Penguin monitoring and conservation activities in the Gulf St Vincent
July 2013 – June 2014

Report to the Adelaide and Mt Lofty Natural Resources Management Board
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inspiring achievement

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I. SUMMARY

Over the past decade, populations of little penguins (*Eudyptula minor*) have been seriously declining across the Gulf St Vincent for reasons still not fully understood. In this study, we investigated breeding performance, adult mortality and blood parasites of little penguins on three islands in the Gulf St Vincent (Granite, Troubridge and Kangaroo Islands) in relation to patterns of population decline. Granite Island population had the highest breeding success with 1.50 (± 0.34) fledglings per pair (n=6) compared to Kangaroo Island with 0.95 (± 0.15) fledglings per pair (n=52) and Troubridge Island with 0.73 (± 0.18) fledglings per pair. We also found that 31% of the individuals sampled had evidence of parasite presence, and that both predation and parasite intensity negatively impacted breeding success. Penguins formed a significant part of fur seal diet and were found in 33% of fur seal scats collected. 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 a causative factor in the decline. Future studies need to investigate parasites and predation pressure from fur seals further to understand their full impact on population declines.

II. INTRODUCTION

Understanding the reasons for population declines is a critical first step to being able to implement effective conservation management approaches. However, the task to identify the causes of decline can be difficult, especially when the study system interfaces both marine and terrestrial environments, such as seabirds. Indeed, seabirds can be particularly affected by environmental and anthropogenic changes, such as variation in predation pressure (both on land and at sea) (David et al. 2003; Jones et al. 2008), variation in food availability (Dann et al. 2000; Furness 2003; Crawford et al. 2006), climate change (Chambers 2004; Barbraud et al. 2011), habitat destruction or alteration (Long et al. 2011; Miller et al. 2012), and/or oil spills (Goldsworthy et al. 2000; Moreno et al. 2013). Such environmental and anthropogenic changes can then lead to population decline, especially if they affect reproductive success, survival and/or recruitment.

In addition, to fully understand population decline, it is important to disentangle the different selection pressures on adults versus offspring (Lack 1954; Brooks et al. 1991). Indeed, there is now evidence that reduced breeding success can also impact population decline by suppressing population dynamics and limiting population growth (e.g., Böhning-Gaese et al. 1993; Schmidt 2003). For example, low reproductive success was responsible for population declines in the marbled murrelet (*Brachyramphus marmoratus*) (Gutowsky et al. 2009). Similarly, population variation in common terns (*Sterna hirundo*) was more dependent on sub-adult survival (48%) and on breeding success (10%) than on adult survival (Ezard et al. 2006).

This study focuses on little penguins (*Eudyptula minor*), a seabird species showing drastic population declines in various parts of the Gulf St Vincent, South Australia (Wiebkin 2011). On Granite Island, penguin numbers plummeted from 1548 individuals in 2001 to 26 in 2012 (Nathalie Bool, pers. comm.). Similarly, populations in other areas whose status was considered 'unsure' or 'stable' until recently – such as Kangaroo Island – are now showing declining trends according to recent 2011-2012 census data. A number of factors have been suggested to explain this decline such as the recent increase in New Zealand fur seal numbers (Bool et al. 2007; Wiebkin 2011); predation on land by introduced predators such as foxes, rats, cats and dogs (Dann 1992; Perriman et al. 2000; Preston 2008; Wiebkin 2011); limited prey availability or starvation (Dann et al. 2000; Wiebkin 2011); low reproductive success (Bool et al. 2007); as well as parasite presence (Cannell et al. 2013). However, the interactions between all these variables and their impacts on each penguin population are still not fully understood.

III. AIMS

The purpose of this study was to quantify predictor variables for patterns of population decline in Little Penguins in the Gulf St Vincent. This project focused on three main issues: reproductive failure, adult mortality caused by predation or parasites, and population genetic structure and gene flow. We first asked whether patterns of reproductive failure differed across colonies. In particular, we investigated the impact of hatching failure, predation, abandonment, parasite intensity and chick starvation on breeding and fledgling success. We also used tracking tunnels and motion camera monitoring to investigate the impact of rodents on breeding success and identify any terrestrial predators at burrows. We then estimated marine predation risk by analysing fur seal scats at penguin colonies and by recording presence/absence of marine predators at each of the colonies. We also compared blood parasite intensity and prevalence in relation to body condition. Finally, we collected blood samples in order to quantify population genetic structure and patterns of gene flow between the penguin colonies in the Gulf St Vincent.

IV. MATERIALS AND METHODS

Study sites

This project was conducted during the 2013-breeding season between August and January on three islands in the Gulf St Vincent: (1) Granite Island (35°37'S, 138°36'E). Granite Island is dominated by indigenous grasses with patchy woodlands and shrubs covering the lower areas of the island with a granite rocky coastline. The island is accessed by a bridge causeway open to pedestrians from Encounter Bay; (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, and is accessible by ferry. The island is 150km long and includes several penguin colonies. Colonies at Antechamber Bay, Emu Bay, Penneshaw, Kingscote, and Vivonne Bay were included in our study. Tracking tunnels and motion cameras were only used at Granite Island, Troubridge Island and Emu Bay (KI).

Causes of reproductive failure

Burrow monitoring

To quantify reproductive success, search for active burrows started around mid-August. Monitoring was carried out until the end of November on Kangaroo and Troubridge Islands and until the end of January on Granite Island. Every burrow was checked every 2-4 weeks during the monitoring period and a burrow was recorded as active if it contained eggs, chicks or adults, or 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. Fledgling success was defined as the number of chicks that fledged compared to the number of eggs that hatched. Breeding success was defined as the number of chicks that fledge per breeding pair. Predation was scored as suspected if eggs or chicks were damaged or removed between visits of known burrow contents and nesting phase. 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 and fledgling success.

Parasites and chick starvation

When present, adults and chicks were captured by hand and removed from their burrow for DNA and parasite sampling. We collected blood samples (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 and measure parasite intensity. Blood smears were air-dried, fixed in 99% ethanol for ~5 min, and later stained with Wright–Giemsa. We microscopically examined all smears under a 100 x oil immersion lens for presence of blood parasites. Chicks were also weighed to the nearest 10g just before fledging, at ~7–8 weeks of age.

Tracking tunnels

We assessed rodent and other mammalian activity once a month between August and October 2013 using tracking tunnels across penguin breeding areas on Granite Island, Troubridge Island and at Emu Bay (KI). Tracking tunnels are rectangular tunnels, commonly made of polyethylene, designed to track animal locations and estimate their abundance (see King & Edgar 1977). Animals are lured into the tunnels with peanut butter wrapped in baking paper; the animal stands on the inkpad after being lured into the tunnel and leaves footprints/marks on absorbent white cardboard attached to the inkpads as they leave the tunnel. We used twenty tracking tunnels placed along two 150m transects that each had 10 tunnels for each of the three colonies (60 tunnels in total). Tunnels were placed approximately 10-15 meters from each other and the ink cards were left for three consecutive nights before collection.

Motion Camera monitoring

Between August and December 2013, we installed small motion sensor-activated cameras to identify potential terrestrial predators or disturbance and monitor activity around active burrows. A total of 17 motion-sensitive cameras were installed in front of 17 burrows: 5 on Granite Island, 6 on Troubridge Island and 6 at Emu Bay (KI). We used two different cameras: (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 ~3 weeks. A total of ~15,218 hours of video images was analysed for evidence of predatory or disturbance activity at burrows.

Adult mortality

New Zealand fur seal observations

Between August and October 2013, we observed the presence of New Zealand fur seals (*Arctocephalus forsteri*) at the following penguin colonies: Granite Island, West Island, Troubridge Island, Kingscote, Penneshaw, Antechamber Bay, Vivonne Bay, and Emu Bay. Seal Island, an island offshore Granite Island, was also included in the observations, using binoculars to estimate fur seal presence. Seals observed on Seal Island were considered as part of the Granite Island numbers. Observations were made twice a month for one hour each time. At each colony, we recorded the number of fur seals observed on shore and at sea. We also report New Zealand fur seal numbers recorded opportunistically on Seal and West islands between 1993 and 2013. These observations were done by a local school teacher (Ian Milnes) during repeated boat surveys of the islands. Observations on West Island were done once per month in August 1993, July 1999, August 2004, August 2006, August 2009, September 2010, October 2011, August 2012 and August 2013. Observations on Seal Island were done once per month in August 2009, September 2010, October 2011, August 2012 and August 2013.

New Zealand fur seal scat collection

In 2013, we also searched for fur seal scats during each of our visits to the penguin colonies as part of our for fur seal observations. Any scats that were found were collected in labelled zip lock bags and stored at -20°C until appropriate analysis was carried out. We conducted the analysis of the fur seal scats at Flinders University Laboratory, where scats were soaked in hot soapy water for 24 hours, then individually sieved with warm to hot water through a 0.5mm and 1.0mm sieve. Contents were sorted through; where 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).

Mortality Register
Carcasses were collected (when found) during visits to the penguin colonies. Additionally, three members of the public volunteered to collect dead penguins from coastal areas and surveyed beaches regularly. 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.

Parasite risk and adult body condition
To estimate parasite presence and intensity, blood samples were collected as described above for all adults present in their burrows during the monitoring period (August-November 2013). In addition, we measured head length with callipers as an indicator of body size (Miyazaki & Waas 2003) and bill depth 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 were also weighed to the nearest 10g at the beginning of breeding.

Dispersal and gene flow

Blood samples (0.01ml per bird) were collected with a 25G needle from the foot vein and stored on FTA paper (Smith & Burgoyne 2004). Genetic samples were collected from both adults and chicks (when present), and chicks were only sampled just prior to fledging, when their mass was 90% of adult mass. The full genetic analyses are part of a separate project, and therefore details of the analyses are not reported here.

Statistical Analysis

All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). Tracking tunnel results were analysed using a Chi-square analysis. We used an ANOVA to test for variation in chick mass between colonies. We used multiple linear regression analyses to investigate the impact of predation and parasite intensity on hatching, fledgling and breeding success. Parasite presence and intensity between colonies was tested using ANOVA. Number of fur seals observed between Kangaroo Island and Encounter Bay was tested using ANOVA, and linear regression analysis was used to test for an increase of fur seals across the decade. We tested for a sex difference in parasite intensity and presence using ANOVA analysis. We used linear regression to investigate the impact of parasite presence and intensity on adult body condition (head length) and mass.

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 was provided to DEWNR on 31/3/2014.

V. RESULTS

Causes of reproductive failure

Breeding and fledgling success

Between August and January 2013, we monitored a total of 130 burrows on Granite Island (n=7), Kangaroo Island (n=76) and Troubridge Island (n=47) (see Table 1). Out of the 130 monitored burrows, 93 showed signs of breeding activity (72%) such as eggs or chicks present in the burrow. Breeding success on Granite Island was the highest with 1.50 (± 0.34) fledglings per pair (n=6) compared to Kangaroo Island with 0.95 (± 0.15) fledglings per pair (n=52) and Troubridge Island with 0.73 (± 0.18) fledglings per pair (n=34; see Table 2). Specifically, Troubridge Island and Antechamber Bay (KI) had the lowest breeding success in 2013 (Figure 1).

Penguin colonies	Adults seen	Burrows monitored	Breeding burrows	Eggs	Chicks	Fledglings	Groups with 2nd clutch	Burrows predated
Antechamber Bay	38	22	15	26	16	5	0	1
Penneshaw	12	10	4	7	4	4	0	0
Vivonne Bay	20	10	10	16	15	11	0	0
Kingscote	19	11	5	8	6	3	0	0
Emu Bay	41	23	19	41	28	13	5	2
Troubridge	85	47	34	72	36	19	5	5
Granite	14	7	6	12	9	9	0	0
Total	**229**	**130**	**93**	**182**	**114**	**64**	**10**	**8**

Table 1. Number of eggs, chicks and fledglings produced in total per penguin colony. The table also presents the total number of adults sighted during the monitoring period, as well as the number of burrows with suspected predation.

Penguin Colonies	Eggs/ Pair (SE)	Chicks/ Pair (SE)	Hatching success (SE)	Breeding success (SE)	Fledgling Success (SE)
Antechamber Bay	1.73 (0.12)	1.14 (0.23)	0.61 (0.12)	0.50 (0.27)	0.42 (0.20)
Penneshaw	1.75 (0.25)	1.00 (0.58)	0.50 (0.29)	1.00 (0.58)	1.00 (0.00)
Vivonne Bay	1.60 (0.16)	1.50 (0.17)	0.95 (0.05)	1.38 (0.26)	0.88 (0.12)
Kingscote	1.60 (0.24)	1.20 (0.37)	0.80 (0.20)	1.00 (0.58)	1.00 (0.00)
Emu Bay	2.28 (0.23)	1.65 (0.17)	0.75 (0.08)	1.00 (0.25)	0.60 (0.14)
Troubridge	2.12 (0.13)	1.06 (0.17)	0.54 (0.08)	0.73 (0.18)	0.86 (0.10)
Granite	2.00 (0.26)	1.50 (0.34)	0.83 (0.17)	1.50 (0.34)	1.00 (0.00)

Table 2. Breeding and fledgling success for each penguin colony for the 2013-breeding season.

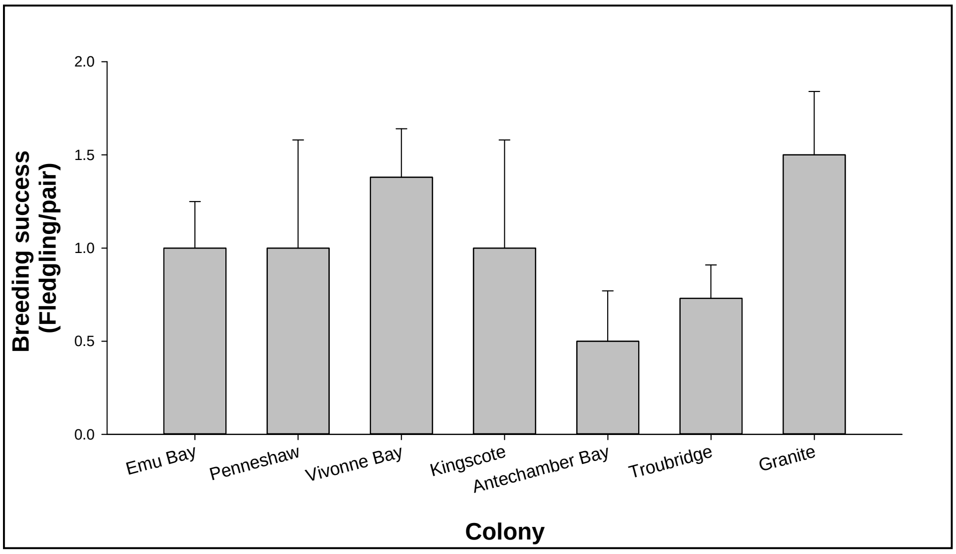

Figure 1. Breeding success across all the penguin colonies monitored in 2013

Tracking Tunnels

Analysis revealed four main types of footprints on the cards: mice, two species of rats and a species of possum (see Figure 2). Identification of mouse species was not possible due to the similarity of mice footprints in general. However, we suspect that prints were from the house mouse (*Mus musculus*), because of evidence found on our camera video recordings on Granite Island in front of the tracking tunnels in 2012. We identified footprints left by rats as water rats (*Hydromys chrysogasterchrysogastes*) and black rats (*Rattus rattus*). Video Camera images in front of the tracking tunnels (from our 2012 data) supported this conclusion. Black rats were found both at Granite Island and Emu Bay, while water rats were only found on Granite Island. We identified the possum marks as common bush-tail possums (*Trichosurus Trishosurus vulpecular*), which was also confirmed by video camera recordings at tracking tunnels from our 2012 data. We compared rodent activity across the three colonies. No rodent marks were found on Troubridge Island, and therefore the data were excluded from the analysis. Overall rodent activity ($\chi2 = 4.97$, df = 3, n = 120, P = 0.17), rats activity ($\chi2 = 0.71$, df = 1, n = 120, P = 0.40) and mice activity ($\chi2 = 1.71$, df = 1, n = 120, P = 0.19) did not differ between Emu Bay and Granite Island. Possums were only present near burrows on Granite Island ($\chi2 = 34.84$, df = 1, n = 120, $P < 0.0001$).

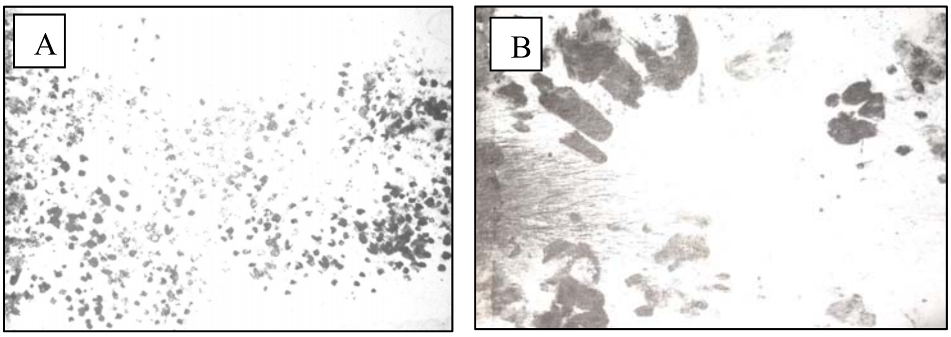

Figure 2.Tracking tunnel marks produced from (a) black rats (*Rattus rattus*) and (b) brush-tail possums (*Trishosurus vulpecular*) on Granite Island. Rodent tracks from Granite Island were identified with the aid of video images recorded in 2012.

Motion Camera monitoring

We monitored 22 burrows across the three colonies; only one case of predation was recorded using the motion cameras. At one burrow at Emu Bay, a goanna (*Varanus rosenbergi*) was seen entering the burrow and the chicks were never seen after that visit (Figure 3a). That same burrow was visited three times by a cat during the incubation stage (Figure 3b) and the Guarding period (the first 2-3 weeks of the chick stage) as well as by a rat during the Guarding period but it was not predated during those times. Another burrow was visited twice by a goanna during the Guarding period but it was not predated. On Granite Island, rats were seen at three burrows and possums at four burrows. Rats were only seen during the incubation period during 2013. However, our 2012 data showed one burrow on Granite Island with young chicks visited by a rat. On Troubridge Island, no potential predator was seen entering the burrows and the recordings only showed evidence of Silver Gulls (*Chroicocephalus novaehollandiae*) and Buff-banded Rails (*Gallirallus philippensis*) passing in front of the cameras.

Figure 3: (a) Picture of a burrow at Emu Bay predated by a goanna (*Varanus rosenbergi*) at the chick stage; (b) a feral cat (*Felix catus*) visiting the same burrow during the incubation stage.

Chick starvation

To investigate any potential effect of chick starvation on breeding success, we weighed a total of 33 chicks. Chick mass did not vary significantly between colonies (ANOVA: $F_{5, 32} = 1.29$, $P = 0.30$), and was within the normal range for little penguins (Table 3; see Bool & Wiebkin 2013).

Penguin Colonies	No. Individuals sampled	Chick Mass (mg ± SE)
Antechamber Bay	4	1037.50 ± 12.50
Emu Bay	4	1137.50± 42.70
Vivonne Bay	7	1200.00 ± 21.82
Kingscote	2	1125.00 ± 125.00
Troubridge	15	1196.67 ± 42.39
Althorpe	1	1196.67 ± 42.39

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

Blood parasites

To investigate the possible fitness costs and impact of blood parasites in little penguins, we collected blood samples from 104 penguins in total (see Table 4 for sample size). We found evidence of parasite presence in 32 (31%) out of the 104 individuals sampled: 13 chicks and 19 adults (14 males and 5 females). We consulted with two specialised Veterinarians as well as Dr James Herbert (Flinders University), who has intensive knowledge of blood parasites and *Plasmodium* spp in particular. Dr Herbert confirmed the presence of a protozoan pathogen (potentially an oocyst-type parasite development that is usually seen in a gut lining but sometimes found in the blood stage; Figure 4a) and an apicomplexan, which could be either *Plasmodium*, *Shellakia*, *Trypanosoma*, *Hepatozoon*, or *Leucocytozoon* (Figure 4b).

Penguin Colonies	No. Individuals sampled	No. with parasites	No. Chicks sampled	No. Adults sampled	No. Males sampled	No. Females sampled
Antechamber Bay	16	11 (69%)	7	9	6	3
Emu Bay	17	6 (35%)	6	11	8	3
Penneshaw	1	0 (0%)	0	1	1	0
Vivonne Bay	8	1 (13%)	7	1	1	0
Kingscote	6	0 (0%)	0	6	3	3
Troubridge	45	12 (27%)	21	24	13	11
Althorpe	4	1 (25%)	2	2	0	2
Granite	7	1 (14%)	2	6	1	4
TOTAL	104	32 (31%)	45	60	33	26

Table 4. Number of individuals, chicks, adults (males, females) sampled for blood parasites at eight colonies in 2013

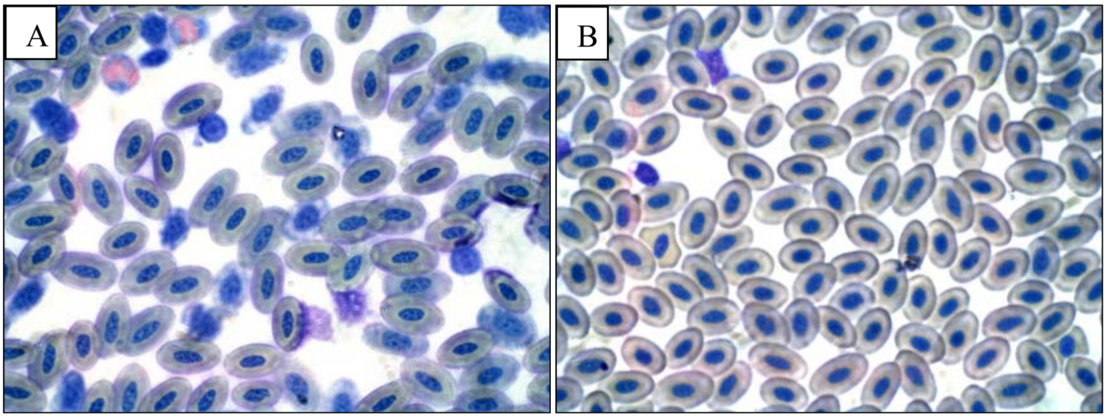

Figure 4. (a) Picture of the apicomplexan and (b) of the protozoan pathogens found in little penguin blood smears collected in 2013.

Factors influencing breeding success

We tested for the impact of predation and parasites on breeding success. Only two burrows had evidence of abandonment, one on Granite Island and one on Troubridge Island. Therefore, the impact of abandonment on breeding success was not tested. Seven burrows were suspected of predation: four on Troubridge, two at Emu Bay and one at Antechamber Bay. Predation negatively affected the number of fledglings produced (Linear regression: $\beta = -0.29$, $t = -2.61$, $P = 0.011$), but not the number of chicks ($\beta = -0.05$, $t = -0.40$, $P = 0.69$). Parasite intensity significantly varied between colonies (but not parasite presence), and was the highest at Antechamber Bay (KI) and on

Troubridge Island (ANOVA – parasite presence: $F_{1,8}$ = 1.53, P = 0.16; parasite intensity: $F_{1,8}$ = 2.35, P = 0.024). In addition, breeding success in colonies with the highest parasite intensity was the lowest (Linear Regression: β = -0.79, *t* = -2.91, P = 0.034; Figure 5). Specifically, parasite intensity tended to influence hatching success (β = -0.68, *t* = -2.11, P = 0.089), but not fledgling success (β = -0.58, *t* = -1.60, P = 0.17).

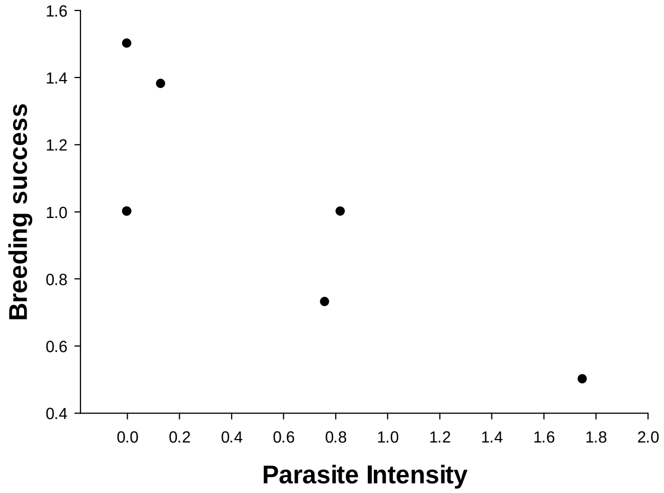

Figure 5. Breeding success (number of fledglings per breeding pair) and parasite intensity at seven South Australian little penguin colonies in 2013

Adults Mortality

New Zealand fur seal observations

In 2013, a total of 488 New Zealand fur seals were observed near penguin colonies. Of these fur seal sightings, with 451 were observed in the Fleurieu Peninsula (118 on Granite and Seal Islands, and 333 on West Island) and 37 observed on Kangaroo Island. There was no observation of No New Zealand fur seals was observed on Troubridge Island. Overall, more fur seals were observed in the Fleurieu Peninsula than on Kangaroo Island (ANOVA: F1, 40 = 50.41, P < 0.0001; Figure 6); and the number of fur seals decreased across the three months for all locations (Figure 6). The number of New Zealand fur seals on Seal Island did not increase between 2009 and 2013 (Linear regression: β = 0.50, t = 0.09, P = 0.94; Figure 7). However, there was a significant increase of fur seal numbers on West Island since 1993 (Linear regression: β = 0.77, t = 3.20, P = 0.01; Figure 8).

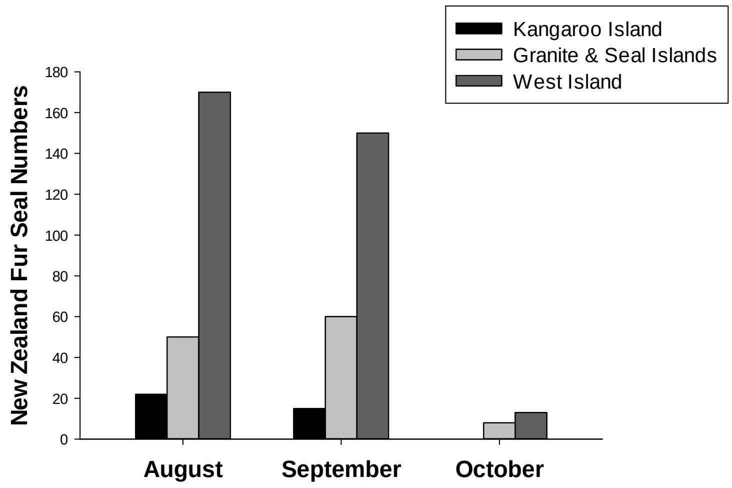

Figure 6: The total number of New Zealand fur seals observed on Granite Island (including Seal Island), West Island and Kangaroo Island across August, September and October 2013

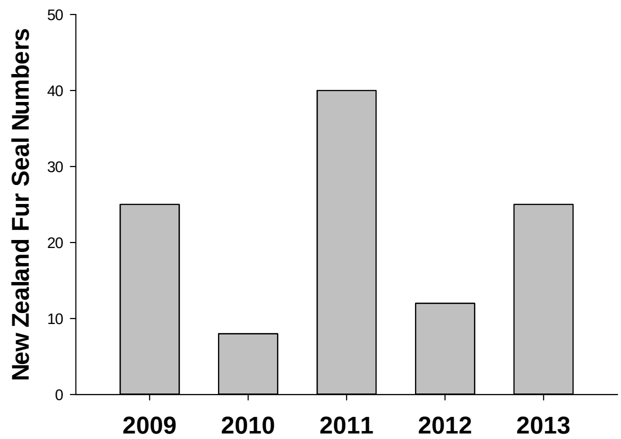

Figure 7: The observed number of New Zealand fur seals on Seal Island between 2009 and 2013

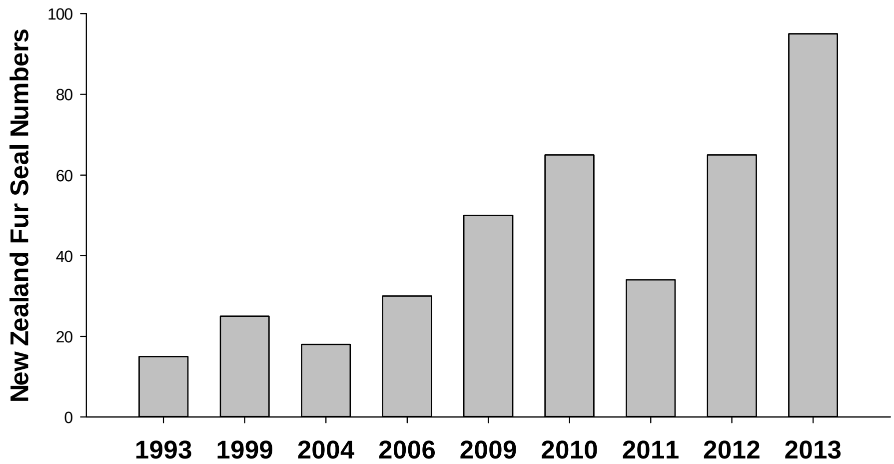

Figure 8: The observed number of New Zealand fur seals on West Island between 1993 and 2013

New Zealand fur seal scat analysis

A total of 12 fur seal scat samples were collected in 2013, with eight collected on Fleurieu Peninsula and four collected on Kangaroo Island. Out of the 12 scat samples analysed, four (33%) had evidence of penguins. The largest portion was unidentified and categorized as unknown prey type (50%), which consisted of bone, tissue or tube like fragments (Figure 9). Fish and cephalopods were the next most abundant prey types in the scat samples, respectively 15.22% and 13.04% (Figure 9). The fourth most abundant prey type was penguin (10.87%), with other avian species and crustaceans making <10% of the fur seal diet overall (Figure 9).

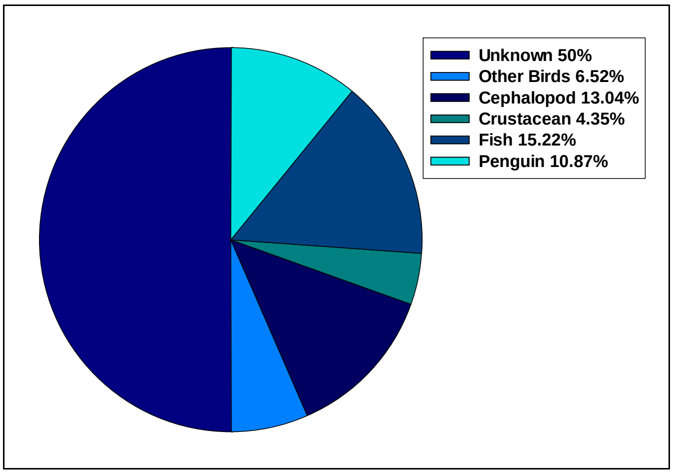

Figure 9: The total percentage of prey types found in the New Zealand fur seal scat samples

Comparing the two locations, there was a higher percentage of all prey types (except cephalopods and crustaceans) present in the Fleurieu Peninsula scat samples (Figure 10a) compared to those collected on Kangaroo Island (Figure 10b). The percentage of crustaceans (2.17%) was similar at both locations, while cephalopods were found only in the Kangaroo Island samples (13.04%) and not in the Fleurieu Peninsula samples. There was a higher percentage of penguin remains in the Fleurieu Peninsula samples (6.52%) than in those collected on Kangaroo Island (4.35%; Figure 10). Similarly, other unidentified avian species were found in the Fleurieu Peninsula scat samples (6.52%), while none were present in the Kangaroo Island samples (Figure 10).

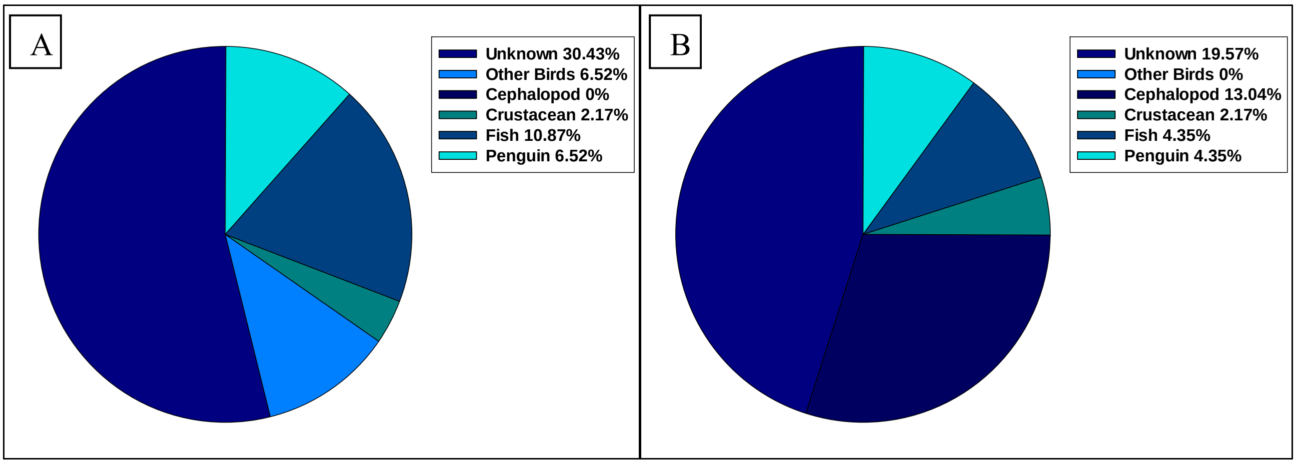

Figure 10: The percentage of prey type found in the New Zealand fur seal scat samples on (a) Fleurieu Peninsula and (b) Kangaroo Island

Parasite risk and adult body condition
We investigated the relationship between parasite prevalence (presence), intensity, and penguin body condition. We found that males had significantly more parasites than females (ANOVA parasite intensity: $F_{1, 59} = 4.56$, P = 0.037; parasite presence: $F_{1, 59} = 2.02$, P = 0.16). We found no correlation between parasite presence or intensity and body condition (head length) (Linear regression presence: β = -0.16, *t* = -1.20, P = 0.23; intensity: β = -0.01, *t* = -0.09, P = 0.92). However, birds with parasites tended to weigh less (β = -0.23, *t* = -1.68, P = 0.099) than those without parasites.

Dispersal and gene flow

We collected blood samples from 115 penguins in 2013: Granite Island (N=9), Troubridge Island (N=51), Althorpe Island (N=4), Emu Bay (N=17), Antechamber Bay (N=17), Kingscote (N=8), Penneshaw (N=1) and Vivonne Bay (N=8). Out of the nine penguins sampled on Granite Island, eight were from the Penguin Centre. Honours student Steffi Graf has been analysing the samples since March 2014. Additionally, Phillip Island Nature Parks Research Manager, Peter Dann has agreed to collect blood samples from 30 birds on Philip Island and Sandra Vogel (PhD Student at the University of NSW) has agreed to provide us with 15-30 samples from the NSW populations for the analysis.

VI. POPULATION CENSUS

Methods

In 2013, penguin censuses were carried out at Althorpe Island (Yorke Peninsula), Troubridge Island (Yorke Peninsula), West Island (Fleurieu Peninsula), Wright Island (Fleurieu Peninsula), Seal Island (Fleurieu Peninsula), Pullen Island (Fleurieu Peninsula), and Granite Island (Fleurieu Peninsula). All censuses were conducted by a team of volunteers and the Penguin Ecologist. The censuses were conducted in October and November in 2013 to align with the community census dates for Granite Island, which were directed in the past four weeks after the first burrow was found with eggs. Each island 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 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 burrows were marked with talcum powder to avoid double counting. In addition, all active burrows on Troubridge Island and Althorpe Island were marked with GPS. Censuses on Kangaroo Island were carried out in August, September and October 2013 and were coordinated by Natural Resources Kangaroo Island, and therefore the data are not presented here.

Results

Granite Island
A preliminary census was carried out on 26th of August 2013 by a team of six people (Penguin Ecologists Diane Colombelli-Négrel and Vanessa Owens accompanied by a research assistant and three volunteers). We found seven burrows definitively active with clear evidence of penguin presence and sometimes even adults in the burrow (one with an adult sitting on 2 eggs); another six burrows were potentially active but the signs were less clear. This would bring the numbers to 14-26 based on previous estimations (1 active burrow = 2 adult penguins). The community censuses were conducted over two days (14th and 21st of October 2014) by 39 volunteers (80% Flinders students,

20% community volunteers) and two penguin researchers. On the first day, 93 burrows were found, out of which 17 were active. Seven penguins were physically present in their burrows on the day, and four chicks (ready to fledge) were encountered. On the second day, 22 burrows were found active. The average number of active burrows for Granite Island is therefore 19 burrows (38 penguins estimated to be present on the island; Figure 11). Four burrows were found on the second day with chicks ready or close to fledging (seven chicks in total). Prior to the census, only five burrows were considered active on the island. Therefore, Penguin Ecologist Diane Colombelli-Négrel and one research assistant spent several days searching for the additional active burrows based on the description and location described by the volunteers (see Figure 12 for the gps location of the active burrows). However, only seven of those 17 burrows defined as active during the community census were confirmed as active after the census. The remaining burrows showed presence of old bird droppings around the entrance, but none had fresh dropping or a strong penguin smell, and most exhibited spider webs inside the burrows.

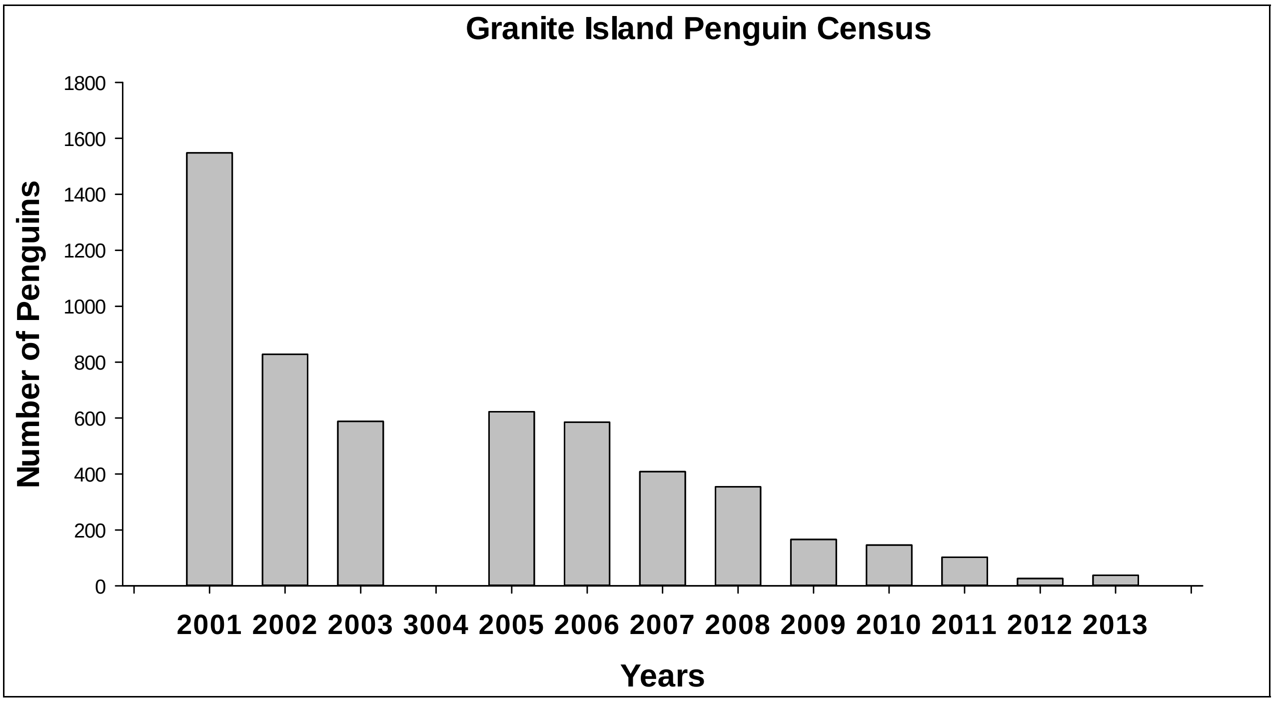

Figure 11. Estimated population size of little penguins on Granite Island between 2001 and 2013.

Figure 12. Map of Granite Island showing the distribution of the 7 active burrows that were gps marked during the 2013 breeding season.

Troubridge Island

The census was conducted over 3 days (16^{th}-18th of October 2013) by a team of three people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and one volunteer). The island was divided into five sections, and each section was walked in transects to count the total numbers of burrows found and record their status (active, not active). A small area of the island (approx. 15%) was completely inaccessible due to the vegetation – but showed signs of penguin tracks – and therefore was not surveyed. The total number of active penguin burrows was extrapolated to account for the whole island. A total of 245 burrows were found out of which 115 showed signs of penguin activity such as fresh droppings, penguin smell, or the presence of adults, eggs or chicks (see Figure 13 for the gps location of the active burrows). Out of the 115 active burrows, only 20% showed signs of breeding activity (41 burrows; Table 5). Extrapolating for the whole island, the total number of active burrows in October was 135, with 270 penguins estimated to be present on the island.

Penguin Colonies	No. Active Burrows	No. Burrows Breeding	No. Burrows with Adults	No. Burrows with Eggs	No. Burrows with Chicks
Troubridge	115	41 (21%)	71 (62%)	26 (23%)	12 (10%)
Althorpe	21	5 (24%)	4 (19%)	1 (5%)	4 (19%)
Granite	19	5 (26%)	3 (16%)	1 (5%)	4 (21%)

Table 5. Percentage of burrows showing signs of breeding activity and number of burrows with adults, eggs and chicks for the 2013-census on Granite, Troubridge and Althorpe Island

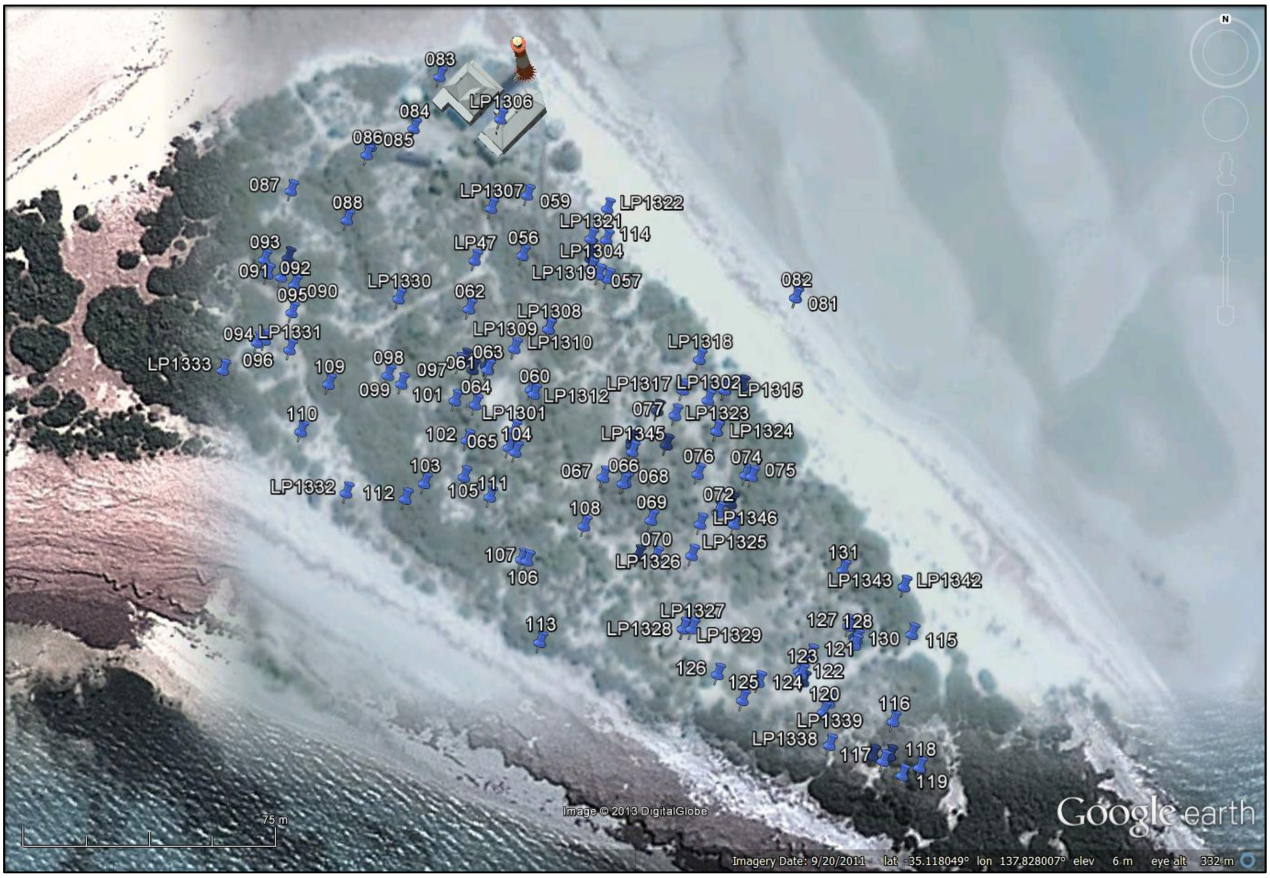

Figure 13. Map of Troubridge Island showing the distribution of the 135 active burrows that were gps marked in October 2013.

Althorpe Island

The census was conducted on the 15th of October 2013 by a team of four people (Penguin Ecologist Diane Colombelli-Négrel accompanied by a research assistant and two volunteers). The island was divided in sections and 50% of the breeding area was searched for the presence of penguin burrows. A total of 40 burrows was found, out of which 21 were active (see Figure 14 for the gps location of the active burrows). Out of the 21 active burrows, only 5 showed signs of breeding activity (24%; see Table 5). Extrapolating for the whole Island, the number of active burrows is estimated to be 42. Assuming that an active burrow indicates two penguins, the population can be estimated to be approximately 84 individuals. Compared with previous censuses, the population of little penguins on Althorpe Island appears to be relatively stable, with a tendency for decline (Figure 15).

Figure 14. Maps of Althorpe Island showing the distribution of the 21 active burrows that were gps marked in November 2013.

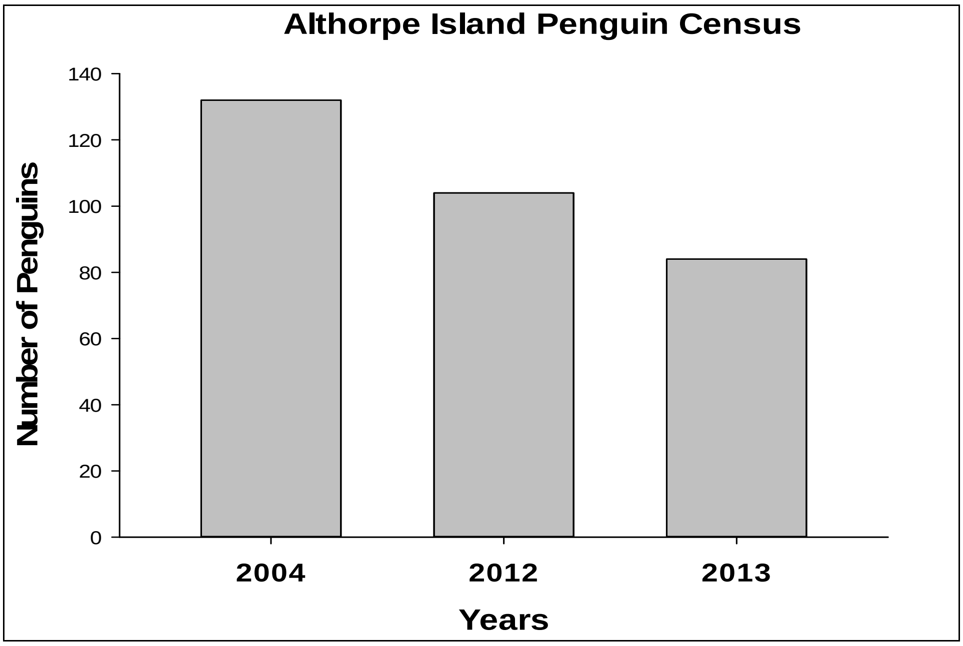

Figure 15. Estimated population size of little penguins on Althorpe Island between 2004 and 2013.

Wright & West Islands

The census was conducted 24th of November 2013. Two people (Penguin Ecologist Diane Colombelli-Négrel and local volunteer) walked along the coastal area of each island searching for burrows. A total of five burrows were found on West Island, but none was determined to be active. On Wright Island, a total of eight burrows were found, but none was active. All 13 burrows found on both islands were used by pigeons and seagulls for nesting.

Seal & Pullen Islands
The census was conducted 25th of November 2013. Three people (Penguin Ecologist Diane Colombelli-Négrel, a research assistant, and District Ranger Seiji Iwao) walked along the coastal area of each island searching for burrows. A total of 15 burrows were found on Seal Island, but none was determined to be active. On Pullen Island, only two burrows were found, again not active. All 17 burrows found were used by pigeons and seagull chicks as refuge.

VII. DISCUSSION

The main findings of this study are: (1) Granite Island population had the highest breeding success, while Antechamber Bay (KI) and Troubridge Island populations had the lowest breeding success; (2) predation significantly reduced breeding success and may be an issue on Kangaroo Island and Troubridge Island in particular; (3) parasite intensity negatively impacted breeding success and could play a larger role than suspected for population decline; and (4) there was a significant increase in fur seal numbers in Encounter Bay over the last 10 years which could have impacted on penguin numbers as evidenced by the fact that penguins formed a significant part of the fur seal diet and were found in 33% of the fur seal scats collected.

Census
In Encounter Bay, population census found no penguins or signs of penguin presence on West, Pullen, Wright or Seal Islands. According to Weibkin (2011), the population at Pullen Island has been declining since 1980s but Seal Island penguins were considered common in 1982, and Wright Island had a population of over 200 penguins in 1992. Additionally, West Island had a population estimated to be approx. 4000 penguins in 1990s (reviewed in Wiebkin 2011), and at least 120 active burrows were found in 2006 (Bool et al. 2007). The fact that no penguin signs were found on any of these three islands in 2013 suggests that the penguin populations did not recover from previous population crashes. On Granite Island, the penguin census showed 38 penguins present on the island in 2013 compared to the 26 recorded in 2012. Numbers seem to be stabilising, but further monitoring across the next years is necessary to identify the population trends. In addition, the difference between the numbers of active burrows recorded during the population census and the numbers of burrows confirmed through monitoring indicates that these population numbers might be lower than indicated.

In the Yorke Peninsula, our 2013 census data estimate the Althorpe population at 84 penguins. This represents a slight decrease compared with the 132 penguins found in 2004 (see Wiebkin et al. 2012), but in general the population is somewhat stable. In addition, it should be noted that the census was carried out in October to align with the census on Granite Island (while most censuses in the past were conducted in June), and therefore the smaller number of penguins found could be the result of the timing difference. To confirm this result, a census conducted on Troubridge Island in October 2013 found lower numbers (approx. 270 penguins estimated to be present on the island; our study) than the census conducted in June of the same year (approx. 1966 penguins; Bool & Wiebkin 2013). In their study, Bool and Wiebkin (2013) found that 20% of the active nests had at least one adult present (possibly on eggs), but only 0.03% (3 nests) had chicks, and therefore suggested that this was the start of the first breeding season. During our October census, we found that 21% of the active burrows were in a breeding stage, with 23% with eggs and 10% with chicks, indicating that this was likely the second breeding season as described by (Wiebkin 2010).

Variation in reproductive success
Although the numbers of active burrows decreased significantly since 2001, the overall breeding success on Granite Island was higher than for any other colonies in 2013. Antechamber Bay (KI) and

Troubridge Island populations however – two supposedly stable colonies – showed the lowest breeding success. Long-term studies on Phillip and Bowen Islands showed that annual variation in breeding success is common in little penguins (Reilly & Cullen 1981; Fortescue 1999; Nisbet & Dann 2009). For example, annual breeding success on Philip Island greatly varied from 0.08 to 1.38 fledglings per pair (Reilly & Cullen 1981; Nisbet & Dann 2009). But comparing with breeding data from previous years, low breeding success at Antechamber Bay and Troubridge Island does not seem to be the result of inter-annual variation (Table 6). In addition, 2013 census data at Antechamber Bay showed a decline in population numbers by nearly 50% (Kinloch unpublished data presented at the Penguin Monitoring Overview Meeting in November 2013). Therefore, further monitoring is required to fully understand whether low breeding performance at Antechamber Bay is a major cause of population decline.

Location	Period	No. Years	Breeding Success	Fledgling Success
Granite Island	1990-2013	17	0.96	0.55
Granite Island	2013	1	1.50	1.00
Antechamber Bay	2012	1	0.52	0.57
Antechamber Bay	2013	1	0.50	0.42
Emu Bay	2012	1	1.04	0.62
Emu Bay	2013	1	1.00	0.60
Troubridge	2004-2009	4	0.77	0.82
Troubridge	2013	1	0.73	0.86

Table 6. Comparison of breeding performance among little penguin colonies in South Australia: fledgling success is the number of chicks that fledged compared to the number of eggs that hatched and breeding success is the number of chicks that fledge per breeding pair.

Predation at burrow

Despite the fact that only one burrow out of the 22 video-monitored burrows had evidence of predation, we found that predation negatively affected breeding success, particularly on Kangaroo and Troubridge Islands. On Kangaroo Island, recent study showed the importance of predation by feral cats (Achurch et al. 2013). Specifically, they found that breeding success was greater for colonies where cats had been trapped and removed than where no cat control had been carried out (Achurch et al. 2013). The impact of cats on penguin population declines is not well documented, but cats are suspected predators of penguins (e.g., Massaro & Blair 2003; King et al. 2012). In yellow-eyed penguins (*Megadyptes antipodes*), for example, the number of breeding pairs was significantly higher in cat-free areas than in areas with many cats, supposedly due to predation (Massaro & Blair 2003). In 2012, Achurch et al. (2013) reported that cats were detected on 50 and 51 nights at Emu Bay and Antechamber Bay respectively over a three month period, coinciding with intensive cat trapping at those sites. In our 2013 study, we only recorded three cat visits at Emu Bay over the three month period, which demonstrates a positive impact of the cat control conducted in 2012. However, further monitoring of predation on Kangaroo Island is required to understand its full impact on breeding success and thus population decline.

On Troubridge Island, the video cameras did not reveal any predators, but four burrows had suspected predation. Out of the four burrows, three had signs of predation at the egg stage (eggs disappeared before hatching date), while the remaining burrow had signs of predation when young chicks were present. Tracking tunnels confirmed that no rodents were present on the island, and therefore predation cannot be attributed to rodents. However, seagulls are known predators of penguin eggs and chicks (e.g., Emslie et al. 1995; Stokes & Boersma 2000), and could be potential predators on Troubridge Island. Additionally, on five tracking tunnel cards, we found marks that could be attributed to reptiles (Figure 16). Reptiles are also well-known predators of seabird eggs and chicks (e.g., Meathrel & Klomp 1990; Carey 2010; Fracasso & Branco 2012), and therefore (if present on Troubridge Island) might be responsible for the eggs and chicks disappearance recorded there. However, reptiles' presence on Troubridge Island remains to be confirmed. It should be noted that the only predation recorded on video in our study was by a goanna on Kangaroo Island. Although it there is common knowledge that goannas may predate on little penguins, this is the first evidence that such predation does occur.

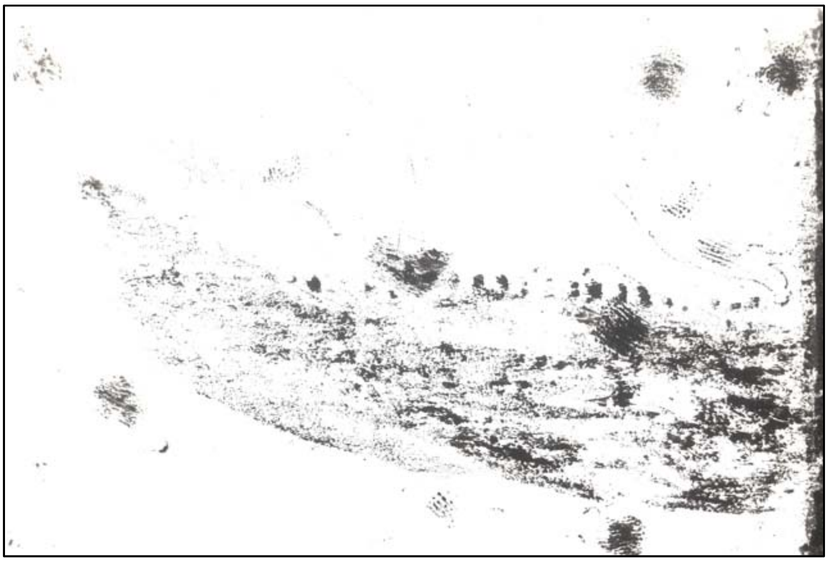

Figure 16: Tracking tunnel marks suspected to be produced by reptiles on Troubridge Island

Contrary to previous studies, we found no evidence of predation at burrows on Granite Island. It was suggested that water rats (*Hydromys chrysogastes*) and black rats (*Rattus rattus*) might be the main predators at little penguin burrows on Granite Island (Bool et al. 2007; Preston 2008). Specifically, Bool et al. (2007) found that black rats preyed principally on small chicks rather than on eggs. Predation by rats has been shown to negatively impact breeding of many seabird species around the world (e.g., Thibault 1995; Pascal et al. 2008), including little penguins (Perriman et al. 2000; Johannesen et al. 2002). An analysis of breeding success on Granite Island since 1990 showed that predation particularly influenced the number of fledglings produced per pair, confirming the results found by Bool et al. (2007) that predation pressure mainly occurred on chicks but not on eggs (Colombelli-Négrel in review). In addition, the study showed that the percentage of burrows with suspected predation significantly decreased while breeding success increased following extensive rat baiting in 2006 (Colombelli-Négrel in review). Therefore, coordinated rat management on Granite Island should continue to maintain high breeding performance on the island.

Blood Parasites

Blood parasites have been identified in six species of penguins so far: (1) *Plasmodium* in crested penguins (*Eudyptes pachyrhynchus*), yellow-eyed penguins (Laird 1950), African penguins (*Spheniscus demersus*), Rockhopper penguins (*Eudyptes chrysocome*) (Fantham & Porter 1944) and magellanic penguin (*Spheniscus magellanicus*) (Silveira et al. 2013); (2) *Leucocytozoon* in crested penguins and yellow-eyed penguins (Fallis et al. 1976; Argilla et al. 2013); (3) *Babesia* in African penguins (Earle et al. 1993); (4) *Trypanosoma* (Jones & Woehler 1989); and (5) *Haemoproteus* in little penguins (Cannell et al. 2013). While morbidity or mortality were only found in captivity in the past (reviewed in Jones & Shellam 1999), there is now growing evidence of mortality in wild penguins caused by blood parasites (Argilla et al. 2013; Cannell et al. 2013; Silveira et al. 2013).

In this study, we found evidence of blood parasite infections in 31% of the individuals sampled. We also found a direct correlation between breeding success and parasite intensity, and colonies with a higher incidence of parasites (higher parasite prevalence) (specifically, Antechamber Bay and Troubridge Island) also had the lowest breeding success. Blood parasites have been shown to decrease breeding success in many bird species, including penguins. For example, in house martin (*Delichon urbica*), treated individuals significantly increased their clutch size and hatching success (potentially via egg quality and incubation behaviour) (Marzal et al. 2005). In blue tits (*Parus caeruleus*), blood parasites reduced the ability of parents to feed their chicks, and hence lowered food intake for the young (Merino et al. 2000). In penguin species, blood parasites affected chick growth (Palacios et al. 2012) and caused chick death (King et al. 2012). Antechamber Bay and Troubridge Island are two colonies considered stable, with large numbers of breeding pairs returning to the sites each year. Our results here imply that the numbers of young produced per year could be quite low, which could in turn impact on population numbers, suggesting a need to improve nesting conditions. It is also suggested that mortality of sub-adults in their first and second years occurs principally due to the combined effects of parasites and starvation (Harrigan 1992), which could in turn drive population declines.

While it was possible to detect the presence of the parasite via visual inspection of the blood smears, it was not sufficient to ascertain parasite species. Therefore, future studies should use molecular techniques to confirm the species and strain of parasites present in little penguins in order to understand their full impact on the population declines. This is particularly relevant since a recent pathology report showed that 22% of the little penguin carcasses found had evidence of various diseases and parasites (Tomo 2014).

Marine Predation

There is growing concern that penguin population declines are due to the recent increase in New Zealand fur seal numbers in the Gulf St Vincent. In yellow-eyed penguins, New Zealand sea lions (*Phocarctos hookeri*) are considered a probable cause for the decline in penguin numbers at Campbell Island (Moore & Moffat 1992; Moore 1992) and threaten the viability of populations at Otago Peninsula (Lalas et al. 2007). Similarly, predation by Cape fur seals (*Arctocephalus pusillus*) threatens the survival of small populations of African penguins (*Spheniscus demersus*) in South Africa (Crawford et al. 2001; David et al. 2003). On Kangaroo Island, there is evidence for the impact of predation by New Zealand fur seals on little penguin populations (Page et al. 2005).

Here, we found that fur seal numbers on West Island increased at least five-fold over the last 10 years. While the exact extent of marine predation is still unknown, little penguins definitively form a significant part of fur seal diet and were found in 33% of the scats collected. This finding aligns with previous studies showing penguin remains in 13-41% of fur seal scats or regurgitates (Page et al. 2005; Bool et al. 2007; Wiebkin et al. 2012). In our study, we found that penguins were the fourth most abundant prey type (10.87%), and that there was a higher percentage of penguin remains in the Fleurieu Peninsula samples (6.52%) than in those collected on Kangaroo Island (4.35%). However, these results should be treated carefully due to the small sample size (12 scats). Additional studies are therefore necessary to estimate the full impact of fur seal predation on little penguin population declines and whether fur seal predation pressure in Encounter Bay is higher than on Kangaroo Island (see also 2012 pathology report showing difference in trauma type between the two areas; (Tomo 2012).

Conclusion

This study showed that Island populations in Encounter Bay are not recovering from previous little penguin population crashes. While little penguin numbers on Granite Island seem to be stable, further monitoring across continuous years is necessary to confirm this result. This study highlights the importance of the timing and methodology used to estimate population numbers, and underscores the need to have a uniform methodology across colonies to ensure that the data are comparable. Breeding success on Granite Island was the highest, likely due to the reduced predation by rats. Therefore, coordinated rat control should be maintained to preserve the high breeding performance on the island. However, predation remains an issue for the other colonies studied here, and further monitoring is required in order to implement appropriate predator controls. Fur seal numbers in Encounter Bay definitely increased over the last 10 years, but despite the fact that little penguins definitively form a significant part of fur seals diet, the exact extent of the impact of marine predation is still unknown and requires further investigation. Finally, the most significant result was the discovery of blood parasites in 32% of the individuals sampled. Blood parasite intensity was negatively linked with breeding success, and could play a larger role than suspected on population declines, maybe via reduced survival of sub-adults.

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. We suggest targeting the following colonies because of the existence of long-term monitoring data: 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 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).

IX. COMMUNITY ENGAGEMENT

Thirty-nine volunteers participated in the Granite Island penguin census in October 2013. An additional 13 volunteers participated in field trips to collect the data, and helped with penguin censuses on KI and Troubridge Island. Two third-year university students conducted a research project on fur seal predation and analysed the fur seal scats. Three volunteers living in the Victor Harbour area regularly scanned the beaches for dead penguins. Katharina Peters gave a presentation to the public at Penneshaw on 22nd of September 2013 and at Kingscote on 29th September 2013 (both attended by approx. 30 members of the public). An article calling for community volunteers to join the Granite Island Penguin count was released on 14/10/13 in the Victor Harbour Times.

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

1. Interview for ABC Channel (filmed on 24/4/14)
2. The advertiser (Australia, 9/12/13) http://www.adelaidenow.com.au/news/south-australia/penguintoursscrapped-as-kangaroo-island-numbers-dwindle/story-fni6uo1m-1226779267711
3. Victor Harbour Times (Australia, 24/10/13) http://www.coastlines.com.au/news/penguin-decline-goes-beyond-granite-island
4. Channel 7 News (Australia, aired on 22/10/13) http://au.news.yahoo.com/sa/a/19493209/more-penguins-found-in-granite-island-count/
5. ABC Radio Adelaide (Australia, aired on 22/10/13) http://www.abc.net.au/news/2013-10-22/more-penguins-found-in-granite-island-count/5037034
6. The Advertiser (Australia, 21/10/13) http://www.adelaidenow.com.au/news/south-australia/flinders-university-researchers-and-volunteers-confirm-there-are-just-38-fairy-penguins-left-on-sas-granite-island/story-fni6uo1m-1226743932583
7. ABC Radio (Australia, aired on 15/10/13)
8. ABC Radio Victoria (Australia, aired on 31/7/13) http://www.abc.net.au/radionational/programs/bushtelegraph/penguins/4855708

X. ACKNOWLEDGEMENT

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