

# Assessing genomic variability linked to fitness in the New Zealand sea lion (*Phocarctos hookeri*)



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## Introduction

Many indigenous animal species have been reduced to small, isolated populations. Their insular nature may lead to inbreeding effects and a reduction in genetic diversity, compromising the ability of populations to evolve and persist long term.

The focal species for my project, the New Zealand sea lion (*Phocarctos hookeri*), is the rarest sea lion in the world, classed as 'Vulnerable' by the IUCN and 'Nationally Critical' by the Department of Conservation (NZ). Epizootic events in 1997, 2002 and 2003 were responsible for the deaths of approximately 40% of pups born during these breeding seasons, with increasing mortality among adult females.

Neutral genetic variability may accurately represent the overall genetic diversity of many species and may be a good indicator of fitness. However, neutral markers may not always accurately reflect the diversity of loci that have important functional roles in growth, inbreeding avoidance and disease resistance. Here we use 21 neutral microsatellite markers in concert with several candidate genes (MHC and NRAMP1, expanding to include others) in which variability is associated with fitness, to test if neutral genetic variability is useful for predicting individual fitness, specifically disease resistance and growth/survivorship in NZ sea lions.

This approach has the capacity to be applied to other species of conservation significance, and may enable improved prediction of the future evolutionary potential of threatened species, aiding species conservation.

## 1. Background



'Vulnerable'

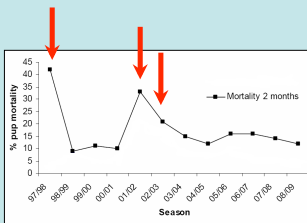
Three seasons of very high mortality - epizootics



Only found here...



## 2. Epizootics and Sampling

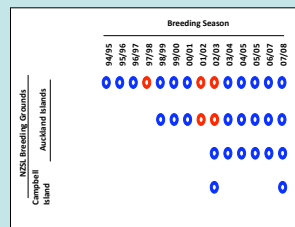


3 seasons of unusually high mass mortality indicated by arrows (mortality more than 20%)

Epizootics

- *Campylobacter* (97/98)
- *Klebsiella* (01/02 and 02/03)

Intensive monitoring since 1998 of Auckland Islands and Campbell Island breeding colonies

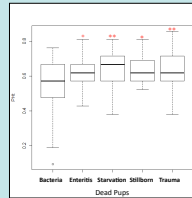


Samples from *before, during and after* the mass mortality events

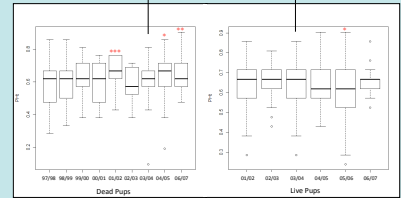
Powerful data set providing information on:

- heterozygosity/variation
- gene evolution
- association of genes with disease resistance and survivorship

## 3. Results

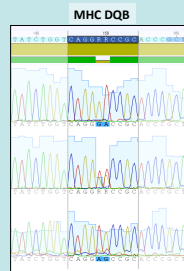


Pups dying from bacterial infection have a significantly lower level of mean PHT (mean heterozygosity) than pups dying from other causes. Red statistical significance scores are relative to bacterial infection



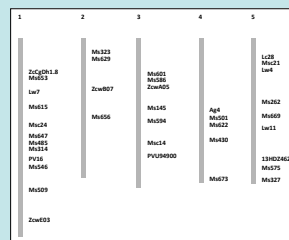
Relative to 1997/98, dead pups (left) have significantly higher mean PHT in 2001/02, 2004/05 and 2006/07  
 Relative to 2001/02, live pups (right) have significantly higher PHT in 2005/06

Dead pups have a significantly lower level of heterozygosity than live pups in 2003/2004



MHC: immune function  
 200 bp MHC DQB exon 2 sequenced. Non-synonymous amino acid changes as a result of two adjacent SNPs

NRAMP1: intracellular pathogen resistance  
 500bp promoter region sequenced. Majority of live pups are GG homozygous, while most A/G heterozygotes are dead pups.



Example of microsatellite map of pinniped genome, assembled based on observed synteny between carnivore genomes against a dog genome scaffold - five canine chromosomes shown.

Single-locus associations between heterozygosity and fitness can now be readily investigated if the location of these microsatellites are known, even in a non-model organism.

Marshall et al. 2010 (in review)

## 4. Conclusions

Some things we know:

1. Pups succumbing to bacterial infection have lower mean heterozygosity (PHT) than pups dying from other causes
2. Following the 01/02 and 02/03 epizootics, dead pups show a significantly lower level of heterozygosity than live pups in 2003/2004
3. Per year, dead pups only differ significantly in PHT from live pups in 2003/2004
4. We have produced a microsatellite map of the pinniped genome for use in the investigation of single-locus associations between microsatellites and fitness traits

Some things we are working on:

1. Heterozygosity at the only polymorphic locus in 500bp of NRAMP1 is more common in dead pups than live pups
2. Two adjacent non-synonymous polymorphisms in MHC DQB exon 2 change the base composition and therefore may have an effect on protein folding and antigen presentation and subsequent immune response
3. We are investigating genes involved in growth rate and fat metabolism, as these are known to be implicated in survival through to weaning and therefore long-term survival