

**Conservation genomics of the 'Endangered' long-nosed bandicoot  
(*Perameles nasuta*) population at North Head, Sydney, Australia**

Author

Nelson, Holly V, Frankham, Greta J, Leo, Viyanna, Anson, Jennifer R, Eldridge, Mark DB, de Bruyn, Mark

Published

2021

Journal Title

Conservation Genetics

Version

Accepted Manuscript (AM)

DOI

[10.1007/s10592-021-01356-z](https://doi.org/10.1007/s10592-021-01356-z)

Rights statement

© 2021 Springer Netherlands. This is an electronic version of an article published in Conservation Genetics, Conservation Genetics, 2021, 22 (5), pp. 745-756. Conservation Genetics is available online at: <http://link.springer.com/> with the open URL of your article.

Downloaded from

<http://hdl.handle.net/10072/417332>

Griffith Research Online

<https://research-repository.griffith.edu.au>

Conservation genomics of the ‘Endangered’ long-nosed bandicoot (*Perameles nasuta*) population at North Head, Sydney, Australia

Holly V Nelson<sup>1</sup>, Greta J Frankham<sup>2</sup>, Viyanna Leo<sup>3</sup>, Jennifer Anson<sup>3</sup>, Mark D B Eldridge<sup>2</sup> and Mark de Bruyn<sup>1</sup>

<sup>1</sup> The University of Sydney, School of Life and Environmental Sciences, NSW 2006, Australia (HN, MdB)

<sup>2</sup> Australian Museum Research Institute, Australian Museum, Sydney, NSW 2010, Australia (ME and GF)

<sup>3</sup> Australian Wildlife Conservancy, 5/280 Hay St Subiaco, Western Australia 6008, Australia (VL and JA)

Corresponding author

Mark de Bruyn

[mark.debruyn@sydney.edu.au](mailto:mark.debruyn@sydney.edu.au)

## **Acknowledgements**

Thanks to Australian Wildlife Conservancy, NSW National Parks and Wildlife Service, Taronga Conservation Society and the Mammal Collection, Australian Museum for providing samples. Thanks to the Sydney Harbour Federation Trust for funding. Thanks also to M. Hall, E. Lee, L. de Gail, and J. Dargan for long term sample collection, the Australian Centre for Wildlife Genomics (Australian Museum) staff for assistance with laboratory work, and C. Van Der Wal, K. Yi Tea, M. Lott and K. Ewart for assistance with data analysis. Funding for this work was provided by the Sydney Harbour Federation Trust and Australian Wildlife Conservancy.

**Abstract:** Wildlife species impacted by habitat loss and fragmentation often require conservation efforts to maintain populations. Long-nosed bandicoots (*Perameles nasuta*) still persist within the highly urbanised matrix of northern Sydney (Australia). One population at North Head, Sydney, is currently listed as an *Endangered* population due to its small size, apparent isolation and other threats. To support future management, we used 1,446 single nucleotide polymorphism markers (SNPs) from 167 bandicoots to: i) assess the assumption of isolation and determine if genetic structuring is present between North Head and individuals from 11 other localities in northern Sydney, and ii) investigate genetic diversity over time in the North Head population from 2002 to 2018. Analyses confirmed population structuring and genetic divergence between North Head and greater northern Sydney. Three distinct populations were identified that corresponded to geographic localities (North Head, northern Sydney and Mosman). All populations were significantly differentiated ( $F_{ST} = 0.171-0.345$ ), suggesting local genetic drift between localities. North Head genetic diversity indices estimated between 2002 to 2018 showed relatively constant levels of allelic richness (1.90-2.00) and observed heterozygosity ( $H_O = 0.231-0.310$ ) along with minor levels of inbreeding ( $F_{IS} 0.020-0.052$ ). The identification of some individuals sampled on North Head that were assigned to other populations suggests some sporadic geneflow into the population has occurred and may have assisted with maintaining genetic diversity. These data suggest that the North Head population is distinct from other northern Sydney populations and has relatively constant levels of genetic diversity.

**Keywords:** Habitat fragmentation, *Perameles nasuta*, North Head, conservation genomics, single nucleotide polymorphisms

**Declarations**

**Funding:** This study was funded by the Sydney Harbour Federation Trust and Australian Wildlife Conservancy.

**Conflicts of interest/Competing interests:** The authors have no relevant financial or non-financial interests to disclose.

**Availability of data and material:** Genomic data has been uploaded to DRYAD. Accession number: doi:10.5061/dryad.f7m0cfxvk

**Ethics approval:** The methods were performed in accordance with the following ethics approvals and licensing.

Animal Research Authority (ARA) – Secretary’s Animal Care and Ethics Committee approval for *Biodiversity Conservation Restoration, North Head*. Department of Primary Industry (DPI)

Scientific Licence 101333 - *Biodiversity conservation and restoration at North Head*. Office of Environment and Heritage (OEH)

Animal Research Authority (ARA) – Secretary’s Animal Care and Ethics Committee approval for Long-nosed Bandicoot Recovery Program AEC 000214/05 (OEH).

**Consent for publication:** All authors have reviewed the manuscript and have provided consent to publish.

## 1. Introduction

Widespread habitat loss and fragmentation has isolated wildlife populations around the world (Saunders et al., 1991; Fischer and Lindenmayer, 2007; Frankham et al., 2014). Since European settlement, these anthropogenic disturbances have resulted in significant declines in Australian biodiversity, especially mammals (Lacy, 1987; Frankham et al., 2014). The fragmentation of populations, particularly along the highly urbanised east coast of Australia, has resulted in a loss of genetic connectivity, leaving populations vulnerable to loss of diversity and increased differentiation via genetic drift (Lacy, 1987; Keyghobadi, 2007; Lancaster et al., 2011; Frankham et al., 2014). The extinction risk of isolated remnant populations is also amplified, as these populations are more susceptible to increased inbreeding, genetic erosion and impacts from environmental factors (Spielman et al., 2004; Frankham et al., 2014).

Recent advances in molecular technology can complement field-based monitoring methods and provide increased resolution for investigating fine-scale population processes (Piggott et al., 2006). Furthermore, a genome-wide approach using single nucleotide polymorphisms (SNPs), for example using DArTseq (Kilian et al., 2012), can provide a greater understanding of the evolutionary, genetic and demographic processes within and between populations (Piggott et al., 2006; Fuentes-Pardo and Ruzzante, 2017). The use of thousands of genetic markers from across the genome provides enhanced capacity to investigate fine-scale and complex patterns of genetic variation compared to more traditional markers (Luikart et al., 2003; Novembre and Stephens 2008; Ewart et al., 2019). As the number of conservation and population genetics studies grow in response to increased habitat loss, genome-wide approaches have yet to be widely incorporated into routine conservation management strategies (Frankham, 2010; Lott et al., 2020).

One species of Australian mammal that can benefit from the application of conservation genetic approaches is the long-nosed bandicoot (*Perameles nasuta* Marsupialia: Peramelidae)

(Chambers and Dickman, 2002; Dowle et al., 2013). As a habitat generalist, long-nosed bandicoots have a broad distribution along the east coast of Australia, from far north Queensland to southwest Victoria (Chambers and Dickman, 2002, Dexter et al., 2011). They inhabit open forests, shrub land, coastal heath and overgrown urban land (Scott et al., 1999; Chambers and Dickman 2002; Dowle, 2012). Their short gestation period, rapid maturity, and large litter size compared to other marsupials can result in rapid population increases. Shifts in breeding patterns when conditions are favourable can result in large variances in abundance over short periods of time (Short et al., 2016; Winnard and Coulson, 2008; Dexter et al., 2011). This flexible reproductive strategy and their omnivorous diet have contributed to the long-nosed bandicoot's persistence throughout much of their distribution, including in fragmented environments (Piggott et al., 2018). However, some populations are threatened by habitat loss due to increased urban and agricultural development, competition with introduced herbivores and the impact of introduced predators such as the European Red Fox (*Vulpes vulpes*) and domestic/feral cat (*Felis catus*) (Banks, 2004).

Although the long-nosed bandicoot has become locally extinct throughout much of the Sydney urban area, it is one of the last ground dwelling marsupial species to maintain remnant populations within urban Sydney (Fig 1) (Dowle and Dean, 2009). In the northern Sydney suburbs, these populations are concentrated in and around national parks and other areas of remnant native vegetation, including North Head, Ku-ring-gai Chase National Park, Bradley's Head in the suburb of Mosman, Garigal National Park, Lane Cove National Park, Dobroyd Head and the northern Beaches (Dowle and Dean, 2009), but also extend into proximate urban areas. In the past few decades, implementation of stricter fox control practices by NSW National Parks and Wildlife Service (NPWS) and northern Sydney councils has led to some recovery of long-nosed bandicoot numbers in these urban and peri-urban areas (V. Leo 2019, pers comm. 30 October).

Currently, two populations of *P. nasuta* are listed as ‘*Endangered*’ under the New South Wales *Biodiversity Conservation Act 2016*; a small inner western Sydney population (NSW TSSC, 2008) and a larger population inhabiting North Head Sydney (NSW TSSC, 1997). The North Head population is of substantial interest to conservation practitioners as one of the largest remaining long-nosed bandicoot populations within the highly urbanised Sydney region (NSW TSSC, 1997; Scott et al., 1999; Chambers and Dickman, 2002; Banks, 2004; Anson, 2015). The population was listed as ‘*Endangered*’ in 1997, due to the risk of extinction from environmental factors events caused by its isolation, small population size ( $n = \sim 100$ ), and high incidence of road mortalities and predation pressures (e.g. foxes and cats) (NSW TSSC, 1997). North Head is a  $\sim 300$  ha coastal headland at the entrance to Sydney Harbour with remnant woodland and heathland habitats, as well as cleared areas and limited urban development. The site is currently managed by Sydney Harbour Federation Trust (SHFT) and National Parks and Wildlife Service (NPWS; Department of Planning Industry and the Environment) with active management and monitoring conducted by Australian Wildlife Conservancy (AWC). Although the North Head population is believed to be disjunct from remnant populations in neighbouring suburbs and thus potentially at risk from loss of diversity and inbreeding (NSW TSSC, 1997), to date there have been no genetic studies to assess how isolated the population is from neighbouring populations, and to determine the genetic health of the North Head population.

This study represents the first population genomic analysis of the long-nosed bandicoot populations on North Head and other northern Sydney suburbs. This research has two primary aims: i) to identify levels of inter-population connectivity and structure between North Head and other remnant northern Sydney populations, and ii) to determine changes in genetic diversity of the North Head population over time. This study provides insight into patterns of genetic connectivity in small mammal populations confined to remnant habitats in urban areas

and provides recommendations for the conservation management of genetically isolated populations.

## **2. Materials and methods**

### ***2.1 Sample Collection***

Long-nosed bandicoot samples obtained for this study were from three collections (Fig 1, Fig 2 and Table S1). Firstly, ear biopsies from North Head samples were collected during biannual trapping surveys carried out by AWC and NPWS between 2002 to 2018 under ethics approval and scientific licensing. Samples from four-year intervals were used for analyses 2002 (n = 7), 2006 (n = 26), 2010 (n = 60), 2014 (n = 46), 2018 (n = 52). Secondly, liver, muscle and ear tissue samples were obtained from the Australian Museum Mammal collection (n = 23), from northern Sydney suburbs sampled between 2001-2019, including Manly (n = 1)(2008), Ingleside (n = 1)(2011), Oxford Falls (n = 1)(2010), Collaroy (n = 1)(2008), Elanora Heights (n = 5)(2001- 2014), Turramurra (n = 2)(2010), Newport (n = 1)(2019), and Ku-ring-gai Chase National Park (n = 12)(2001-2019) as well as samples from the inner west suburbs of Petersham (n = 1)(2007) and Dulwich Hill (n = 1)(2006). Thirdly, samples from Bradley's Head, Mosman (here after referred to as Mosman) were collected from bandicoots trapped by Taronga Zoo (n = 13)(2018) monitoring program.

### ***2.2 DNA Extraction and Genotyping and SNP filtering***

Genomic DNA extractions were performed using a high salt method (Sunnucks and Hales, 1996). Following quantification using a Qubit fluorometer and Invitrogen™ Qubit™ dsDNA+ Broad Range Assay Kit (Life Technologies, Carlsbad, USA), samples with DNA concentrations below 5ng/μl were excluded from the analysis.



Reduced genome sequencing and SNP discovery was then undertaken at Diversity Arrays Technology Pty Ltd. (Canberra, Australia). This involved genome complexity reduction using the restriction enzyme combination *PstI-SphI*, along with the exclusion of fragments without cut sites (Kilian et al., 2012). High-throughput DNA sequencing was then run using HiSeq 2500 (Illumina) next-generation sequencing and standard DArTseq protocols. The sequences obtained by DArTseq, containing the filtered SNP markers that had been mapped against the Tasmanian Devil (*Sarcophilus harrisii*) genome (RefSeq Accession GCF\_000189315.1), were generated using a Diversity Arrays Technology pipeline (Kilian et al., 2012).

Genomic DNA SNP data were quality-filtered in R STUDIO v.1.2.1335 (RStudio Team, 2015) using the DARTR package v1.1.11 (Gruber et al., 2018). Filtering was carried out on 3 subsets of samples for different downstream analyses: i) all samples, ii) samples from North Head and the northern Sydney suburbs only, and iii) North Head samples only. SNPs with a repeat average <100%, loci with a call rate <70% and individuals with a call rate <75% were removed from the analysis to ensure quality of the resulting datasets (Gruber et al., 2018). Markers with minor allele frequencies of <0.05 were removed to prevent interference of population structure (Gruber et al., 2018). SNPs in linkage disequilibrium (LD) and monomorphic loci were also removed across all individuals during the filtering process. After filtering, 167 individuals were genotyped for 1,446 SNPs in the ‘all samples’ dataset; from North Head (n = 132), Mosman (n = 12), Manly (n = 1), Inner West (n = 1), Oxford Falls (n = 1), Collaroy (n = 1), Elanora Heights (n = 5), Turramurra (n = 2), Newport (n = 1), Ingleside (n = 1) and Ku-ring-Gai Chase National Park (n = 10). Secondly, 1,200 SNPs from 155 sampled individuals remained for the ‘North Head and northern Sydney’ data set, and finally, 997 SNPs were genotyped from 136 individuals for the ‘North Head only’ dataset, these samples were collected in 2002 (n = 4), 2006 (n = 12), 2010 (n = 30), 2014 (n = 44), and 2018 (n = 46).

### ***2.3 Inter-population structure***

Three population structure analyses were performed. Firstly, a principal coordinates analysis (PCoA) was conducted on the filtered ‘all samples’ dataset (1,446 SNPs) using the R packages DARTR v.1.1.11 and ADEGENET v2.1.1 (Jombart, 2008; Jombart et al., 2010) to initially visually infer putative genetic clusters (Melville et al., 2017; Ewart et al., 2019). A second PCoA was then generated using the 1,200 SNPs dataset to further explore relationships between North Head and northern Sydney suburbs. Secondly, inter and intra-population structure was explored using a Bayesian method implemented in STRUCTURE v2.3.4 (Pritchard et al., 2000). Markov chain Monte Carlo simulations were carried out with 10 replicates for values of  $K$  from 1-10, with  $10^5$  iterations after a  $10^4$  burn-in. Maximum delta log likelihood ( $\Delta K$ ) (Evanno et al. 2005) and the maximum posterior probability (L(K)) (Pritchard et al., 2000) were used to identify the optimal number of genetic clusters using Cluster Markov Packager Across  $K$  (CLUMPAK) v1.1 (Kopelman et al., 2015) and STRUCTURE HARVESTER (Earl and Von Holdt, 2012). Owing to the known issue of inferring population clusters from  $\Delta K$  (Evanno et al., 2005; Janes et al., 2017), the optimal  $K$  value was inferred using a hierarchical approach by sequential STRUCTURE analyses of the clusters identified at each step (Tea et al., 2019). Replicate runs of the STRUCTURE output were summarised using CLUMPAK and plotted using DISTRUCT v1.1 (Rosenberg, 2004).

Thirdly, a discriminant analysis of principal components (DAPC) was performed in the R package ADEGENET, to confirm the population clusters observed in previous analyses and to infer genetic relationships among sampling localities (Jombart, 2008; Jombart et al., 2010). Bayesian Information Criterion were used to define the number of genetic clusters (Lott et al., 2020). The data were transformed using a PCA with 50 principal components, which explained >70% of the variance.

## ***2.4 Inter-population genetic divergence***

To estimate genetic divergence among identified populations ( $K = 3$ ), pairwise fixation indices ( $F_{ST}$ ) were calculated (Weir and Cockerham, 1984) (Table 1) using the R package STAMPP (Pembleton et al., 2013), with statistical significance calculated using 1,000 bootstrap replicates. The Manly sample was included in the North Head population for this analysis as previous results showed no distinction between these localities.

## ***2.5 Intra-population genetic diversity***

To assess temporal changes in genetic diversity, the North Head samples were subdivided into five ‘populations’ based on collection year from 2002 to 2018. The R packages ADEGENET (Jombart, 2008; Jombart et al., 2010), DIVERSITY (Keenan et al., 2013), PEGAS (Paradis, 2010), and HIERFSTAT (Goudet, 2005) were used to calculate the expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity’s, allelic richness, deviation from HWE, and the mean individual inbreeding coefficient ( $F_{IS}$ ) (Weir and Cockerham, 1984). Genetic diversity indices were also calculated on the Mosman and Ku-ring-Gai Chase National Park samples, for comparative analysis with the North Head population.

# **3. Results**

## ***3.1 Inter-population structure***

The principal coordinates analysis (PCoA) using 1,446 high quality filtered SNPs revealed 3 clusters that for the most part corresponded with geographic localities (Fig 3A). Up to 11.7% of the variation, primarily separating out North Head individuals, was explained by PCo I, while PCo II (3.8% of variation) separated Mosman and northern Sydney individuals. The single Manly individual clustered within the North Head population. Six North Head samples

appear as outliers and cluster more closely with northern Sydney and Mosman with one outlier falling tightly within the Mosman cluster, and two tightly within the northern Sydney cluster (Fig 3A). The second PCoA analysis (Fig 3B), which excluded samples from Mosman, showed strong separation between North Head and northern Sydney with up to 9.6% of variance explained by PCo I and 3.4% by PCo II, with the aforementioned North Head outliers remaining within the northern Sydney cluster. Within this cluster, there was also some structuring of samples based on locality, which suggests local population structure occurs throughout northern Sydney (Fig 3B). For the DAPC analysis, the Bayesian Information Criterion determined  $K = 3$  genetic clusters (Fig 5). Similar to the PCoA, DAPC separated northern Sydney and North Head into separate genetic clusters, with Mosman as a highly distinct third cluster.

The results from the hierarchical STRUCTURE clustering analyses using the full 1,446 SNP dataset determined  $K = 3$  as the best fit, clustering samples from North Head, Mosman, and North Sydney, respectively (Fig 4B).  $K = 3$  was also suggested by the PCoA (Fig 3A) and DAPC analyses (Fig 5) (Meirman, 2015). Initial analysis suggested  $K=2$  but with three major clusters apparent, comprising Mosman and North Head and with north Sydney appearing as admixed (Fig 4A and Fig S1). When the combined North Head and northern Sydney individuals were rerun, two clusters ( $K=2$ ) were resolved comprising each of these populations (Fig 4C and Fig S2). The STRUCTURE analysis was consistent with the PCoA and DAPC, in that the Manly individual was assigned to the North Head population. The North Head outliers that clustered towards Mosman and northern Sydney in the PCoA analysis, were also found to be more genetically similar to Mosman and northern Sydney individuals in the STRUCTURE analysis.

### ***3.2 Inter-population genetic divergence***

Amongst the three identified populations,  $F_{ST}$  values were significant and ranged from 0.171-0.345 (Table 1). North Head and Mosman were most divergent ( $F_{ST} = 0.345$ ), followed by Mosman and northern Sydney ( $F_{ST} = 0.232$ ). Northern Sydney and North Head were the most genetically similar ( $F_{ST} = 0.171$ ).

### ***3.3 Intra-population genetic diversity***

Of the 1,446 SNP loci used for analysis, most were in HWE (~90%) ( $P < 0.01$ ). Reanalysis of the dataset based on sample origin and year reduced the number of SNPs not in HWE to 3.5% for all loci, subsequently all loci were retained for analysis. Allelic richness for North Head ranged between 1.94 (2006) to 2.00 (2010) which was similar to Mosman (1.97) and Ku-ring-Gai Chase National Park (1.92) (Table 2). Observed heterozygosity for temporal North Head populations ranged from 0.231 (2006) to 0.310 (2010) (Table 2). Consistently lower observed heterozygosity to expected heterozygosity was significant for all years aside from 2010 and 2018 (Table 2). Observed heterozygosity was similar for Mosman (0.334) and Ku-ring-Gai Chase National Park (0.267). Inbreeding coefficients ( $F_{IS}$ ) across all temporal populations were small ranging from 0.020 (2014) to 0.052 (2002) (Table 2).

## **4. Discussion**

As habitat fragmentation continues to threaten biodiversity, the conservation of remnant populations is increasingly dependent on the maintenance of gene flow and genetic viability. This study has demonstrated population structure among remnant long-nosed bandicoot populations across northern Sydney, as well as relatively constant levels of genetic diversity within the North Head population through time. The current study significantly increases the genetic data on long-nosed bandicoots, while providing critical information on populations

dynamics of small to medium-sized mammals in an urban matrix in order to inform conservation management.

#### ***4.1 Inter-population structure and genetic divergence***

The genetic differentiation of the North Head population confirms its effective genetic isolation and limited connectivity with remnant long-nosed bandicoot populations sampled in the adjacent northern Sydney suburbs. Genotyped bandicoots were found to have strong population structure across the greater northern Sydney sampling area, with three main genetic clusters ( $K = 3$ ) identified by model-based, distance-based and multivariate analyses. Two of the clusters are highly localised to relatively isolated headlands (North Head and Mosman), while the third is more dispersed across a suburban landscape (remainder of sites throughout northern Sydney).

As a result, continued management of the North Head long-nosed bandicoot population as an isolated population should be considered with appropriate management actions (see section 4.4) implemented to mitigate the loss of genetic diversity in the future. This restriction of gene flow amongst the sampled populations is likely a result of physical barriers to dispersal including habitat loss via urbanisation, natural landscape features (e.g. waterways) and artificial structures (e.g. roads). The high level of differentiation between North Head and Mosman is likely a consequence of a natural barrier created by Middle Harbour and an extensive urban matrix (Fig 1). Similar to the North Head population, Mosman shows an independent clustering, suggesting other long-nosed bandicoot populations in remnant habitat on headlands around Sydney Harbour may also require similar management to North Head. However, further sampling of individuals around Mosman and of other harbour headland populations (e.g. Dobroyd Head) should be carried out to confirm this pattern.

The PCoA and Structure analyses identified a small number of individuals collected at North Head that genetically clustered with or closer to the Mosman and northern Sydney populations, suggesting some geneflow between the populations (Fig 3A). There are two potential explanations for these anomalies. Firstly, limited connectivity may exist between northern Sydney and North Head, allowing long-nosed bandicoots to disperse. However, this is unlikely due to the limited dispersal capabilities of this species and the aforementioned physical barriers to movement. Secondly, interbreeding may have occurred following the movement of individuals facilitated by human intervention, taking animals from Mosman to North Head. Translocation of individuals may have occurred either via the release of injured and rehabilitated individuals by wildlife carers, or the relocation of bandicoots from backyards by humans that perceive bandicoots to be a pest, or a vector for tick transmission for pets (Dowle and Dean, 2009).

Although long-nosed bandicoot populations throughout northern Sydney would have once had continuous natural habitat, the impact of habitat fragmentation and the resultant isolation and genetic drift is likely to have resulted in population differentiation. Our analyses show population differentiation and discrete sub-structuring within northern Sydney over relatively small geographic distances (suburbs) (Fig 3B). It is likely that the hostility of the urban matrix, including the presence of invasive predators (e.g. cats, dogs and foxes), and small pockets of remnant vegetation (i.e. parks, backyards), coupled with the presence of increased physical barriers have restricted long distance dispersal causing localised population structure.

A recurring result of our analyses was the consistent clustering of the Manly individual with those sampled within the *Endangered* North Head population boundary (Fig 3, Fig 4). The listed *Endangered* population sits within the suburb of Manly and includes the North Head Headland and remnant bush (which is managed by AWC, NPWS and SHFT). The listing extends into the surrounding urban areas to a designated boundary at Addison Road, Manly,

which bisects the isthmus connecting to North Head (Fig 1). This urban part of the *Endangered* North Head population is separately managed by the Northern Beaches Council (Fig 2). The Manly individual was collected outside the boundary of the *Endangered* population listing, suggesting this boundary is not a biological boundary and individuals are moving beyond this. Ongoing management of the *Endangered* North Head population would benefit from analysis of individuals sampled from across the suburb of Manly (both within and outside of the designated boundary) and surrounding areas, including Freshwater and Balgowlah, in order to determine a more biologically relevant boundary for the *Endangered* North Head population. Additionally, expansion of monitoring to include animals within the surrounding Northern Beaches Council Local Government Area (Fig 2) would provide further insight into the population dynamics of the *Endangered* North Head population. Tight clustering of samples from the *Endangered* North Head population suggests that if there is gene flow beyond the range of the *Endangered* listing (Fig 2), it is likely spatially limited, consistent with the small home range of this population (~4.4 ha) (Scott et al., 1999). This effect, combined with limited fox control and less vehicle signage beyond the listed area, is likely to create a barrier to gene flow onto North Head (Banks, 2004).

While not the focus of this research, one sample from the Inner Western Sydney *Endangered* population (NSW TSSC, 2008) was successfully genotyped. This individual was sampled in Dulwich Hill, located on the southern side of Sydney Harbour and a 10 km straight line distance from Mosman, which were the geographically most proximate samples included in the analysed dataset. However, the sample clustered closely with the northern Sydney long-nosed bandicoot population (Fig 1, Fig 3A). This may suggest that the long-nosed bandicoot sampled from the Inner West population may have originated from northern Sydney, although testing of more individuals from the Inner West would need to be sampled to confirm this.



### ***4.3 Intra-population genetic diversity***

In this study, the temporal genetic diversity of the long-nosed bandicoot population on North Head was characterised. Slight fluctuations in genetic diversity across years was observed however, there was no evidence or noticeable trend to suggest a major decline in genetic diversity over the approximate 12 generations between 2002 and 2018 (Banks, 2004). As the continual decline of genetic diversity in isolated populations is primarily a function of effective population size ( $N_e$ ) and length and degree of isolation (Frankham, 1997), these results indicate that  $N_e$  is high enough to maintain relatively constant levels of heterozygosity and/or that limited natural/artificial gene flow, that was identified in the PCoA and Structure analyses, is sufficient to offset any genetic diversity loss through genetic drift. North Head shows similar allelic richness to Mosman, and to Ku-ring-gai Chase National Park, a sampling locality where adequate population level sampling was available (Table 2), which also displayed relatively constant genetic diversity levels across temporal populations (2002-2018). This pattern suggests minor levels of gene flow occurs from Manly, or from potential translocations based on outliers in our results.

Slight fluctuations in genetic diversity may be a consequence of environmental factors such as adverse weather events (e.g. drought) or the infiltration of predators (e.g. foxes) onto the headland. The sampling period 2002-2018 spanned two major drought periods; the Millennium Drought (2001-2010) and the most recent drought (2017-2020) (NSW DPI, 2020). During drought, bandicoot reproductive output can decrease to aid chances of individual survival, and similar to the effects of predation, can result in a decrease in population numbers which has implications on genetic diversity (Short, 2016).

The second objective of our research determined that the intensively managed North Head population has retained relatively constant levels of genetic diversity under current management practices. Current management does not include active translocation of

bandicoots, however some gene flow has been identified which may have assisted current management strategies (e.g. predator control and habitat protection) in maintaining a healthy bandicoot population and preventing loss of genetic diversity on the headland. However, in future, translocation decisions underpinned by best scientific evidence, including genetic data such as this study, would help to alleviate outbreeding.

#### ***4.4 Management implications***

The identification of three genetic clusters with significant  $F_{ST}$  values provides evidence that there is population structure within the long-nosed bandicoot populations throughout the northern Sydney suburbs, and supports the initial listing of the North Head population based on perceived isolation. However, to ensure ongoing maintenance of genetic diversity, the appropriate agencies should consider augmenting the North Head population via translocation of bandicoots from other populations (Frankham, 2015; Goossens et al., 2016), provided the carrying capacity ( $K$ ) on the headland has not been reached. Periodically introducing new genetic material can provide a stronger buffer against environmental factors (e.g. predation and drought) and offset any issues associated with small population size (Menges, 1991; Frankham et al., 2017). While potential outbreeding effects and introduction of novel pathogens should always be considered when translocating animals, outbreeding should not be an issue if other northern Sydney suburban populations are used as source populations. These nearby populations occupy comparable environments, share the same karyotype, and have previously experienced gene flow among them (Frankham, 2010; Frankham et al., 2017; Lott et al., 2020). The Ku-ring-gai Chase National Park population would be a suitable source population for any future translocations with 1-10 migrants every 2 generations theoretically sufficient to increase genetic diversity, as has previously been recommended (Allendorf, 1983; Lacy, 1987, Lande and Barrowclough, 1987; Backus et al., 1995; Lott et al., 2020).

The current boundary of the *Endangered* North Head population is unlikely to represent a true biological boundary, and should be further investigated and expanded if necessary, to better protect the entirety of this population. Conservatively, it is postulated that the *Endangered* North Head population is likely to include all bandicoots on the headland side of the Manly shopping strip (Fig 2). In addition, the *Endangered* population is currently managed by two entities, therefore a continued holistic and coordinated management approach throughout greater northern Sydney is recommended to benefit the entire *Endangered* North Head long-nosed bandicoot population into the future.

## References

- Anson, J., 2015. Long-nosed bandicoot (*Perameles nasuta*) monitoring, North Head Sanctuary, Manly. Australian Wildlife Conservancy, Sydney.
- Allendorf, F.W., 1983. Isolation, gene flow and genetic differentiation among populations. Genetics and Conservation: A Reference for Managing Wild Animal and Plant Populations. (eds. C.M., Schonewald-Cox, S.M., Chambers, B., MacBryde, W.L., Thomas), 51–65. Menlo Park, California.
- Backus, V.L., Bryant, E.H., Hughes, C.R., Meffert, L.M., 1995. Effect of migration or inbreeding followed by selection on low-founder-number populations: Implications for captive breeding programs. Conservation Biology 9, 1216–1224.
- Banks, P.B., 2004. Population viability analysis in urban wildlife management: Modelling management options for Sydney's quarantined bandicoots. Urban Wildlife: More Than Meets the Eye. (eds. D., Lunney and S., Burgin), 70–77. Royal Zoological Society of New South Wales: Sydney, Australia.
- Chambers, L.K., Dickman, C.R., 2002. Habitat selection of the long-nosed bandicoot, *Perameles nasuta* (mammalia, peramelidae), in a patchy urban environment. Austral Ecology 27, 334–342.
- Dennison, S., Frankham, G., Neaves, L., Flanagan, C., FitzGibbon, S., Eldridge, M., Johnson, R., 2017. Population genetics of the koala (*Phascolarctos cinereus*) in north-eastern New South Wales and south-eastern Queensland. Australian Journal of Zoology 64, 402–412.
- Dexter, N., Hudson, M., Carter, T., Macgregor, C., 2011. Habitat-dependent population regulation in an irrupting population of long-nosed bandicoots (*Perameles nasuta*). Austral Ecology 36, 745–754.
- Dowle, M., 2012. A comparison of two species of bandicoots (*Perameles nasuta* & *Isoodon obesulus*) influenced by urbanisation: Population characteristics, genetic diversity, public perceptions, stress and parasites. Macquarie University, Sydney, New South Wales.
- Dowle, M., Deane, E.M., 2009. Attitudes to native bandicoots in an urban environment. European Journal of Wildlife Research 55, 45–52.
- Dowle, M., Webster, K.N., Deane, E., 2013. Faecal glucocorticoid metabolite concentrations in the free-ranging bandicoots (*Perameles nasuta* and *Isoodon obesulus*) of northern Sydney. Australian Mammalogy 35, 1–7.
- Earl, D.A., Von Holdt, B., 2012. STRUCTURE HARVESTER: A website and program for visualizing structure output and implementing the evanno method. Conservation Genetics Resources 4, 359–361.
- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14, 2611–2620.
- Ewart, K.M., Johnson, R.N., Ogden, R., Joseph, L., Frankham, G.J., Lo, N., 2019. Museum specimens provide reliable SNP data for population genomic analysis of a widely distributed but threatened cockatoo species. Molecular Ecology Resources 19, 1381–1688.
- Fischer, J., Lindenmayer, D.B., 2007. Landscape modification and habitat fragmentation: A synthesis. Global Ecology and Biogeography 16, 265–280.
- Frankham, G.J., Handasyde, K.A., Norton, M., Murray, A., Eldridge, M.D., 2014. Molecular detection of intra-population structure in a threatened potoroid, *Potorous tridactylus*:

- Conservation management and sampling implications. *Conservation Genetics* 15, 547–560.
- Frankham, R., 2015. Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology* 24, 2610–2618.
- Frankham, R., 2010. Where are we in conservation genetics and where do we need to go? *Conservation Genetics* 11, 661–663.
- Frankham, R., 1997. Do island populations have less genetic variation than mainland populations? *Heredity* 78, 311–327.
- Fuentes-Pardo, A.P., Ruzzante, D.E., 2017. Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations. *Molecular Ecology* 26, 5369–5406.
- Goossens, B., Sharma, R., Othman, N., Kun-Rodrigues, C., Sakong, R., Ancrenaz, M., Ambu, L.N., Jue, N.K., O'Neill, R.J., Bruford, M.W., 2016. Habitat fragmentation and genetic diversity in natural populations of the Bornean elephant: Implications for conservation. *Biological Conservation* 196, 80–92.
- Goudet, J., 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* 5, 184–186.
- Gruber, B., Unmack, P.J., Berry, O.F., Georges, A., 2018. DARTR: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* 18, 691–699.
- Janes, J.K., Miller, J.M., Dupuis, J.R., Malenfant, R.M., Gorrell, J.C., Cullingham, C.I., Andrew, R.L., 2017. The  $K = 2$  conundrum. *Molecular Ecology* 26, 3594–3602.
- Jombart, T., 2008. ADEGENET: A r package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405.
- Jombart, T., Devillard, S., Balloux, F. 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics* 11, 94.
- Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., Prodöhl, P.A., 2013. DIVERSITY An r package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4, 782–788.
- Keyghobadi, N., 2007. The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology* 85, 1049–1064.
- Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Uszynski, G., 2012. Diversity arrays technology: A generic genome profiling technology on open platforms. *Methods Mol Biol* 888, 67–89.
- Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across  $K$ . *Molecular Ecology Resources* 15, 1179–1191.
- Lacy, R.C., 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology* 1, 143–158.
- Lancaster, M.L., Taylor, A.C., Cooper, S.J., Carthew, S.M., 2011. Limited ecological connectivity of an arboreal marsupial across a forest/plantation landscape despite apparent resilience to fragmentation. *Molecular Ecology* 20, 2258–2271.
- Lande R., Barrowclough G.F., 1987. Effective population size, genetic variation, and their use in population management: Viable Populations for Conservation. (eds. M.E., Soule), 87–123. Cambridge University Press, Cambridge, England.

- Lott, M., Wright, B., Kemp, L., Johnson, R., Hogg, C., 2020. Genetic Management of Captive and Reintroduced Bilby Populations. *The Journal of Wildlife Management* 84, 20–32.
- Luikart, G., England, P.R., Tallmon, D., Jordan, S., Taberlet, P., 2003. The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics* 4, 981–994.
- Meirmans, P.G., 2015. Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology* 24, 3223–3231.
- Melville, J., Haines, M.L., Boysen, K., Hodkinson, L., Kilian, A., Smith Date, K.L., Potvin, D.A., Parris, K.M., 2017. Identifying hybridization and admixture using SNPs: Application of the DArTseq platform in phylogeographic research on vertebrates. *Royal Society Open Science* 4, 161061.
- Menges, E.S., 1991. The application of minimum viable population theory to plants. *Genetics and Conservation of Rare Plants* 45, 158–164.
- Moyle, D., Hume, I., Hill, D., 1995. Digestive performance and selective digesta retention in the long-nosed bandicoot, *Perameles nasuta*, a small omnivorous marsupial. *Journal of Comparative Physiology B* 164, 552–560.
- New South Wales, Department of Primary Industries, 2020. Drought in NSW <https://www.dpi.nsw.gov.au/climate-and-emergencies/drougthub/drought-in-nsw>
- New South Wales, Threatened Species Scientific Committee, 1997. Long-nosed bandicoot (*Perameles nasuta*) population, North Head - endangered population listing <https://www.environment.nsw.gov.au/topics/animals-and-plants/threatened-species/nsw-threatened-species-scientific-committee/determinations/final-determinations/1996-1999/long-nosed-bandicoot-population-north-head-endangered-population-listing>
- Novembre, J., Stephens, M., 2008. Interpreting principal component analyses of spatial population genetic variation. *Nature Genetics* 40, 646.
- Paradis, E., 2010. PEGAS: An r package for population genetics with an integrated–modular approach. *Bioinformatics* 26, 419–420.
- Pembleton, L.W., Cogan, N.O., Forster, J.W., 2013. STAMPP: An r package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources* 13, 946–952.
- Piggott, M., Banks, S.C., MacGregor, C., Lindenmayer, D.B., 2018. Population genetic patterns in an irruptive species, the long-nosed bandicoot (*Perameles nasuta*). *Conservation Genetics* 19, 655–663.
- Piggott, M., Banks, S.C., Taylor, A.C., 2006. Population structure of brush-tailed rock-wallaby (*Petrogale penicillata*) colonies inferred from analysis of faecal DNA. *Molecular Ecology* 15, 93–105.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data *Genetics* 155, 945–959.
- RStudio Team, 2015. RStudio: Integrated Development for R. RStudio, Inc. Boston, MA. <http://www.rstudio.com/>.
- Ralls, K., Ballou, J.D., Dudash, M.R., Eldridge, M.D., Fenster, C.B., Lacy, R.C., Sunnucks, P., Frankham, R., 2018. Call for a paradigm shift in the genetic management of fragmented populations. *Conservation Letters* 11, e12412.
- Rosenberg, N.A., 2004. DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes* 4, 137–138.
- Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., Hanski, I., 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392, 491–494.

- Saunders, D.A., Hobbs, R.J., Margules, C.R., 1991. Biological consequences of ecosystem fragmentation: A review. *Conservation Biology* 5, 18–32.
- Scott, L., Hume, I., Dickman, C., 1999. Ecology and population biology of long-nosed bandicoots (*Perameles nasuta*) at North Head, Sydney Harbour National Park. *Wildlife Research* 26, 805–821.
- Short, J., 2016. Predation by feral cats key to the failure of a long-term reintroduction of the western barred bandicoot (*Perameles bougainville*). *Wildlife Research* 43, 38–50.
- Spielman, D., Brook, B.W., Briscoe, D.A., Frankham, R., 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics* 5, 439–448.
- Sunnucks, P., Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase i-ii in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* 13, 510–524.
- Tea, Y.K., Van Der Wal, C., Ludt, W.B., Gill, A.C., Lo, N., Ho, S.Y., 2019. Boomeranging around Australia: Historical biogeography and population genomics of the anti-equatorial fish *Microcanthus strigatus* (Teleostei: Microcanthidae). *Molecular Ecology* 28, 3771–3785.
- Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358–1370.
- Winnard, A.L., Coulson, G., 2008. Sixteen years of eastern barred bandicoot *Perameles gunnii* reintroductions in Victoria: A review. *Pacific Conservation Biology* 14, 34–53.

## Figure and Table Legends

**Fig 1:** Collection localities across Sydney, Australia, for genotyped long-nosed bandicoots (*Perameles nasuta*) examined in this study including an image of a long-nosed bandicoot trapped on North Head. In this study ‘Northern Sydney’ refers to all sample localities north of Sydney Harbour.

**Fig 2:** Map of the suburb of Manly, including North Head Headland. The designated boundary of the long-nosed *Endangered* North Head population is outlined in pink, and the area currently managed and surveyed by the AWC, NPWS and SHFT is highlighted in blue.

**Fig 3:** (A) Principal coordinates analysis (PCoA) of 167 long-nosed bandicoots genotyped for 1,446 SNPs generated using DArTSeq. (B) Principal coordinates analysis (PCoA) of long-nosed bandicoots from North Head (North Head) (n = 132) and northern Sydney (northern Sydney) (n = 23) genotyped using 1,200 SNPs generated using DArTSeq.

**Fig 4:** Bayesian clustering STRUCTURE plots depicting individual assignment probabilities for genotyped long-nosed bandicoots. (A) Individuals from all sampled populations when  $K = 2$ ; (B) Individuals from all sampled populations when  $K = 3$ ; (C) northern Sydney (NS) and North Head (NH) populations when  $K = 2$ . Each column represents an individual and colours represent the relative probability of assignment into a cluster ( $K$ ). Population labels represent North Head (NH), Manly (MN), Ku-ring-gai Chase National Park (KRG), Newport (NP), Ingleside (IS), Turramurra (TM), Elanora Heights (EH), Inner West (IW), Oxford Falls (OF), Collaroy (CR) and Mosman (MM).

**Fig 5:** DAPC analysis yielding a best fit of  $K = 3$ . Each dot represents an individual long-nosed bandicoot genotype and each colour and circle depicts a genetic cluster. Eigenvalue plots show the number of PCAs retained and amount of genetic variation contained in each discriminant factor.

**Table 1:** Genetic differentiation ( $F_{ST}$ ) among the three inferred long-nosed bandicoot populations based on 1,446 SNPs.

**Table 2:** Genetic diversity indices for the North Head (North Head), Mosman (Mosman) and Ku-ring-gai Chase National Park (Ku-ring-Gai) long-nosed bandicoot populations. The number of individuals ( $N$ ), mean allelic richness ( $R_A$ ), mean observed heterozygosity, ( $H_O$ ) mean expected heterozygosity ( $H_E$ ), and mean inbreeding coefficients ( $F_{IS}$ ) are shown.



**Table 1**

	northern Sydney	North Head	Mosman
northern Sydney	-		
North Head	0.171*	-	
Mosman	0.232*	0.345*	-

Significant values are indicated \*

**Table 2**

Population	Year	<i>N</i>	<i>R<sub>A</sub></i>	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>
North Head	2002	6	1.90	0.296*	0.340	0.052
North Head	2006	18	1.94	0.231*	0.338	0.049
North Head	2010	47	2.00	0.310	0.346	0.050
North Head	2014	43	1.99	0.305*	0.340	0.020
North Head	2018	46	1.99	0.300	0.338	0.050
Mosman	2018	11	1.97	0.334*	0.366	-0.011
Ku-ring-gai Chase National Park	2001-19	9	1.92	0.267*	0.327	0.015

Significant differences between *H<sub>O</sub>* and *H<sub>E</sub>* are indicated \*

## Supplementary Material

**Fig S1:** Delta  $K$  graph from the STRUCTURE HARVESTER analysis to determine the optimal number of population clusters among all long-nosed bandicoot samples ( $n = 167$ ) sourced from Sydney between 2001 and 2019.

**Fig S2:** Delta  $K$  plot from the STRUCTURE HARVESTER analysis to determine the optimal number of population clusters among North Head and northern Sydney long-nosed bandicoot samples ( $n = 155$ ) sourced from Sydney between 2001 and 2019.

**Table S1.** Information for all successfully genotyped long-nosed bandicoot samples collected in Sydney, Australia from 2001 to 2019. Collectors include the Australian Museum (AM), Taronga Zoo (TZ), NSW National Parks and Wildlife Service (NPWS), & Australian Wildlife Conservancy (AWC).

**Table S1**

<b>Specimen Identifier</b>	<b>Location</b>	<b>Collector</b>	<b>Collection year</b>
M.42738	Collaroy	AM	2008
M.47931	Elanora Heights	AM	2014
M.43651	Elanora Heights	AM	2011
M.43652	Elanora Heights	AM	2011
M.43653	Elanora Heights	AM	2011
M.35482	Elanora Heights	AM	2001
M.45895	Ingleside	AM	2011
M.42779	Inner West (Petersham)	AM	2007
M.42781	Inner West (Dulwich Hill)	AM	2006
M.51042	Ku-ring-gai Chase National Park	AM	2019
M.47412	Ku-ring-gai Chase National Park	AM	2013
M.45591	Ku-ring-gai Chase National Park	AM	2012
M.46992	Ku-ring-gai Chase National Park	AM	2010
M.46996	Ku-ring-gai Chase National Park	AM	2010
M.47012	Ku-ring-gai Chase National Park	AM	2010
M.47000	Ku-ring-gai Chase National Park	AM	2009
M.47005	Ku-ring-gai Chase National Park	AM	2005
M.46302	Ku-ring-gai Chase National Park	AM	2001
M.46303	Ku-ring-gai Chase National Park	AM	2001
M.46304	Ku-ring-gai Chase National Park	AM	2001
M.46305	Ku-ring-gai Chase National Park	AM	2001
M.43641	Manly	AM	2008
M.43215	Oxford Falls	AM	2010
M.42739	Turrumurra	AM	2010
M.42789	Turrumurra	AM	2010
M.51043	Newport	AM	2019
M.51693	Mosman	TZ	2018
M.51694	Mosman	TZ	2018
M.51660	Mosman	TZ	2018
M.51681	Mosman	TZ	2018
M.51761	Mosman	TZ	2018
M.51614	Mosman	TZ	2018
M.51638	Mosman	TZ	2018
M.51642	Mosman	TZ	2018
M.51721	Mosman	TZ	2018
M.51649	Mosman	TZ	2018
M.51670	Mosman	TZ	2018
M.51752	Mosman	TZ	2018
M.51734	Mosman	TZ	2018

M.51661	North Head	NPWS & AWC	2018
M.51891	North Head	NPWS & AWC	2018
M.51639	North Head	NPWS & AWC	2018
M.51858	North Head	NPWS & AWC	2018
M.51848	North Head	NPWS & AWC	2018
M.51731	North Head	NPWS & AWC	2018
M.51763	North Head	NPWS & AWC	2018
M.51632	North Head	NPWS & AWC	2018
M.51643	North Head	NPWS & AWC	2018
M.51750	North Head	NPWS & AWC	2018
M.51730	North Head	NPWS & AWC	2018
M.51668	North Head	NPWS & AWC	2018
M.51873	North Head	NPWS & AWC	2018
M.51872	North Head	NPWS & AWC	2018
M.51648	North Head	NPWS & AWC	2018
M.51650	North Head	NPWS & AWC	2018
M.51757	North Head	NPWS & AWC	2018
M.51682	North Head	NPWS & AWC	2018
M.51849	North Head	NPWS & AWC	2018
M.51667	North Head	NPWS & AWC	2018
M.51677	North Head	NPWS & AWC	2018
M.51745	North Head	NPWS & AWC	2018
M.51652	North Head	NPWS & AWC	2018
M.51666	North Head	NPWS & AWC	2018
M.51867	North Head	NPWS & AWC	2018
M.51654	North Head	NPWS & AWC	2018
M.51823	North Head	NPWS & AWC	2018
M.51728	North Head	NPWS & AWC	2018
M.51727	North Head	NPWS & AWC	2018
M.51743	North Head	NPWS & AWC	2018
M.51700	North Head	NPWS & AWC	2018
M.51726	North Head	NPWS & AWC	2018
M.51711	North Head	NPWS & AWC	2018
M.51883	North Head	NPWS & AWC	2018
M.51824	North Head	NPWS & AWC	2018
M.51832	North Head	NPWS & AWC	2018
M.51765	North Head	NPWS & AWC	2018
M.51709	North Head	NPWS & AWC	2018
M.51755	North Head	NPWS & AWC	2018
M.51857	North Head	NPWS & AWC	2018
M.51618	North Head	NPWS & AWC	2018
M.51874	North Head	NPWS & AWC	2018

M.51890	North Head	NPWS & AWC	2018
M.51621	North Head	NPWS & AWC	2018
M.51762	North Head	NPWS & AWC	2018
M.51749	North Head	NPWS & AWC	2018
M.51631	North Head	NPWS & AWC	2018
M.51779	North Head	NPWS & AWC	2018
M.51740	North Head	NPWS & AWC	2018
M.51669	North Head	NPWS & AWC	2018
M.51841	North Head	NPWS & AWC	2018
M.51619	North Head	NPWS & AWC	2018
M.51845	North Head	NPWS & AWC	2014
M.51616	North Head	NPWS & AWC	2014
M.51662	North Head	NPWS & AWC	2014
M.51871	North Head	NPWS & AWC	2014
M.51657	North Head	NPWS & AWC	2014
M.51692	North Head	NPWS & AWC	2014
M.51646	North Head	NPWS & AWC	2014
M.51863	North Head	NPWS & AWC	2014
M.51680	North Head	NPWS & AWC	2014
M.51691	North Head	NPWS & AWC	2014
M.51637	North Head	NPWS & AWC	2014
M.51634	North Head	NPWS & AWC	2014
M.51672	North Head	NPWS & AWC	2014
M.51647	North Head	NPWS & AWC	2014
M.51674	North Head	NPWS & AWC	2014
M.51655	North Head	NPWS & AWC	2014
M.51685	North Head	NPWS & AWC	2014
M.51640	North Head	NPWS & AWC	2014
M.51862	North Head	NPWS & AWC	2014
M.51561	North Head	NPWS & AWC	2014
M.51689	North Head	NPWS & AWC	2014
M.51656	North Head	NPWS & AWC	2014
M.51742	North Head	NPWS & AWC	2014
M.51820	North Head	NPWS & AWC	2014
M.51887	North Head	NPWS & AWC	2014
M.51664	North Head	NPWS & AWC	2014
M.51636	North Head	NPWS & AWC	2014
M.51635	North Head	NPWS & AWC	2014
M.51659	North Head	NPWS & AWC	2014
M.51665	North Head	NPWS & AWC	2014
M.51821	North Head	NPWS & AWC	2014
M.51829	North Head	NPWS & AWC	2014

M.51880	North Head	NPWS & AWC	2014
M.51663	North Head	NPWS & AWC	2014
M.51645	North Head	NPWS & AWC	2014
M.51671	North Head	NPWS & AWC	2014
M.51651	North Head	NPWS & AWC	2014
M.51658	North Head	NPWS & AWC	2014
M.51568	North Head	NPWS & AWC	2014
M.51613	North Head	NPWS & AWC	2014
M.51675	North Head	NPWS & AWC	2014
M.51701	North Head	NPWS & AWC	2010
M.51624	North Head	NPWS & AWC	2010
M.51715	North Head	NPWS & AWC	2010
M.51747	North Head	NPWS & AWC	2010
M.51705	North Head	NPWS & AWC	2010
M.51738	North Head	NPWS & AWC	2010
M.51676	North Head	NPWS & AWC	2010
M.51833	North Head	NPWS & AWC	2010
M.51723	North Head	NPWS & AWC	2010
M.51615	North Head	NPWS & AWC	2010
M.51710	North Head	NPWS & AWC	2010
M.51725	North Head	NPWS & AWC	2010
M.51852	North Head	NPWS & AWC	2010
M.51878	North Head	NPWS & AWC	2010
M.51633	North Head	NPWS & AWC	2010
M.51744	North Head	NPWS & AWC	2010
M.51686	North Head	NPWS & AWC	2010
M.51716	North Head	NPWS & AWC	2010
M.51684	North Head	NPWS & AWC	2010
M.51718	North Head	NPWS & AWC	2010
M.51758	North Head	NPWS & AWC	2010
M.51622	North Head	NPWS & AWC	2010
M.51828	North Head	NPWS & AWC	2010
M.51836	North Head	NPWS & AWC	2010
M.51837	North Head	NPWS & AWC	2010
M.51885	North Head	NPWS & AWC	2010
M.51830	North Head	NPWS & AWC	2010
M.51888	North Head	NPWS & AWC	2010
M.51840	North Head	NPWS & AWC	2010
M.51822	North Head	NPWS & AWC	2010
M.51846	North Head	NPWS & AWC	2010
M.51864	North Head	NPWS & AWC	2010
M.51847	North Head	NPWS & AWC	2010

M.51870	North Head	NPWS & AWC	2010
M.51865	North Head	NPWS & AWC	2010
M.51678	North Head	NPWS & AWC	2010
M.51688	North Head	NPWS & AWC	2010
M.51737	North Head	NPWS & AWC	2010
M.51683	North Head	NPWS & AWC	2010
M.51707	North Head	NPWS & AWC	2010
M.51623	North Head	NPWS & AWC	2010
M.51708	North Head	NPWS & AWC	2010
M.51818	North Head	NPWS & AWC	2010
M.51886	North Head	NPWS & AWC	2010
M.51826	North Head	NPWS & AWC	2010
M.51827	North Head	NPWS & AWC	2010
M.51699	North Head	NPWS & AWC	2010
M.51856	North Head	NPWS & AWC	2010
M.51842	North Head	NPWS & AWC	2010
M.51816	North Head	NPWS & AWC	2010
M.51825	North Head	NPWS & AWC	2010
M.51875	North Head	NPWS & AWC	2010
M.51817	North Head	NPWS & AWC	2010
M.51819	North Head	NPWS & AWC	2010
M.51851	North Head	NPWS & AWC	2010
M.51703	North Head	NPWS & AWC	2010
M.51850	North Head	NPWS & AWC	2010
M.51831	North Head	NPWS & AWC	2010
M.51876	North Head	NPWS & AWC	2010
M.51835	North Head	NPWS & AWC	2010
M.51834	North Head	NPWS & AWC	2010
M.51861	North Head	NPWS & AWC	2010
M.51868	North Head	NPWS & AWC	2010
M.51859	North Head	NPWS & AWC	2010
M.51889	North Head	NPWS & AWC	2006
M.51625	North Head	NPWS & AWC	2006
M.51751	North Head	NPWS & AWC	2006
M.51629	North Head	NPWS & AWC	2006
M.51753	North Head	NPWS & AWC	2006
M.51714	North Head	NPWS & AWC	2006
M.51410	North Head	NPWS & AWC	2006
M.51746	North Head	NPWS & AWC	2006
M.51717	North Head	NPWS & AWC	2006
M.51764	North Head	NPWS & AWC	2006
M.51687	North Head	NPWS & AWC	2006



M.51754	North Head	NPWS & AWC	2006
M.51401	North Head	NPWS & AWC	2006
M.51713	North Head	NPWS & AWC	2006
M.51733	North Head	NPWS & AWC	2006
M.51704	North Head	NPWS & AWC	2006
M.51724	North Head	NPWS & AWC	2006
M.51732	North Head	NPWS & AWC	2006
M.51653	North Head	NPWS & AWC	2006
M.51719	North Head	NPWS & AWC	2006
M.51617	North Head	NPWS & AWC	2006
M.51729	North Head	NPWS & AWC	2006
M.51423	North Head	NPWS & AWC	2006
M.51839	North Head	NPWS & AWC	2006
M.51408	North Head	NPWS & AWC	2006
M.51641	North Head	NPWS & AWC	2006
M.51741	North Head	NPWS & AWC	2002
M.51644	North Head	NPWS & AWC	2002
M.51760	North Head	NPWS & AWC	2002
M.51690	North Head	NPWS & AWC	2002
M.51739	North Head	NPWS & AWC	2002
M.51720	North Head	NPWS & AWC	2002
M.51722	North Head	NPWS & AWC	2002