Australian Marine Mammal Centre

Final Report

(subclause 9 and Schedule Item 5 of the Funding Agreement)

- **Project No**. – 09/38
- **Title** - 'Structure and subdivision of the Australian sea lion - defining species-wide management units using ecological and genetic information'
- **Chief Investigator** – A/Prof Simon Goldsworthy
- **Organisation** – SARDI Aquatic Sciences

Activity Period – January 2010 – May 2011

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1. Activity Summary

A clear summary of approximately 500 words outlining the work undertaken and any significant findings (for publication on the Department's web site)

A previous population genetic study conducted on the threatened Australian sea lion (ASL), *Neophoca cinerea* (Campbell 2003, Campbell et al. 2008), found little or no interchange of females among breeding colonies, even those separated by only 20 km, though the majority of their samples were from Western Australia (WA) where only 20% of the species breeds. These levels of subpopulation structure are the most extreme seen in any seal species, however as only two widely spaced breeding colonies from South Australia (SA) were included by Campbell (2003) and Campbell et al. (2008), the generality of their conclusions across the range of the species has been unclear. Current management units for the species in South Australia where the species has been subject to significant bycatch in the Commonwealth managed shark gillnet fishery, have relied on proxies for subpopulation structure, that may not reflect the true structure of populations (Goldsworthy et al. 2007, 2010). Finer-scale knowledge of genetic subdivisions has been identified as a high priority/key knowledge gap.

This project aimed to build on stable isotope analyses that has identified ecological substructuring of ASL populations based on foraging ecotypes (AMMC 0708/26 and 0809/27) by examining genetic population structure to address the following objectives: 1) characterise the genetic population structure and assign meaningful population boundaries by fine scale mitochondrial DNA (mtDNA); 2) estimate the contemporary effective population size for the species.

Hair samples were collected from ASL pups (aged <3mths) at 17 colonies along the southern coast of SA between January 2008 and October 2009 and three colonies in WA during February 2010. Mitochondrial DNA (mtDNA) was extracted from hair samples using the GentraPuregene© DNA purification kit (Qiagen Pty Ltd, Victoria Australia). All laboratory work was conducted at the University of Adelaide's Evolutionary Biology Unit (EBU).

<u>Diversity within the mitochondrial DNA control:</u>
The final edited alignments provided sequence of 464 bp of the mtDNA control region from 478 individuals across the South Australian range. The sequenced region contained 21 polymorphic sites of which 19 were informative under parsimony. 21 individual haplotypes were identified of which 11 were unique to a particular colony, with

one haplotype (A) being shared between seven colonies ranging from The Pages Islands to Olive Island (Figure 1). Half of the colonies (8 out of 16 – The Pages combined) contained unique haplotypes not shared with other colonies. Haplotype (h) and nucleotide (π) diversities ranged from 0 to 0.86 (mean 0.53 ±0.239) and 0 to 0.0069 (mean 0.0028 ± 0.002), respectively, with two colonies being fixed for a single (same) haplotype (Blefuscu and Lounds Islands) (Figure 1). There was no evidence for a departure from neutrality or evidence of population expansion (Tajima's D =-0.86 to 2.77; Fu's Fs>0.62, P>0.23 at all colonies for both parameters).

Strong genetic partitioning among colonies:
Overall fixation among colonies was high (Φ_{st}= 0.75, P<0.001). Maximising significant between-group Φ_{ct} variation clustered ASL breeding colonies into 10 putative groups (Φ_{ct}=0.66, P<0.001).The Pages Islands, Dangerous Reef and English Island (Spencer Giulf), and the Nuyts Archipelago (Blefuscu, Lilliput, West, Fenellon, Purdie and Lounds Islands) formed three clusters, with all other colonies being characterised as individual subpopulations (Figure 1). Between-group corrected pair-wise Φ_{st} comparisons generally supported these clusters. Spatial autocorrelation suggested significant genetic structuring occurred at a distance of approximately 40km.

Of the three clusters, The Pages Islands (North and South Page) were the most similar to each other in terms of haplotype frequency. Genetic data indicate that the two islands (separated by only 1.8 km) are a single colony, sharing only two haplotypes with very similar frequencies. One haplotype is not shared with any other colony (Figure 1). Dangerous Reef and English Island demonstrate much greater haplotype diversity with one colony having a unique haplotype not shared with the other colony. This suggests that despite being clustered together, migration rates between colonies are low. Within the six colonies sampled in the Nuyts Archipelago, two haplotypes (C and E) predominate, but representation of these varies markedly among colonies (Figure 1). Lilliput and Purdie Islands have unique haplotypes not shared with any other colony. Blefuscu and Lounds Islands are fixed for the same haplotype (C). Differences in haplotype frequencies suggest that migration rates among colonies within the Nuyts Archipelago are low (Figure 1).

Campbell (2003) and Campbell et al. (2008) analysed a smaller mtDNA control region fragment (360 bp vs 456 bp this study) in their study of ASL genetics, but identified a similar number of variable sites. For 10 colonies sampled across the range of the species, Campbell et al. (2008) noted 18 haplotypes, 16 (89%) of which were unique to individual colonies. This compares to our study of 16 colonies, 21 haplotypes, 11 (52%) of which were unique to an individual colony. Both studies noted high fixation among colonies Φst= 0.93 vs. 0.75, respectively. The generally higher levels of unique colony haplotypes and fixation levels among colonies in Campbell (2003) and Campbell et al. (2008) reflect their study covering the entire geographic range of the species, with greater average geographic distance among sampled colonies. Despite this, our data strongly corroborate the findings of Campbell (2003) and Campbell et al. (2008), identifying strong genetic subpopulation structure of ASL in SA. Although two breeding sites (North and South Page Islands) are clearly the same colony, the two other clusters each had one or more colonies with unique haplotypes, and marked variation in haplotype frequency. Together, these results suggest low rates of mtDNA exchange (migration) among colonies. Among all other colonies, a high degree of population subdivision was supported.

Western Australian subpopulations:
In addition to the data generated in SA, mtDNA samples were collected from three spatially-close colonies in WA: Beagle Is. (n=10), North Fisherman Is. (n=20) and Buller Is. (n=31) in collaboration with Macquarie University under AMMC Project 0809/12. Analyses indicate the same colonies are almost completely monohaplotypic suggesting all three colonies could be clustered as a single breeding subpopulation. Notably, the single haplotype recovered in WA does not occur in SA subpopulations, supporting the findings of Campbell et al (2008).

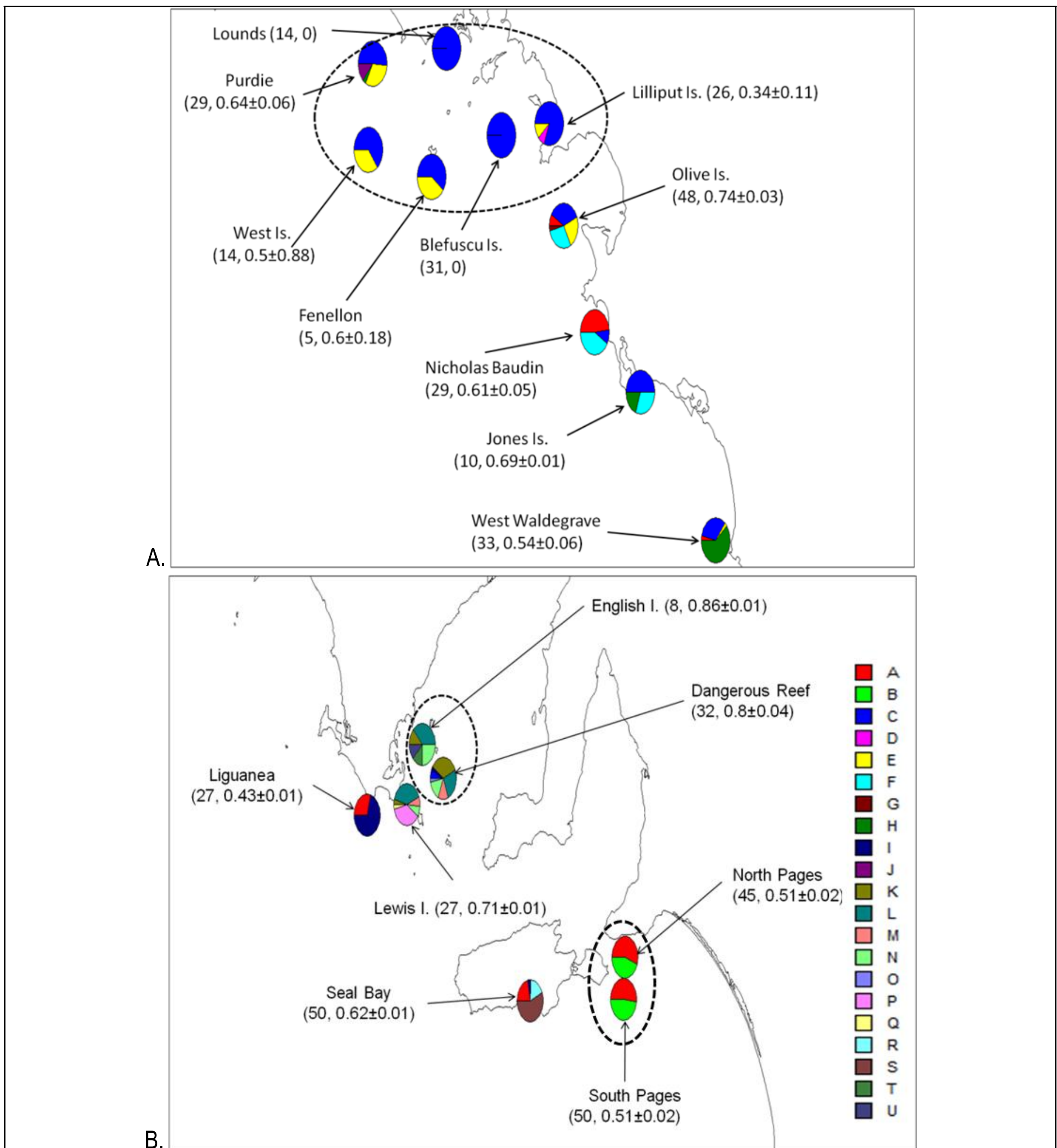

Figure 1. Haplotype distribution maps of ASL colonies of A) Western Eyre Peninsula and Nuyts Archipelago and B) southern Spencer Gulf and Kangaroo Island. Pie charts depict relative frequency of haplotypes (see legend). Dotted lines surround groups of colonies clustered together by SAMOVA. Numbers after colony names represent sample size, haplotype diversity and ± S.E.

Effective population size:

Estimates of the population parameter θ were derived using a Bayesian Monte Carlo Markov Chain (MCMC) approach in the coalescent geneaology sampler LAMARC 2.1.5 (Likelihood Analysis using Metropolis Algorithm and Random Coalescence) (Kuhner 2006; Kuhner 2009). When using mtDNA sequence data, the population parameter θ can be related to effective population size using $\theta = 2N_f\mu$, where μ represents the mutation rate per site per generation. The validity of using estimated or non species-specific mutation rates to determine historical and contemporary effective population estimates is still strongly debated with flawed analyses having significant conservation implications (Ho and Larson 2006). As such, we report only θ estimates. Contemporary effective adult female population parameter (θ) estimates generally followed a priori expectations of reflecting genetic diversity with the exception of The Pages Islands which displayed one of the lowest parameter estimates despite

being the second largest breeding colony of the species (Table 1).

Table 1. Estimates of the population parameter θ (± Credibility Interval) for each colony sampled. Census estimate is based on mark-recapture estimates or total counts of pup production at each colony. Note the low θ estimate for North and South Pages Islands.

Colony	Census estimate	θ	CI
NP & SP	550	0.021	0.0024 - 0.0479
SB	250	0.0371	0.0027 - 0.0832
DR & EI	1000	0.172	0.0631 - 0.2942
LI	80	0.0424	0.0029 - 0.1051
LIG	30	0.0011	0.0001 - 0.0437
WW	120	0.067	0.0142 - 0.1383
JI	20	0.0492	0.0078 - 0.0924
NB	120	0.055	0.0097 - 0.117
OI	200	0.0657	0.00145 - 0.124
L & LIL	160	0.011	0.005 - 0.04
WI & FEN	50	0.004	0.0004 - 0.028
PU & LO	130	0.055	0.004 - 0.07

References:

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2. The Outcomes/Objectives

List of the Project Objectives

The main objectives of this project were to:
1). Characterise genetic population structure and assign meaningful population boundaries by fine scale mitochondrial DNA (mtDNA) and microsatellite analysis of at least ten colonies spanning the breeding range of the species in South Australia.
2). Collect samples at several Western Australian breeding sites (in conjunction with project AMMC 0809/12 led by Professor Rob Harcourt), and describe population management units for the entire species and increase the applied value of this project.
3). Estimate the contemporary effective population size for the species.

The degree to which the Activity has achieved each of the objectives

1). Characterise genetic population structure and assign meaningful population boundaries by fine scale mitochondrial DNA (mtDNA) and microsatellite analysis of at least ten colonies spanning the breeding range of the species in South Australia.

mtDNA analysis:
Achieved – details for 16 sampled colonies in SA provided above.

Microsatellite analysis:
At the time of writing, raw microsatellite data has been generated for 478 individuals at 18 separate loci across the SA colonies sampled. The time taken to process, score and analyse 8,604 individual loci is considerable, with analysis of all samples needing to be completed before population-level conclusions can be drawn. Consequently, at this stage we are unable to report on results based on microsatellite markers at this stage. Once data have been analysed, the dataset will be made available through the peer-reviewed scientific literature. Microsatellite analyses are being undertaken in collaboration with Macquarie University.

2). Collect samples at several Western Australian breeding sites (in conjunction with project AMMC 0809/12 led by Professor Rob Harcourt), and describe population management units for the entire species and increase the applied value of this project.

Samples from ASL colonies in WA were all collected from three spatially-close colonies off the west coast WA (Beagle, North Fisherman and Buller Islands) in collaboration with Macquarie University under AMMC Project 0809/12. mtDNA analysis indicated these colonies to be almost completely monohaplotypic (i.e. effectively a single breeding subpopulation). Samples were not obtained from the Abrolhos Islands further north, or from the numerous colonies off the south coast in the Recherche Archipelago. These colonies were sampled as part of the Campbell et al. (2008) study, which detected complex subpopulation structure similar to that found in our analyses of SA ASL populations. Analyses are currently underway of the population wide subpopulation structure of the species; however, we recommend further sampling along the Bunda Cliffs in SA and along the southern WA coastline, to better resolve the connectivity between SA and WA populations.

A report on the SA ASL subpopulation structure was prepared for the Sustainable Fisheries Section of DSEWP&C and the Australian Fisheries Management Authority (AFMA) in December 2010 to assist in developing management strategies/policies to mitigate ASL bycatch in the demersal gillnet fishery off South Australia. The results have therefore had significant applied value.

Goldsworthy, S.D., and Lowther, A.D. (2010). Genetic population structure and bycatch: assessment of management measures for reducing the bycatch of Australian sea lions in the demersal gillnet fishery off South Australia. Report to the Department of Sustainability, Environment, Water, Population and Communities. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000979-1.SARDI Research Report Series No. 515.32 pp.

3). Estimate the contemporary effective population size for the species.

The delay in scoring microsatellite data precludes species-level contemporary effective population estimates being made. We strongly recommend further investigation of mutation rates specific to Australian sea lion enetic markers to avoid drawin erroneous conclusions.

3. Appropriateness

The appropriateness of the approaches used in the development and implementation of the Activity

The sampling and methodological approaches used in the development and implementation of the Activity all proved to be appropriate.

4. Effectiveness

The degree to which the Activity has effectively met its stated objectives

As detailed in 1 (Activity Summary} and 2 (Outcomes/Objectives} most of the objectives of this project have been met, and work is progressing well on the remaining outstanding expected outputs. This project forms the basis of a PhD study being undertaken by Andrew Lowther (Adelaide University}. He is presently in the process of writing up his thesis, and should submit before the end of 2011. He already has one of his thesis chapters published in *Marine Mammal Science.* I do not envisage any major delays in his completion.

Lowther, AD, Goldsworthy SD. (in press} Detecting alternate foraging ecotypes in Australian sea lion *(Neophoca cinerea}* colonies using stable isotope analysis. *Marine Mammal Science.* Article first published online: 26 OCT 2010 | DOI: 10.1111/j.1748-7692.2010.00425.x