Penguin monitoring and conservation activities in the Gulf St Vincent

July 2015 – June 2016

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

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inspiring achievement

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I. SUMMARY

This project further investigated little penguins (*Eudyptula minor*) population declines in the Gulf St Vincent by focusing on breeding performance and blood parasites on three islands (Granite, Troubridge and Kangaroo Islands). Population censuses showed stabilizing trends for both Granite and Troubridge islands, with 22 penguins present in 2015 on Granite Island and 313 penguins present on Troubridge Island. Population census on Kangaroo Island however showed declining trends with 84% decline since 2011. Granite Island continues to have the highest breeding success (1.00 ± 0.45 fledgling per pair; n=5) while Emu Bay had the lowest (0.29 ± 0.13 fledgling per pair; n=20). On Kangaroo Island, predation at burrows remained an issue at Emu Bay with 25% of the burrows showing signs of predation. Blood parasites (*Haemoproteus* and *Plasmodium spp*.) were identified in 86.4% of the individuals sampled. There was no impact of blood parasite presence on Hb concentrations or Hematocrit levels, but individuals with multiple infections had longer bills and smaller bill depths and widths than those with single infection or non-infected individuals. Finally, this report further explored population differentiation and found that there were substantial morphological variation for bill measurements (except bill length) and body mass among the different breeding colonies.

II. INTRODUCTION

This project further investigated potential explanations for the decline of little penguins (*Eudyptula minor*) in the Gulf St Vincent as a response to the population censuses conducted on Granite Island (Encounter Bay) and Kangaroo Island over the last few years indicating drastic population declines. The overall project aims to monitor targeted populations to collect baseline information and increase awareness of little penguin conservation issues with a particular focus on three main issues: (1) factors impacting breeding success, (2) factors impacting adults and sub-adults survival and (3) the distinctiveness of the populations and their interactions. The following report outlines the data collected between July 2015 and June 2016.

The 2015 report 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.

The 2015 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 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.

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, Granite Island and at four colonies on Kangaroo Island (Emu Bay, Antechamber bay, Kingscote, Vivonne Bay). Genetic data were also analysed to identify blood parasites (and their potential impacts) in little penguins and additional morphological data were analysed to further investigate differentiation between the colonies.

IV. MATERIALS AND METHODS

Study sites

This project was conducted during the 2015-breeding season between August 2015 and January 2016 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 for breeding monitoring and colonies at Antechamber Bay, Emu Bay, Kingscote and Vivonne Bay were included for population surveys.

Aim 1: Breeding monitoring and survival

Search for active burrows started around mid-August and monitoring was carried out until November on Kangaroo and Troubridge Islands and until the end of January on Granite Island. 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. Once found active, burrows were checked every 2 weeks.

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 in 2014 to assess survival rate of adults and sub-adults using mark-recapture methods. When present and accessible, 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 nostrils 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 2015a).

Aim 2: Population Census

Penguin censuses were carried out on Troubridge Island (Yorke Peninsula), Granite Island (Fleurieu Peninsula) and Kangaroo Island. All censuses were conducted by a team of volunteers and the Penguin Ecologist. The censuses were all conducted at the end of September and in October to align with previous censuses conducted in 2013 and 2014.

Each surveyed colony was divided into separate smaller sections and each section was searched along transects for presence or absence of penguin burrows. Once a burrow was found, its status 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 if 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.

Not all sections of the colonies were surveyed on Kangaroo Island. Therefore, in the results section, estimated number of little penguins across years, in each colony, are only presented for the surveyed sections. The details of the section surveyed are as follow: (1) Emu Bay: Boat Ramp and Whittle; (2) Antechamber Bay: Cowry Beach, Post Point and Cape Coults; (3) Kingscote: Hospital Beach and Tidal Pool and (4) Vivonne Bay: Point Ellen North.

As per previous years, about 20% of Troubridge Island was completely inaccessible due to dense vegetation cover – but showed signs of penguin tracks. As a result, some areas could not be surveyed and population estimates for these areas had 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 sections were surveyed to have robust estimates. The extrapolated data in 2015 was comparable for that done in 2014 and 2013, as the same areas were inaccessible for census data collection during all three years.

In addition to the population census, an acoustic survey was conducted on Troubridge Island to confirm the density of little penguins observed during the day. The acoustic survey was conducted on the night of the 13th of October 2015, 2 hours after dark. The acoustic survey consisted of two eight point counts transects (*see* Bibby et al. 2012; Colombelli-Négrel 2015b) conducted in two different sections (total transects = 4; total points survey = 32). Along each transect, each consecutive point was spaced 10 m apart. Once at a point transect, one observer started a selected playback of full bray calls 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 Troubridge Island were used. 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.

Additional data

Blood parasites

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/or a potential apicomplexan, which could be *Plasmodium*, *Shellakia*, *Trypanosoma*, *Hepatozoon*, or *Leucocytozoon.* This report follows on this results and analysed data collected during two breeding seasons (2013 and 2014) using molecular techniques to identify the genus and/or species of the blood parasites. These data were analysed as part of Tamara Burt's Honours project (2014-2016) entitled 'Blood 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 was funded by Flinders University, DEWNR, NCSSA Conservation Biology and the Lirabenda Endowment Fund. The aims of Tamara's project were: (1) to use molecular analyses to identify blood parasites to the genus level and (2) to identify their potential impacts on little penguin fitness in order to assess their influence on the observed population declines in the Gulf St Vincent.

To estimate blood parasite species and presence, blood samples were collected (0.01ml per bird) with a 25G needle from the foot vein and stored on FTA paper (Smith & Burgoyne 2004). To test for potential impacts of blood parasites on fitness (adults only), morphological measurements, haemoglobin (Hb) concentration and haematocrit (HCT) data were correlated with parasite presence. To obtain Hb concentration (g dL-1) and haematocrit data, a small portion of the blood collected was placed in a portable HemoCue HB 201+ haemoglobinometer (see also Dudaniec et al. 2006; Colombelli-Negrel and Kleindorfer 2008) while another portion (10 µL in total) was placed into a heparinised capillary tube centrifuged in the field for 2 minutes at 15,000g. The following morphological measurements were also taken at the time of capture: (1) bill length head (measured from the back of the head to the tip of the bill); (2) bill length (measured from the tip of the bill to the base of the bill, where the feathers start); (3) bill depth base (measured as the vertical thickness of the bill at the base of the bill); (4) bill depth nostrils (measured as the vertical thickness of the bill at the nostrils); (5) bill width (measured at the base of the bill); and (6) body mass (weighed to the nearest 10 g).

Morphological differentiation between colonies

The 2015 report identified some genetic differentiation between Troubridge Island and the other monitored colonies. To further investigate potential divergence between the little penguin populations, this report focused on morphological differentiation. Geographic variation in bill size and body mass across the five breeding colonies was investigated in relation to environment parameters (air temperature, sea surface temperature and sea depth) and geographic distances between the colonies. All morphological measurement were recorded as above. The sex of the individuals was determined using bill depth nostrils measurements as previously described for little penguins (see Arnould et al. 2004; Overeem et al. 2006; Wiebkin 2012). Environmental data were obtained for the last 10 years (2003–2014). Air temperature data were obtained from the Australian Bureau of Meteorology database (http://www.bom.gov.au/climate/data/) and the data on sea surface temperature were sourced from the Integrated Marine Observing System (IMOS) http://imos.org.au/home.html. Mean water depths (WD) were obtained from the Geoscience Australia website: http://www.ga.gov.au (Whiteway 2009). Distances between the colonies were estimated using the measurement tool in Google Earth 7.1 (http://earth.google.com).

Ethics

This project was approved by the Flinders University Animal Welfare Ethics Committee (Project number No. E388) and Flinders University Social and Behavioural Research Ethics Committee (Project number No. 7085). It is also 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 30/6/2016.

V. RESULTS

Aim 1: Breeding monitoring and survival

Between August and January, a total of 84 burrows was monitored on Granite Island, Kangaroo Island and Troubridge Island (Table 1). Out of the 84 monitored burrows, 62 showed signs of breeding activity (74%) such as eggs or chicks present in the burrow. Breeding success on Granite Island was the highest with 1.00 (± 0.45) fledglings per pair (n=5) while breeding success at Emu Bay (KI) was the lowest with 0.29 (± 0.13) fledglings per pair (n=20) (Table 2; Figure 1).

Two burrows had evidence of abandonment, one at the Kingscote colony and one on Troubridge Island, and both of them happened during the incubation period. Eight burrows were suspected of predation, with five of them at Emu Bay, one at Antechamber Bay and two on Troubridge Island. An additional burrow was seen by the researchers predated by a goanna (*Varanus rosenbergi*) at Emu Bay, supposedly during the chick stage (however, this was not one of the monitored burrows). At all burrows predated at Emu Bay and at Antechamber Bay, dead chicks were found at the entrance. The two burrows on Troubridge Island were predated during the incubation period.

Penguin colonies	Burrow monitored	Breeding burrows	Eggs	Chicks	Fledglings	Groups with 2nd clutch	Burrows predated
Troubridge	**37**	**28**	**60**	**28**	**9**	**3**	**2**
Antechamber Bay (KI)	**5**	**4**	**10**	**6**	**2**	**1**	**1**
Kingscote (KI)	**6**	**6**	**14**	**6**	**2**	**1**	**0**
Emu Bay (KI)	**24**	**20**	**44**	**31**	**4**	**2**	**5**
Granite	**12**	**5**	**10**	**5**	**5**	**0**	**0**
Total	**84**	**62**	**136**	**76**	**22**	**7**	**8**

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	2015 Eggs/ Pair (SE)	2015 Chicks/ Pair (SE)	2015 Breeding success (SE)	2014 Eggs/ Pair (SE)	2014 Chicks/ Pair (SE)	2014 Breeding success (SE)
Troubridge	2.14 (0.10)	1.04 (0.19)	0.43 (0.16)	2.28 (0.12)	1.24 (0.14)	0.61 (0.12)
Antechamber Bay (KI)	2.50 (0.50)	1.50 (0.29)	0.40 (0.50)	2.00 (0.26)	1.57 (0.31)	1.13 (0.30)
Kingscote (KI)	2.33 (0.33)	1.20 (0.37)	0.50 (0.50)	2.00 (0.12)	1.40 (0.24)	0.82 (0.30)
Emu Bay (KI)	2.20 (0.14)	1.55 (0.23)	0.29 (0.13)	2.50 (0.12)	1.06 (0.17)	0.60 (0.18)
Granite	2.00 (0.00)	1.00 (0.45)	1.00 (0.45)	2.00 (0.26)	1.67 (0.24)	1.67 (0.24)

Table 2. Breeding success for each penguin colony monitored during the 2015 and 2014-breeding seasons

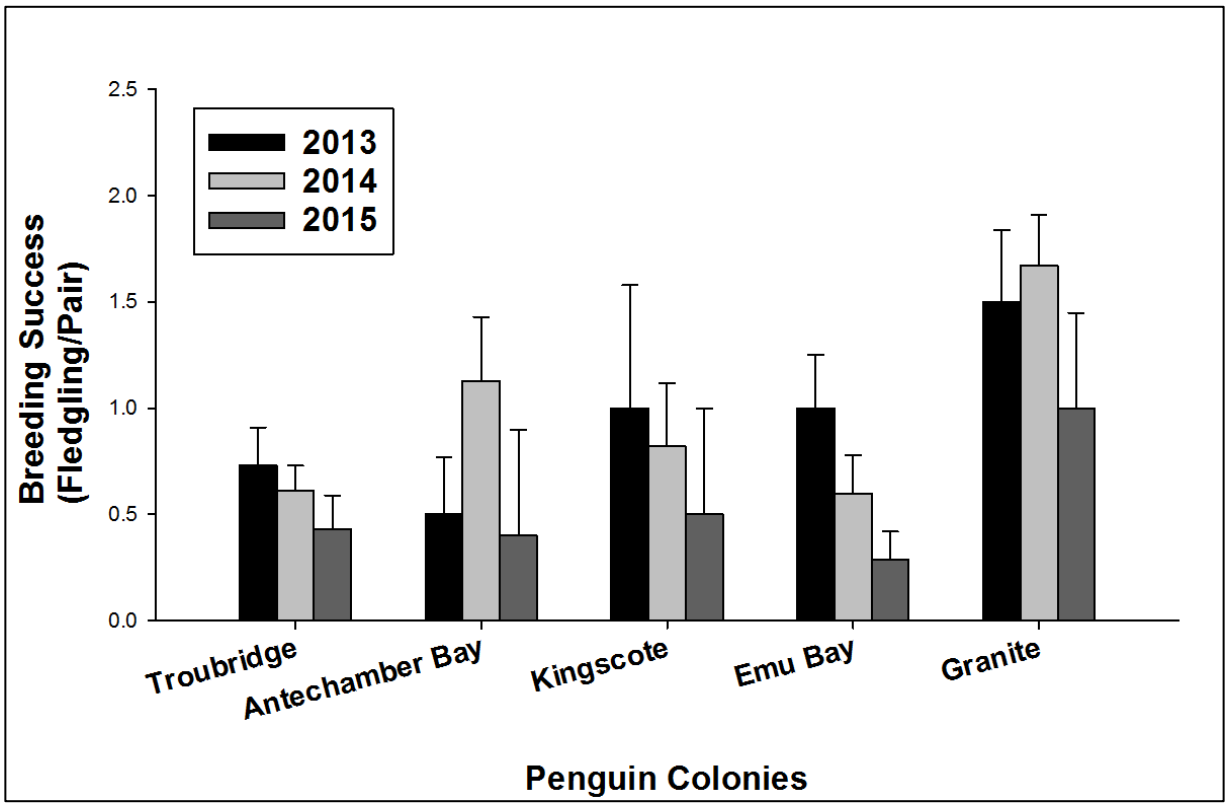

Figure 1. Breeding success across all the penguin colonies monitored in 2013, 2014 and 2015

Only five additional little penguins (all adults) could be captured and microchipped in 2015. Seven individuals that were previously microchipped were re-sighted in 2015: three on Troubridge Island, two at Emu Bay (previously microchipped in 2014) and two on Granite Island (previously microchipped in 2010 and 2011). The full list of microchipped individuals (included re-sighted individuals) is presented in Table 1 in Appendix.

Aim 2: Population Census

Granite Island

The community censuses were conducted over two days (12th and 19th of October 2015) by 34 volunteers and two penguin researchers. On the first day, a total of 10 active burrows (mostly along the north side of the island) were found and eight adult penguins were seen in their burrows. On the second day, a total of 12 active burrows (again mostly on the north side of the island) were found and six adult penguins were seen in their burrows. The two new burrows found during the second day were definitively not active the previous week. Therefore, the average number of active burrows for Granite Island is therefore 11 burrows (22 penguins estimated to be present on the island; Figure 2). It should be noted that out of those 11 burrows, all were being monitored regularly for signs of breeding attempt and only five of those showed signs of breeding activity.

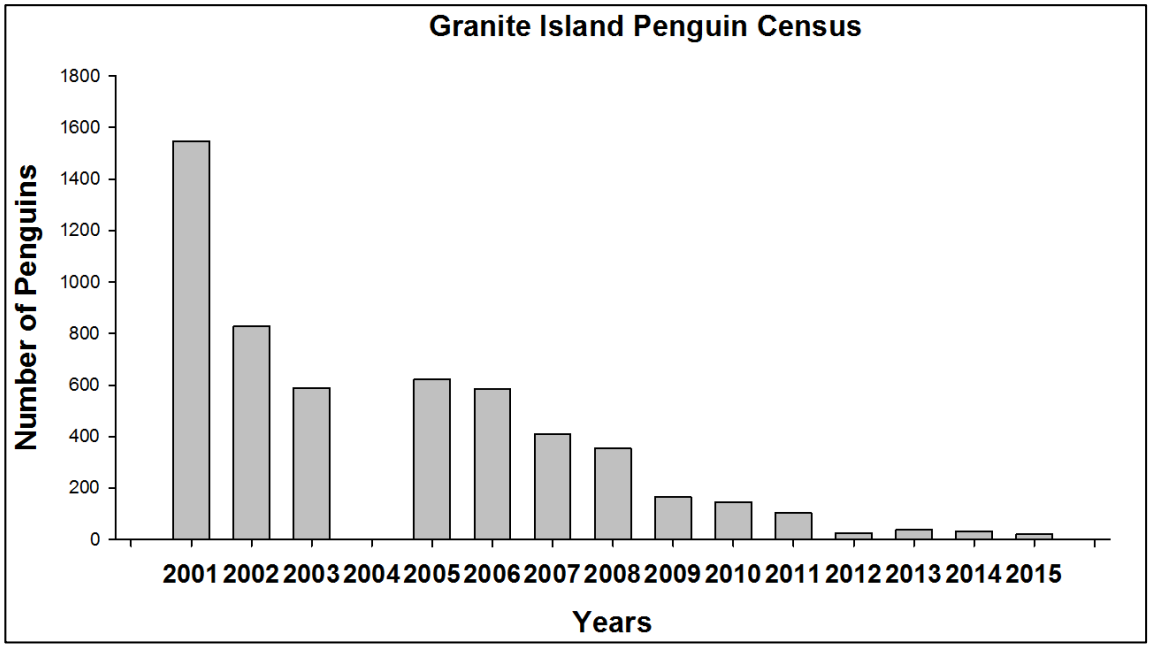

Figure 2. Estimated population size of little penguins on Granite Island between 2001 and 2015

Troubridge Island

Population census on Troubridge Island was conducted on over three days (13th-15th of October 2015) by a team of four people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant, a student and Deborah Furbank, Natural Resources Northern and Yorke). A total of 143 occupied burrows and 621 empty burrows were found. As per previous years, 20% of the island was completely inaccessible due to the vegetation (but showed marks for penguin tracks) and therefore had to be extrapolated. With the estimation, this brings the population census to 157 occupied burrows (313 penguins present on the island at the time of the census) and 778 empty burrows.

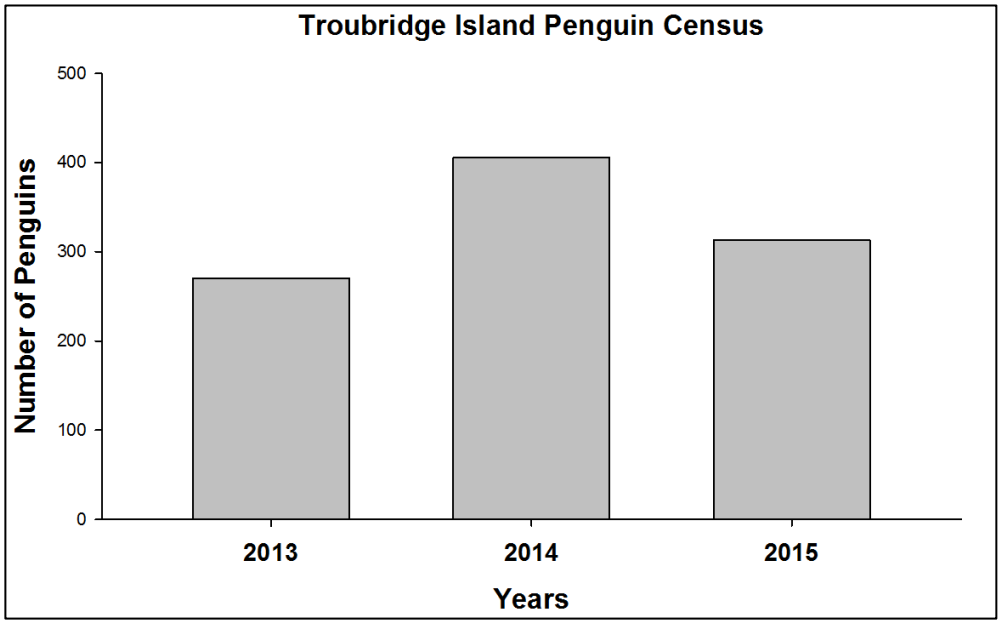

Figure 3. Estimated population size of little penguins on Troubridge Island between 2013 and 2015

Out of the 157 active burrows, 15% showed signs of breeding activity (22 burrows; Table 3). The sex of the adults was identified at 93 burrows: 30 (32%) burrows had males only, 41 (44%) burrows had females only and 22 (24%) 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	157	22 (15%)	119 (76%)	12 (8%)	15 (10%)
Granite	11	5 (45%)	6 (54%)	5 (45%)	0 (0%)

Table 3. Percentage of burrows showing signs of breeding activity and number of burrows with adults, eggs and chicks for the 2015-census on Granite and Troubridge Islands

A total of 32 playback point surveys were conducted on Troubridge Island on the night of the 13th of October in two different sections (16 point surveys in each section). During the population census conducted on the same day, eight and one burrows respectively 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 two areas selected for the acoustic survey (total nine active burrows for the two sections). In response to the acoustic survey, a total of 15 and nine little penguins from different burrows were heard (total of 24 adults heard over the two sections). Therefore, the acoustic survey suggested that, on average, penguin numbers increased by three fold during night time.

Kangaroo Island

Population census at Emu Bay (sections Boat Ramp and Whittle) was conducted on the 20th of September by a team of three people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and a volunteer). A total of 21 active and 8 inactive burrows were found (42 penguins estimated to be present in the colony; Figure 4).

Population census at Antechamber Bay (sections Cowry Beach, Post Point and Cape Coults) was conducted on the 21st of September by a team of three people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and a volunteer). A total of 5 active and 69 inactive burrows were found (10 penguins estimated to be present in the colony; Figure 4).

Population census at Vivonne Bay (Point Ellen North section) was conducted on the 21st of September by a team of three people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and a volunteer). A total of 5 active and 40 inactive burrows were found (10 penguins estimated to be present in the colony; Figure 4).

Population census at Kingscote was conducted over two days: (1) census at the Hospital Beach section was conducted on the 20th of September by a team of three people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and a volunteer). A total of 6 active and 88 inactive burrows were found; and (2) census at the Tidal Pool section was conducted on the 6th of October by a team of three people (Research assistant and two volunteers). A total of 6 active and 23 inactive burrows were found. A total of 24 penguins were estimated to be present in the colony.

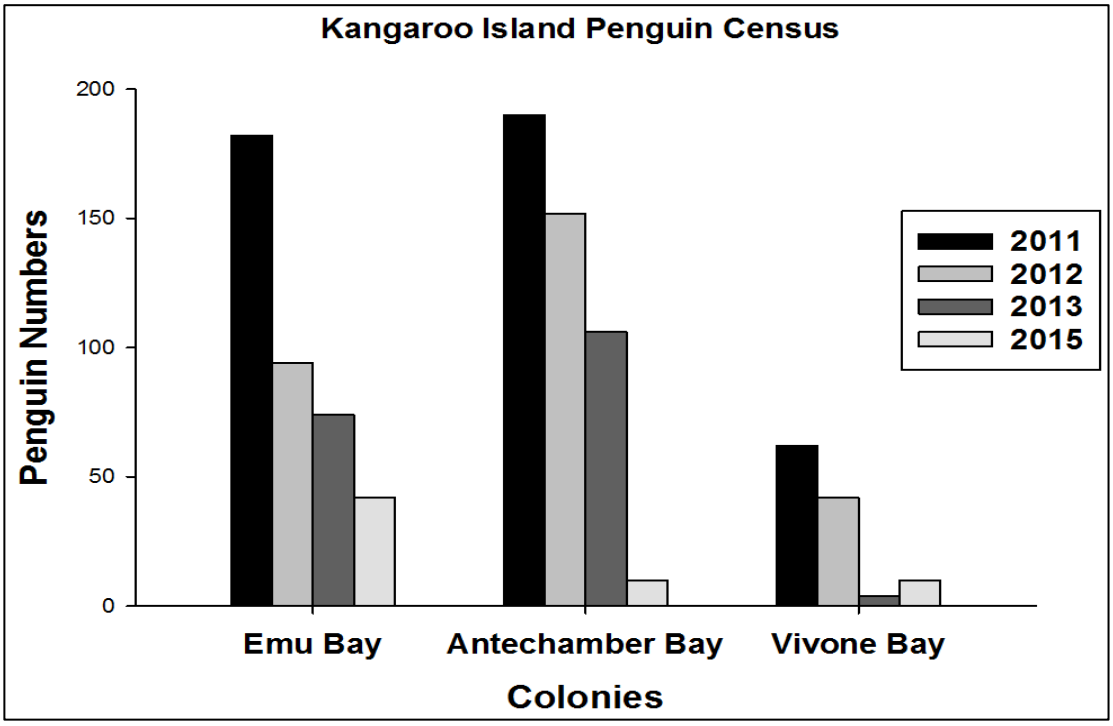

Figure 4. Estimated population size of little penguins at three colonies on Kangaroo Island between 2011 and 2015

Additional data

Blood parasites

A total of 162 little penguins (94 adults, 68 chicks) were sampled between 2013 and 2014 for blood parasite presence. Molecular analysis identified blood parasites in 86.4% of the individuals sampled (85 adults, 55 chicks): 98 % of the infected individuals were infected with *Haemoproteus spp.* and 82 % with *Plasmodium spp.* In addition, 80% of the infected individuals had multiple infections and were infected with both *Haemoproteus* and *Plamodium spp*. Blood parasite presence did not differ between years or sexes. However, there was a higher prevalence of *Haemoproteus* and *Plamodium* infections in adults than in chicks. There were also significant variation in blood parasite presence between the colonies: 100% of individuals were parasitised on Althorpe Island and at Antechamber Bay compared to only 44% on Granite Island. There was no relationship between blood parasite presence and either Hb concentration or Hematocrit levels. However, individuals with multiple infections had longer bills and smaller bill depths and widths than those with single infection or non-infected individuals.

Morphological differentiation between the colonies

There was substantial morphological variation among the different colonies for body mass and bill measurements (except bill length). Additionally, colonies further located from each other were more different morphologically than adjacent colonies. Morphological traits were also correlated to environment parameters: (1) birds at colonies surrounded by hotter sea surface temperatures were heavier with longer and larger bills and (2) birds with larger and longer bills were also found at colonies surrounded by shallower waters.

VI. DISCUSSION

The main findings of this study are: (1) Granite Island population continues to have the highest breeding success, while Emu Bay (KI) population had the lowest; (2) predation at burrows remained an issue at Emu Bay with 25% of the burrows showing signs of predation and dead chicks being found regularly in the colony; (3) population censuses showed stabilizing trends for both Granite and Troubridge Islands; (4) blood parasites (*Haemoproteus* and *Plasmodium spp*.) were identified in 86.4% of the individuals sampled; and (5) there were substantial morphological variation among the different breeding colonies for bill depth base and body mass.

Population census

Population censuses on Granite and Troubridge Islands showed stable trends. On Granite Island, the penguin census estimated 22 penguins present in 2015, compared to 32 adults in 2014 (Colombelli-Négrel & Kleindorfer 2014). The number of birds present during the 2015-census was further confirmed by the fact that 12 active burrows (in which adult penguins were detected) were continuously monitored throughout the breeding season. On Troubridge Island, the population census estimated 313 penguins present in 2015, compared to 406 penguins in 2014 (Colombelli-Négrel 2015b). Similar to the 2014 results, the acoustic survey suggested that a higher number of individuals might be present on Troubridge at night. Specifically, the acoustic survey suggested that penguin numbers increased by three fold during night time, which would bring the population on Troubridge Island to 939 individuals. While population censuses detected less birds in 2015 than in 2014 on both Granite and Troubridge islands, the data show some fluctuations around similar values since 2012 (see Figures 2 and 3). Fluctuations in numbers for breeding populations reflect a trade-off between reproductive effort and survival due to the interactions with environmental variability (Stearns 1992; see also Jenouvrier et al. 2003). Therefore, temporal variation (variation between years) is expected for long-lived species, like little penguins.

Kangaroo Island, on the contrary, showed decreasing trends. Both Emu Bay and Antechamber Bay showed drastic declines since 2011 (Figure 4): 182 and 190 adult penguins respectively were found during the censuses in 2011 compared to only 42 and 10 in 2015. While previous data for Kingscote were not available for this report, over the three colonies, this represents a decline of 84%. It should be noted, however, that Vivonne Bay seems to show a small increase in numbers since 2013 (Figure 4), but further monitoring across additional years is necessary to confirm this trend.

Reproductive success

All colonies showed a decrease in breeding success in 2015 compared to 2014 (Table 2; Figure 1). As found in previous studies (Colombelli-Négrel & Kleindorfer 2014; Colombelli-Négrel 2015b), breeding success on Granite Island was the highest despite population decline. Higher breeding success on Granite Island may be explained by the small sample size (only five breeding pairs) or by variation in individual quality, if only the strongest individuals returned to the island (and were therefore more likely to be successful breeders). In Adélie penguin (*Pygoscelis adeliae*), for example, Lescroël et al. (2009) showed that successful breeders also exhibited higher survival rates than unsuccessful breeders. Future studies should therefore investigate factors that predict successful breeding across colonies.

Breeding success at Emu Bay was the lowest, with five out 20 burrows (25%) showing signs of predation. While video monitoring at burrows showed evidence of predation by goannas (Colombelli-Négrel & Kleindorfer 2014; Colombelli-Négrel 2015b), recent necroscopy analysis of carcasses showed that cats were also predators of juveniles at Emu Bay (Colombelli-Négrel and

Tomo, unpublished data). The study suggested that cats did not prey on little penguins while they were sitting inside their burrows but instead predated adults and juveniles once they ventured out; hence, explaining why no predation event by cats was caught on camera (Colombelli-Négrel and Tomo, unpublished data). Considering the small number of birds present at Emu Bay, continued predation will likely have a significant impact on long-term population trends.

Blood parasites

As found in previous studies (e.g., Altay et al. 2008; Vanstreels et al. 2015), molecular analyses were more sensitive in detecting blood parasites than traditional methods and showed that 86% of the little penguins sampled were infected with blood parasites, compared to only 31% when using microscopic examination of blood smears (Colombelli-Négrel 2015b). The study identified two genus (*Haemoproteus* and *Plasmodium*) and revealed that 80% of the infected individuals had multiple infections. *Haemoproteus* was recently found as one cause of mortality in little penguins in Western Australia (Cannell et al. 2013). However, while *Plasmodium* has been formerly detected in five other species of penguins (crested penguins (*Eudyptes pachyrhynchus*), yellow-eyed penguins (*Megadyptes antipodes*) (Laird 1950), African penguins (*Spheniscus demersus*), Rockhopper penguins (*Eudyptes chrysocome*) (Fantham & Porter 1944) and magellanic penguin (*Spheniscus magellanicus*) (Silveira et al. 2013)), this is the first evidence of its presence in little penguins.

Recent studies suggested that haemoglobin concentration is one of the most reliable measure to assess the impacts of blood parasites (O'Brien et al. 2001). Blood parasites can consume and destroy mature red blood cells, leading to more immature red blood cells in parasitised individuals and a decrease in haemoglobin concentration (as immature cells cannot synthesise as much haemoglobin as mature cells) (O'Brien et al. 2001). In this study, there was no relationship between parasite presence and haemoglobin concentration or Hematocrit levels, suggesting that blood parasites may not affect the fitness of the parasitised individuals.

However, this study also found that individuals with multiple infections had longer bills and smaller bill depths and widths than those with single infection or non-infected individuals. In little penguins, Wiebkin (2012) showed that individuals with higher food availability produced heavier and larger fledglings with larger bills. Hence, it is possible that young chicks that were better fed (and hence had longer bills) developed into more resistant adults and the correlation between blood parasites and bill measurements may only emerge due to multicollinearity. Blood parasite presence was also higher in adults than in chicks, maybe because infected chicks were more likely to die before being sampled. Studies in humans have shown that malaria infections generally kill young infants (reviewed in Murray et al. 2012). Together, these data suggest that only the strongest individuals may have survived with the parasite infections. Additional studies are clearly necessary to further investigate the impacts of blood parasites in little penguins.

Morphological differentiation between the colonies

This report showed morphological variation between the breeding colonies. Difference between morphological traits were correlated to geographic distances and changes in environment parameters. Such variation may raise questions regarding the conservation management of these populations. However, the 2014 report investigating genetic variation of the same colonies identified only two genetically distinct populations: the first population included Emu Bay, Kingscote, Penneshaw, Antechamber Bay and Vivonne Bay (all on Kangaroo Island), as well as Granite and Althorpe Islands while the second population consisted solely of Troubridge Island (Graff S, Gardner M and Colombelli-Négrel D, unpublished data; see also Colombelli-Négrel 2015b). This therefore suggests that the loss of local populations (except for Troubridge Island) should not result in major losses of genetic variability.

VII. SUMMARY OF LITTLE PENGUIN RESEARCH MEETING

Date: 30th of November 2015.
Venue: Adelaide and Mt Lofty Ranges Natural Resources Management Board Meeting room

Present: Diane Colombelli-Négrel (Penguin Ecologist, Flinders University), Renate Velzeboer (Ecologist, Marine Interaction and Wildlife Biosecurity, DEWNR), Valerie Lawley (Conservation and Sustainability Unit, DEWNR), Peter Copley (Senior Ecologist, Threaten species and Conservation and Sustainability Unit, DEWNR), Simon Goldworthy (Endangered Species Unit, SARDI), Sarah-Lena Reinhold (SARDI and Flinders University), Peter Dann (Research Manager, Phillip Island Nature Parks), Michelle Power (Parasitologist, Macquarie University), Anna Dutkiewicz (Senior Policy Officer Conservation and Sustainability, DEWNR), Tony Flaherty (Manager, Coastal and Marine, NRM)

Focus of meeting: (1) key research questions being investigated or to investigate in the future and (2) necessary steps to be undertaken to fill out knowledge gaps in overall little penguin population trends.

OPEN DISCUSSIONS

Population Trends
Mortality & Predation Pressures
Nesting burrows & Breeding success
Moult
Parasites

STEPS TO FILL OUT THE EXISTING KNOWLEDGE GAPS

Potential Outcomes
- Presence/absence
- Relative estimation of size class for each site: common, abundant, etc…
- Actual counts

Potential Methods
- Transect counts over small section of colony and extrapolation
- Automated acoustic recorders or video monitoring
- Citizen Science

Collect data for SA - Develop a 3-Years Action Plan
- Select strategic sites
- Identify one method across all colonies to obtain comparable data
- Identify the timing of breeding for each region (i.e., identify local champions)
- Investigate costs for monitoring each region

OTHER PARAMETERS TO BE RECORDED ACROSS RESEARCH GROUPS

- Death records and survival
- Breeding success and timing of breeding
- Timing of moulting
- Presence/absence of predators (marine or terrestrial)
- Parasites
- ***One unique measure of fitness (still to be determined) that could be compared between populations***

VIII. DIRECTIONS FOR FUTURE RESEARCH

1) Continue long-term annual monitoring of 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) Develop an Action Plan for estimating population trends in South Australia to get a better understanding of the little penguin status

3) Continue monitoring breeding success across several targeted populations for inter-annual variation and further investigate the impact of terrestrial predation for breeding success and long term population trends.

4) Continue rat control on Granite Island to maintain high breeding performance

5) Consider cat control and workshops to increase public awareness about the impact of pets on Kangaroo Island.

6) 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 and long-term population trends.

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

8) Further test whether reproductive isolating mechanisms exist between the two genetic populations identified.

9) 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

Volunteers and public awareness

Thirty four volunteers participated in the Granite Island penguin census in October 2015. An additional fifteen volunteers participated in field trips to collect the data and helped with penguin census on Troubridge Island. One Honours student (Tamara Burt) worked on a little penguin related project (specifically investigating presence and impacts of blood parasites).

Diane Colombelli-Négrel gave a presentation to the public on Granite Island on the 12th and on the 19th of October for the penguin island wide census. Diane Colombelli-Négrel and research assistant Sarah-Lena Reinhold both gave a presentation to the KI NRM Board on 23rd of October 2015. Diane Colombelli-Négrel was invited to give a talk to the Friends of Althorpe group on the 20th of March 2016 and to the Commercial Tour Operators meeting on Kangaroo Island on the 30th of March 2016. Research assistant Vanessa Owens raised public awareness about little penguin conservation issues and presence of little penguins on Granite Island to 93 people (which included two school groups of 15 students).

Sarah-Lena Reinhold (presenting her Honours work on long-nosed fur seal diet) and Diane Colombelli-Négrel gave a presentation to the public during two penguin night events on Kangaroo Island (22nd of October 2015) and at Victor Harbour (27th of November 2015). Diane Colombelli-Négrel tested the impact of the two community nights on public awareness by mean of a questionnaire distributed before and after the presentations to determine the extent of attitudinal change among people. The questionnaire is presented as Appendix 2. A total of 38 participants answered the questionnaire: 53% felt that their knowledge of penguin conservation was increased by the presentations and 100% felt that their expectations were met during the events. 92% would like to hear more about little penguin research and conservation and 42% would like to get an update of the research via another presentation in the future.

Media output

An article calling for community volunteers to join the Granite Island Penguin count was released on the 1st of October 2015 in the Victor Harbour Times. Another media release regarding the penguin night presentation on Kangaroo Island was released in November 2015. A media release to raise public awareness regarding little penguins' presence on Granite Island was published in The Times Victor Harbour (24/3/16) and in the Advertiser (25/3/16). Diane Colombelli-Négrel gave an interview for ABC Radio Australia, which aired on 28th of March 2016.

X. ACKNOWLEDGEMENT

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XII. APPENDIX 1

(a) List of individuals that were captured and microchipped in 2015

Island	Reference Number	Age Category
Troubridge Island	982000063643867	Adult
Troubridge Island	982000063644169	Adult
Troubridge Island	982000063644956	Adult
Troubridge Island	982000063644479	Adult
Troubridge Island	982000063644112	Adult

(b) List of microchipped individuals that were re-sighted in 2015

Island	Reference Number	Year Microchipped
Granite Island	157468226	2010
Granite Island	157468261	2011
Troubridge Island	88746157	unknown
Troubridge Island	157468403	unknown
Troubridge Island	1450150249	unknown
Emu Bay	982000063643845	2014
Emu Bay	982000063644601	2014

XIII. APPENDIX 2 – Questionnaire

LITTLE PENGUINS CONSERVATION - INITIAL SURVEY QUESTIONS

What is your gender?
Male Female

Please select your age group.
•18-24 •25-34 •35-44 •45-54 •55-64 •65-74 •75+

How would you describe yourself? Please select all that apply.
•Student •Retired
•Full-time employment •Part-time employment
•Conservationist

Have you volunteered in a conservation project(s) before?
Yes No

If you have volunteered please select the appropriate level of activity.
•Once
•Between 1-5 occasions
•More than 5 occasions

If you have volunteered, why did you participate? Please select all that apply.
•Went with a friend •Member of local volunteer group
•Member of local friends group •Hobby ornithologist/ Conservationist
•Professional ornithologist/ Conservationist •Other please state _____

How would you describe your level of knowledge about little penguin conservation?

1	2	3	4
Poor	Average	Good	Very Good

To your knowledge, what is the status of the little penguins?

1	2	3	4	5
Critically endangered	Endangered	Vulnerable	Near Threatened	Least Concern

How did you gain your current knowledge on the status of the little penguins? Please select all that apply.
•Television •Radio •News
•Publications •Books •Friends
•Conservation groups •Volunteer groups •Research paper
•Zoo visit •Penguin Centre visit •Other – please state _____

How concerned are you about the conservation of the little penguins?

1	2	3	4
Not at all	A Little Concerned	Fairly Concerned	Very Concerned

If research or conservation efforts towards little penguins stopped tomorrow how concerned would you be?

1	2	3	4
Not at all	A Little Concerned	Fairly Concerned	Very Concerned

What are your expectations of tonight presentation?
•Learn more about little penguins in general
•Learn more about the conservation of the little penguins
•Learn more about the research conducted
•Have the opportunity to ask questions to the researchers
•Others please state _____

LITTLE PENGUINS CONSERVATION - POST PRESENTATION SURVEY QUESTIONS

Has your knowledge of little penguin conservation been increased by the presentation tonight?
Yes No

How useful were the following activities in helping you gain your new knowledge?

a. Presentation/Talk

1.	2.	3.	4.
Not at all useful	A little useful	Fairly useful	Very useful

b. Interpretive display

1.	2.	3.	4.
Not at all useful	A little useful	Fairly useful	Very useful

c. Discussions with researchers/experts

1.	2.	3.	4.
Not at all useful	A little useful	Fairly useful	Very useful

How would you describe your current level of knowledge on little penguin conservation?

1	2	3	4
Poor	Average	Good	Very Good

How concerned are you about the conservation status of the little penguins?

1	2	3	4
Not at all	A Little Concerned	Fairly Concerned	Very Concerned

If research or conservation efforts towards the little penguins stopped tomorrow how concerned would you be?

1	2	3	4
Not at all	A Little Concerned	Fairly Concerned	Very Concerned

How interested would you be in learning more about little penguins?

1.	2.	3.	4.
Not interested	A little interested	Fairly interested	Very interested

If you are interested in learning more about little penguins, how would you search for more information? Please select all that apply.

•Join Friends Group	•Join Volunteer Group	•Brochure
•Email newsletter	•Field trip	•Museum visit
•Research paper	•Research/update presentation	•Website
•Zoo visit	•Penguin Centre visit	•Other please state

Were your expectations of tonight presentation met? Yes No
If not, what would you like to have seen presented or discussed_____
_____

Other Comments? Please state.
_____
_____
_____

Thank you for your time