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

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Klebsiella pneumoniae in NZ sea lions

- 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

Risk factors involved in *K. pneumoniae* infection may be amenable to active management

Research Questions

- 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?

- **How can pup mortality be mitigated?**

Components of the 16/17 field season

- Monitoring of pupping to determine pup birthdate and maternal ID
- Permanent individual identification of Sandy Bay pups
- Ivermectin treatment trial to investigate hookworm carriage as risk factor for death
- Necropsy of dead pups to designate cause of death
- Case control study to investigate risk factors for pup mortality and *K. pneumoniae* infection
- Prospective cohort study to investigate morbidity and pup response to disease
- Investigation of avian reservoirs of *K. pneumoniae* by sampling skuas
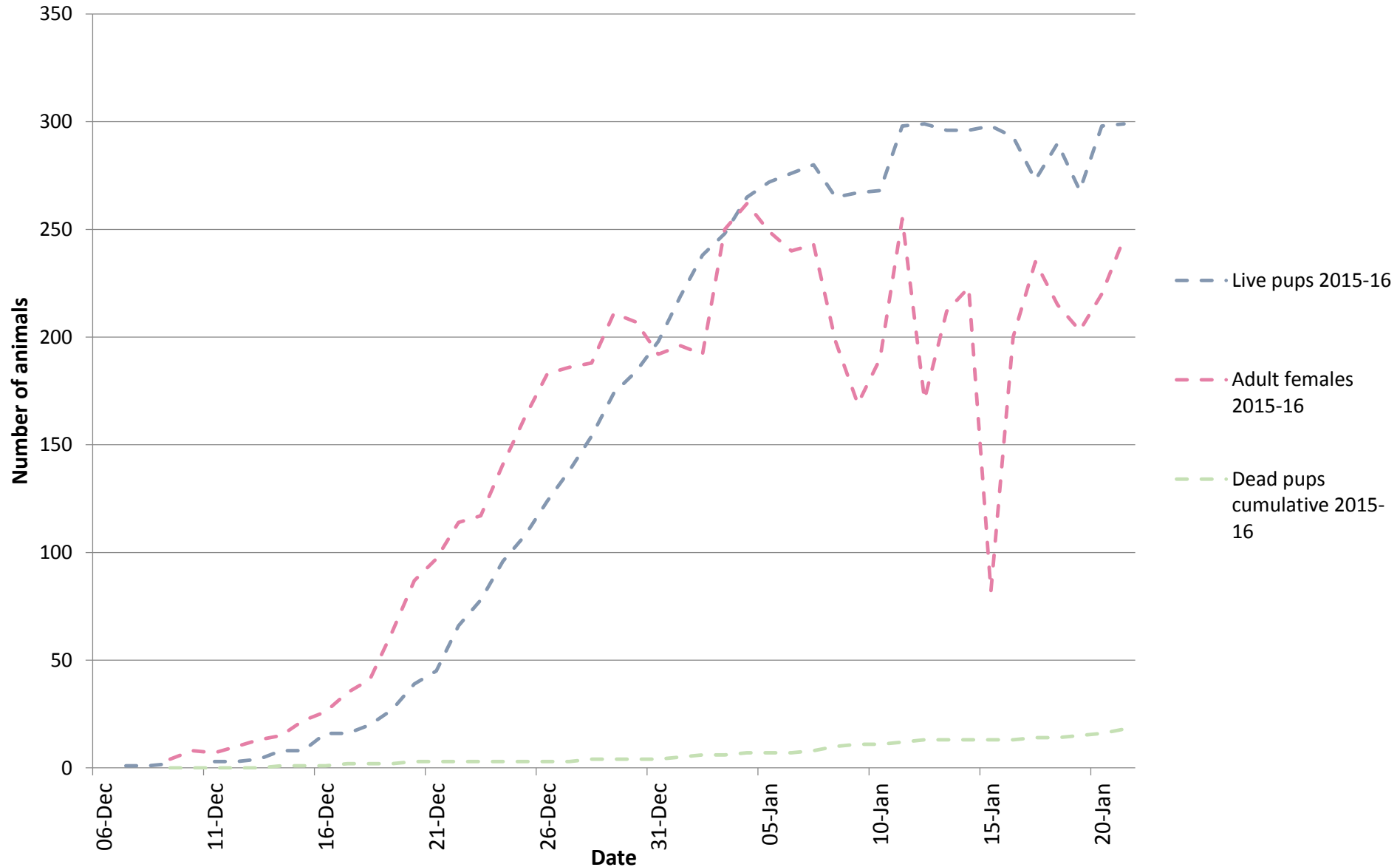- Investigation of environmental reservoirs of *K. pneumoniae*
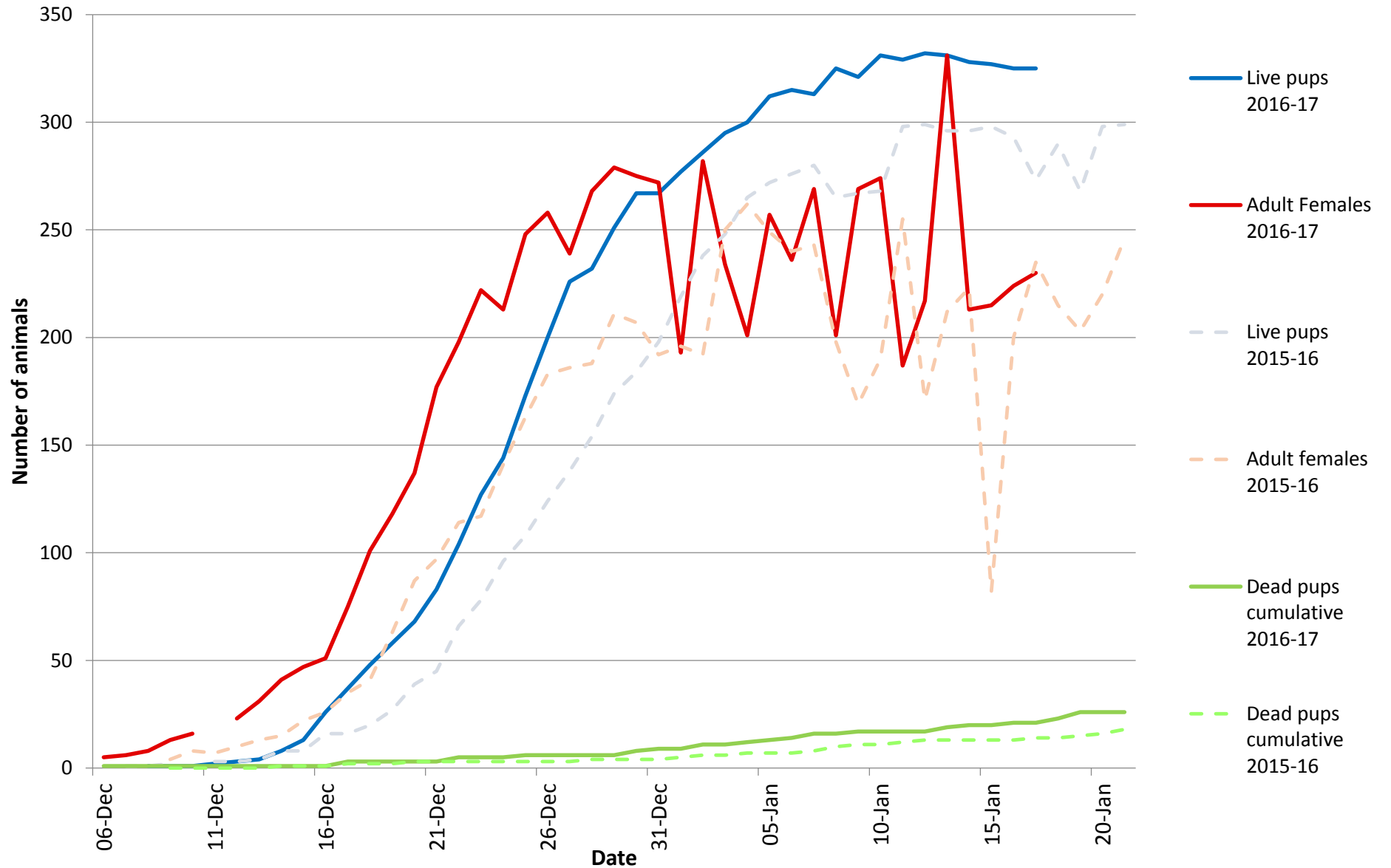
Season Summary

- 11 December 2016 – 13 March 2017 at Sandy Bay, Enderby Island
- Collection of 1236 oral and rectal swabs, 306 blood smears, 153 serum samples from NZ sea lion pups for control and cohort studies
- 77 NZ sea lion post mortem examinations completed yielding 282 tissue samples
- 44 sub-Antarctic skuas captured, banded and sampled
- 61 substrate samples from around Enderby Island at several time points
- Three complete Enderby Island surveys and five partial surveys

NZ sea lion counts – Sandy Bay 2015-16

Legend:
- Live pups 2015-16
- Adult females 2015-16
- Dead pups cumulative 2015-16

Y-axis: Number of animals

X-axis: Date (06-Dec, 11-Dec, 16-Dec, 21-Dec, 26-Dec, 31-Dec, 05-Jan, 10-Jan, 15-Jan, 20-Jan)

2015-16 counts courtesy of Blue Planet Marine, NZ

NZ sea lion counts – Sandy Bay 2016-17

2015-16 counts courtesy of Blue Planet Marine, NZ

New Years Storm

- Southeast storm between 31 December 2016 and 1 January 2017
- Average wind speeds over 40 knots (74km/h) causing storm surge at Sandy Bay
- Many adults and pups moving up to the sward earlier than usual

New Years Storm

- 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

Aerial photos courtesy of Barry Baker

Individual marking of pups

- **To enable estimation of birth date as a risk factor**

- As soon as possible after birth
- Initially with stock marker but did not last long with sand abrasion and weather
- Afterwards by resighting of females giving birth (flipper tags, PIT tags or where no other identification was present, distinctive scars), so they could later be associated with their pups once marked

Linking females and pups by distinctive scars

First captures

- Weight, length and girth, full physical examination, oral and rectal swabs
- Temporary vinyl cap with identification number glued to the rump
- PIT tag inserted subcutaneously in the dorsal pelvic region
- Pups randomly allocated to ivermectin treatment or control groups

- **341 pups processed for first capture from 16 December 2016 to 15 January 2017**
- **163 pups randomly allocated to the ivermectin treatment group and 178 controls**

Photo courtesy of Thomas Burns

Flipper tagging

- Tag application as part of the CSP segment of the field season was altered due to incorrect printing of tags
- Many tags became almost unreadable due to number being printed over an embossing mark
- Subjectively increased tag loss in first 2 months (opportunistic sightings)
  - 26 pups with one tag lost
  - 7 pups with both tags lost
  - Tags lost by tearing through trailing edge of flipper
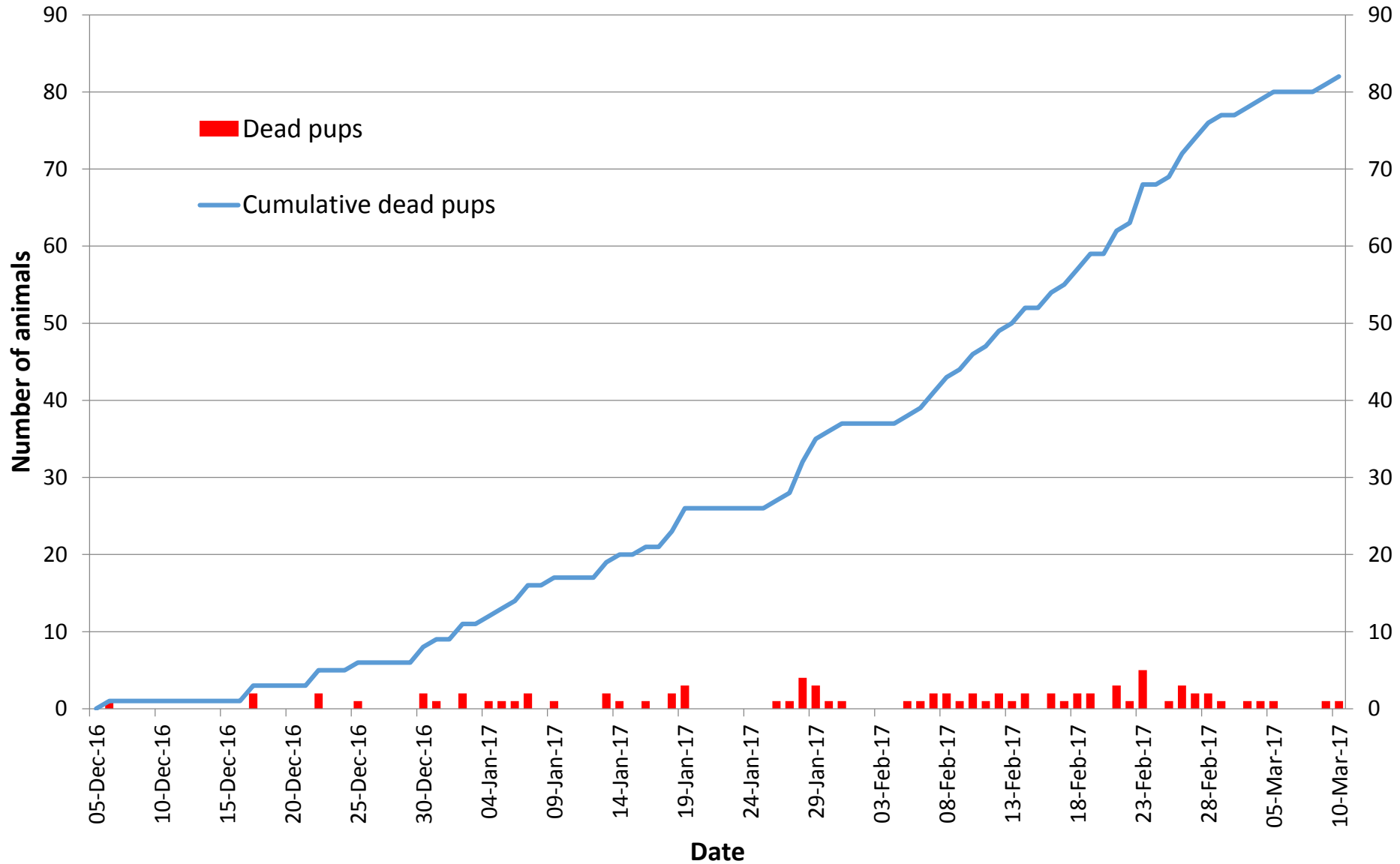- Implications for demographic and survival assessments of this cohort in future years

Pup mortality

- **Total dead pups between 6 December 2016 and 12 March 2017 was 82**

- Of these, 75 were in adequate condition for necropsy
- All died at Sandy Bay, except for one found at Teal Lake in a decomposed state

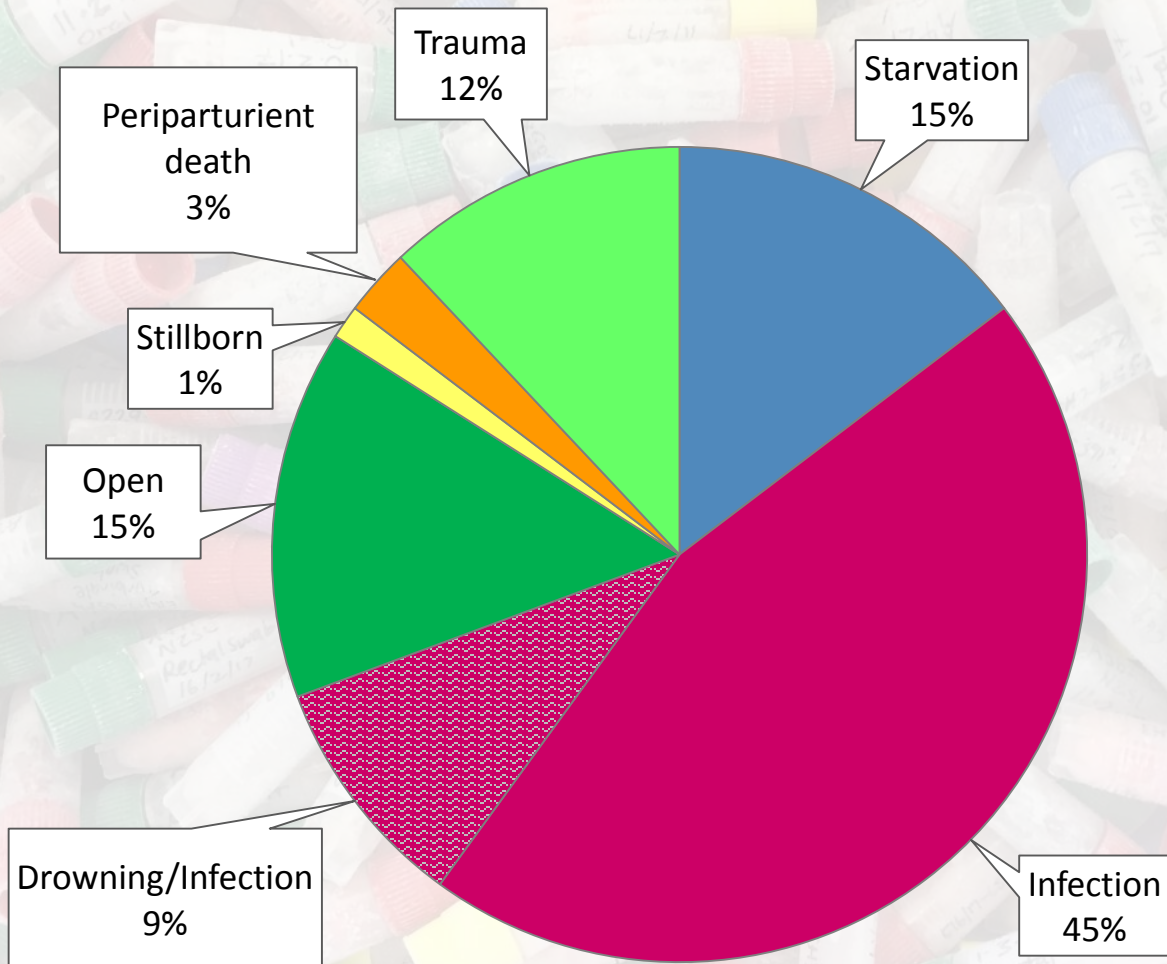# Daily and cumulative counts of pup mortality

Pup mortality

- Female pup deaths over-represented
  - 32 males : 43 females
- All dead pups were born on Enderby Island except for eight confirmed or suspected Dundas Island-origin pups

- Of Enderby born pups that died, 21 had received ivermectin compared with 41 that were controls
  - Increased immune capacity in absence of hookworm infestation?
  - *K. pneumoniae* transmissions through the intestinal tract potentiated by damage by hookworms?

Photo courtesy of Thomas Burns

Provisional diagnosis based on gross necropsy

Trauma
12%

Starvation
15%

Periparturient
death
3%

Stillborn
1%

Open
15%

Drowning/Infection
9%

Infection
45%

Pup mortality

- Seven pups 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 and the consistent post mortem findings of milk aspiration in all seven indicated that he most likely suffocated them by the same means.

Pup mortality

- Seven dead pups found in small pools or wedged in shallow streams that should normally be escapable or had ramps installed

- All pups had gross necropsy findings of drowning but also those consistent with *K. pneumoniae* infection

- Pups 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 cause of death will be carried out with histopathology and microbiology analysis.

Case control study

PUP IS FOUND DEAD

To understand risk factors for pup mortality and interaction between predisposing factors

Dead pup data collected eg. GPS coordinates, habitat, substrate and circumstances of death

Pup brought back to base for necropsy

TWO LIVE CONTROL PUPS RANDOMLY SELECTED

Pup identified by tag and/or chip

Pups identified by tag and/or chip and captured by hand or net

Pup weighed, sexed and measured

Pups weighed, sexed and measured, given physical examination

Throat/rectal swabs for later culture to determine *K. pneumoniae* carriage

Necropsy undertaken with collection of routine samples as well as any abnormal lesions

Faeces collected for assessment of hookworm presence or absence

Preliminary diagnosis assigned

Data collected about risk factors eg. location, weather, pup and maternal variables

Random selection of control pups

- A grid system was successfully used for random selection of control pups

- In the early season a strip of north-south grid squares (10 metres wide) was randomly selected allowing marking of the boundaries, a count of all available pups within the swathe, a random number to be selected and a pup chosen

- Once pups had dispersed from the beach, the 20 metre Sandy Bay grid was used. 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
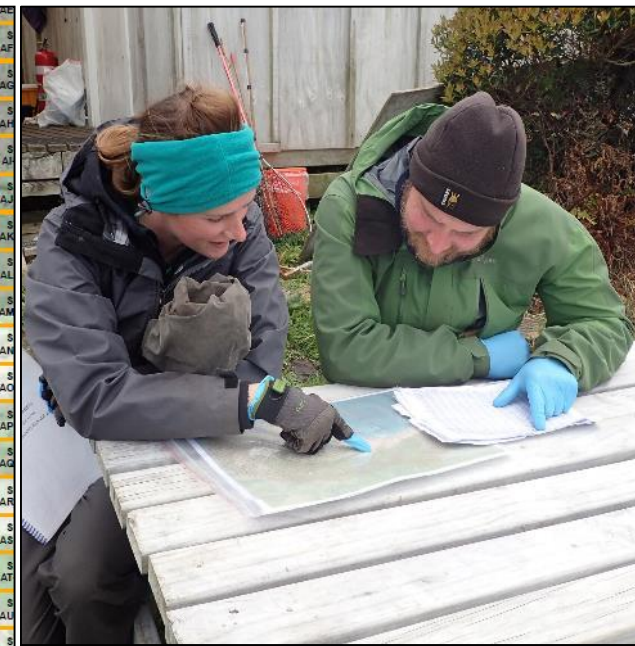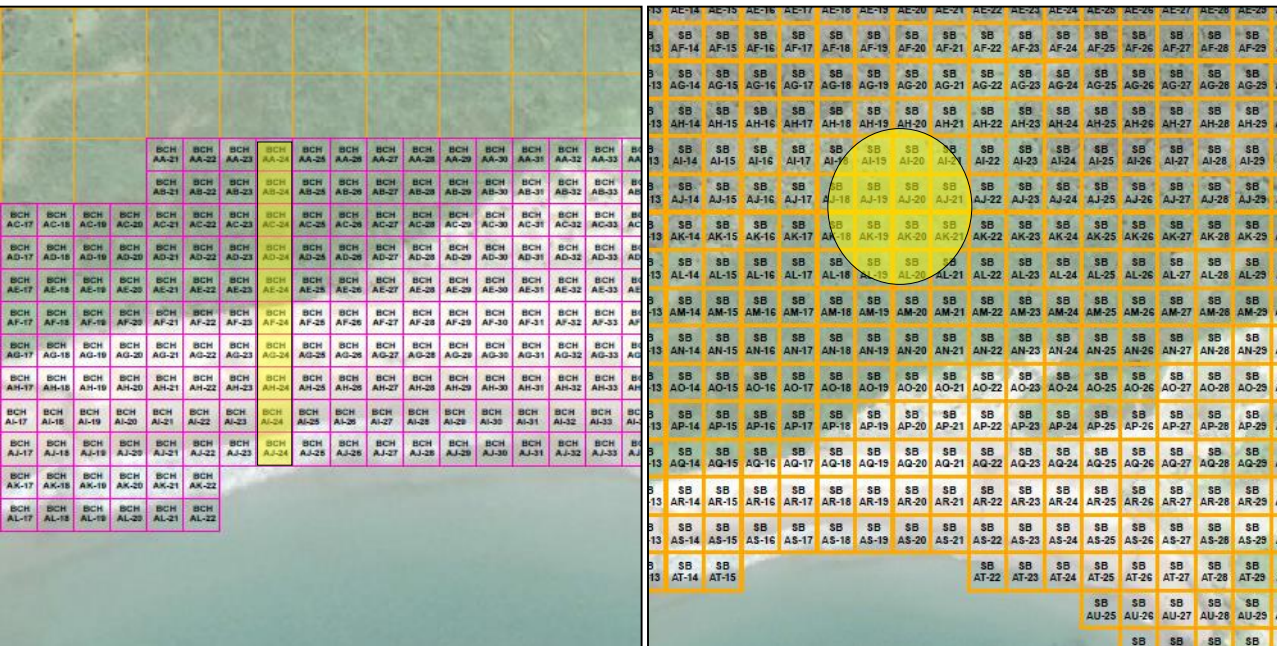
The other criteria included:

- Pups that are nursing are excluded from selection
- If one pup is present in the circle, it is selected
- 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
  - To select from a pod, a fraction is 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
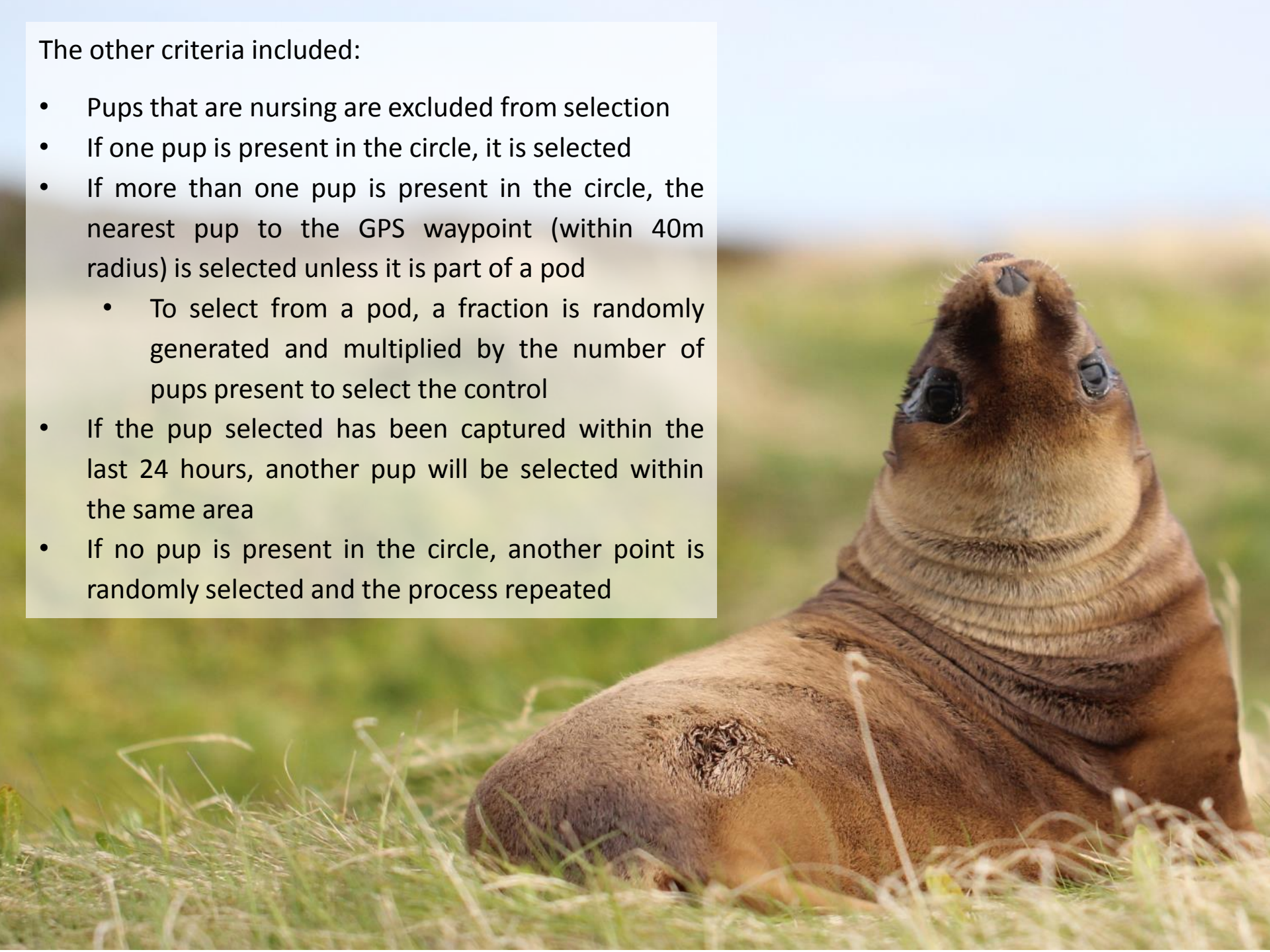
Case control study
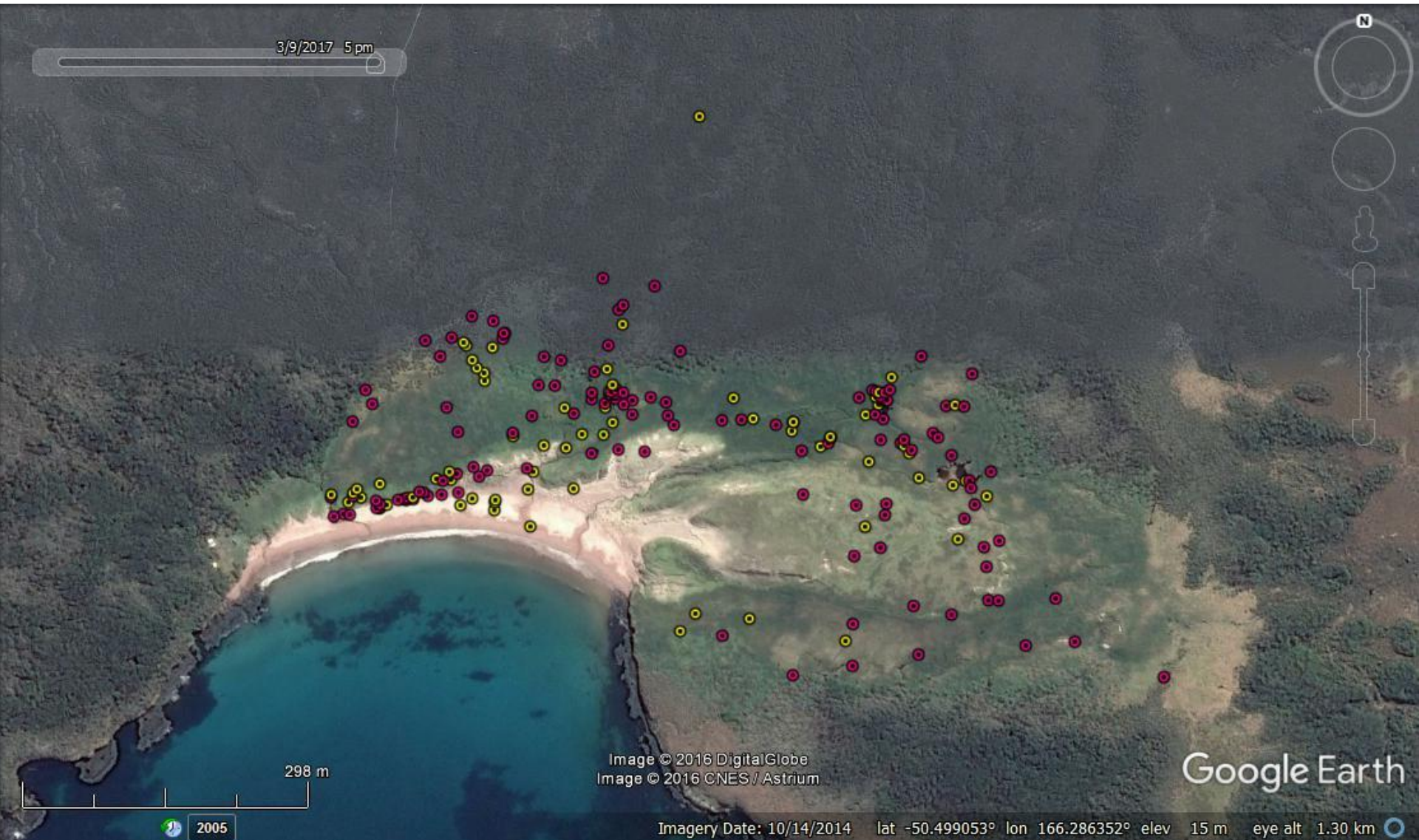
Control captures

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

Risk factors assessed:
- Location, substrate, habitat
- Pup variables (eg. recent nursing, age, number of times handled)
- Environmental variables (eg. weather, skua presence, tourist interactions)
- Clinical variables (eg. wounds, conjunctivitis, umbilical infection)

Samples collected:
- Oral and rectal swabs to assess *K. pneumoniae* carriage
- Faeces for hookworm determination
- Toe clip for genetics

Prospective cohort study

To investigate the temporal sequence of events leading to pup death including the role of multiple concurrent risks, in order to calculate the incidence of disease.

- Relatively 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.
- Pups caught approximately fortnightly for assessment

Photo courtesy of Thomas Burns

Prospective cohort study

Each capture involved:

- Weight and morphometric measurements

- Full physical examination

- Assessment of risk factors

- Rectal temperature

- Samples collected:
  - Oral and rectal swabs
    - Frozen for later *K. pneumoniae* determination
  - Faecal collection
    - Examined in the field for hookworm presence
  - Blood collection via brachial vein
    - Processed in the field for PCV, total protein and blood smears, serum separated and frozen

Prospective cohort study

- 50 pups were recruited to the study at first capture by selection of every sixth pup
  - 28 male (11 ivermectin, 17 control)
  - 22 female (11 ivermectin, 11 control)
- Twelve cohort pups died throughout the season (3 ivermectin, 9 control)

Prospective cohort study

Early and widespread dispersal of pups deep into the rata forest and by sea to other islands, made locating specific pups for serial sampling difficult
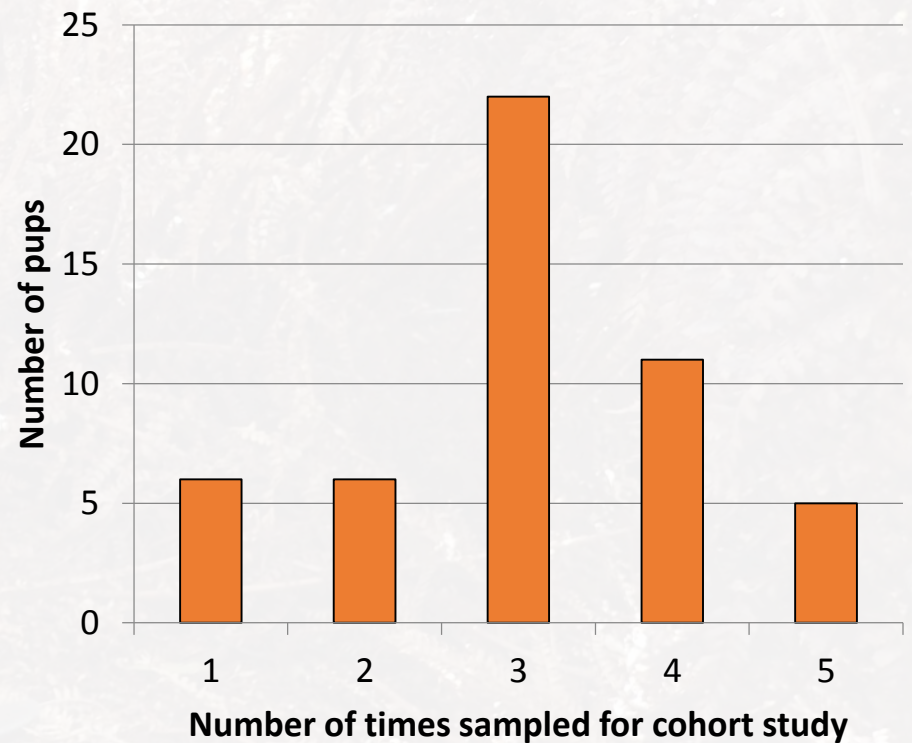
Avian reservoirs of *K. pneumoniae*

- Sub-Antarctic skuas (*Catharacta antarctica lonnbergi*) scavenge from carcasses of pups infected with *K. pneumoniae*
- Skuas are capable of apparent asymptomatic carriage of *K. pneumoniae* in their digestive tracts and may act as reservoirs of infection in the immediate area and potentially as vectors during dispersal to distant locations

Collection of samples from permanently identified birds was required to evaluate prevalence

Avian reservoirs of *K. pneumoniae*

- Forty four sub-Antarctic skua were successfully captured by hand net at Sandy Bay and leg banded with a uniquely numbered metal band between 20 December 2016 and 17 February 2017

- Cloacal swabs (and voided faeces where present) were collected from all skuas following banding and frozen for later culture for *K. pneumoniae*

Only eight repeat (voided) samples were collected as banded birds had left the area following dispersal of the sea lions and the birds remaining were unbanded

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

Investigation of substrate for *K. pneumoniae*

- In order to fill this knowledge gap, substrate 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)
   - late season (mid-March)

Investigation of substrate for *K. pneumoniae*

Wider Enderby substrate sampling at sites representative of known sea lion congregation areas
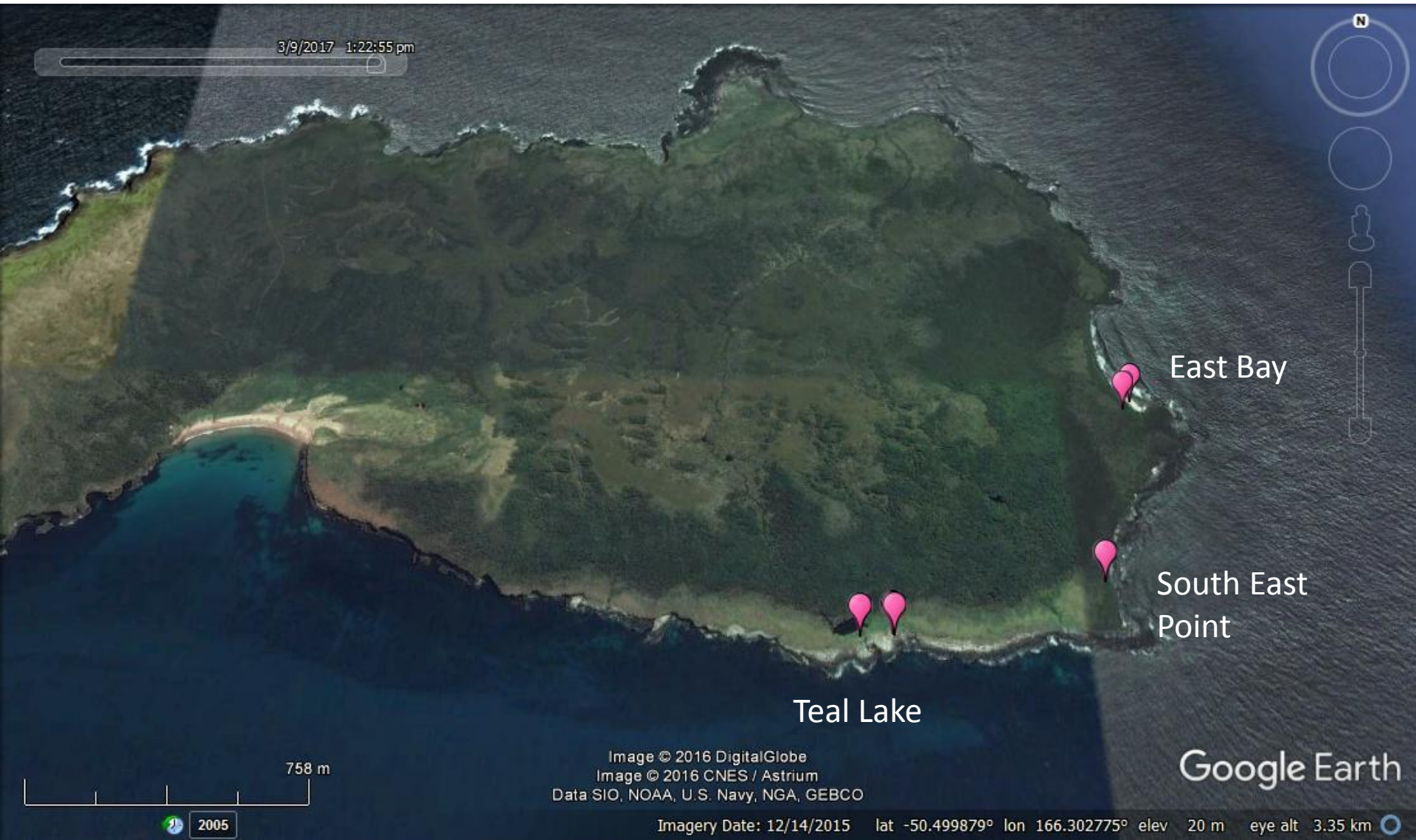
Port Ross Islands Survey

- 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
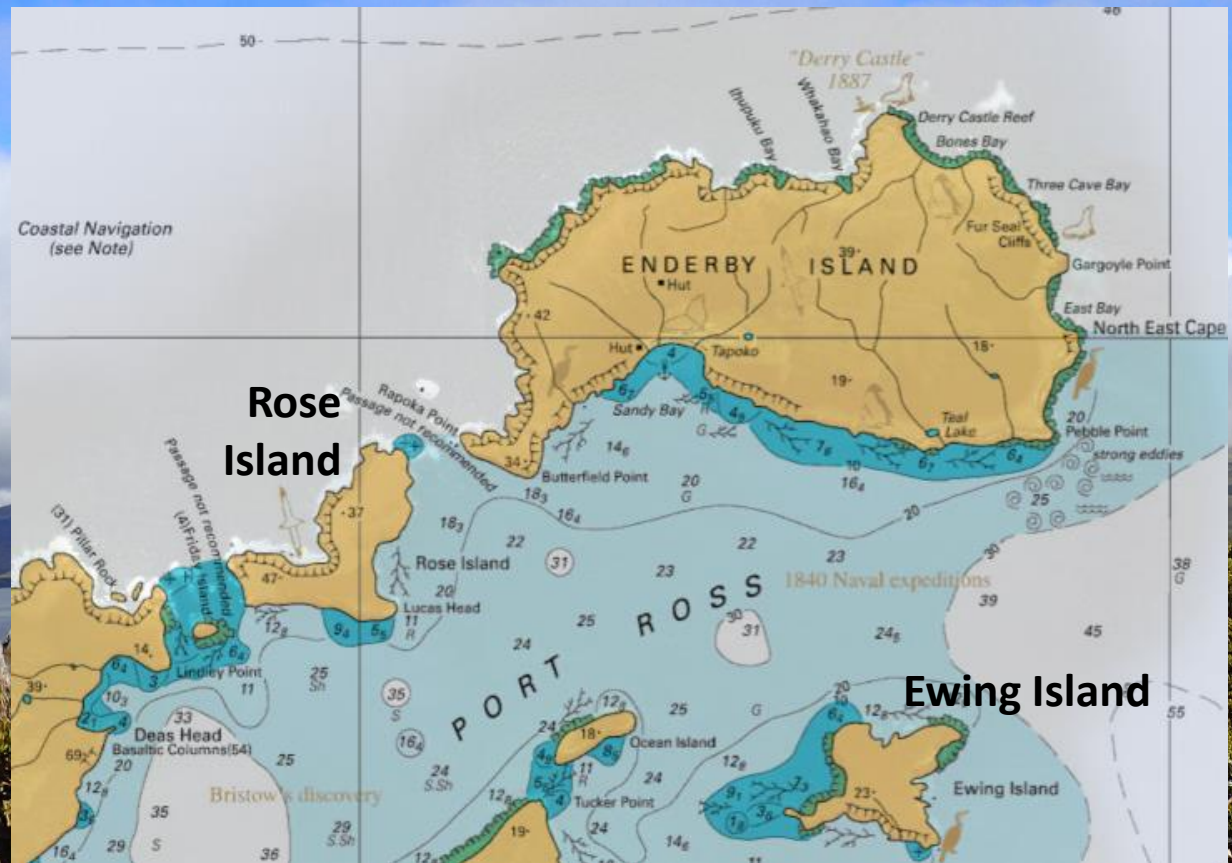
Port Ross Islands Survey

- Rose Island: 3 hours survey time, 26 pups found, 14 of Sandy Bay origin
- Ewing Island: 2 hours survey time, 13 pups found, none of Sandy Bay origin
- No dead pups found
- Lower pup counts, likely due to only partial coverage of islands with less people and two weeks earlier than previous survey

This field study has generated a substantial number of samples from pups 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 my PhD project
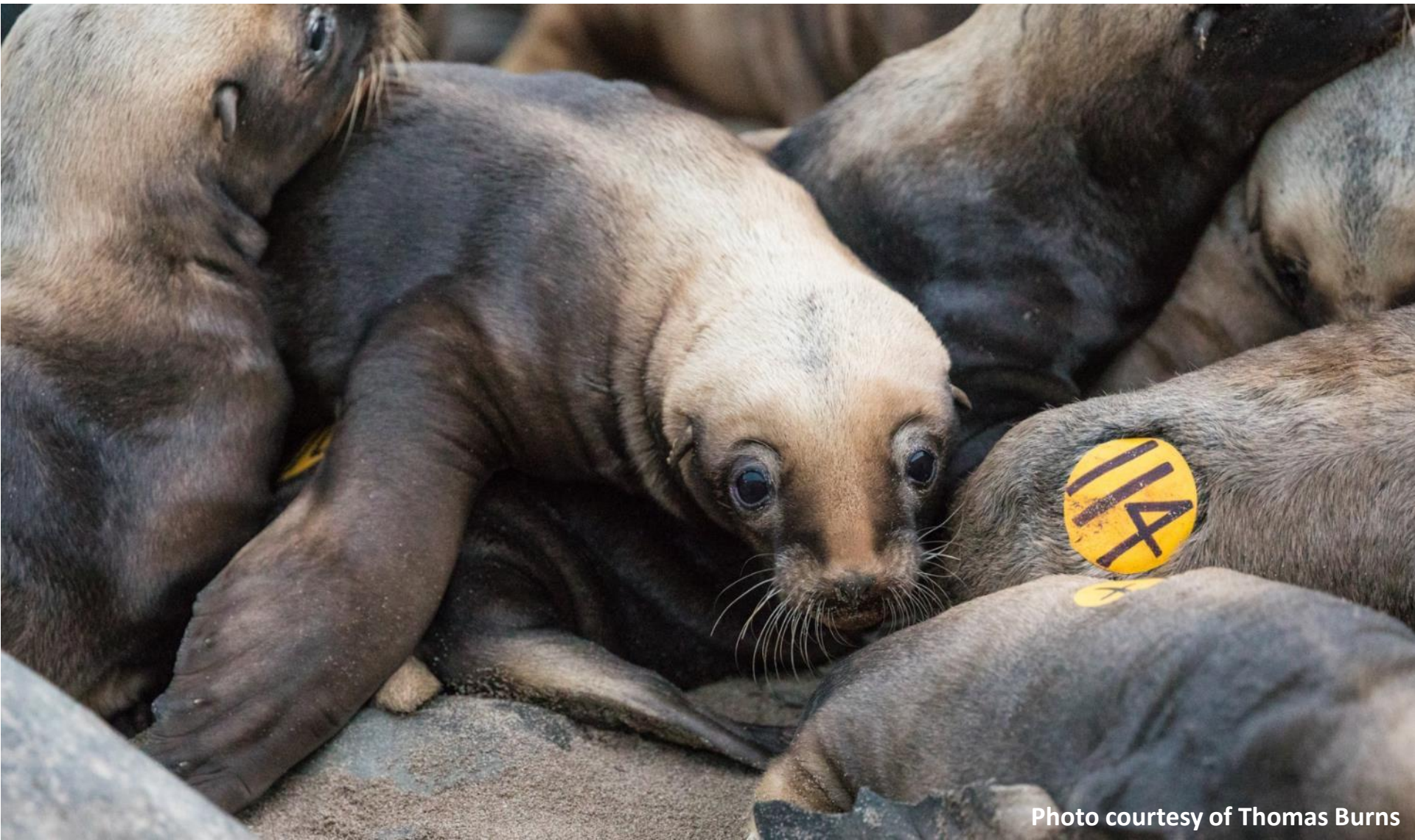
Results of lab analysis will feed back into risk factor data to determine the most important risk factors for pup mortality. Following this, options for mitigation can be assessed.

Acknowledgements

Field Team: Tom Burns, Shannon Taylor, Rachael Gray

Acknowledgements

- Steve Kafka and crew of RV Evohe
- Andy Whittaker and crew of RV Baltazar
- Personnel on Enderby Island for assistance and support in the field
- Helicopter team : Barry Baker, Mark Hayes and Katie Clemens-Seely
- DOC Southland Murihiku staff : John Peterson, Rhuari Hannan, Jo Hiscock, Sharon Trainor, Joseph Roberts, Kathryn Pemberton, Tony Preston
- Rakiura Stewart Island Visitor Centre staff
- DOC Wellington : Laura Boren, Katie Clemens-Seely, Ian Angus
- Bird banding : Michelle Bradshaw, Sandy Taylor, Graeme Taylor (DOC Wellington); Colin Miskelly (Te Papa, Wellington)
- Enderby Island grids: Andrea Sward (DOC Geospatial Services, Wellington)
- Equipment and procurement: Tony Russell, Mike Hogan, Craig Thomas, Anne Midwinter, Kristene Gedye, Peter Wildbore (Massey University)
- Haematology: Margaret Anderson (NZVP, Palmerston North)
- Database development: Ahmed Fayaz (Massey University)