Short Note

A Mass Stranding Event of Long-Finned Pilot Whales (*Globicephala melas*) in Southern Chile

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Cetacean strandings have historically been of great interest for scientists because they provide opportunities to study typically inaccessible marine species and supply information on their spatiotemporal distribution (Maldini et al., 2005), biology (Fernández & Hohn, 1998; Thayer et al., 2003), population ecology (Santos et al., 2014), and health (Fernández et al., 2008; Peltier et al., 2013). Occasionally, data collected from cetacean strandings can reveal the anthropogenic or natural causes of the mortality event (e.g., Evans et al., 2005; Johnston et al., 2012; Peltier et al., 2013).

Every year, hundreds of marine mammals strand (Servicio Nacional de Pesca [Sernapesca], 2018) along the 8,000-km coastline of Chile (Andrade, 1991), much of which is composed of isolated, high latitude islands and fjords that are difficult to access. The long-finned pilot whale (*Globicephala melas*, hereafter LFPW) has been recorded along much of the Chilean coast from the north in Iquique (20° 12' S to 79° 8' W) to the extreme south in Navarino Island (55° 15' S to 67° 30' W), including the island of San Ambrosio (26° 20' S to 79° 53' W; Aguayo-Lobo et al., 1998). This vast and remote area poses problems for

stranding response since rapid collection of data from these events is crucial to determine the cause of stranding or death. To reach as many strandings as possible, the National Fisheries Service of Chile (Sernapesca), the Chilean Navy, and certain research-based conservation organizations (e.g., universities and nonprofit organizations) share stranding response jurisdiction in the country. Despite these efforts, numerous events are not rapidly studied, and many probably go unreported.

The LFPW is a widely distributed cetacean which exhibits the largest number of reported mass strandings worldwide (Olson, 2018). In some cases, strandings involve what appear to be healthy animals (Olson, 2018). However, the causes behind such strandings are often undetermined, even when fresh specimens are available for investigation (Moore et al., 2018). The majority of strandings, however, are likely to present decomposed individuals, making the cause of death even more difficult to establish (Kemper et al., 2005).

Historically, identification of beached LFPW has been based on body size and specific skin coloration patterns (e.g., saddle patch located posterior to the dorsal fin, postorbital eye blaze, and

anchor-shaped throat patch; Sergeant, 1962; Bloch et al., 1993). Sometimes the level of decomposition of stranded animals makes it difficult to confirm the species, thus LFPW could be confused with its congener *G. macrorhynchus* (the short-finned pilot whale, hereafter SFPW). Although both *Globicephala* species are documented in Chile, the distributions of the two species have not been fully established, in part because of the difficulties in identifying free-ranging individuals when observed in the field. It has been suggested that their distribution overlaps in the north-center of the country (between 20 to 33° S): SFPW is located at lower latitudes of Chile between Arica and Valparaiso, and LFPW is present in both pelagic and coastal areas throughout the country (Sanino & Yáñez, 2001).

Previous to this report, only four LFPW group stranding events have been reported in Chile. Venegas & Sielfeld (1980) reported the first mass stranding of 125 LFPW individuals in Windhond Bay in Navarino Island, Magallanes Region (55° 15' S to 67° 30' W) in 1979. The second occurred in Posesión Bay in the Strait of Magellan (52° 17' S to 69° 13' W) in August 1982 and involved 61 individuals (Mansilla et al., 2012). Finally, 13 and 45 individuals stranded in the Beagle Channel (54° 56' S to 67° 15' W) in 2006 and 2013, respectively (Mansilla et al., 2012). In addition, some LFPW strandings involving one or two individuals have been reported to beach sporadically along the Chilean coast (Table 1). Herein, we report a recent LFPW mass stranding in Chile, the largest to date, and use molecular tools to confirm the species identity and sex of stranded individuals.

On 18 July 2016, local fishermen filmed dozens of stranded cetaceans on a beach on the northwest coast of Clemente Island (45° 35' S to 74° 34' W) in the Chonos Archipelago of southern Chile (Figure 1A & B). This is a remote area composed of fjords and channels with only a few human settlements in a 200-km radius. A sandy beach of 900 m in length is located on the northwest part of the island with a low slope of approximately 3°. Based on an aerial inspection on 19 July, and a first short visit on 24 July to the stranding event, at least 100 cetaceans were confirmed to be dead. On 24 August, a 2-day expedition to the site confirmed the stranding of 124 medium-sized cetaceans in an advanced state of decomposition.

Multi-angle and drone photographs of all individuals were taken and georeferenced to document their positions on the beach. Positions were plotted using *QGIS* software (Quantum GIS Development Team, 2017; Figure 1C). Due to the advanced state of decomposition, the degree of disarticulation, and the position of carcasses (some buried in the sand), only 78 individuals were suitable for examination and sampling. For each individual, we (1) conducted an external examination to evaluate body condition (following Pugliares-Bonner et al., 2007) and document evidence of trauma by fishery bycatch and/or vessel collision (i.e., muscle or skin injuries, propeller marks, and/or net scars); (2) measured total length: straight line measured from the tip of the rostrum to end of the tail (± 1 cm; Norris, 1961; Figure 1D); (3) counted the number of mandibular teeth (*n* = 48 animals); (4) sampled tissues for sex identification (following Pugliares-Bonner et al., 2007); and (5) estimated age class based on the length at which LFPW reach sexual maturity: approximately 3.7 m at 6 to 8 years of age in females, and 4.9 m at 11 years in males

Table 1. Historical strandings of long-finned pilot whales (*Globicephala melas*) reported in Chile; sites, dates, localities, geographical coordinates, and number (*N*) of stranded individuals.

| Date	Locality	Coordinates	*N*	Reference
March 1979	Windhond Bay, Navarino Island	55° 15' S–67° 30' W	125	Venegas & Sielfeld, 1978
August 1982	Posesión Bay, Strait of Magellan	52° 17' S–69° 13' W	61	Mansilla et al., 2012
January 1999	Chañaral de Aceituno	29° 4' W–71° 29' W	2	Sanino & Yáñez, 2001
August 2006	Holger Island, Beagle Channel	54° 56' S–67° 15' W	13	Mansilla et al., 2012
January 2009	Carelmapu	41° 44' 36.2" S–73° 43' 43" W	1	Sernapesca, 2018
August 2009	Tocopilla	22° 5' 2.6" S–70° 11' 48.6" W	1	Sernapesca, 2018
May 2010	La Ligua	32° 20' 34" S–71° 27' 18.1" W	1	Sernapesca, 2018
February 2013	Strait of Magellan	53° 28' 51" S–70° 46' 60" W	45	Sernapesca, 2018
March 2015	Los Vilos	32° 14' 22" S–71° 30' 42.1" W	1	Sernapesca, 2018
April 2015	Ancud Bay, Chiloe Island	41° 52' 44" S–73° 52' 32.6" W	1	Sernapesca, 2018
July 2016	Clemente Island, Chonos Archipelado	45° 35' 58.4" S–74° 34' 35.4" W	124	This report
February 2018	Iloca	34° 56' 13" S–72° 10' 59" W	1	Sernapesca, 2018
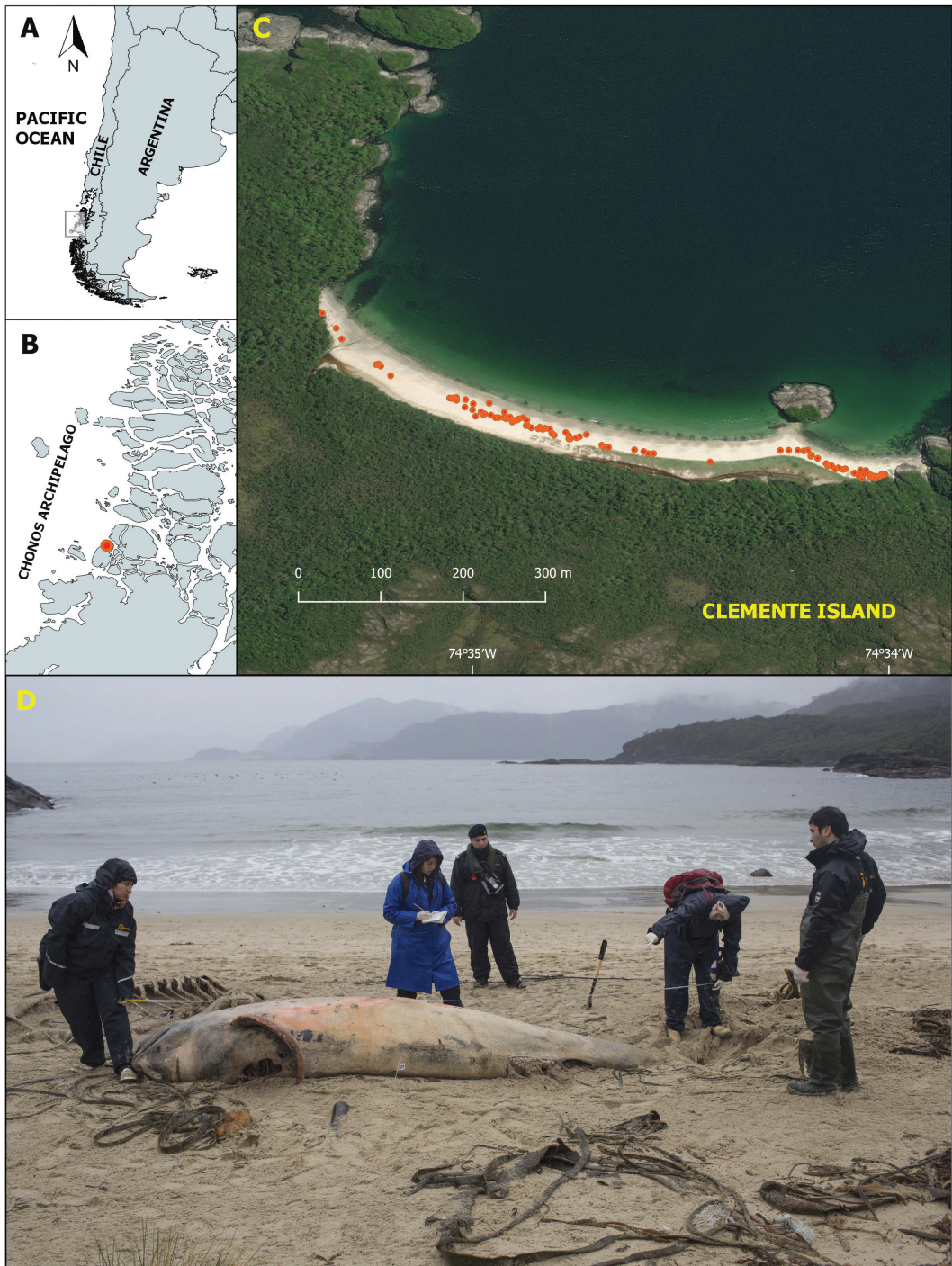
Figure 1. (A) Map of southern South America showing the location of a mass stranding of a long-finned pilot whale (*Globicephala mela*s) that occurred in 2016 on (B) Clemente Island, Chonos Archipelago, southern Chile (red dot). (C) Position of each of the 124 stranded individuals on the beach (red dots); and (D) field work obtaining morphological measurements of a dead *G. melas* individual.

(Sergeant, 1962). Calves are born with an average body length of 1.75 m and grow rapidly during the first 2 years of lactation. Therefore, individuals with total body length < 3 m were considered to be calves (Sergeant, 1962).

We initially identified all individuals as LFPW based on morphological measurements and color patterns of the skin (Sergeant, 1962; Bloch et al., 1993). However, due to the advanced state of decomposition, only a subset of animals were skin sampled (*n* = 52) for molecular confirmation of the species and sex. Skin samples (~1 g) were obtained by scalpel using a standard sterile technique (Pugliares-Bonner et al., 2007). Samples were then fixed in 95% ethanol and stored at 4°C. DNA was extracted from approximately 25 mg of each sample using a genomic DNA minikit (Geneaid®, New Taipei, Taiwan) following manufacturer's instructions. A phylogenetic reconstruction was carried out with one stranded LFPW to confirm species identity. A fragment of the 16S rRNA was amplified using the universal primers 16Sar/br (Palumbi et al., 1991). Amplicons were purified and sequenced at Macrogen (South Korea). Both sequence directions were determined, using the individual primers from the original reaction.

The DNA sequence was edited using *Proseq*, Version 2.91 (Filatov, 2002). The new sequence has been deposited in GenBank under Accession Number MF600311. Sequences from other odontocete relatives were obtained from GenBank (JF339972-82) for phylogenetic comparisons. All nucleotide sequences were aligned using *MAFFT*, Version 7 (Katoh & Standley, 2013) under the iterative method of global pairwise alignment (G-INS-I; Katoh et al., 2005), and default settings were used for all parameters involved. Phylogenetic reconstruction was performed using the criteria of Maximum Likelihood (ML) and Bayesian Inference (BI). The model of nucleotide substitution was selected using Bayesian Information Criterion (BIC) implemented in *PartitionFinder*, Version 1.1.0 (Lanfear et al., 2012), applying the greedy algorithm, and ML was performed using *GARLI*, Version 2.0 (Bazinet et al., 2014). Statistical support for the nodes was estimated by the nonparametric bootstrap (BS; Felsenstein, 1985) with 1,000 replicates. Bayesian analysis was performed with *MrBayes*, Version 3.2.4 (Ronquist et al., 2012). Markov chains were started from a random tree and run for 10^7 generations, sampling every 1,000 generations. The stationary phase was checked following Nylander et al. (2004) using *Tracer*, Version 1.5 (Rambaut & Drummond, 2007). Sample points prior to the plateau phase were discarded as burn-in, and the remaining trees were combined to find the maximum *a posteriori* estimated probability of the phylogeny. Branch support was estimated by Bayesian Posterior Probabilities (PP).

Sex of individuals was determined using a duplex PCR of two primer pairs: one that amplifies a ~400 bp portion of the ZFX/ZFY gene (present in both sex chromosomes) and one that amplifies a ~200 bp portion of the SRY gene (only present in the Y-chromosome). For this purpose, the F 5′ CCC ATG AAC GCA TTC ATT GTG TGG 3′ and R 5′ ATT TTA GCC TTC CGA CGA GGT CGA TA 3′ primers were used to amplify a segment of the SRY gene (Augusto et al., 2013), and the F 5′ATA ATC ACA TGG AGA GCC ACA AGC T 3′ and R 5′ GCA CTT CTT TGG TAT CTG AGA AAG T 3′ primers were used to amplify a segment of the ZFX/ZFY gene (Aasen & Medrano, 1990). All PCR products were scored on 1.2% agarose gels stained with SYBR® Safe DNA and photographed under a blue-light transilluminator (Invitrogen).

Stranded animals had an average total length of 4.06 m (adult male = 5.25 ± 0.33 m; adult female = 4.13 ± 0.38 m); age composition was 24.4% adults (*n* = 19), 24.4% juveniles (*n* = 19), 11.5% calves (*n* = 9), and 39.7% could not be categorized (*n* = 31) (Table 2). The dental formula was on average ten mandibular teeth (range: 8 to 12), which is within the range of nine to 12 teeth described for LFPW (Olson, 2018); however, four animals had only eight teeth (three of undetermined age and one adult). One calf presented only three well-defined mandible teeth (specimen 61 in Table S1; Table S1 is available in the Supplementary Material section of

Table 2. Age class, number of animals (*N*), total length (m), and number of mandibular teeth (range) of examined stranded LFPW on Clemente island, Southern Chile, 2016

Age class	*N*	Average total length (m) ± SD	Mode of mandibular teeth (range)
Calf	9	2.55 ± 0.30	10 (3-11)
Juvenile	19	3.68 ± 0.57	10 (8-12)
Adult	19	4.84 ± 0.70	11 (8-12)
Undetermined	31	4.25 ± 0.30	10 (8-11)
Total	78	4.06 ± 0.84	10 (3-12)

the *Aquatic Mammals* website: https://www.aquatic
mammalsjournal.org/index.php?option=com_
content&view=article&id=10&Itemid=147).

No carcasses of other species (e.g., other ceta-
cean, pinnipeds, birds, or fishes) were observed in
this stranding event. Most of the animals exam-
ined were highly decayed and, therefore, clas-
sified in Categories 4 ($N = 57$) and 5 ($N = 21$),
according to Pugliares et al. (2007). As a conse-
quence, full necropsies could not be performed.
Carcasses in Category 4 were characterized by
completely discolored skin; presence of abundant
postmortem marks caused by birds; strong odor,
with most individuals presenting liquefied blub-
ber; autolysis of internal organs; and sand within
the abdomen. Individuals in Category 5 had whole
skeletons covered by dry skin and scarce tissue
remains. The external examination of 78 animals
did not show signs of anthropogenic interven-
tion (e.g., marks of cuts, entanglement, or colli-
sion with vessels). Due to the advanced stages of
decomposition, specific tests for common marine
mammal infectious diseases were not feasible
(e.g., *Morbillivirus* and *Brucella* spp.). One of
the examined individuals in which a partial nec-
ropsy was performed revealed a pregnant female
with a fetus of 153 cm total length. LFPW calves
are born with a mean length of 145 to 175 cm
(Sergeant, 1962), so it is estimated that the fetus
was in the last third of its gestation. These results
confirm LFPW birth events occurring in Chile
in summer-autumn as reported in the Northern
Hemisphere (Olson, 2018).

Examined animals were lying in left lateral
position ($n = 27$), right lateral position ($n = 33$),
ventral position ($n = 10$), or were buried ($n = 8$).
Carcasses were found in various orientations along
the shoreline, which made it impossible to deter-
mine if animals stranded alive or dead. However,
the low variation in decomposition stages of the
animals and the limited area of the event are consis-
tent with a synchronous mass stranding. The LFPW
decomposition state indicated a *postmortem* inter-
val-since-death of less than 6 months (Liebig et al.,
2003, 2007; Häussermann et al., 2017). Therefore,
we estimated the stranding happened 3 to 4 months
before the arrival of our first expedition.

Given this stranding window between April
and May 2016, we systematically reviewed poten-
tial causes of this mass stranding. The LFPW is
well known for its highly cohesive behavior
(Ottensmeyer & Whitehead, 2003; Visser et al.,
2014), potentially contributing to its propensity
for mass stranding. In the reports of most strand-
ings, LFPW have no known pathology, and it
is not understood why apparently healthy ani-
mals strand together (Moore et al., 2018; Olson,
2018). However, in some cases, pathogens (e.g.,

Morbillivirus) have been reported to be enzootic
in LFPW (Van Bressem et al., 2001), and strand-
ings associated with disease outbreaks have
also been described (Fernández et al., 2008, and
refences therein). The carcasses from this report
were too degraded to establish pathology, empha-
sizing the need for more rapid sampling.

Due to the extensive decomposition of the car-
casses, we found no definitive cause of mortality
in this event. Also, we found no evidence of direct
human interaction. It is worth noting, however, that
during the time frame of this stranding, there were
several large marine storms in the region with sub-
stantial swells (3 to 4 m) and winds (40 kts). Mass
strandings are frequently the result of a sequence
of events; therefore, it is plausible that a combi-
nation of entrapment in regionally strong tides
(differences between tides is 7 m; Fierro, 2008)
and adverse climatic conditions (Irvine et al.,
1979; Venegas & Sielfeld, 1980; Sigurjónsson
et al., 1993; Raghunathan et al., 2013; Huertas &
Lagueux, 2016) contributed to this stranding.

The phylogenetic tree derived from the 16S
(mtDNA) sequences is shown in Figure 2. The
analysis confirmed that the stranded individuals
corresponded to LFPW. The ML and BI analyses
revealed the same tree topology with strong overall
support (PP > 0.95 and BS > 70%; Hillis & Bull,
1993; Huelsenbeck & Rannala, 2004). Our ML
analysis generated a phylogenetic tree (with In L =
-958.93), indicating reciprocal monophyly between
the species LFPW and SFPW (Figure 2). The
close phylogenetic relationship between the two spe-
cies of *Globicephala* has been reported in previ-
ous studies (i.e., LeDuc et al., 1999; Cunha et al.,
2011; McGowen, 2011; Vilstrup et al., 2011), and it
is because these Globicephalinae species were the
most recently diverged (Cunha et al., 2011; Vilstrup
et al., 2011); the genetic distance between these spe-
cies was 0.8%. Similar values have been estimated
in other mitochondrial loci (e.g., Cytochrome b:
LeDuc et al., 1999). The mitochondrial genes (i.e.,
Cyt b and D-loop or 16S) show a high taxonomic
sensitivity in Delphinidae (LeDuc et al., 1999;
Archer et al., 2017). Therefore, they can be used
successfully as diagnostic markers to identify spe-
cies in samples that come from carcasses of ceta-
ceans with an advanced state of decomposition.

Advanced stages of decomposition precluded
sex determination by external inspection, but tissue
from 28 animals successfully amplified the SRY
and ZFX/ZFY genes in which sex could be deter-
mined (see Table S1). The sex ratio on sampled
individuals ($N = 52$) were skewed towards males
(20 animals = five adults, ten juveniles, and five
calves) over females (eight animals = all adults).
Conversely, literature indicates LFPW populations
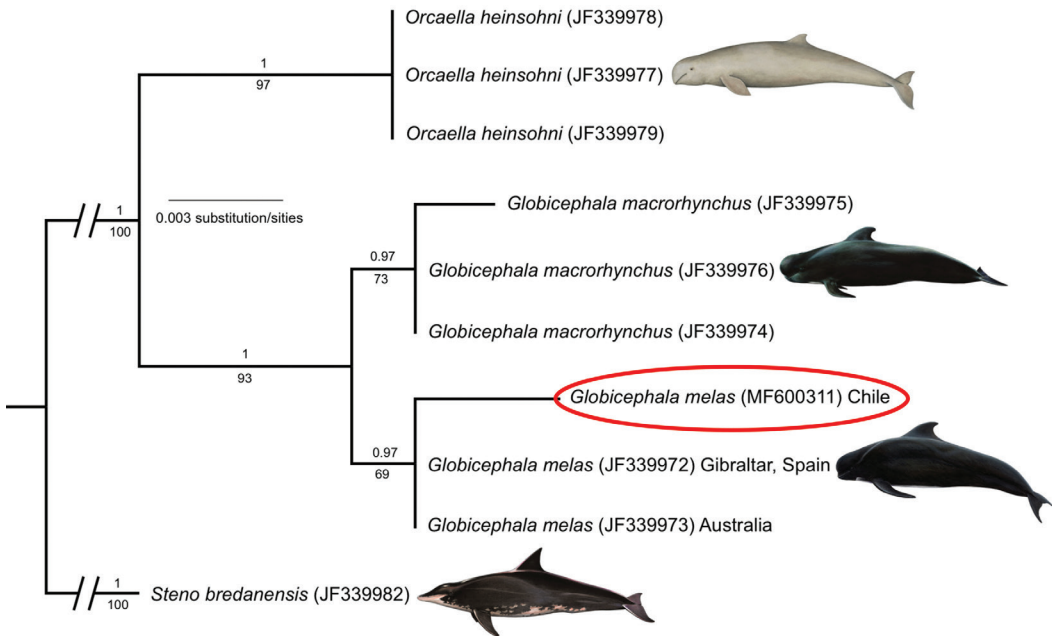are generally skewed towards females (Amos et al.,

Figure 2. Bayesian phylogenetic reconstruction of pilot whales (*Globicephala* spp.) and selected relatives based on analysis of the 16S rRNA dataset (nucleotide substitution model = K80). Bayesian analysis yielded the same topology. Numbers above the branches are Bayesian Posterior Probability/maximum likelihood bootstrap. Numbers at nodes present the posterior probabilities for nodes with values less than 0.9. Tips are labeled with species, GenBank accession numbers, and country of origin (only for *G. melas*).

1993; Ottensmeyer & Whitehead, 2003). Due to tissue degradation, our samples were too limited to establish a sex ratio for the entire group; however, this finding is important as it relates to the population structure of LFPW and other cetaceans.

Adequate management and investigation of marine mammal strandings requires a rapid response. To accomplish this, trained personnel and financial resources (including land, sea, or air transportation), along with coordination to facilitate decisions and authorizations, are all key aspects. For instance, the greatest whale mass mortality recorded to date, in which over 300 sei whales (*Balaenoptera borealis*) stranded in the fjord district of southern Chile, was discovered several months after it occurred (Häussermann et al., 2017). Therefore, the development of a marine mammal sighting and stranding network in Chile, coordinated between government agencies, universities, research centers, nongovernmental organizations, and including the general public, is highly recommended. This may stimulate the allocation of resources towards more effective monitoring and surveillance of these events in Chile, including in remote regions.

Although nearly half of the 89 cetacean species described worldwide can be found inhabiting Chilean waters (Aguayo-Lobo et al., 1998; Viddi et al., 2010), little information is available about stranding events. By utilizing newly developed technologies and improved molecular techniques, we learned valuable information about LFPW; however, with a more rapid response by multidisciplinary teams, we could have learned a great deal more about their population structure and ecology. Rapid, coordinated responses in the future will provide biological information on cryptic marine mammals and can elucidate the causes of strandings and mass mortalities, which are expected to occur more frequently under the current scenario of global change (Azzellino et al., 2017; Nash et al., 2017; Sousa et al., 2019).

Acknowledgments

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