



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

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Male reproductive biology is described for the Southern Hemisphere long-finned pilot whale (*Globicephala melas edwardii*), a subspecies that regularly mass strands along the New Zealand coastline. Ten mass stranding events sampled over a 7-year period enabled assessments of key life history parameters. 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 years) and length (450–490 cm) at which individuals attained sexual maturity. Using Bayesian cumulative logit regression models, we estimated the average age and length at the attainment of sexual maturity 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 nonhistological 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 years and 570 cm), and at a younger age and smaller body length than previously reported for *Globicephala melas melas* in the North Atlantic. Given the ease of collection, minimal processing, and applicability to suboptimal material collected from stranding events, future studies should assess 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 conservation management of the subspecies, which is subject to high levels of stranding-related mortality.

Key words: age, body length, cetacean, male reproduction, New Zealand, pilot whale, sexual maturity, testes

Knowledge of life history is the basis 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 behavioral 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 long-finned pilot whale (*Globicephala melas edwardii*). Further, most research on cetacean reproduction to date has focused on females, primarily because female reproductive parameters are required for population modeling. 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). 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.

Male cetaceans attain sexual maturity once they produce mature male gametes through spermiogenesis (Plön and Bernard 2007). Plasma and blubber testosterone levels have been used as markers for assessment of sexual maturity in delphinids by delineating sexually immature from mature individuals (e.g., Kellar et al. 2009). However, Desportes et al. (1994) reported that in the North Atlantic long-finned pilot whale subspecies (*Globicephala melas 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. 1994). 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. 1993). 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 center 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 long-finned pilot whales, the core of the testis matures last and thus 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 (1962) carried out a more thorough investigation of *G. m. melas* taken in the drive fishery off Newfoundland, but limitations of the data set only allowed for an approximate estimate of ASM. Also, problems with the methodology employed for estimating age rendered Sergeant's earlier (1962) estimates of life history parameters to be inaccurate (Kasuya et al. 1988). Extensive, and comparable, reproductive studies were later conducted on both long- and short-finned pilot whales in the North Hemisphere, based on large numbers of samples collected from the *G. m. melas* drive fishery in the Faroe Islands (Desportes et al. 1993, 1994; Desportes 1994) and the short-finned pilot whale (*Globicephala macrorhynchus*) 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 ca. 17 years, and the cessation of testis growth at ca. 25 years, with larger mature males having heavier testes than smaller ones (Kasuya and Marsh 1984; Desportes et al. 1993; 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 long-finned pilot whale frequently mass-strands alive on the coast of New Zealand (Brabyn 1991; Oremus et al. 2009; Betty 2019). 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 long-finned pilot whales. Here, we used tissues opportunistically collected from carcasses of *G. m. edwardii* mass-stranded on the New Zealand coast to provide 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), we examine 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 attainment of sexual maturity are compared with previous estimates

for the North Atlantic subspecies, and mating strategies used by male long-finned pilot whales are considered.

MATERIALS AND METHODS

Sexual maturation was assessed for 98 male *G. m. edwardii* involved in 10 live MSEs on the New Zealand coast between January 2010 and February 2017 (Table 1). Teeth, gonadal samples (testes and associated epididymides), total body length (TBL), and an assessment of decomposition were obtained through standard postmortem 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 millimeter. 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 h' postmortem. Where testes were large, 3 × 1 cm³ blocks were dissected from the center 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 field locations, many of the samples could not 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 predicted average length at 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 dentinal growth layer groups (GLGs) in the teeth, as defined by Perrin and Myrick (1980), and revealed by decalcified and stained longitudinal sections. Tooth preparation methods for this study were adapted from the protocol described by Lockyer (1993). Teeth were ground down on both sides (using a faceting machine equipped with a 600 grit wheel) to obtain

a 3- to 5-mm longitudinal section through the center of the tooth, and then decalcified with hydrochloric acid (RDO, Apex Engineering Products Corporation, Aurora, Illinois) until they were slightly pliable (1–36 h). Teeth from neonatal whales were decalcified whole. Decalcified teeth were sectioned at approximately 25 µm on a carbon dioxide freezing stage of a sledge microtome, stained with hematoxylin, and then blued in a weak ammonia solution. The best sections (i.e., those cut through the center of the pulp cavity) were mounted permanently on glass slides using DPX mounting medium.

Age in years was estimated by counting GLGs in the dentin using a dissecting microscope at 10–40× magnification. All sections were read by two or three experienced readers. 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. If readers disagreed on the age of the animal, the sections were examined again. If the difference was greater 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, minimum age, or an age range. 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 dentin, were classified as newborns (i.e., age 0).

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. (1993). The tissues were prepared using standard histological techniques, i.e., embedded in paraffin wax, sectioned at 5 µm, stained with hematoxylin 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

Table 1.—Details of long-finned pilot whale (*Globicephala melas edwardii*) mass stranding events on the New Zealand coast from which male gonads were collected for this study.

Location	Date	Total no. stranded	Confirmed males	Males sampled
Port Levy, Banks Peninsula	23 January 2010	54	5	4
West Ruggedy Beach, Stewart Island	14 February 2010	28	10	9
Ruapuke, Waikato	18 June 2010	20	8	3
Spirits Bay, Far North	22 September 2010	49	17	5
Port Pūponga, Golden Bay	4 February 2011	84	5	5
Mason Bay, Stewart Island	20 February 2011	107	36	27
Farewell Spit, Golden Bay	14 November 2011	65	22	12
Farewell Spit, Golden Bay	6 January 2014	39	18	18
Farewell Spit, Golden Bay	14 January 2014	99	13	8
Farewell Spit, Golden Bay	10 February 2017	600	62	7
Total		1,145	196	98

cells, and germinal cells (e.g., spermatogonia, spermatocytes, spermatids, and spermatozoa), activity of the epididymis, and the presence and relative proportion of spermatozoa within the epididymis. In slightly autolyzed tissues, some areas of the basement membrane detached from the germinal epithelium. As Neimanis (1996) reported the diameter of the tubules in slightly autolyzed harbor porpoise (*Phocoena phocoena*) testes did not differ significantly from fresh testes, 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 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. 1993; 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. (1993), 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 categorized into maturity stages based on the earlier studies of Kasuya and Marsh (1984), Desportes et al. (1993), and Kasuya and Tai (1993), and the proportion of seminiferous tubules that were mature: immature = 0% of the tubules mature; maturing = both immature and mature tubules found, less than 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, normalizing 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 millimeters (Hohn et al. 1985; Desportes et al. 1993). This index variable was then included in a data set 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 diameter of the seminiferous tubules). 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.

We modeled progression through the three maturity stages with Bayesian cumulative logit models fit with the “brms” package (Bürkner 2017) for R (R Development Core Team 2018). We wished to compare the utility of each of the individual-level variables for predicting maturity stage, so 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 modeled 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 (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 summarized 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. We refit models 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 data sets; namely, age versus TBL ($n = 90$) and mean diameter of the seminiferous tubules versus 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, we fit two models: 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, we also tested whether the mean diameter of the seminiferous tubules differed among months of the year using univariate analysis of variance (ANOVA).

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 nonreproductive individuals (i.e., immature; Table 2). Two of the individuals identified as reproductive (based on the presence of spermatozoa) were too autolyzed to

Table 2.—Mean (SD), range, and number of samples obtained for each variable (total body length [TBL], age, combined testes weight, combined testes length, an index of testicular development [index], and seminiferous tubule diameter) for each stage of sexual maturation in male long-finned pilot whales (*Globicephala melas edwardii*) stranded on the New Zealand coast, 2010–2017. Note: two males were identified as reproductive (based on the presence of spermatozoa) but were too autolyzed to permit further classification as maturing or mature; parameters for these two individuals are included in the totals only.

Stages	<i>n</i>	TBL (cm)	Age (years)	Combined testes weight (g)	Combined testes length (mm)	Index (g/mm)	Seminiferous tubule diameter (μm)
Immature	65	337 (82) 184–482 (<i>n</i> = 65)	4.9 (4.0) 0–13.5 (<i>n</i> = 62)	117 (121) 15–515 (<i>n</i> = 48)	259 (102) 129–570 (<i>n</i> = 60)	0.42 (0.30) 0.09–1.26 (<i>n</i> = 48)	42.1 (9.7) 29.2–75.3 (<i>n</i> = 59)
Maturing	5	456 (30) 420–490 (<i>n</i> = 5)	12.8 (1.3) 12–15 (<i>n</i> = 5)	1,530 (1,188) 790–2,900 (<i>n</i> = 3)	595 (187) 419–900 (<i>n</i> = 5)	2.63 (1.70) 1.54–4.58 (<i>n</i> = 3)	102.1 (26.8) 66.2–137.8 (<i>n</i> = 5)
Mature	26	518 (36) 450–573 (<i>n</i> = 26)	17.0 (3.4) 11–25 (<i>n</i> = 23)	7,606 (3,669) 3,000–13,020 (<i>n</i> = 8)	963 (146) 645–1,340 (<i>n</i> = 21)	7.94 (3.16) 4.09–12.50 (<i>n</i> = 8)	179.5 (29.3) 126.4–236.4 (<i>n</i> = 15)
Total	98	393 (106) 184–573 (<i>n</i> = 98)	8.5 (6.5) (0–25) (<i>n</i> = 92)	1,204 (2,885) 15–13,020 (<i>n</i> = 59)	450 (325) 129–1,340 (<i>n</i> = 86)	1.55 (2.85) 0.09–12.50 (<i>n</i> = 59)	72.0 (56.7) 29.2–236.4 (<i>n</i> = 79)

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, and mature). Descriptions of the histological appearance of the testis and epididymis at each reproductive stage are given below.

Immature testes (Figs. 1A and 1B; *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 to 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 (Figs. 1C and 1D; *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, and ranged from 66.2 to 137.8 μm (\bar{x} = 102.1).

In mature testes (Figs. 1E and 1F; *n* = 26), all cell types involved in spermatogenesis were observed in the tubules. Full testicular activity is characterized 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 epididymal ducts were 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; Fig. 2). Although males ranged in age from 0 to 25 years, 21% of the sample for which age was estimated comprised individuals younger than 2 years old, and 54% younger than 6 years old. The high proportion of young males in the sample reflects the composition of the stranded groups, as most mass stranded groups were composed primarily of sexually immature males (Fig. 3). Age and TBL increased with maturity stage, though there was some overlap in values between stages (Table 2; Fig. 4). Between 11 and 13.5 years of age and length classes 450 to 480 cm, both nonreproductive (immature) and reproductive (maturing and mature) male pilot whales were observed. The estimated average age and length of the onset of maturity (i.e., transition from immature to maturing) was 12.2 years and 454 cm, respectively (Table 3). The estimated average age and length at attainment of full sexual maturity for males was 13.5 years and 472 cm, respectively (Table 3; Fig. 5). 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.—All demographic and testicular variables were highly positively correlated (Fig. 4). The testicular variables (i.e., combined testes weight, combined testes length, index of testicular development, and mean seminiferous tubule diameter) followed the general pattern of increasing size with maturity stage, although, as with the demographic variables, there was some overlap between maturity stages (Table 2; Fig. 4). A sharp increase in combined testes length was observed at approximately 13 years of age and 470 cm TBL, although 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 (Fig. 4). At approximately 11 years of age and 450 cm TBL, a rapid increase in combined testes weight was observed (see Fig. 4). Sexually

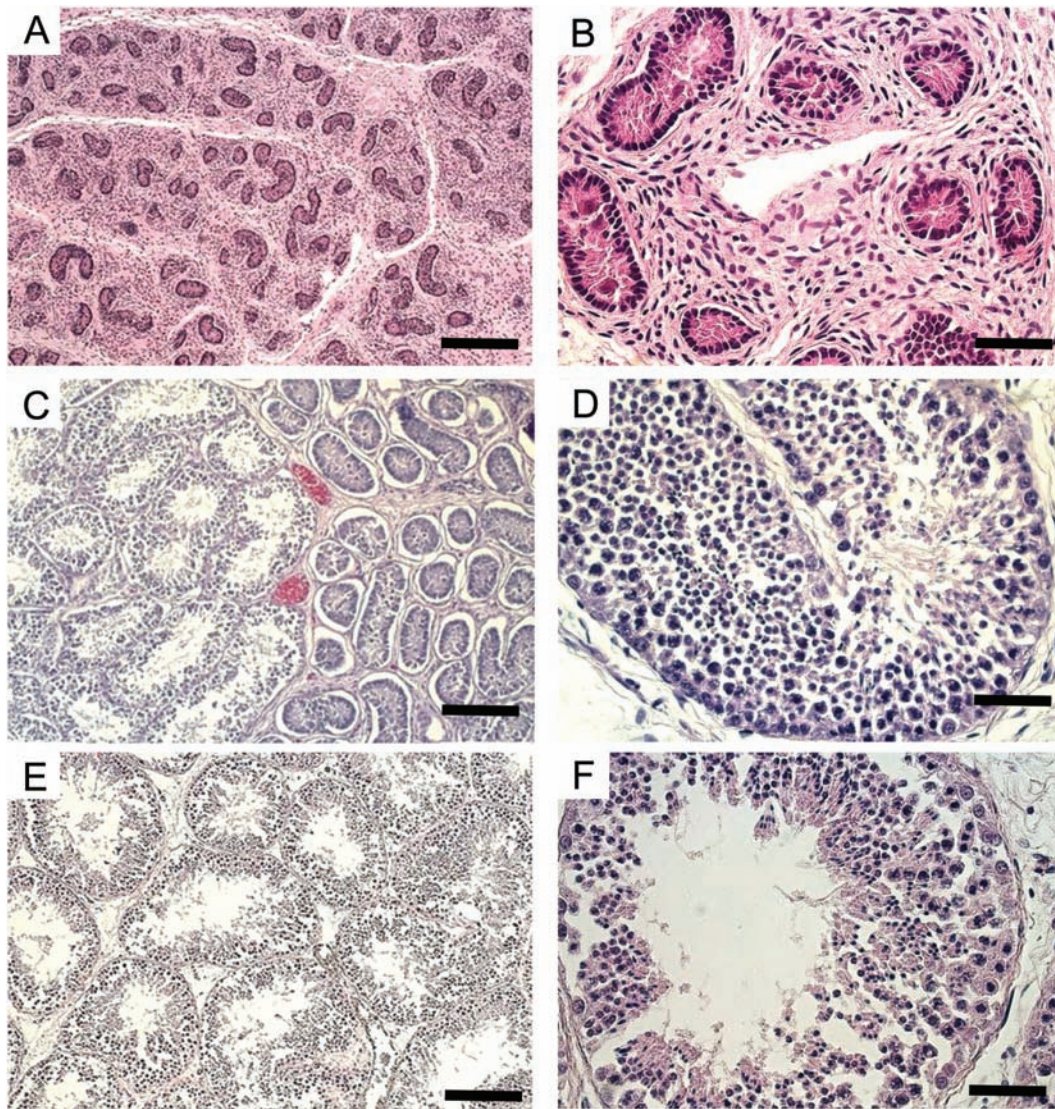


Fig. 1.—Histological appearance of immature, maturing, and mature testes of long-finned pilot whales (*Globicephala melas edwardii*) sampled from mass strandings on the New Zealand coast, 2010–2017. (A) and (B) Immature neonate (GM241; age 0 years, TBL 213 cm; combined testes weight 17.7 g, mean seminiferous tubule diameter 32.6 μ m) showing abundant interstitial tissue, neatly aligned Sertoli cells lining the tubules, and spermatogonia the only germinal cells present. (C) Early-maturing male (GM530; age 12 years, TBL 420 cm, combined testes weight 900 g, mean seminiferous tubule diameter 90.2 μ m, 27% tubules mature) showing adjacent immature and mature zones (note the immature tubules have detached from the basement membrane as a result of autolysis). (D) Early-maturing male (GM530) mature zone showing a multilayered seminiferous epithelium with all cell types involved in spermatogenesis, including spermatozoa. (E) and (F) Mature and very reproductively active male (GM475; age 15 years, TBL 527 cm, combined testes weight 13,020 g, mean seminiferous tubule diameter 236.4 μ m) showing little interstitial tissue and multilayered seminiferous epithelium with all cell types involved in spermatogenesis, including spermatozoa, and a well-developed lumen. Scale bar is 200 μ m in (A), (C), and (E) and 50 μ m in (B), (D), and (F).

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 (Fig. 4). 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. We 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 a mean seminiferous tubule diameter of 125.3 μ m (Table 3; Fig. 5). Of all the modeled 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 3). All testicular variables were better indicators of maturity stage than age or TBL (Table 3).

Reproductive seasonality.—Most MSEs sampled in this study occurred during the first quarter (austral summer period; Fig. 3), which is the peak stranding season for the species in New Zealand (Brabyn 1991; Betty 2019). No mature males were included in the study from the austral autumn or winter seasons (March–August). The largest male sampled during

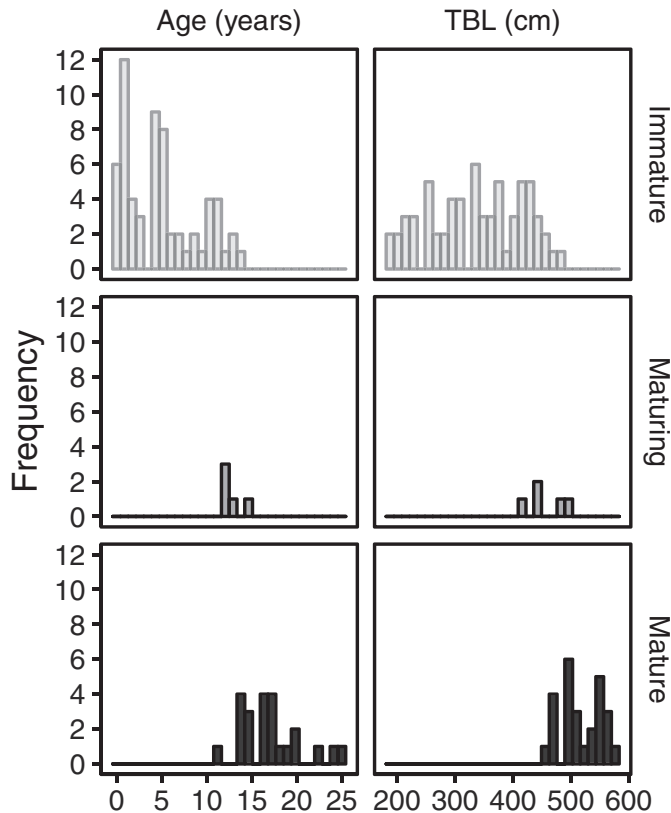


Fig. 2.—Age ($n = 92$) and total body length (TBL; $n = 98$) frequency distributions of immature, maturing, and mature male long-finned pilot whales (*Globicephala melas edwardii*) sampled from mass stranding events on the New Zealand coast between 2010 and 2017.

this period (TBL 450 cm, sampled in June, austral winter period) was identified as reproductive based on the presence of spermatozoa, but was too autolyzed to permit further classification into maturing or mature categories and thus was not included in the statistical analysis. Spermiogenesis 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 recorded in all months sampled, and there was no significant difference in the mean seminiferous tubule diameters of mature males among months (ANOVA, $F_{3,11} = 0.82$, $P > 0.05$; [Supplementary Data SD1](#)).

DISCUSSION

This study describes male sexual maturation in the Southern Hemisphere subspecies of the long-finned pilot whale and evaluates the utility of various testicular and demographic variables as indicators of sexual maturation in the subspecies. 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. \(1993\)](#). 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 characterized 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 short-finned ([Kasuya and Marsh 1984](#)) and long-finned pilot whale (*G. m. melas*—[Desportes et al. 1993](#)). 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. 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. \(1993\)](#) and short-finned pilot whales by [Kasuya and Marsh \(1984\)](#). A rapid increase in testicular size (e.g., length and 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 [Fig. 4](#)). [Desportes et al. \(1993\)](#) reported an apparent extended male maturing stage in *G. m. melas*, with individuals ranging in age from 11 to 22 years, compared to 11 to 15 years in *G. m. edwardii*. The low proportion of maturing *G. m. edwardii* 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, we consider it to be unlikely that maturing animals are under-represented in the current study based on the observed age (11–15 years) and length class (420–490 cm) ranges (see [Fig. 2](#)). 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 long-finned pilot whales ([Desportes et al. 1993](#)), and it has also been suggested for short-finned pilot whales ([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. 1993](#)). Future sampling efforts should 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.

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

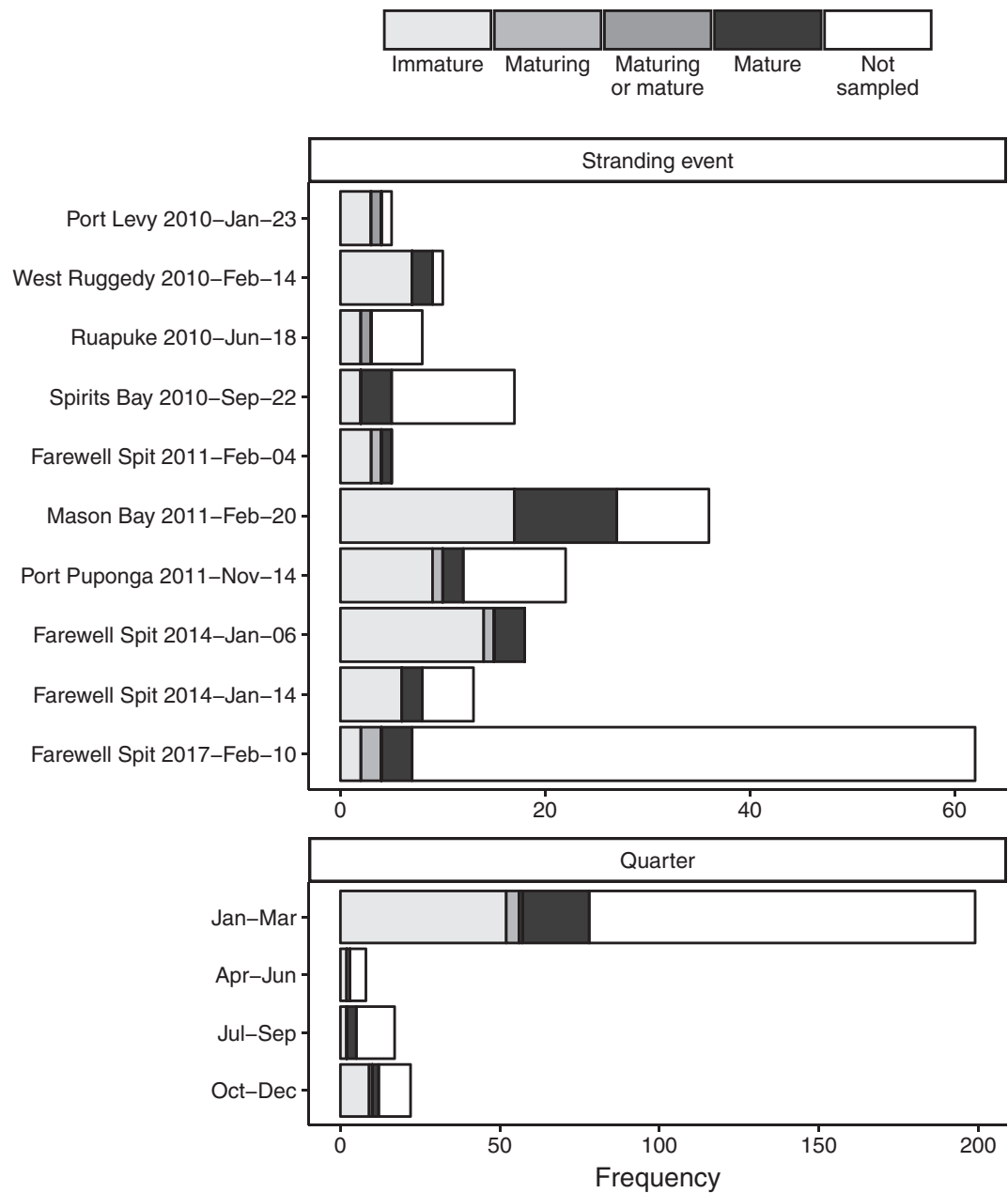


Fig. 3.—Number of male long-finned pilot whale (*Globicephala melas edwardii*) gonads collected from mass stranding events on the New Zealand coast between 2010 and 2017, by stranding event, and quarter (Total, $n = 98$; Quarter 1: Jan–Mar, $n = 78$; 2: Apr–Jun, $n = 3$; 3: Jul–Sept, $n = 5$; 4: Oct–Dec, $n = 12$).

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. 1993) 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 h postmortem) for histological examination is challenging (Kemper et al. 2014). Thus, there is a need for the development of nonhistological 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 whale studies (e.g., Desportes et al. 1993). Age tended to be 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. 1993) 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. (1993) to be the best nonhistological indicator of sexual maturity in *G. m. melas*, overlapped with both the immature and mature categories.

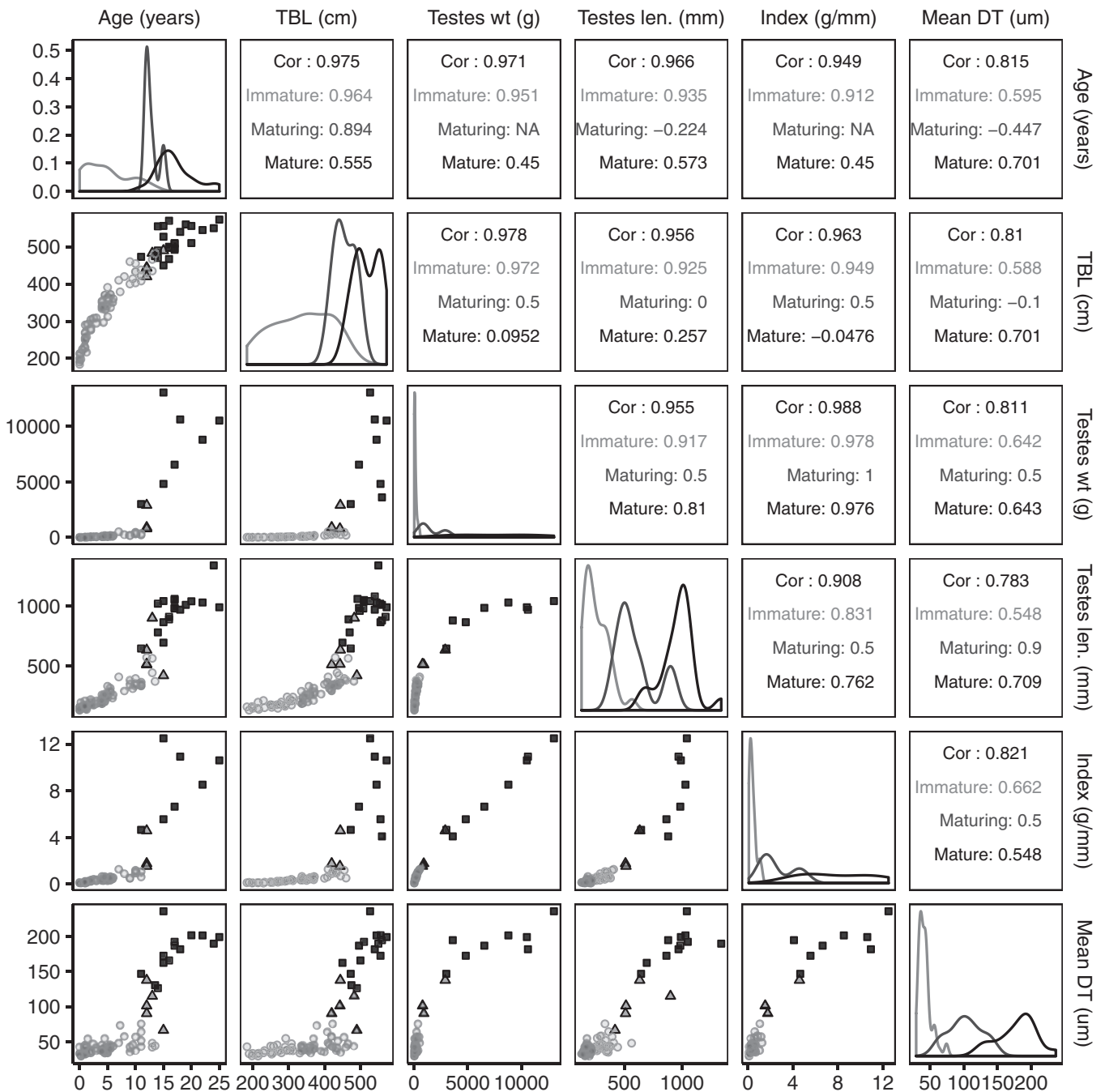


Fig. 4.—Demographic variables (age and total body length [TBL]) versus testicular variables (combined testes weight [age: $n = 57$, TBL: $n = 59$], combined testes length [age: $n = 80$, TBL: $n = 86$], index of testicular development [age: $n = 57$, TBL: $n = 59$] and mean diameter of seminiferous tubules [mean DT; age: $n = 76$, TBL: $n = 79$]) for male long-finned pilot whales (*Globicephala melas edwardii*) stranded on the New Zealand coast, 2010–2017. Point types and colors represent the individuals' sexual maturity stage: immature = light gray circle, maturing = medium gray triangle, mature = black square. The lower-left panels show scatterplots for each pair of variables. The panels along the diagonal are kernel density estimates for each variable in each of the three stages of maturity. The upper-right panels show the Spearman's rank correlation coefficients for each pair of variables, across all data ("Cor") and within each maturity stage, as indicated.

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 nonhistological 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*.

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

Table 3.—Estimates of the average age, total body length (TBL), combined testes weight, combined testes length, index of testicular development (index), and mean diameter of seminiferous tubules (mean DT) of male long-finned pilot whales (*Globicephala melas edwardii*) at the attainment of the two stages of sexual maturity (maturing and mature). Calculated using Bayesian cumulative logit regression. *CrI* = credible interval; LOOIC = Leave-One-Out Information Criterion (all variables together; *n* = 55); *SE* = standard error. Data were collected from mass stranding events on the New Zealand coast between 2010 and 2017.

Indicator	Maturing mean (95% <i>CrI</i>)	Mature mean (95% <i>CrI</i>)	LOOIC (<i>SE</i>)
Age (years)	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)

G. m. melas off the Faroe Islands (Desportes et al. 1993). The maturing stage was attained, on average, approximately 2 years earlier (at an ASM of 12.2 versus 13.9 years, and LSM of 454 versus 486 cm), while the mature stage was attained approximately 3 years earlier (at an ASM 13.5 versus 16.8 years, and LSM of 472 versus 516 cm; Desportes et al. 1993; this study). Comparison with other studies of North Atlantic (e.g., Sergeant 1962; Martin et al. 1987; Sigurjonsson et al. 1993) or Southern Hemisphere (Schroder and Castle 1998) long-finned pilot whale populations is difficult since they are based on small sample sizes, or did not attempt to estimate ASM or LSM using comparable methods. Comparisons are possible with studies of the southern and northern forms of the related short-finned pilot whale (Kasuya and Marsh 1984; Kasuya and Tai 1993), however, where correlations of age and body length with sexual maturation were observed. The ASM was reported to occur at approximately 17 years of age in both forms of the short-finned pilot whale, and the LSM was 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 a relative reduction in the size of a population. 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, 1977) and exploitation (e.g., Kasuya 1985) in small delphinids. Long-finned pilot whales around the Faroe Islands have been hunted since at least the 9th century (Sanderson 1992). Catches of long-finned pilot whales 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). 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 stocks, 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 (Wade et al. 2012), and is considered sustainable by the North Atlantic Marine Mammal Commission (NAMMCO 2018). The abundance of long-finned pilot whale population(s) in the Southern Hemisphere is not well understood, but, except possible isolated incidents in the 19th century, during the peak whaling period, *G. m. edwardii* does not have a history of significant exploitation (Reeves et al. 2003).

Possible explanations for nonexploited long-finned pilot whales off New Zealand having a lower ASM and LSM than exploited long-finned pilot whales off the Faroe Islands are: 1) frequent, large MSEs may have a significant impact on the local population of *G. m. edwardii*; 2) favorable 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* (Betty 2019); 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). Long-finned pilot whales 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 a delayed attainment of sexual maturity in some individuals, which may explain the protracted maturing stage (11–22 years) reported in *G. m. melas*. Alternatively, the Faroese sample may have been composed of individuals from multiple stocks, exhibiting different reproductive traits. Pollutant burdens of long-finned pilot whales in New Zealand waters are not well understood, but a preliminary study reported PCB concentrations to be 2 to 3 orders of magnitude lower than in North Atlantic long-finned pilot whales (Schroder and Castle 1998).

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

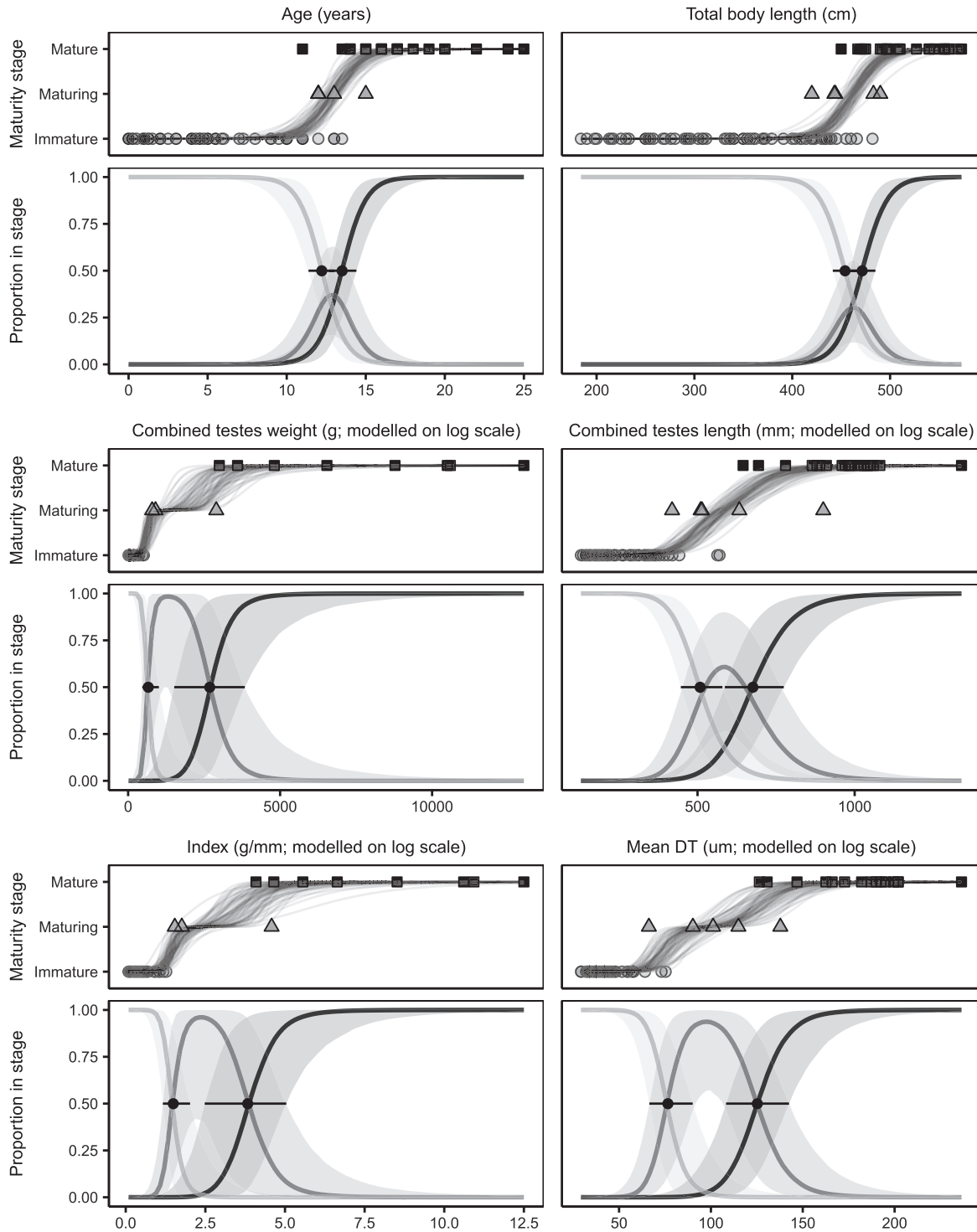


Fig. 5.—Results from Bayesian cumulative logit regression models of the sexual maturation of male long-finned pilot whales (*Globicephala melas edwardii*) through three stages (immature, maturing, and fully mature) as a function of one of six individual measures: age, total body length, combined testes 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. For each of the six variables, the upper panel shows the data values observed within each stage, with lines representing posterior predictions of the mean maturity stage. On the lower panel, the thick lines show the estimated mean proportion of whales in each of the three maturity stages across the observed values of the predictor variables, with ribbons showing 95% credible intervals for the means (the three maturity stages are indicated by increasingly dark lines and ribbons, going left to right). The black points and thin horizontal lines on the lower plots 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 fit to the log-transformed testicular variables, as indicated. Data were collected from mass stranding events on the New Zealand coast between 2010 and 2017.

to the seasonally limited sampling of MSEs (austral spring and summer; see [Supplementary Data SD1](#)), which occurred during the proposed peak mating period ([Betty 2019](#)). 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. 1993](#)). However, 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. 1993](#)).

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](#)). Sexual bimaturism was observed in *G. m. edwardii* off New Zealand where females attained sexual maturity 6.8 years, on average, earlier than males ([Betty 2019](#); this study). Physical maturity is not obtained until well after sexual maturity in *G. m. edwardii* (asymptotic TBL 570 cm and age > 40 years—[Betty 2019](#)), and a pattern of protracted growth is suggested, similar to that reported for *G. m. melas* off the Faroe Islands ([Bloch et al. 1993](#)). 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](#)). Whereas in Atlantic spotted dolphins (*Stenella frontalis*), reproductive success appears to be based on social standing, or attainment of a 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](#)).

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 non-copulatory traits (e.g., body size, armaments, and ornaments) and copulatory traits (e.g., testis size and spermatogenic efficiency—[Dines et al. 2015](#)). If males can monopolize access to females, they will place greater investment in traits that are used for defence or aggressive interactions ([Parker et al. 2013](#); [Lüpold et al. 2014](#); [Dines et al. 2015](#)). Thus, it has been suggested that selection for different types of sexual traits can indicate the mating system of a species for which observations of mating behavior are rare ([MacLeod 2010](#); [Dines et al. 2015](#)). Long-finned pilot whales 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. 1993](#)). Prominent noncopulatory 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](#); [Betty 2019](#)). Most authors assumed that long-finned pilot whales are polygynous, drawing attention to the sexual dimorphism observed in the species (e.g., [Sergeant 1962](#); [Evans 1987](#)). However, there is limited evidence for male-male combat in pilot whales; “their dimorphic characters appear to function as ornaments, rather than armaments” ([Dines et al. 2015](#):1568). After taking into account body mass, the long-finned pilot whale has the fourth largest residual testes mass among cetacean species ([MacLeod 2010](#)). The lack of a trade-off with testis size suggests that male pilot whales are not able to monopolize access to females to the same extent as those species with male-male combat, and therefore also need to invest in traits such as larger testis size ([Dines et al. 2015](#)), indicating that sperm competition is of importance within its mating strategy ([MacLeod 2010](#)).

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 in an almost linear relationship with TBL in mature individuals ([Fig. 4](#)). 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 reproductive organs, as previously reported for *G. m. melas* by [Desportes et al. \(1993\)](#) and short-finned pilot whales by [Kasuya and Marsh \(1984\)](#). This is in contrast to what has been suggested for some mammalian 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 short-finned pilot whales ([Kasuya and Marsh 1984](#)).

Although *G. m. edwardii* attains 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 is 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 length 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. 1993](#)). 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 ca. 560-cm male *G. m. melas* (Desportes et al. 1993).

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 unhealthy 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 versus 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 differences in mating strategy between the two subspecies, with *G. m. edwardii* investing more in sperm competition, 2) *G. m. edwardii* is not as resource-limited as *G. m. melas*, 3) *G. m. edwardii* suffers less from disease, or 4) *G. m. edwardii* has lower exposure to endocrine disrupting chemicals, and thus can invest more heavily in reproduction.

In summary, 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 behavioral observations of individuals are needed to confirm mating strategies, and when social maturity is attained in *G. m. edwardii* of New Zealand.

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SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—(A) Combined testes weight versus Julian date ($n = 11$), and (B) mean seminiferous tubule diameter versus Julian date ($n = 20$) of maturing and mature male long-finned pilot whale (*Globicephala melas edwardii*) specimens collected from mass stranding events on the New Zealand coast between 2010 and 2017. Point types represent the individuals' sexual maturity stage: maturing = triangle, mature = square.

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