Proposal and Methodology for New Zealand Sea Lion Disease Research, Auckland Islands 2016-17

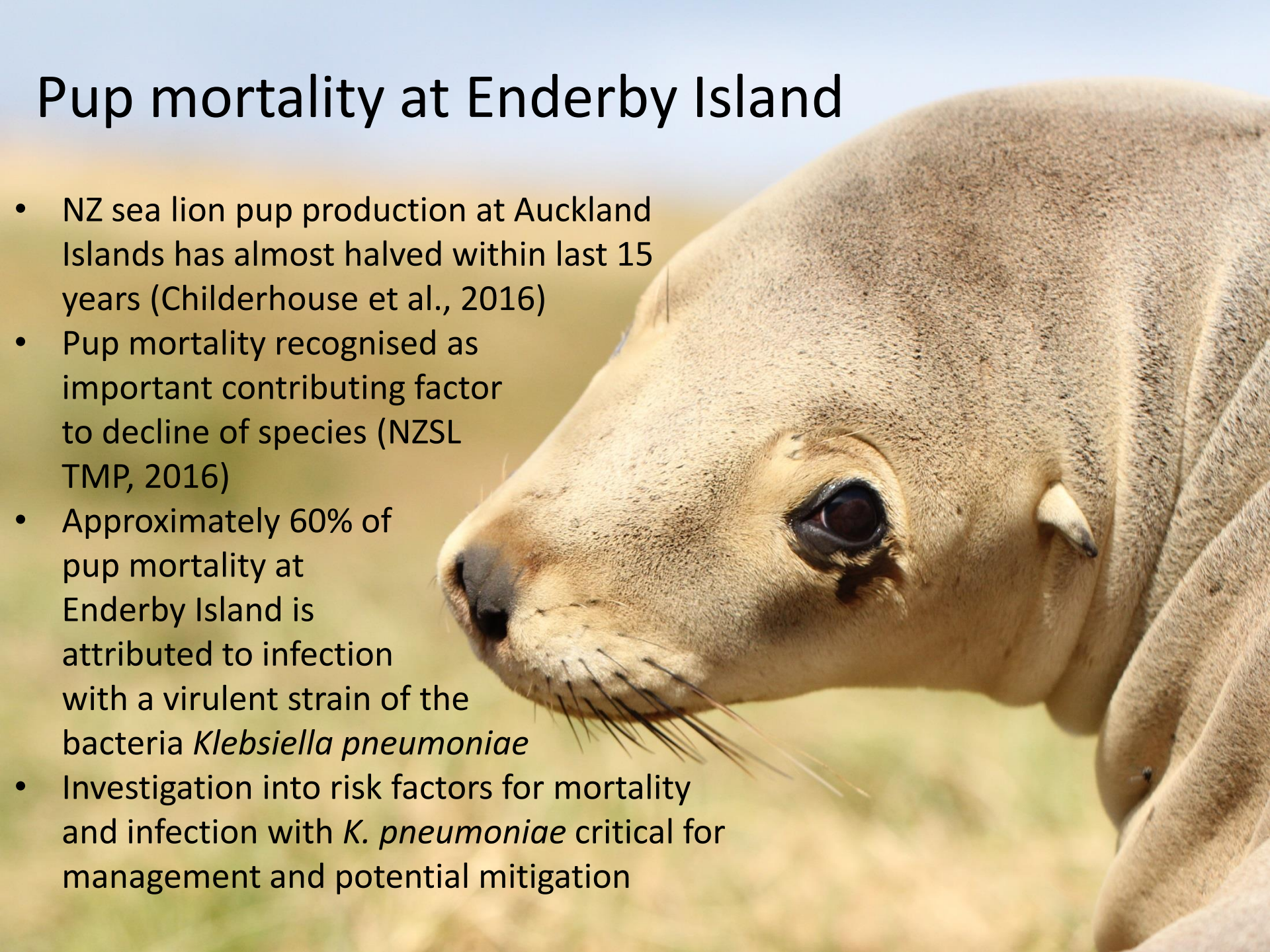
Sarah Michael, Rachael Gray, David Hayman and Wendi Roe

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

THE UNIVERSITY OF SYDNEY

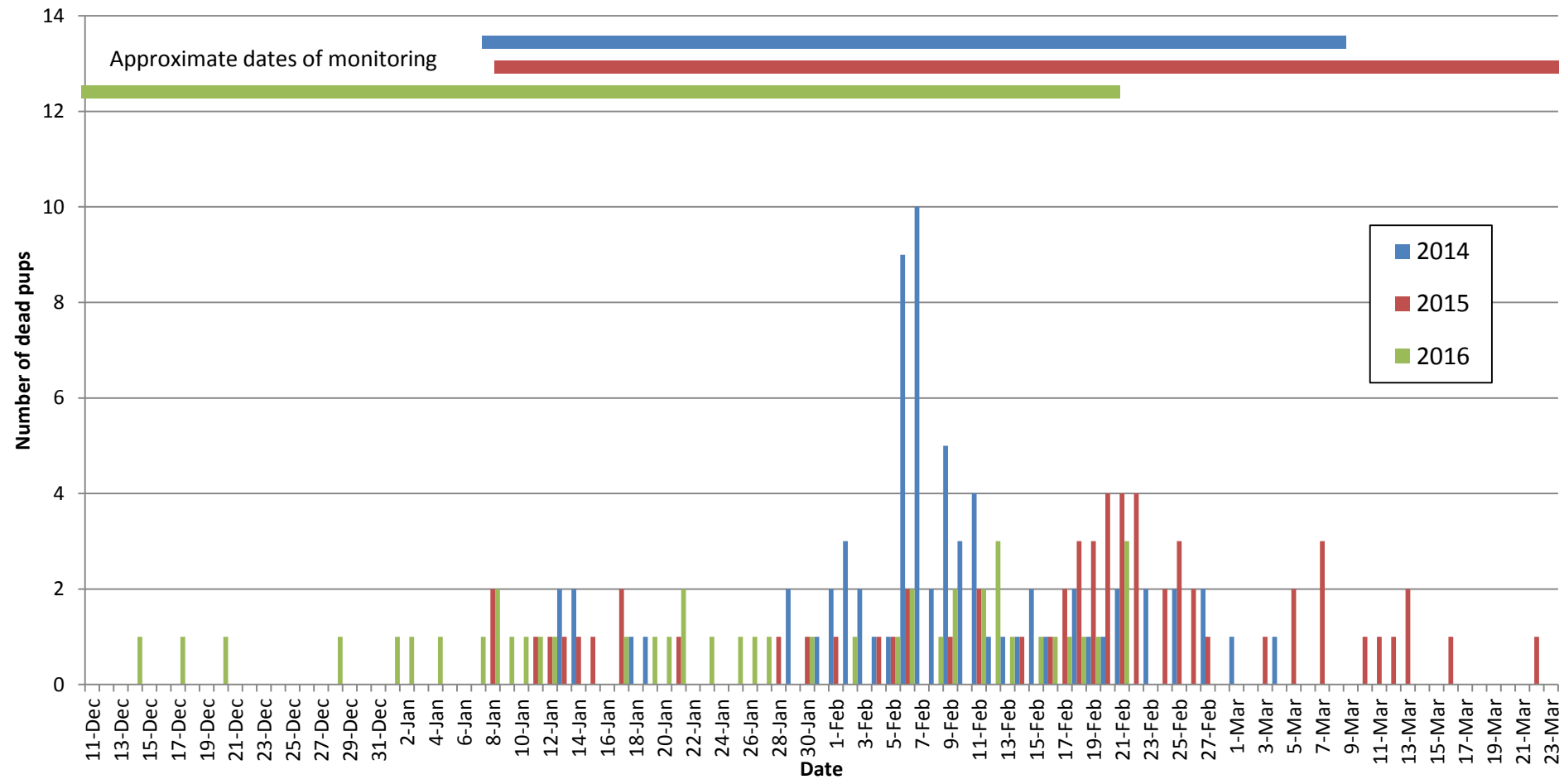
Department of Conservation
Te Papa Atawhai

Pup mortality at Enderby Island



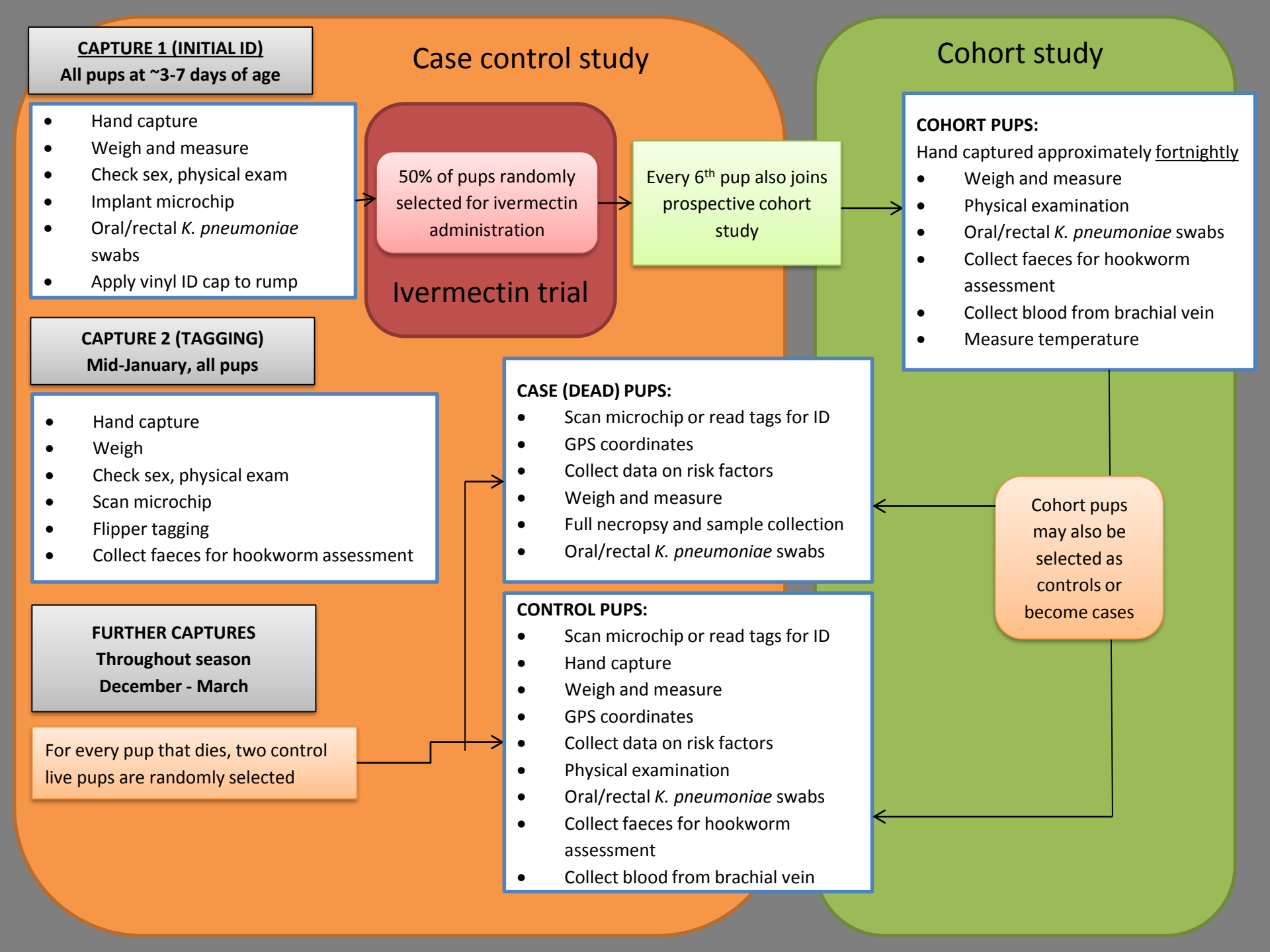
- NZ sea lion pup production at Auckland Islands has almost halved within last 15 years (Childerhouse et al., 2016)
- Pup mortality recognised as important contributing factor to decline of species (NZSL TMP, 2016)
- Approximately 60% of pup mortality at Enderby Island is attributed to infection with a virulent strain of the bacteria *Klebsiella pneumoniae*
- Investigation into risk factors for mortality and infection with *K. pneumoniae* critical for management and potential mitigation

Pup deaths at Enderby Island by season 2014-2016



Project components

- Case control study
  - To understand risk factors for pup mortality and interaction between predisposing factors
- Ivermectin treatment trial
  - To understand impact of hookworm carriage on mortality and *K. pneumoniae* infection
- Prospective cohort study
  - To understand pup health status and body's response to progression of disease


Case control study
Cohort study
CAPTURE 1 (INITIAL ID)
All pups at ~3-7 days of age
Hand capture
Weigh and measure
Check sex, physical exam
Implant microchip
Oral/rectal K. pneumoniae swabs
Apply vinyl ID cap to rump
50% of pups randomly selected for ivermectin administration
Ivermectin trial
Every 6th pup also joins prospective cohort study
COHORT PUPS:
Hand captured approximately fortnightly
Weigh and measure
Physical examination
Oral/rectal K. pneumoniae swabs
Collect faeces for hookworm assessment
Collect blood from brachial vein
Measure temperature
CAPTURE 2 (TAGGING)
Mid-January, all pups
Hand capture
Weigh
Check sex, physical exam
Scan microchip
Flipper tagging
Collect faeces for hookworm assessment
CASE (DEAD) PUPS:
Scan microchip or read tags for ID
GPS coordinates
Collect data on risk factors
Weigh and measure
Full necropsy and sample collection
Oral/rectal K. pneumoniae swabs
Cohort pups may also be selected as controls or become cases
FURTHER CAPTURES
Throughout season
December - March
For every pup that dies, two control live pups are randomly selected
CONTROL PUPS:
Scan microchip or read tags for ID
Hand capture
Weigh and measure
GPS coordinates
Collect data on risk factors
Physical examination
Oral/rectal K. pneumoniae swabs
Collect faeces for hookworm assessment
Collect blood from brachial vein

Field logistics 2016/17

- On island from approximately 12 December 2016 – 16 March 2017
- Team of minimum 3 project personnel at a time throughout season

Identification of pup date of birth

- Monitoring of colony from arrival at island to identify pups as they are born
- Initial mark of day old pups where possible with non-toxic stock paint on fur with colour or pattern specific to date of birth
- Applied from end of ~3m pole to minimise disturbance to pup and mother

First capture

- **All pups, hand capture at ~3-7 days of age or when unattended by mother**
- Determine date of birth based on colour mark on fur
- Measurement of weight, length, girth and check sex
- Physical examination for wounds, injuries, signs of infection
- Implantation of microchip in dorsal lumbar region and ID cap glued to rump
- Oral and rectal swabs to detect *K. pneumoniae* carriage
- 50% pups randomly chosen to join ivermectin treatment group
- Every 6th pup (to a total of 50) joins prospective cohort study

Ivermectin treatment trial

- Broad spectrum anti-parasitic
- NZ sea lion pups have up to 100% prevalence of hookworm (*Uncinaria* sp.) carriage at Sandy Bay (Castinel et al., 2007)
- Ivermectin is safe and effective in NZ sea lions and other pinnipeds
- Treatment efficacy tested by collection of faeces at tagging
- **Results will feed into case control study to determine associations between hookworm infestation and *Klebsiella* infection**

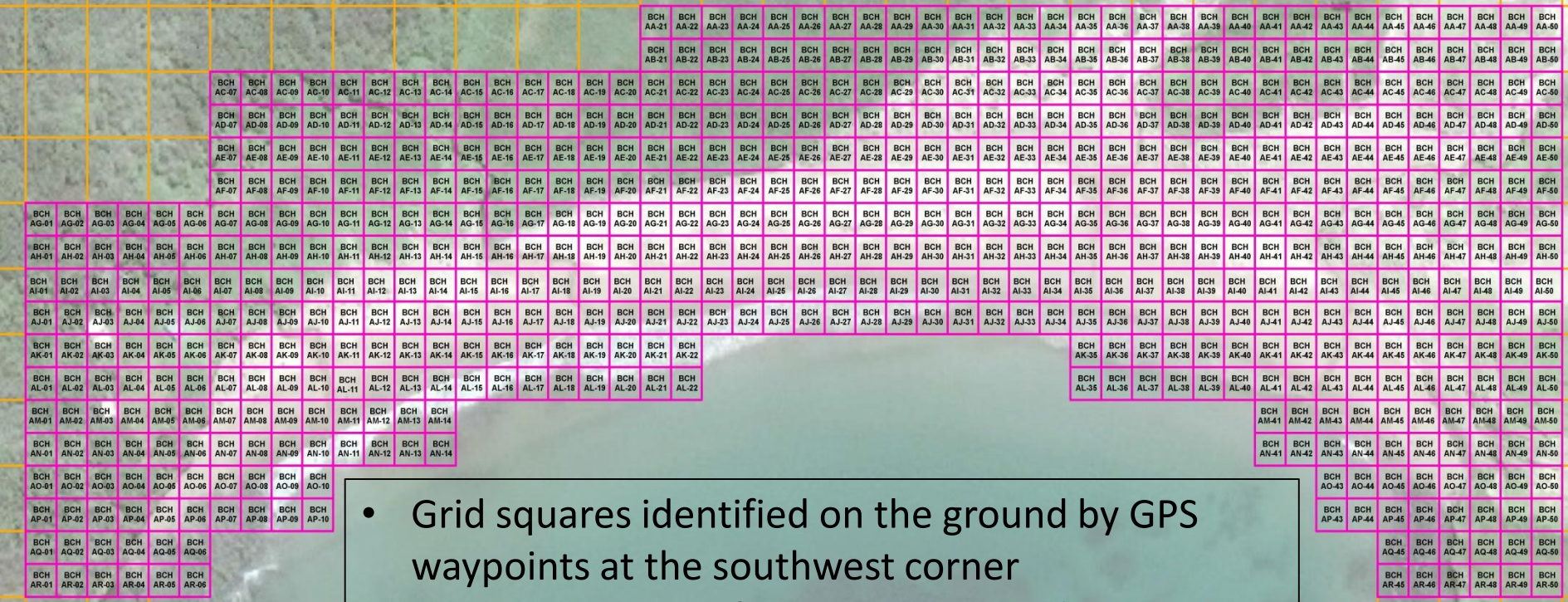
Flipper tagging

- All pups, permanent external identification
- Routinely carried out approximately 16 January at Sandy Bay, Enderby Is.
- Correlate tags applied with already implanted microchip identification
- Weigh and confirm sex
- Collect faeces from all pups where possible
  - Faecal smear examined with microscope to determine presence or absence of hookworm eggs, and therefore ivermectin treatment efficacy

Case control study

- For every pup that is found dead throughout the season, 2 live pups will be randomly selected through a grid system within 24 hours
- A full necropsy will be conducted on all dead pups
- Risk factors and samples will be collected from the dead pup and the two controls

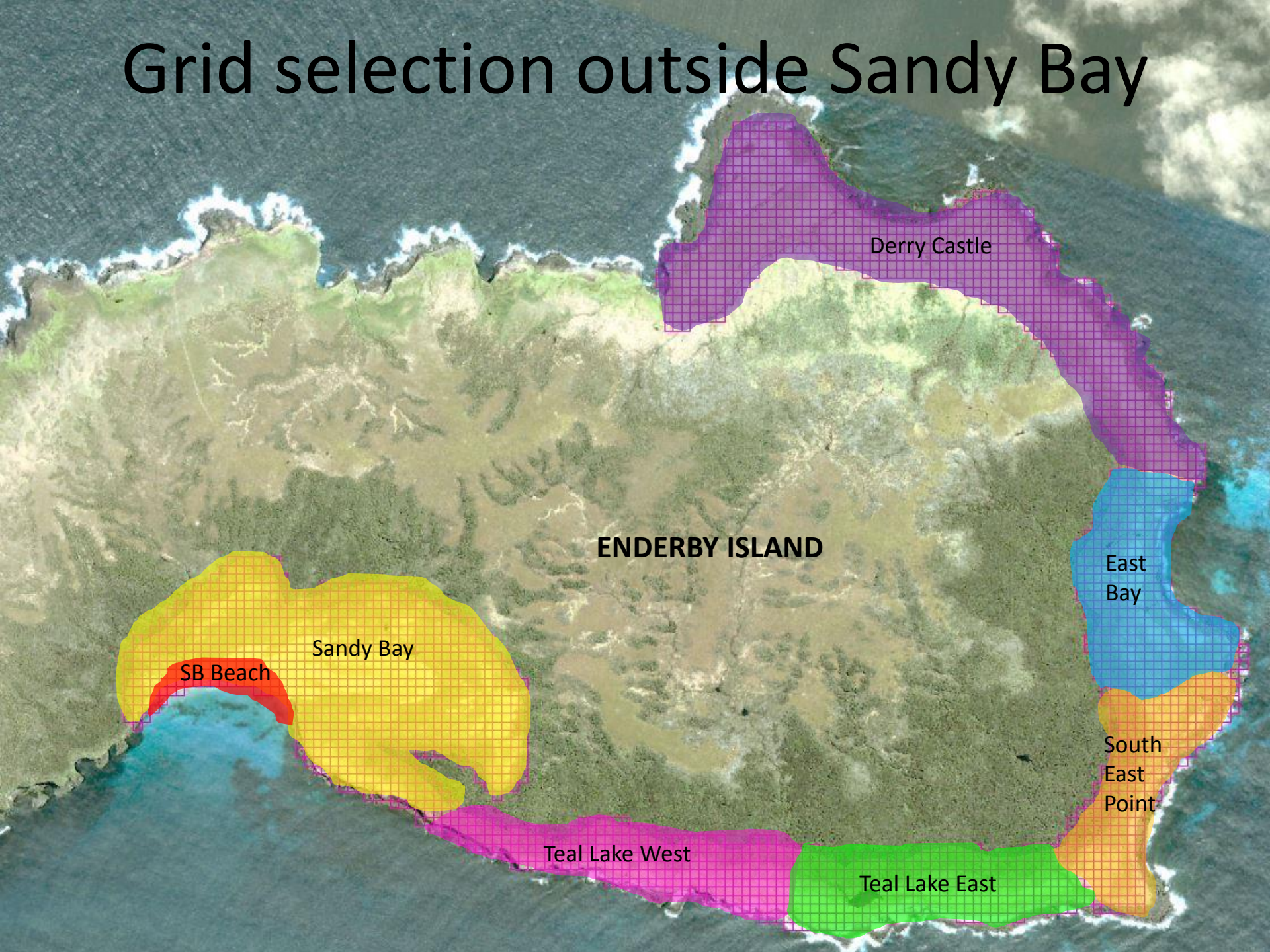
Sandy Bay grid selection



- Grid squares identified on the ground by GPS waypoints at the southwest corner
- 10x10m squares of Sandy Bay area where pupping occurs
- Move to island-wide 20x20m grid once animals have dispersed from the Sandy Bay grid

Map courtesy of Andrea Sward, DOC Geospatial Services

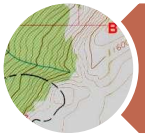
Grid selection outside Sandy Bay



Random control pup selection

- Pups that are nursing are excluded from selection
- If one pup is present in the square, it is selected as a control
- If more than one pup is present in the square, a fraction will be randomly generated and multiplied by the number of pups present to select the control
- If no pups are present in the square, either:
  - The nearest pup to the GPS waypoint (within 40m radius) is selected
  - Another square is randomly selected and the process repeated

PUP IS FOUND DEAD



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

Pup brought back to base for necropsy

Pup identified by tag and/or chip

Pup weighed, sexed and measured

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

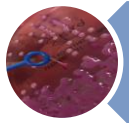
Intestine flushed to quantify hookworm infection intensity

Preliminary diagnosis assigned

<u>TWO</u> LIVE CONTROL PUPS RANDOMLY SELECTED

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

Pups weighed, sexed and measured, given physical examination



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

Faeces collected for assessment of hookworm presence or absence

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

Risk factor data collected from all pups

Pup morphometrics	Weight
	Length
	Axillary Girth
	Body condition

Environmental variables	Air temperature
	Rainfall
	Wind speed
	Tourist interactions
	Skua presence

Pup variables	Colony of origin
	Age
	Sex
	Pup status
	Recent suckling
	Rescue from sink hole
	Handling events

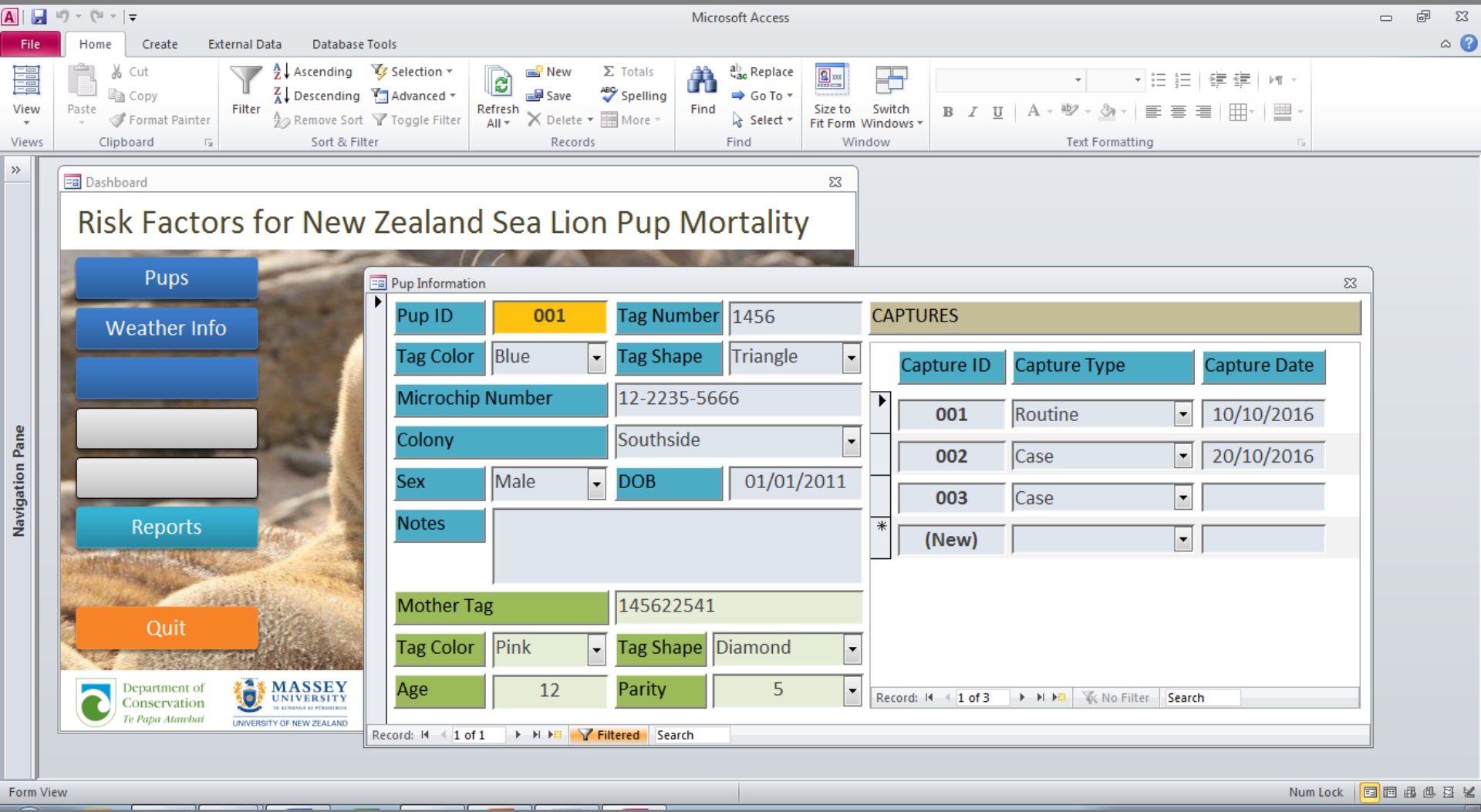
Location	GPS
	Site
	Habitat type
	Substrate type

Maternal variables	Age of mother
	Parity of mother

Clinical variables	Physical examination
	K. pneumoniae carriage
	Ivermectin treatment
	Hookworm infestation
	Clinical signs
	Clinical sign type

Data management

Database being developed by Ahmed Fayaz, mEpiLab, Massey University



Microsoft Access

ternal Data Database Tools

Filter | Ascending | Descending | Remove Sort | Selection | Advanced | Toggle Filter — Sort & Filter

Refresh All | New | Save | Delete | Totals | Spelling | More — Records

Find | Replace | Go To | Select — Find

Size to Fit Form | Switch Windows — Window

Text Formatting

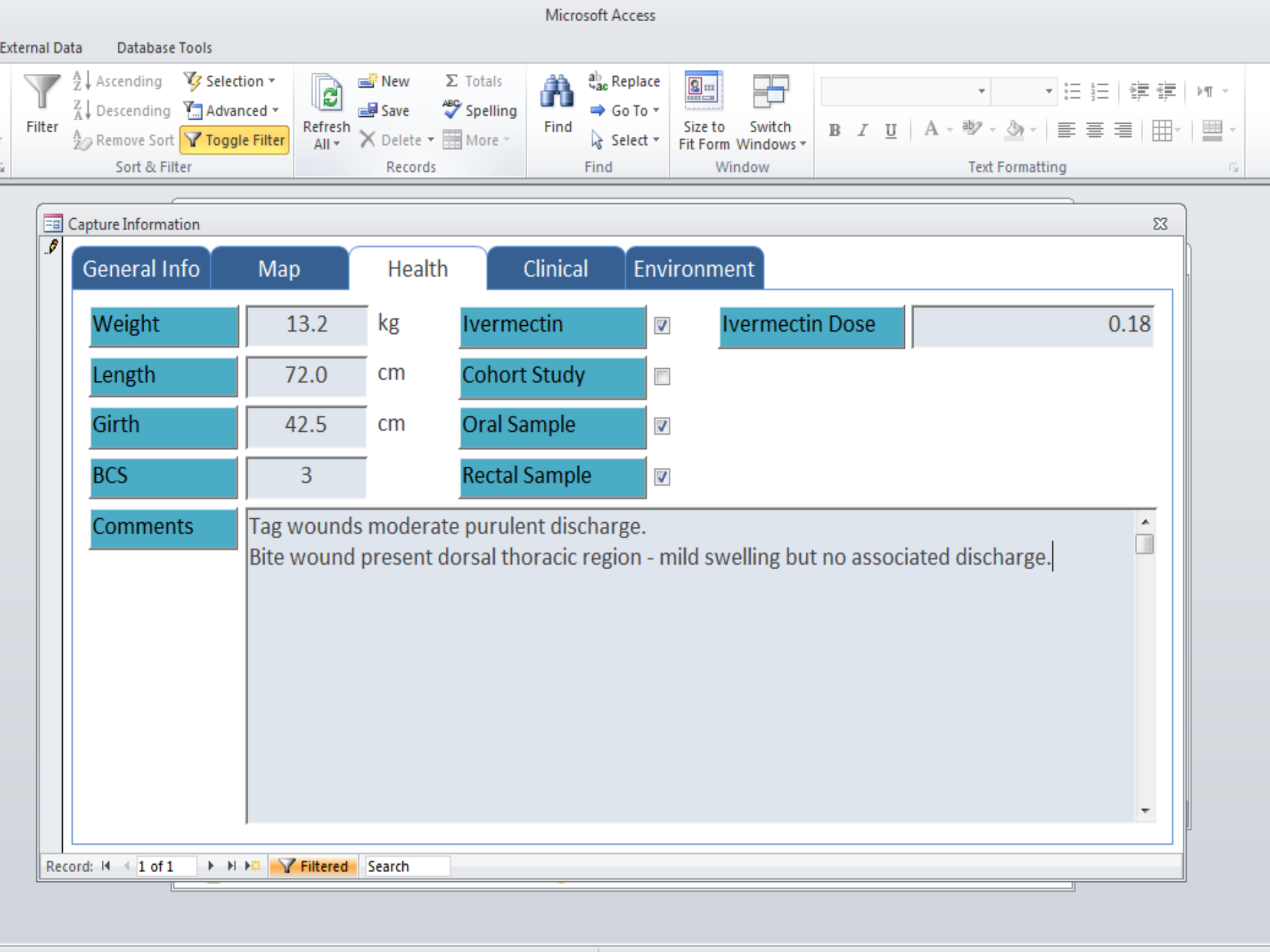
Capture Information

General Info | Map | Health | Clinical | Environment

Capture ID	002
Capture Type	Case
Capture Date	20/10/2016
Site	
Habitat Type	
Substrate Type	
Pup Status	
Recent Suckling	
Rescued	
Rescued Date	
Rescued Place	
Preservation	
Scavenging	

Photo Number

Record: 1 of 1 | Filtered | Search


Microsoft Access
External Data
Database Tools
Filter
Ascending
Descending
Remove Sort
Selection
Advanced
Toggle Filter
Sort & Filter
Refresh All
New
Save
Delete
Totals
Spelling
More
Records
Find
Replace
Go To
Select
Find
Size to Fit Form
Switch Windows
Window
Text Formatting
Capture Information
General Info
Map
Health
Clinical
Environment
Weight
13.2
kg
Length
72.0
cm
Girth
42.5
cm
BCS
3
Ivermectin
Cohort Study
Oral Sample
Rectal Sample
Ivermectin Dose
0.18
Comments
Tag wounds moderate purulent discharge.
Bite wound present dorsal thoracic region - mild swelling but no associated discharge.
Record:
1 of 1
Filtered
Search

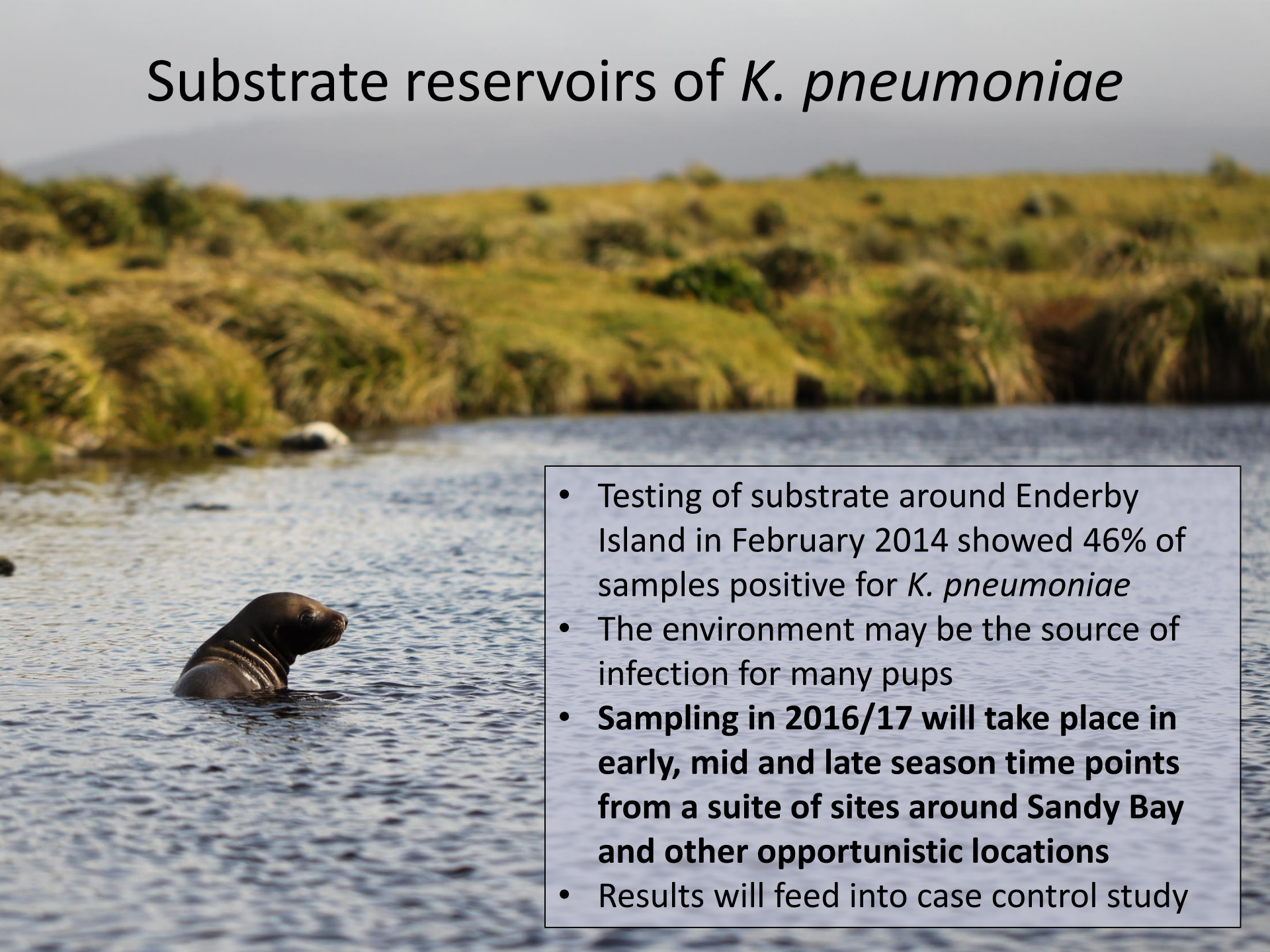
Prospective Cohort Study

- Up to 50 pups selected at first capture
- Pups then caught approximately fortnightly (two groups in alternate weeks)
- Risk factor data and samples collected
  - Blood: health status, anaemia, inflammatory response, immune function
  - Faeces: hookworm carriage
  - Oral and rectal swabs: *K. pneumoniae* carriage
  - Temperature: body's inflammatory response to disease

Avian reservoirs of *K. pneumoniae*

- Sub-Antarctic skuas commonly scavenge pup faeces and sea lion carcasses
- Sampling in 2014/15 showed that skuas can carry the same form of *K. pneumoniae* that causes disease in pups, in their intestinal tracts
- No apparent clinical effect on skua health
- Skuas could potentially spread the bacteria to other NZ sea lion colonies and potentiate numbers of environmental bacteria at high density sites
- 5/33 (15.1%) samples positive on Enderby Island in 2014/15 but birds not individually identified
- **Sampling in 2016/17 will include leg banding of skuas to more accurately determine prevalence of *K. pneumoniae* carriage**

Substrate reservoirs of *K. pneumoniae*



- Testing of substrate around Enderby Island in February 2014 showed 46% of samples positive for *K. pneumoniae*
- The environment may be the source of infection for many pups
- **Sampling in 2016/17 will take place in early, mid and late season time points from a suite of sites around Sandy Bay and other opportunistic locations**
- Results will feed into case control study

Acknowledgments

- Blue Planet Marine: Simon Childerhouse
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