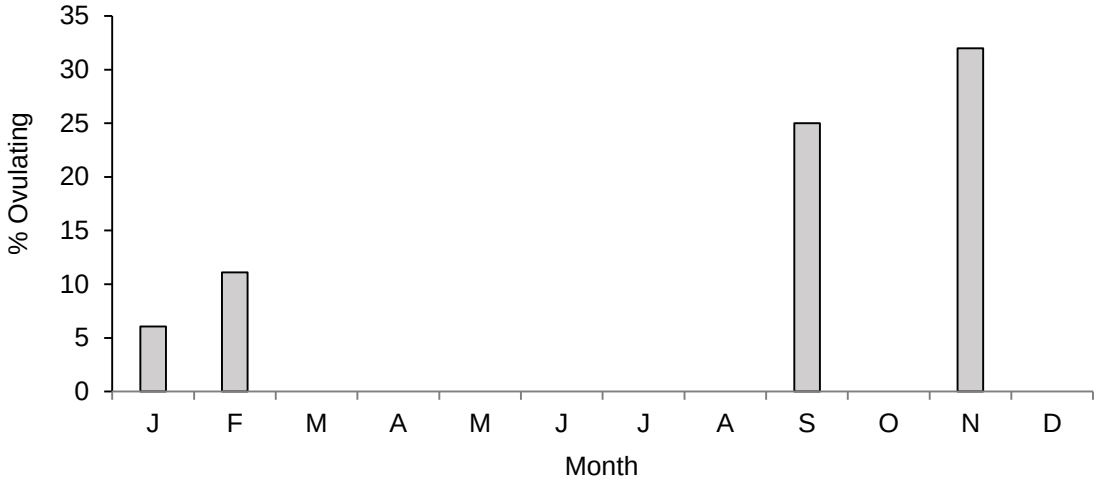


Figure 6.12. Proportion of mature females ovulating, by month (as indicated by the presence of a CL but no observed foetus; *n* = 19/130).

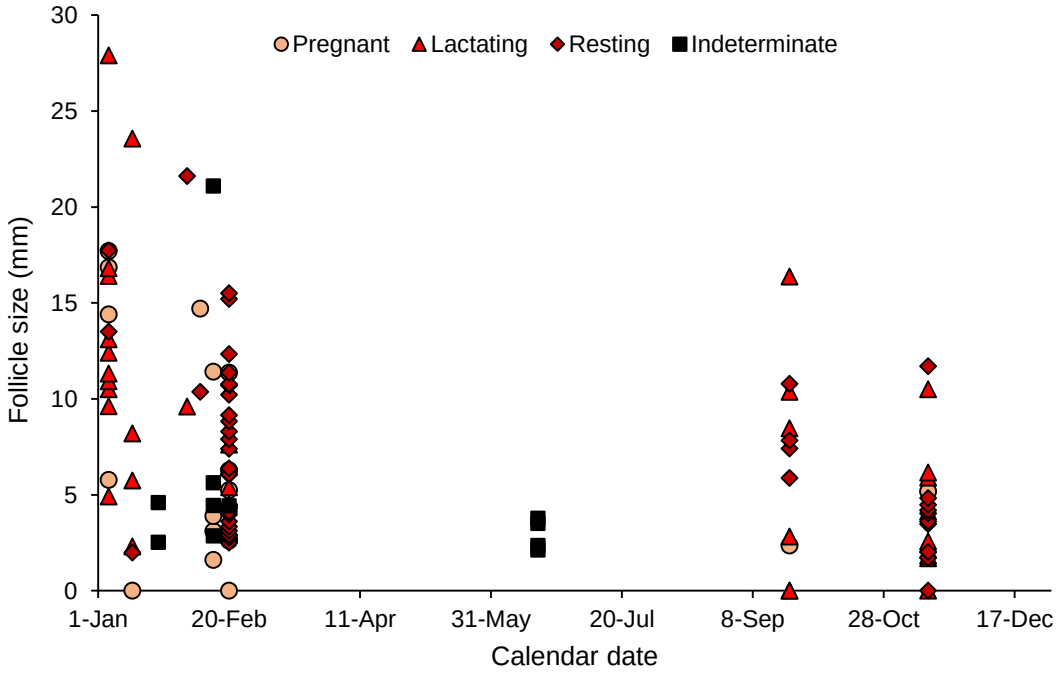


Figure 6.13. Seasonal changes in the mean diameter of the largest (≥ 10mm) follicle on either ovary of indeterminate mature, resting mature, lactating and pregnant females vs calendar date (*n* = 108).

Back-calculating the date of conception for each foetus (*n* = 27) and yearling calf (*n* = 115), by subtracting the estimated foetal age from the date stranded, gives conception dates in every month of the year with a primary peak in September and October (austral spring) and a smaller, secondary peak in April (austral autumn; Figure 6.14a). Estimated birth dates of the foetuses and calves strongly suggest that births also occur in every month of the year, with a primary calving peak in December (austral summer), and a smaller, secondary peak in May (austral autumn; Figure 6.14b).

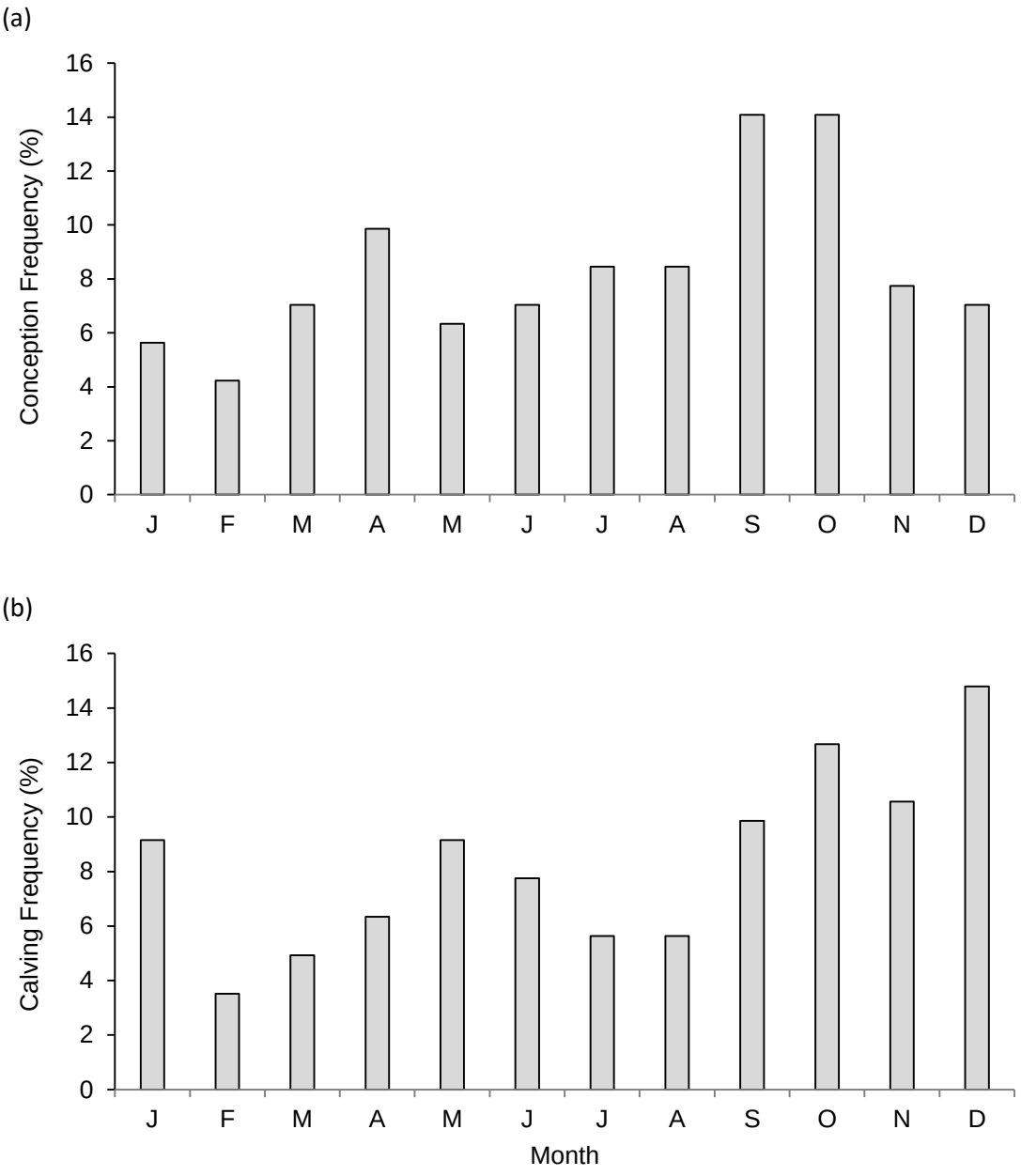


Figure 6.14. Monthly distribution of estimated (a) conception months and (b) for foetuses (*n* = 27) and calves ≤ 1 yr of age (*n* = 115).

Note the peaks of conception September and October (austral spring) and calving in December (austral summer).

6.5 Discussion

Average age and body length at the attainment of sexual maturity

Our estimated mean length of sexually mature female *G. m. edwardii* (356 cm) was consistent (± 10 cm) with those observed in two previous studies of the species in both the North Atlantic (Sergeant 1962a) and Southern Hemisphere (Soto et al. 2017; Table 6.2). However, it is almost 20 cm lower than the most reliable LSM estimate (375 cm) for *G. m. melas* to date (Martin and Rothery 1993). Similarly, the estimated ASM of 6.7 years was found to correspond to estimates of 6 to 7 years for populations off Newfoundland (Sergeant 1962a, Kasuya et al. 1988b) and Britain (Martin et al. 1987) but is 1.6 years lower than reported from the Faroe Islands (Martin and Rothery 1993), and 1.3 years lower than previously estimated for *G. m. edwardii* from Argentina (Soto et al. 2017; Table 6.2).

Although individual variation in the ASM is common among mammals, evidence for density-dependence has also been found in this parameter, with population-level declines in ASM over time associated with relative reductions in population abundance (Fowler 1984, Wade 2018). In populations at a level well below carrying capacity, females become sexually mature and start reproducing at an earlier age than females from a population at a level close to carrying capacity; presumably due to access to greater resources, e.g. prey (Wade 2018). For example, relative declines in the ASM in baleen whales have been correlated with declining population size due to exploitation in baleen whales (Lockyer 1984, Boyd et al. 1999) and small delphinids (Perrin et al. 1976, Perrin et al. 1977, Kasuya 1985). Sergeant's (1962a) sample of female *G. m. melas* used to assess ASM was collected during a period of intensive exploitation off Newfoundland, though the level of previous exploitation was low. Martin and Rothery's (1993) sample of female *G. m. melas* was taken from the Faroe Islands where fishery pressure is continuous at a relatively low level (see Chapter 5 and references therein). In contrast, *G. m. edwardii* samples from Argentina (Soto et al. 2017) and New Zealand (this study) were taken from populations with no significant history of exploitation. Theory predicts that a reduction in population size due to exploitation would (in the absence of density-dependent limiting factors) trigger a subsequent density-dependent compensatory increase in reproductive rate, resulting in a lower ASM (and LSM) relative to populations that do not have a significant history of exploitation (e.g. New Zealand, Argentina). However, attainment of sexual maturity is complex and is influenced by the general health of the animal, though factors such as hierarchical position, mate availability, genetics, quality and quantity of available food, and consumption of food high in contaminant levels (especially endocrine-disrupting chemicals) may also have potentially confounding effects (Murphy et al. 2005).

In the absence of accurate estimates of population size before and after exploitation, as well as comparable measures of ASM before exploitation, it is unknown as to why (non-exploited) New Zealand *G. m. edwardii* have a lower ASM (and LSM) than (exploited) Faroese *G. m. melas*. Possible explanations are: (1) frequent, large MSEs in New Zealand (and Australia) may have more of an impact on the *G. m. edwardii* population in the South Pacific than the Faroese drive fisheries have on the *G. m. melas* population in the North Atlantic; (2) favourable conditions in temperate South Pacific waters could potentially support further population growth (i.e. the carrying capacity has not been reached); (3) real population-level differences between the two subspecies such as smaller mean body size and shorter lifespan in *G. m. edwardii*; (4) smaller sample sizes for *G. m. edwardii* leading to an underestimation of ASM and LSM; (5) high pollutant burdens of *G. m. melas* in Faroese waters leading to reproductive dysfunction; and (6) any combination of the above (see Chapter 5 and references therein).

The higher ASM estimated for *G. m. edwardii* in Argentinian waters compared to New Zealand (Soto et al. 2017) may be due to the limited sample examined from Argentina (i.e. n = 27 females from a single MSE). However, given the unparalleled frequency of MSEs on the New Zealand coast, the difference in ASM between the South Pacific and South Atlantic could also be a result of localised impacts on population abundance due to relatively high mortality rates in New Zealand. Any trends that might indicate that density-dependent mechanisms are operating in the New Zealand population would require monitoring of ASM and LSM estimates over time.

Interestingly, the average age at attainment of sexual maturity in female New Zealand *G. m. edwardii* of 6.7 years, is considerably lower than the estimated 13.5 years for males (see Chapter 5). The most reliable corresponding figures for *G. m. melas* are 8.3 years (female) and 17 years (male) (Desportes et al. 1993b, Martin and Rothery 1993), and for SFPWs are 9 and 16 years for females and males, respectively (Kasuya and Marsh 1984). Therefore, in both pilot whale species, there is agreement that males mature about 6 to 7 years later than the females, which also concurs with the general delphinid pattern of bimaturism (Perrin and Reilly 1984). This delay in male maturation is undoubtedly related to the sexually dimorphic larger body size attained by males and possible polygynous breeding behaviour (see Chapter 5 and references therein).

Table 6.2. Average length (LSM) and age (ASM) at the attainment of sexual maturity, mean ($\bar{x}$) ovulation rate, gestation period (t_g), annual pregnancy rate (APR), calving interval (CaI), peak birth months, maximum reported female age, and proportion of senescent females (using criteria from Marsh and Kasuya 1984) estimated for New Zealand *Globicephala melas edwardii*, with comparisons to published data from other populations of *G. melas.*

Location	Data Source	LSM (cm)	ASM (yrs)	$\bar{x}$ ovulation rate (yr^{-1})	t_g (yrs)	APR (%)	CaI (yrs)	Peak birth months	Max. length	Max. age	Senescent (%)	Reference
G. m. melas												
NW Atlantic (Newfoundland)	Drive fishery	356[a]	*c.* 6 – 7[a]	0.3 – 0.5 (*n*=23)	1.3	24.6 (*n*=529)	3.3	May to November	511 (*n*=1,9)	56.5[b] (*n*=437)	≤ 5 (*n*=529)	Sergeant (1962), Kasuya et al. (1988)[c]
NE Atlantic (Britain)	Stranding	c. 300 – 400[a] (*n*=34)	*c.* 7[a] (*n*=17)					No seasonality detected	546	25 (*n*=31)	14 (*n*=22)	Martin et al. (1987)
NE Atlantic (Iceland)	Stranding	NA	6 – 10[a] (*n*=37)						475 (*n*=119)	34 (*n*=92)		Sigurjonsson et al. (1993)
NE Atlantic (Faroe Islands)	Drive fishery	375 (*n*=1,402)	8.3 (*n*=1,402)	0.25 (*n*=851)	1	28.6[d] (*n*=1,097)	5.1	April to September	512 (*n*=1,635)	59 (*n*=1,482)	< 5[e] (*n*=1,070)	Martin & Rothery (1993), Bloch et al. (1993)
G. m. edwardii												
SW Atlantic (Argentina)	Stranding	366 (*n*=27)	8 (*n*=27)	0.4 (*n*=23)		37[f] (*n*=23)	2.4[g]		479 (*n*=31)	35 (*n*=31)	0 (*n*=23)	Soto et al. (2017)
SW Pacific (New Zealand)	Stranding								470 (*n*=37)	35 (*n*=19)		Schroder & Castle (1998)
SW Pacific (New Zealand)	Stranding	356 (*n*=161)	6.7 (*n*=150)	0.4 (*n*=107)	1.1	19 (*n*=130)	5.3	December	500 (*n*=781)	38 (*n*=227)	0 (*n*=130)	This study (Chapters 3 & 6)

[a] Estimated from evidence of first ovulation. [b] Considered an outlier; next oldest females were 42.5 and 33.5 yrs. [c] Sergeant (1962) material re-examined. [d] Pregnancy rate was considered to be positively biased in this sample. [e] Ovulation continued through life in many females, but inter-pregnancy interval increases with age and pregnancy is rare after 40 yrs. [f] Estimated as proportion of mature females pregnant. [g] Estimated directly from ovulation rate.

Ovarian symmetry

The asymmetry of *corpora* scars in the ovaries, with a 35% increase in the mean number of scars on the left ovary compared to the right, suggests that there is a dominance of activity in the left ovary. This concurs with both LFPWs (Soto et al. 2017) and SFPWs (Marsh and Kasuya 1984) internationally but is less pronounced than what has been observed in sperm whales (*Physeter macrocephalus*; Best 1967), harbour porpoises (*Phocoena phocoena*; Murphy et al. 2010), and other delphinids (Perrin et al. 1976, Perrin et al. 1977, Murphy 2004, Danil and Chivers 2007, Kemper et al. 2019), where very strong asymmetry is observed.

Persistence of corpora and ovulation rate

The wide scatter in the plot of total *corpora* count *vs* age for all mature females (Figure 6.8a) demonstrates variation in the ASM, and high individual variability in ovulation rates in *G. m. edwardii*. Such variability has also been documented in other populations of LFPW (Martin and Rothery 1993, Soto et al. 2017), the closely related SFPW (Marsh and Kasuya 1984), and other delphinids (e.g. Myrick et al. 1986, Danil and Chivers 2007, Larese and Chivers 2009, Murphy et al. 2010, Photopoulou et al. 2017, Kemper et al. 2019) and suggests the certain females in the population reproduce more regularly than others (hence have a lower number of *corpora* scars). The continued linear increase in *corpora* count with age (Figure 6.8) indicates that these scars persist as a macroscopic body on the ovary and offers insight into female reproductive history. However, the interpretation of this reproductive history requires further research to determine whether CA scars of ovulation can be distinguished from those of pregnancy (Danil and Chivers 2007). As identified by Marsh and Kasuya (1984), even assuming that CAs persist as a record of ovulation, estimating the rate of accumulation is difficult. There is considerable evidence that CAs persist throughout life in at least some cetaceans (Perrin and Donovan 1984). Marsh and Kasuya (1984) studied the rate of regression in SFPWs and concluded that some CL regressed to OCAs within two years, but the rate of regression probably varies with hormonal status.

Reproductive phases, fecundity, and calving interval

Although gestation of 13.6 months is an approximation; it is well within the range (12 – 15 mo.) of what has been estimated for *G. m. melas* (Sergeant 1962a, Martin and Rothery 1993; see Table 6.2). Estimated APR of 19% for New Zealand *G. m. edwardii* is lower (see Table 2) than the APR estimated for *G. m. edwardii* off Argentina (37%; Soto et al. 2017), and *G. m. melas* off both Newfoundland (24.6%; Sergeant 1962a) and the Faroe Islands (28.6%; Martin

and Rothery 1993). The *apparent* pregnancy rate (not APR) estimated for *G. m. edwardii* off Argentina is reported by Soto et al. (2017) to be considerably higher than estimated for other LFPW populations; the discrepancy may be due to the small sample size (*n* = 23) of the study, and the fact that all samples were collected from a single MSE, presumably during the breeding season (although the date of stranding was not reported). For *G. m. melas* off the Faroe Islands, the proportion of mature females pregnant was 36.6% in summer and 20.7% in winter (Martin and Rothery 1993) indicating that season of sample collection can have an impact on the *apparent* pregnancy rate, due to possible foetal mortality, and must be considered when estimating APR. As the majority of sampling in the current study occurred during the summer months (presumably during the first trimester of pregnancy when foetal mortality is highest), then it is likely the APR is overestimated and is actually lower than 19% for *G. m. edwardii* off New Zealand.

Negative density-dependence theory suggests that the mechanism for the regulation of increasing populations would follow a sequence, with density-dependence first affecting the rate of immature survival, followed by age at sexual maturity, then birth rate, and finally adult survival rate (Eberhardt and Siniff 1977, Wade 2018). Females are under selective pressure to produce more progeny at a reduced population level since individuals that contribute most to population growth also add the most to the genetic composition of succeeding generations (Fowler 1981). Therefore, the proportion of sexually mature females is likely to be a reliable index of changing population status, followed by the proportion of mature females that are simultaneously pregnant and lactating (Chivers and DeMaster 1994). Thus, the lower APR and no observation of simultaneously pregnant and lactating females in *G. m. edwardii* off New Zealand, compared with higher APR and presence of simultaneously pregnant and lactating female *G. m. melas* off the Faroe Islands could indicate: (1) an increased APR in *G. m. melas* as a response to exploitation, or (2) a decreased APR in *G. m. edwardii* as a density-dependent response to decreasing prey availability. In killer whales (*Orcinus orca*), periods of reduced abundance of preferred prey have been correlated with periods of reduced survivorship and fecundity (Ward et al. 2009, Ford et al. 2010). In North Atlantic fin whales (*Balaenoptera physalus*), blubber thickness was found to decline at low per capita prey availability, and in breeding-age females, pregnancy rate declined with low blubber thickness, demonstrating a density-dependent response of pregnancy to prey limitation mediated through body condition (Williams et al. 2013).

The calving interval is calculated directly from the APR, therefore the estimated calving interval of 5.3 years for *G. m. edwardii* off New Zealand is taken to be tentative as it also does not

account for pre- and post-natal mortality and undetectable early pregnancies, i.e. pregnancy rate is not equivalent to birth rate (Danil and Chivers 2007). If *G. m. edwardii* in New Zealand waters experience high foetal mortality rates similar to those reported for *G. m. melas* (Desportes et al. 1994a), the observed proportion of pregnant females may be higher (depending on when females are sampled in relation to increased foetal mortality) than the number of observed calves born – thus underestimating the calving interval. Conversely, if there are high calf mortality rates and females conceive again at the next available opportunity, then more females would be classified as 'pregnant', and the calving interval would, in fact, be shorter than estimated. Some foetal and calf mortalities almost certainly occur in the population, and it is not known when pregnancy first becomes macroscopically visible in pilot whales (Kasuya and Marsh 1984), so the calculated calving interval is considered to be an average.

The oldest female examined in this study (33 yrs), was pregnant with a 23.5 cm foetus. Assuming a normal gestation, parturition would have been expected when the mother was *c.* 34 years old. As ASM is estimated to occur at 6.7 years, followed by first parturition at approximately eight years, the average breeding longevity is estimated to be a minimum of 26 years for New Zealand LFPWs. Given an estimated calving interval of 5.3 years, the average reproductive output amounts to approximately five calves by 34 years of age. This figure will be upwardly biased if pregnant females are over-represented in the seasonally biased samples, as suspected.

Reproductive senescence

The observation that pregnancy rate decreases and the duration of the resting period increases in animals older than 10 years (Figure 6.11) suggests that female *G. m. edwardii* show a reduction in fecundity with age, which concurs with that reported for *G. m. melas* (Martin and Rothery 1993). In contrast to *G. m. melas*, reproductive senescence was not evident for New Zealand *G. m. edwardii*. However, the maximum age estimated for female *G. m. edwardii* in the current study (33 yrs) was much lower than that recorded for *G. m. melas* in the Faroe Islands (59 yrs; Martin and Rothery 1993) and Newfoundland (56.5 yrs; Sergeant 1962a, Kasuya et al. 1988b), where longevity exceeded 50 years (see Table 6.2 and Chapter 3). The smaller sample size in the current study (n = 150), compared with the availability of much larger datasets from North Atlantic drive fisheries (e.g. n = 1,402; Martin and Rothery 1993), would have decreased our probability of sampling the rare older (possibly senescent) females. However, even if true reproductive senescence does occur in a small proportion of the oldest

females, so few live long enough to enter this phase (e.g. approximately 10% of females reach 40 yrs of age in Faroese LFPWs; Bloch et al. 1993) that it is unlikely to represent a significant and functional part of the life history of social ecology of this species (Martin and Rothery 1993, Ellis et al. 2018b). Less than 5% of female *G. m. melas* are reported to become reproductively senescent, and pregnancy can potentially continue throughout life (oldest pregnant female 55 yrs; Martin and Rothery 1993). This is in contrast to the closely related SFPW and killer whale, for which there is evidence that the post-reproductive adult lifespan is substantial (Marsh and Kasuya 1984, Croft et al. 2015, Ellis et al. 2018). It has also been suggested that false killer whales have a significant post-reproductive lifespan (Photopoulou et al. 2017), though more data are needed to establish the extent and frequency of post-reproductive life in false killer whales (Ellis et al. 2018).

It is considered that both resident killer whales and SFPWs have selected for an extension of the post-reproductive lifespan, whereas an acceleration in the mortality rate is observed in LFPWs (Foote 2008). The observed variation in life history strategies between the two pilot whale species may in part be due to the social organisation within stable social groups and the benefits of cooperative foraging and multigenerational transfer of information (Marsh and Kasuya 1984, Whitehead 2015). In resident killer whales, increasing inclusive fitness, due to 'late-life' helping by fulfilling a mother and grandmother role has been observed in the interactions between post-reproductive females and younger females in the group (Croft et al. 2017). Further, increased mortality of calves in older generational females compared to younger females has been reported, resulting from intergenerational reproductive conflict (Croft et al. 2017). The social structure of pilot whale pods is similar to that of killer whales (Olson 2018), however, the reason for the observed differences in the mortality rate acceleration between the two pilot whale species has not been established (Foote 2008) and requires further investigation.

Reproductive seasonality

In this study, *G. m. edwardii* exhibited weak patterns of bimodal reproductive seasonality. Back-calculating conception dates of foetal and yearling calf specimens suggested a likely peak of conceptions (28%) in September and October (austral spring; Figure 6.14a), with a secondary peak (10%) in April (austral autumn), though the estimated conception dates occurred in all months of the year. Estimated birth dates of the foetuses and yearling calves suggest that some births also occur in every month of the year, with a primary peak of estimated births in December (early austral summer), and a smaller, secondary peak eight

months later, in May (austral autumn; Figure 6.14b). This breeding pattern is consistent with the that reported for *G. m. melas* (Martin and Rothery 1993), and with information presented on male *G. m. edwardii* (see Chapter 5), in which active spermatogenesis was observed in all sexually mature individuals, although the sample was seasonally biased. The indications of the timing of conceptions and births presented herein must be interpreted as approximate estimations only, since the true patterns may be masked by a limited understanding of both pre- and post-natal growth of pilot whales (Martin and Rothery 1993). The presence of a foetus provides evidence that conception occurred, but as the rate of embryonic development in cetaceans is not well understood, the size and date of death can only be used to estimate conception date with an accuracy of approximately one month (Martin and Rothery 1993). Uncertainty also results from assuming a uniform growth rate from birth (Miyazaki 1977, Barlow 1984, Martin and Rothery 1993). The calf growth rate probably slows down in the first year after birth as it does in most mammals, including other small cetaceans (Hohn and Hammond 1985), but there is no specific information available for pilot whales.

While typically rare in mammals, a bimodal breeding pattern has also been proposed for *G. m. melas* in the North Atlantic (Martin and Rothery 1993), whereas a multimodal breeding pattern was reported for *Stenella* spp. on the Pacific coast of Japan (Kasuya 1972, Miyazaki 1977) and the eastern tropical Pacific (Barlow 1984). A bi or multimodal distribution of breeding events could be induced by changes in the length of the reproductive cycle, perhaps as a density-dependent result of high mortality rates (Barlow 1984). Such an explanation is plausible in a situation where females were ready to conceive mid-way between breeding seasons, and where giving birth outside the 'normal' calving period did not significantly disadvantage either mother or calf. An alternative explanation is that most of the animals calving during the second peak were doing so after aborting a foetus conceived during the main peak; assuming it is advantageous to conceive again in the same season after early pregnancy termination (Martin and Rothery 1993). A further explanation for a bimodality could be that samples were taken from two pilot whale populations, each with a unimodal breeding pattern but with peaks several months apart. There is evidence that two or more subpopulations of *G. m. melas* are present in the North Atlantic and occur around the Faroe Islands (Wade et al. 2012), with a similar situation existing for SFPWs in the North Pacific (Kasuya et al. 1988a, Kasuya and Tai 1993). Given the observed weak breeding synchrony within the New Zealand *G. m. edwardii* population, all of the above scenarios are consistent with the fact that selective pressure to calve at a particular time of the year must be low.

The utility of strandings data for estimating reproductive parameters

Stranding data might not always provide a representative sample for estimating the life history parameters of a population, however, MSEs of pilot whales generally comprise mixed sex and age groups of apparently 'healthy' animals (Martin et al. 1987) and are therefore expected to be representative of the population. Post-mortem sampling of stranded animals also often restricts the type of analyses that can be performed due to small sample sizes. However, the high incidence of MSEs in New Zealand (see Chapter 7) resulted in a substantial sample size for this study (i.e. *n* = 150 females with age and reproductive status). Therefore, this study has enabled the estimation of most standard reproductive parameters not previously possible with more limited datasets.

Data and specimens collected from female carcasses provide information on age (tooth GLGs), sexual maturity (ovaries and mammary glands), and fecundity (ovarian *corpora* and scars). The limitations of these methods include a degree of subjectivity in reading teeth and ovarian scars. In addition, only a proportion of females involved in any one MSE are often retrieved, and these individuals may not be representative of the population as a whole. Longitudinal studies (i.e. long-term observational studies in which recognisable animals and their offspring are monitored over many years) have sometimes discovered errors in life history parameters determined by post-mortem studies (Mann and Karniski 2017). Such longitudinal studies, when carried out for a length of time adequate for the longevity of the species, can provide answers to questions which cannot be addressed from cross-sectional studies of animals just once, i.e. at their death (Martin and Rothery 1993). Conversely, observational studies in the field cannot, for example, provide information about ovulatory activity or foetal growth, or provide more than an upper bound on gestation period (Martin and Rothery 1993). Complementary observational and post-mortem studies applied to the same population may offer the opportunity to characterise age and reproductive characters more precisely and would help to account for the limitations of each approach (Kemper et al. 2019). Such complimentary studies have not yet been attempted for pilot whales, presumably due to the difficulties in conducting field studies on such long-lived and typically pelagic species.

6.6 Conclusions

Parameters such as LSM, ASM, fecundity, calving interval, senescence and reproductive seasonality are important in the life history of any species and enable us to study the social and demographic structures that help to understand population dynamics. This study has provided some of the first insights on the reproductive biology of female *G. m. edwardii* in the

Southern Hemisphere and allowed us to draw comparisons with other LFPW populations. *G. m. edwardii* calving appears to be diffusely seasonal with some births occurring throughout the year and peaking in December (early austral summer). Females were estimated to produce a calf every 5.3 years, after an average gestation period of 13.6 months, an average lactation period of 19.3 months, and an average resting period of approximately two years. In contrast to *G. m. melas*, no evidence of reproductive senescence was found. Although the overall breeding pattern is comparable between the two subspecies, estimated length and age attainment of sexual maturity is reported to be lower in *G. m. edwardii* than best estimates reported for *G. m. melas* (Sergeant 1962a, Bloch et al. 1993a, Desportes et al. 1993b, Martin and Rothery 1993), indicating that geographic variation in life history occurs for this species, which likely reflects population-specific adaptations to local habitats. LFPW strandings tend to involve large groups of mixed ages and sexes (see Chapter 7), and these MSEs should be recognised as valuable opportunities to collect further behavioural, genetic and life history data in order to fully comprehend the biology and population dynamics of *G. m. edwardii*. Future research to assess reproductive dysfunction and disease and their associations with exposure to anthropogenic pollutants is also recommended.

Chapter 7

Using stranding records to inform conservation management of a data-poor cetacean species; the long-finned pilot whale (*Globicephala melas edwardii*) in New Zealand waters

Mass stranding of c. 600 long-finned pilot whales, Farewell Spit, Golden Bay, February 2017.

Photograph credit: Project Jonah New Zealand.

In this chapter, spatial and temporal patterns of LFPW strandings on the New Zealand coast are examined using all long-finned pilot whale (*Globicephala melas edwardii*) data held in the NZWSDB (1874 – 2017) but with emphasis placed on strandings between 1978 and 2017. Following the establishment of the New Zealand Marine Mammal Protection Act in 1978, cetacean strandings were more actively recorded from this point on. This chapter achieves the fifth research objective of this thesis:

Objective 5: Identify spatiotemporal trends in the New Zealand long-finned pilot whale stranding record.

This chapter is a reformatted version of the following manuscript:

Betty EL, et al. (in review). Using stranding records to inform conservation management of a data-poor cetacean species. Biodiversity and Conservation.

7.1 Abstract

Conservation monitoring of highly mobile species in relatively inaccessible habitats presents a considerable challenge to wildlife biologists. Effective conservation strategies require knowledge of cetacean ecology that is often challenging and expensive to obtain. Despite their caveats, stranding data represent an underused resource to study the long-term dynamics of cetacean populations. Using long-finned pilot whale (LFPW; *Globicephala melas edwardii*) strandings on the New Zealand coast as a case study, the utility of stranding data to provide crucial insights into the ecology of data-poor species is demonstrated. A total of 8,571 LFPWs stranded on the New Zealand coast within a 40-year period between January 1978 and December 2017. Strandings occurred in all months, though significant seasonal variation was evident, with 66% of stranding events reported during austral spring and summer months (October – February). The majority of individuals stranded at hot spot locations Golden Bay, Great Barrier Island, Stewart Island and the Chatham Islands, with emerging hot spot analysis (ArcGIS) used to identify spatiotemporal trends. While no significant trend in the overall numbers of stranded LFPWs was evident, the numbers of individuals stranded have declined in areas of the Far North, Coromandel, Canterbury, Otago, and the Chatham Islands but increased in Golden Bay and Stewart Island. When combined with other contextual information, such trends help identify the most significant clusters of LFPW strandings on the New Zealand coast, provide baseline ecological data on a poorly understood subspecies, and can be used to guide conservation management of *G. m. edwardii* in New Zealand waters.

7.2 Introduction

Conservation management of highly mobile species that cover vast areas, or are rarely encountered, is often hindered by a lack of accurate data on their ecology, life history parameters, abundance, distribution, and population status (Magera et al. 2013). Marine mammals often fit this description yet have been identified as useful indicators of ecosystem change (Hammond et al. 2002) and a priority for sustained conservation effort (Schipper et al. 2008, Kaschner et al. 2011). While marine mammals are sentinel species that can reflect cumulative impacts of stressors and facilitate our overall understanding of ocean health (Wells et al. 2004, Evans et al. 2005, Moore 2008), monitoring marine mammal populations long-term, and at fine-scale, is financially prohibitive (Peltier et al. 2013), especially when approaches such as capture-mark-recapture or distance sampling is required (Meager and Sumpton 2016). As a consequence, information on significant threats and estimates of abundance and distribution are, for many pelagic species at least, generally unavailable in most geographic regions (ten Doeschate et al. 2018). However, long-term opportunistically collected sighting and/or stranding records are often available.

In recent years, it has been increasingly recognised that opportunistic data collected from cetacean strandings can offer a relatively low-cost monitoring method. Stranding records have the potential to provide critical insights into species distribution (Maldini et al. 2005), demography (Mannocci et al. 2012), and population structure (Bilgmann et al. 2011, Stockin et al. 2014), particularly if they cover a large area and are collected over long time periods (Leeney et al. 2008, Pikesley et al. 2011, Meager and Sumpton 2016). Stranding data have helped establish past distribution and declines of common dolphin (*Delphinus delphis*) in various Mediterranean Sea areas (Bearzi et al. 2003), and proven value as an early warning system for identifying fisheries impacts in species such as the common bottlenose dolphin (*Tursiops truncatus*; Byrd et al. 2008). Analyses of cetacean stranding data have also identified changes in cetacean communities attributed to increased sea temperature (MacLeod et al. 2005) and provided information on species that are otherwise rarely encountered (Thompson et al. 2013). Over long time series (> 100 yrs), stranding records have further revealed higher species richness than line-transect survey methods for a given area (Pyenson 2010). Stranding records have also exhibited the potential to detect population declines sooner than survey data (Gerrodette 1987, Gulland 2006). For example, increased in gray whale (*Eschrichtius robustus*) strandings were observed along the west coast of the U.S. and Mexico in 1999, but a decline in the population was not detected by survey effort until 2001 (Gulland et al. 2005).

Monitoring of cetacean strandings is encouraged by multiple intergovernmental organisations and agreements (e.g. International Whaling Commission, and multiple agreements under the Convention for Migratory Species). Despite this, stranding data remain relatively underused as an indicator to assess cetacean population status or broader marine ecosystem conservation (ten Doeschate et al. 2018). This is in part due to uncertainty around the relationship between the stranding record and the at-sea population, which can be confounded by the complexity of stranding events including unusual stranding events (e.g. epizootics or mass strandings; Williams et al. 2011), environmental variation (Evans et al. 2005), carcass drift (Peltier et al. 2012), or reporting effort. It is also arguable whether strandings records are representative of population demography since mortality risks vary with ontogeny (Perrin and Geraci 2002, Meager and Sumpton 2016).

Periodicity in cetacean strandings has been linked to large-scale climate events, resulting from long-term shifts in sea-pressure gradients (Evans et al. 2005, Pierce et al. 2007). The biological signal, however, can be confounded by the various physical and social factors that influence stranding events (Peltier et al. 2012), complicating the interpretation of stranding data at a population level. For example, physical factors, such as coastal topography and oceanography, have been linked to the occurrence of stranding events (Hamilton 2018), and tight social cohesion of mass stranding cetaceans (e.g. pilot whales) is thought to be a significant contributing factor for groups stranding and re-stranding after being refloated by human-initiated efforts (Sergeant 1982, Perrin and Geraci 2002). Nonetheless, long-term stranding records permit analysis of historical patterns and trends, providing a means to define baseline stranding rates in addition to biological and ecological metrics (ten Doeschate et al. 2018). These include cause of death (e.g. Gulland 2006, Stockin et al. 2009, Jepson et al. 2013), nutritional condition (e.g. Lockyer 1995, Gómez-Campos et al. 2011), disease burden (e.g. Gulland and Hall 2007, Arbelo et al. 2013, Sierra et al. 2016), diet (e.g. Beatson and O'Shea 2009, Santos et al. 2014, Beasley et al. 2019), life history (e.g. Murphy et al. 2009, Jefferson et al. 2012, this thesis) and environmental contaminant levels (e.g. Stockin et al. 2010, Jepson et al. 2016, Murphy et al. 2018). These metrics can assist in identifying changes, pressures and threats; in terms of both acute effects and long-term loss of population viability (Leeney et al. 2008).

Long-finned pilot whales (LFPWs; *Globicephala melas*) are known for stranding in large numbers of mixed ages and sexes, and mass stranding events (MSEs) often recur in a specific geographic location e.g., Cape Cod, Massachusetts, USA (McFee 1990, Wiley et al. 2001, Sweeney et al. 2005); Tasmania, southern Australia (Evans et al. 2005, Gales et al. 2012,

Beasley et al. 2019); and Farewell Spit, Golden Bay, New Zealand (Gaskin 1968, Baker 1981, Brabyn 1991). Despite their propensity to mass strand, LFPWs have a wide-ranging oceanic distribution and typically prefer deep water beyond the shelf edge (Taylor et al. 2008). As a result, data collected from strandings are the primary data available for LFPWs throughout most of their range, yet few analyses of pilot whale stranding patterns have been conducted (e.g. Brabyn and McLean 1992, Brabyn and Frew 1994, Hamilton 2018). Although the global International Union for the Conservation of Nature threat classification listing for LFPWs has recently been updated from 'Data Deficient' to 'Least Concern', some populations, especially *G. m. edwardii* populations in the Southern Hemisphere, undoubtedly remain data-poor (Minton et al. 2018).

Using LFPWs as a case study, the utility of strandings data to provide insights into the ecology of a data-poor species is demonstrated. The LFPW (*G. m. edwardii*) is described by the New Zealand government as a "poorly known migrant species in New Zealand waters" that is "occasionally implicated in mass stranding events" (Suisted and Neale 2004). However, the LFPW is, in fact, the most common mass stranding cetacean recorded on the New Zealand coast (New Zealand Whale Stranding Database [NZWSDB], extracted on 20/07/2018). Stranding records held in the NZWSDB were used to assess spatiotemporal patterns in the incidence of LFPW strandings on the New Zealand coast, with emphasis placed on strandings between January 1978 and December 2017. Specifically, the following are examined: (1) the sex and maturity composition of stranded individuals, (2) if the incidence of LFPW MSEs has increased over time, (3) if MSEs are more likely to occur at certain times of the year, (4) if there are geographic hot spots of MSEs, and (5) if there have been temporal shifts in hot spot locations. How improved understanding of the trends in the stranding record can contribute to the utility of stranding data as an ecological indicator for a data-poor species is discussed.

7.3 Material and methods

Data collection

All data presented here on LFPW stranding locations, dates, and composition of stranded pods held in the NZWSDB to December 2017, have been collated, checked for transcription errors, and verified against original sources if they were accessible. Sampling units used are (1) the number of stranding events and (2) the number of stranded individuals. Stranding events were not further categorised by cause of stranding. Accurately identifying causation of a stranding event is often not possible, for example, disease or poor health may lead to navigational errors and subsequent stranding (MacLeod et al. 2004), but necropsies to determine the health

status of stranded individuals are not routinely undertaken. There was also no separation of records into live or dead strandings. There are regions of New Zealand with very remote coastlines, for example, the far end of Farewell Spit and most of Stewart and Chatham Islands, where strandings may not be detected for several days or more, post-event. Therefore, although the condition of the animal when it was first observed is known, it can not always be confirmed if it was alive or dead when it stranded.

An MSE was defined as two or more animals stranded together, excluding mother-calf pairs (Geraci and Lounsbury 2005). Following MacLeod et al. (2004), unless individuals were known to have stranded together at the same place and time, they were considered separate records to avoid any possible difficulties in trying to ascertain whether individuals who stranded nearby in space and/or time were related in any way. There is a chance that some stranding events have been counted twice, but the number of such possible double counts was low (< 5% of all cases). Finally, although LFPW records in the NZWSDB date back to 1874, stranding events were not consistently reported until the introduction of the New Zealand Marine Mammal Protection Act in 1978, when it became government policy to record all cetacean stranding events. The distribution of all New Zealand LFPW stranding records (January 1874 – December 2017) were mapped by stranding event using geographical mapping software, ArcMap 10.6.1 (Esri Inc., Redlands, CA). However, only stranding records from 1978 onwards (January 1978 – December 2017) are included in the following analyses.

Sex and maturity composition

Whenever possible, individuals were classified as calves (≤ 1 yr), juveniles (> 1 yr but sexually immature) or adults (sexually mature). Individuals were considered calves if they had a total body length (TBL) ≤ the estimated length at one year of age using sex-specific growth curves for LFPWs in New Zealand waters, i.e. 237 cm for females and 248 cm for males (see Chapter 3). Juveniles were classified as individuals with a TBL greater than calves but less than adults; defined using estimated length at sexual maturity (LSM) values of 472 and 356 cm for males (see Chapter 5) and females (see Chapter 6), respectively. Sex ratios were estimated for the dataset (1978 – 2017), using maturity groups: calves, juveniles, adults. Sex ratios were estimated using the empirical logistic transform method (Murphy 2004). Exact binomial tests (two-tailed) were used to test for significant departure from the expected sex ratio of unity, i.e. the ratio of males to females 1:1.

Temporal patterns

Trends in the annual and monthly frequency of occurrence of stranding events, and numbers of animals involved in each event, were examined. First, the Mann-Kendall trend test (Mann 1945, Kendall and Gibbons 1990) was applied using the 'Create Space-Time Cube' geo-processing tool in ArcMap 10.6.1 (Esri Inc., Redlands, CA) to test whether statistically significant temporal trends in strandings exist throughout the 40-year time series (1978 – 2017). A negative binomial generalised linear model with a log link was used to compare the likelihood of stranding events on the New Zealand coast by month of the year (1978 – 2017), with statistical significance defined as $p \leq 0.05$.

Spatial patterns

To investigate spatial patterns in strandings (1978 – 2017), a 15 km (linearly spaced in *X* and *Y*) grid was placed over the New Zealand coastline, and kernel density analysis in ArcMap 10.6.1 (Esri Inc., Redlands, CA) was used to investigate the density of LFPWs stranded within each grid square. Spatial statistics tools in ArcMap 10.6.1 (Esri Inc., Redlands, CA) were used to further analyse spatial patterns in the stranding data. First, Global Moran's I (Ord and Getis 1995) was applied to compute spatial autocorrelation in numbers of LFPWs stranded on the New Zealand coast between 1978 and 2017. Using the location, distance, and values of cells, Moran's Index was calculated with values ranging between -1 (dispersed pattern) and +1 (clustered pattern), and values near zero indicating random distribution. Several distance classes (including 5, 15, 50, 100, 150 and 200 km) were tested to determine the distance band where autocorrelation and clustering patterns occur within LFPW stranding distribution. This approach evaluates whether strandings across New Zealand occur non-randomly and if so, then whether they are dispersed or clustered. Fixed distance band and Euclidean distance were used for the autocorrelation analysis.

Once the stranding patterns across New Zealand were determined, a spatial cluster analysis was conducted to determine whether there were spatial hot spots in strandings between 1978 and 2017. The term 'hot spot' has been used across many disciplines to describe a region or value that is generally higher relative to its surroundings (Lepers et al. 2005, Aben et al. 2012, Isobe et al. 2015). Here, a hot spot is defined as a location that exhibits statistically significant clustering (local autocorrelation) in the spatial pattern of strandings. Using the 'Hot Spot Analysis' geoprocessing tool in ArcMap 10.6.1 (Esri Inc., Redlands, CA), the Getis-Ord Gi* statistic (Getis and Ord 1992) was computed for each stranding location to determine areas where non-random concentrations of high and low numbers of LFPWs stranded along the New

Zealand coast. A fixed distance band of 50 km was used for calculating neighbourhood statistics following the analysis of Moran's autocorrelation where this distance band resulted in high *z*-score values as an indication of clustering patterns in the stranding data. The Gi* statistic returned for each stranding location in the dataset is a *z*-score (standard deviation) with an associated *p*-value (statistical probability) and Gi_Bin (confidence level bin). For statistically significant positive *z*-scores, the larger the *z*-score is, the more intense the clustering of high values (hot spot). For statistically significant negative *z*-scores, the smaller the *z*-score is, the more intense the clustering of low values (cold spot). Hot spots (higher numbers of stranded LFPWs than expected by chance) were identified as locations with an associated Gi_Bin > 1 (90% confidence), > 2 (95% confidence), or > 3 (99% confidence) while cold spots (lower numbers of stranded LFPWs than expected by chance), were identified as locations with an associated Gi_Bin < -1 (90% confidence), < -2 (95% confidence), or < -3 (99% confidence).

Spatiotemporal patterns

The spatial cluster analysis (described above) was extended to incorporate information about the temporal dimension of the data using the 'Emerging Hot Spot Analysis' geoprocessing tool in ArcMap 10.6.1 (ESRI Inc. Redlands, CA). The emerging hot spot analysis tool was used to evaluate spatiotemporal patterns of LFPW strandings on the New Zealand coast using a combination of two statistical measures: (1) the Getis-Ord Gi* statistic to identify the location and size of stranding events (as above), and (2) the Mann-Kendall trend test to detect temporal trends at each location. While the emerging hot spot analysis tool has been used to investigate emerging hot spots of malaria infections (Chihanga et al. 2016), fatal landslides (Haque et al. 2016), forest loss (Harris et al. 2017), and Florida manatee mortality (*Trichechus manatus latirostris*; Bass 2017), it has not yet been applied to cetacean stranding analyses.

Before statistical analysis, data were transformed into netCDF (network common data form) 'space-time cube' structure by aggregating stranding locations in space-time 'bins' with a spatial resolution of 15 km. The value of each bin was assigned as the number of individuals stranded in a given year. The netCDF structure stores space as latitude and longitude (*X*, *Y*) coordinates and time (*Z*), i.e. year the stranding occurred, as another dimension (Figure 7.1). The decision to aggregate data into 15 km bins was made after empirically testing bin sizes ranging from 1 to 50 km; the final selection of 15 km preserved a varied frequency distribution of numbers of whales stranded. Small adjustments to bin size did not significantly impact the final results.

In the first stage of the statistical analysis, the emerging hot spot analysis tool uses the Getis-Ord Gi* statistic to measure the intensity of clustering of high or low values (i.e. numbers of stranded whales) in a bin relative to its neighbouring bins in the data cube. The sum for a bin and its neighbours is compared proportionally to the sum of all bins. When the sum of a bin is different than expected, and that difference is too large to be the result of random chance, the result is a statistically significant *z*-score. The Getis-Ord Gi* statistic generates *z*-scores (standard deviations) and *p*-values (statistical probabilities) for each bin that indicate whether strandings are statistically clustered compared to strandings in neighbouring bins, as well as strandings across New Zealand. A *z*-score above 1.96 or below -1.96 means that there is a statistically significant hot or cold spot of strandings at a significance level of $p < 0.05$. The larger a bin's *z*-score, the more intense the clustering of values (hot spot). Due to the cube structure of the data, neighbouring bins exist both in time and in space. A fixed distance band of 50 km was used to define neighbourhood size in space, and temporal neighbours were defined using a time-step interval of one year.

Secondly, the Mann-Kendall statistic was used to test whether a statistically significant temporal trend exists throughout each bin's 40-year time series of *z*-scores resulting from the Getis-Ord Gi* statistic. To evaluate temporal trends for each bin, each time-step (i.e. year) was compared to the one directly after it. If the *z*-score in the second time-step was larger than the first, the result was +1 (increasing trend). If the *z*-score in the second time-step was smaller than the first, the result was -1 (decreasing trend). Each pair of time-steps was compared over the 40-year time series, generating the Mann-Kendall statistic with associated *z*-score and *p*-value for each bin. The expected sum is zero, indicating no temporal trend. Based on the variance for the values in the bin time series and the number of time-steps, the observed sum is compared to the expected sum (zero) to determine if the difference is significant ($p < 0.05$).

7.4 Results

12,592 LFPWs stranded in 351 independent events (including 168 MSEs) on the New Zealand coast between 1874 and 2017 (Figure 7.2). The spatial distribution of all *G. m. edwardii* strandings recorded in the NZWSDB over the 144 years between 1874 and 2017 are shown in Figure 7.3, a – d). The largest MSE recorded on the New Zealand coast occurred at Chatham Island in 1918 and involved an (estimated) 1000 individuals (Figure 7.3, 7.3c). Other sizeable MSEs comprised 450 individuals stranded on Great Barrier Island in August 1985 (Figure 7.3, 7.3a), and more recently, *c.*600 LFPWs stranded on Farewell Spit, Golden Bay in February 2017 (Figure 7.3, 7.3b).

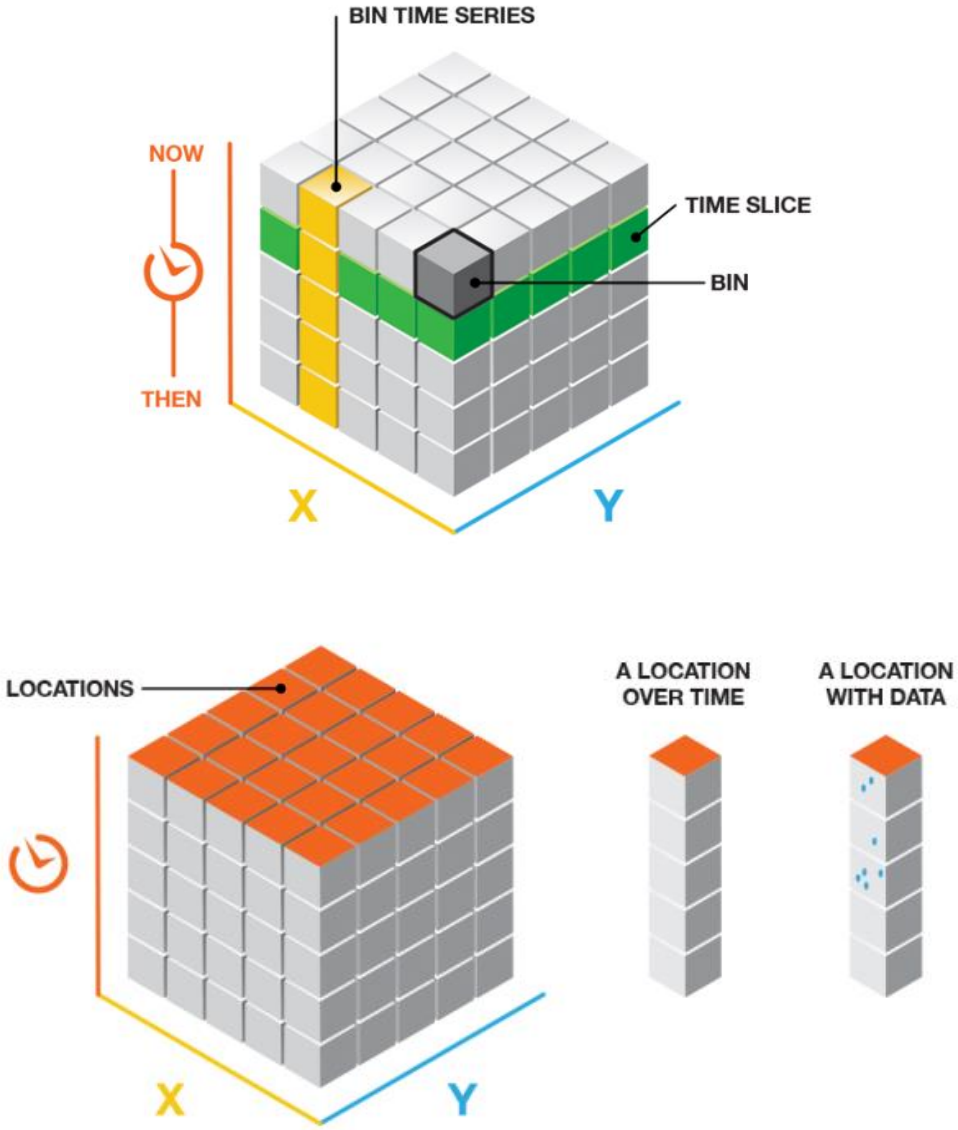


Figure 7.1. Space-Time Cube.

Each time slice represents a time increment or time-step interval (*Z*; 1-yr time increment). These time steps in sequential order create a bin time series. The *X*- and *Y*-axis of each bin is the geographic location of each bin (*X*, *Y*; 15 km spatial resolution). Source: modified from http://desktop.arcgis.com/en/arcmap/10.3/tools/space-time-pattern-mining-toolbox/emerginghotspots.htm.

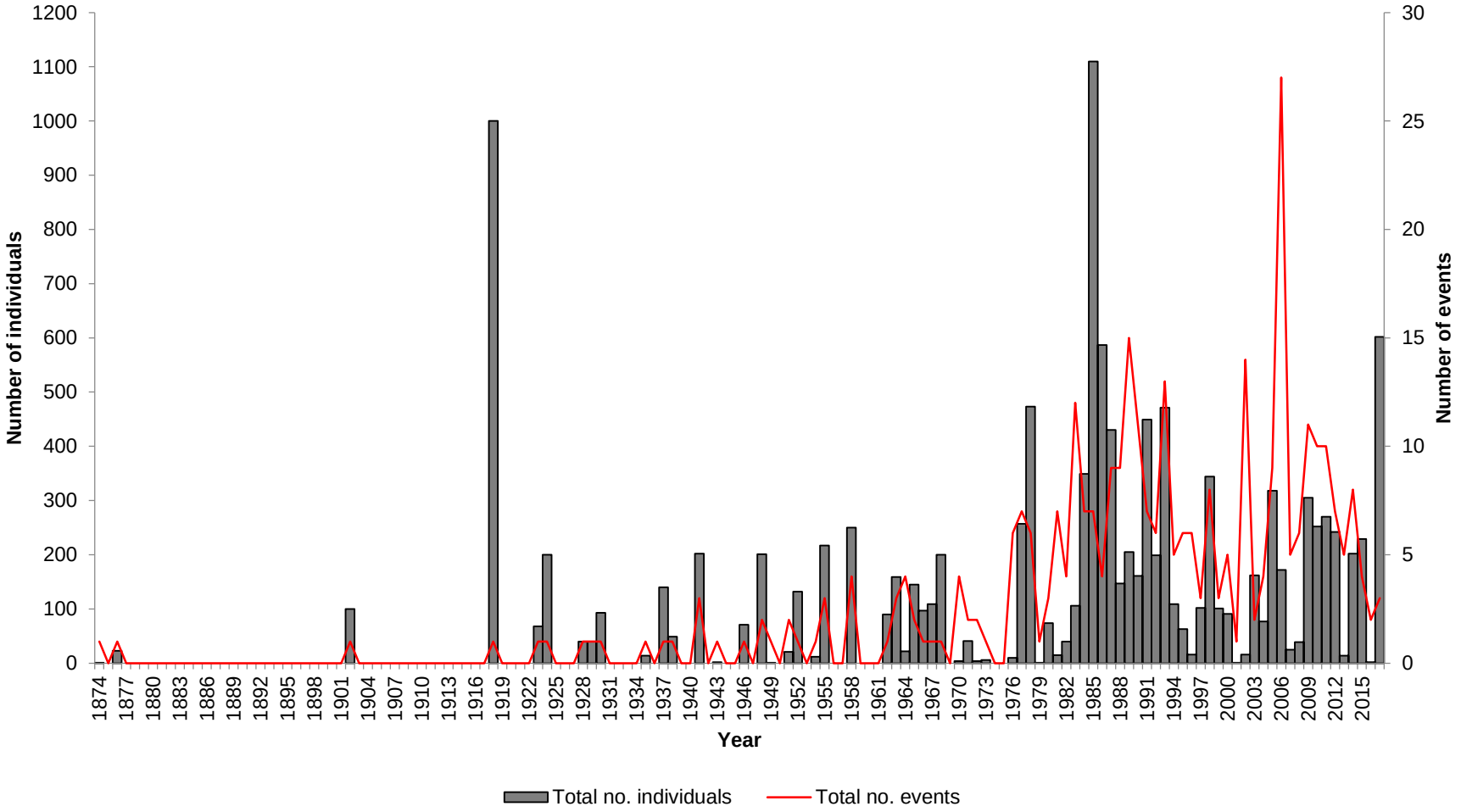


Figure 7.2. Total number of LFPW stranding events (*n* = 351), and total number of stranded individuals (*n* = 12,592) recorded in the New Zealand Whale Stranding Database (1874 – 2017).

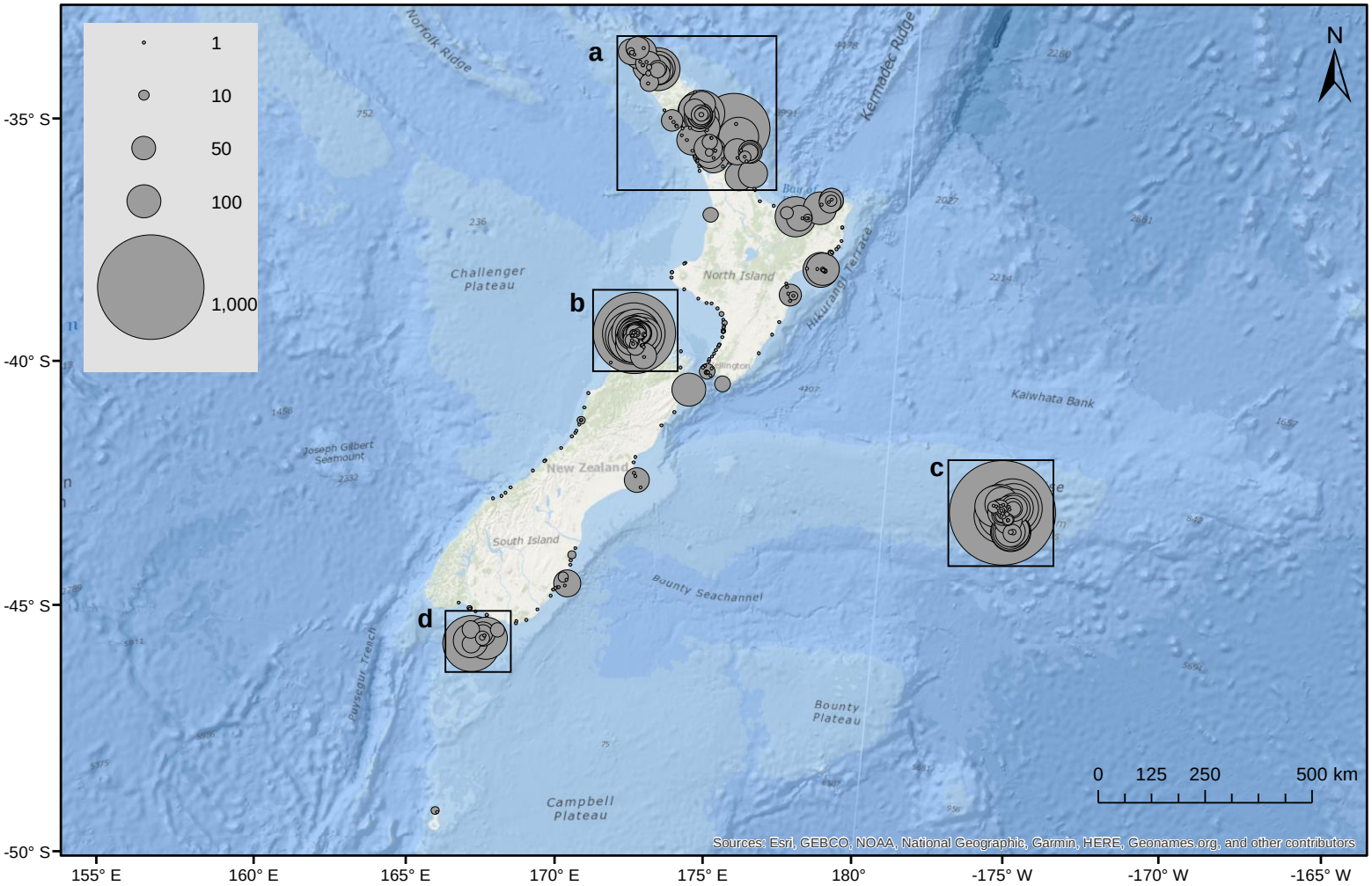


Figure 7.3. Spatial distribution of all New Zealand LFPW stranding events recorded in the New Zealand Whale Stranding Database (1874 – 2017).

Proportional symbols represent the number of individual pilot whales in each stranding event. Boxes indicate areas with frequent, recurrent stranding events: (a) Northland and Auckland (including Great Barrier Island), (b) Golden Bay, (c) the Chatham Islands, (d) Stewart Island (further detail shown in Figs. 7.3a, b, c, d).

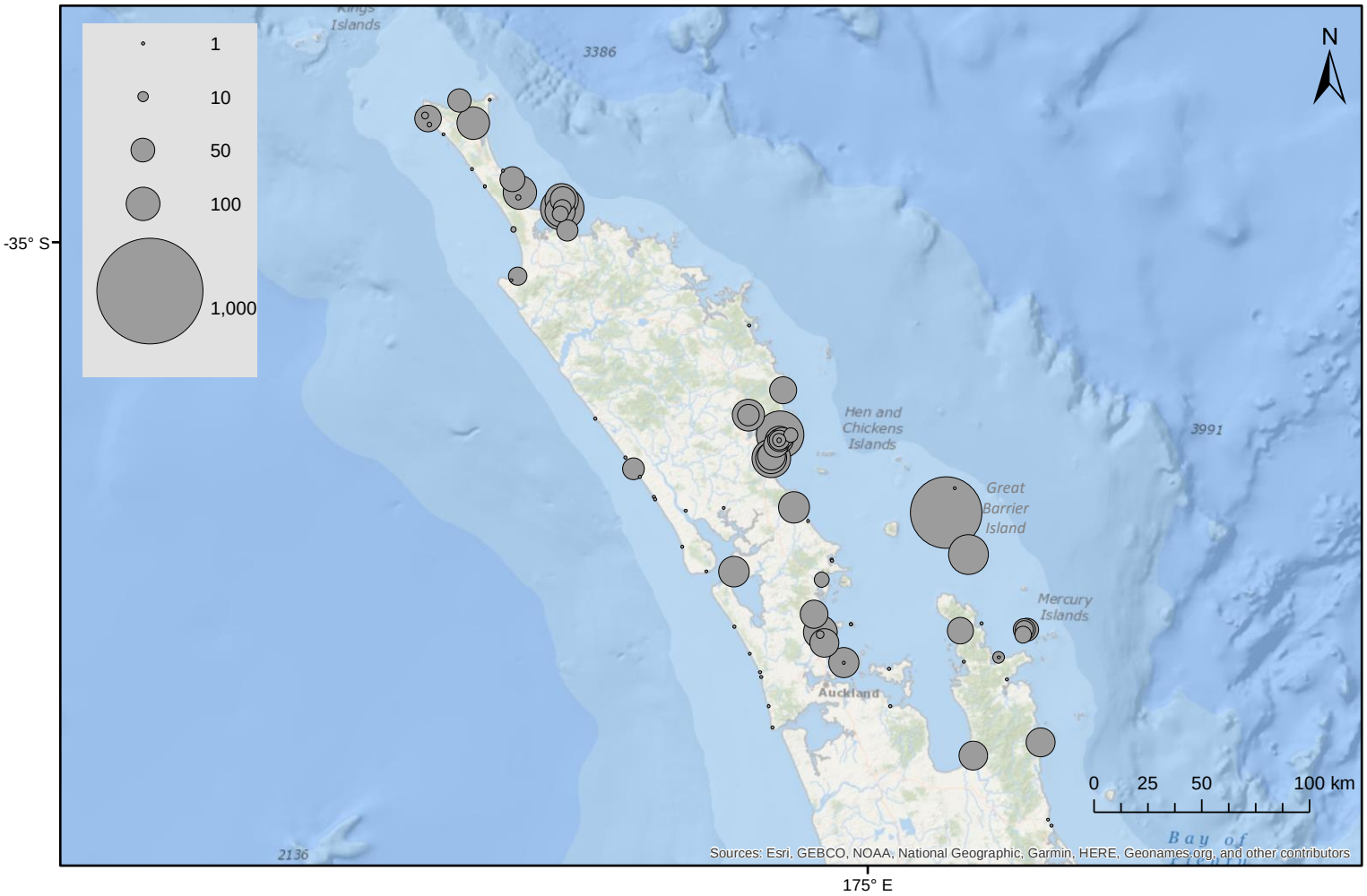


Figure 7.3a. Spatial distribution of all New Zealand LFPW stranding events in northern New Zealand 1874 – 2017.

Proportional symbols represent the number of individual pilot whales in each stranding event.

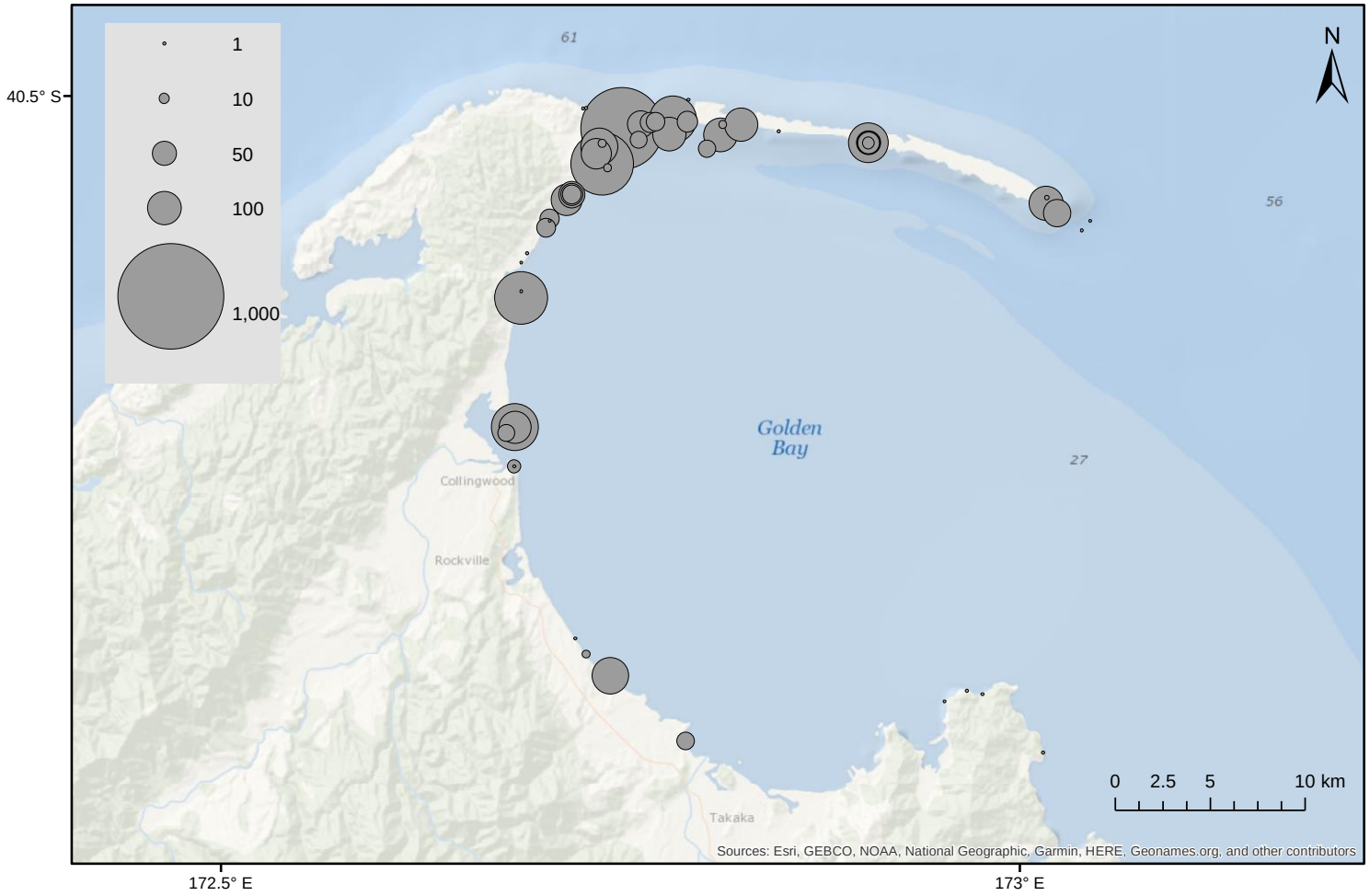


Figure 7.3b. Spatial distribution of all New Zealand LFPW stranding events in Golden Bay 1874 – 2017.

Proportional symbols represent the number of individual pilot whales in each stranding event.

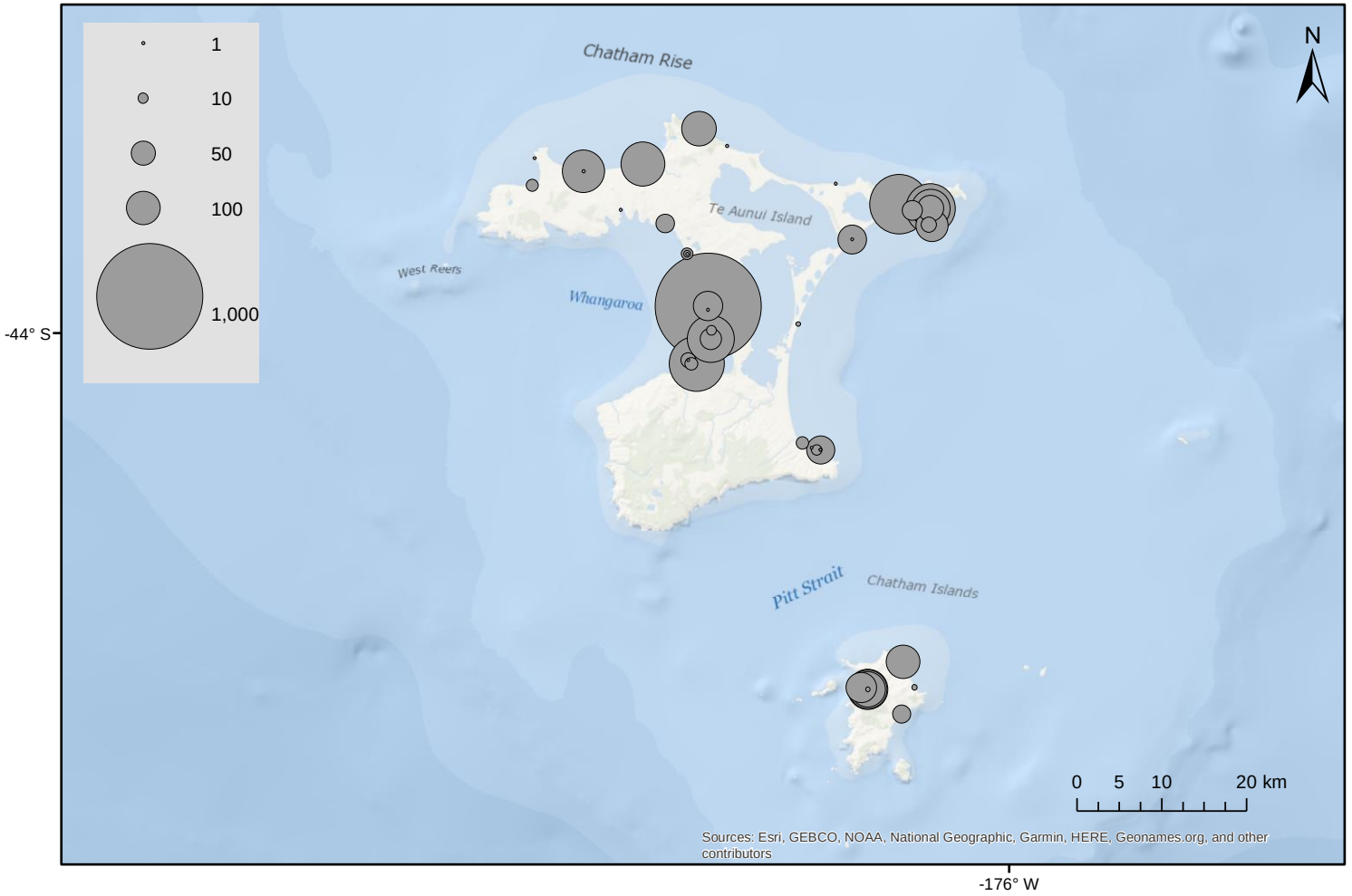


Figure 7.3c. Spatial distribution of all New Zealand LFPW stranding events on the Chatham Islands 1874 – 2017.

Proportional symbols represent the number of individual pilot whales in each stranding event.

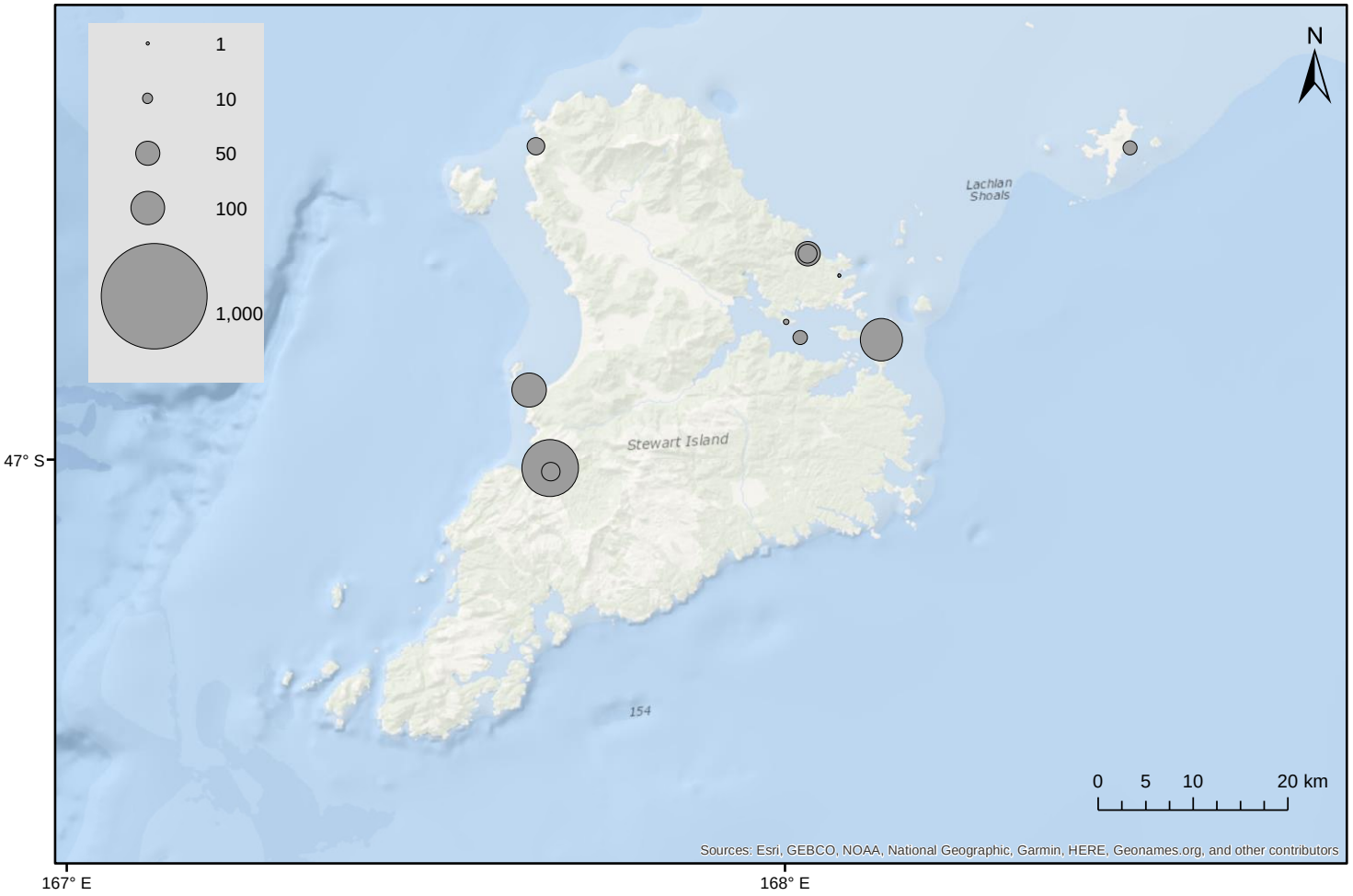


Figure 7.3d. Spatial distribution of all New Zealand LFPW stranding events on Stewart Island 1874 – 2017.

Proportional symbols represent the number of individual pilot whales in each stranding event.

Sex and maturity composition

Between 1978 and 2017, records indicate that 8,571 LFPWs stranded along the New Zealand coastline during 285 independent events. Data on sex, age, and length were available for a subset of the LFPWs stranded between 1978 and 2017 (Table 7.1). Significant female bias (0.68 male: 1 female) was noted in the 1,407 individuals for which sex information was reported ($p < 0.001$; Table 7.1). Data on the maturity class (estimated from TBL, see Methods) were available for 1,467 stranded animals. Overall, the number of adult LFPWs stranded (65.2%) exceeded that of juveniles and calves combined (34.8%; Table 7.1). Calves did not significantly depart from a sex ratio of unity (1:1; $p = 1.000$), while males dominated the juvenile class (sex ratio 2.16 males: 1 female, $p < 0.001$, Table 7.1) and the adult class was significantly biased towards females (sex ratio 0.39 male: 1 female, $p < 0.001$, Table 7.1). For further information on age and sex-specific survivorship and mortality rates, see (Chapter 4).

Table 7.1. Demographic data on stranded LFPWs on the New Zealand coast (1978 – 2017) and results of exact binomial tests comparing the number of males to females overall, and in all maturity classes.

*statistically significant departure from a sex ratio of unity, $p < 0.001$.

Demographic	Number (% of reported)	Total reported	Sex ratio (±SE)
Sex		1,407	0.68 (±0.003)
Female	840 (59.7)*		
Male	567 (40.3)*		
Maturity category		1,467	
Calf	126 (8.6)		0.98 (±0.037)
Juvenile	384 (26.2)		2.16 (±0.013)
Adult	957 (65.2)		0.39 (±0.005)
Sex-maturity category		1,407	
Female calf	54 (3.8)		
Male calf	53 (3.8)		
Female juvenile	116 (8.2)*		
Male juvenile	251 (17.8)*		
Female adult	670 (47.6)*		
Male adult	263 (18.7)*		
Median total body length	425 cm (range 165 – 622)	1,519	

Temporal patterns

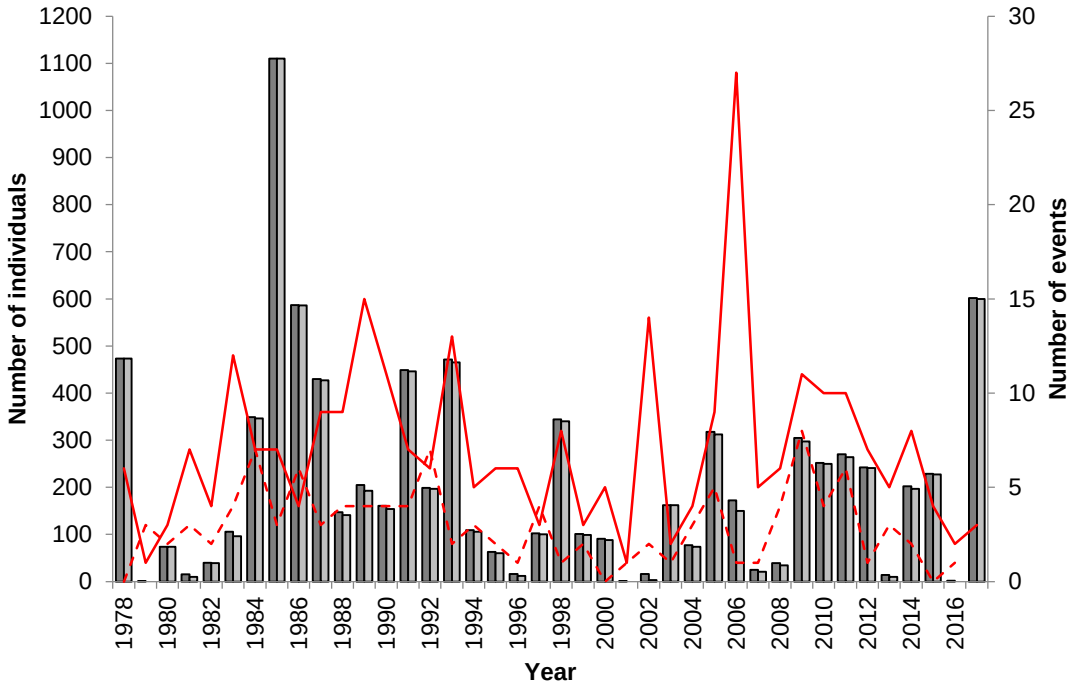
Of the 285 reported stranding events between 1978 and 2017, 42.1% (*n* = 120) were MSEs comprising 98% (*n* = 8,404) of all LFPWs stranded during this time period (Figure 7.4a). Of the 8,404 individuals that mass stranded, at least 5,559 mortalities (66%) occurred *in situ*. On average, there were approximately 46 *in situ* mortalities per MSE (range 0 – 310 individuals). Stranding events were recorded every year but with considerable annual variation in both the number of events and the total number of individuals stranded (Figure 7.4a). Data from 1978 – 2017 suggest that, on average, seven independent LFPW stranding events involving 214 individuals are reported each year in New Zealand – this includes three MSEs involving 210 individuals. Neither increasing nor decreasing trends in the annual frequency of stranding events (Mann-Kendall statistic = -0.32, $p = 0.75$), or numbers of individuals stranded (Mann-Kendall statistic = -0.43, $p = 0.67$) on the New Zealand coast between 1978 and 2017 were detected.

During the 40-year period (1978 – 2017), strandings of LFPWs were recorded on the New Zealand coast in all months, with the highest number of stranding events (16.5%, *n* = 47) reported in January and the highest number of individuals (21.8%, *n* = 1,872) stranded in February (Figure 7.4b). A significantly higher frequency of stranding events occurred in the austral (late) spring and summer months from October through to February (66%, *n* = 189) when compared with other months of the year ($p < 0.01$; Figure 7.4b). The lowest number of stranding events (1.8%, *n* = 5) and lowest number of stranded individuals (0.2%, *n* = 16 individuals from a single MSE) were recorded in June and May, respectively (Figure 7.4b).

Spatial patterns

The spatial distribution of all LFPWs stranded on the New Zealand coast between 1978 and 2017 reveals the highest density on the north-east coast of the North Island (between Cape Reinga and East Cape), Golden Bay, Stewart Island and the Chatham Islands (Figure 7.5). Strandings on the New Zealand coast are spatially aggregated, with high clustering observed at the 50km distance band ($z = 7.4$, $p < 0.001$). 50km was therefore used to fix the distance band in the subsequent hot spot analysis. The observed clustering patterns or hot spots of LFPW strandings were caused by the frequency of stranding events involving high numbers of stranded individuals in the following regions, in order of importance: Golden Bay ($p < 0.01$), Great Barrier Island ($p < 0.01$), Chatham Islands ($p < 0.05$), Stewart Island ($p < 0.05$; Figure 7.6).

(a)



(b)

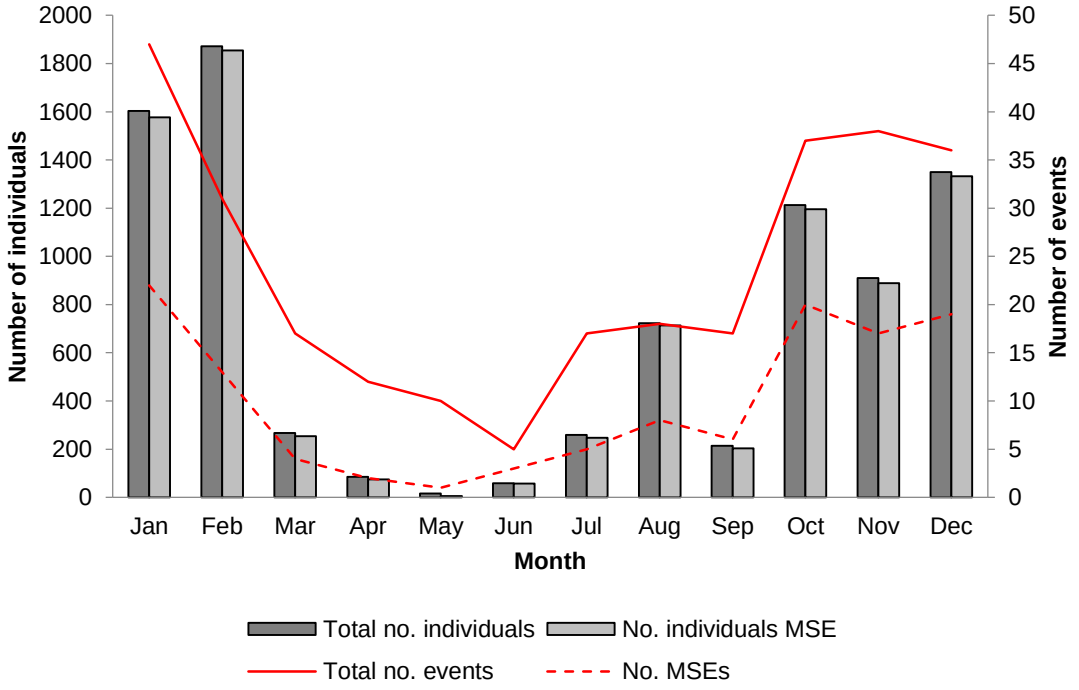


Figure 7.4. (a) Annual and (b) monthly variation in the total number of LFPW stranding events (*n* = 285), number of mass stranding events (MSEs) (*n* =120), total number of individuals stranded (*n* = 8,571), and total number of individuals stranded in MSEs (*n* = 8,404) on the New Zealand coastline between 1978 and 2017.

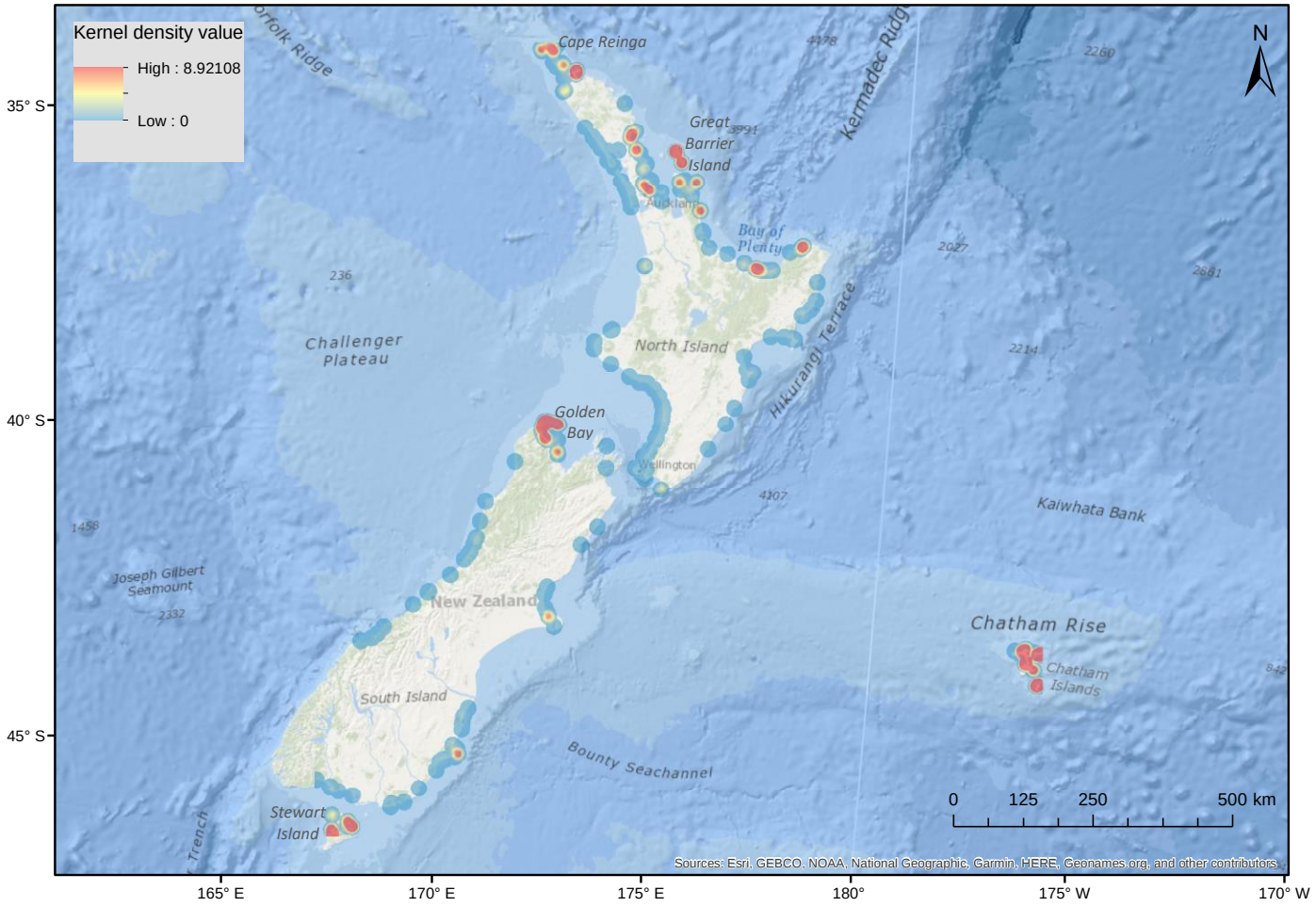


Figure 7.5. Kernel density analysis of LFPWs stranded per square kilometre on the New Zealand coast, 1978 – 2017.

Cell size: 1 km, search radius: 15 km, geodesic method (ArcGIS 10.5.1).

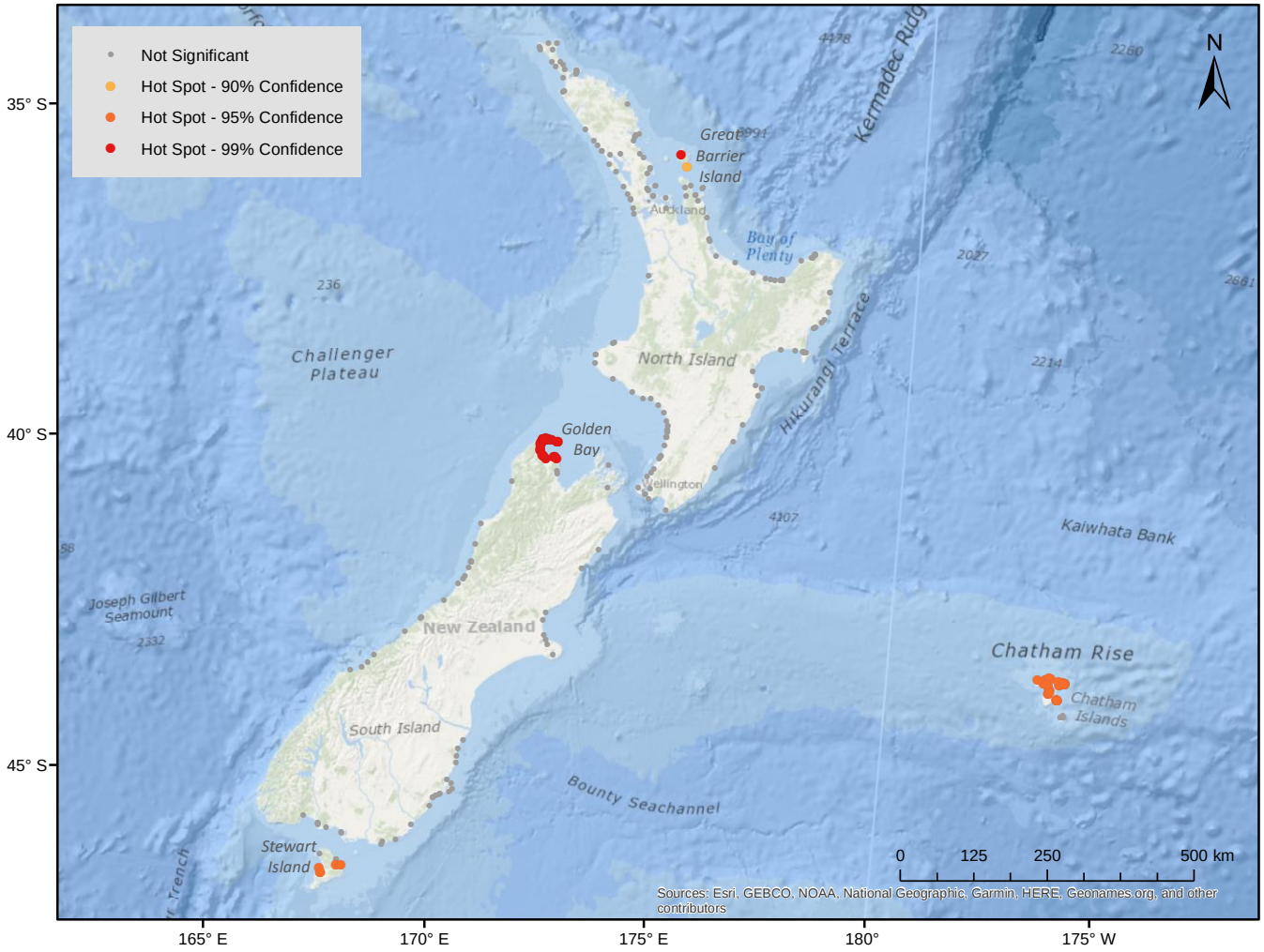


Figure 7.6. Hot spot analysis of New Zealand LFPW stranding events, 1978 – 2017.

Distribution of statistically significant spatial clusters of high (hot spot) numbers of stranded individuals, using the Getis-Ord Gi*statistic (ArcGIS 10.5.1).

Spatiotemporal patterns

While there was no indication of an increasing or decreasing trend in the overall number of LFPW stranding events or number of individuals stranded on the New Zealand between 1978 and 2017, the emerging hot spot analysis did identify locally significant temporal trends throughout the 40-year time series (Figure 7.7). Statistically significant increasing trends in numbers of stranded LFPWs were identified in Golden Bay and Stewart Island ($z > 2$, $p < 0.05$), while statistically significant decreasing trends were identified in the Far North, Coromandel, Canterbury, Otago and the Chatham Islands ($z < -2$, $p < 0.05$; Figure 7.7 and Figure 7.8).

7.5 Discussion

Concern regarding the conservation status of marine mammals has resulted in the growth of citizen science-based recording schemes for strandings, supported by government-sponsored investigations into the causes of mortality (see Chan et al. 2017 for a review). Our analyses of a long-term dataset, comprising 40 years of public records, have identified clear spatiotemporal trends that provide significant insight into patterns of LFPW strandings on the New Zealand coast, and highlight possible changes in distribution.

Sex and maturity composition

The value and utility of a long-term marine mammal stranding database are strengthened considerably by the addition of life history data to assign both sex and maturity class accurately. The results of this study show that, where sex was recorded, mass stranded adults were significantly biased towards females. Although the mating strategy of pilot whales is unknown, limited genetic evidence indicates that male LFPWs remain in their natal group but do not father calves in that group (Amos et al. 1993a). Therefore, mating must occur when two or more groups meet, or when adult males visit other groups (Desportes et al. 1993b; see Chapter 5 for further information). There is some evidence from the North Atlantic that maturing and young mature males may, at least temporarily, move away from their natal groups to aggregate in other groups and/or form non-breeding groups (Desportes et al. 1993b). Formation of such non-breeding groups could potentially explain some of the bias towards females in the sex ratio of adult animals observed within groups of both captured (North Atlantic) and mass stranded (this study) whales but is probably less important than the higher male mortality described for both *G. m. melas* (Martin et al. 1987, Bloch et al. 1993a, Desportes et al. 1994a) and *G. m. edwardii* (Chapter 4).

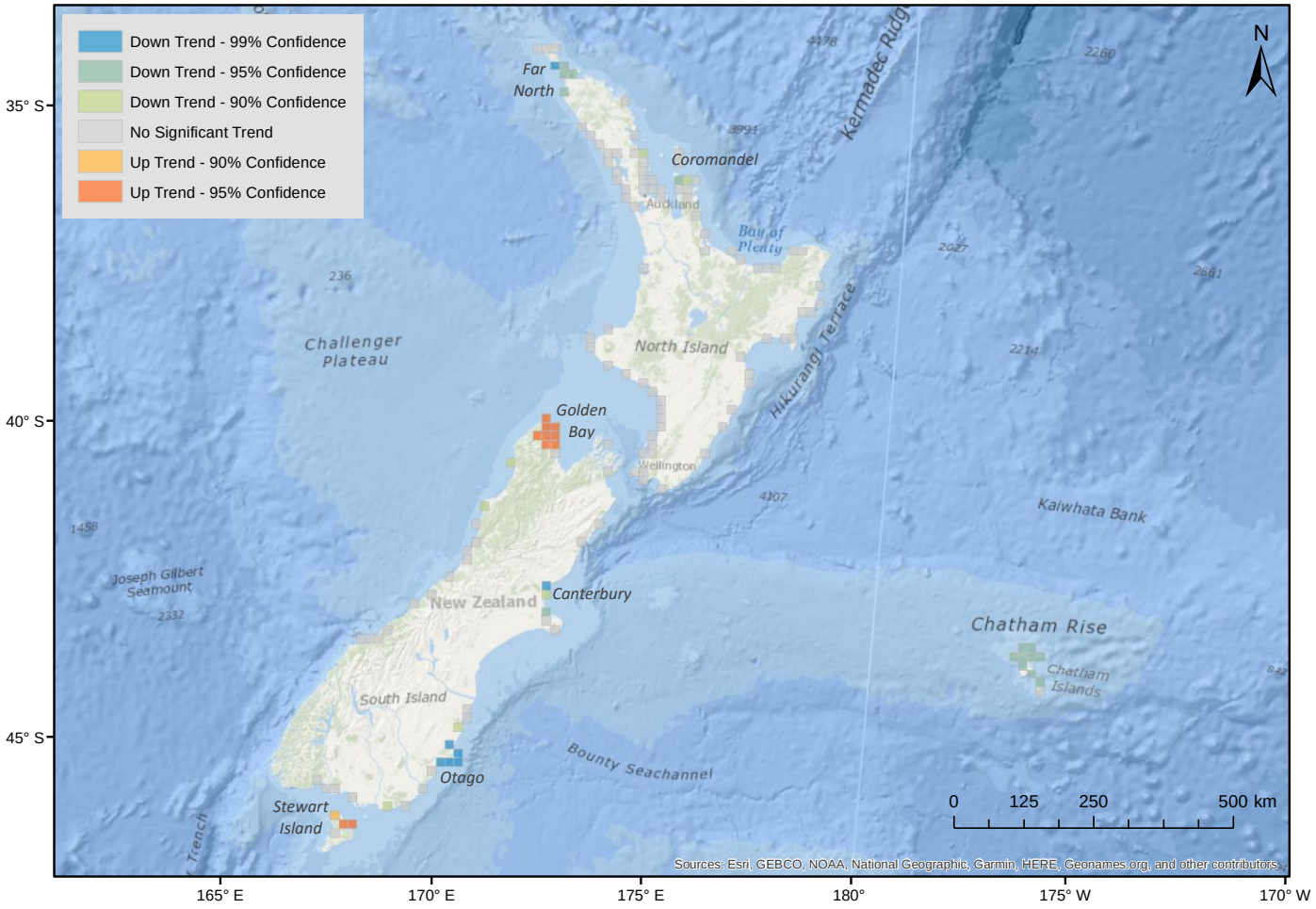


Figure 7.7. Emerging hot spot analysis of New Zealand LFPW stranding events, 1978 – 2017 identifies spatiotemporal trends in numbers of stranded individuals.

Locations of trends (> 90% confidence) are identified using the Getis-Ord Gi*statistic and Mann-Kendall trend test (ArcGIS 10.5.1). Bin size: 15 km. Neighbourhood distance: 50 km.

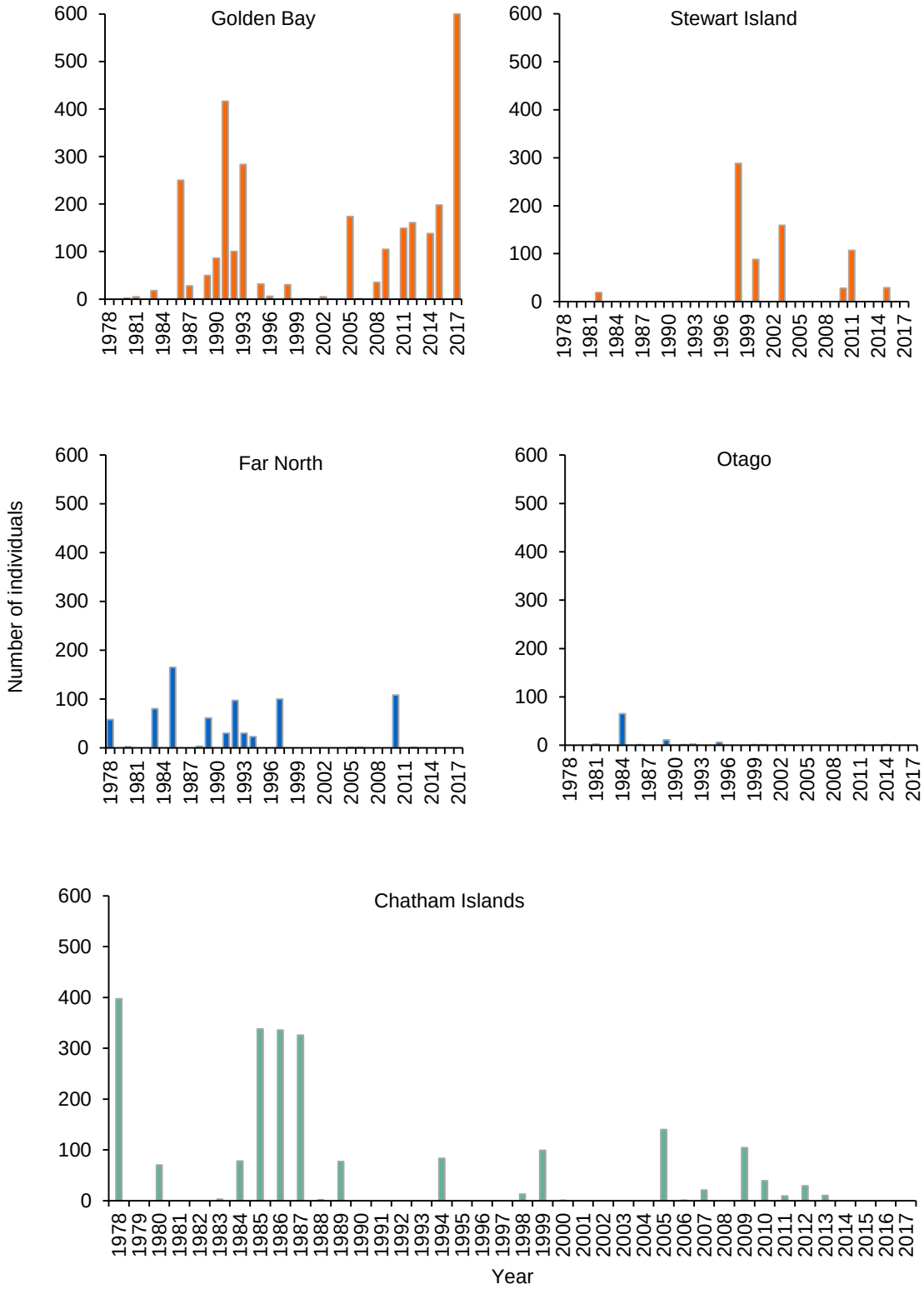


Figure 7.8. Annual variation in the total number of LFPW individuals stranded on the New Zealand coastline, in locations of clustered (> 95% confidence) up trends (i.e. Golden Bay, Stewart Island) and down trends (i.e. Far North, Otago, Chatham Islands).

Trends are identified using the Getis-Ord Gi*statistic and Mann-Kendall trend test (ArcGIS 10.5.1). Bin size: 15 km. Neighbourhood distance: 50 km.

Temporal patterns

Globally, cetacean stranding patterns suggest that broad changes in prey availability resulting from variations in climate and oceanography may cause these animals to follow prey close to land, increasing their probability of stranding (Evans et al. 2005, Bradshaw et al. 2006, Hamilton 2018). In the western North Atlantic, for example, LFPWs have been shown to exhibit some inshore movement in summer and autumn when they follow their target prey into coastal areas and continental shelf waters (Taylor et al. 2008). While strandings of the species occur on the New Zealand coast year-round, the examination of 40 years of stranding data has identified a strong seasonal peak during austral summer months (Figure 7.4b). Similarly, the highest number of LFPW strandings in nearby Tasmania are recorded during austral spring and summer (September – December), while no events have been recorded in the austral autumn/winter months of May and June (DPIPWE unpublished data cited in Beasley et al. 2019). This apparent seasonality of strandings could be a result of long-distance or inshore migration during summer, and/or reflect seasonal changes in prey distribution (Beasley et al. 2019). Seasonal changes in phytoplankton are known to occur during early summer on the continental shelf adjacent to north-east New Zealand, where strong upwelling significantly enriches coastal productivity within this area (Chang et al. 2003). Although pilot whales are not generally encountered in shallower waters (Berkenbusch et al. 2013), the observed summer peak in strandings may be a result of more frequent mixing of the water column and increased temperatures resulting in these animals following their prey closer to shore, increasing the number available to strand.

The peak stranding season for LFPWs (austral summer) also coincides with the peak calving season for the species in New Zealand waters (Chapter 6), implying groups with calves may be using habitat closer to shore during this time. This potential inshore movement may relate to foraging behaviour or prey preferences of groups with young calves. For example, an observed foraging behaviour of deep-diving cetaceans is to leave calves at the surface for the duration of foraging dives (Whitehead 1996, Gero et al. 2009). Female pilot whales caring for young calves may prefer to make shallower and less time-consuming dives, and the presence of young calves is expected to peak in austral summer months (Chapter 6).

Knowledge of seasonal patterns of stranding events may be used to guide resource allocation and stranding response efforts (Barbieri et al. 2013) and is also crucial to detect any unusual event in the short term. However, understanding potential multi-decadal trends is equally important to enable detection and interpretation of possible long-term changes in the

population (ten Doeschate et al. 2018). Recent MSEs of several cetacean species, including LFPWs, in the Northern Hemisphere, have been linked to anthropogenic activities such as military sonar (e.g. Parsons et al. 2008, Jepson et al. 2013, Brownlow et al. 2015, Bernaldo de Quirós et al. 2019). Despite increasing anecdotal public perceptions that MSEs are a more frequent phenomenon in the Anthropocene, results from this study show that while there were fluctuations in total stranding numbers per year, there was no increasing trend in LFPW strandings on the New Zealand coast during the 40-year study period.

Spatial patterns

Sightings of pilot whales in New Zealand waters are reported from northern to southern waters, including offshore areas and subantarctic islands (Berkenbusch et al. 2013). The density of New Zealand LFPW strandings (strandings per km) was highest on the north-eastern coast of the North Island, Golden Bay, the Chatham Islands, and Stewart Island. Insufficient data are available to infer that this represents a higher relative abundance of LFPWs in these areas, but it does suggest that the offshore waters to the northeast of the North Island, and waters off Golden Bay, the Chatham Islands, and Stewart Island represent (or have historically represented) important habitat.

The primary prey species of LFPW*s* in New Zealand waters are the commercially targeted 'arrow squid' (*Nototodarus* spp.) of the family Ommastrephidae (Beatson et al. 2007a, Beatson et al. 2007b, Beatson and O'Shea 2009). Both species of *Nototodarus* occur over the continental shelf in water up to 500m depth but are most prevalent in water less than 300m (Fisheries New Zealand 2018). Catch and effort data from the mainland New Zealand arrow squid fishery (SQU1T) show that the catch has been taken from the Snares shelf on the south coast of the South Island through to the Mernoo Bank off the east coast of the South Island and extending out to the Chatham Islands (Fisheries New Zealand 2018). The SQU1T catch data also reveal that the fishery operates between December and May, with peak harvest from January to April (Fisheries New Zealand 2018), coinciding with the peak stranding season for LFPWs in the austral summer months.

In the eastern North Atlantic, tracking studies have shown LFPWs have a preference for areas along the edge of the continental shelf (Bloch et al. 2003a). The main prey items of LFPWs in the North Atlantic are cephalopods of the families Octopodidae and Ommastrephidae (Desportes and Mouritsen 1993, Santos et al. 2014). The examination of LFPW whaling data from the Faroe Islands (Desportes and Mouritsen 1993, Bloch et al. 2003a) and Newfoundland (Rumage 1983) has shown a positive correlation between good squid years and years with high

LFPW catch rates in the drive fishery, suggesting a relationship between squid and pilot whale numbers. Moreover, there is a correlation between the annual position of the polar front in the Faroese area and the occurrence of LFPWs and blue whiting (*Micromesistius poutassou*), another main prey species of the whales (Desportes and Mouritsen 1993, Hoydal and Lastein 1993, Zachariassen 1993, Bloch and Lastein 1995). While the abundance and seasonal movements of LFPWs in New Zealand waters are currently unknown, presumably the movements of pilot whales are in some way linked to their pelagic prey resources (Bloch et al. 2003a). Increasing the understanding of such links will require data on the spatiotemporal distribution and behaviour of the prey species as well as data on the movements and foraging behaviour of the whales (Bloch et al. 2003a).

Spatiotemporal patterns

Although no overall long-term trend in numbers of LFPWs stranded on the New Zealand coast was evident, local increases were identified in Golden Bay and Stewart Island, while decreases were identified in the Far North, Coromandel, Canterbury, Otago and the Chatham Islands. Such trends could reflect shifts in the distribution of LFPWs in New Zealand waters, potentially as a consequence of prey availability. Predator demography is expected to be affected by prey, with changes in prey distribution or abundance preceding shifts or declines in predator populations (Simmonds and Issac 2007). As described previously, pilot whales feed mostly on pelagic cephalopods (Beatson and O'Shea 2009, Santos et al. 2014, Beasley et al. 2019) and the distribution of pilot whales is known to be strongly affected by the distribution of their prey (Desportes and Mouritsen 1993, Würsig et al. 2001). For example, in the El Niño year of 1982, short-finned pilot whales (*G. macrorhynchus*) left the southern California area following the departure of their primary prey species; 'market squid' (*Loligo opalescens*; Würsig et al. 2001). Similarly, strandings data have suggested a change in sperm whale (*Physeter macrocephalus*) distribution in the north-east Atlantic, and this has been related to shifts in the North Atlantic Oscillation possibly affecting cephalopod prey species (Robinson et al. 2005). Except for (highly variable) annual catch data for 'arrow squid' (*Nototodarus* spp.; Fisheries New Zealand 2018), very little is known about the distribution and movements of cephalopod prey species in New Zealand waters.

Golden Bay is the primary, increasingly prevalent, hot spot for LFPW strandings in New Zealand, and is located immediately adjacent to the South Taranaki Bight. The South Taranaki Bight is New Zealand's most industrialised marine region, with active oil and gas extraction and exploration, potential seabed mining, heavy vessel traffic, and commercial fishing (Torres et al.

2017). For centuries, people have generated sounds to induce mass strandings of otherwise healthy small cetaceans in drive fisheries (Brownell et al. 2008) and a growing number of cetacean MSEs have been linked, though not always casually, to acoustic disturbance from anthropogenic activities (e.g. Southall et al. 2006, Jepson et al. 2013, Brownlow et al. 2015). These include 145 LFPWs which stranded and died in a series of three events in the Marion Bay region, south-eastern Tasmania, 25 to 27 October 2005 (Department of the Environment and Heritage 2005) and a mass stranding of 39 LFPWs in the Kyle of Durness, northern Scotland, 22 July 2011 (Brownlow et al. 2015). Given the potential importance of the Golden Bay region to LFPWs, and the proximity to direct (e.g. auditory damage from seismic operations, toxicity from oil spills) and indirect (e.g. lost foraging opportunities, acoustic masking) threats associated with industrial activity, there is a need for further research to determine: (1) the significance of Golden Bay as a potential foraging and calving area for LFPWs, and (2) any links between increasing anthropogenic activities in the South Taranaki Bight and increasing MSEs in Golden Bay. Investigation of potential causes of pilot whale MSEs was outside the scope of the current study.

7.6 Conclusions

This study has demonstrated the value of establishing long-term infrastructure to facilitate opportunistic data collection for data-poor species. The NZWSDB has provided baseline ecological data on a poorly understood cetacean species in New Zealand waters, *G. m. edwardii*. The east coast of the North Island, Golden Bay, Chatham Islands and Stewart Island were identified as stranding hot spots and likely areas of importance for pilot whales in New Zealand waters, especially during the summer months. Notably, spatiotemporal analyses indicate the numbers of LFPWs stranded on Golden Bay and Stewart Island have been increasing in recent years, while numbers have declined in areas such as the Far North, Otago and the Chatham Islands.

Information derived from cetacean strandings is opportunistic and usually cannot be gathered by other means. As a consequence, tools to improve and expand the utility of these data offer significant benefit (ten Doeschate et al. 2018). A more detailed baseline with regards to the composition of the stranded population would further contribute to the value of a long-term database, and enable detection of substantial variations in the biological components of a population such as mortality trends, life history, genetics, and threats to conservation (e.g. incidental capture in fisheries, contaminant exposure, infectious disease). These are all factors that can be used as indicators of change in the status and health of a population (Norman et al.

2004, McFee et al. 2006, Nemiroff et al. 2010, Hohn et al. 2013), and are likely to improve the qualitative and quantitative applicability of stranding records as a population indicator for future monitoring (ten Doeschate et al. 2018). Collectively, the diversity of information gathered from stranded cetaceans is essential in guiding conservation measures and population management (Barbieri et al. 2013).

Chapter 8

Conclusions and recommendations

Stranded long-finned pilot whale, Te Horo Beach, Spirit's Bay, northern New Zealand, September 2010.

This thesis aimed to improve current understanding of the life history of long-finned pilot whales (LFPWs; *Globicephala melas edwardii*) in New Zealand waters and to identify any relationships between LFPW mass stranding events and life history characteristics that may have implications for conservation. There were five key research objectives to achieve this aim:

Objective 1: Describe the growth rates, allometric relationships and sexual dimorphism of LFPWs stranded on the New Zealand coast.

Objective 2: Examine the age structure and construct age and sex-specific life tables, survivorship curves, and mortality schedules for LFPWs in New Zealand waters, using age-at-death data from stranded animals.

Objective 3: Classify the stages of sexual maturation in male *G. m edwardii*, and define indicators of sexual maturity.

Objective 4: Estimate the reproductive parameters of female *G. m edwardii*, and investigate evidence of reproductive senescence and seasonality.

Objective 5: Identify spatiotemporal trends in the New Zealand LFPW stranding record.

In this chapter, the key contributions this doctoral research makes to science are outlined by describing key results in context to their conservation and management implications. Recommendations for management, in addition to suggested priorities for future research, are further detailed.

8.1 Summary of original research contributions

This thesis addressed critical gaps in the biological knowledge of long-finned pilot whales (LFPWs) by defining key life history parameters for the Southern Hemisphere subspecies (*Globicephala melas edwardii*) using samples and data obtained from post-mortem examinations of individuals stranded on the New Zealand coast (Table 8.1). Estimates of such life history parameters allowed the first comprehensive population assessment of LFPWs in New Zealand waters to be presented, and comparisons to be drawn with other pilot whale populations.

The first descriptions of growth rates, allometric relationships and sexual dimorphism of *G. m. edwardii* are presented in Chapter 3, using data collected from MSEs on the New Zealand coast. Age-related changes in growth rates between the sexes and strong evidence of sexual size dimorphism were demonstrated, with males attaining a larger body size than females. Sexual shape dimorphism was also evident, with males having considerably longer pectoral flippers, wider flukes and taller dorsal fins than females. Estimated length-at-birth and maximum ages for *G. m. edwardii* (Chapters 3 and 4; Table 8.1) were considerably lower than previously reported for the North Atlantic subspecies (*G. m. melas*), which may indicate subspecies or population-level differences in morphology, longevity and sociality. The potential difference in longevity between the two subspecies is further supported by estimates of other life history parameters, with *G. m. edwardii* attaining sexual maturity at a younger age and smaller body size (Chapters 5 and 6) and exhibiting an accelerated adult mortality rate (Chapter 4).

In Chapter 4, sex-specific life tables were constructed for *G. m. edwardii* stranded on the New Zealand coast. Survival and mortality curves revealed distinct differences in the age and sex-specific survival and mortality rates, with females having greater longevity than males (Table 8.1). Overall (total) average annual mortality is estimated to be approximately 8.8% for male and 6.8% for female *G. m. edwardii* in New Zealand waters. The mortality curve resembles that of other large mammals, with high calf mortality and an exponentially increasing risk of senescent mortality. An accelerated mortality rate was observed in adult female LFPWs (Chapter 4), in contrast to the closely related short-finned pilot whale (SFPW; *G. macrorhynchus*) – a species which selects for an extension to the post-reproductive life span (Kasuya and Marsh 1984, Ellis et al. 2018b). The observed variation in life history strategies between the two pilot whale species may in part be due to the social organisation within

stable social groups and the benefits of cooperative foraging and multigenerational transfer of information (see Chapter 6).

Chapters 5 and 6 contributed to current understanding of the reproductive biology of LFPWs by providing many of the first estimates of reproductive parameters for both male (Chapter 5) and female (Chapter 6) *G. m. edwardii*. The first description of sexual maturation in immature, maturing, and mature males was presented in Chapter 5 using morphological data and histological examination of testicular tissue. The low proportion of maturing males examined suggests that sexual maturation occurs rapidly, or maturing animals are under-represented in the stranded groups examined, potentially as a result of temporary male dispersal from natal groups. The utility of various testicular and demographic variables as indicators of sexual maturation in the subspecies were evaluated, with combined testes length found to be the best non-histological indicator of sexual maturation for *G. m. edwardii*. All testicular measures were better indicators of sexual maturity than age or total body length.

Female reproductive parameters such as the average age and length at sexual maturity, ovulation rate, gestation period and foetal growth rate, average date of conception, lengths of lactation and resting periods, annual pregnancy rate, and calving interval were estimated in Chapter 6 (see Table 8.1). Ovarian symmetry, senescence, and indicators of seasonality were also assessed. Calving is reported to be diffusely seasonal with some births occurring throughout the year and peaking in December (early austral summer; Table 8.1). Females were estimated to produce a calf every 5.3 years, after an average gestation period of 13.6 months, an average lactation period of 19.3 months, and an average resting period of approximately two years (Table 8.1). Contrary to *G. m. melas* and SFPWs, no evidence of reproductive senescence was observed in *G. m. edwardii*. Further, estimated length-at-birth (Chapter 3), maximum size and age (Chapters 3 and 4), survivorship (Chapter 4) and average length and age at attainment of sexual maturity (Chapters 5 and 6), were all lower in *G. m. edwardii* than best estimates reported for the North Atlantic subspecies (*G. m. melas*; Sergeant 1962a, Bloch et al. 1993a, Desportes et al. 1993b, Martin and Rothery 1993). Such biological differences indicate that geographic variation in life history occurs for this species, likely reflecting population-specific adaptations to local habitats.

A major factor influencing the population of LFPWs in New Zealand waters is the frequent occurrence of MSEs (Chapters 4 and 7). However, the establishment of long-term infrastructure to facilitate opportunistic data collection from MSEs has considerable ecological value. This is especially true for data-poor species, subspecies and populations, as

demonstrated in Chapter 7 of this thesis. In Chapter 7, the New Zealand Whale Stranding Database (NZWSDB) was used to provide baseline ecological data on LFPWs; a poorly understood cetacean species in New Zealand waters. Spatial and temporal patterns of LFPW stranding events on the New Zealand coast were examined using all LFPW data held in the (NZWSDB; 1874 – 2017) but with emphasis placed on strandings between 1978 and 2017. Following the establishment of the New Zealand Marine Mammal Protection Act in 1978, cetacean strandings were more systematically recorded from this point on. A total of 8,571 LFPWs stranded on the New Zealand coast within the 40-year period between January 1978 and December 2017. Strandings occurred in all months, though significant seasonal variation was evident, with two-thirds of stranding events reported during austral spring and summer months (October – February; Table 8.1). The east coast of the North Island, Golden Bay, Chatham Islands and Stewart Island were identified as stranding hot spots (Table 8.1) and likely areas of importance for pilot whales in New Zealand waters, especially during the austral summer months. While no significant trend in the overall numbers of stranded LFPWs was evident, emerging hot spot analysis (ArcGIS) indicated the numbers of LFPWs stranded on Golden Bay and Stewart Island have been increasing in recent years, while numbers have declined in the Far North, Canterbury, Otago, and the Chatham Islands. When combined with other contextual information, such trends help identify the most significant clusters of LFPW strandings on the New Zealand coast, provide baseline ecological data on a poorly understood subspecies, and can be used to guide conservation management of *G. m. edwardii* in New Zealand waters.

Despite known biases with data derived from stranded individuals, the frequent MSEs of LFPWs on the New Zealand coast (in large groups of mixed ages and sexes; see Chapters 4 and Chapter 7) provides important opportunities to collect data on the demography and ecology of this species in New Zealand waters. These data, when collected in a standardised and comprehensive manner, offer an ability to assess changes in population parameters over time, providing essential information for the conservation management of LFPWs in New Zealand waters. The approaches used in this study are also broadly applicable to data gathered by stranding networks in other areas. With dedicated collection of life history samples, similar models could be developed for other populations of marine mammals.

Table 8.1. Key life history parameters for LFPWs stranded on the New Zealand coast. TBL = total body length; [a] = 0.9 × asymptotic values obtained using von Bertalanffy growth curves.

Life history parameter	Male	Female	Reference
Min TBL	165 cm	160 cm	Chapter 3
Median TBL at birth	163 cm	163 cm	Chapter 3
Max TBL	622 cm	500 cm	Chapter 3
Min age	neonate	neonate	Chapters 3, 4
Max age	31 yrs	38 yrs	Chapters 3, 4
TBL at physical maturity[a]	513 cm	394 cm	Chapter 3
Age at physical maturity[a]	16 yrs	10 yrs	Chapter 3
Mean length of physically maturea individuals	550 cm	432 cm	Chapter 3
Asymptotic length	570 cm	438 cm	Chapter 3
Age at asymptotic length	c. 40 yrs	c. 30 yrs	Chapter 3
Annual average mortality	8.8%	6.8%	Chapter 4
Life expectancy at birth	11.3 yrs	14.7 yrs	Chapter 4
TBL range of sexually immature individuals	184 – 490 cm	160 – 375 cm	Chapters 5, 6
TBL range of sexually mature individuals	450 – 573 cm	346 – 485 cm	Chapters 5, 6
Age range of sexually immature individuals	0 – 15 yrs	0 – 8.5 yrs	Chapters 5, 6
Age range of sexually mature individuals	11 – 25 yrs	5 – 33 yrs	Chapters 5, 6
Average age at attainment of sexual maturity	13.5 yrs	6.7 yrs	Chapters 5, 6
Average TBL at attainment of sexual maturity	472 cm	356 cm	Chapters 5, 6
Combined testes weight range in mature males	3000 – 13020 g		Chapter 5
Reproductive senescence	No	No	Chapters 5, 6
Gestation period		1.1 yrs	Chapter 6
Annual pregnancy rate		19%	Chapter 6
Calving interval		5.3 yrs	Chapter 6
Lactation period		1.6 yrs	Chapter 6
Resting period		2 yrs	Chapter 6
Reproductive season		Diffuse – peak calving in December (austral summer), but births occur year-round	Chapter 6
Stranding season	Peak October – February (late austral spring & summer), but some strandings occur year-round		Chapter 7
Stranding 'hot spots'	Golden Bay, Great Barrier Island, Stewart Island, Chatham Islands		Chapter 7

8.2 Conservation and management

Species-led conservation management usually focuses on species with high extinction risk. This approach typically results in conservation priority lists dominated by species that have small population sizes and/or restricted geographical ranges (Stockin 2008). Alternatively, efforts have been focused on keystone (Payton et al. 2002), flagship (Smith et al. 2012) or indicator species (Hutcheson et al. 1999), with the rationale that the conservation of surrogate species may retain more biodiversity over the long-term by proxy (Caro 2010). However, although they generally receive much less attention, common and widespread species are also of significant conservation importance for three reasons: (1) a number of species that are currently threatened or extinct could previously have been described as common and widespread; (2) there is growing evidence that large numbers of currently common and widespread species are undergoing massive declines, with major ramifications for ecosystem functions and services, and (3) the processes that underlie such declines seem likely to intensify with time (Gaston and Fuller 2007). While it is recognised that rare species may have important roles, it is common species that are the service providers of most ecosystems (Gaston and Fuller 2008). Therefore, in addition to threatened species, conservation biologists and managers should pay more attention to the depletion of common species (Stockin 2008), including long-term monitoring to identify and manage any significant negative impacts.

New Zealand is recognised as an important hotspot of biodiversity (Myers et al. 2000), and home to many endemic species. Unfortunately, New Zealand has also suffered considerable biodiversity loss, with nearly one-third of land and freshwater bird species driven to extinction by anthropogenic activity over the last *c*. 700 years (Wilson 2004). However, comparatively little is known about the extent of biodiversity loss within the marine environment. Usually, research effort is focused on commercially important (e.g. snapper, *Pagrus auratus*) or threatened endemic species (e.g. Hector's and Maui's dolphins, *Cephalorhynchus hectori*). Preventing the extinction of New Zealand's biodiversity remains a critical component of the New Zealand Government's Biodiversity Strategy. This is primarily facilitated through the New Zealand Threat Classification System (Townsend et al. 2008), which is administered by the New Zealand Department of Conservation (DOC) with reference to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.

Threat classification

Despite the lack of research undertaken on the Southern Hemisphere LFPW, the IUCN Red List of Threatened Species has recently updated the global threat classification for LFPWs from

'Data Deficient' (Taylor et al. 2008) to 'Least Concern' (Minton et al. 2018). However, it is recognised that Southern Hemisphere populations are especially 'data-poor', indicating that more research is needed to adequately determine their conservation status (Minton et al. 2018). LFPWs are also currently treated by the IUCN as a single species, even though there is evidence that they may comprise a complex of two or more species (Minton et al. 2018). If so designated, the IUCN classification may change, and some potential new species may warrant listing under a higher category of risk (Minton et al. 2018). As an immediate priority, consideration should be given to the separate assessment of the two subspecies; *G. m. melas* in the North Atlantic and *G. m. edwardii* in the Southern Hemisphere (Minton et al. 2018).

According to the official New Zealand Threat Classification System (NZTCS; Townsend et al. 2008; Figure 8.1), LFPWs are listed as 'Not Threatened $_{DP, S?O}$' within New Zealand waters (Baker et al. 2016). The 'Not Threatened' classification, described by Baker et al. (2016) as "resident native taxa that have large, stable populations", was assigned to LFPWs by a marine mammal 'expert' panel convened in 2013 (Baker et al. 2016). Qualifiers 'DP' (Data Poor) and 'S?O' (uncertainty as to whether the overseas taxon is secure) were also added to this listing (Baker et al. 2016). The qualifier 'Data Poor' indicates "confidence in the listing is low due to there being only poor data available for assessment" (Townsend et al. 2008). Remarkably, this classification appears to have been assigned to LFPWs in the absence of any abundance, density or life history data rather than "poor data". Townsend et al. (2008) define 'Not Threatened' as "taxa that are assessed and do not fit any of the other categories". Where information is so lacking that there is "not sufficient information available to assess the (conservation) threat", taxa should instead be assigned to the 'Data Deficient' category (Townsend et al. 2008; Figure 8.2). Classification of any species as 'Not Threatened' without such data is arguably erroneous on the basis of there being no science on which to validate such an assumption (Stockin 2008). Cetacean populations whose abundance, distribution, habitat use and reproductive biology remain unknown could be considered most at risk since population declines are likely to go unnoticed (Stockin and Orams 2009). Failure to monitor and recognise local population declines can threaten the national (and eventually the international) status of once-common species, for example, the bottlenose dolphin (Vermeulen and Bräger 2015). Townsend et al. (2008) confirm that "collection of sufficient demographic data to allow an evaluation is a high priority for 'Data Deficient' taxa". As highlighted throughout this thesis, the lack of empirical data is certainly a risk for LFPWs in New Zealand waters, and *G. m. edwardii* in general. The current study has filled a number of critical knowledge gaps by providing the first baseline data on aspects of the demography

(Chapter 3, 4, and 7), mortality (Chapter 4), reproductive biology (Chapters 5 and 6) and trends in the stranding record (Chapter 7). However, there remains very limited information available related to their contaminant load (Schroder and Castle 1998), and no information on abundance, density, or population trends. The lack of empirical data, alongside numerous potential anthropogenic impacts faced by this species, exemplifies why LFPWs should be reclassified as 'Data Deficient' within New Zealand waters.

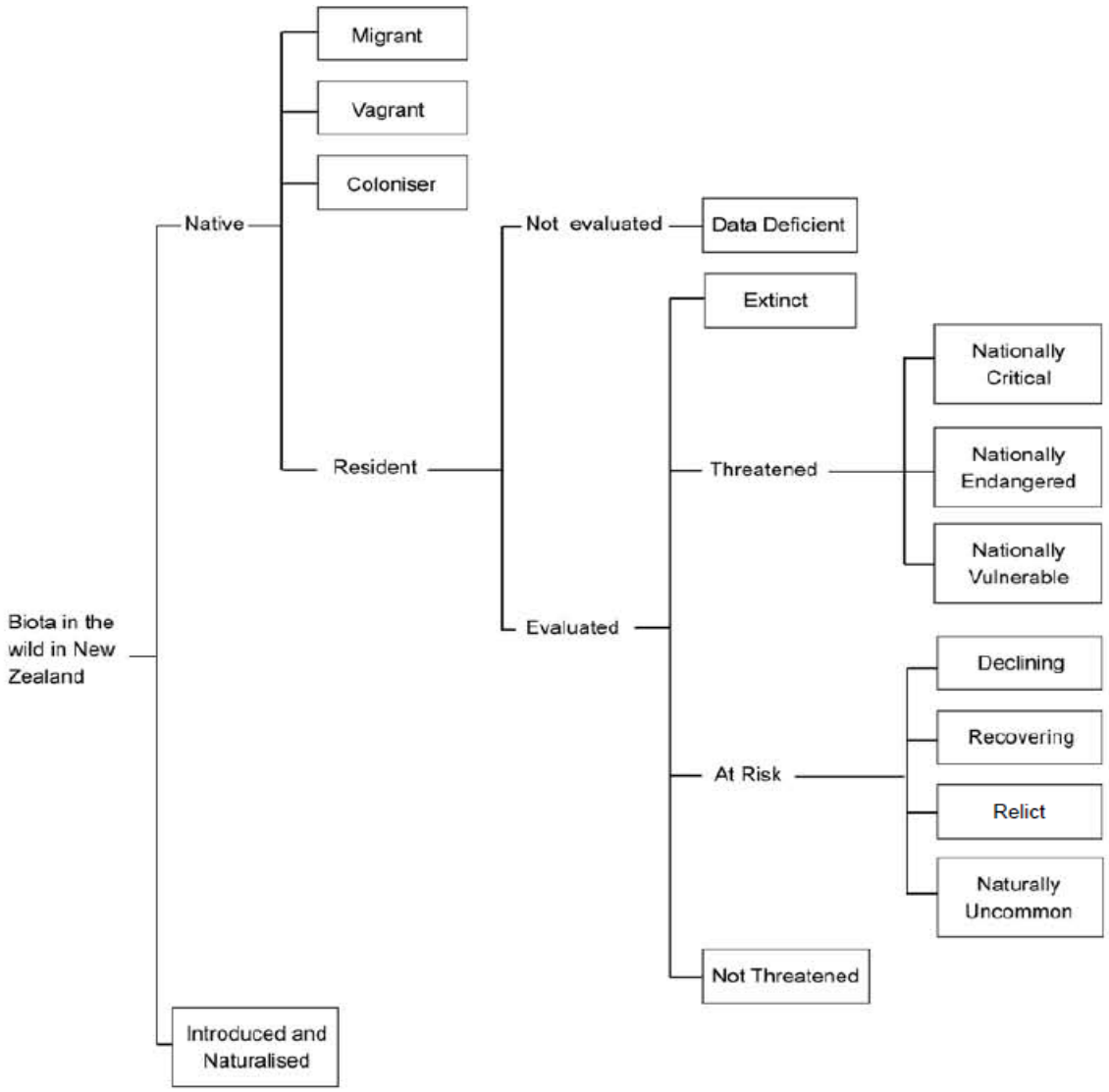


Figure 8.1. Structure of the New Zealand threat classification system.

Source: Townsend et al. (2008).

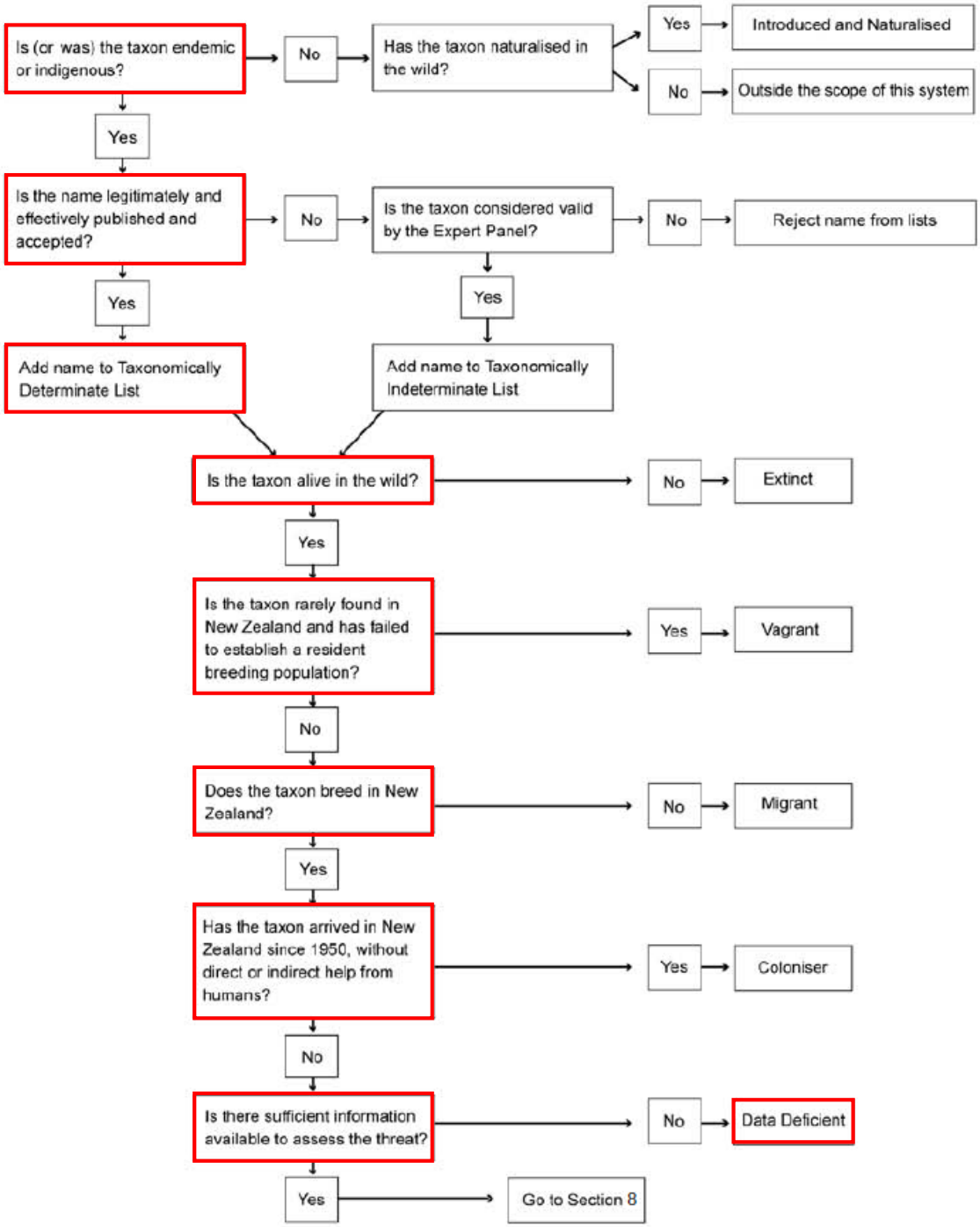


Figure 8.2. Flow chart for defining 'Introduced and Naturalised', 'Vagrant', 'Coloniser', 'Migrant', 'Extinct' and 'Data Deficient' categories of the New Zealand Threat Classification System.

Source: Townsend et al. (2008). Red boxes indicate the recommended classification for LFPWs in New Zealand waters, i.e. 'Data Deficient'.

Management

As the government agency responsible for the management of marine mammals within New Zealand waters, DOC issued a Marine Mammal Action Plan (MMAP) covering the period 2005 to 2010 (Suisted and Neale 2004). Despite the intention for the MMAP to be "a dynamic document, reviewed on an ongoing basis, but at least every five years" (Suisted and Neale 2004), there has been no updated version of this plan to cover the period post-2010, hence this remains the most recent management plan for New Zealand marine mammals. The MMAP contains species-specific action plans for most resident cetacean species, except common dolphins (*Delphinus* sp.), which erroneously feature under section '2.16 Other toothed cetaceans', and LFPWs, which are grouped with SFPWs under section '2.15 Pilot whale' (Suisted and Neale 2004). Pilot whales (*Globicephala* spp.) are described by Suisted and Neale (2004) as a "poorly known migrant species in New Zealand waters" that are "occasionally implicated in mass stranding events". Key objectives for the management of pilot whales in New Zealand waters are to: (1) "better understand the population ecology, key habitat requirements and threats to the species" and (2) "manage pilot whale stranding events safely, effectively and humanely" (Suisted and Neale 2004).

Managing marine mammal populations is challenging, especially when dealing with a species, subspecies or population for which limited biological information is available (Stockin 2008). Effective conservation management is dependent on an accurate understanding of the species being managed. In particular, an understating of reproduction is important, since life history traits may ultimately influence population stability, growth and/or recovery (Slooten 1991). Management strategies for populations subject to exploitation often use estimated rates of increase in population size based on life history parameter estimates (Danil and Chivers 2007). Such an approach would also apply to populations recovering from high levels of stranding related mortality. In delphinid populations, rates of increase for vital rates examined are most sensitive to calving interval and non-calf survival rate, followed by age at first birth, and are insensitive to changes in calf survival rate (Reilly and Barlow 1986). In the event of increasing stranding-related mortality, or increased anthropogenic impacts on LFPWs in waters, expected recovery rates can be modelled using reproductive parameters estimated in this thesis, such as calving interval and ASM (see Chapter 6).

Conservation management is generally aimed at species and population levels, with biological information used to define conservation units as the species, evolutionary significant units (ESUs), or demographically independent populations (DIP; Morin and Dizon 2018). However, as

highlighted throughout this thesis, there is a lack of empirical data relating to the biology of the Southern Hemisphere subspecies of LFPW. Such constraints hamper our knowledge of 'ESU' and 'DIP' definitions within New Zealand waters. This thesis has contributed to this need by providing the first substantive biological data on LFPWs in New Zealand waters. Through providing new insights into the life history of LFPWs, it is hoped that the results of this study may help inform future conservation management of the species and ensure MSE management capability at identified stranding hotspots such as Farewell Spit, Stewart Island and the Chatham Islands (Chapter 7).

8.3 Future research

The research presented in this thesis has provided an important contribution to our scientific understanding of *G. m. edwardii*, but also serves as a base of fundamental biological data from which further work on this species can be undertaken. To better understand the Southern Hemisphere LFPW, it is important to bridge some of the remaining critical knowledge gaps. As LFPW MSEs occur frequently and tend to involve large groups of mixed ages and sexes (see Chapters 4 and 7), they should be recognised as valuable opportunities to collect further behavioural, genetic and life history data to fully comprehend the biology and population dynamics of *G. m. edwardii*. These data are important for the conservation management of this subspecies within New Zealand waters. Proposed areas of recommended research include:

Taxonomy, population and social structure

It is recommended that further molecular studies be undertaken on *G. m. edwardii* to clarify issues of taxonomy, population and social structure. The reason for the observed differences in mortality rate acceleration between the two pilot whale species and social implications of stranding-related mortality have not been established and warrant further investigation. Previous genetic studies on stranded pilot whales have helped determine genetic differences between ocean basins, and have begun to assess elements of population and social structure at a regional level (e.g. Amos et al. 1993a, Oremus et al. 2009, Oremus et al. 2013). However, further investigation using samples collected from complete mass stranded groups (including live whales) is required to enable a thorough assessment of the social structure of *G. m. edwardii* and to examine relatedness between different groups. Samples from stranded pilot whales that are used to evaluate genetic variation will also have important management implications if the scientific community determines that *G. melas* should be split into multiple species (Taylor et al. 2008).

Abundance and distribution

Obtaining population estimates and assessing trends is typically the first step to understanding a population. However, abundance and distribution patterns remain unknown for LFPWs in New Zealand waters, with no dedicated conventional distance sampling or capture-mark-recapture study in the published literature. In light of research findings detailed herein, it is recommended that a population estimate for New Zealand LFPWs be undertaken. Suggestions for methods to obtain such an population estimate include: (1) dedicated sighting surveys and collaboration with 'platforms of opportunity' (e.g. vessels involved in offshore exploration); (2) recent advances in acoustic detection of cetacean species have allowed researchers to conduct surveys of their density and distribution using acoustic methods (Marques et al. 2009) — dedicated surveys in New Zealand using passive acoustic monitoring would provide valuable and complementary evidence of cetacean distributions in New Zealand waters; (3) capture-mark-recapture models should be applied to genetic data extracted from tissue samples collected from MSEs on the New Zealand (samples available on request from the New Zealand Cetacean Tissue Archive [NZCeTA, curated by the University of Auckland], and the New Zealand Pilot Whale Tissue Archive [NZPWTA, curated by E. L. Betty – see Appendix 1A), to generate an abundance estimate for this region.

Reproductive biology

Further sampling of maturing male *G. m. edwardii* is recommended to refine estimates of ASM, LSM, duration of the maturing stage, and indicators of sexual maturity based on testicular size. Additionally, temporal monitoring of ASM and LSM, alongside assessments of environmental and anthropogenic stressors that may impact those parameters would provide some insight into the condition of the population and its relative carrying capacity. An examination of reproductive seasonality in addition to further information from genetic studies, detailed analysis of group structure (in MSEs and at sea), and behavioural observations of individuals are needed to confirm mating strategies, and when social maturity is attained in *G. m. edwardii* off New Zealand. Complementary observational and post-mortem studies applied to the same population may offer the opportunity to characterise age and reproductive characters more precisely. Future research is also recommended on reproductive dysfunction and disease, including that related to toxic contaminants.

Contaminants

Marine mammals are widely considered important sentinels for the implications of marine pollution (Nelms et al. 2019). Exposure to endocrine-disrupting contaminants such as

organochlorines has been reported to cause a decline in fertility, and alter or delay sexual maturation in marine mammals (Murphy et al. 2018). In addition, plastic pollution is recognised as an increasing threat to marine mammals and there is a need for further research to understand: (1) the extent to which microplastics (< 5mm) are ingested by marine mammals, and (2) the potential chronic effects of microplastic exposure (Nelms et al. 2019). With the exception of an unpublished report describing PCB burdens (Schroder and Castle 1998), very little information is available on the pollutant burden of LFPWs in New Zealand waters. Further investigation is required to examine current contaminant levels of LFPWs off New Zealand and to identify any population-level implications.

Diet

Preliminary diet research has suggested that the primary prey species for LFPWs in New Zealand waters are the commercially targeted 'arrow squid' (*Nototodarus* spp.; Beatson et al. 2007a, Beatson et al. 2007b, Beatson and O'Shea 2009). A comprehensive understanding of potential pilot whale-fisheries interactions and movements in response to prey distribution can be provided by the expansion of diet research. Thus, it is recommended that further dietary research is undertaken on LFPWs in New Zealand waters. Specifically, stable isotopes should be used in conjunction with stomach contents to examine feeding history over a larger temporal scale and to detect any dietary shifts.

Emerging hot spots

Emerging hot spot analysis (see Chapter 7) indicated that the numbers of LFPWs stranded on Golden Bay and Stewart Island have been increasing in recent years, while numbers have declined in areas such as the Far North, Otago and the Chatham Islands. It is recommended that further research is undertaken to attempt to identify potential factors contributing to such spatiotemporal shifts in LFPW strandings. For example, given the potential importance of the Golden Bay region to LFPWs, and the proximity to direct (e.g. auditory damage from seismic operations, toxicity from oil spills) and indirect (e.g. lost foraging opportunities, acoustic masking) threats associated with industrial activity, there is a need for further research to determine (1) the significance of Golden Bay as a potential foraging and calving area for LFPWs and (2) any links between increasing anthropogenic activities in the South Taranaki Bight and the trend of increasing LFPW strandings in Golden Bay.

New Zealand Whale Stranding Database (NZWSDB)

As with other opportunistic wildlife data, information derived from cetacean strandings usually cannot be gathered by other means. Thus, tools to improve and expand the utility of NZWSDB would offer significant benefit. A more detailed baseline with regards to the composition of stranded cetaceans would contribute to the value of a long-term database, and facilitate the detection of substantial variations in the biological components of a population such as mortality trends, life history, genetics, and threats to conservation (e.g. incidental capture in fisheries, contaminant exposure, infectious disease). These are all factors that can be used as indicators of change in the status and health of a population and are likely to improve the qualitative and quantitative applicability of stranding records as a population indicator for future monitoring. Collectively, the diversity of information gathered from stranded cetaceans is essential in guiding conservation measures and population management (Barbieri et al. 2013).

Given that New Zealand is a stranding hot spot, four specific recommendations are made that would develop the NZWSDB and expand our knowledge of cetacean species in general in New Zealand waters: (1) it is suggested that post-mortem sampling of as many whale carcasses as possible be undertaken. Data such as age at attainment of sexual maturity, diet, disease burden and contaminant loads for most cetacean species stranding on New Zealand coasts are, at best, scant and such vital information will provide a valuable contribution to our knowledge of these animals; (2) further studies, with improved data collection and more detailed observations of behaviour, could provide a better understanding of MSEs and recommendations for improved efficiency of rescue efforts and animal welfare practices during stranding events; (3) development of tagging or marking techniques of live stranded cetaceans would permit determination of the survival rate of refloated animals, as well residency, movements and behaviour of pods; and (4) construction of a publically accessible and interactive online stranding database would help to facilitate further research on stranded cetaceans.

8.4 Conclusion

This thesis makes a significant contribution to the current scientific understanding of the LFPW in New Zealand waters by providing critical data on demographics, life history, and spatiotemporal stranding patterns. However, a vast paucity in our knowledge of LFPWs in New Zealand waters remains, with fundamental data required for management (e.g. population structure, abundance, diet) still absent for this population. LFPWs are the most frequent mass

stranding cetacean on the New Zealand coast but are considered the lowest conservation priority of all marine mammals within New Zealand waters. Although neither endemic nor rare, LFPWs are subject to ecological, morphological, and genetic differentiation and require, as a highly gregarious species, to be conserved in large biomasses. The status of New Zealand LFPWs requires careful review to ensure the long-term conservation of this subspecies. It is highly recommended that the classification of LFPWs within New Zealand waters be reviewed and the absence of scientific data carefully considered in relation to the threats posed to this poorly known and frequently mass stranded species. As a consequence of the present study, it is hoped that LFPWs within New Zealand waters become the subject of further research and pro-active management in the immediate future.

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Appendices

Appendix 1A. The New Zealand Pilot Whale Project (NZPWP)

The New Zealand Pilot Whale Project (NZPWP) was instigated in 2010 by E.L. Betty (nee Beatson), with support from K.A. Stockin, under the auspices of the School of Science, Auckland University of Technology (AUT) and the Coastal-Marine Research Group, School of Natural and Computational Sciences, Massey University. Established as a long-term research programme, the NZPWP aims to: (1) establish baseline data on the biology and ecology of New Zealand pilot whales, and (2) raise awareness about the conservation issues that affect pilot whales in New Zealand waters. Research efforts have primarily focused upon aspects considered to be of most benefit to conservation (e.g. life history, mass stranding events, human-impacts). Selected parts of this research feature as part of the present PhD study, specifically: age, growth, survivorship, reproduction, and spatiotemporal trends in the stranding record. However, considerable data not included in the present thesis also exist as a result of the NZPWP. Such data include the New Zealand Pilot Whale Database (NZPWD) and the New Zealand Pilot Whale Tissue Archive (NZPWTA).

Currently, the NZPWD features *c.* 1,500 individuals that stranded on the New Zealand coast and the NZPWTA features *c.* 700 individuals which have undergone sampling and/or post-mortem examination. Data and extracted tissues collected during post-mortem examination are currently being used to investigate: (1) diet, (2) toxicology, (3) pathology, (4) tooth growth chronologies, (5) foetal growth, (6) population structure, (7) kinship and social structure of mass stranded groups, and (8) welfare implications of mass stranding events. Both the NZPWD and NZPWTA are held at the Coastal-Marine Research Group, Massey University. Since the materials in the database and tissue archive were supported through funding from public non-profit organisations, these databases are not strictly proprietary. However, it is recognised that E.L. Betty was both the instigator and principal investigator of the NZPWP and thus, remains curator of both databases in addition to the datasets presented as part of the presented PhD study. Requests for non-conflicting purposes will require the written permission of E.L. Betty. Assuming no conflict is evident, access to data and/or samples will be granted. E.L. Betty reserves the right to be included as a co-author on scientific publications and/or reports that have resulted from the use of these data and/or samples.

Appendix 3A. Department of Conservation Whale & Dolphin Stranding/Accident/Death Report

Key contacts	Wendi Roe: 027 270 8982 (Massey Uni) Stu Hunter: 027 266 2204 (Massey Uni)	Hannah Hendriks: 027 201 3258 (DOC) Project Jonah: 0800 494253 (stranding assistance)

DEPARTMENT OF CONSERVATION **DOCDM-870555**
Te Papa Atawhai
WHALE & DOLPHIN – STRANDING / ACCIDENT / DEATH REPORT

DETAILS
Observer's name: _____ Agency: _____
Vessel details: _____
Address: _____ Ph, email etc: _____
_____
Time of observation: _____ Date of observation: _____

RESPONSE
DOC Area: _____ Action taken: _____
Response by: _____ Ph, email etc: _____
Report completed by: _____ Ph, email etc: _____

LOCATION
Name/Description: _____
_____ (with photocopy of map)
☐ Map or ☐ GPS reference: _____, _____
What map grid did you use? ☐ NZMS260, ☐ Topo50, ☐ Other: _____
Where was animal? ☐ On land or ☐ At sea

ENVIRONMENT
Depth: _____ Habitat: _____
Sea condition (Beaufort scale): _____ Wind Direction: _____ Strength: _____
Swell (circle): None / Light (0-1m) / Moderate (1-2m) / Heavy (>2m)
Tides (circle): H / M/ L Incoming / Outgoing

ANIMAL DETAILS Name/tag/ID number: _____

Animal type: ☐ Hector's / Maui's dolphin ☐ Other dolphin ☐ Beaked whale ☐ Small toothed whale
☐ Large toothed whale ☐ Small baleen whale (<10m) ☐ Large baleen whale (>10m)
Number of animals (total): _____ Species: _____
ID confidence (definite/probable/unsure): _____ ☐ Used key

Condition: ☐ Live ☐ Weak ☐ Emaciated ☐ Diseased ☐ Freshly dead ☐ Decomposed
Type: ☐ Stranded ☐ Beach cast ☐ Floating at sea ☐ Catch incidental to fishing ☐ Accident/Collision
☐ Entangled (please specify material, where on the animal it is): _____
_____ If alive, released? ☐ Yes ☐ Partially ☐ No

☐ Deliberately killed (please specify rifle and bullets used, number of shots fired, time until death): ___
_____

Strand ID (National Office) _____ **Local Stranding No:** _____

MEASUREMENTS All measurements should be taken in a straight line parallel to the body axis, not around the curve of the body. Measurements to be in cm, see reverse for diagram

1. Total Length (from tip of UPPER jaw to deepest part of fluke notch) _____cm
2. Tip of upper jaw to tip (or rear attachment for Hector's dolphin) of dorsal fin _____cm
3. Tip of upper jaw to anus _____cm
4. Tip of upper jaw to genital slit _____cm
5. Tip of upper jaw to front (forward insertion) of flipper _____cm
6. Tip of upper jaw to blowhole _____cm
7. Length of Flipper (external) _____ cm, (internal) _____ cm
8. Greatest width of flipper _____cm
9. Greatest width tail flukes (If undamaged) _____cm
10. Length of rostrum (beak) _____cm
11. Length of gape (tip lower jaw to corner of mouth) _____cm
12. Height of dorsal fin _____cm
13. Axillary girth (immediately behind flippers, around body) _____cm
14. Length of: Throat grooves (beaked whales) Left _____ Right _____cm
    Throat pleats (baleen whales) _____cm
15. Length of genital slit _____cm
16. Sex: ☐ Male ☐ Female ☐ Unknown If female, pregnant? ☐ Yes ☐ No ☐ Unknown

PHOTOS Use a scale and ID label in each photo
Yes☐ No ☐ Date taken: _____ Negatives/originals held by _____

☐(L) ☐(R) Entire animal from side (L&R) ☐ Entire animal from Below
☐ Detail of Genital area ☐(L) ☐(R) Head from side (L&R)
☐ Head from below ☐ Head from above ☐(L) ☐(R) Dorsal fin from side (L&R)
☐ Detail of teeth or Baleen ☐ Detail of jaws ☐☐(L) ☐☐(R) Flippers (L&R,top&bot.)
☐(T) ☐(B) Tail flukes (top & bottom view) ☐ Throat pleats ☐ Useful media photos
☐ Details of any (all) injuries/ net marks ☐ Other features/ site photos
Reason for incomplete set: _____

REFLOAT DETAILS

Number of animals refloated: _____ Date/Time animals refloated: _____
Number of refloat attempts: _____ Refloating technique: _____
Location of refloat attempt: _____
Number of animals restranded: _____ Date/Time animals restranded: _____
Location animals restranded: _____

SAMPLING Always collect skin samples from dead animals

☐ Cut skin sample (e.g. 1cm² 'wedge' from trailing edge of the tail fluke)
☐ Preserve in 70% ethanol (preferably) or freeze in tinfoil
☐ Label sample (pencil on waterproof label inside sample container)
☐ Send skin samples to Auckland University (Rochelle Constantine, Thomas Building, School of Biological Sciences, University of Auckland, 3A Symonds St, Auckland 1010)
☐ Contact Marine Species & Threats to confirm if other samples are required

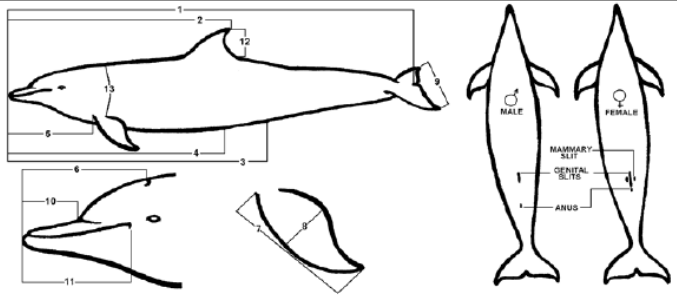
For Hector's and Maui's dolphins

☐ Contact Marine Species & Threats for advice or assistance
☐ Recover carcass and refrigerate/chill immediately after all measurements, samples and photos have been taken. DO NOT FREEZE
☐ Send carcass to Massey University for necropsy

For Common, dusky, and striped dolphins: contact Karen Stockin (021 423 997)

DISPOSAL

☐ Natural decay ☐ Burning ☐ At sea ☐ Unknown ☐ Other _____
☐ Sent for necropsy (items, storage, destinations, dates): _____
_____
☐ Burial (detailed location): _____
_____
☐ Iwi approval if sending material ☐ Material to be returned to iwi
Iwi contact details: _____
_____



Note: Mammary slits may also occur in males

DESCRIPTION OF CIRCUMSTANCES & REMARKS (Injuries Old/New, Suspected cause of death (& evidence), circumstances, iwi & cultural notes etc) [*Use extra pages for detailed notes*]

_____
_____
_____
_____
_____
_____
_____

FINAL CHECKLIST

☐ Attach this form to all samples that are being sent
☐ Save copies of this form, photos, maps and other relevant information to the DOCCM and email the link to Marine Species & Threats team (marinemammals@doc.govt.nz & CC hhendriks@doc.govt.nz)
☐ Send copies of this form to relevant iwi

ID GUIDE Below are some species that you may come across.

Acknowledgements: Food and Agriculture Organization of the United Nations. ID images from: **Jefferson, T.A., S. Leatherwood**, and **M.A. Webber. FAO species identification guide. Marine mammals of the world.** Rome, FAO. 1993. 320 p. 587 figs

Hector's dolphin <1.6m

Rounded dorsal fin. No beak. Light grey upper. White throat and chest, extending towards tail. Black head and dorsal fin

Gray's beaked whale <5.6m

Small head, extremely long narrow beak. Grey body, white patches in the genital region, beak becomes white in adults

Pygmy sperm whale <3.4m

Robust body. Squarish head. Tiny dorsal fin. Tiny, underslung lower jaw. Grey upper, light belly. False gill behind eye

Dusky dolphin <2m

Short, dark-coloured beak. Dark grey-blue upper. Pale face and belly, light blazes along flank. Two-toned dorsal fin

Cuvier's beaked whale <7.5m

Short, poorly defined beak. Mouthline upcurved at rear. Top of head slightly concave. Lighter colouration around head and belly

Pilot whale (image sp. Long-finned) <6.5m

Dark brown/black, white streak behind eye. Long, low dorsal fin. Blunt head, bulging forehead. Often mass strands

Common dolphin <2.3m

Sleek body. Prominent beak. Dark upper and dorsal fin. Tan or yellow sides, "hourglass" pattern. Cream or white belly

Strap-toothed whale <6.2m

Adult males have long tusks near the middle of lower jaw that curl backward and inward. Mostly grey or black, white underside and band around head

False killer whale <6m

Black body. Erect dorsal fin, rounded tip. Rounded head. Often breaches. Can mass strand. Seasonal visitor to NZ

Appendix 3B. Histological preparation of pilot whale teeth for ageing

Adapted from:

Evans et al. (2007)

Kasuya and Matsui (1984)

Lockyer (1993a)

Murphy (2004)

Perrin and Myrick (1980)

Pierce and Kajimura (1980)

Before entering the laboratory

The importance of keeping a lab book for recording the various processes involved in preparing tissues for age determination cannot be emphasised enough. This will enable you to track the processes (e.g. acid-etching and decalcification times) and help develop guides relating to them for particular species. It will also be a record of each specimen that can be later used to trouble-shoot problems (e.g. over- or under- decalcified teeth) and work out the best practices (e.g. temperature settings, thin-section thickness). In addition, a lab book can be used as a means of keeping track of the status of the equipment and consumables used.

Sample Collection

Equipment required:

- Tyvek coveralls
- Gloves
- Dental forceps
- Scalpel or knife (chisel tip works well)
- Lopers (if removing section of jaw)
- Plastic sample bags
- Permanent markers
- Ice-cream containers (for maceration)

Select the least worn/damaged and least curved teeth from mid-way along the upper or lower jaw. Obtain several teeth from each animal to ensure sufficient material for replication.

NOTE: Removing cetacean teeth from a fresh carcass is not recommended, particularly for young animals because the base of the tooth is delicate and can easily be broken.

If there is no requirement to keep the skull intact or the whole skull is not required to be collected, cut a small section of the lower jaw from the animal and macerate it for a week or two to allow the teeth to loosen.

Storing Teeth

Ideally, it is best to use teeth as soon as possible after removal from the animal, however, in many situations, this is not possible (e.g. museum collections often store teeth for many years in various types of containers).

Equipment required:

- Specimen containers
- Plastic sample bags
- Permanent markers
- ID labels/ waterproof paper
- Pencil
- 70% ethanol

For cleaned, dry teeth, optimal conditions for storage are 10 – 20˚C and 40 – 70% relative humidity, without rapid changes in either temperature or humidity. Fresh teeth removed with tissue still attached can be stored in 70% ethanol or frozen. It is important not to use chemicals such as formalin and strong degreasing agents to prepare teeth.

Preparation (Measuring, cleaning and photographing)

Equipment required:

- Distilled water
- Ruler and vernier callipers
- Pencil
- Data sheets
- Camera
- ID labels/waterproof paper
- Scalpel and blades (or tooth extractor)
- Specimen containers
- Plastic sample bags
- Permanent markers
- ID labels
- 70% ethanol

1. If frozen, defrost and rehydrate teeth in distilled water overnight.
2. If required, remove excess tissue from teeth VERY GENTLY using a scalpel or with a tooth extractor. Maceration is preferred.
3. Measure length, width, depth, max diameter of apical foramen (pulp cavity opening), and length above the gumline.
4. Photograph each tooth (include whale ID label and scale bar in all photographs).
5. If not grinding/sectioning immediately, label each tooth and store in individual containers (inside one ziplock bag for each whale). Store as above, i.e. dry or with 70% ethanol.

G**rinding**

Pilot whale teeth need to be wafered prior to decalcification to ensure that an even decalcification of the tooth is achieved and in doing so that partial over- or under-decalcification is avoided.

Wafering: both sides are removed, leaving a 'wafer' that contains the centre of the tooth.

NOTE: Juvenile teeth with a large pulp cavity are too fragile to be ground and so are decalcified whole instead.

Occupational Health and Safety:

- Wear safety glasses.
- Keep hands away from grinding plate.
- Wear gloves and do not touch melted thermoplastic cement or hot plate...it will burn!
- Use large forceps.

Equipment required:

- Lab coat
- Safety glasses
- Gloves
- Pencil
- Ruler and/or vernier callipers
- Data sheets
- Camera
- ID labels/waterproof paper
- Microscope slides (clear)
- Crystalbond™ mounting media
- Water bath
- Distilled water
- Hot plate
- GEMMASTA GF4 faceting machine with slow drip attachment
- 300 – 600 grit diamond grinding wheels
- Histological cassettes
- 5 ml containers with holes drilled in them
- Elastic bands
- 70% ethanol
- Large specimen jars (at least 4)

1. Choose 1 – 3 of the straightest, least-worn teeth from each individual (cleaned of flesh, preferably not cracked). If possible, prepare more than one tooth per individual to allow for back-ups in case of a poor-quality tooth, mistakes etc.
2. Mark out the centre line on the tooth with a pencil (crown to root axis), and measure 2 mm either side of this line.
3. Mount the tooth longitudinally in the centre of a clear slide. Re-mountable mounting media such as Crystalbond™ is required. Melt a small amount of glue on a hot plate by holding a stick of the glue onto a clean slide (ensure the hot plate is just hot enough to melt the glue, but not too hot to cause bubbling or burning of the glue, e.g. setting 4 – 5). Ensure adequate glue is used to hold the tooth in place and is left to harden and/or placed in a water bath to harden. Check that the tooth is secure and will not move while it is being ground.
4. Before you start grinding, ensure there is sufficient water dripping onto the grinding wheel – NEVER TRIM TEETH USING A DRY WHEEL.
5. Grind the tooth almost in half – grind down to approx. 2 mm off centre parallel to the centre line (crown and pulp cavity apices) using a GEMMASTA GF4 faceting machine equipped with a 300 – 600 (grade sandpaper) grit wheel. Start grinding at a medium speed and slowly work to a faster speed, being very careful to use enough water and not cause too much friction heat.
6. Remove the half-tooth from the mounting media, ensuring the freshly ground edge is not damaged. (In the case of Crystalbond™, re-heat the slide to melt the mounting media.)
7. Place the ground edge downwards on the slide, and allow the media to harden
8. Similarly grind off the other side of the tooth, leaving a 3 – 5mm thick section on the slide. Remove section from the slide by re-heating.
9. Photograph each ground tooth wafer (include whale ID label and scale bar in all photographs).
10. Measure the post-grind width and depth of each tooth wafer.
11. Place tooth wafers in a labelled histological cassette (labelled on at least two sides of the cassette). Wrap an elastic band around each container so that it won't accidentally open.
12. Divide teeth into crude age categories at this point (e.g. no pulp, small pulp, large pulp, juvenile) because decalcification times will vary. Store in 70% ethanol if not being

decalcified immediately. Place any unground juvenile teeth into 5 ml containers with holes drilled in them (they won't fit into histology cassettes).

NOTE: Use only quality, sharp diamond grinding wheels. Worn wheels will need to be replaced to avoid damaging teeth.

Decalcification

Teeth can either be decalcified in histology cassettes or if the trimmed tooth or whole tooth is too large for histological cassettes they can be decalcified in clear plastic vials which have had several small holes drilled into the sides and bottom. A small drill bit can be used to make approx. 20 holes in the sides and bottom of the vial. Ensure that there are enough holes in the vial to adequately mix the decalcification fluid around the tooth and that any rough bits remaining from the drilling process are cleaned off.

Occupational Health and Safety:

- Use RDO only under a fume hood.
- Make sure used RDO is clearly labelled and stored under a fume hood.
- Wear gloves when handling RDO.
- Do not use metal/stainless steel containers as they will corrode

Equipment required:

- Lab coat
- Safety glasses
- Fume hood
- Gloves
- Pencil
- Decalcifying agent (RDO, Apex Engineering Products Corporation, Aurora, Illinois)
- Large glass jars for decalcified teeth (at least 4)
- Filter paper
- Large glass beakers
- Plastic funnel
- pH strips
- Ammonium hydroxide
- Ammonium oxalate
- 30ml vial
- Tap water for rinsing
- Distilled water or 70% ethanol
- Magnetic stirrer (optional)

1. If teeth have been stored in ethanol, they will need to be rinsed in tap water prior to decalcification. Place containers in a large glass jar or beaker and using a plastic tube extender or hose (ensures that water is directed to the bottom of jar and that there is adequate mixing) on a cold-water tap rinse the teeth for a minimum of 2 h.
2. In the meantime, under a fume hood filter the decalcification agent (RDO) using a large funnel and filter paper into a wide-mouth glass jar with a screw-on lid. Filtering removes the dark precipitate in the RDO.

3. Teeth of juvenile pilot whales should be kept in RDO for approx. 4 h, adults for approx. 24 – 36 h.
4. Place the cassettes in a plastic/glass sealed container of RDO at a tissue to volume ratio of no more than about 20 teeth per 2 l of RDO. Screw the lids on and agitate each jar to ensure circulation of the RDO through the containers holding the teeth specimens. Agitate the jar again every 1 – 2 h to ensure proper circulation of the RDO around the tooth specimens. Mechanical agitation of tissues in the solutions is recommended during the decalcification process. Can use magnetic stirrer if available.
5. Decalcification times may vary, and the teeth should be checked regularly towards the end of the procedure. When checking the tooth specimens remove the containers from the RDO using long forceps or tongs, and place them into a large glass jar or beaker. Using a plastic tube extender or hose, rinse the containers under running water for 5 min. Once rinsed, remove each tooth from its container and check the state of the tooth specimen using your bare hands (do this individually so you don't mix up teeth and cassettes), taking care not to damage the tooth, especially with fingernails. When fully decalcified, the tooth should be pliable (rubbery) throughout its whole length and be reasonably translucent when held up to the light. DON'T BEND TEETH TOO MUCH AS THEY CAN FRACTURE! Spots of opaque material and rigidity within the tooth indicate that it is not fully decalcified. Care must be taken not to over-decalcify, which will result in damage to the growth layers in the dentine or cementum.
6. If the tooth specimens are not fully decalcified, replace the teeth into their containers, securing the cassettes with an elastic band. Return the containers into the RDO and repeat step 4.
7. Once fully decalcified, place the containers into a large glass jar or beaker. Using a plastic tube extender or hose on a cold-water tap, rinse the teeth for at least 3 h (preferably overnight) to ensure that all the RDO is removed. Take care that the tap is on enough to ensure adequate rinsing, but not enough that the containers float out of the jar/beaker.
8. If the decalcified tooth specimens are not to be sectioned and stained immediately, they can be stored in distilled water for a few days, making sure that the water is replaced with fresh distilled water each day. If the decalcified tooth specimens are to be stored indeterminately they can be stored in 70% ethanol. To use ethanol stored-teeth/wafers/tooth halves, just place back in baskets/vials and rinse in running tap water for 2 – 3 h.

9. If it becomes necessary to stop the process at any time before the tooth specimens are fully decalcified, they need to be rinsed under running water for a minimum of 3 h (e.g. overnight) so that decalcification process is fully stopped.
10. Transfer cassettes/teeth to running water overnight.
11. Transfer cassettes/teeth to distilled water for 1 h.

A chemical endpoint test can be carried out to check the effectiveness of the RDO solution – see http://www.rdo-apex.com/t_procedures.html

The ammonium oxalate turbidity endpoint test:

1. Add 5 ml of the decalcifying solution from the bottom of the specimen container (avoiding picking up particles in the pipette) to a 30 ml tube containing a pH indicator strip.
2. Pipette approximately 5 ml of ammonium hydroxide into the tube, neutralising the solution as indicated by the pH strip.
3. Add 5 ml of ammonium oxalate to the tube.

If the test aliquot is clear after 30 minutes, decalcification is completed. If cloudy, the solution is exhausted, indicated by the precipitated calcium oxalate, decalcification is not complete and the solution must be changed (RDO, Apex Engineering Products Corporation, Aurora, Illinois).

Thin Sectioning

Thin sectioning, staining and mounting are usually carried out in sequence. If you do not plan to stain/mount your thin sections immediately after sectioning/staining, and want to store sections for only a short amount of time (a few days at most) ensure that you have pre-prepared small storage jars containing distilled water and an identification label into which the baskets containing the thin sections can be placed prior to staining/mounting. Again, replace the distilled water each day.

Occupational Health and Safety

- Operators are required to be trained in the techniques involved in thin-sectioning before they use the cryostat or slide microtome.
- Keep hands away from the knife at all times.
- Take the knife blade out of the stage when the machine is not in use, e.g. overnight, lunch break.
- Move the blade stage to the rear when changing or manipulating the specimen/chuck.
- When cleaning the blade, use a small paintbrush or low-lint wipes and a motion working away from the blade edge.
- When cleaning out the dish of waste sections, make sure the blade is towards the rear.
- Avoid contact between skin and very cold parts of the machine as this may result in fingers becoming stuck to the cold metal.

Equipment required:

- Lab coat
- Safety glasses
- Sledge microtome with freezing stage or cryostat
- Low profile new microtome blades
- OCT embedding agent (e.g. Tissue-Tek)
- 7 kg CO_2 cylinder with siphon
- Paintbrush
- Petri dishes
- Distilled water
- 70% ethanol
- Low-lint wipes
- Forceps
- Pantyhose
- Specimen jars (70 ml)
- Pencil
- Permanent marker
- Elastic bands

1. If decalcified specimens have been stored in ethanol, rinse in running tap water for 2 – 3 h.
2. Set up 2 – 3 Petri dishes with distilled water for collecting sections in a position where they can be easily reached (e.g. on a bench nearby or on top of the cutting machine). Label each dish with the animal number, section thickness, and sequence of groups of sections.
3. Turn on the CO_2 cylinder and set the microtome thickness to 25 µm.
4. Remove decalcified specimen from its container and let dry for a few seconds on paper towel. Before mounting the tooth onto the cryostat disc or the freezing slide (sledge) microtome stage, ensure that the blade head is moved to a position well away from the cryostat stage clamp or the freezing slide (sledge) microtome stage and take extreme care to avoid the blade.
5. Place enough embedding compound (OCT) onto the disc or stage to allow the tooth to be embedded (usually the size of a New Zealand 10 cent piece). Avoid the formation of bubbles in the medium. Feather the CO_2 until the OCT becomes somewhat opaque (i.e. the bottom layer is frozen) before placing the tooth in position. Make sure the tooth is as level as possible and that the orientation of cutting will be from crown to root tip.
6. Open the CO_2 valve to freeze the tooth onto the microtome stage. If the tooth is not level, close the CO_2 valve and allow the OCT to thaw, after which the tooth can be repositioned, re-open the CO_2 valve and allow the tooth to re-freeze onto the stage.
7. Once the specimen is satisfactorily frozen in place, add additional OCT around the tooth to enclose tip and root, building up the sides with OCT. If a large specimen is being cut, cover the entire specimen and allow this to freeze.
8. Be careful not to over-freeze the specimen as this will result in scrape marks being left on the section by the blade. Under-freezing will result in the specimen falling off the mount.
9. Start sectioning. There will be several layers of OCT to shave off before getting to the decalcified tooth. Stop when you reach the tooth and discard the sections out of the collection tray. This ensures that if a tooth section falls into the tray it can be easily retrieved. Section teeth at 25 µm. Collect the thin-sections produced by the freezing slide (sledge) microtome as you section through the tooth with a fine-haired paintbrush and place these in a petri dish with distilled water (the OCT will dissolve off). Place those sections away from the tooth centre and those from the centre into different Petri dishes. Take care not to freeze the brush to the blade and avoid touching the mounted

tooth with the brush (particularly if the tooth has partially unfrozen because there is a chance you will dislodge it). Use a smooth, steady motion when sectioning, particularly at the centre of the tooth and just each side of it. The midline of the tooth is the most important part for aging and includes the centre of the crown, the pulp cavity and the centre of the root. In teeth that still have a pulp cavity, this will include the widest portion of the tooth cavity. If ice or tissue builds up on the blade remove using a low-lint wipe and ethanol, wiping in an upward direction and taking extreme care of the blade. Remove all tissue from blade and machine between samples and reposition the blade to use a fresh section of the blade (to ensure that the sharpest part of the blade is used each time).

10. Once you have finished sectioning, allow the OCT to thaw from the remaining part of the tooth and remove it from the microtome stage. Wipe down any remaining OCT from the stage or disc with a paper towel or cloth.
11. Select the most central and complete sections from each tooth. It is advisable to check thin-sections under a dissecting microscope for scrape marks (associated with a blunt or damaged blade or over-freezing) and other problems early in the sectioning process.
12. Using a pair of smooth forceps, remove the sorted sections from the Petri dishes, place into the container you will be using for staining (cassette or vial) and clearly label the outside with a pencil or permanent marking pen. Do not crowd the container with too many sections or they will overlap during the staining process, and the teeth will be unevenly stained. Wrap the container in a small square or bag of stocking (try and cover with only one layer to allow liquid to circulate) securing this with an elastic band (this stops small sections sliding through the holes in the container).
13. Place the containers in large glass jar or beaker and using a plastic tube extender or hose on a cold-water tap rinse under running tap water for 5 min to remove any remaining OCT. Clean the Petri dishes and replace the distilled water. Repeat steps above steps for additional teeth.
14. Once the thin-sections have been rinsed, remove them from the jar/beaker and let the containers drain on paper towel (to avoid excess water diluting the stain). If you are not staining the tooth sections immediately, they can be stored in distilled water for a few days, making sure that the water is replaced with fresh distilled water each day.

Staining thin sections

Occupational Health and Safety

- Wear safety glasses and gloves.

Equipment required

Lab coat

- Safety glasses
- Prepared haematoxylin stain
- Timer with alarm
- Large glass jars or beakers (x2)
- Gloves
- Tap water for rinsing
- Forceps
- Petri dishes
- Distilled water
- Dissecting microscope
- 2% ammonia solution

NOTE: Using the histology cassettes for staining can result in uneven staining even when the mixture is agitated. It may be necessary to place tooth sections into small plastic vials, fill with haematoxylin, and cover with pantyhose to strain for rinsing and blueing.

1. Stain sections for 25 – 40 mins in haematoxylin
2. Rinse in distilled water (approx. 3 minutes, until water runs clear)
3. Blue in alkaline water (approx. 5 drops of ammonia in a 250 ml beaker of distilled water; approx. 3 minutes)
4. Rinse in tap water (acidic) and transfer to distilled water (approx. 3 minutes)

Mounting thin sections

Occupational Health and Safety

- Wear surgical gloves and safety glasses when handling chemicals.
- The coverslip mounting medium must be used under a fume hood.

Equipment required:

- Safety glasses
- Lab coat
- Petri dishes
- Slides (preferably gelatin coated)
- Coverslip mounting medium
- Glass coverslips
- Forceps
- Pencil/permanent marker
- Air-drying rack
- Wooden mounting sticks
- Warming plate
- Slide box

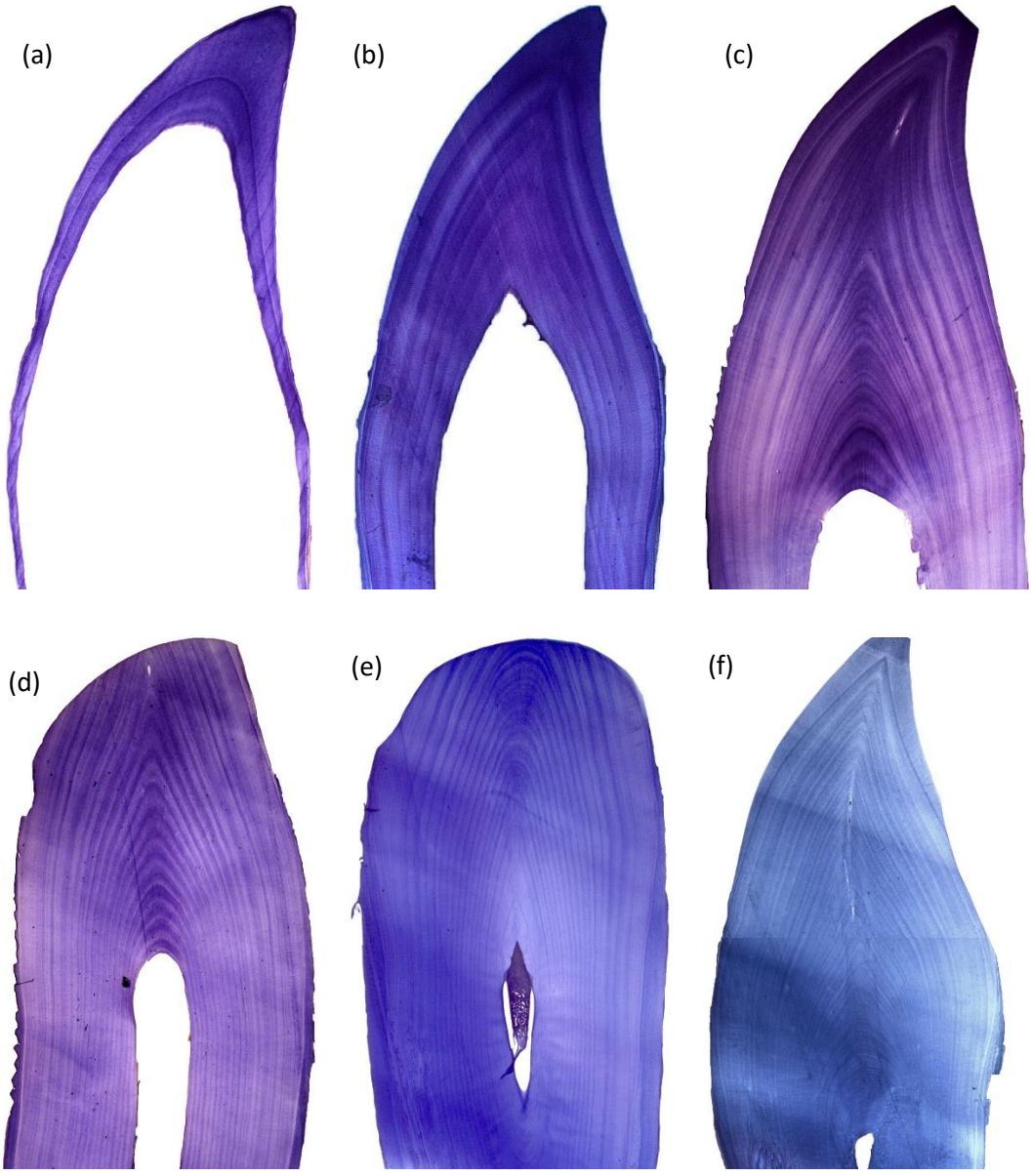
1. Use a magnifying lamp to select the best sections to mount.
2. Mount teeth on gelatin-coated histological slides under water. Leave slides to dry for at least 15 – 20 mins. Can blot dry with kitchen towel/blotting paper. Should be able to fit two teeth per slide.
3. Cover sections with a glass coverslip using DPX and dry on a slide warmer for a few days.

Reading

1. Examine sections under a binocular compound microscope using plain transmitted light, counting growth layer groups in the dentine (10 – 40×) and the cementum (100 – 400×).
2. Assess tooth sections for quality, after Luque et al. (2009b): good quality (when GLGs were distinct, and easily counted), satisfactory (GLGs could be distinguished, but there was some uncertainty about how many GLGs were present), and poor quality (a reasonable estimate of the number of GLGs was impossible).
3. All age estimation reading should be carried out without access to other data on the animals, and replicate counts by at least two readers
4. If readers disagree on the age of the animal, the sections are examined again. If a difference is greater than 1 GLG both readers re-read the teeth, and if no agreement is reached, another tooth is sectioned and re-read by both.
5. While examining the teeth sections all anomalies, and the degree of occlusion of the pulp cavity should also be recorded.

Appendix 3C. Example tooth sections

Decalcified, thin-sectioned, and stained long-finned pilot whale (*Globicephala melas edwardii*) tooth sections from (a) 1-yr-old female, 237 cm TBL, GM66; (b) 5.75-yr-old female, 350 cm TBL, GM252; (c) 11-yr-old male, 445 cm TBL, GM46; (d) 18-yr-old male, 497 cm TBL, GM100; (e) 31-yr-old male, 563 cm TBL, GM84 (f) 33-yr-old female, 434 cm TBL, GM280. Note open pulp cavities.



Appendix 3D. Total body length range at age

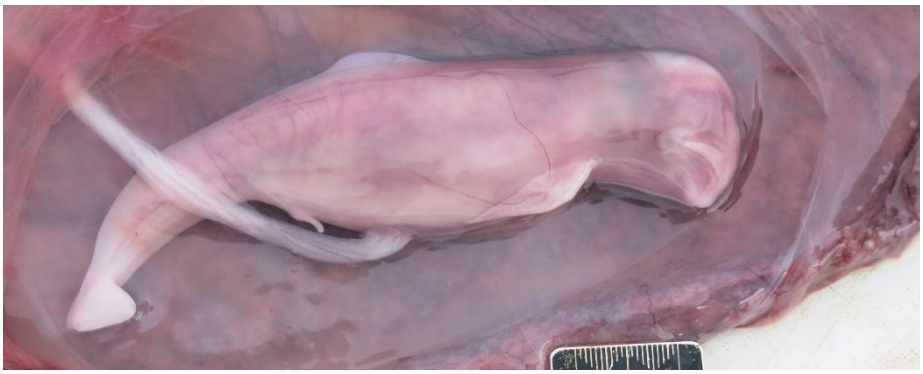
Age and total body length (TBL) range of 304 *Globicephala melas edwardii* stranded on the New Zealand coast between 2006 and 2017. Including data from 227 females and 154 males.

	All data		Female		Male	
Age	***n***	**TBL (cm)**	***n***	**TBL (cm)**	***n***	**TBL (cm)**
0	21	176 – 250	12	176 – 222	9	180 – 250
1	23	201 – 292	11	201 – 287	12	217 – 292
2	13	270 – 324	5	270 – 315	8	271 – 324
3	12	306 – 347	8	306 – 326	4	294 – 347
4	15	310 – 370	5	310 – 365	10	296 – 370
5	17	312 – 391	8	312 – 386	9	312 – 391
6	10	334 – 449	8	334 – 382	2	360 – 449
7	7	346 – 412	4	346 – 396	3	379 – 412
8	11	375 – 440	5	375 – 407	5	390 – 440
9	14	350 – 460	8	350 – 401	6	400 – 460
10	13	355 – 455	7	355 – 440	6	403 – 455
11	17	352 – 473	7	384 – 435	10	352 – 473
12	16	400 – 451	10	380 – 460	6	420 – 451
13	15	364 – 483	10	364 – 458	5	435 – 483
14	14	390 – 555	7	390 – 460	6	470 – 555
15	19	383 – 622	8	383 – 443	11	450 – 622
16	23	380 – 570	16	380 – 461	7	467 – 570
17	11	390 – 554	4	390 – 478	7	492 – 554
18	14	401 – 547	9	401 – 475	5	497 – 547
19	16	410 – 581	9	410 – 463	7	511 – 581
20	18	410 – 556	13	410 – 460	4	510 – 556
21	6	408 – 547	4	408 – 482	2	536 – 547
22	9	398 – 560	6	398 – 460	3	545 – 560
23	10	417 – 575	8	417 – 473	2	575
24	8	422 – 562	6	422 – 460	2	550 – 562
25	9	418 – 574	7	418 – 485	2	573 – 574
26	6	419 – 460	6	419 – 460	0	
27	1	426	1	426	0	
28	4	424 – 475	4	424 – 475	0	
29	2	426 – 431	2	426 – 431	0	
30	5	423 – 453	5	423 – 453	0	
31	1	563	0		1	563
32	2	430 – 451	2	430 – 451	0	
33	1	434	1	434	0	
34	0		0		0	
35	0		0		0	
36	0		0		0	
37	0		0		0	
38	1	459	1	459	0	

Appendix 6A. Example foetal images.

Foetal specimens of *Globicephala melas edwardii*, (a) male foetus, 13.9 cm TBL, GM521a; (b) female foetus, 47 cm TBL, GM247a; (c); male foetus, 207 cm TBL, GM374a.

(a)



(b)

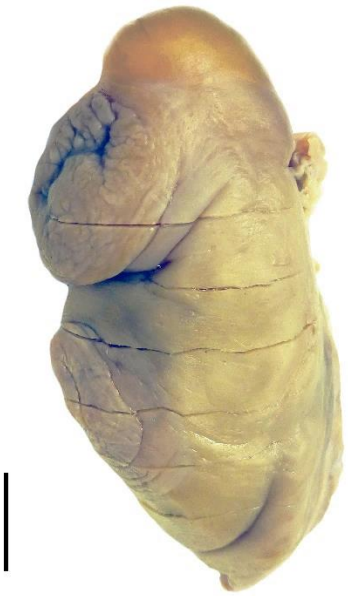
(c)

Appendix 6B. Example gross external ovarian images.

Ovaries of *Globicephala melas edwardii*: (a) left ovary of sexually immature female (GM109, 0 yrs of age, 198 cm TBL) no ovarian scars; (b) left ovary of a resting mature female (GM221, 20 yrs of age, 423 cm TBL), a large fluid-filled follicle and a young (YCA), medium (MCA), and old (OCA) *corpora albicantia* are visible; (c) left ovary of a lactating female (GM420, 18 yrs of age, 420 cm TBL), YCA and two MCAs are visible; (d) right ovary of a pregnant female (GM477, 28 yrs of age, 425 cm TBL), *corpora luteum* of late pregnancy (CLP), MCA and OCA visible, foetus 176 cm TBL. All ovaries were formalin-fixed. Scale bar = 1 cm.

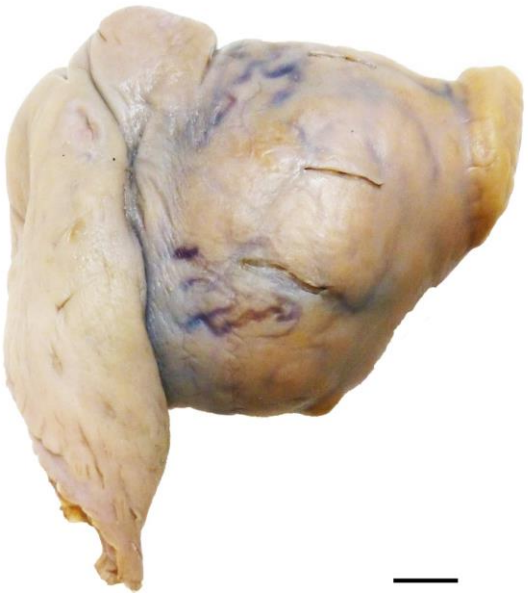
(a)

(b)



(c)

(d)

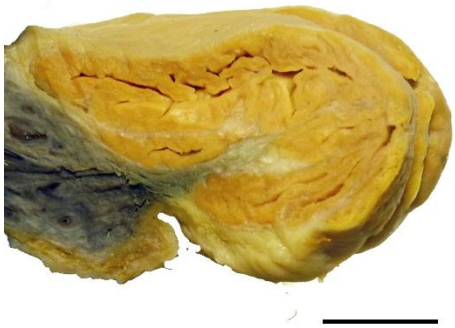


Appendix 6C. Example gross internal ovarian images.

Ovarian cross-sections of *Globicephala melas edwardii*: (a) median slice through left ovary of a lactating female (GM334, 11 yrs of age, 413 cm TBL), multiple follicles visible; (b) median slice through a young *corpus luteum* (CL) of ovulation on left ovary of a resting female (GM426, 25 yrs of age, 428 cm TBL); (c) median slice through a CL of late pregnancy on right ovary of a pregnant female (GM477, 28 yrs of age, 425 cm TBL), note jelly-filled cavity in centre, foetus 176 cm TBL; (d) median slice through young *corpora albicantia* (CA) on left ovary of a resting female (GM386, 16 yrs of age, 425 cm TBL); (e) median slice through medium CA on the left ovary of a lactating female (GM478, 24 yrs of age, 436 cm TBL); (f) median slice through an old CA on the left ovary of a resting female (GM396, 30 yrs of age, 440cm TBL). All ovaries were formalin-fixed. Scale bar = 1 cm.

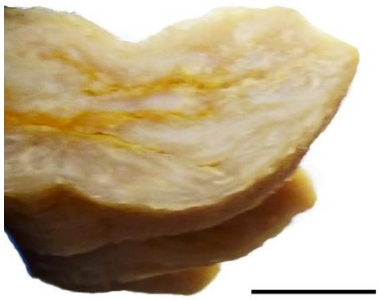
(a)

(b)



(c)

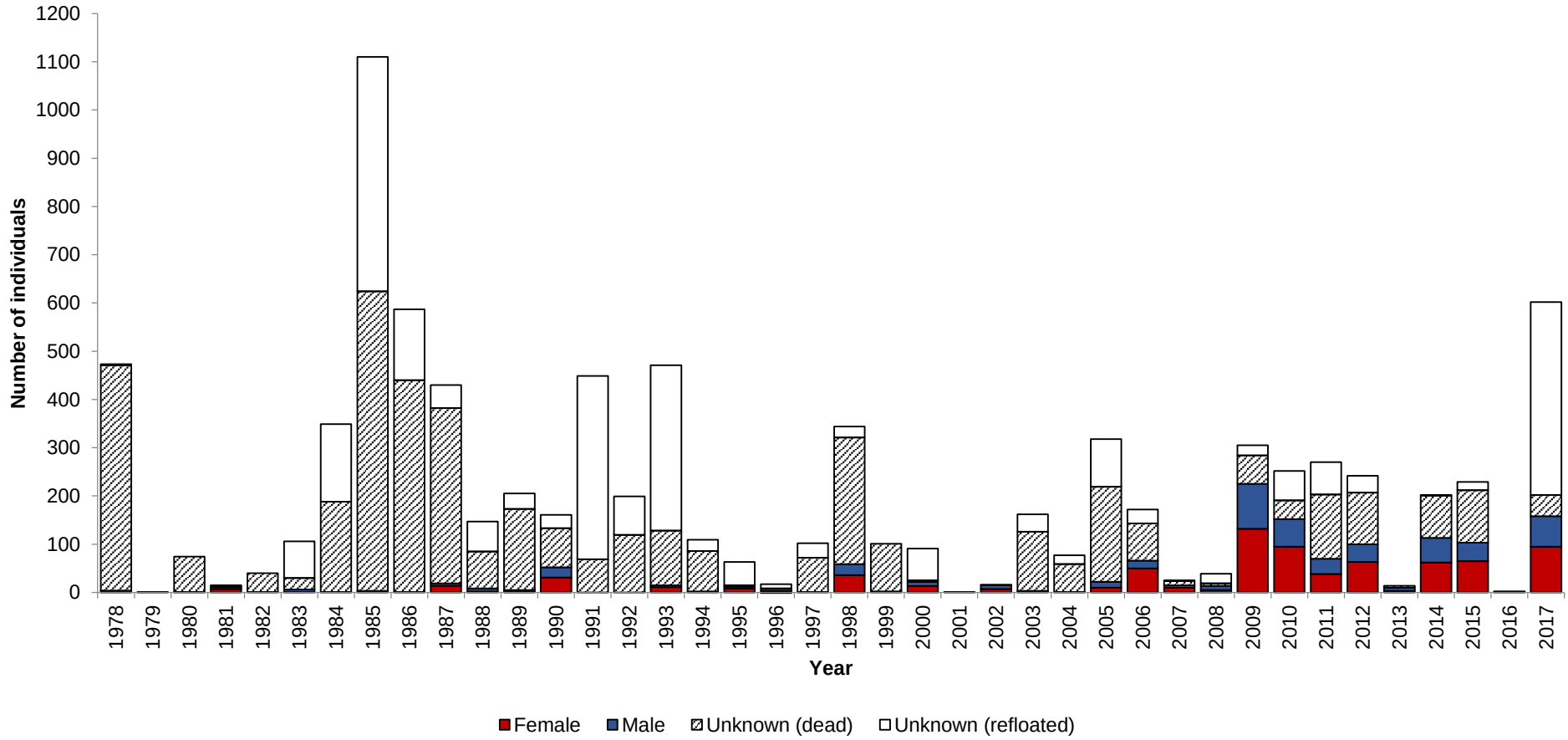
(d)



(e)

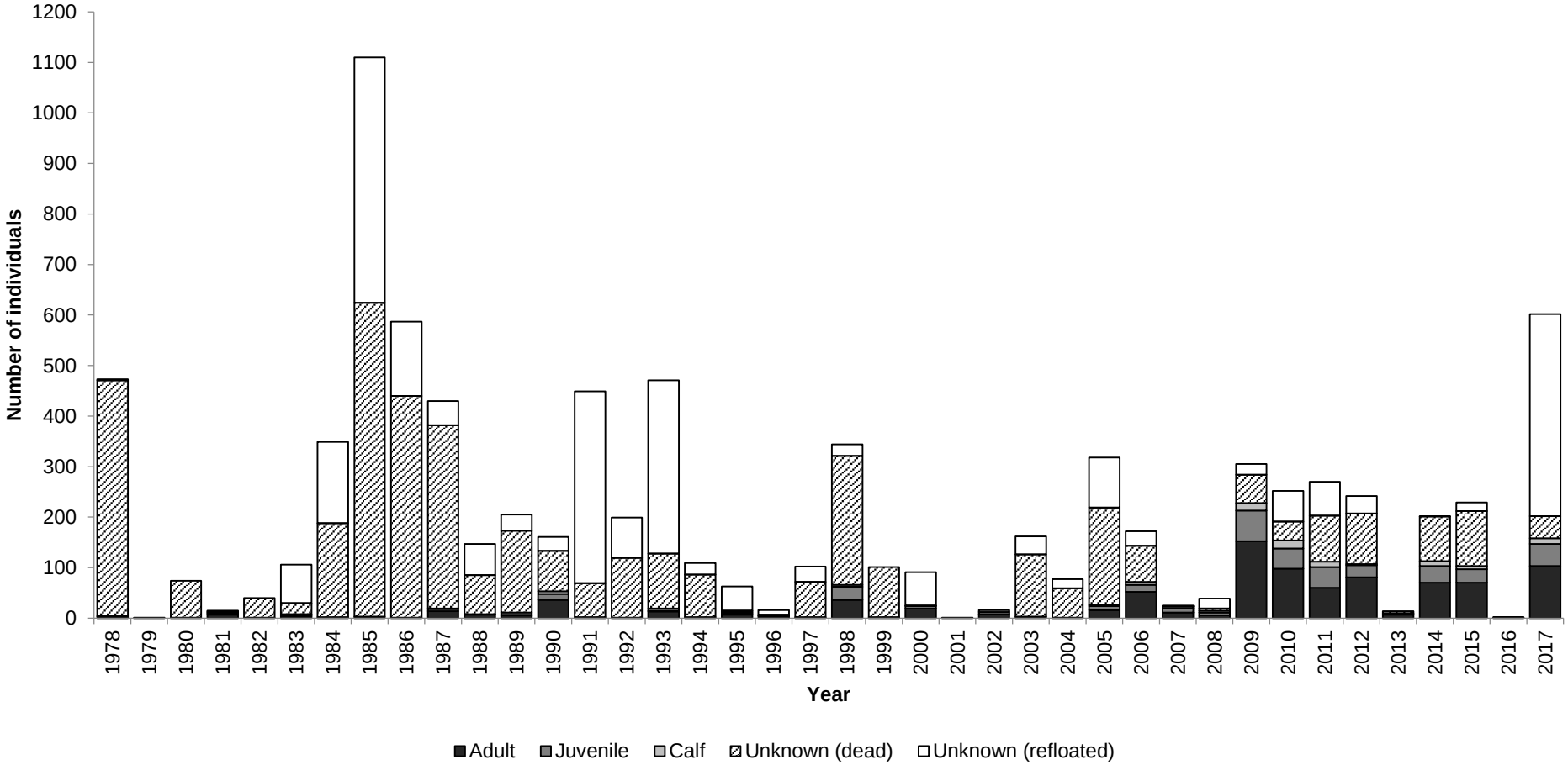
(f)

Appendix 7A. Sex composition of stranded long-finned pilot whales (1978 – 2017)



Sex composition of *Globicephala melas edwardii* stranded on the New Zealand coast between 1978 and 2017 (*n* = 776 females, 527 males, 7,268 unknown sex).

Appendix 7B. Maturity composition of stranded long-finned pilot whales (1978 – 2017)



Number of *Globicephala melas edwardii* adults (total body length [TBL] > estimated length at sexual maturity [LSM]), juveniles (TBL > estimated TBL at 1 yr but < estimated LSM), and calves (TBL < estimated TBL at 1 yr) stranded on the New Zealand coast between 1978 and 2017 (*n* = 902 adults, 375 juveniles, 126 calves, and 7,168 unknown).