Penguin monitoring and conservation activities in the Gulf St Vincent

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Report to the Adelaide and Mt Lofty Ranges Natural Resources Management Board

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inspiring achievement

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I. SUMMARY

Some populations of little penguins (*Eudyptula minor*) have been drastically declining across the Gulf St Vincent for reasons still not fully understood. This study investigated breeding performance, adult mortality and genetics of little penguins on three islands in the Gulf St Vincent (Granite, Troubridge and Kangaroo Islands) in relation to patterns of population decline. Population census data showed stabilizing trends for Granite Island with 32 penguins present in 2014 compared to the 38 and 26 individuals estimated in 2013 and 2012 respectively. On Troubridge Island, the 2014 population census showed an increase in numbers with 406 penguins compared to 270 penguins recorded in 2013, but further monitoring is required to assess the long-term trends. Granite Island population had the highest breeding success with 1.67 (± 0.24) fledglings per pair (n=9) compared to Kangaroo Island with 0.85 (± 0.26) fledglings per pair (n=39) and Troubridge Island 0.61 (± 0.12) fledglings per pair (n=26). On Kangaroo Island, 31% of the burrows showed signs of predation, likely by goannas (*Varanus rosenbergi*) on older chicks. The presence of little penguin remains in long nosed fur seal diets varied from 40% in the Fleurieu Peninsula to 10% in the Yorke Peninsula and 4% on Kangaroo Island, suggesting that penguin presence within the regions may not be the main driver for predation rates. Finally, subtle genetic population structure analysis revealed that Troubridge Island showed genetic differentiation compared with other colonies in the Gulf St Vincent. However, medium level of gene flow still occurred between the colonies, which could be promising for natural re-colonisation or potential translocations.

II. INTRODUCTION

Population censuses conducted in the Gulf St Vincent (South Australia) and on Granite Island in particular (Fleurieu Peninsula) indicate that little penguin numbers are declining, for reasons still not fully understood. In response to this pattern of stark decline, the Adelaide and Mt Lofty Ranges Natural Resources Management Board undertook a study on Conservation Management Priorities for Little Penguin Populations in Gulf St Vincent (Weibkin 2011). The NRM Board has been supporting work to implement key recommendations from this report in collaboration with the Kangaroo island NRM Board. The NRM Board provided funding to Flinders University to further investigate potential explanations for the decline of penguins, and to monitor the populations to collect baseline information and increase awareness of little penguin conservation issues. This project follows up on a previous project (2013-breeding season) investigating three main issues: (1) reproductive failure, (2) mortality caused by predation or parasites, and (3) population genetic structure and gene flow. The 2014 report showed regional variation in breeding success, whereby the Granite Island population had the highest breeding success on land despite strong population decline across years. The study also found that 31% of individuals sampled had evidence of parasite presence, and that both predation and parasite intensity negatively impacted breeding success. In the 2014 study, penguins formed a significant part of the long nosed fur seal (*Arctocephalus forsteri*) diet and were found in 33% of collected scats. In addition, there was a significant increase in fur seal numbers in Encounter Bay over the past 10 years, which corresponds with a decrease in penguin numbers and may be one of the causative factors in the decline.

The 2014 report recommended the following directions for future research:

1) Continue long-term annual monitoring of several targeted populations to record penguin numbers and trends across the Gulf St Vincent. Given the existence of long-term monitoring data for comparison, the following sites are suggested: Troubridge Island, Granite Island, Antechamber Bay (KI) and Emu Bay (KI).

2) Monitor breeding success across several targeted populations for inter-annual variation and to investigate the impact of terrestrial predation. Continue rat control on Granite Island to maintain high breeding performance on the island.

3) Monitor return rates of adults and sub-adults at different populations using micro-chipped individuals to determine survival after the breeding season.

4) Investigate the impact of marine predation with a special focus on the impact of long nosed fur seal predation on penguin population decline.

5) Identify parasite species with molecular methods and investigate the impacts of blood parasites for biological fitness. Consider parasite treatment to decrease the impact of parasites on adults and increase breeding success.

6) Resolve inconsistencies in little penguin genetic findings to date to determine whether the Gulf St Vincent populations still form a single genetic population; investigate inbreeding within and between colonies considering the impact that inbreeding has on survival, reproduction, and disease resistance in other bird species (see Keller & Waller 2002).

III. AIMS

The current funded project had two main objectives: (1) To continue breeding monitoring on Granite Island, Troubridge Island, and at three colonies on Kangaroo Island (Emu Bay, Antechamber bay, Kingscote); and (2) To conduct population surveys on Troubridge Island in October and organise a public census over 2 days on Granite Island.

IV. MATERIALS AND METHODS

Study sites

This project was conducted during the 2014-breeding season between August 2014 and January 2015 on three islands in the Gulf St Vincent: (1) Granite Island (35°37'S, 138°36'E), in the Fleurieu Peninsula. Granite Island is a small island off Victor Harbour connected to the mainland by a bridge causeway open to pedestrians; (2) Troubridge Island (35°06'S, 137°49'E), in the Yorke Peninsula. Troubridge Island is a small sandy island about 7 km east of Sultana Point, which is only accessible by boat with restricted access; and (3) Kangaroo Island (35°47'S, 137°13'E), 112 km south-west of Adelaide. The island is accessible by ferry, 150km long and includes several penguin colonies. Colonies at Antechamber Bay, Emu Bay and Kingscote were included in this study.

Aim 1: Breeding monitoring and survival

Search for active burrows started around mid-August and monitoring was carried out until early December on Kangaroo and Troubridge Islands and until the end of December on Granite Island. Every burrow was checked every 2 weeks during the monitoring period and a burrow was recorded as active if it contained eggs, chicks or adults, or clear evidence of penguin presence, such as fresh droppings or a strong penguin smell.

During each visit, the number of adults, eggs and chicks present in each burrow was recorded in order to assess breeding success. A chick was recorded as fledged when it disappeared from the burrow at about eight weeks of age and was not found depredated nor in any of the other burrows. Breeding success was defined as the number of chicks that fledged per breeding pair. Predation was scored as suspected if eggs or chicks were damaged or removed between visits before the eggs were ready to hatch or the chicks were close to fledgling but adults were still attending their burrows and therefore had not abandoned the nest. Eggs were considered as abandoned if they were found unattended during two consecutive visits and felt cold to the touch. If the outcome of a burrow was unknown at the end of the monitoring period (e.g., the burrow still had eggs and therefore it was unknown whether those eggs hatched and produced fledglings), it was excluded from the analysis for breeding success.

Micro-chipping of individuals was re-initiated to assess survival rate of adults and sub-adults using mark-recapture methods. When present, adults and chicks were captured by hand and removed from their burrow for micro-chipping and measurements. Head length was measured with callipers as an indicator of body size (Miyazaki & Waas 2003) and bill depth was measured to determine the sex of the individual (Arnould et al. 2004; Overeem et al. 2006; Wiebkin 2012). Head length was measured from the tip of the bill to the back of the skull. Bill depth was measured as the vertical thickness of the bill at the nostrils. Adults and chicks were weighed to the nearest 10g. Chicks were only microchipped and weighed just before fledging, at ~7–8 weeks of age (*see also* Dann et al. 2014; Colombelli-Négrel 2015).

Aim 2: Population Census

Penguin censuses were carried out at Troubridge Island (Yorke Peninsula) and on Granite Island (Fleurieu Peninsula). All censuses were conducted by a team of volunteers and the Penguin Ecologist. The censuses were conducted in October to align with censuses conducted in 2013.

Each island was divided into separate smaller sections, and each section was searched along transects for presence or absence of burrows. Once a burrow was identified, the status of the burrow was recorded as active or not active. A burrow was recorded as active if it contained eggs, chicks or adults, or clear evidence of penguin presence such as fresh droppings, a strong penguin smell or recent burrow excavation. A burrow was recorded as inactive if none of the above criteria was found or it had evidence of cobwebs at the entrance. All active burrows were marked with GPS. On Granite Island, all burrows were marked with talcum powder to avoid double counting by different team of volunteers.

On Troubridge Island, about 23% of the island was completely inaccessible due to dense vegetation cover – but showed signs of penguin tracks; therefore, some areas could not be surveyed and population estimates for these areas needed to be extrapolated. The extrapolations were not done using the burrow counts for the whole island but rather using the smaller sections where at least half of the section was surveyed to have robust estimates. The extrapolated data in 2014 was comparable for that done in 2013, as the same areas were inaccessible for census data collection during both years.

In addition to the population census, an acoustic survey was conducted to confirm the presence and density of little penguins within a selected section. The acoustic survey consisted of two eight point counts transects (*see* Bibby et al. 2012) using playback of full bray calls. The survey was conducted on 29th of September 2014 on Granite Island and on 15th of October 2014 on Troubridge Island, 2 hours after dark. Along a transect, each consecutive point was spaced 10 m apart. Once at a point transect, one observer started a selected playback stimuli using an Apple iPod (Apple Inc., USA) connected to a Moshi Bass burger speaker (Moshi Corporation, USA). A second observer then recorded the number of individuals that responded to the stimuli within a 5m radius.

The playback stimuli consisted of one call followed by 10 s of silence, repeated three times for one minute. To create the stimuli, recordings from eight different males from the tested island were used (calls from Granite Island were played on Granite Island and calls from Troubridge Island were played on Troubridge Island). All males were recorded in 2013, when alone in their burrow, using a Zoom Handy Recorder H4n (Zoom Corporation, Australia). All recorders had integrated stereo microphones and were placed outside the burrows (approx. 30-50cm away), facing the entrance of the burrow and hidden in the vegetation. All sound files were recorded as broadcast wave files (44.1 kHz sampling rate, 16-bit depth). The playback stimuli were normalised at -15 db, saved as uncompressed 16 bit 44.1 kHz broadcast wave files (.wav) using Amadeus Pro 1.5 (Hairersoft Inc, Switzerland), and transferred onto the iPod.

The census on Kangaroo Island (carried out in September 2014) was coordinated by Natural Resources Kangaroo Island, and therefore the data are not presented here.

Additional data collected to assess mortality patterns

Motion Camera monitoring

Between August and December 2014, small motion sensor-activated cameras were installed in front of active burrows to continue monitoring potential predators or disturbance. A total of 10 cameras

were installed in front of 10 burrows: five on Granite Island and five at Emu Bay (KI). Two different cameras were used: (1) Scout Guard KG680V Faunatech cameras (Faunatechaustbat, Australia) 140 x 102 x 74 mm, and powered by eight internal AA batteries; and (2) Buckeyes Orion 5030-2 XIR Cameras (BuckEye Cam, Australia) powered by 6V 12A rechargeable battery. Both camera types were weatherproof and could capture 3 megapixels photos with full colour in daytime and monochrome at night via a LED infrared illuminator array. Images were stored on SDHC memory cards upon activation of the motion sensor every 10s. Each burrow was recorded continuously from the day it was found until fledging or until the burrow's failure (either abandonment or predation). The cameras were placed approx. 30-50 cm above the ground, 0.5-1 m from the burrows. The cameras monitored the burrows 24 h/day and were checked every 2 weeks. A total of ~13,049 hours of video images were analysed for evidence of predatory or disturbance activity at burrows.

Mortality Register

Carcasses were collected (when found) during visits to the penguin colonies. All carcasses were stored in -20C freezer and given to Dr Ikuko Tomo, the veterinary pathologist based at the South Australian Museum for analysis. The monitoring of little penguin mortalities is a separately reported project supported by the AMLR NRM Board and other partners.

Parasites and body condition

The 2014 report identified a potential protozoan pathogen (potentially an oocyst-type parasite that is usually seen in gut lining but sometimes found in the blood stage) and a potential apicomplexan, which could be *Plasmodium*, *Shellakia*, *Trypanosoma*, *Hepatozoon*, or *Leucocytozoon.* Therefore, data were collected during the field season 2014-2015 to identify the genus and/or species of the parasites. These data were collected as part of Tamara Burt's Honours project (2014- Nov 2015) entitled 'Parasites and their impacts on fitness in little penguins (*Eudyptula minor*)' under the supervision of A/Prof Ian Menz (Flinders University) and Dr Diane Colombelli-Négrel. This project is funded by Flinders University, DEWNR, NCSSA Conservation Biology and the Lirabenda Endowment Fund. The aims of Tamara's project are: (1) to use molecular and phylogenetic analyses to identify parasites to the genus and potentially species level and (2) to identify the impacts of parasites on the fitness of the little penguins in order to assess their influence on the observed declines of the little penguin populations in the Gulf St Vincent.

To estimate parasite presence, blood samples were collected (0.01ml per bird) with a 25G needle from the foot vein and placed one drop of blood on a slide to prepare blood smears. Blood smears were air-dried, fixed in 99% ethanol for ~5 min, and later stained with Wright-Giemsa. All smears are to be microscopically examined under a 100 x oil immersion lens for presence of parasites back in the laboratory. Few additional drops of blood were stored on FTA paper (Smith & Burgoyne 2004) to identify parasite species via PCR. Body condition was examined by measuring; head length for body size indication (Miyazaka & Waas 2003), bill depth for sex determination (Arnould et al. 2004; Overeem et al. 2006; Wiebkin 2012) and weight to the nearest 10g for adults at the end of the breeding season, and chicks at ~7-8 weeks of age prior to fledging. A small portion of the blood sample was used to measure Haemoglobin concentration using a Haemocue HB 201+ portable haemoglobinometer to measure the direct effect of parasites on Haemoglobin (*see* Dudaniec et al. 2006; Colombelli-Négrel & Kleindorfer 2008).

New Zealand fur seal scat collection

Fur seal scats were collected as part of Sarah-Lena Reinhold's Honours project (2014-April 2015) entitled 'An investigation of long nosed fur seals diet: identifying the importance of commercially fished species and predation on little penguins' under the supervision of Dr Diane Colombelli-Négrel and Prof Simon Goldsworthy (SARDI). This project was funded by Flinders University, DEWNR, BSSA, and the Nature Foundation. The aims Sarah-Lena's project were to: (1) list the prey taxa consumed by the long nosed fur seal in South Australian waters, (2) identify spatial variability in

their diet, (3) compare the dietary profiles between fur seals at breeding colonies versus haulouts, and (4) identify the importance of commercially fished species and little penguins in long nosed fur seal diets.

To estimate fur seal diet, scats were collected between July and September 2014 at the following sites: (1) Granite Island (35.550°S, 138.617°E; FP), (2) West Island (35.608°S, 138.592°E; FP), (3) Seal Island (35.577°S, 138.644°E; FP); (4) Cape du Couedic (36.052°S,136.706°E; KI, breeding site); (5) Cape Gantheaume (35.934°S, 137.445°E; KI, breeding site); (6) Kersaint (36.031°S, 137.132°E; KI); (7) Penneshaw (35.723°S, 137.986°E; KI); (8) Ballast Head (35.723°S, 137.779°E; KI); (9) Kingscote (35.653° S, 137.634° E; KI);(10) Hummocky (35.606°S, 137.235°E KI); (11) Pissy Boy Rock (35.686°S, 136.881°E; KI); and (12) Port Giles (35.033° S, 137.767° E; YP).

Due to the small number of samples collected on Granite Island and its proximity with Seal Island (< 1.5 km), the scats collected on both islands were pooled and collectively referred to as Seal Island scats. A total of 326 scats were collected, averaging approximately 30 scats per study site, with the exception of Ballast Head (KI) (26 scats). Any scats that were found were collected in labelled zip lock bags and stored at -20°C until appropriate analysis in the laboratory was carried out. Prior to analysis, scats were soaked in hot soapy water for 24 hours and then individually sieved with warm to hot water through a 0.5mm and 1.0mm sieve. Contents were sorted as follow: any otoliths, feathers, vertebrae, bone fragments or crustacean carapaces were stored and dried while cephalopod beaks were kept in 70% ethanol. This allowed taxa present in scats to be easily identified and the frequency of different prey types to be calculated and statistically analysed. Scat analysis was based on procedures carried out by Bool et al. (2007). Full details of the methodology can be found in Sarah-Lena Reinhold's thesis.

Report on data collected to assess dispersal and gene flow between colonies

Data on gene flow between penguin colonies were collected as part of Steffi Graff's Honours project (2014-April 2015) entitled 'Conservation genetics of the little penguin: Does potential exist for translocation?' under the supervision of Dr Diane Colombelli-Négrel and Dr Mike Gardner (Flinders University, SA Museum). This project was funded by Flinders University, AMLR NRM Board, and the Nature Conservation Society. The aims Steffi's project were: (1) to quantify genomic sequence diversity and population structure to identify patterns of gene flow between little penguin colonies in the Gulf St Vincent, and (2) to test for inbreeding within colonies.

A total of 91 individuals were used for genetic analysis from eight colonies (n=5-27) in South Australia: (1) Granite Island (Fleurieu Peninsula), (2) Antechamber Bay (Kangaroo Island), (3) Emu Bay (Kangaroo Island), (4) Penneshaw (Kangaroo Island), (5) Kingscote (Kangaroo Island), (6) Vivonne Bay (Kangaroo Island), (7) Troubridge Island (Yorke Peninsula), and (8) Althorpe Island (Yorke Peninsula). These consisted of 75 blood samples and 16 liver/muscle samples. Blood samples were collected (0.01ml per bird) with a 25G needle from the foot vein and were stored on FTA paper (Smith & Burgoyne 2004). The liver/muscle samples were provided by Dr Ikuko Tomo (South Australian Museum) and obtained from deceased chicks collected between 2011 and 2014 on Granite Island, Althorpe Island, and at Kingscote and Penneshaw.

Genetic analysis was conducted using the highly innovative Restriction site associated DNA (RAD) sequencing techniques. RAD sequencing uses digestion sites that occur randomly and frequently to associate genetic markers between individuals and unique nucleotide barcodes linked to the digested DNA fragments (*see also* Booy et al. 2000; Davey & Baxter 2011). Sequences between 130-400bp were identified and used to make comparisons between individuals within a population. Full details of the methodology can be found in Steffi Graff's thesis.

Ethics

This project was approved by the Flinders University ethics committee (E388) and is supported by a scientific permit to conduct the research (Y26040). Permit allows access to Encounter Bay Islands, Kangaroo Island, Troubridge Island and Althorpe Island. Progress report on the numbers of animals that were used will be provided to DEWNR on 31/8/2015.

V. RESULTS

Aim 1: Breeding monitoring and survival

Between August and January, a total of 95 burrows were monitored on Granite Island, Kangaroo Island and Troubridge Island (Table 1). Out of the 95 monitored burrows, 77 showed signs of breeding activity (81%) such as eggs or chicks present in the burrow. Breeding success on Granite Island was the highest with 1.67 (± 0.24) fledglings per pair (n=9) while breeding success at Emu Bay (KI) and on Troubridge Island were the lowest, with 0.60 (± 0.18) fledglings per pair (n=15) and 0.61 (± 0.12) fledglings per pair (n=26) respectively (Table 2; Figure 1).

Three burrows had evidence of abandonment, all at the Kingscote colony. Two burrows were abandoned during the eggs stage, and one was abandoned during the Guarding period (the first two weeks of chick rearing). Five burrows were suspected of predation, all at Emu Bay. One burrow was predated by a goanna (*Varanus rosenbergi*) during the chick stage. At two other burrows, dead chicks were found at the entrance of the burrow showing marks of potential terrestrial predation.

Penguin colonies	Burrow monitored	Breeding burrows	Eggs	Chicks	Fledglings	Groups with 2nd clutch	Burrows predated
Troubridge	35	29	66	36	16	4	0
Antechamber Bay (KI)	11	8	16	11	9	1	0
Kingscote (KI)	16	15	30	21	9	0	0
Emu Bay (KI)	18	16	40	17	9	4	5
Granite	15	9	18	15	15	0	0
Total	**95**	**77**	**168**	**98**	**56**	**9**	**5**

Table 1. Number of eggs, chicks and fledglings produced in total per penguin colony. The table also presents the number of burrows with suspected predation.

Penguin Colonies	2014 Eggs/ Pair (SE)	2014 Chicks/ Pair (SE)	2014 Breeding success (SE)	2013 Eggs/ Pair (SE)	2013 Chicks/ Pair (SE)	2013 Breeding success (SE)
Troubridge	2.28 (0.12)	1.24 (0.14)	0.61 (0.12)	2.12 (0.13)	1.06 (0.17)	0.73 (0.18)
Antechamber Bay (KI)	2.00 (0.26)	1.57 (0.31)	1.13 (0.30)	1.73 (0.12)	1.14 (0.23)	0.50 (0.27)
Kingscote (KI)	2.00 (0.12)	1.40 (0.24)	0.82 (0.30)	1.60 (0.24)	1.20 (0.37)	1.00 (0.58)
Emu Bay (KI)	2.50 (0.12)	1.06 (0.17)	0.60 (0.18)	2.28 (0.23)	1.65 (0.17)	1.00 (0.25)
Granite	2.00 (0.26)	1.67 (0.24)	1.67 (0.24)	2.00 (0.26)	1.50 (0.34)	1.50 (0.34)

Table 2. Breeding success for each penguin colony monitored during the 2014 and 2013-breeding seasons

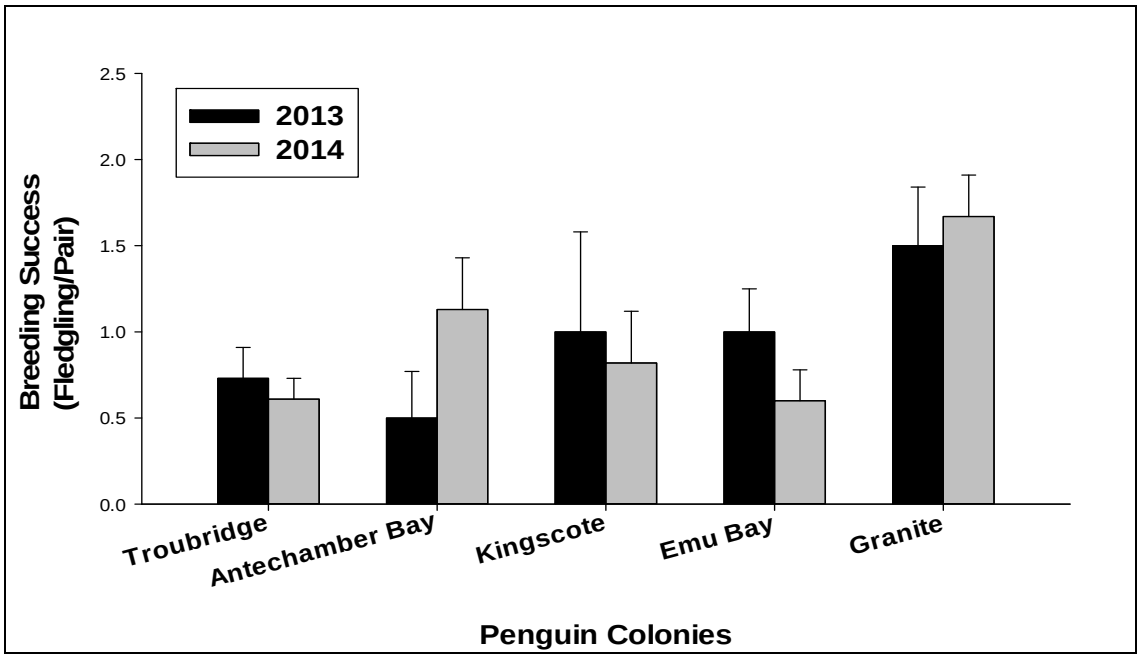

Figure 1. Breeding success across all the penguin colonies monitored in 2013 and 2014

A total of 45 little penguins were captured and 45 individuals (31 adults and 10 chicks) were microchipped in 2014. Table 3 presents chick mass between colonies, which was within the normal range for little penguins (*see* Bool & Wiebkin 2013). The full list of microchipped individuals is presented in Table 1 in Appendix.

Penguin Colonies	No. Individuals sampled	Chick Mass 2014 (mg ± SE)	Chick Mass 2013 (mg ± SE)
Antechamber Bay	4	950.00 ± 88.98	1037.50 ± 12.50
Emu Bay	1	1500.00	1137.50± 42.70
Kingscote	4	1275.00 ± 87.80	1125.00 ± 125.00
Troubridge	9	1277.78 ± 110.59	1196.67 ± 42.39

Table 3. Mass (mg ± SE) of little penguin chicks (aged between 7and 8 weeks) in 2013 and 2014

Aim 2: Population Census

Granite Island

The community censuses were conducted over two days (13th and 22nd of October 2014) by 47 volunteers and two penguin researchers. On the first day, 18 burrows were found active, and five adult penguins and six chicks were seen in their burrows. On the second day, 14 burrows were found active, and nine adult penguins and five chicks (older than 3 weeks or close to fledging) were seen in their burrows. The average number of active burrows for Granite Island is therefore 16 burrows (32 penguins estimated to be present on the island; Figure 2). The average number of active burrows for Granite Island is therefore 16 burrows (32 penguins estimated to be present on the island; Figure 2). It should be noted that out of those 16 burrows, fifteen were being monitored regularly for signs of breeding attempt and nine of those produced eggs.

A total of 16 playback point surveys were conducted on Granite Island on the night of the 29th of September. A total of nine little penguins were heard or seen at those point transects, confirming the results of the community censuses.

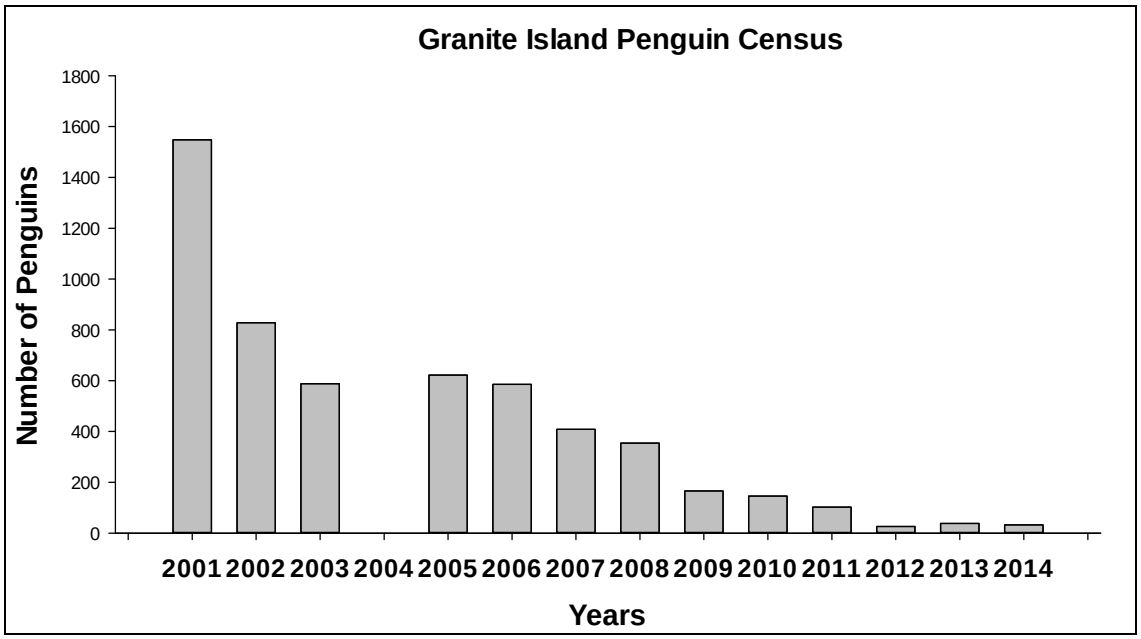

Figure 2. Estimated population size of little penguins on Granite Island between 2001 and 2014

Troubridge Island

The census was conducted over 3 days (14th-16th of October 2014) by a team of four people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and two students). A total of 23% of the island was completely inaccessible due to the vegetation but showed marks for penguin tracks and adult penguins were heard in this area during the census. The cormorants breeding area (the far end of the island) did not show any physical sign of penguin presence. A total of 165 occupied burrows were found and 518 empty burrows. With the estimation, this brings the population census to 203 occupied burrows (406 penguins present on the island at the time of the census) and 628 empty burrows.

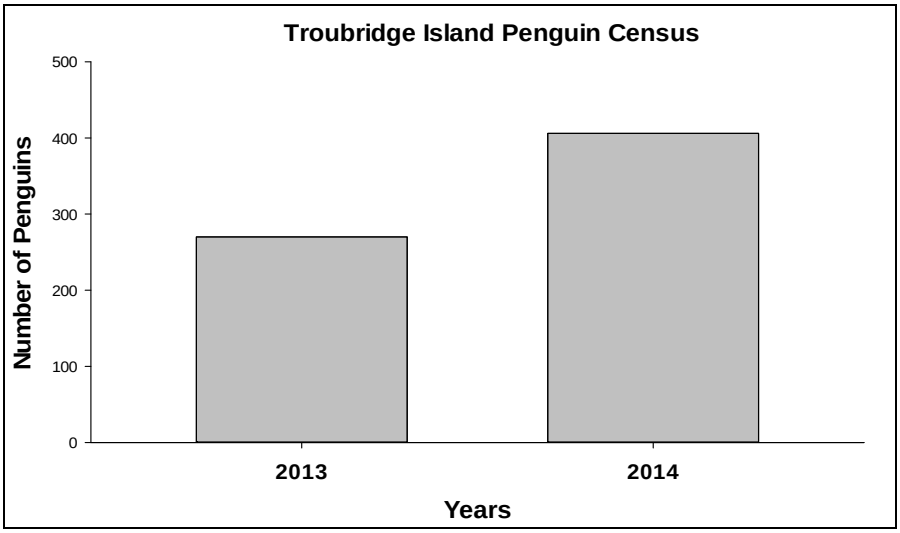

Figure 3. Estimated population size of little penguins on Troubridge Island between 2013 and 2014

Out of the 203 active burrows, 14% showed signs of breeding activity (29 burrows; Table 4). The sex of the adults was identified at 41 burrows: 15 (36.5%) burrows had males only, 11 (27%) burrows had females only and 15 (36.5%) burrows had two adults.

Penguin Colonies	No. Active Burrows	No. Burrows Breeding	No. Burrows with Adults	No. Burrows with Eggs	No. Burrows with Chicks
Troubridge	203	29 (14%)	130 (64%)	14 (7%)	15 (7%)
Granite	16	9 (56%)	6 (37%)	1 (6%)	5 (31%)

Table 4. Percentage of burrows showing signs of breeding activity and number of burrows with adults, eggs and chicks for the 2014-census on Granite and Troubridge Islands

A total of 16 playback point surveys were conducted on Troubridge Island on the night of the 15th of October. During the population census conducted on the same day, only five burrows were found active (with clear signs of penguin presence such as droppings in the burrow, penguins smell, and/or presence of adults, eggs or chicks) in the area selected for the acoustic survey. In response to the acoustic survey, a total of 19 little penguins from 19 different burrows were heard, therefore suggesting that a higher number of little penguins were present during the acoustic survey at night than during the population census during the day.

Additional data collected to assess mortality patterns

Motion Camera monitoring and carcasses

Ten burrows were recorded across the two colonies. A total of four different species of intruders and potential predators were recorded: goannas, feral cats (*Felix catus)*, black rats (*Rattus rattus*), and common brush-tail possums (*Trichosurus vulpecula*) (Figure 4). Only one case of predation was recorded using the motion cameras. Similar to the predation event recorded in 2013, predation by a goanna (*Varanus rosenbergi*) on ~ 6-7 weeks old chicks was recorded during day time at Emu Bay (Figure 5). Goannas were also seen scavenging on fresh little penguin carcasses around active burrows (Figure 5). No predation event was recorded on Granite Island.

Figure 4: Video images from sensor-activated camera installed on Granite Island (GI) and at Emu Bay (EB) in 2014 of four different species of intruders and potential predators: goanna (top left corner; EB), feral cat (top right corner; EB), black rat (bottom left corner; GI), and common brush-tail possums (bottom right corner; GI)

Figure 5: Pictures of (a) a burrow predated by a goanna at the chick stage and (b) scavenging activities by goannas on carcasses at Emu Bay

Mortality Register
A total of 14 carcasses were found during penguin surveys (two at Antechamber Bay, 11 at Emu Bay and one on Granite Island). Nine were collected for necroscopy analysis. At Emu Bay, it should be noted that most carcasses were found near the border of the grasses, where little penguins become visually exposed to predators. Analysis of little penguin carcasses is a separately reported project supported by the AMLR NRM Board and other partners.

Parasites and body condition
A total of 150 individuals (105 in 2013 and 45 individuals in 2014) were sampled for parasite identification. Molecular analyses to ascertain the identity of the parasites found in little penguins are still under investigation. Tamara Burt's project is expected to be finished in November 2015.

New Zealand fur seal scat collection
A total of 326 scats were collected in 2014 to assess long nosed fur seal diet. In total, 21 taxa (species or genera) were identified. Fish remains were found in 305 (92%) scats, cephalopod remains in 155 (47%) scats and bird remains in 43 (13%) scats. Evidence of little penguin remains was found in 38 of the 43 scats containing bird remains, which represents 88% of the bird remains. Key prey species varied regionally. Leatherjackets (*Meuchenia spp.*) and garfish (*Hyprohamphus melanochir*; a commercially fished species) were the most common taxa consumed by fur seals on Kangaroo Island and in the Yorke Peninsula, while little penguins were the most important preys in terms of estimated biomass for Fleurieu Peninsula. Little penguin remains were present in 40% of scats collected in the Fleurieu Peninsula, but in only 4% of the scats collected on Kangaroo Island and in 10% of the scats collected in the Yorke Peninsula. Full details of the results can be found in Sarah-Lena's thesis.

Dispersal and gene flow between colonies

The study found subtle genetic structuring amongst the eight colonies, suggesting that individuals on Troubridge Island (Yorke Peninsula) were genetically distinct from individuals living at the other colonies (Emu Bay, Kingscote, Penneshaw, Antechamber Bay and Vivonne Bay on Kangaroo Island, and Granite and Althorpe Islands). However, low population differentiation indicated moderate levels of gene flow between these two groups and confirmed that Kangaroo, Granite, and Althorpe Island colonies were panmixed. Analyses showed little evidence of inbreeding depression within colonies. However, the inbreeding coefficient for Granite Island was relatively high compared to the other colonies. Full details of the results can be found in Steffi's thesis.

VII. DISCUSSION

The main findings of this study are: (1) Granite Island population continue to have the highest breeding success, while Emu Bay (KI) and Troubridge Island populations had the lowest in 2014; (2) predation at burrows remained an issue on Kangaroo Island with 31% of the burrows showing signs of predation, likely by goannas; (3) population censuses showed stabilizing trends for both Granite and Troubridge Islands; (4) the importance of little penguins in long nosed fur seal diets varied regionally from 40% in the Fleurieu Peninsula to 10% in the Yorke Peninsula and 4% on Kangaroo Island; and (5) individuals sampled on Troubridge Island were somewhat genetically different from individuals belonging to Granite, Kangaroo and Althorpe Islands.

Population census

On Granite Island, the penguin census showed 32 penguins present on the island in 2014 compared to the 38 and 26 individuals estimated in 2013 and 2012 respectively (*see* Colombelli-Négrel & Kleindorfer 2014). The similar numbers of active burrows recorded during the population census and through monitoring confirmed that these numbers were a good estimate of the current population on the island for 2014. However, although numbers seem to be stabilising on Granite Island, ongoing monitoring across several years is still necessary to confirm the long-term population trends.

In the Yorke Peninsula, the 2014 population census estimated the Troubridge population at 406 penguins, which was higher than the 270 penguins recorded in 2013 (Colombelli-Négrel & Kleindorfer 2014). While numbers seem to be increasing, further monitoring across the next years is necessary to confirm this trend. In addition, the results of the acoustic survey suggested that a higher number of individuals might be present on the island at night. If the 628 empty burrows also found during the daytime census were included, this would bring the number of individuals to 1662 penguins, which is comparable to what was found by previous studies (Wiebkin 2010; Bool & Wiebkin 2013). The different results across years and by different researchers clearly highlight the need for standardised methods to ensure comparable results, and also highlight the advantages of using more than one monitoring method when clear signs (such as fresh droppings, a strong penguin smell, recent burrow excavation or presence of adults or chicks) are not present. In addition, this study suggests that more penguins may be resting at night than found during daytime censuses. It should be noted that out of the 203 burrows found active, only 14% showed signs of breeding activity, thus suggesting that all adult penguins may not be present at one time on the island.

Reproductive success

As found in the 2013 study (Colombelli-Négrel & Kleindorfer 2014), breeding success on Granite Island was higher than at any of the other colonies, despite population decline. Breeding success on Troubridge Island, on the contrary, remained the lowest, and has remained the lowest since 2004 (Table 5). Such differences in breeding success between colonies may be explained by a variety of reasons, including variation in prey items and food availability. Indeed, Fortescue (1999) showed that colonies located at lower latitudes along the Australian east coast had higher breeding success than those located along the west coast, which he attributed to differences in foraging ranges, oceanographic characteristics and local food availability. Therefore, further investigation into factors influencing lower reproductive success on Troubridge Island may be required, with a focus on prey availability and differences in foraging effort between colonies.

Location	Period	No. Years	Breeding Success
Granite Island	1990-2013	17	0.96
Granite Island	2013	1	1.50
Granite Island	2014	1	1.67
Antechamber Bay	2012	1	0.52
Antechamber Bay	2013	1	0.50
Antechamber Bay	2014	1	1.13
Emu Bay	2012	1	1.04
Emu Bay	2013	1	1.00
Emu Bay	2014	1	0.60
Troubridge	2004-2009	4	0.77
Troubridge	2013	1	0.73
Troubridge	2014	1	0.61

Table 5. Comparison of breeding performance among little penguin colonies in South Australia: breeding success is calculated as the number of chicks that fledged per breeding pair.

Contrary to previous years (Table 5), breeding success at Antechamber Bay more than doubled from 2012 and 2013 (on average 0.51 chicks fledged per breeding pair) to 2014 (1.13 chicks fledged per breeding pair). However, while population census was not done in 2014, there was evidence for severe decline: in 2014, only 11 burrows were considered active compared with 22 active burrows in 2013. This finding further supports the 2013 census trends showing a decline in population numbers by nearly 50% (Kinloch unpublished data, presented at the Penguin Monitoring Overview Meeting in November 2013).

Similar to the 2013 study (Colombelli-Negrel & Kleindorfer 2004), this study found no evidence of predation at burrows on Granite Island. While previous studies suggested that water rats (*Hydromys chrysogastes*) and black rats may have impacted breeding success in the past (Bool et al. 2007; Preston 2008), this study clearly highlights the positive and long term impacts of the coordinated rat management that occurred in 2006. On Kangaroo Island, however, goannas appeared to be regular predators at little penguin burrows. While only one predation event was recorded with the motion cameras, 5 (31%) burrows out of the 16 monitored for breeding showed evidence of predation during the chick stage. It should be noted that most colonies on Kangaroo Island, which were considered unsure or stable until now, are now showing declining trends (Natural Resources Kangaroo Island 2014). Predation at such a noteworthy rate on a small colony may have significant impact on long-term population trends and, therefore, further monitoring of predation on Kangaroo Island is required to understand its full impact on breeding success and population decline.

Marine Predation

As found in other studies, the importance of little penguins in long nosed fur seal diets within South Australia showed regional variation, ranging from 37-47% in the Fleurieu Peninsula (Bool et al. 2007; Reinhold 2015) to 10% in the Yorke Peninsula (Reinhold 2015) to 2-5% on Kangaroo Island (Baylis and Nichols 2009; Reinhold 2015). Interestingly, while located near one of the largest penguin colony in the Gulf St Vincent, only 10% of scats from Yorke Peninsula site had little penguin remains, while the Fleurieu Peninsula sites had the highest percentage of penguin remains (located near the smallest penguin population). Therefore, the results of Sarah-Lena Reinhold's Honours thesis suggest that little penguin availability within the regions alone may not be driving higher predation rates, but instead may be a response by the seals to the absence or reduced availability of other prey species. In support for this hypothesis, Daneri et al. (2008) suggested that Antarctic fur seal (*Arctocephalus gazella*) shift their preferred prey item and only preyed on chinstrap penguins (*Pygoscelis antarctica*) in periods of low krill abundance, when the energetic cost

of foraging for krill became too high. In addition, it should be noted that seal foraging may not occur exactly at the site where they haul out, and therefore little penguin prey could come from other colonies around the hauling sites and not just from the most adjacent penguin colonies. Indeed, previous studies have shown that fur seals travel between 37 and 1154 km away from their haul out site when foraging (e.g., Harcourt et al. 1995; Page et al. 2006). Finally, robust prey species that can survive digestion quiet well, such as penguins, may be present in several scats and could be over-represented (Gales et al. 1993; Fea and Harcourt 1997). In this study, it was assumed that the presence of feathers in one scat represented an entire bird consumed. If the remains of a single consumed penguin were actually spread across multiple successive scats (potentially deposited over multiple days), then the extent and importance of birds and penguins in the long nosed fur seal diet is likely to have been over estimated. Therefore, while increasing fur seal populations and increasing predation pressure from fur seals may partly explain recent declines in some little penguin populations, its significance and interaction with other factors (such as prey availability, terrestrial predation and/or parasites for example; reviewed in Wiebkin 2011) still require further investigation.

Population genetic structure

Fine-scale population genetic structure analysis identified two genetically distinct populations. The first population included seven of the eight colonies sampled and consisted of Emu Bay, Kingscote, Penneshaw, Antechamber Bay and Vivonne Bay (all on Kangaroo Island), as well as Granite and Althorpe Islands. The second population consisted solely of Troubridge Island, suggesting that this colony was genetically more isolated. However, low population differentiation analysis indicated that moderate levels of gene flow still occurred between the two populations. The isolation of Troubridge Island may be a result of recent genetic divergence as suggested by Wiebkin (2010), which could have occurred, for example, due to the location of Troubridge on a low sand island (Burridge et al. 2015) and/or local adaptation. Indeed, morphological variation between populations, such as in bill for example, is determined by selective pressure linked to foraging, and larger bills increase efficiency of obtaining food (Agnew & Kerry 1995; Weibkin 2012). In support for this hypothesis, other studies have indicated that individuals living on Troubridge Island have larger body size and longer bills than those living on other islands (Wiebkin 2012; Colombelli-Négrel unpublished data). In addition, differences in breeding phenology, which have been observed among other colonies in South Australia (Peucker et al. 2009), may also have contributed to the different population structure found by Graff (2015).

VIII. DIRECTIONS FOR FUTURE RESEARCH

1) Continue long-term annual monitoring of several targeted populations to record penguin numbers and trends across the Gulf St Vincent with a specific focus on Troubridge Island, Granite Island, Antechamber Bay (KI) and Emu Bay (KI).

2) Continue monitoring breeding success across several targeted populations for inter-annual variation and further investigate the impact of terrestrial predation on Kangaroo Island. Continue rat control on Granite Island to maintain high breeding performance.

3) Assess annual survival rates of adults and sub-adults (using micro-chipped individuals) and continue to measure the impacts of predation, parasites and diseases on survival.

4) Investigate variation in food availability, foraging effort and resource use between colonies.

5) Test whether reproductive isolating mechanisms exist between the two genetic populations identified.

6) Develop population viability analysis models to explore how variation in each of the parameters listed above affect population trends and population vulnerability to environmental change.

IX. COMMUNITY ENGAGEMENT

Forty seven volunteers participated in the Granite Island penguin census in October 2014. Four volunteers participated in field trips to collect the data, and helped with penguin census on Troubridge Island. One third-year university student and three Honours students worked on little penguin related projects (specifically investigating NZ fur seal predation, identifying blood parasites and analysing genetic diversity between populations). Dr Diane Colombelli-Négrel gave a presentation to the public at Kingscote on 21st of September, and on Granite Island on 13th and 22nd of October. Dr Diane Colombelli-Négrel gave a presentation to the KI NRM Board on 22nd of September and to the Biology Society of SA on 2nd of October. An article calling for community volunteers to join the Granite Island Penguin count was released in October in the Victor Harbour Times.

Below is a list of the media releases for the project for 2014:

1. The Times Victor Harbour (13 November 2014)

   http://www.victorharbortimes.com.au/story/2694165/little-penguin-census-shows-numbers-stabilise-on-granite-island/?cs=1537
2. The advertiser (Australia, 28/09/14)
3. The Squadron Quarterly (Australia, Spring Edition 2014, Volume 28 Issue 3)

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XII. APPENDIX – List of individuals that were captured and microchipped in 2014

Island	Reference Number	Age Category
Troubridge	982000063644562	Adult
Troubridge	982000063643774	Adult
Troubridge	982000063645151	Adult
Troubridge	982000063644952	Adult
Troubridge	982000063645392	Adult
Troubridge	982000063644781	Adult
Troubridge	982000063644121	Adult
Troubridge	982000063643767	Adult
Troubridge	982000063644894	Adult
Troubridge	982000063644673	Adult
Troubridge	982000063643896	Adult
Troubridge	982000063645432	Adult
Troubridge	982000063643812	Adult
Troubridge	982000063643994	Adult
Troubridge	982000063644186	Adult
Troubridge	982000063644453	Adult
Troubridge	982000063644209	Chicks
Troubridge	982000063644735	Chicks
Troubridge	982000063643750	Chicks
Troubridge	982000063645172	Chicks
Troubridge	982000063645196	Chicks
Troubridge	982000063645083	Chicks
Kingscote	982000063643848	Adult
Kingscote	982000063643849	Chicks
Emu Bay	982000063644580	Adult
Emu Bay	982000063644657	Adult
Emu Bay	982000063644593	Adult
Emu Bay	982000063644601	Adult
Emu Bay	982000063644350	Adult
Emu Bay	982000063645362	Adult
Emu Bay	982000063644842	Adult
Emu Bay	982000063644178	Adult
Emu Bay	982000063645004	Adult
Emu Bay	982000063644934	Adult
Emu Bay	982000063644475	Adult
Emu Bay	982000063644977	Adult
Emu Bay	982000063643845	Adult
Emu Bay	982000063645026	Chicks
Antechamber Bay	982000063615775	Adult
Antechamber Bay	982000063643598	Chicks
Antechamber Bay	982000063644815	Chicks