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Structure and subdivision of the Australian sea lion - defining species-wide management units using ecological and genetic information

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SARDI Publication No. F2011/000421-1
SARDI Research Report Series No. 576

SARDI Aquatic Sciences
PO Box 120 Henley Beach SA 5022

September 2011

Final report to the Wildlife Conservation Fund, Department of Environment and Natural Resources, South Australia

Government of South Australia

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This publication may be cited as:
Lowther, A.D.[1 2], Goldsworthy, S.D.[2], Harcourt, R.[3], Donnellan, S.[4], and Page, B[5] (2011). Structure and subdivision of the Australian sea lion - defining species-wide management units using ecological and genetic information. Final report to the Wildlife Conservation Fund, Department of Environment and Natural Resources, South Australia. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2011/000421-1. SARDI Research Report Series No. 576. 15pp.

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Printed in Adelaide: September 2011

SARDI Publication No. F2011/000421-1
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Date:	16 September 2011
Distribution:	Department of Environment and Natural Resources, SAASC Library, University of Adelaide Library, Parliamentary Library, State Library and National Library
Circulation:	Public Domain

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1 EXECUTIVE SUMMARY

This project builds on our understanding of the mechanisms of population structuring by characterising genetic population structure and assign meaningful population boundaries by fine scale mitochondrial DNA (mtDNA) and microsatellite analysis of 17 colonies spanning the breeding range of the species in South Australia.

Preliminary analyses of genetic data for 478 individual ASL are presented.

Analyses of 468 bp from the mtDNA control region identified 21 individual haplotypes, of which 11 were unique to a particular colony. Half of the colonies contained haplotypes not shared with another colony, and overall fixation among colonies was high (Φ_{st}= 0.75, P<0.001).

There was strong genetic partitioning among colonies, with most being characterised as individual populations, however, there was support for three clusters: The Pages islands, Dangerous Reef and English Island in Spencer Gulf; and the Nuyts Archipelago.

Results of this study build on the findings of Campbell (2003) and Campbell et al.2008, identifying strong genetic subpopulation structure within the SA ASL population.

2 INTRODUCTION

The Australian sea lion (ASL) (*Neophoca cinerea*) is Australia's least-abundant and only endemic seal species. Reproductive behaviour in the ASL is most unusual and unique amongst seals. Females produce one pup approximately every 17.6 months, instead of an annual cycle typical of other seal species, resulting in low fecundity. Furthermore, breeding is asynchronous, even among geographically close colonies. The total population size has been estimated at 14,700 individuals, and is distributed over numerous, small subpopulations, making the ASL vulnerable to extinction (Goldsworthy and Page 2007). Recent population genetic studies have indicated there is little or no interchange of females among breeding colonies, even those separated by short distances (Campbell et al. 2008). The important conservation implication is that each breeding colony represents a closed population. As such, the ASL poses significant conservation and management challenges. The species is listed as Vulnerable under the threatened species category of the Commonwealth *Environment Protection and Biodiversity 1999 Act* (EPBC Act), vulnerable under the South Australian *National Parks and Wildlife Act* (1972), and was recently upgraded to Endangered by the IUCN Red List (www.iucnredlist.org).

Eighty percent of the ASL population exists within South Australian (SA) waters, with the region hosting 39 breeding colonies, of which only 16 produce more than 30 pups per breeding season (Goldsworthy et al. 2009) These factors combine to make the species vulnerable to the impact of both Commonwealth and State-managed demersal gillnet fishery because fishing effort occurs on the continental shelf, in SA where ~ 20,000 km of net is set per year, and occurs year-round in proximity to most ASL breeding colonies (Goldsworthy et al. 2009). Critically, a recent fishery independent observer program identified significant levels of ASL bycatch, which varied considerably among locations depending on the proximity of fishing effort and the relative proportion of inshore and offshore foraging by ASL (Goldsworthy et al. 2009). Our research using stable isotope analysis revealed strong subdivision of ASL breeding colonies into onshore and offshore foraging ecotypes, which is likely to pre-dispose particular breeding colonies to elevated probabilities of bycatch. Our project will resolve appropriate management units for at least 80% of the ASL, and provide data to Government agencies to address important management issues relating to mitigating ASL bycatch in commercial fisheries.

The only previous population genetic study on ASL (Campbell et al. 2008) found little or no interchange of females among breeding colonies, even those separated by only 20 km, although the majority of samples were from Western Australia where only 20% of the species breeds. These levels of population differentiation are the most extreme seen in any seal species (Campbell et al. 2008) however as only two widely spaced breeding colonies from SA

were included, the conclusions cannot be generalised to SA. Many SA colonies were subjected to heavy harvest pressure in the 18th and 19th centuries (Ling 2002) and thus many of the smaller SA breeding colonies may have been re-established from a small number of large breeding colonies that survived the harvest. If colonies have only recently re-established, they may not be as genetically isolated as WA colonies appear to be. These alternative scenarios have the potential to have differential impacts on the design of spatially explicit management units. Current management units have relied on proxy data that may not reflect the genetic structure of the population(s) (Goldsworthy et al. 2007). Finer-scale knowledge of genetic subdivisions was identified as a high priority/key knowledge gap, and is one of eight targets to address critical management needs for the species in a recent report (McKenzie et al. 2005).

This project investigated the genetic population structure of ASL to assign meaningful population boundaries by fine scale mitochondrial DNA (mtDNA) and microsatellite analysis of 17 colonies spanning the breeding range of the species in South Australia. Additionally, we collected samples at three Western Australian breeding sites to assess population structure across the known geographical range of the species.

3 METHODS

a. Genetic data on the SA and WA ASL population

Hair samples were collected from ASL pups (aged <3mths) at 17 colonies along the southern coast of South Australia (Figure 1) between January 2008 and October 2009, and three colonies in Western Australia during February 2010. Approximately 60% of all pups produced in each colony were sampled. Total genomic DNA was extracted from five to 50 hair samples using the Gentra Puregene© DNA purification kit (Qiagen Pty Ltd, Victoria Australia) and the mitochondrial DNA (mtDNA) control region was amplified using PCR. The PCR reactions were performed in 12.5microL volumes. PCR conditions were: 15 minutes denaturing at 95^{o}C followed by 35 cycles of 95^{o}C for 30s, 45^{o}C for 30s and 68^{o}C for one minute. All laboratory work was conducted at the University of Adelaide's Evolutionary Biology Unit (EBU). Amplification of a 650bp fragment of the mtDNA control region was performed using the following specifically designed primer pair: forward 5' ACACCCAAAGCTGACGTTCTC 3' (EBU nomenclature: M1214) and reverse 5' TGAAATGCACCTCATGGTTGTA 3' (EBU nomenclature: M1216). Cleaned PCR product was sent to the Australian Genome Research Facility (AGRF) for sequencing on an AB 3730 DNA Sequencer (Applied Biosystems Inc).

Sequences were aligned manually using BioEdit 7.0.5.3 (Hall 1999). Kimura's two-parameter model of nucleotide substitution (Kimura 1980) was selected as the most appropriate model of nucleotide substitution using the Akaike Information Criterion (AIC) in jModelTest v0.1.1 (Posada 2008). Haplotype composition, haplotype diversity (h) and nucleotide diversity (π) were estimated, with Tajima's D (Tajima 1989). Fu's F_s (Yun-xin 1997) tests were employed to detect deviations from neutrality and recent population expansion events in DNAsp v5.10.01 (Librado and Rozas 2009). Geographical distance was calculated as the shortest swimming distance in kilometres between colonies (Gonzalez-Suarez et al. 2009).

The use of Spatial Analysis of Molecular Variance (SAMOVA) to detect clusters of populations that are maximally differentiated can be confounded in the presence of isolation by distance. In response, the analytical software SAMOVA 1.0 (Dupanloup et al. 2002) was employed to develop hypotheses about clustering of populations (from k=2 to k=16 clusters), and each hypothesis was tested using Analysis of Molecular Variance (AMOVA) in Arlequin (Manier and Arnold 2006). Optimal cluster selection was based on maximised between-cluster significant differences (Φ_{ct}) as recommended by Dupanloup et al. (2002).

Possible effects of isolation by distance were assessed using a plot of Slatkins linearised Φ_{st} (calculated in Arlequin v3.5.1.2) (Excoffier et al. 2005) with geographic distance, and a Mantel Test (Mantel 1967) was conducted in Arlequin (significance being determined after 10,000 permutations).

Estimates of the population parameter θ was derived using a Bayesian Monte Carlo Markov Chain (MCMC) approach in the coalescent genealogy sampler LAMARC 2.1.5 (Likelihood Analysis using Metropolis Algorithm and Random Coalescence) (Kuhner 2006, 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.

4 RESULTS AND DISCUSSION

a. Genetic data on the SA ASL population

Diversity within the mitochondrial DNA control

The final edited alignments reduced useable sequence from 650bp to 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. A total of 21 individual haplotypes were identified of which 11 were unique to a particular colony (Table 1, Figure 2), with one haplotype (A) being shared between seven colonies ranging from The Pages islands to Olive Island. 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) (Table 1, Figure 2). There was no evidence for a departure from neutrality or evidence of population expansion (Tajima's D =-0.86 to 2.77; Fu's F_S >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; Figure 4). The Pages islands, Dangerous Reef and English Island (Spencer Gulf), 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 3). 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 (Figure 4).

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 1.8 km) are a single colony, sharing only two haplotypes with very similar frequencies (Table 1). One haplotype is not shared with any other colony (Figure 3).

Dangerous Reef and English Island demonstrate much greater haplotype diversity, with each colony having a unique haplotype not shared with the other (Table 1). 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 (Table 1, Figure 3). Lilliput and Purdie Islands have unique haplotypes not shared with any other colony. Blefuscu and Lounds Islands were fixed for the same haplotype (C). Differences in haplotype frequencies suggest that migration rates among colonies within the Nuyts Archipelago are low (Table 1, Figure 3).

Campbell (2003) and Campbell et al. (2008) analysed a smaller mtDNA control region fragment (360 bp vs 456 bp this study) in their studies of ASL genetics, but identified a similar number of variable sites. For ten 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 a recent study of 16 colonies, 21 haplotypes, 11 (52%) of which were unique to an individual colony (Lowther et al. unpublished data). Both Campbell et al. (2008) and Lowther (unpublished data) noted high fixation among colonies Φ_{st}= 0.93 vs. 0.75, respectively). The higher levels of unique colony haplotypes and fixation levels among colonies in Campbell (2003) and Campbell et al.(2008), reflects that their study covered the entire geographic range of the species, with greater average geographic distance among sampled colonies. Despite this, Lowther's (unpublished) 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 identified 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. These colonies are almost completely monohaplotypic, suggesting all three 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). However, given the significantly different and complex subpopulation structure detected in SA in comparison to Campbell et al. (2008), it is reasonable to assume that further subpopulation structure may exist amongst colonies along the southern coast of WA. As such, we recommend further sampling along the Bunda Cliffs in South Australia and along the southern coastline of Western Australia.

Effective population size 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 2). The delay in scoring microsatellite data precludes species-level contemporary effective population estimates being made, however, our data highlight which colonies require prioritisation of conservation effort. We strongly recommend further investigation of mutation rates specific to ASL genetic markers to avoid drawing erroneous conclusions.

Microsatellite data

At the time of preparation of this report, raw microsatellite data had been generated for 478 individuals at 18 separate loci across every South Australian colony studied. However it takes considerable time to score and analyse 8,604 individual loci and this needs to be completed before population-level conclusions can be drawn; this was beyond the scope and time frame of this report. Once data have been analysed, the dataset will be made available through the peer-reviewed scientific literature.

Conclusion

We conclude that the degree of population structure of adult female ASL is substantially more complex than previously described by Campbell et al. (2008). There is considerable evidence of haplotype sharing, with one haplotype spanning a large portion of the South Australian geographical range, and only two colonies displaying fixation for a single control region haplotype. Commensurate with this, we report a lower degree of population subdivision than previously reported by Campbell et al. 2008 (Φ_{st} 0.93 c.f. 0.75 this study). Nonetheless, this more comprehensive assessment seems to support the earlier contention that ASL exhibit the most restricted effective migratory movement of any marine mammal (Campbell et al. 2008). As such our data supports the current approach of considering each breeding colony a closed population for management purposes.

Critically, information pertaining to adult male ASL foraging and reproductive ecology is absent and must be gathered before a comprehensive overview of population structure can be generated. Once the effects of male-mediated gene flow are quantified, breeding colony connectivity should be reassessed. Such information will be important to the ongoing development of management strategies to assist in the recovery of the species.

Table 1. Haplotype frequency of ASL colonies sampled throughout South Australia. Colonies are ordered East-West and those clustered by SAMOVA are highlighted. Eleven of 21 haplotypes (bold) were unique to a particular colony (B, D, G, J, O, P, Q, R, S, T and U).

(Abbrev)	South Page (SP)	North Page (NP)	Seal Bay (SB)	Dangerous Reef (DR)	English (EI)	Lewis (LI)	Liguanea (LIG)	West Waldegrave (WW)	Jones (JI)	Nicholas Baudin (NB)	Olive (OI)	Blefuscu (BL)	Lilliput (LIL)	West (WI)	Fenellon (FEN)	Purdie (PU)	Lounds (LO)
n	**50**	**45**	**50**	**32**	**10**	**27**	**27**	**33**	**10**	**29**	**48**	**26**	**31**	**14**	**5**	**29**	**14**
Haplotype																	
A	0.52	0.556	0.22	0	0	0	0.296	0.030	0	0.483	0.083	0	0	0	0	0	0
B	**0.48**	**0.444**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0.063	0	0	0	0.333	0.5	0.103	0.354	1	0.808	0.643	0.6	0.517	1
D	0	0	0	0	0	0	0	0	0	0	0	0	**0.077**	0	0	0	0
E	0	0	0	0	0	0	0	0.030	0	0	0.229	0	0.115	0.357	0.4	0.31	0
F	0	0	0	0	0	0	0	0	0.3	0.414	0.292	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	**0.041**	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0.606	0.2	0	0	0	0	0	0	0.034	0
I	0	0	0.04	0.0312	0	0	0.704	0	0	0	0	0	0	0	0	0	0
J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.138**	0
K	0	0	0	0.344	0.125	0.037	0	0	0	0	0	0	0	0	0	0	0
L	0	0	0	0.25	0.375	0.407	0	0	0	0	0	0	0	0	0	0	0
M	0	0	0	0.125	0	0.074	0	0	0	0	0	0	0	0	0	0	0
N	0	0	0	0.156	0.25	0.074	0	0	0	0	0	0	0	0	0	0	0
O	0	0	0	**0.031**	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	**0.370**	0	0	0	0	0	0	0	0	0	0	0
Q	0	0	0	0	0	**0.037**	0	0	0	0	0	0	0	0	0	0	0
R	0	0	**0.18**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	**0.56**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	**0.125**	0	0	0	0	0	0	0	0	0	0	0	0
U	0	0	0	0	**0.125**	0	0	0	0	0	0	0	0	0	0	0	0

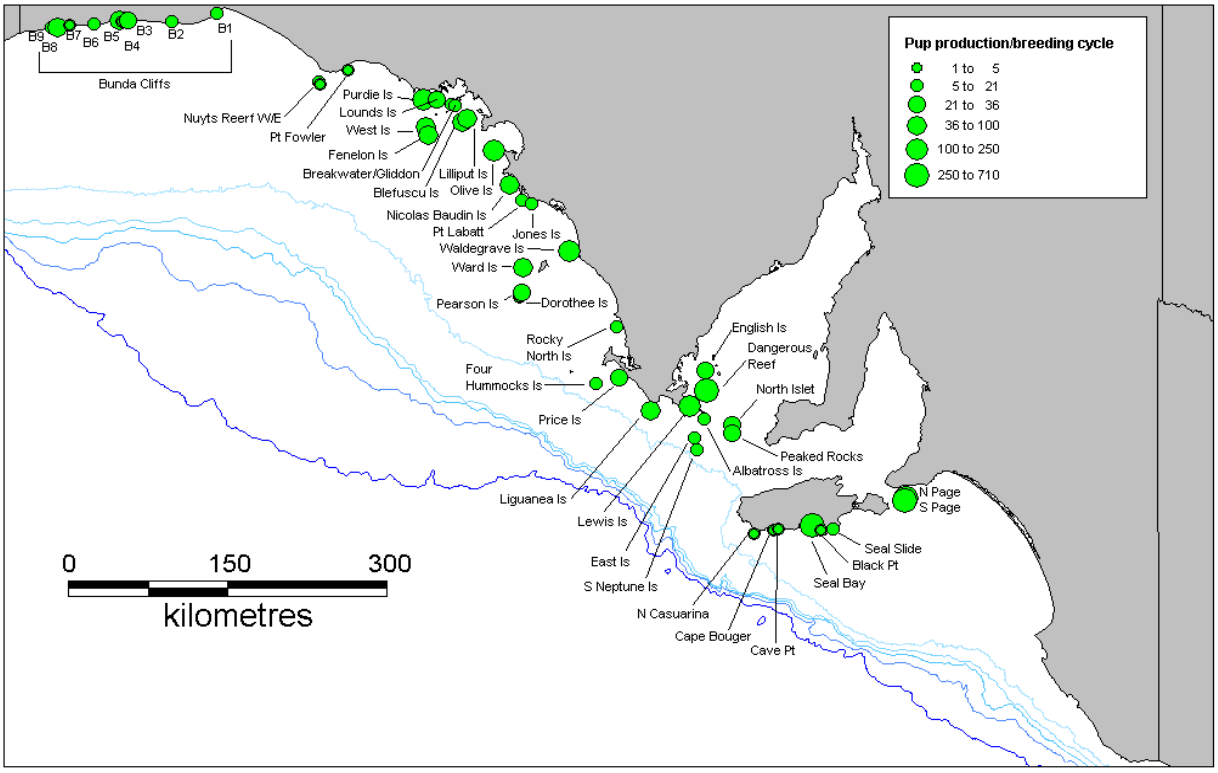

Figure 1. The location of Australian sea lion breeding sites in SA and their relative pup production per breeding cycle. Bathymetry lines are indicated from light to dark blue (100, 200, 500, 1000, 2000m). From Goldsworthy et al. (2010).

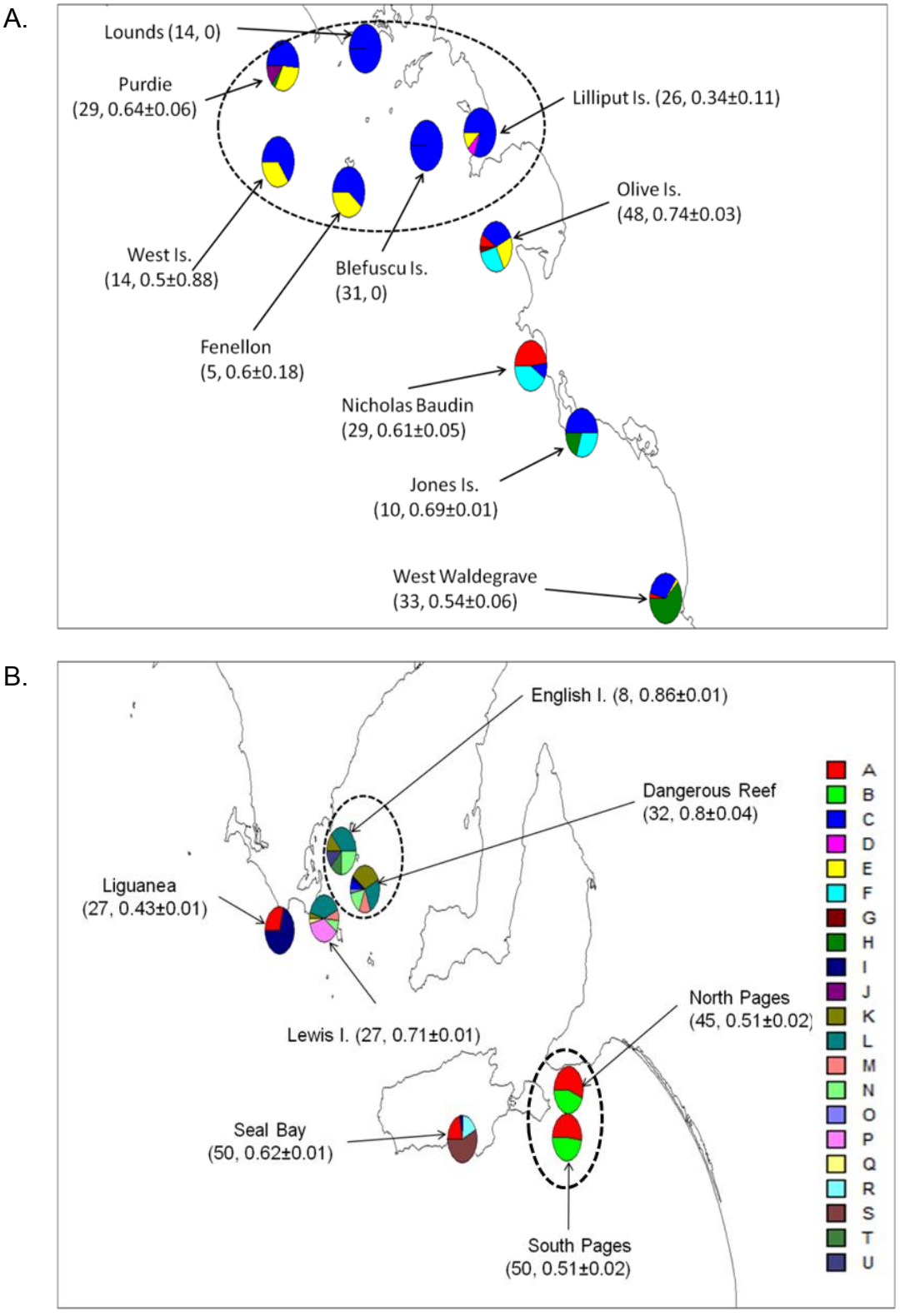

Figure 2. Haplotype distribution maps of ASL colonies of A) Western Eyre Peninsula and Nuyts Archipelago and B) southern Spencer Gulf and Kangaroo Island. Pie charts depict relative frequency of haplotypes (see legend). Dotted lines surround groups of colonies clustered together by SAMOVA. Numbers after colony names represent sample size, haplotype diversity and ± S.E. (source Lowther unpublished data).

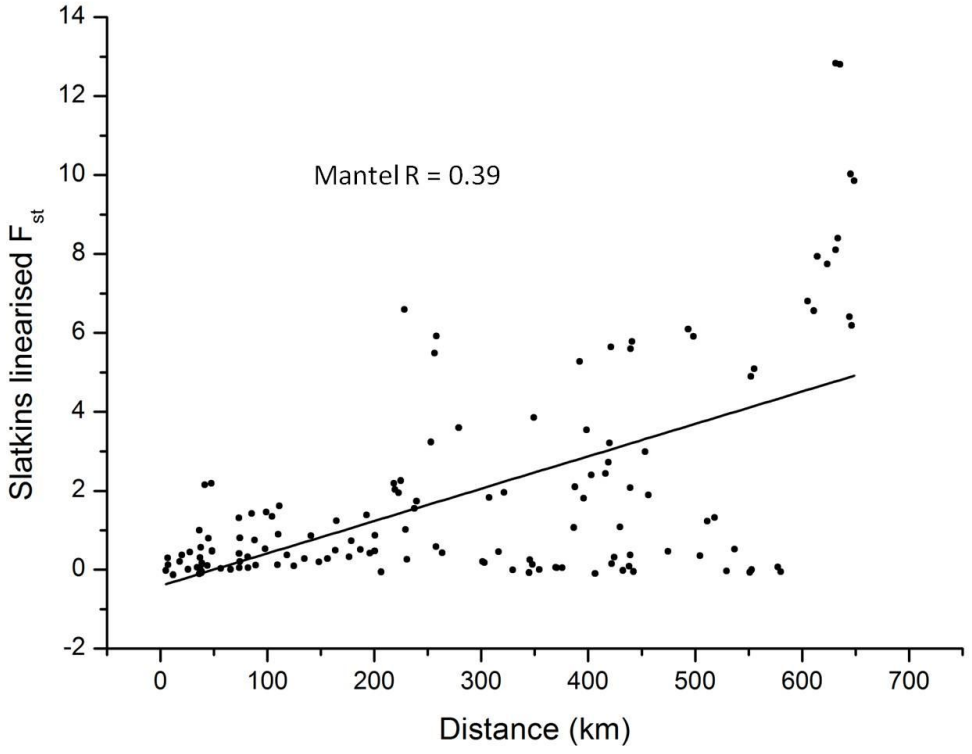

Figure 3. Plot of Slatkins Linearised Φ_{st} on geographic distance. Overall there was evidence of significant Isolation by distance (Mantel R=0.39 P<0.001) (source Lowther unpublished data).

Table 2. 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
North and South Pages Is.	550	0.021	0.0024 - 0.0479
Seal Bay (Kangaroo Is.)	250	0.0371	0.0027 - 0.0832
Dangerous Reef and English Is.	1000	0.172	0.0631 - 0.2942
Lewis Is.	80	0.0424	0.0029 - 0.1051
Liguanea	30	0.0011	0.0001 - 0.0437
West Waldegrave	120	0.067	0.0142 - 0.1383
Jones Is.	20	0.0492	0.0078 - 0.0924
Nicholas Baudin	120	0.055	0.0097 - 0.117
Olive Is.	200	0.0657	0.00145 - 0.124
Blefuscu and Lilliput	160	0.011	0.005 - 0.04
West Is. and Fenelon	50	0.004	0.0004 - 0.028
Purdie and Lounds	130	0.055	0.004 - 0.07

5 ACKNOWLEDGMENTS

Research on the genetic population structure of ASL presented in this report has been funded by the Department of Environment and Natural Resources Wildlife Conservation Fund grants program. We thank Jason Tanner, Nathan Bott and Paul Rogers for reviewing a draft of this report.

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