

Aggregation of mummified adult crabeater seals (Pinnipedia: Phocidae) in the eastern Antarctic Peninsula: age and sex structure, taphonomy and cause of death

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Abstract: In Antarctica, crabeater seals tend to strand as immature animals with disorientation, due to their inexperience, given as the probable cause. In 2012 and 2013, we examined a group of 80 mummified crabeater seals on Seymour Island (Marambio). The age and gender of 28 seals was determined, and virology and stomach content analyses were performed in order to determine the cause of stranding. Around 82% of the seals examined were adults and 79% were females, some of which were pregnant. All of the seals sampled tested negative for *Morbillivirus*, suggesting that the stranding was not related to the mass mortality event reported in the 1950s in the region. Most seals had empty stomachs and thin blubber suggesting that they died from starvation. The state of the carcasses suggests multiple stranding events. Most of the seals were located along an ice-covered stream, suggesting that this may act as a 'natural trap', isolating the seals from the open ocean. This is exceptional as it is the first report of mostly adult female seals to strand in Antarctica and refutes the theory that only young animals are prone to stranding.

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Introduction

Marine mammal strandings have occurred for millions of years and mass strandings of marine mammals are documented in contemporary and historical times (Pyenson 2010, 2011, Pyenson et al. 2014). In the arid, desiccating conditions in Antarctica, the low temperatures and precipitation levels combined with strong winds, mummify stranded marine mammals. This has resulted in the preservation of many marine mammal stranding events. The crabeater seal, Lobodon carcinophaga (Hombron & Jacquinot), is the marine mammal species most frequently found stranded in the Antarctic and sub-Antarctic (see Péwé et al. 1959, Barwick & Balham 1967, Dort 1975, 1981, Gordon & Harkness 1992, Nelson et al. 2008, Negrete et al. 2011). Although other species, such as Weddell (Leptonychotes weddellii (Lesson)), leopard (Hydrurga leptonyx (de Blanville)) and southern elephant (Mirounga leonina (L.)) seals, have also been reported mummified, they have been found in far fewer numbers (Péwé et al. 1959, Nichols 1966, Barwick & Balham 1967, Banks et al. 2010). Stirling & Kooyman (1971) proposed that the crabeater seal is more likely to strand and become mummified because they are both more agile and more numerous. Live crabeater seals have been found over a hundred kilometres from the ocean (Stirling & Rudolph 1968) and the crabeater seal is by far the most abundant Antarctic seal species (Southwell et al. 2012). There are no global estimates for the population size but approximately one million individuals have been estimated for the eastern Antarctic sector (Southwell et al. 2008), although higher densities are reported for the Antarctic Peninsula than any other region (Forcada et al. 2012). Crabeater seals are primarily found on the pack ice of Antarctica, where they represent 80-95% of the seals observed (Erickson & Hanson 1990, Bester & Odendaal 1999). Higher densities are recorded at marginal or peripheral pack ice (Siniff et al. 1970) and they may travel up to 1900 km searching for suitable pack ice conditions (Bornemann & Plötz 1999).

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Fig. 1. The crabeater seal at Cierva Cove, Danco Coast, Antarctica. Photography J. Negrete.

The crabeater seal (Fig. 1) is a krill feeding specialist. Crabeater seal body mass ranges from 200-300 kg (Kooyman 1981). On average, females are slightly larger than males. Breeding takes place during spring on the pack ice, where males gather with females (Siniff & Reichle 1976, Siniff et al. 1979, Laws 1981). Females give birth to a single pup. A male will remain close to the female until she becomes receptive, thus family groups of a male, female and usually one pup are commonly observed (Siniff et al. 1979). Other social groups seen on the pack ice are male-female pairs, i.e. two adults in close contact after weaning, and female-pup pairs (Shaughnessy & Kerry 1989). In general, the peak mating season for crabeater seals is late October-early November and gestation lasts c. 11 months (Siniff et al. 1979, Southwell et al. 2003), probably including a period of delayed implantation (Laws et al. 2003a). However, at the latitude of Seymour Island (Marambio) mating occurs between late September and mid-October (Jorge Lusky, personal observation 2011).

Mummified crabeater seals (MCS) have been reported from disparate eastern and western Antarctic locations. In the eastern Antarctic, there have been reports from McMurdo Sound (Péwé et al. 1959) and nearby southern Victoria Land (Barwick & Balham 1967, Dort 1975, 1981). In the western Antarctic, MCS have been reported on James Ross (Nelson et al. 2008) and Seymour (Negrete et al. 2011) islands off the Antarctic Peninsula. Seymour Island presents low temperatures and annual precipitation levels with prevalent strong winds (mostly from the south-west). The climate is similar to an arid sub-polar region with < 200 mm of annual precipitation and mean annual temperatures of -8.5°C (Reynolds 1981), and the surface is mostly free of snow. The topographical and climatic characteristics contribute to the mummifying process of dead seals.

Many hypotheses have been proposed for why these marine mammals strand, including disease (Nelson *et al.* 2008), changes in sea level, herding behaviour (Stirling &

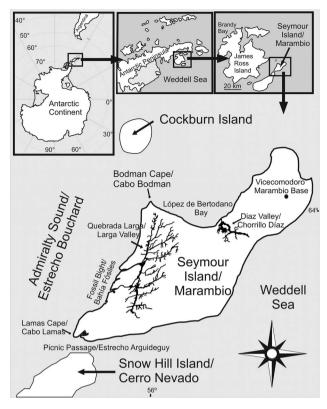


Fig. 2. Location map of the study area. Map of Seymour Island with details of Larga Valley stream.

Kooyman 1971), navigation failure (Stirling & Kooyman 1971), and disorientation (Barwick & Balham 1967, Stirling & Kooyman 1971, Nelson *et al.* 2008). Malnutrition of young seals under the age of one and harmful algal blooms (Pyenson *et al.* 2014) are additional causes linked to marine mammal stranding in other regions. To date most of the MCS have stranded as juvenile or sub-adults (Barwick & Balham 1967). Nelson *et al.* (2008) observed that most (*c.* 90%) of the MCS on James Ross Island were immature individuals and proposed that disorientation, due to inexperience, had caused their stranding. Disorientation has also been proposed as a causal factor for stranding in other seal species including the leopard seal (Banks *et al.* 2010).

In this paper new information is presented regarding the age structure of part of a group of MCS at Bodman Cape, Seymour Island. Here we will discuss how the strandings occurred (i.e. one event versus multiple events), the age and sex structure of the stranded MCS, and the possible causes of death. The drivers for this crabeater seal stranding event will also be examined.

Materials and methods

Study area

The study was conducted on Seymour Island in an area near Bodman Cape located on the south-western coast of

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Fig. 3. Mummified crabeater seal at Bodman Cape showing the desiccation and soft tissue preservation, and tags used for individual identification. Photography L.H. Soibelzon.

Bertodano Bay (Lopez de Bertodano) (64°15'S, 56°48'W) (Fig. 2).

More precisely the study area, known as Larga Valley (Quebrada Larga) (Fig. 2), represents one of the major present-day courses of ephemeral streams in the region. The Larga Valley ice-covered stream is ~ 8 km long and drains south-west into Fossil Bight (Bahía Fósiles) on the eastern coast of Admiralty Sound (Estrecho Bouchard); an ocean channel separating Seymour Island and James Ross Island (Fig. 2). The altitude at the headwaters of the stream is c. 30 m above sea level (a.s.l), decreasing to < 3 m a.s.l. at the mouth. The land is hardly distinguishable from the sea ice pack when the sea surface is frozen (May–December). Following Nozal $et\ al.\ (2007)$, the area corresponds to the relief unit number two dominated by valleys and hills of the Lopez de Bertodano Formation.

Spatial disposition of mummified crabeater seal carcasses

In October–November 2012 and August–September 2013 a group of 78 adult and two pup MCS observed in the

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study area was examined. Data regarding the gender, age and taphonomic aspects (including the determination of state of mummification) of a large number of the group was obtained. The geographical location of the carcasses was recorded with Garmin[®] Map62stc GPS. The orientation and disposition on the ground in relation to the topography of the study area of each MCS was registered.

Necropsies, age and gender determination

Necropsies were performed on 33 MCS. For each seal, where the state of decay allowed, the following procedures were conducted: the gender was determined, the blubber thickness measured, the digestive tract contents noted, the whole body inspected (including skull and long bones) to identify evidence of trauma, and the specimen was identified with a numbered plastic tag (Fig. 3). In a subset of seals (n = 28), where desiccation had ensured soft tissue preservation, samples of one or two post-canine teeth, lung, liver and skin were obtained in order to estimate age and to detect evidence of possible disease. Reproductive state was identified in 14 females by examining the uterus.

Age was determined by counting cementum layers in post-canine teeth (División Paleontología Vertebrados of the Museo de La Plata). After boiling in a solution of water and hydrogen peroxide for removal of organic material, the teeth were sectioned sagittally and the incremental growth layers in the cementum were counted following the protocol provided by Laws *et al.* (2002).

Virology analysis

To detect the presence of *Morbillivirus* the total RNA was extracted (virology laboratory, Facultad de Veterinaria of the Universidad Nacional de La Plata) from samples of lung, liver and skin. Tissue (50 mg) samples were mixed with 1 ml of Trizol (Invitrogen) reagent. The mixture was extracted with 220 μl of chloroform (Merck, Darmstadt). After centrifugation at 10 000 g for 10 minutes, 400 μl of RNA in aqueous solution was precipitated by adding an equal volume of isopropanol (Wako Pure Chemical Industries, Osaka). The precipitated RNA was collected by centrifugation at 10 000 g for 20 minutes, washed by 70% ethanol (Merck) and dissolved in 50 μl of RNase-free water.

Reverse transcription was carried out using 500 ng of total RNA, random hexamer and Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI) under conditions specified by the manufacturer. The PCR was performed by adding 5 μ l cDNA to a reaction mixture containing a final concentration of 200 μ M of each dNTP, 1.5 mM of MgCl₂, 0.25 U of Taq DNA polymerase (Fermentas, Glen Burnie, MD) and 10 pM of each primer: P1 5' TTCTG AGGCA GATGA GTTCT TC 3' and



Fig. 4. Group of several crabeater seals (marked with red plastic tags) showing the same state of decay. Photography L.H. Soibelzon.

P2 5'CTTGG ATGCT ATTTC TGACA CT 3' to amplify a conserved sequence (372–1200) within the N gene (Onderstepoort strain). The amplification was performed on a Mastercycler gradient (Eppendorf, Hamburg). After denaturation at 94°C for 2 minutes, the reactions were cycled 35 times at 94°C for 45 sec, 52.2°C for 45 sec and 72°C for 45 sec. This was followed by a final elongation step at 72°C for 5 minutes (Wang et al. 2011). The PCR product was run on a 1.5% agarose gel dyed with ethidium bromide.

Results

Taphonomy and spatial disposition of mummified seals

Most of the MCS were lying on the surface, the state of decay varied from disarticulated bones to entirely desiccated with perfect soft tissue preservation. This diversity constitutes strong evidence that this aggregate did not occur in a single event, but over several years. Furthermore, one specimen was presumably recently dead since the internal tissues were fresh.

It was possible to distinguish two main groups of specimens at the study area: one group (the most numerous) at the top of the Larga Valley stream, and the other group is chaotically dispersed downhill in the moraines that face the south-west flank of Bertodano Bay just beyond the Larga Valley cliff edge. Several MCS were found on small Larga Valley tributary streams.

Remarkably, the majority of the carcasses at the Larga Valley were oriented SW–NE, parallel to the predominant strong icy winds from the south-west. Some of the MCS were scattered along the first third of Larga Valley stream (mostly buried and disarticulated), but the largest aggregation was located on a flat area near the edge of the cliff.

Most of the specimens at Larga Valley were found in a prone position suggesting that this had been the place they had died and that the carcasses had not been moved by

Table I. Coordinates, age and gender of the mummified crabeater seals sampled at Bodman Cape in 2012.

Sample ID	Gender	Age (years)	Latitude (S)	Longitude (W)
1	Female	0	64°14'47.3"	56°47'15.8"
2	Female	3	64°14'48.4"	56°47'16.7"
3	Undetermined	3	64°14'48.4"	56°47'16.7"
4	Female	4	64°14'48.4"	56°47'16.6"
5	Male	4	64°14'48.4"	56°47'16.8"
6	Female	5	64°14'48.3"	56°47'16.4"
7	Female	5	64°14'45.8"	56°47'15.2"
8	Female	5	64°14'47.5"	56°47'16.6"
9	Female	5	64°14'48.5"	56°47'16.5"
10	Male	5	64°14'48.8"	56°47'17.1"
11	Female	6	64°14'47.3"	56°47'16.5"
12	Female	6	64°14'48.6"	56°47'16.6"
13	Female	6	64°14'46.8"	56°47'18.3"
14	Female	6	64°14'47.4"	56°47'16.1"
15	Female	6	64°14'48.4"	56°47'16.3"
16	Female	7	64°14'45.8"	56°47'15.2"
17	Female	7	64°14'47.3"	56°47'16.7"
18	Female	7	64°14'52.2"	56°47'17.3"
19	Undetermined	7	64°14'52.2"	56°47'17.3"
20	Female	8	64°14'48.3"	56°47'16.6"
21	Undetermined	8	64°14'47.4"	56°47'16.6"
22	Male	8	64°14'52.4"	56°47'18.6"
23	Female	10	64°14'48.4"	56°47'16.6"
24	Female	10	64°14'50.6"	56°47'16.3"
25	Female	12	64°14'47.3"	56°47'16.6"
26	Female	15	64°14'51.2"	56°47'17.7"
27	Male	15	64°14'48.4"	56°47'16.5"
28	Male	19	64°14'48.4"	56°47'16.5"

the wind (or some other secondary transport). The specimens did not appear to have been transported as they lay on top of a 10–30 cm soil 'pedestal' scoured by the wind from the surrounding substrate. Other highly desiccated MCS (flat and light) had been transported by the wind and re-deposited.

Small groups of two or three seals showed similar stages of decay (Fig. 4) suggesting that these seals had stranded and moved inland together.



Fig. 5. Mummified crabeater seal female and her pup found at Bodman Cape. Photography P. Campana.

Age and gender

The mean age of the crabeater seals was 7.2 years (standard deviation = 4.0, n = 28). Sex was determined for 25 MCS; of those, the majority were female (80%, n = 20) (Table I). Most of the female seals were at least 5 years of age (85%, n = 17). Necropsies showed that four of the adult females were pregnant with a single foetus almost on term, and another had recently given birth to a single pup that was found next to her (Fig. 5). Of the remaining females examined, four specimens showed their uterus in regressive phase, and eight specimens did not show signs of gestation.

Virology

All the processed samples were negative for phocine distemper virus (*Morbillivirus*).

Blubber thickness and digestive tract contents

From the seven specimens in which blubber thickness could be measured, only two had blubber thickness > 3.3 cm, which is the mean value for this species (Laws *et al.* 2003b).

In six specimens at Larga Valley, sediment and fossil mollusc (soil constituents at this locality) were identified in the digestive tract, these individuals had no subcutaneous blubber. Seven specimens showed empty stomachs. Only one specimen had liquid stomach contents; this seal had 3.5 cm of subcutaneous blubber.

Signs of trauma

The specimens distributed downhill in the moraines (n = 6) had multiple fractures to the skull, mostly in the mandible and head region, presumably derived from falling from the cliff. None of these seals presented sediment in the digestive tract and blubber thickness was normal.

Discussion

Mummified crabeater seals have been observed in several places in the Antarctic (Péwé et al. 1959, Barwick & Balham 1967, Dort 1975, 1981, Gordon & Harkness 1992, Nelson et al. 2008, Negrete et al. 2011). Approximately 70 km from Bodman Cape, in an area known as Abernethy Flats in the northern flank of James Ross Island, Nelson et al. (2008) described 154 randomly distributed MCS. The carcasses were in varying states of decay, and the authors suggested numerous events were involved. Our observations reinforce the hypothesis of Nelson et al. (2008), we found various states of decay ranging from highly weathered bones to entirely desiccated bodies with soft tissue preservation. Moreover, the observation of a recently deceased seal at Bodman Cape, not yet mummified, is evidence that strandings are still happening today as Nelson et al. (2008) proposed.

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Fig. 6. Mummified crabeater seal at the edge of the cliff in Bodman Cape, *c*. 30 m a.s.l. Photography E. Soibelzon.

In contrast to other MCS, the group at Seymour Island are not an aggregation of disoriented young animals. The majority of the seals were at least 5 years of age meaning that most were sexually mature (Hårding & Härkönen 1995). Many of the female seals were pregnant or showed evidence of having recently given birth. This high incidence of pregnant seals suggests the MCS at Seymour Island were probably stranded during the breeding season.

In this region, crabeater seals reach the pack ice off Admiralty Sound from the ice-free waters of the Weddell Sea by two different routes: from the east through the Picnic Passage (which separates Seymour Island from Snow Hill Island) and from the north through Bertodano Bay (see Fig. 2). We hypothesize that once the seals are at Admiralty Sound, some climb to the mainland at Fossil Bight, probably confusing the land with ice pack. The seals may be seeking shelter from the cold south-west winds as Fossil Bight is partially protected by the elevated cliff at Lamas Cape (south-west tip of Seymour Island, see Fig. 2).

The distribution of the mummified seals along the Larga Valley stream (from Fossil Bight to Bodman Cape, and on small tributary streams) indicates that the seals became stranded while moving inland following the course of the frozen stream until they reached the cliff edge at Bodman Cape. Once the seals arrived at the headwaters of the Larga Valley stream some attempted to descend downhill; based on the signs of trauma observed (multiple fractures of the mouth and head region) they died as a result of falling from the 30 m cliff. We do not discount that some seals may survive the 'jump' from the Larga Valley cliff edge, but we do not have evidence of that. Other seals remained at the flat area, near the edge, hesitating to jump probably for many days until they died (Fig. 6). Based on our results, two hypotheses regarding the cause of death for these seals could be proposed: i) Some specimens showed < 2 cm of blubber thickness and their digestive tracts were either completely empty or full of sediment; these seals probably died of starvation. ii) Other specimens had normal blubber thickness and

since there was no evidence of disease (virology results were negative for all samples) they most probably died as a result of low temperatures during storms. In this area during spring temperatures below -20°C are frequent, in addition to strong winds (> 40 knots) from the southwest. Normal seal behaviour is to find shelter in the water when temperatures are below -5°C; the seals stranded on Larga Valley would have been exposed to freezing temperatures and/or strong storms that would lead to death within a few hours.

It appears that Larga Valley is a natural trap for seals, which has been active for hundreds of years, and continues to be active. Moreover, the occurrence of mummified seals in the Larga Valley area was noticed by the Alferez José Maria Sobral who spent 20 months in the area between 1901 and 1903 as part of the group led by Otto Nordenskjöld. Sobral (1904 p. 299) stated that: "In the gully part of Seymour Island you could see several carcasses of seals pretty high over sea level and although they seem very old they conserved the skin and soft tissues, all of them belong to Lobodon carcinophaga". This is evidence that the MCS recorded at Seymour Island were observed 110 years ago and, taking into account that the seals observed by Sobral were already mummified, the initial strandings must have occurred long before Sobral made his observations.

Finally, the role of disease in these strandings was examined. All samples analysed were negative for morbillivirus. Nelson *et al.* (2008) speculated that phocine distemper virus had played a part in the nearby James Ross Island crabeater seal stranding, and antibodies to canine distemper virus have been found in crabeater seals in the Antarctic Peninsula region previously (Bengtson *et al.* 1991); this disease was linked to a mass crabeater seal mortality event reported in the 1950s at the nearby Prince Gustav Channel where these seals had shown clinical signs of distemper (Laws & Taylor 1957). Our negative virology results for phocine distemper virus suggest that the crabeater seals at Seymour Island were not affected by the mass mortality event reported in the 1950s (Laws & Taylor 1957) but we cannot discount other types of disease.

Conclusions

The aggregation of MCS on Seymour Island described here corresponds to an exceptional stranding record. This is the first report of a large number of mummified adult seals, some even pregnant or with pups. These findings allow us to conclude that the seals used the Admiralty Sound area to breed in the past, and refutes the theory that only young animals are prone to stranding. The predominance of healthy mature adult seals in the aggregation of mummified seals suggests that age, disease or hunger have not solely played a part in these stranding events. Adult crabeater seals have stranded in this region through multiple events and not from a single

mass mortality. The Larga Valley, on Seymour Island, may act as a natural trap for crabeater seals; trapping seals for hundreds of years.

The mummified seals at Bodman Cape travelled for c. 5 km following the Larga Valley stream and climbed from 3 m a.s.l to around 30 m a.s.l, reinforcing previous ideas about the agility of this species on land.

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Author contribution statement

Dr Javier Negrete, Dr Leopoldo H. Soibelzon and Dr Esteban Soibelzon: participated in the field trips and wrote the article. Bioq María Elba Isabel Márquez and Lic Mara Cleopatra Loza: determined the age of the seals by counting the cement layers of the post-canine teeth. Med Vet Walter Acosta and Jorge Lusky: performed most of the necropsies and provided logistical support during field trips. Dr Marcelo Pecoraro: carried out all the virological analyses.

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