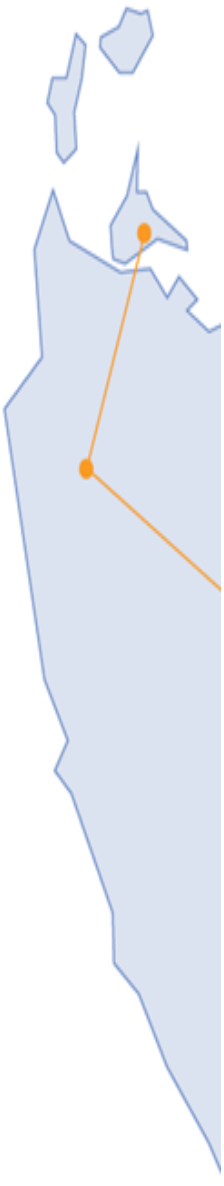
UPC AC Renewables AUSTRALIA

Jim's Plain & Robbins Island
Renewable Energy Parks



Robbins Island Renewable Energy Park

Appendix D

Tasmanian Devil Survey

UPC Robbins Island Pty Ltd

Robbins Island Tasmanian Devil Survey
17–27 May 2018

Final Report
7 October 2018

Prepared for

GHD Pty Ltd

Prepared by

The Carnivore Conservancy Ltd

23 King Edward Street
Ulverstone, Tasmania 7315
Channing Hughes
Executive Director
channing@carnivores.co
0468 328 262

THE CARNIVORE CONSERVANCY

INTRODUCTION

At the request of GHD Pty Ltd, The Carnivore Conservancy (TCC) conducted an 11-day research expedition to Robbins Island, off the north-western coast of Tasmania, to survey the population of the endangered Tasmanian devil *(Sarcophilus harrisii)*. The trip took place from 17 to 27 May 2018.

The goals of the expedition were to determine the distribution and relative density of Tasmanian devils on the island, collect DNA samples for genotype analysis, and examine captured animals for any visible signs of Devil Facial Tumour Disease (DFTD), a contagious cancer that has caused dramatic declines in devil populations across much of the Tasmanian mainland.

TRAP SITES

A total of 40 trap sites (labelled 01 to 40) were initially identified to achieve as comprehensive a coverage of the entire island as possible, within the limitation that each trap site needed to be accessible by vehicle. An additional 5 traps sites (labelled 41 to 45) were subsequently identified for use during the latter part of the trip, to make up for incomplete trap deployment early in the trip. (Logistical difficulties on the initial day of trap-setting, including the breakage of two traps, meant that only 34 traps were deployed on night 1, 38 traps on nights 2–4, and [after successful repair of one of the broken traps] 39 traps on nights 5–7. An additional 6 traps were brought from the Tasmanian mainland on day 8, allowing the deployment of 45 traps on nights 8–10, thus bringing the total overall number of trap-nights for the trip to the agreed-upon 400.)

Figure 1 shows the location of all 45 traps sites utilised during the survey. Table 1 provides the coordinates (in both latitude/longitude and easting/northing) for each trap site as well as the number of trap-nights each trap site was utilised.

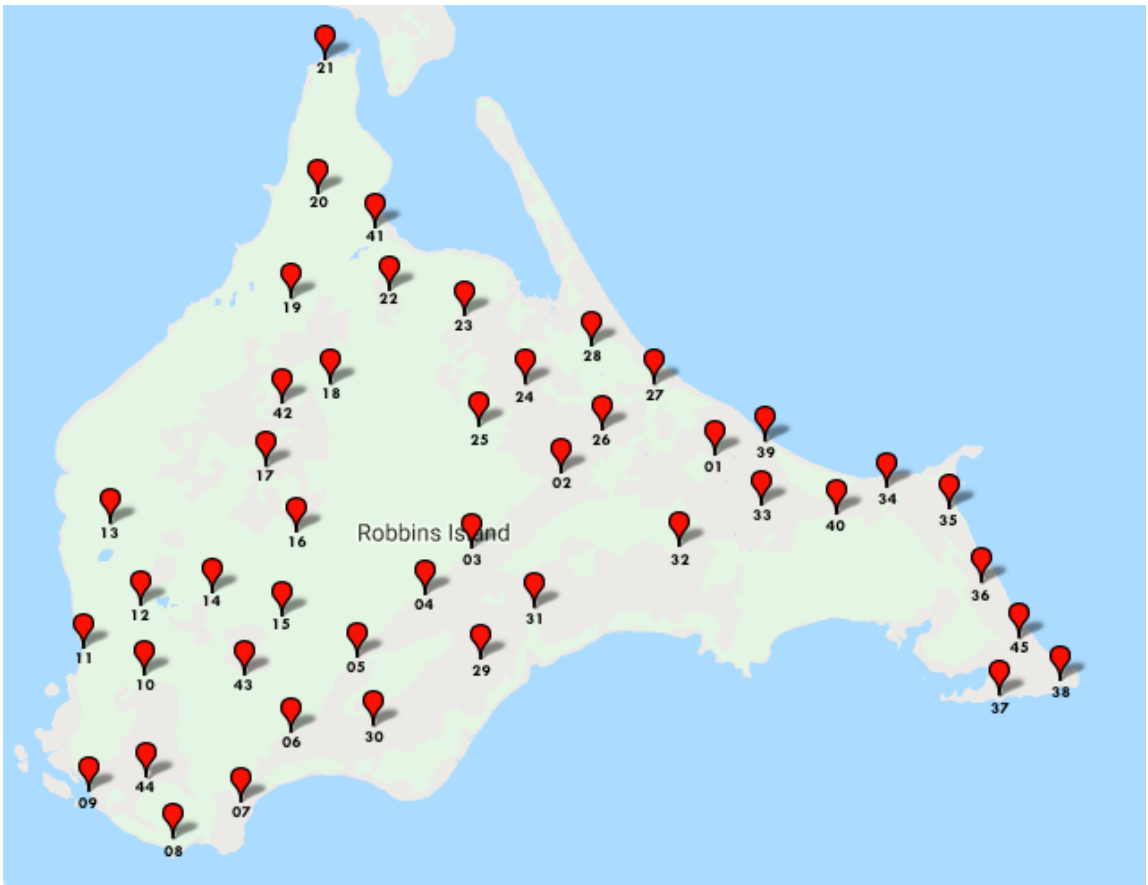


Figure 1. Location of all trap sites

Trap	Latitude	Longitude	Easting	Northing	Start Night	End Night	Total Nights
01	-40° 40' 54.0"	144° 59' 51.5"	330786	5494652	1	10	10
02	-40° 41' 02.9"	144° 58' 05.5"	328303	5494321	1	10	10
03	-40° 41' 43.2"	144° 57' 04.4"	326898	5493044	1	10	10
04	-40° 42' 06.6"	144° 56' 31.5"	326143	5492305	1	10	10
05	-40° 42' 40.2"	144° 55' 44.5"	325064	5491243	1	10	10
06	-40° 43' 18.8"	144° 54' 59.4"	324034	5490027	1	10	10
07	-40° 43' 56.2"	144° 54' 25.1"	323257	5488855	1	10	10
08	-40° 44' 15.3"	144° 53' 37.6"	322157	5488239	1	10	10
09	-40° 43' 49.5"	144° 52' 40.0"	320786	5489002	1	10	10
10	-40° 42' 49.1"	144° 53' 18.0"	321633	5490886	1	10	10
11	-40° 42' 35.0"	144° 52' 36.4"	320646	5491298	1	10	10
12	-40° 42' 12.7"	144° 53' 16.1"	321562	5492008	1	10	10
13	-40° 41' 30.1"	144° 52' 55.3"	321042	5493310	1	10	10
14	-40° 42' 06.3"	144° 54' 04.9"	322702	5492233	1	10	10
15	-40° 42' 17.9"	144° 54' 53.1"	323842	5491902	1	10	10
16	-40° 41' 34.3"	144° 55' 02.8"	324037	5493252	1	10	10
17	-40° 40' 59.9"	144° 54' 41.6"	323515	5494301	1	10	10
18	-40° 40' 17.1"	144° 55' 26.5"	324538	5495646	1	10	10
19	-40° 39' 31.8"	144° 54' 58.6"	323849	5497027	1	10	10
20	-40° 38' 36.8"	144° 55' 17.7"	324258	5498734	1	10	10
21	-40° 37' 26.8"	144° 55' 23.1"	324334	5500895	1	10	10
22	-40° 39' 28.0"	144° 56' 07.6"	325467	5497182	1	10	10
23	-40° 39' 40.8"	144° 56' 59.2"	326688	5496816	1	10	10
24	-40° 40' 17.1"	144° 57' 41.4"	327705	5495720	1	10	10
25	-40° 40' 38.9"	144° 57' 08.7"	326953	5495030	1	10	10
26	-40° 40' 41.4"	144° 58' 34.0"	328957	5494999	1	10	10
27	-40° 40' 17.0"	144° 59' 09.9"	329783	5495771	1	10	10
28	-40° 39' 57.0"	144° 58' 26.5"	328750	5496364	1	10	10
29	-40° 42' 41.3"	144° 57' 10.5"	327083	5491256	1	10	10
30	-40° 43' 15.8"	144° 55' 56.1"	325362	5490151	1	10	10
31	-40° 42' 14.1"	144° 57' 47.7"	327936	5492115	1	10	10
32	-40° 41' 42.2"	144° 59' 27.8"	330263	5493153	1	10	10
33	-40° 41' 19.9"	145° 00' 25.0"	331590	5493871	1	10	10
34	-40° 41' 10.7"	145° 01' 51.6"	333616	5494201	1	10	10
35	-40° 41' 22.3"	145° 02' 33.5"	334608	5493865	2	10	9
36	-40° 42' 01.0"	145° 02' 57.0"	335186	5492684	2	10	9
37	-40° 43' 00.1"	145° 03' 08.9"	335506	5490868	2	10	9
38	-40° 42' 51.6"	145° 03' 51.4"	336497	5491152	2	10	9
39	-40° 40' 47.3"	145° 00' 26.7"	331607	5494878	5	10	6
40	-40° 41' 24.7"	145° 01' 16.0"	332791	5493750	8	10	3
41	-40° 38' 54.7"	144° 55' 57.6"	325208	5498204	8	10	3
42	-40° 40' 26.9"	144° 54' 53.0"	323758	5495325	8	10	3
43	-40° 42' 49.0"	144° 54' 27.3"	323259	5490928	8	10	3
44	-40° 43' 42.6"	144° 53' 20.0"	321720	5489238	8	10	3
45	-40° 42' 29.9"	145° 03' 22.3"	335800	5491806	8	10	3
					Total		**400**

Table 1. Coordinates and number of trap-nights for each trap site

TRAPPING PROTOCOL

Devils were captured using purpose-built devil traps constructed of lengths of PVC piping 90 cm long and 30 cm in diameter. Each trap was baited with approximately 300 g of fresh meat — either Tasmanian pademelon *(Thylogale billardierei)* or red-necked wallaby *(Macropus rufogriseus)*. Traps were positioned in shaded locations and stabilised with a "cradle" fashioned of small rocks or chunks of wood, to prevent trapped animals from being able to shift the trap from its position.

Each trap was checked daily for the presence of an animal, and all trapped animals were processed and released at the trap site. Trap-checking began at dawn and continued until all traps had been checked and all trapped animals processed. In preparation for the subsequent night's trapping, all visited traps were cleaned, disinfected and rebaited; unvisited traps were left as is.

ANIMAL PROCESSING PROTOCOL

Upon initial capture, each animal underwent the following processing steps:

- transfer from the trap into a hessian sack
- scanning for an existing microchip
- recording of gross weight of animal in hessian sack
- subcutaneous insertion of a microchip in the loose skin between the scapulae (unless the animal had previously been microchipped)
- thorough examination to identify visible or palpable lumps or lesions that might be indicative of Devil Facial Tumour Disease (DFTD)
- determination of birth year, based primarily on an assessment of the animal's dentition
- determination of sex
- (for females): determination of reproductive status based on an assessment of pouch condition
- (for females with dependent young present in the pouch): tallying the number of pouch young present
- recording of the following morphometric values:
    - head width
    - tail diameter
    - canine length (tip to gum-line)
    - canine length (tip to dentine/enamel-junction)
    - (for males): length, width and depth of left testis
- collection of a small tissue sample from the right pinna (external ear flap) for genotyping (DNA analysis)
- collection of blood sample
- collection of whisker sample
- collection of a faecal sample from the trap, if present
- assessment of ectoparasite load and collection of ectoparasite samples
- assessing the degree of anterior and posterior wounds and scarring
- assigning an overall condition score between 1 and 5
- characterising the animal's overall demeanour during handling
- noting any agonistic or stress-related behaviours that occurred during handing
- photographing, measuring and characterising the degree of asymmetry of the white blazes (if any) on chest, shoulders/sides and rump
- taking photographs or video recordings of the animal on release
- recording of hessian sack weight and calculation of net mass

Animals recaptured subsequently during the trip were weighed and released without further processing.

NUMBER OF CAPTURES

Tasmanian devils

In 400 trap-nights, a total of 191 Tasmanian devil captures were recorded. That number included one individual previously captured (at TCC's nearby mainland Tasmanian study site in June 2017) and either 108 or 109 individuals never previously captured. (One animal was a suspected trip recapture; it had what appeared to be a very recent ear biopsy, consistent with the DNA sample collection technique, but no detectable microchip. This animal was [re?-]microchipped and an [-other?] ear biopsy was collected. Genotyping will hopefully determine whether this animal was a distinct individual or a trip recapture.) The remaining 81 captures were trip recaptures.

The demographic breakdown of the captured animals is shown in Table 2.

Birth year	Male	Female	Total
2012	3	1	4
2013	10	3	13
2014	7	8*	15*
2015	17	18	35
2016	2	10	12
2017	14	17	31
Total	**53**	**57***	**110***
** May be overstated by 1 individual, if the suspected trip recapture is confirmed as such.*			

Table 2. Demographic breakdown of captured animals

Spotted-tailed quolls

Despite the presence of spotted-tailed quolls *(Dasyurus maculatus)* in north-western mainland Tasmania (verified through frequent capture of quolls at TCC's nearby Buckby's Road study site), not a single quoll was captured during the Robbins Island survey, suggesting that the species is not present on the island. This comports with anecdotal evidence from Robbins Island's landholders, who report frequent sightings of devils but have never observed a quoll on the island.

RELATIVE POPULATION DENSITY

In order to estimate absolute population density, it would be necessary to establish trap sites that are either regularly distributed or randomly distributed, neither of which was possible given the limitation that trap sites needed to be accessible by vehicle, limiting locations to the existing network of roads and tracks on the island. It is, however, possible to compare capture numbers relative to TCC's eight study sites on mainland Tasmania (shown in Figure 2).

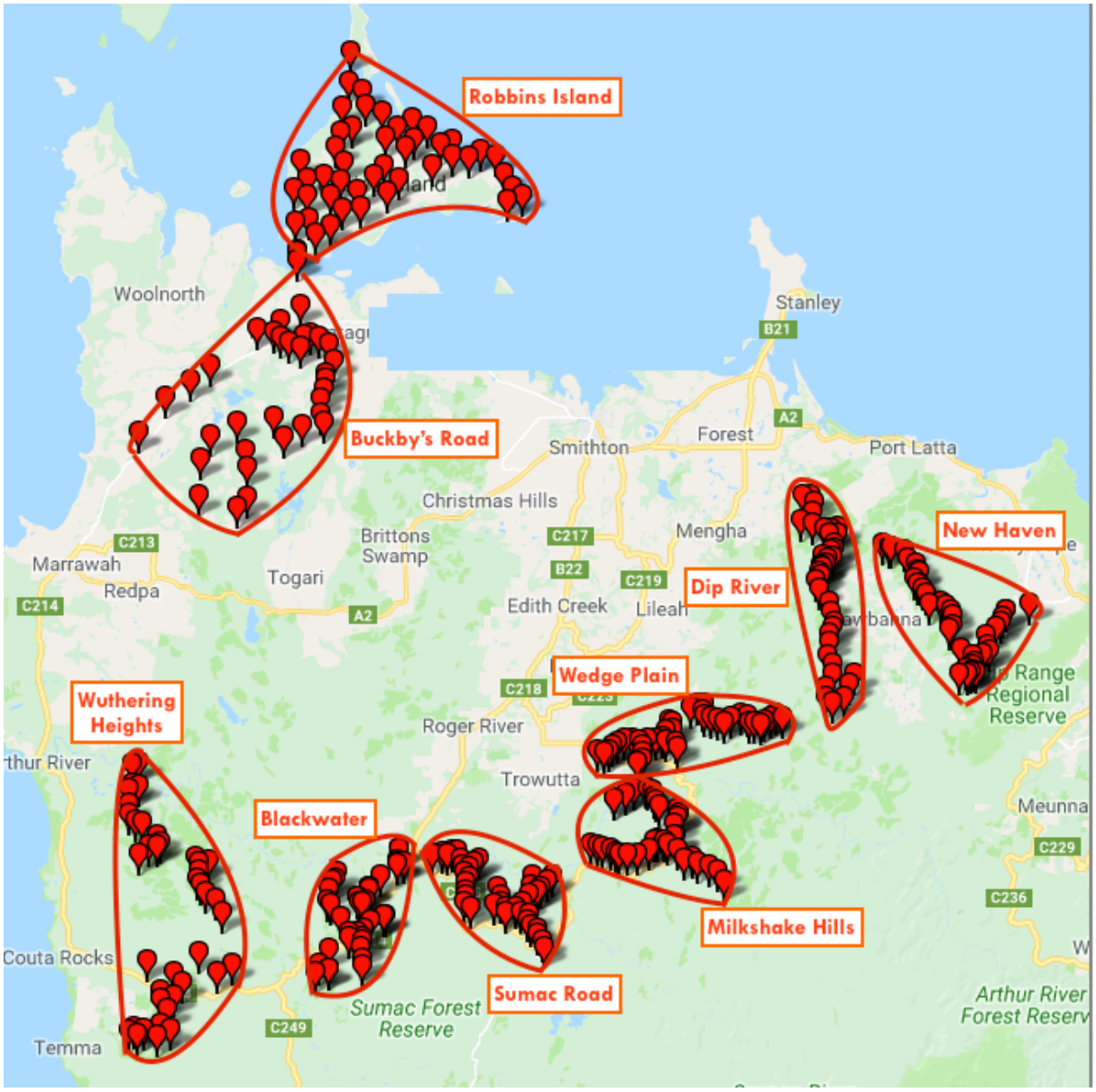


Figure 2. Location of all TCC study sites

Table 3 compares Robbins Island capture numbers with TCC's mainland study sites. Data shown include all surveys conducted since the beginning of 2017. For between-site comparisons to be valid, the following factors must be taken into account:

- TCC's study sites differ in area. To adjust for that fact, the population figure used for comparison is the number of individuals per 10 km^2. (Area of sites is shown to the nearest 10 km^2.)
- TCC's usual surveys run for 7 nights rather than 10 nights. To adjust for that fact, Robbins Island capture data include only individuals captured during the first 7 nights of the survey.
- While the goal of any 7-day survey is to include 280 trap-nights, logistical factors often make it impossible to achieve that goal. For a valid comparison, average number of individuals is adjusted as follows for each site, yielding an average number of individuls per 280 trap-nights:

Average number of individuals / Average number of trap-nights x 280

Site	Area (km^2)	Number of trips since 2017	Average individuals per trip	Average trap-nights per trip	Average individuals per 280 trap-nights	Average individuals per 10 km^2
Wedge Plain	30	3	49.3	257.7	53.6	17.9
Sumac Road	30	3	35.0	263.0	37.3	12.4
Robbins Island*	80	1	92.0*	265.0*	97.2*	12.2
Blackwater	30	2	30.5	273.5	31.2	10.4
Dip River	30	2	27.5	264.0	29.2	9.7
Milkshake Hills	30	3	28.3	272.0	29.2	9.7
Buckby's Road	110	3	99.0	269.3	102.9	9.4
Wuthering Heights	60	2	45.0	256.0	49.2	8.2
New Haven	40	3	25.7	265.7	27.1	6.8
**Figures include only captures from the first 7 nights of the Robbins Island survey.*						

Table 3. Location of all study sites

Although the Robbins Island site ranks third in average number of individuals per 10 km^2, the differences among sites are not statistically significant ($\chi^2 = 7.65$, d.f. = 8, $p = 0.47$).

DISTRIBUTION

Figure 3 shows the trap success rate for each trap site used during the survey.

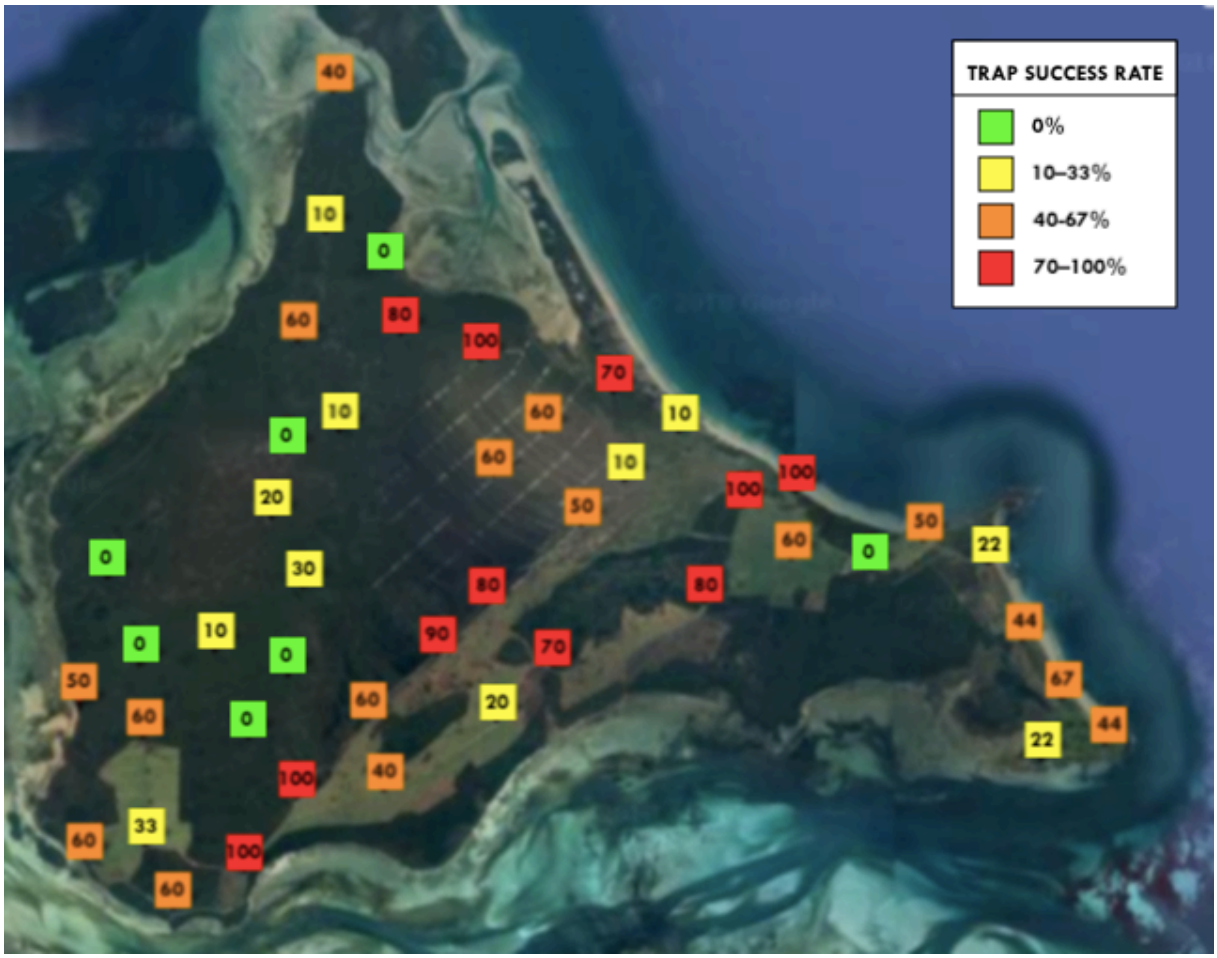


Figure 3. Trap success rate (%) of all deployed traps

Differences in trap success rates were statistically significant ($\chi^2 = 83.16$, d.f. = 44, $p < 0.001$), suggesting that the devil population is not evenly distributed across Robbins Island. The west-central section of the island (particularly traps 12–18 and 42–43) was less likely than average to yield captures. The central corridor along Main Road and Inland Track (particularly traps 03–07 and 31–33) and the north-central section of the island (particularly traps 01, 22–25, 28 and 39) were more likely than average to yield captures. Other sections had mixed results, with some traps more successful than average and other traps less successful than average.

Highest levels of trap success tended to occur at trap sites along ecotones between scrub and paddock, and lowest levels of trap success tended to occur in areas of scrub more distant from paddock. Given the landholders' herbivore control programme, which entails the shooting macropods (Tasmanian pademelons and red-necked wallabies) primarily in and around paddocks, the observed higher densities in proximity to paddocks is not surprising.

POSSIBLE DFTD CASES

The likelihood that a devil has DFTD is assessed using a 5-point scale, outlined in Table 4.

Score	Description
1	No visible or palpable indications of DFTD
2	Animal has one or more lumps or lesions that bear watching, but seem unlikely to be DFTD
3	Animal has one or more lumps or lesions that look/feel consistent with DFTD but could be something else; 50/50 chance of being DFTD
4	Animal has one or more lumps or lesions that look/feel strongly consistent with DFTD; a likely DFTD case, but requires confirmation via laboratory analysis
5	DFTD confirmed in the lab through histological or cygogenetic analysis of one or more tumour biopsies

Table 4. Explanation of DFTD assessment system

Four of the 110 devils captured were assigned a DFTD score of 2 or higher. To assess the suspicious lump(s) or lesion(s) in these cases, one or more biopsies were collected if the position of the lump/lesion permitted:

- For an ulcerated lesion, two "punch" biopsies were collected: one fixed in 10% buffered formalin for histological analysis of the cell structure, and one preserved in RNALater buffer for cytogenetic analysis (both methods can be used to determine whether a tumour is DFTD).
- For a non-ulcerated lump or lesion, a fine-needle-aspiration (FNA) biopsy was collected in a liquid FNA buffer for cytogenetic analysis.

Case 1

- *Date observed:* 23 May 2018
- *Sex:* Female
- *Birth year:* 2013
- *Observations:* The animal presented with two suspicious lesions. The first (Figure 4a) was an ulcerated lesion on the top of the head, measuring 22 mm x 22 mm x ~5mm. Tissue substrate of the lesion was cutaneous. Secondary infection was present. Appearance was strongly consistent with DFTD and the lesion was assigned a DFTD score of 4. The second (Figure 4b) was a non-ulcerated lump on the neck/throat, measuring 22 mm x 20 mm x ~10 mm. Tissue substrate of the lesion was cutaneous. No secondary infection was present. Palpation was consistent with DFTD and the lesion was assigned a DFTD score of 3.
- *Action taken:* Two 6 mm punch biopsies of the ulcerated lesion were collected, one fixed in 10% buffered formalin and one preserved in RNALater. A fine-needle-aspiration biopsy of the non-ulcerated lesion was collected and preserved in a liquid FNA buffer. All biopsies were submitted to the Animal Health Laboratory of the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE).
- *Results:* Histological analysis of the formalin-fixed punch biopsy diagnosed the ulcerated lesion as a poorly differentiated mesenchymal (soft/connective tissue) tumour inconsistent with DFTD. Subsequent cytogenetic analysis of the RNALater-preserved punch biopsy, using the polymerase chain reaction (PCR) technique, ruled out both variants of DFTD (DFT1 and DFT2). (The Animal Health Laboratory report outlining these results is attached as Appendix A.) The FNA biopsy of the non-ulcerated lesion was not analysed. (The Animal Health Laboratory processes FNA biopsies according to a different protocol to that followed by TCC's cytogenetic research partner at Cambridge University, and the *locum tenens* pathologist who received the biopsy misunderstood it to be an empty vial and discarded it.)

Case 2

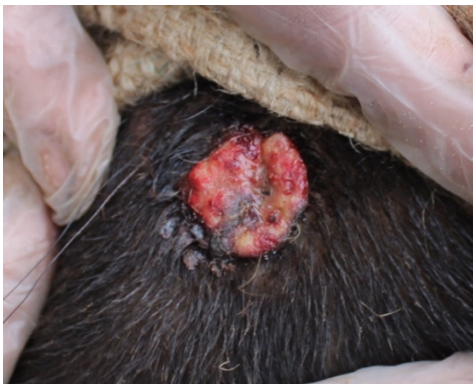
- *Date observed:* 26 May 2018
- *Sex:* Male
- *Birth year:* 2013
- *Observations:* The animal presented with a non-ulcerated lesion on the upper chest (Figures 4c and 4d), measuring 38 mm x 36 mm x ~20 mm, plus a raised "nub" measuring 11mm x 11mm x ~10mm. Tissue substrate of the lesion was cutaneous. No secondary infection was present, but some apparent pus (or possibly sebum) was aspirated during biopsy collection. Appearance was consistent with DFTD, but the apparent pus or sebum was consistent with an abscess or sebaceous cyst, and the lesion was assigned a DFTD score of 3.
- *Action taken:* A fine-needle-aspiration biopsy of the lesion was collected and preserved in a liquid FNA buffer. This biopsy was submitted to DPIPWE's Animal Health Laboratory.
- *Results:* The FNA biopsy was not analysed. (As with the FNA biopsy in case 1, the *locum tenens* pathologist who received the biopsy misunderstood it to be an empty vial and discarded it.)

Case 3

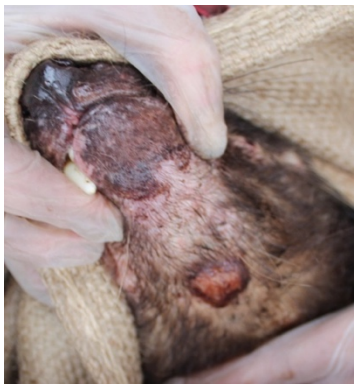
- *Date observed:* 23 May 2018
- *Sex:* Male
- *Birth year:* 2013
- *Observations:* The animal presented with large amounts of scar tissue on the face and neck, which is not unusual for a 5-year-old male. However, one lump on the lower-left jaw (Figure 4e), measuring 33 mm x 23 mm x ~20 mm, was somewhat more spherical in shape than is typical for scar tissue. Tissue substrate of the lesion was cutaneous. No secondary infection was present. Palpation was more consistent scar tissue than with DFTD, and the lesion was assigned a DFTD score of 2.
- *Action taken:* A fine-needle-aspiration biopsy of the lesion was collected and preserved in a liquid FNA buffer. This biopsy was submitted to DPIPWE's Animal Health Laboratory.
- *Results:* The FNA biopsy was not analysed. (As with the FNA biopsies in cases 1 and 2, the *locum tenens* pathologist who received the biopsy misunderstood it to be an empty vial and discarded it.)

Case 4

- *Date observed:* 18 May 2018
- *Sex:* Female
- *Birth year:* 2017
- *Observations:* The animal presented with a small non-ulcerated lesion under the tongue (Figure 4fr), measuring ~5 mm x ~5 mm x ~5 mm. Tissue substrate of the lesion was mucosal. No secondary infection was present. Appearance was not consistent with DFTD, and DFTD very rarely presents in such a young animal. Accordingly the lesion was assigned a DFTD score of 2.
- *Action taken:* None. (The position of the lesion made it impracticable to collect a biopsy.)

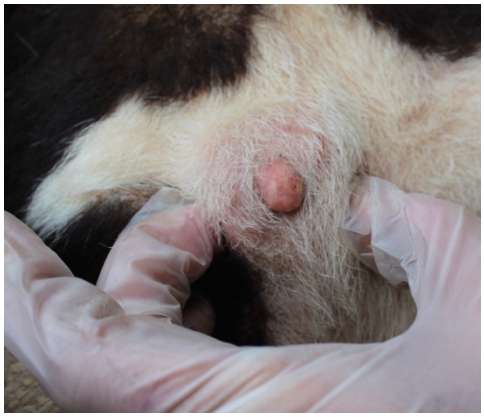


(a)

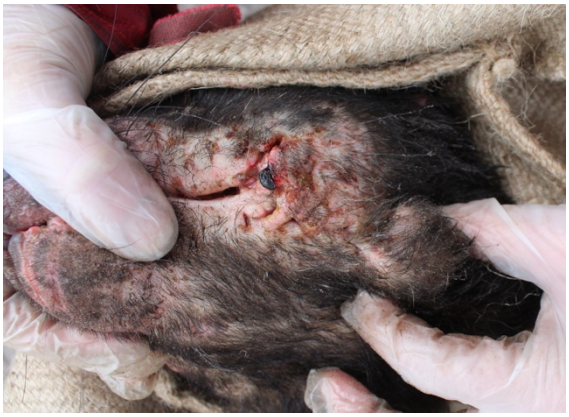


(b)

(c)



(d)



(e)

(f)

Figure 4. Suspicious lesions on four individual devils
(a) Ulcerated lesion on the top of the head of a 5-year-old female (Case 1)
(b) Non-ulcerated lesion on the neck/throat of a 5-year-old female (Case 1)
(c, d) Non-ulcerated lesion on the chest of a 5-year-old male (Case 2)
(e) Non-ulcerated lesion on the left jaw of a 5-year old male (Case 3)
(f) Non-ulcerated lesion on the soft palate of a 1-year-old female (Case 4)

Overall conclusions regarding DFTD

The loss of the FNA biopsies of the non-ulcerated tumours in Cases 1–3 is unfortunate, as biopsy results might have definitively ruled out DFTD in those cases. Despite that loss, however, we can be reasonably optimistic that DFTD has not arrived on Robbins Island, considering the following:

- Case 1 was the most worrisome of the four potential DFTD cases observed. The fact that this animal's ulcerated tumour was negative for DFTD is thus reassuring, since that tumour was more worrisome than the non-ulcerated tumour.
- There are plausible alternate explanations for Case 2 (abscess or sebaceous cyst) and Case 3 (scar tissue).
- Case 4 seems unlikely to have been DFTD, given the atypical appearance of the lesion and the animal's young age.

The demographics of the Robbins Island devil population are consistent with a non-diseased site. As DFTD becomes established at a site, the population begins to acquire a pronounced skew toward younger animals, and after a few years of disease presence, it becomes extremely rare to see adult animals older than age 3. No demographic skew toward juveniles was observed on Robbins Island, and adults of every age class were well represented in the population.

The fact that neither the Save the Tasmanian Devil Program nor TCC has observed any cases of DFTD at the Buckby's Road or Woolnorth sites (the closest sites on mainland Tasmania to have been surveyed) further supports the belief that DFTD has not arrived on Robbins Island.

Nevertheless, the observation of anomalous lumps or lesions in 4 individuals — none of which have been definitively ruled out as DFTD cases — suggests that the situation bears watching. Ongoing surveying, perhaps annually, may be advisable.

RESULTS OF GENETIC ANALYSIS

The Australasian Wildlife Genomics Group in the School of Life and Environmental Sciences at the University of Sydney genotyped 60 of the 110 DNA samples collected during this expedition. (Their report is attached as Appendix B.) They found a low number of alleles per locus, limited heterozygosity and an elevated inbreeding coefficient, all of which were comparable to levels in the nearby Woolnorth population and indicate the same limited genetic diversity found in Tasmanian devil populations around the state.

OTHER FINDINGS

Three interesting findings about the Robbins Island devil population emerged during the survey:

- Ectoparasites were much less prevalent than in the mainland populations TCC has surveyed. Not a single flea was observed on any animal. It may be that the founder individual(s) of the Robbins Island devil population happened to be free of fleas at the time they initially colonised the island; perhaps fleas abandoned their host animals if devils need to swim any distance in salt water while crossing to the island. While ticks were present on some devils, they occurred in much lower numbers than on mainland animals, and many individuals were tick-free.
- On average, Robbins Island devils were more resistant during handling than is the case with devils on the mainland. In particular, several animals vocalised during processing, which very rarely happens with animals at TCC's mainland sites. This difference in behaviour may stem from Robbins Island devils' relative familiarity with human presence. Farm workers are present throughout the year at Robbins Island, whereas human activity at TCC's mainland study sites is much more limited. (In general, wild devils are extremely docile during handling, but captive devils tend to lose their fear of humans and develop agonistic behaviours toward humans.)

- Three devils had some degree of unusual coloration, exhibiting white spots on the back. In the most pronounced case (Figure 5), several large white spots were distributed across the entire back. In the other cases, just a few smaller white spots occurred on the shoulders. These spots were not consistent with senescent greying (the animal with the most pronounced spots was only 2 years old) and were not associated with any scar tissue (which can cause fur to grow back white). Rather, it appeared to be the animal's normal coloration.

Figure 5. Unusual color pattern in a 2-year-old Tasmanian devil

Tasmania
Explore the possibilities

Animal Health Laboratory

Department of Primary Industries, Parks, Water and Environment
ABN 58 259 330 901

165 Westbury Road
Prospect TAS 7250

Enquiries Phone:(03) 6777 2111
Fax: (03) 6344 3085

S PECK
VETERINARY OFFICER
DPIPWE
HOBART TAS 7000

Case Report

Case Id:	18/1996		
Revision:		**Submitter:**	HUGHES
Serial No:		**Collected:**	23/05/2018
Reference No:	991001001114140	**Received:**	04/06/2018
Order No:		**Finalised:**	18/06/2018

Extra Copy: DR Graeme KNOWLES

Owner:	WILD TASMANIAN DEVIL Robbins Island TAS 7330	**Species:**	Tasmanian Devil
		Sex:	Female
PIC:	Not supplied	**Age:**	5 Years

PATHOLOGIST'S SUMMARY COMMENTS:
PCR excludes DFT1 and DFT2. Based on the histology, poorly differentiated mesenchymal tumour, cytogenetics is strongly recommended to further investigate this tumour.

PATHOLOGY REPORT

Animal ID (tag number, etc): None provided
Date collected: 23/5/18
Time of death or time found:
Sex: Female
Age: 5 years
Location it was collected from: Robbins Island
Collected by: Dr C Hughes
Type of examination: biopsy

CLINICAL HISTORY
Please refer to laboratory advice note

Punch biopsy of lesion #1
Animal name Quinticlave

SAMPLES SUBMITTED
Formalin fixed tissue

GROSS SUMMARY
Slides A contains a 3mm biopsy

HISTOPATHOLOGY DESCRIPTION
Skin: There are pleomorphic cells varying from spindle to polygonal to irregular, often forming bundles, set within a fine fibrovascular stroma, effacing the normal dermis. The cells have indistinct cell borders, moderate to scant amount of eosinophilic cytoplasm, oval nucleus with prominent nucleolus (often multiple) and coarse to vesicular chromatin pattern. There is moderate anisocytosis, karyomegaly, increased nuclear to cytoplasmic ratio. The mitotic rate was 10-20 mitotic figures per 10 high powered fields (HPF). There is mild multifocal mixed inflammation, lymphocytes, plasma cells, neutrophils, scattered through the bundles of neoplastic cells.

DIAGNOSIS
Skin: Poorly differentiated mesenchymal tumour not consistent with DFT1

PATHOLOGIST'S COMMENTS:
There was no suitable sample for PCR testing. Resampling is recommended to include a larger biopsy for histology and also tissue in RNAlater for DFT1/DFT2 PCR. In addition, given the poorly differentiated nature of the tumour in this wild Tasmanian devil, organizing for samples to be collected in tissue culture media (which can be supplied by the laboratory) for cytogenetics, should be considered.

Please note, unfortunately the FNA samples were not suitable for cytology or PCR testing using the DFT1/DFT2 PCR.

Histopathology reported by Graeme Knowles (veterinary pathologist)

MOLECULAR BIOLOGY REPORT

MOLECULAR BIOLOGY REPORT

SPECIES: Tasmanian devil

TEST REQUESTED: Identification of Tasmanian Devil Facial Tumour (DFTD) DNA by PCR.

SAMPLES SUBMITTED:
1 biopsy in RNAlater

TEST RESULTS

SAMPLE	DFT1 DNA	DFT2 DNA
1	Negative	Negative

CONTROLS: Controls gave expected results.

NATA accreditation does not cover the performance of this service.

AUTHORISED: Kate Swift **DATE:** 18/06/18

Russell Graydon
Veterinary Pathologist - Locum
Ph: 0367772124

NATA ACCREDITATION NO: 384

ATTENTION SUBMITTER: Reports will only be issued to the persons or practice nominated as the submitter on the specimen advice sheet. Test results and findings may be provided to authorised staff and used for statistical, surveillance, extension, certification and regulatory purposes in accordance with Departmental policies. The information assists disease and residue control programs and underpins market access for agricultural products. The source of the information will remain confidential unless otherwise required by law or regulatory policies. Samples submitted and materials generated by laboratory processing and testing remain the property of DPIPWE. Some samples and materials may be held indefinately for reference purposes but all other samples and materials will be discarded in line with the Animal Health Laboratory normal retention times, which will be no more than 30 days.

When completing the Laboratory Advice Note please write the Property Number (M_ _ _ _ _ _ _) on the top right hand side of the sheet. The PIC is very useful in disease control programs and for export certification purposes. Please enquire from the producer if they have a PIC. DPIPWE exercises due care in performing all tests but, takes no responsibility for errors associated with sample collection or freight forwarding. In a continued effort to improve our service we welcome your comments. Please forward any comments or inquires via E-mail to specimenreception@dpipwe.tas.gov.au or call 03 6777 2111

Genetic analysis of Tasmanian Devils on Robbins Island
Final Report for GHD
September 2018

Dr Carolyn Hogg and Riley Ferguson
Australasian Wildlife Genomics Group, SOLES, University of Sydney, Sydney

Executive Summary

A total of 60 Tasmanian devil (*Sarcophilus harrisii*) samples have been successfully genotyped using thirty-three neutral microsatellite loci. The samples were collected by Channing Hughes from Robbins Island (18-27 May 2018) for the purposes of this analysis. Although 110 ear biopsy samples were collected, only 60 samples were genotyped from across the island (Fig. 1) to reflect spatial and demographic differences (29 males, 31 females, age range 1 to 6). The Robbins Island population had on average 2.9 ± 0.2 alleles per locus in the population reflecting an observed heterozygosity of 0.34 and inbreeding coefficient of 0.089. Robbins Island was compared to genotyping data from 58 Tasmanian devils (32 females, 26 males, age range 2 to 5) from Woolnorth. By comparison, the Robbins Island population is not genetically different to the Woolnorth population (Appendix 1). Like most other wild Tasmanian devil populations, Robbins Island Tasmanian devils are genetically depauperate exhibiting a small number of alleles, low heterozygosity and increased inbreeding. Although there were nine private alleles found on Robbins Island compared to Woolnorth, these are at low frequency. In conclusion, although Tasmanian devils on Robbins Island are separated from mainland Tasmania, there appears to be some limited gene flow between Robbins Island and mainland Tasmania (compared to Woolnorth the closest mainland Tasmanian site that genotyping data existed for).

Introduction

Tasmanian devils (*Sarcophilus harrisii*) are the world's largest extant marsupial carnivore and are currently listed as endangered due to an infectious clonal cancer, devil facial tumour disease, DFTD (Jones & McCallum 2007). Due to a number of unexplained population crashes over time, genetic diversity within Tasmanian devil populations is low (Jones *et al.* 2004; Miller *et al.* 2012; Hendricks *et al.* 2017) and these have been associated with the prevalence of DFTD across the state of Tasmania (Siddle *et al.* 2007; Cheng *et al.* 2012). A recent study showed that Tasmanian devils are a highly vagile species where landscape features do little to limit dispersal and gene flow (Storfer *et al.* 2017).

Australasian Wildlife Genomics Group
Faculty of Science
School of Environmental and Life Sciences
Room 219, Gunn Building (B19)
The University of Sydney NSW 2006 Australia

T +61 2 8627 4896
E Carolyn.hogg@sydney.edu.au

ABN 15 211 513 464
CRICOS 00026A

Robbins Island is a 9,900ha island in Bass Strait, lying off the north-west coast of Tasmania. The island is separated from the Tasmanian mainland by a highly tidal area known as Robbins Passage (Gazetteer of Australia).

The aim of this study was to ascertain the genetic composition of Tasmanian devils resident on Robbins Island, and how this population differs from other mainland Tasmania locations, in particular Woolnorth.

Results summary

The Robbins Island Tasmanian devil population had 2.9 ± 0.2 alleles, this is comparable to other contemporary wild Tasmanian devil sites, where the number of alleles range from 2.65 to 2.71 (Grueber *et al.* 2018). The observed heterozygosity was 0.34 which is lower than other contemporary wild populations using the same microsatellite loci, 0.43 to 0.47 (Grueber *et al.* 2018). Although Robbins Island was noted to have nine private alleles compared to Woolnorth, these occurred at low frequencies and not at a rate any higher than other wild sites in Tasmania.

Compared to Woolnorth, the closest mainland Tasmania location that has a demographically similar population that genotyping data existed for, there was little difference in the observed heterozygosity, number of alleles or inbreeding (Gooley *et al.* in prep). The F_{ST}, a measure of genetic difference between the two populations, was 0.025. This F_{ST} is similar to the mean F_{ST} of 0.021 between north-west and north-east Tasmania populations (Storfer *et al.* 2017). By comparison, the Robbins Island population is not genetically differentiated from the Woolnorth population (Appendix 1).

Methods

Sample collection – Tasmanian devils were trapped by Channing Hughes (DPIPWE permit TFA18149; University of Sydney Ethics 2017/1149) using PVC-pipe traps at locations across Robbins Island (Fig. 1). 110 ear biopsies were collected and stored in 70% ethanol and provided to the University of Sydney for genotyping, along with the individual's estimated age, sex and trapping location. Only 60 samples were genotyped for this study, samples were chosen from across the trapping locations, and sex and age matched to the existing Woolnorth data for comparison. As a result of this selection 29 males and 31 females were selected of estimated ages 1 (n = 7); 2 (n = 12); 3 (n = 17); 4 (n = 15); 5 (n = 7); and 6 (n = 2).

Genotyping - DNA was extracted from the 60 ear biopsies using Bioline Isolate II Genomic DNA kits according to manufacturer's instructions. Samples were genotyped with microsatellite analysis using 33 neutral markers (Jones *et al.* 2003; Gooley *et al.* 2017). Polymerase chain reaction (PCR) was used to

amplify the markers under the following conditions: initial denaturation of 90°C for 5 min; 30 cycles of 95°C for 30s, 60°C for 90s, and 72°C for 30s; and final extension of 60°C for 30 min. Capillary electrophoresis was performed in a 3130xl DNA Analyzer (Applied Biosystems, ThermoFisher) and GENEMARKER (SoftGenetics, State College, PA) was used to score the markers. Population genetic analysis was performed using GenAlEx (Peakall & Smouse 2006; Peakall & Smouse 2012). Woolnorth genetic data (Gooley *et al.* in prep) was extracted and genotyped using the same method.

Figure 1: Tasmanian devil trap locations from Robbins Island.

Acknowledgements

Thank you to Channing Hughes and Anna Lewis for their collection of the samples. This work was undertaken as a sub-consultancy with GHD, Hobart, Tasmania. Genotyping data for Woolnorth was produced by Rebecca Gooley for her PhD in collaboration with the Save the Tasmanian Devil Program.

References

Cheng, Y., Sanderson, C., Jones, M. & Belov, K. (2012) Low MHC class II diversity in the Tasmanian devil (Sarcophilus harrisii). *Immunogenetics,* **64,** 525-533.

Gooley, R., Hogg, C.J., Belov, K. & Grueber, C.E. (2017) No evidence of inbreeding depression in a Tasmanian devil insurance population despite significant variation in inbreeding. *Scientific Reports*.

Gooley, R., Hogg, C.J., Fox, S., Pemberton, D., Belov, K. & Grueber, C.E. (in prep) Early signs of inbreeding avoidance in the face of inbreeding depression in the last disease-free population of the endangered Tasmanian devil.

Grueber, C.E., Fox, S., McLennan, E.A., Gooley, R.M., Pemberton, D., Hogg, C.J. & Belov, K. (2018) Complex problems need detailed solutions: harnessing multiple data types to inform genetic management in the wild. *Evolutionary Applications*.

Hendricks, S., Epstein, B., Schönfeld, B., Wiench, C., Hamede, R., Jones, M., Storfer, A. & Hohenlohe, P. (2017) Conservation implications of limited genetic diversity and population structure in Tasmanian devils (Sarcophilus harrisii). *Conservation Genetics***,** DOI 10.1007/s10592-10017-10939-10595.

Jones, M. & McCallum, H. (2007) Managing an emerging disease in a threatened species: Tasmanian devil facial tumour disease. *Australian & New Zealand Journal of Obstetrics & Gynaecology,* **47,** A16-A16.

Jones, M.E., Paetkau, D., Geffen, E. & Moritz, C. (2003) Microsatellites for the Tasmanian devil (Sarcophilus laniarius). *Molecular Ecology Notes,* **3,** 277-279.

Jones, M.E., Paetkau, D., Geffen, E. & Moritz, C. (2004) Genetic diversity and population structure of Tasmanian devils, the largest marsupial carnivore. *Molecular Ecology,* **13,** 2197-2209.

Miller, W., Hayes, V.M., Ratan, A., Petersen, D.C., Wittekindt, N.E., Miller, J., Walenz, B., Knight, J., Qi, J., Zhao, F.Q., Wang, Q.Y., Bedoya-Reina, O.C., Katiyar, N., Tomsho, L.P., Kasson, L.M., Hardie, R.A., Woodbridge, P., Tindall, E.A., Bertelsen, M.F., Dixon, D., Pyecroft, S., Helgen, K.M., Lesk, A.M., Pringle, T.H., Patterson, N., Zhang, Y., Kreiss, A., Woods, G.M., Jones, M.E. & Schuster, S.C. (2012) Genetic diversity and population structure of the endangered marsupial Sarcophilus harrisii (Tasmanian devil) (vol 108, pg 12348, 2011). *Proceedings of the National Academy of Sciences of the United States of America,* **109,** 18625-18625.

Peakall, R. & Smouse, P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes,* **6,** 288-295.

Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. . *Bioinformatics,* **28,** 2537-2539.

Siddle, H.V., Kreiss, A., Eldridge, M.D., Noonan, E., Clarke, C.J., Pyecroft, S., Woods, G.M. & Belov, K. (2007) Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. *Proceedings of the National Academy of Sciences,* **104,** 16221-16226.

Storfer, A., Epstein, B., Jones, M., Micheletti, S., Spear, S.F., Lachish, S. & Fox, S. (2017) Landscape genetics of the Tasmanian devil: implications for spread of an infectious cancer. *Conservation Genetics*.

Appendix One

Data contained in Appendix One is not for public distribution. Comparative analysis between Woolnorth (Gooley *et al.* in prep) and Robbins Island (this study).

Table 1: Microsatellite diversity statistics for Tasmanian devils sampled at Robbins Island and Woolnorth. N_a = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F = inbreeding.

	N	**N_a**	**H_o**	**H_e**	**F**
Robbins Island	60	2.91	0.34	0.37	0.089
Woolnorth	58	3.09	0.38	0.38	0.000

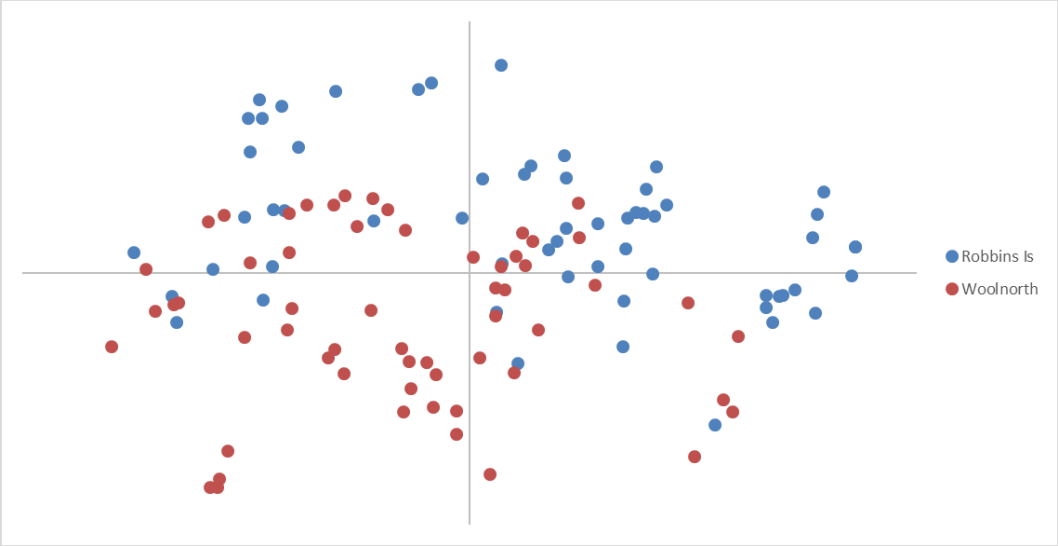


Figure 2: Principal of coordinates analysis of Tasmanian devils from Robbins Island and Woolnorth based on 33 neutral microsatellite loci.