DRAFT Preliminary Report on New Zealand Sea Lion Research, Auckland Islands 2016-17

Prepared for the Department of Conservation, New Zealand

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Executive summary

A successful research field season investigating pup mortality and disease in New Zealand (NZ) sea lions (*Phocarctos hookeri*) was undertaken on Enderby Island between 11 December 2016 and 13 March 2017. The aim of the work was to identify risk factors associated with pup mortality in NZ sea lions including the identification of reservoirs of the pathogenic bacteria *Klebsiella pneumoniae*. This field study has generated a substantial number of samples from pups including 1236 oral and rectal swabs, 306 blood smears, 153 serum samples and 282 post mortem tissue samples. Planned investigation through microbiology, histopathology and epidemiology will be essential in identification of the risk factors important in pup mortality.

- In total, 341 pups were captured at approximately one week of age for PIT tag implantation, morphometric measurements, oral and rectal swab collection for *K. pneumoniae* and random selection for ivermectin treatment. The ivermectin treatment group comprised 163 pups; 178 constituted the control group.
- The total number of dead pups identified during the field season was 82. Of these, 75 were in a suitable state for necropsy. Provisional diagnosis included 45% infection (suspected with *K. pneumoniae*), 9% drowning with lesions consistent with *K. pneumoniae*, 15% starvation, 15% open (scavenged, decomposed or inconclusive on gross necropsy alone), 12% trauma, 3% periparturient death and 1% stillborn. Of Enderby born pups that died, almost twice as many had not been treated with ivermectin, compared to those that had.
- A total of 135 control live pups were sampled to investigate risk factors for mortality as part of the case-control study. A GPS grid system was practical and successful in random selection of controls in a widely distributed colony.
- A total of 50 pups were recruited to the prospective cohort study. Twelve of these pups died during the season including three in the ivermectin treatment group and nine controls. Cohort pups were serially sampled between one and five times for the collection of blood, oral and rectal swabs and faeces with risk factor data collection.
- Forty four sub-Antarctic skua (*Catharacta antarctica lonnbergi*) were captured, leg banded and cloacal swabbed to determine carriage of *K. pneumoniae*. Voided faecal samples from eight individually identified birds were also collected later in the season.
- Substrate samples including sand, water and mud from a suite of sites around Sandy Bay and wider Enderby Island were collected at three time points: early (mid-December, on arrival); mid (early February) and late season (mid-March) to determine *K. pneumoniae* environmental presence.

Background

The New Zealand (NZ) sea lion is the only pinniped endemic to New Zealand. Historically prevalent around the coasts of the NZ mainland, over 99% of the species' breeding is now restricted to the NZ sub-Antarctic islands between latitudes 50-53°S, primarily at colonies in the Auckland Islands and Campbell Island. The second largest breeding colony at Sandy Bay, Enderby Island in the Auckland Island archipelago is also the most well studied with detailed population dynamics data and gross necropsy records from austral summer field seasons since 1998.

The proposed primary driver for declines over the last two decades was adult mortality due to fisheries interactions including bycatch and resource competition (Robertson and Chilvers, 2011). Management decisions have reflected this by focusing on mitigation of adult and subadult mortality within the fisheries. Recently however, analysis has suggested that poor reproductive success and pup survival may be important contributing factors (Roberts *et al*., 2013). The bacteria *Klebsiella pneumoniae* is a common and fatal pathogen in NZ sea lion pups at Sandy Bay, causing mortality by septicaemia with common findings of meningitis and polyarthritis. This pathogen was responsible for two initial epizootic events in 2001-03 and was the cause of on average 58% of pup deaths annually between 2007 and 2010 (Roe *et al*., 2015). However, due to the often cryptic presentation of disease based on gross examination alone, the true extent of *K. pneumoniae*-associated morbidity and mortality may actually be higher than this.

Focus

Whilst many identified threats to adult pinnipeds including fisheries interactions and climate change are unlikely to be abated in the near future (Kovacs et al., 2012), risk factors involved in pup mortality may be amenable to active management. New Zealand sea lion pup mortality requires investigation at this critical time as mortality due to *K. pneumoniae* remains high.

The following questions will begin to be addressed in the 2016-17 Auckland Islands field season:

- What risk factors are associated with pup mortality in NZ sea lions?
- Are there reservoirs of *K. pneumoniae* in the terrestrial and aquatic ecosystems?
- Does *K. pneumoniae*-mediated pup mortality continue after pups leave Enderby Island?

Addressing these questions will aid in answering the most important question:

- How can pup mortality be mitigated?

The primary components of the research during the 2016-17 field season included:

- Monitoring of pupping to determine estimated pup birth dates and maternal identification
- Individual permanent identification of pups born at Sandy Bay
- An ivermectin treatment trial to investigate hookworm carriage as a risk factor for pup mortality
- Necropsy of pups to determine cause of death and risk factor information, including collection of tissue samples to enable later ancillary diagnostic testing
- A case control study to investigate risk factors for pup mortality and *K. pneumoniae* infection
- A prospective cohort study to investigate morbidity and pup response to disease
- Investigation of avian reservoirs of *K. pneumoniae* by sampling sub-Antarctic skuas
- Investigation of environmental reservoirs of *K. pneumoniae*

The field team included Sarah Michael, Thomas Burns, Rachael Gray and Shannon Taylor.

A summary of the key dates of the 2016-17 field season are below:

- 7 December 2016 — Arrived in Invercargill for pre-departure preparation
- 9 December 2016 — Team of three departed Bluff aboard RV Evohe
- 11 December 2016 — Arrived at Enderby Island
- 11 January 2017 — One team member arrived aboard RV Baltazar for scheduled changeover
- 13 January 2017 — One team member evacuated by helicopter
- 13 March 2017 — Partial surveys of Rose and Ewing Islands, Port Ross via RV Baltazar
- 13 March 2017 — Team of three departed Auckland Islands aboard RV Baltazar
- 15 March 2017 — Arrived in Bluff

Pupping at Sandy Bay, Enderby Island

Daily counts of adult females and pups were undertaken from when females began to arrive at Sandy Bay until pups were tagged. As reported in Childerhouse *et al* (2017), pup production at Sandy Bay in 2016-17 was 9% higher than 2015-16. This was seen throughout the pupping period as an earlier increase in pups born, from approximately 16 December (Figure 1). Total numbers of adult females were also generally higher and increased earlier than in 2015-16.

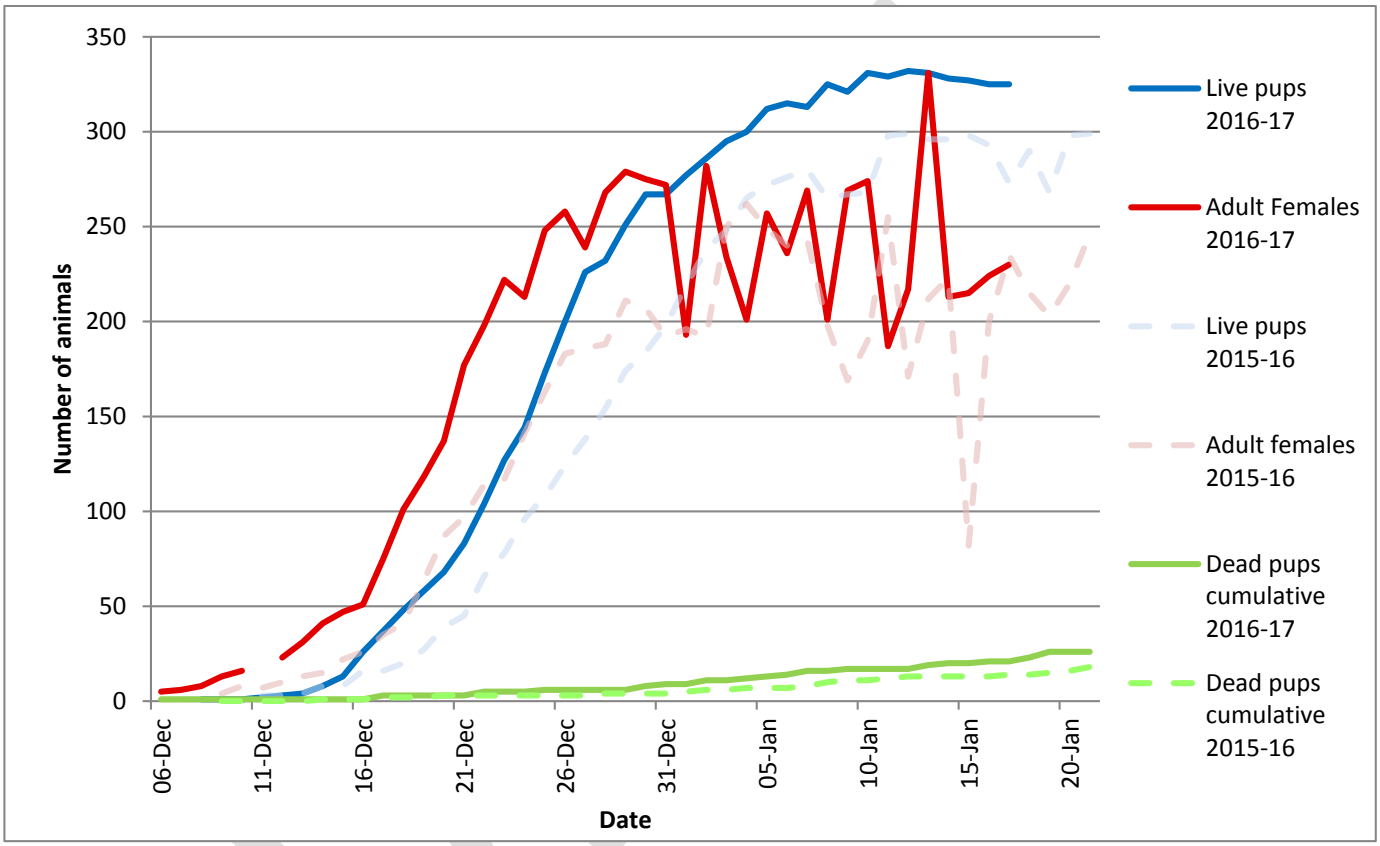

Figure 1. Daily counts of New Zealand sea lion females and pups during the pupping period in 2015-16 and 2016-17. Data for 2015-16 provided by Simon Childerhouse, Blue Planet Marine and data between 6-11 December 2016 by Chris Muller.

Generally, pupping occurs on Sandy Bay beach with females and pups remaining until mid-late January when dispersal begins onto the surrounding sward (Augé *et al*., 2009). This was not the case in 2016-17 following a southeast storm between 31 December 2016 and 1 January 2017. Average wind speeds over 40 knots (74km/h; measured at local Sandy Bay weather station) caused a storm surge at Sandy Bay, obliterating most of the space on the beach on 1 January 2017, resulting in many adults and pups moving up to the sward earlier than usual (Figure 2). This weather event triggered the early dispersal, particularly of animals at the western end of the beach that continued throughout the season in the form of a very widely distributed population in spatial clusters. This increased the time taken for resighting and searches for dead pups for the remaining season.

Figure 2. Sandy Bay beach, Enderby Island in the dissipating storm on 1 January 2017. Many New Zealand sea lions were pushed up the bank and onto the sward, triggering early dispersal for some.

Individual identification of pups and first capture

Individual marking of newborn pups with a stock marker on the end of a pole as proposed was undertaken for the first week of births, until it became evident that the paint was wearing away much faster than expected due to the abrasive action of the sand and ongoing poor weather. As an alternative, birth dates of pups were instead calculated by the resighting of females giving birth, by flipper tags, PIT tags or where no other identification was present distinctive scars (Figure 3), so that they can later be associated with their pups once marked. Many of these resights, particularly of old, worn tags from extended distances due to harem structure were possible only by use of a digital SLR camera and telephoto lens.

Figure 3. Examples of distinctive scars on female New Zealand sea lions that gave birth at Sandy Bay used to correlate pup age. These females were otherwise unidentifiable as they had lost all external individual identification and did not have PIT tags.

When pups were unattended (approximately less than one week of age), they were captured by hand for assessment. First captures took place between 16 December 2016 and 15 January 2017. During the first capture, pups were weighed, their length and girth measured, a full physical examination for wounds or abnormalities undertaken, oral and rectal swabs collected, a vinyl cap with identification number glued to the rump and a PIT tag inserted subcutaneously over the dorsal pelvic region. Pups were also randomly allocated to ivermectin treatment or control groups. Those in the treatment group were dosed with 200µg/kg ivermectin (Ivomec 1% Injection for Cattle, Sheep and Pigs; Merial, Manukau, New Zealand) subcutaneously in the interscapular region. In total 341 pups underwent first capture assessment (179 male, 162 female), with 163 pups randomly allocated to the ivermectin treatment group (81 male, 82 female) and 178 allocated as controls (98 male, 80 female).

Five pups that underwent first capture were not identified at tagging. This may be due to loss of the rump ID cap and PIT tag (less likely as no glue marks were seen on the coats of pups from loss of the rump cap) or the pups died and were buried in the beach or washed out to sea during the New Year's Day storm.

As described in Childerhouse *et al* (2017), tag application as part of the Conservation Services Programme (CSP) segment of the field season was altered this year due to incorrect printing of the tag number on the underside of the female part, so that the female part required deployment upside down. Opportunistic observations of pup tags throughout the season suggest that tag loss was increased this season in the two months after deployment with identification of seven pups having lost both flipper tags (only identifiable by PIT tag) and 26 pups having lost one flipper tag. This is subjective as pup tag loss has not been consistently recorded in previous seasons but appears significant with a 9.8% tag loss rate (of one or both tags) in Sandy Bay tagged pups after two months. All observed tag losses were from tearing of the tags through the trailing edge of the flipper. Additionally, the underside of the tag had an embossed circular logo that is normally out of view facing the flipper skin. This year, having the tag number printed over the embossing caused problems in accurate reading of the number with particularly the second and third digits obscured (Figure 4). These issues may cause ongoing problems for demographic and survival assessments of this cohort in future years.

Figure 4. Examples of New Zealand sea lion pup flipper tag numbers obscured by the embossed logo at Enderby Island 2016-17.

Linking of pups with mothers was undertaken by resighting of flipper tags, PIT tags or distinctive scars of females with indicative behaviours with a marked pup (nursing, call and reply, close contact away from others). A mother

and pup were classified as linked based on observation of at least two instances of such. At the present level of analysis, 169 of 341 initially captured pups (49.6%) have been linked with a permanently identified female so that maternal age can be integrated as a risk factor. Of those females, 56 were observed giving birth, so pup birth date is known. Several additional females only identifiable by scars were observed giving birth so pup birth date is known but maternal age is not. The remainder of pups were either never resighted with a female, resighted less than twice with a female, resighted with unidentifiable females (double tag scars and no PIT tag or females that had never been tagged and had no PIT tag) or the pup died before they could be resighted with their mother. Increased resighting effort in future would be crucial to ensuring a higher proportion of pairs observed.

Necropsy of dead pups

Pup mortality

Mortality of pups at Sandy Bay was monitored throughout the season, with comprehensive searches for dead pups undertaken at least once daily. Other sites around the island were monitored opportunistically. The total number of dead pups identified between 6 December 2016 and 12 March 2017 was 82. Of these, 75 were able to be retrieved in adequate condition that a necropsy could be undertaken. Risk factor information was collected for all pups and samples such as oral and rectal swabs collected from freshly dead pups only. All pups discussed here died at Sandy Bay, except for one found at Teal Lake in a decomposed state. Daily and cumulative dead pup data is presented in Figure 5.

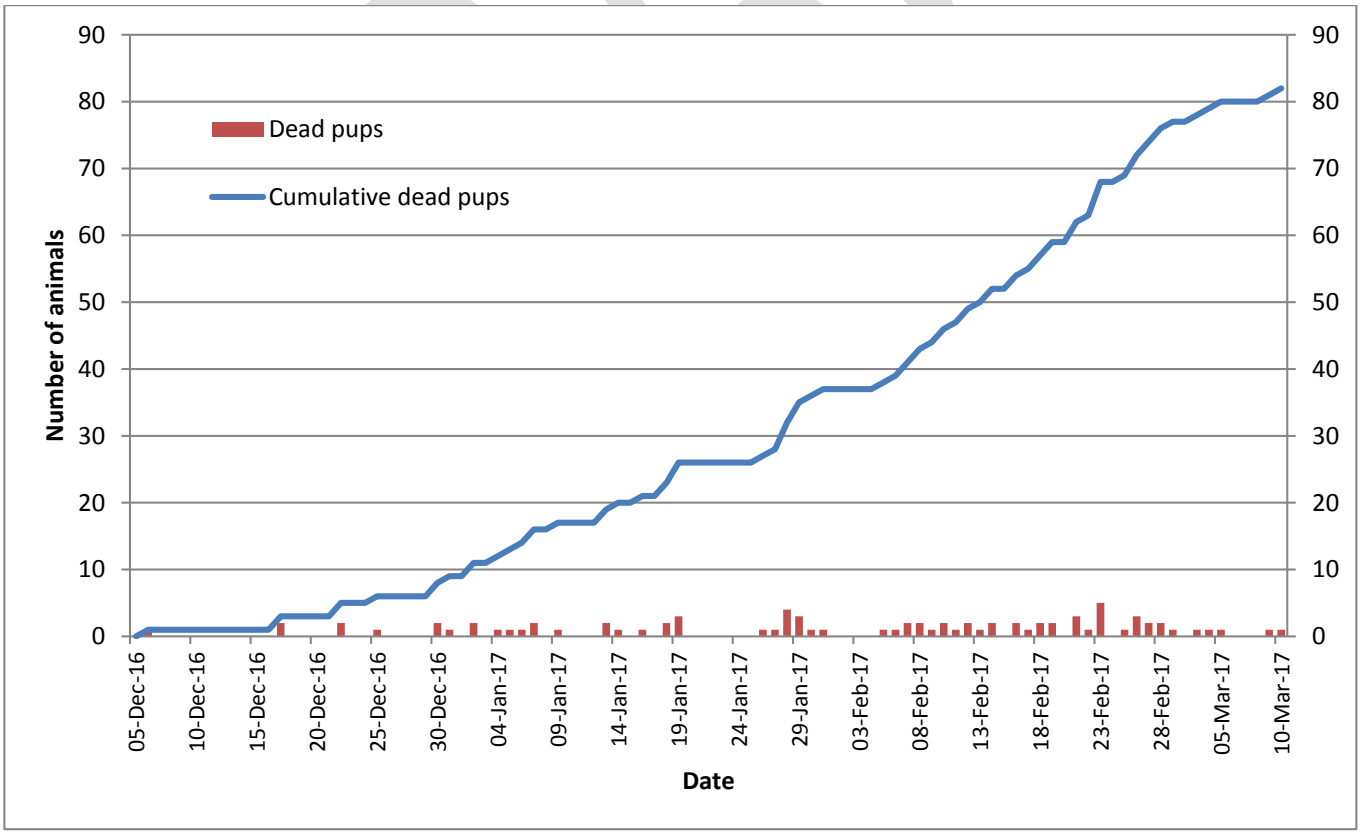

Figure 5. Daily and cumulative counts of New Zealand sea lion pup mortality at Sandy Bay, Enderby Island 2016-17.

A bias toward female pup deaths was seen in 2016-17 with 32 males and 43 females examined at necropsy (male:female sex ratio 0.74, in contrast to 1.11 in all Sandy Bay-born pups). All dead pups were born on Enderby Island except for eight either confirmed or suspected Dundas Island-origin pups (tagged at Dundas or no tags or microchip after Sandy Bay pups had been tagged). Of Enderby born pups that died, 21 had received ivermectin at first capture, compared with 41 that were controls and had not received ivermectin. This almost two-fold difference in survival is interesting and a similar trend has been reported in the species previously with smaller sample sizes involved (Chilvers *et al*., 2009; Michael *et al*., 2015). This difference may reflect the increased immune capacity in those pups that do not have a hookworm infestation or allude to a significant proportion of *K. pneumoniae* transmissions through the intestinal tract that is potentiated by damage to the mucosa by hookworms. Further assessment of other risk factor data is required to determine the significance and potential future management protocols.

Preliminary diagnoses were made based on gross necropsy examinations in the field. Comprehensive formalin fixed and frozen samples were collected during necropsy to allow definitive diagnosis following histopathology and microbiology analysis. Provisional diagnosis for pups at Enderby Island in 2016-17 (Figure 6) included 45% infection (suspected with *K. pneumoniae*), 9% drowning with lesions consistent with *K. pneumoniae*, 15% starvation, 15% open (scavenged, decomposed or inconclusive on gross necropsy alone), 12% trauma, 3% periparturient death and 1% stillborn.

Gross post mortem findings consistent with *K. pneumoniae* septicaemia included suppurative meningitis (pus around the brain), subdural haemorrhage of the cerebrum and cerebellum (blood around the brain), septic arthritis (pus within a joint), pyothorax (pus around the lungs), septic peritonitis (pus in the abdomen), muscle abscesses and umbilical infections. Seven dead pups were found floating in small pools or wedged in shallow streams that should normally be escapable or had ramps installed. These pups had gross necropsy findings of drowning but also those consistent with *K. pneumoniae* infection and may have entered the water in attempt to quell a high fever and could not exit due to neurological or joint symptoms, resulting in drowning. Confirmation of the primary and secondary cause of death in these pups will be carried out with histopathology and microbiology analysis.

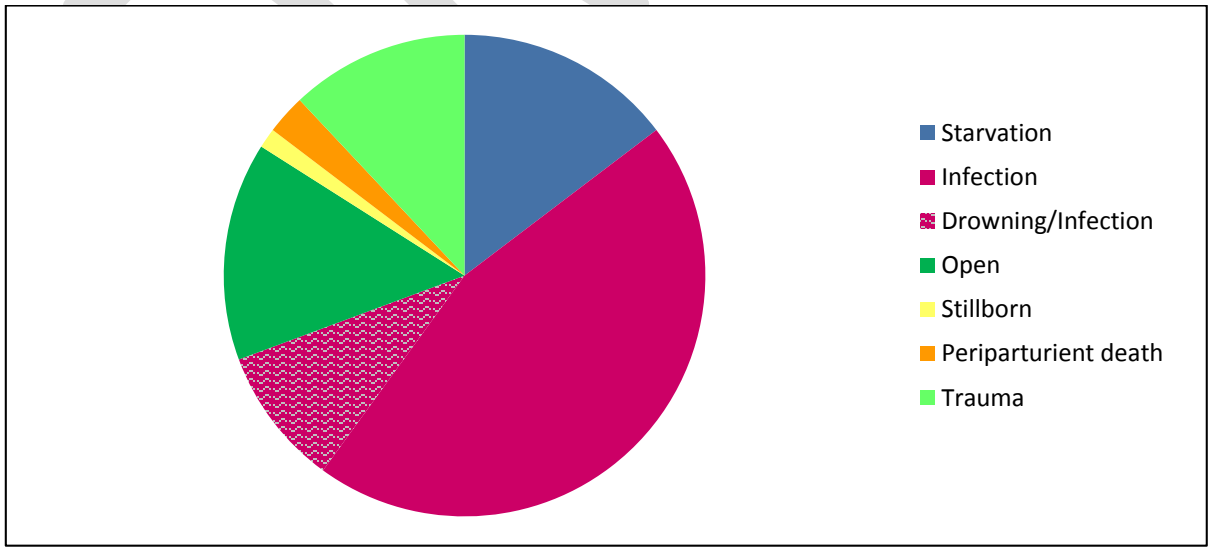

Figure 6. Preliminary cause of death of 75 New Zealand sea lion pups at Sandy Bay, Enderby Island between 17 December 2016 and 10 March 2017.

Seven pups were found dead between 27-28 January 2017 within an approximately 20 metre radius of an untagged territorial adult male. This animal had been seen mounting and attempting to mate a dead pup (Figure 7) and the consistent post mortem findings of milk aspiration in all seven indicated that he most likely suffocated them by the same means. After these two days the male went to sea and although he did not have any external identification to monitor his return, no further pups were found in the same condition.

Figure 7. Territorial adult male New Zealand sea lion mounting a dead pup on the sward at Sandy Bay, Enderby Island.

Adult mortality

During the 2016-17 season, one juvenile female, four adult females and one adult male were found dead on Enderby Island (Table 1). Partial post mortem examinations were performed on two adult females at Sandy Bay where time allowed in conjunction with other sampling. No significant gross findings were identified in these animals, however one was moderately scavenged.

Table 1. Summary of dead adult New Zealand sea lions at Enderby Island 2016-17.

Individual ID	Date found	Origin	Location	Sex	Age
No tags or chip	11/12/16		Sandy Bay	Female	Juvenile
No tags or chip	13/12/16		Sandy Bay	Female	Adult
K011 Green Coffin	24/12/16	Enderby Island	Sandy Bay	Female	Adult
Double tag scar, chip 00-01EF-66B2	1/2/17	Dundas Island	Derry Castle	Male	Adult
No tags or chip	12/2/17		Sandy Bay	Female	Adult
E729 Green Coffin	7/3/17	Dundas Island	East Bay	Female	Adult

Controls

Random selection was based on a grid overlaid on a map of Enderby Island as proposed (Michael *et al*., 2016). The grid was subdivided into zones, with a 10m^2 grid for the Sandy Bay beach area in the early part of the season and a larger 20m^2 grid following dispersal from the beach. The north-oriented grid squares were located in the field using GPS waypoints at the southwest corner of each grid square.

The grid system was successfully used for random selection of control pups at Sandy Bay, with several adjustments to the proposed protocol. Firstly, on the beach at the beginning of the season a strip of north-south grid squares (10 metres wide) was randomly selected instead of a single square (Figure 8a). This allowed marking of the boundaries of the strip, a count of all available pups within the swathe, a random number to be selected and then a pup chosen based on a second count by another researcher blinded to the number chosen. Once pups had dispersed from the beach, the 20 metre Sandy Bay grid was used. Instead of marking out the boundaries of the grid square, a 'distance to destination' function on the handheld GPS allowed simple visualisation of a 40 metre radius from the selected GPS point in the southwest corner of each square (Figure 8b).

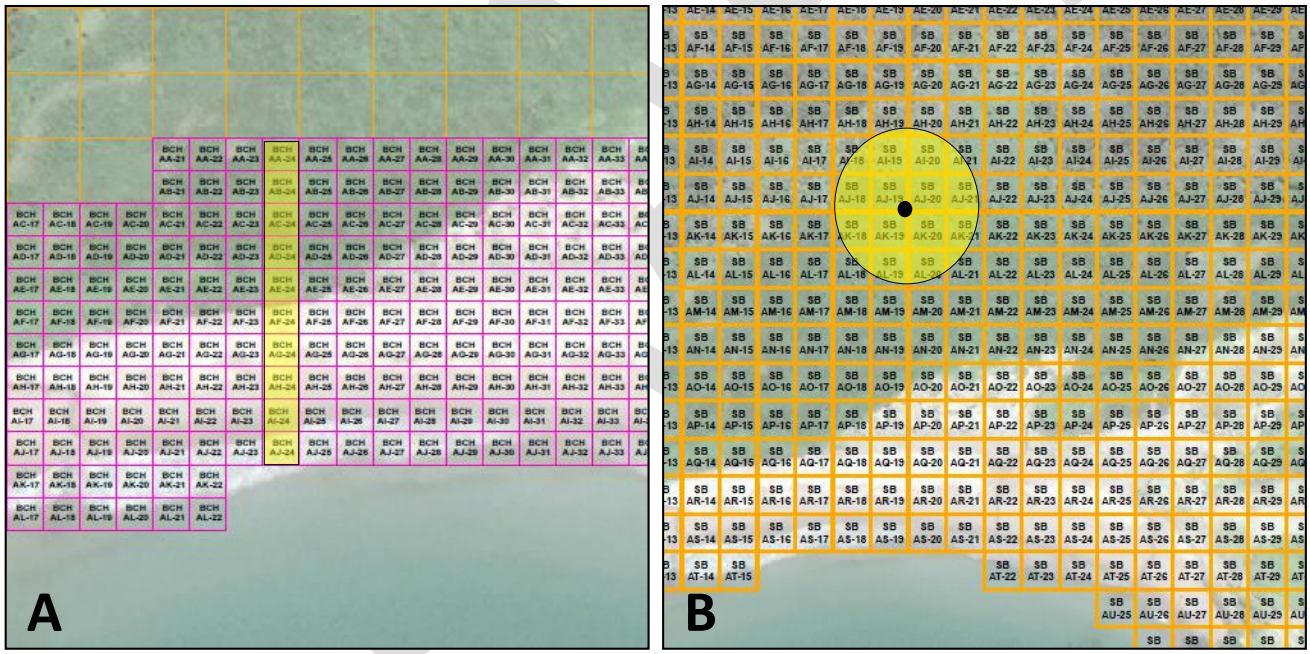

Figure 8. Example of use of grids to randomly select a pup as a control. A. In early season on the beach the 10m grid was used as a north-south strip, here as BCH-24, from which available pups were counted and one randomly chosen from the sample. B. Once pups had begun to leave the beach, a 40m radius from a randomly selected grid point was used as a margin for pup selection, in this case for grid point SB-AJ-20.

The other criteria included:

- Pups that are nursing are excluded from selection
- If one pup is present in the circle, it is selected as a control

- If more than one pup is present in the circle, the nearest pup to the GPS waypoint (within 40m radius) is selected unless it is part of a pod (group)
    - To select from a pod, a fraction will be randomly generated and multiplied by the number of pups present to select the control
- If the pup selected has been captured within the last 24 hours, another pup will be selected within the same area
- If no pup is present in the circle, another point is randomly selected and the process repeated

During control captures, selected pups were identified by flipper or PIT tag, weighed, their length and girth measured, a full physical examination for wounds or abnormalities undertaken and their risk factor information assessed as based on categories in Table 2. Samples collected included oral and rectal swabs, faeces and toe clip for genetics. In total, 135 control captures were undertaken. The spatial distribution of cases and controls is presented in Figure 9. Risk factor data from cohort and first captures will also be able to be evaluated as additional controls.

Table 2. Summary of risk factor information to be collected from every control and case (dead) pup.

	Risk factor	**Variables**
Location	Location	GPS coordinates
	Site	Sandy Bay, Teal Lake, South East Point, East Bay, Derry Castle, Dundas, Port Ross Islands, Figure of Eight
	Habitat type	Beach, rock platform, sward, forest
	Substrate type	Sand, mud, grass, water, soil
Pup variables	Colony of origin	Enderby, Dundas, Unknown (assumed Dundas)
	Age	Based on date of birth to nearest day where possible
	Sex	Male, female, unknown if scavenged
	Pup status	With mother, with pups, alone
	Recent suckling	Based on visual assessment; yes, no, unknown
	Has been rescued from stream or sink hole	Yes, no and when, which hole
	Handling events	Number of times handled previously
Pup morphometrics	Weight	Kilograms to nearest 100g
	Length	Centimetres
	Axillary Girth	Centimetres
	Body condition	BMI based on length and weight
Post mortem variables	Preservation	Fresh, mild, moderate or severe decomposition
	Scavenging	None, mild, moderate, heavy
Maternal variables	Age of mother	Number or unknown
	Parity of mother	Number or unknown
Environmental variables	Air temperature	Daily minimum and maximum
	Rainfall	mm, daily
	Wind speed	km/h, daily maximum
	Tourist interactions	Days since most recent tourist visit
	Skua presence	Within eyesight; none, 1-2, 3+

Clinical variables	Physical examination	Bite wounds, tag wound infections, omphalitis, conjunctivitis, nasal discharge
	K. pneumoniae carriage	Present, absent – oral or rectal
	Ivermectin treatment	Control, treated
	Hookworm infestation	Present, absent, severity of enteritis in PM cases
	Clinical signs	Present, absent, when started
	Clinical sign type	Arthritis/lameness, neurologic , emaciation

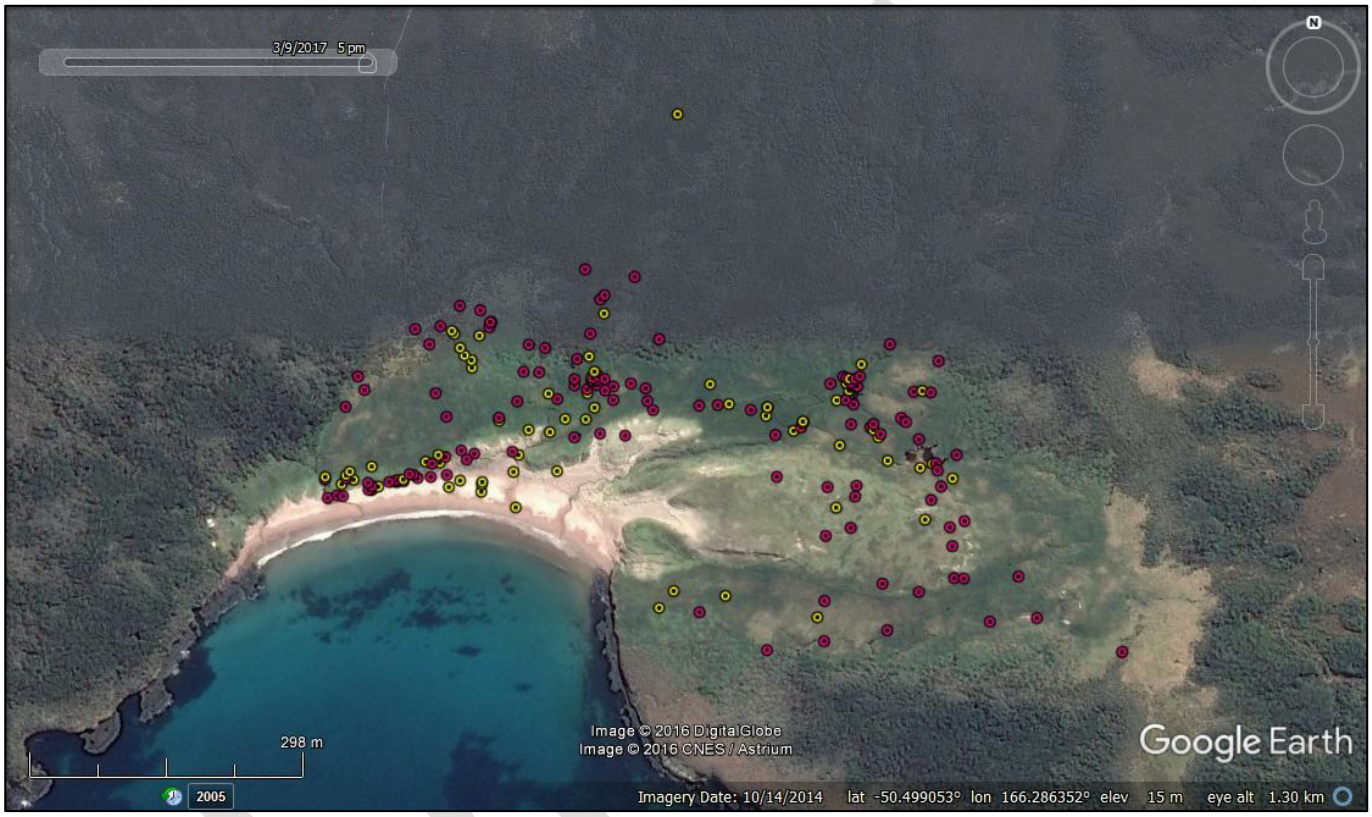

Figure 9. GPS waypoints for cases (yellow circles) and controls (pink circles) at Sandy Bay, Enderby Island.

Prospective cohort study investigating risk factors for *K. pneumoniae* disease

Prospective cohort studies allow us to more clearly elucidate the temporal sequence of events leading to death including the role of multiple concurrent risks, in order to calculate the incidence of disease. The small sample size of 50 pups allows detection of large differences in incidence (eg. 2 to 5-fold) for different risk factors and provides a proof of concept for future studies.

As proposed, 50 pups were recruited to the study at first capture by selection of every sixth pup captured. Twenty eight cohort pups were male, with eleven of these in the ivermectin treatment group and 17 as controls. Twenty two cohort pups were female, with eleven receiving ivermectin and eleven selected as controls. Twelve cohort pups died throughout the season including three that received ivermectin and nine that did not. Due to the early and

widespread dispersal of pups, including deep into the rata forest and by sea to other islands, location of specific pups for serial sampling was time consuming. In summary, six pups were sampled once only, six were sampled twice, 22 were sampled three times, eleven were sampled four times and five were sampled five times (Figure 10). Each capture involved weight and morphometric measurements as for control pups, with additional oral and rectal swabs, faeces collection, rectal temperature measurement and blood collection via the brachial vein. Blood samples were successfully collected from every cohort pup capture. Blood was processed for packed cell volume and total protein as well as blood smears prepared and serum separated in the field.

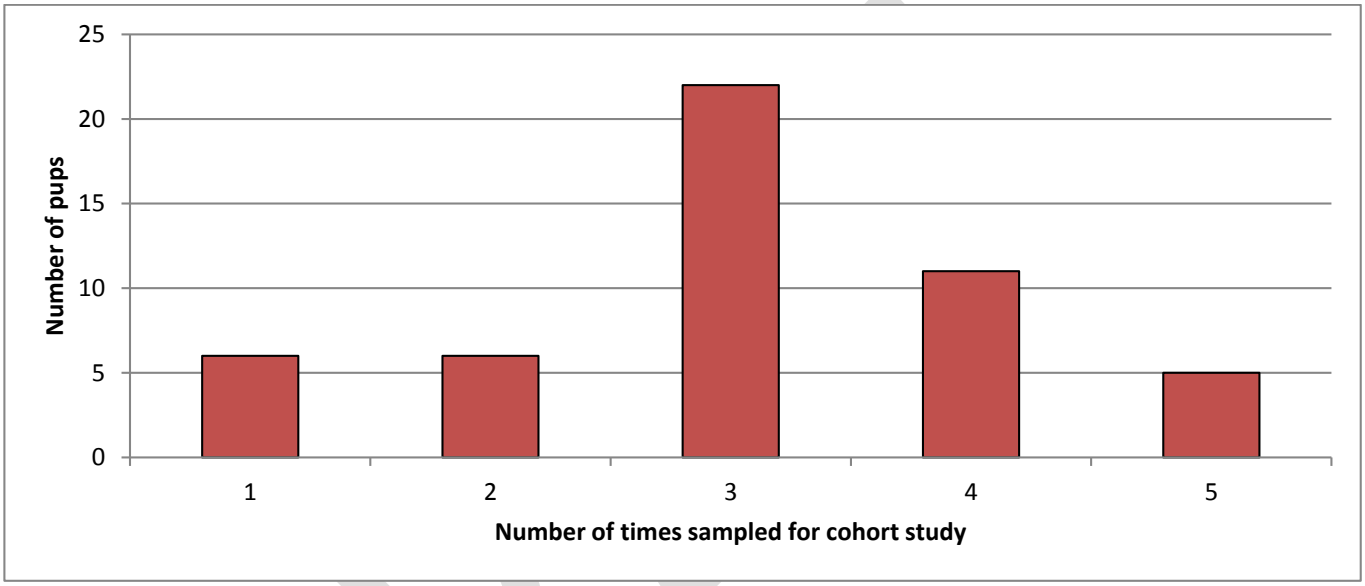

Figure 10. Number of times that pups were located and sampled for the cohort study at Sandy Bay, Enderby Island.

Investigation of avian reservoirs of *K. pneumoniae*

Sub-Antarctic skuas are sympatric with New Zealand sea lions at Sandy Bay. Skuas are well known to scavenge from sea lion carcasses, including those of pups infected with *K. pneumoniae* and may act as reservoirs of infection in the immediate area and potentially as vectors during dispersal to distant locations. Previous studies have shown that skuas are capable of apparent asymptomatic carriage of *K. pneumoniae* in their digestive tracts (S. Michael, unpublished data), so collection of samples from permanently identified birds was required to evaluate prevalence.

Forty four sub-Antarctic skua were successfully captured by hand net at Sandy Bay and leg banded with a uniquely numbered metal band, with a colour pattern applied in reflective tape to enable individual identification from a distance (Figure 11). Banding of skuas was undertaken between 20 December 2016 and 17 February 2017. Cloacal swabs (and voided faeces opportunistically) were collected from all skuas following banding.

As observed in 2014-15, the number of skuas present at Sandy Bay was highest in December and January during pupping and decreased due to dispersal until there were few from February onwards. Repeat sampling was undertaken by collection of voided faeces from identified individuals. Only eight repeat samples were collected as

banded birds had left the area following dispersal of the sea lions and the birds remaining were unbanded. All cloacal and voided samples were frozen in liquid nitrogen for later microbiological culture.

Figure 11. Banded sub-Antarctic skua (yellow/orange) scavenging from an adult NZ sea lion carcass following necropsy at Sandy Bay, Enderby Island.

Investigation of substrate for *K. pneumoniae*

Microbiological analysis of substrate (soil, mud, water and sand) from Sandy Bay late in the season has previously shown the presence of *K. pneumoniae* in the environment. It is currently not known whether *K. pneumoniae* is present in the environment at Sandy Bay before pups are born, serving as a reservoir for infection. In order to fill this knowledge gap, substrate from a suite of sites around Sandy Bay (Figure 12) and wider Enderby Island were collected at three time points: early (mid-December, on arrival); mid (early February) and late season (mid-March). A standard set of twelve sites at Sandy Bay was used, with opportunistic collections from other sites of congregation of pups as required. Analysis of these samples will be undertaken as part of a separate PhD project that is currently underway.

Figure 12. GPS waypoints of substrate sampling sites at Sandy Bay, Enderby Island.

Round island surveys of Enderby Island

The number of round island surveys was limited this season due to the high workload at Sandy Bay and the dramatically reduced CSP resighting period. Over the season, three complete round island surveys (including Teal Lake, South East Point, East Bay and Derry Castle zones) were undertaken with another five partial surveys (Teal Lake and South East Point only) later in the season, targeting resights of dispersing females and pups. As expected, as numbers of animals at Sandy Bay decreased after the pupping and mating period, round island counts increased, particularly at Teal Lake and South East Point (Figure 13).

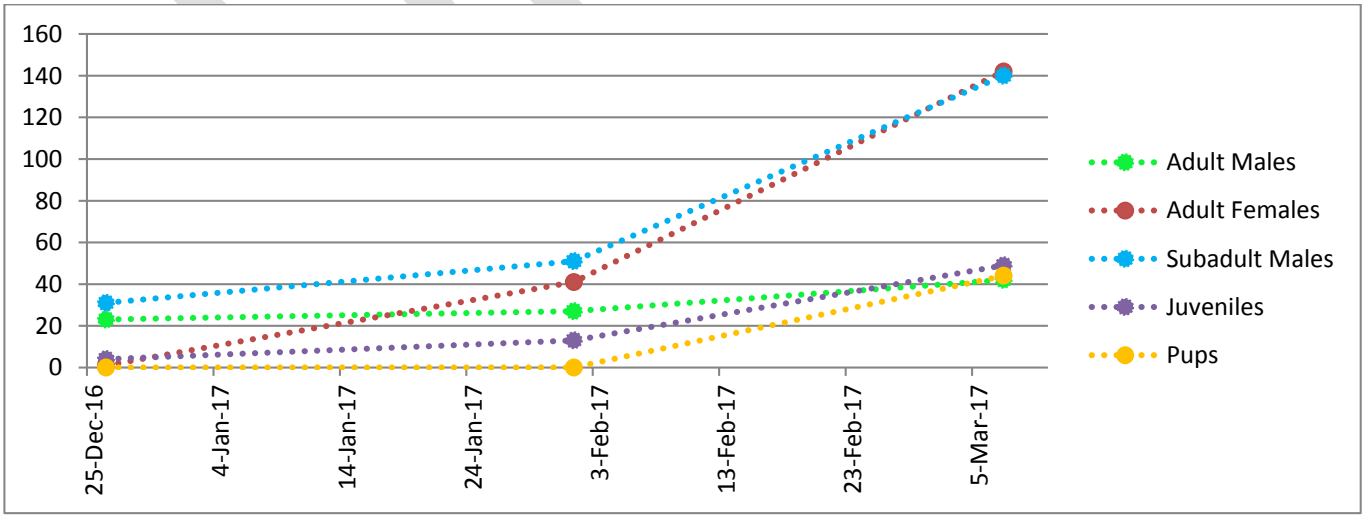

Figure 13. Total counts of New Zealand sea lions from three complete round island surveys at Enderby Island. Counts do not include Sandy Bay.

Survey of Port Ross Islands

Approximately half a day was available before leaving the Auckland Islands to survey Port Ross Islands for dispersal of pups and identification of sick or dead pups. Many pups leave Sandy Bay by sea with their mothers from late January so dispersal around Port Ross is likely. A similar survey was undertaken over two days in late March 2015, where a pup with clinical signs of septic arthritis (possible *K. pneumoniae* infection) and a dead pup were found on Ewing Island.

Partial surveys of Rose and Ewing Islands were undertaken on 13 March 2017 for counts (Table 3), resighting and searches for sick and dead pups. No sick or dead pups were found and fewer total pups were found compared to the last survey. This is not unexpected given the short time period available for the survey (3 hours on Rose Island and 2 hours on Ewing Island), a team of three to cover a large area that is in parts difficult to traverse and the time period being a fortnight earlier than in 2015. Fourteen of the pups located on Rose Island were of Sandy Bay origin, while none of the Ewing Island pups originated from Sandy Bay.

Table 3. Counts of New Zealand sea lions from partial surveys of Rose and Ewing Islands on 13 March 2017. Note that only a partial survey was conducted.

	Adult males	Adult females	Subadult males	Juveniles	Live pups
Rose Island	8	33	3	4	26
Ewing Island	0	4	2	0	13

Processing and analysis of samples

This field study has generated a substantial number of samples from pups including 1236 oral and rectal swabs, 306 blood smears, 153 serum samples, 282 post mortem tissue samples in addition to a large volume of risk factor data. Planned investigation through microbiology, histopathology and epidemiology has begun and findings will become available over the course of Sarah Michael's PhD project.

Acknowledgments

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- Equipment, logistics and procurement: Tony Russell, Mike Hogan, Craig Thomas, Anne Midwinter, Kristene Gedye, Peter Wildbore (Massey University); Margaret Anderson (NZVP, Palmerston North)
- Database development: Ahmed Fayaz (Massey University)

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