Ecology and management of the northern quoll *Dasyurus hallucatus* in the Pilbara

Progress Report

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September 2013

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December 2013

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The recommended reference for this publication is:
Department of Parks and Wildlife, 2013, *Ecology and management of the northern quoll* Dasyurus hallucatus *in the Pilbara*, Department of Parks and Wildlife, Perth.

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Summary

The Department of Parks and Wildlife's (formerly Department of Environment and Conservation - DEC) Pilbara Regional Northern Quoll Monitoring Program has been developed to improve our understanding of the distribution, ecology, population trends and demographics of the northern quoll (*Dasyurus hallucatus*) in the Pilbara region of Western Australia. A priority of the program is to provide baseline data to environmental regulators and resource development companies to inform appropriate management ensuring the persistence of this threatened species in the region.

This document reviews progress on five major tasks set out in the Department of Sustainability, Environment, Water, Population and Communities (SEWPaC) Project Plan.

1 Introduction

Little is known about the ecology and biology of northern quolls in the Pilbara region of WA. Most information on the species comes from studies on the populations throughout the Kimberley, the Northern Territory and Queensland that are genetically distinct from the Pilbara population. Although many surveys are conducted for the mining industry and other developers to detect the presence/absence of quolls in the Pilbara, these studies do not address the gaps in our knowledge of the ecology of northern quoll populations and at present distribution data are not freely available.

In view of the current rapid development of the mining industry in the Pilbara, the need for a regional monitoring project and ecological study of the northern quoll was identified during workshops conducted by Department of Sustainability, Environment, Water, Population and Communities (SEWPaC) in 2010. One of the outcomes of this workshop was the Project Plan (SEWPaC 2010).

The Department of Parks and Wildlife have been working towards five major tasks based on the SEWPaC Project Plan (see Appendix 1);

1. Undertake Literature review
2. Develop a Pilbara wide survey and monitoring program
3. Undertake quoll survey and landholder consultation
4. Implement Pilbara wide monitoring program
5. Undertake ecological and demographic study of quolls in the Pilbara

This document reviews progress on these tasks as at October 2013.

2 Key Achievements

2.1 Undertake literature review

A literature review was completed in October 2011 (Cook, 2010b) providing information from more than 200 unpublished reports with reference to the northern quoll. It is available online within the Naturemap website, under Pilbara northern quoll: http://naturemap.dpaw.wa.gov.au/ - Themes - Pilbara Threatened Fauna.

2.2 Develop a Pilbara wide survey and monitoring program

Ten sites have been identified for annual monitoring of population trends of northern quolls (see 3.3 *Implement Pilbara wide monitoring program*). Information on their ecology and biology will also be obtained across the spread of suitable rocky habitats of the Pilbara bioregion, from Mardie in the west, to Yarrie in the east, and as far south as Karijini National Park and the south eastern extent of the Chichester Ranges. Where possible, these sites are on conservation estate or pastoral leases not identified for mining activities. These will serve as reference sites for monitoring activities on nearby mining sites. Other sites, such as Barlee Range Nature Reserve, Karlamilyi National Park and Meentheena Conservation Park will be opportunistically surveyed to determine quoll presence but are not included as long term monitoring sites at this stage.

The development of the Pilbara wide survey and monitoring program by the Department of Parks and Wildlife coincided with a three-year study of disturbed areas for BHP Billiton. Monitoring was based on the Environment Protection and Biodiversity Conservation Act 1999 referral guidelines for the endangered northern quoll, Dasyurus hallucatus, EPBC Act policy statement 3.25 (DSEWPaC, 2011). The monitoring procedures adopted for the Disturbed Area Monitoring have acted as an assessment tool for the protocols being developed for the Pilbara wide program.
In June 2013 Parks and Wildlife produced the *Northern Quoll Survey and Monitoring Project,*
Pilbara Region of Western Australia (Department of Environment and Conservation, 2013). A Science Project Plan has also been produced for this project (Department of Environment and Conservation, 2011). This includes objectives, methodology, habitat assessment and reporting guidelines, and is in place to ensure scientific rigor for the program.

2.2.1 Monitoring Procedures

The ten long-term sites will be monitored annually. These sites will need to remain free of mining and/or pastoral disturbance as much as possible.

Objectives

- To improve understanding of northern quoll distribution, ecology, and abundance and other demographic parameters in the Pilbara and allow

comparison with published studies in the Kimberley, Northern Territory and Queensland.
- To inform management for the conservation of northern quoll populations in and around mining sites and other developments in the Pilbara.
- To clarify the taxonomic and conservation status of the Pilbara northern quoll population
- To provide a model for other proposed regional fauna projects in the Pilbara

Monitoring parameters
The following parameters will be monitored at each site:
- Body weight
- Morphometrics – short pes, head length, tail diameter at base
- Sex ratio
- Age class
- Reproductive status / condition
- Health / body condition
- Diet
- Abundance
- Genetic diversity
- Fox/cat/dog/dingo presence/abundance

At each monitoring site there will also be opportunities for ecological research projects such as examining den and feeding habitat use / partitioning, movements across the landscape, and impact of introduced predators.

Monitoring Protocols
Because of anticipated low capture rates and seasonal fluctuations in quoll population abundance it will be necessary to monitor sites over a long period (10+ years) to detect any significant changes or trends in abundance and other demographic parameters.

Monitoring at each site will be conducted once per year: Monitoring will occur accordingly;

In April-early May after dispersal on juveniles and prior to the onset of the breeding season.

In September-early October after breeding activity has ceased, and prior to females depositing young in dens, to obtain data on breeding success and male survival.

The aim is to deploy 50 traps per site. Inter-trap spacing must remain consistent across all sites. The configuration applied initially at an individual site must be maintained at that site.

Specific quoll trapping methods:
- Small Sheffield wire cage traps (45 cm x 17 cm x 17 cm)
- 50 traps spaced 50 m apart

- Individual trap locations are fixed and marked (GPS and dropper post) for the duration of the monitoring program.
- Traps opened for 4 consecutive nights at each site (200 trap nights)
- Traps checked and closed within 3 hours of sunrise, rebaited and opened in the late afternoon
- Universal bait (peanut butter, oats, sardines)

Data collection
All captured quolls are implanted with a subcutaneous microchip (PIT) for individual identification. Standard measurements of all captured quolls are taken (body weight, short pes length, head length, age class, sex and breeding condition). A small amount of ear tissue is collected from all individuals at initial capture for genetic analysis. A sample of scats is collected each trapping session for dietary analysis. An estimation of fox/cat/dog/dingo activity at each site will be derived using either sand plots or remote cameras. Morphometric, dietary, breeding and genetic information will be compared with other populations of northern quolls.

Habitat monitoring and modelling
Habitat attributes are being recorded at all sites (including camera survey and monitoring sites) and will be analysed to help predict the spatial distribution of suitable habitat and the probability of quolls occupying locations based on environmental attributes. Fire history and other disturbances such as cattle impact will also be assessed and monitored.

- Data sheets are used to record habitat attributes (Appendix 2)
- Photo points will be established at all long-term monitoring sites
- Digital rainfall gauges and temperature loggers will be installed at all long-term monitoring sites

2.3 Undertake quoll survey and landholder consultation

In addition to the literature review, assistance from the department's Pilbara region staff helped to identify focus areas for landholder consultation and a distribution study using motion sensor cameras. Surveying of up to 100 sites to detect quolls commenced in 2012. At each site, up to 20 remote motion sensor cameras are deployed for at least 2 nights to record presence/absence data, and habitat characteristics of each site is recorded.

Consultation with landholders has provided valuable historical and current information on northern quolls. These consultations and camera surveys have continued since and are an important ongoing tool in habitat assessment and anecdotal observations. In 2013, Parks and Wildlife included targeted trapping surveys as part of follow-up consultations at Indee Station and Millstream-Chichester National Park. Additional information has been gathered through consultation with park rangers, tourism operators and environmental staff from mining and consultancy companies.

The table in Appendix 3 shows historical and current northern quoll records provided by consultations to date. Information gained as part of these consultations has assisted in the choosing of 10 long-term monitoring sites as part of the Pilbara wide monitoring program that will begin in 2014. These records are being added to NatureMap to enhance the species distribution data, past and present, for the northern quoll.

2.3.1 Camera Trapping Procedures

Remote sensor Reconyx Hyperfire PC900 cameras were deployed at areas of potential quoll habitat. Each camera was placed on a sturdy platform or strapped to a secure boulder; facing south-east, south or south-west to reduce shadow triggers. The cameras were positioned approximately 2-3m from an area of interest such as a cave, ledge or animal path. A scent lure of mixed sardines, oil and water was placed in the field of view for increased interest in the target zone for the cameras.

Cameras were set:

- On a sturdy, flat platform supported by rocks to prevent it being moved or knocked over, or secured to a boulder with the strap
- Facing south-east, south or south-west to reduce triggers due to sunrise, sunset or the movement of shadows
- With an area of interest in the field of view, e.g. a cave, ledge, animal thoroughfare
- Without obstructions in the field of view such as moving branches, obscuring boulders or grasses
- Approximately 2-3m from where you predict the animal will go past. Place the scent elon something in this spot, e.g. an elevated rock
- Between 10-30cm above ground level, facing slightly downwards

Each camera is baited with an oily scent lure of sardines, oil and water, placed at the focal point of the field of view.

2.4 Implement Pilbara wide monitoring program

Regional presence / absence surveys have commenced with Parks and Wildlife staff opportunistically deploying remote sensor cameras to detect northern quolls throughout the Pilbara. Monitoring of northern quoll populations over 10+ years will commence in 2014. Ten long-term survey sites will be monitored annually. Sites were selected based on land status, anecdotal or confirmed current records of quolls, presence of suitable habitat, and providing a geographic spread throughout the rocky areas of the Pilbara. The sites chosen (see Figure 1) are as follows;

1. Fortescue River
2. Dolphin Island,
3. Karratha hills,
4. Millstream-Chichester National Park - Python Pool,
5. Mt Florance Station,
6. Karijini National Park – Kalamina Gorge,
7. Indee Station,
8. De Grey Station,
9. Yarrie Station,
10. Chichester Range – Eastern area

In addition to the ten sites that will be monitored by trapping, 100 sites throughout the Pilbara will be assessed for quoll presence/absence using camera trapping and detailed habitat data collected to inform the development of a habitat model and a better understanding of northern quoll distribution. This data will be collected opportunistically from work undertaken by Parks and Wildlife, as well as in collaboration with environmental consultants working in the Pilbara. Records will soon be able to be entered online via the Department of Parks and Wildlife Pilbara Threatened Species Naturebase website.

Landholder consultations and associated camera deployment on pastoral leases will continue as an important aspect of regional surveying, and camera sites will be revisited to observe historical population fluctuations.

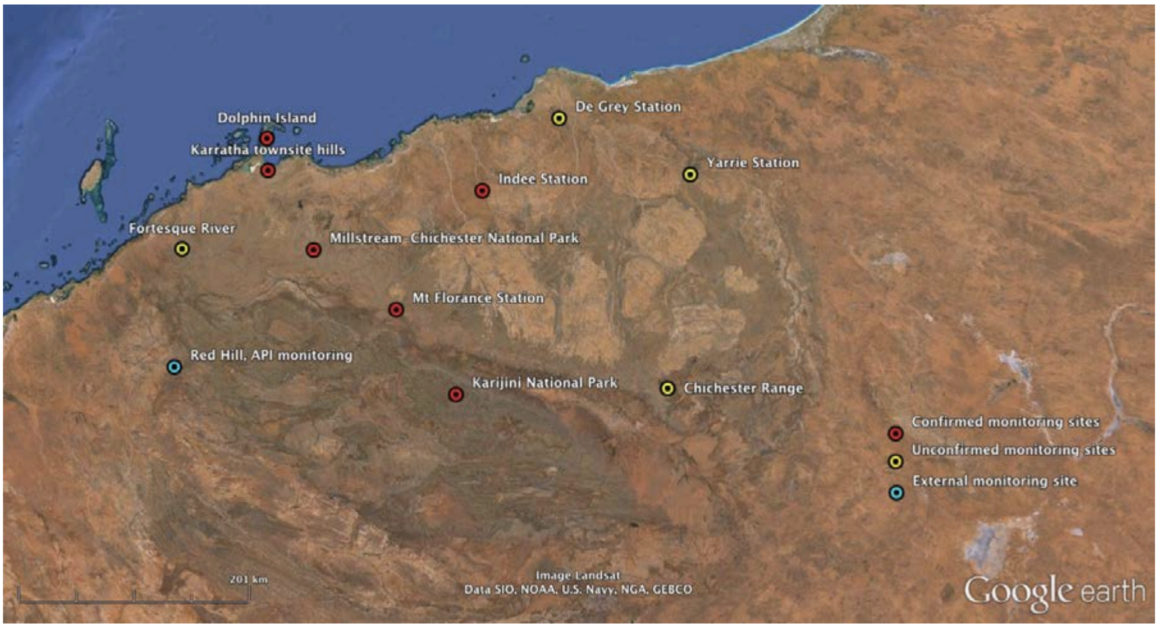

Figure 1. Long-term survey sites for Pilbara-wide northern quoll monitoring

2.5 Undertake ecological and demographic study of quolls in the Pilbara

2.5.1 BHPBIO project 2011-2013; response of northern quoll to mining disturbance

A major focus of work undertaken by Parks and Wildlife between 2011 and 2013 has been Disturbed Area Monitoring in conjunction with BHPBIO. This work was discussed in detail in Department of Parks and Wildlife, 2013, *BHP Billiton Iron Ore Pilbara Northern Quoll Monitoring Project*. A summary of the works follows here.

Biannual monitoring of northern quoll populations at operational and disused BHPBIO mine sites and quarries was conducted from June 2011 to June 2013. This study has provided information relevant to impacts identified in the Northern Quoll EPBC Act Policy Statement (DSEWPaC, 2011). These impacts include: Loss of known or potential habitat critical to the survival of the species; Loss of known or potential foraging/dispersal habitat; Introduction of barriers restricting dispersal opportunities and genetic flow. A brief synopsis of this work is presented here.

The specific objectives of these surveys were to:

- Identify quoll usage of operational and disused BHPBIO mine sites and granite quarry sites, and compare abundance and density with nearby undisturbed areas.
- Examine Population demographics – sex ratios, age structure, breeding etc, and detect ecological differences between Pilbara and other NQ populations.
- Investigate VHF and GPS radio-telemetry methodology for den site identification and spatial use.

Methods

The project was undertaken at a number of mine sites and quarries in the Pilbara. Monitoring sites were paired; one was disturbed by development or mine production activities; the other was located in an analogue habitat at least 5km away but removed from impacts to act as a control (Figure 2). Trapping surveys were conducted at each site twice annually in autumn (May-June) and spring (August-September). Numbers of individuals were estimated using the capture-mark-recapture method.

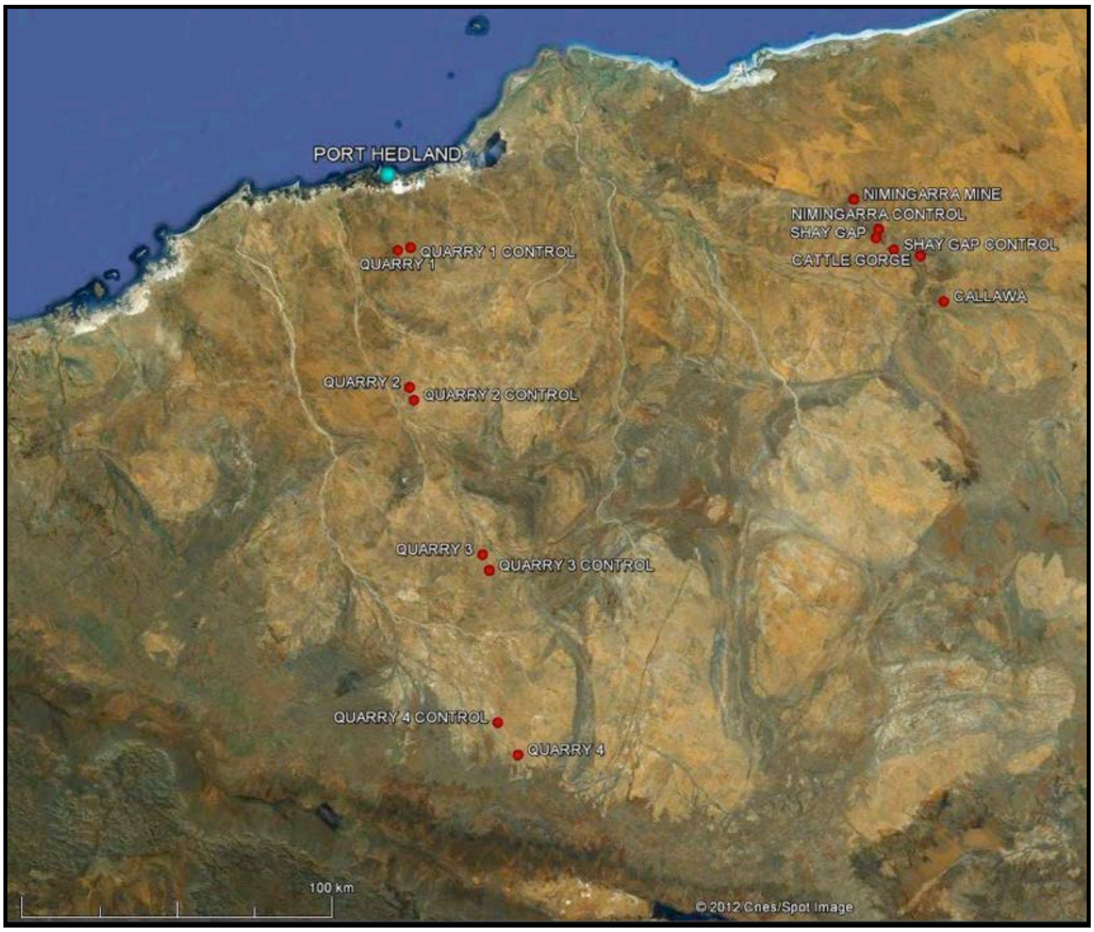

Figure 2. Regional location of paired monitoring sites

The quoll habitat present at each of these sites differed according to the level of disturbance as well as landforms present in the landscape. At the 4 quarry sites along the railway, the sites consisted of open-faced granite cliffs following removal of rock for railway construction. The undisturbed pair for each of these sites were granite boulder islands in a relatively open landscape of spinifex. Cattle Gorge, Callawa and both Nimingara (disturbed and undisturbed) sites were located along a connected ironstone ridge in the East of the Pilbara. The disturbed areas at Nimingarra and Cattle Gorge consisted of mining pits, haul roads and associated laydown areas. Rehabilitation of landform and vegetation was present at Nimingarra mine. The undisturbed sites – Nimingarra control and Callawa were located along similar but undisturbed ridges and drainage gullies.

No quolls were detected at Quarry 4 and Shay Gap (disturbed site) during the trap surveys in 2011, so after discussion with BHPBIO, it was agreed that further monitoring efforts should focus on the established sites with quoll presence. Surveys at Cattle Gorge were not continued due to issues with site accessibility in areas of mine activity. Instead, camera traps were opportunistically deployed at Cattle Gorge (control) in September 2011 and Cattle Gorge mine in June-July 2012; see table 2.

Table 1: Survey locations

SURVEY SITES	DISTURBED	UNDISTURBED	Trapping dates
BHPBIO Rail	Quarry 1	Quarry 1 control	2011-2013
BHPBIO Rail	Quarry 2	Quarry 2 control	2011-2013
BHPBIO Rail	Quarry 3	Quarry 3 control	2011-2013
Nimingarra	Nimingarra mine	Nimingarra control	2011-2013
Callawa		Callawa	2011-2013
DISCONTINUED SITES			
BHPBIO Rail	Quarry 4	Quarry 4 control	2011
Shay Gap	Shay Gap	Shay Gap control	2011
Cattle Gorge	Cattle Gorge ridge		2011
CAMERA DEPLOYMENT			
Cattle Gorge	Cattle Gorge mine	Cattle Gorge (gorge)	2011-2012

Limited VHF radio tracking was conducted to observe individual movements, and to examine den sites and areas of refuge. Observations and measurements collected from trapping were used to examine population demographics and compare to that obtained from Kimberley, Northern Territory and Queensland populations. Camera traps were also used in the operational disturbed area at Cattle Gorge unsuitable for trapping due to access restrictions. These were used to observe continued use and survivorship of quoll populations in disturbed areas of operation.

Results

Trapping

Population demographics

Northern quolls were captured during 37 of the 50 surveys conducted to date (including camera surveys). A total of 299 captures 107 individuals were recorded during the 5351 trap nights across all sites (see table 3). Sex ratio was relatively even with males making up an average of 56.25% of captures, females 43.75% (figure 2). Overall, these ratios were not statistically significantly different ($\mathbf{X}^2_{11}$=2, p=0.1573). There were no significant differences in number of individuals captured according to season ($F_{1,33}$ = 1.113, P = 0.299) or sex ($F_{1,33}$ =0.473, P = 0.496). Mass measurements of Pilbara northern quolls during this study averaged 695.2 ± 18.08 g, (range 420 – 1100 g), n = 72 for males, and 410.9 ± 11.33 g, (range 260 – 630 g), n = 56 for females.

VHF radio tracking surveys

Quarry 1

2011

Over the 10 nights of tracking at Quarry 1 the two collared females denned in three distinct areas. Two areas were inaccessible within broken boulder piles against the blasted quarry wall, and one was located beneath a rock sheet with minimal disturbance at the top of the granite outcrop. Multiple dens were recorded beneath the minimally disturbed rock sheet, as is possible in the inaccessible broken boulder areas. It seemed the same dens were used by both individuals on alternating days, which may not be unusual between maternal relations.

2012

The three quolls radio tracked at Quarry 1 carried radio collars for four days. They were trapped on the forth night and collars were removed the following morning. Because of bad weather quolls were only radio tracked to dens on days 2 and 4. Two separate dens were recorded for each individual. The male was recorded denning in the quarry and in a windrow of small boulders adjacent to Mooka workshop, approximately 400 m from the quarry. A female was tracked to a stock pile of concrete sleepers, also adjacent to the Mooka workshop area. All other dens were recorded in the quarry and were very close to those recorded in May 2011 within boulder piles against the blasted quarry wall and beneath a rock sheet at the top of quarry. Because of the unstable substrate within the quarry and the sheer wall the exact location of the quarry dens could not be accessed.

No accurate locations could be calculated from the night tracking data. However, based on observation of signals at the time of recording none of the three collared individuals crossed the rail infrastructure during the time of the survey. Signals indicated that two of the quolls moved towards or into the workshop/construction area west of the quarry, which is confirmed by the den sites located there.

Quarry 2

2011

Of the four quolls collared at Quarry 2, the two females shed their collars before any dens could be recorded. One collar was retrieved from under a small boulder pile at the top of the quarry close to the quarry wall. The second could not be retrieved due to its location deep within a boulder pile on a granite outcrop located 1 km north of the quarry.

Eight independent den locations were recorded for one male and seven for the other. All dens were located in granite outcrops in undisturbed habitat, as shown in Figure 4, up to 1.5 km from the quarry site with up to 1.5 km distance between consecutive dens. No dens were recorded in the quarry.

Motion Sensing Cameras

During spring 2011, four cameras were opportunistically deployed at the undisturbed Cattle Gorge. Northern quolls and common rock rats (*Z. argurus*) were captured on all four cameras. In autumn 2012, eight cameras were deployed at the active mining area at Cattle Gorge. Images of northern quoll were captured on four of these cameras. We could differentiate 3 individuals. Other species recorded to be present by remote camera survey included common rock rats (6/8 cameras), Rothchild’s rock-wallabies (*P. rothschildi*: 4/8 cameras)), *Pseudantechinus sp.* (likely *P. roryi* or *P. woolleyae*; 2/8 cameras) and a *Macropus sp*. was recorded on 2/8 cameras but poor image quality did not allow identified to species level (likely *M. robustus* or *M. rufus*; 2/8).

During the autumn 2013 survey at Quarry 3 Control, 29 remote sensor cameras were deployed in substitution for Sheffield traps. No quolls were detected using these cameras. Rothschild's rock-wallabies (*P. rothschildii*) were captured on all of the 29 cameras, native rock rats were captured on 21/29 cameras and unidentified *Pseudantechinus* species were captured on 15/29 cameras.

Discussion

Population demographics

Body mass records from BHPBIO Pilbara sites (males: 695.2 ± 18.08 g, range 420 – 1100 g, n = 72, females: 410.9 ± 11.33 g, range 260 – 630 g, n = 56) are larger than Kimberley adult northern quolls reported by Cook (2010) , who reported the mean weight of 537 g (330-718 g; n = 15) for males and 370 g (range 290-458 g; n = 13) for females. Oakwood (1997) found Northern Territory populations to quolls of a similar size to those in the Pilabara, with mean weight of males being 761 g (ranged 540-1120 g; n = 26) and 448 g for females (range 350-690 g; n = 11). Mean weights reported for northern quolls in the Kimberley suggest that there is a difference in size of this species between the two regions. This suggests that the Pilbara northern quolls are generally larger than those in the Kimberley, but comparable to Northern Territory populations. Formal comparisons of the size differences between these populations requires the raw data from the Kimberley and Northern Territory, and we are working on obtaining these from their respective scientists.

For Northern Territory populations, female breeding is synchronised and occurs over a discrete 3-4 week period in May/June (Oakwood, 1997) and female quolls were thought to all give birth within a few weeks of each other, regardless of location (Nelson and Gemmell, 2003). However, northern quolls in the Pilbara appear to breed later in the year and over a more extended period. The Pilbara populations breed across a period of at least 10 weeks, between July and September. Females showed signs of recent mating (bites and scarring) during this time. Evidence of breeding activity (females showing pouch development and males in poor body condition) was recorded at all 5 sites, during July through to September. Presence of pouch young was recorded in August at the disturbed Quarry 1 site, 30km south of Port Hedland,and in September at the disturbed Nimingarra mine site, 150km east of Port Hedland. Females at quarries were generally seen to persist at sites for 2 years, with one individual captured into her 3rd year at Quarry 2 disturbed site.

Male northern quolls from the Kimberley to Queensland experience a post-mating die-off (Dickman and Braithwaite, 1992) and consequently do not live to be older than 1 year. It appears that male die-off is not complete in the Pilbara populations; although no males were captured over the autumn period, some new males caught did seem to have survived the breeding season showing new hair growth and worn teeth.

2.5.2 Pilbara Northern Quoll Project Collaborative/future works

Revision of northern quoll research plan following the July workshop

Genetic structure of northern quoll populations

In collaboration with Murdoch University (Peter Spencer) and other collaborators (Ric How, Linc Schmitt) investigation into the taxonomic status of the NQ and population genetic structure in the Pilbara have commenced using molecular techniques (this study also includes other Pilbara EPBC listed fauna). (see Appendix 4)

Northern Quoll ecology PhD: 2012-2015

PhD student Lorna Hernandez Satin began field work 2013 aims to investigate population dynamics, demography and threats to Northern Quolls, focusing on the Python Pool and Yarrie populations.

Population distribution modeling

Amy Whitehead (DSEWPAC) will be using all available presence/absence data for Pilbara northern quolls to more accurately model the distribution of the species.

Department of Parks and Wildlife group future works
Studies on dietary preference (scat analysis) underway.
GPS radio collars being investigated for use in this project for home range studies.
Karlamilyi National Park site to be investigated in 2014 following recent NQ records

2.6 Project outputs

Pilbara Threatened Species Northern Quoll Workshop

Funded by Roy Hill, Stephen Van Leeuwen (Department of Parks and Wildlife) conducted the workshop bringing together environmental regulators, resource development companies, environmental consultancy companies and university contributors.

ABC Radio

ABC local radio interview with Department of Parks and Wildlife Northern Quoll Research Scientist Judy Dunlop.

Landscope article

Cook, A., 2013. ‘Spotting Quolls in the Pilbara’ Landscope 29:1, Spring edition

Pilbara Threatened Species webpage

Developed with Department of Parks and Wildlife Pilbara Bilby Project team and Gaia Environmental Consultancy allowing external records to be logged.

Appendices

Appendix 1: Northern Quoll Project performance tracking as at September 2013

Project	Timeline	Progress	Funding - committed
Undertake Literature review	October 2011	Completed, report available	Species Information section, DSEWPaC.
Develop a Pilbara wide survey and monitoring program	by July 2012	Completed in June 2013. May need update to include the use of camera traps	BHPBIO sponsorship ($750k over 3 years) Offset funding Fortescue (EPBC 2010/5567): $100k/annum for 10 years
Undertake quoll survey & landholder consultation	Started by July 2013	In progress. . Data from these records to be uploaded to the Pilbara Threatened Fauna Naturemap website	Offset funding Fortescue (EPBC 2010/5567): $100k/annum for 10 years
Implement Pilbara wide monitoring program	July 2012-2022	In progress. Annual monitoring of 10 region wide sites commencing 2014. Monitoring protocol conforms to *Quoll Survey and Monitoring Project, Pilbara Region of Western Australia*.	BHPBIO sponsorship ($750k over 3 years) Offset funding Fortescue (EPBC 2010/5567) : $100k/annum for 10 years
Undertake ecological and demographic study of quolls in the Pilbara	July 2011-2016	In progress. Completed BHP Billiton Iron Ore (BHPBIO) monitoring of rail quarries and rehabilitation sites in the east Pilbara (Yarrie, Shay Gap) 2011-2013 as pilot study. Monitoring informed adjustments to future DSEWPaC survey guidelines.	Offset funding Atlas (EPBC 2009/5167) - $50K/annum for 7 years Fortescue (EPBC 2010/5567) - $100k/annum for 10 years

Appendix 2: Habitat Assessment Sheet

Time:

C	Crest	R	Ridge	F	Flat	D	Closed depression
H	Hillock	S					

Limited clearing .

ining, quarry

VEGETATION

Cover Class:	**<2%**	**2 - 10%**	**10 - 30%**
	over 30	10 - 30m	under 10m
Life Form			
Cover class %			
Dominant species			

	over 2m	1 - 2m	under 1m
Life Form			
Cover class %			
Dominant species			

	Hummock	Tussock	Bunch
Life Form			
Cover class %			
Dominant species			

Veg growth stage

1 Early regeneration
2 Advanced regeneration
5 Senescent phase

Evidence of fire

Frequency	**Intensity**		**Distance to large unburnt habitat**
0 Long unburnt	0 No damage		
Burnt	1 Minor, scars	%	1 < 100 m

Appendix 3: Presence/absence data from pastoralist observations and camera trapping

Date	Site	Species	Observation Type	Survey type	Observer	Comments
Jun-07	Millstream-Chichester NP	*Dasyurus hallucatus*	Sighting	opportunistic	Scott Godley, former DEC snr ranger	George River area
Jun-07	Millstream-Chichester NP	*Dasyurus hallucatus*	scat	opportunistic	Scott Godley, former DEC snr ranger	George River area
Aug-07	Millstream-Chichester NP	*Dasyurus hallucatus*	sighting	opportunistic		Pillingilli Creek area
Dec-11	Millstream-Chichester NP	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	Python Pool
2004	Millstream-Chichester NP	*Dasyurus hallucatus*	sighting	opportunistic		Millstream homestead
2005 – 2011	Mt Florance Station	*Dasyurus hallucatus*	Tracks	opportunistic	Robyn and Tony Richardson (Mt Florance)	Sherlock River
May-13	Mt Florance Station	*Dasyurus hallucatus*	Remote camera	Camera survey	Annette Cook, Julia Lees	Euro Springs
2008	Mt Florance Station	*Dasyurus hallucatus*	sighting	opportunistic	Robyn and Tony Richardson (Mt Florance)	in homestead kitchen drawers
1930s	Mt Florance Station	*Macrotis lagotis*	carcass	opportunistic	Robyn and Tony Richardson (Mt Florance)	hunted for food; later disappeared
2001	Karijini NP	*Macrotis lagotis*	sighting	opportunistic	Robyn and Tony Richardson (Mt Florance)	on road at Hamersley Gorge turnoff

1970s	Pigeon Camp/Salt Creek	*Macrotis lagotis*	sighting	opportunistic	Robyn and Tony Richardson (Mt Florance)	
2006	Karijini NP	*Dasyurus hallucatus*	carcass	opportunistic	Johlene Shalders (DEC Snr Ranger)	on track in Kalamina Gorge
2011	Karijini NP	*Dasyurus hallucatus*	absence record	opportunistic	Bevan Drage (local grader driver)	
1985-2011	Karijini NP	*Dasyurus hallucatus*	absence record	opportunistic	Margie and Maitland Parker (snr ranger)	
1996	Indee Station	*Dasyurus hallucatus*	sighting	opportunistic	Bevan Drage (local grader driver)	
Dec-11	Karijini NP	*Dasyurus hallucatus*	absence record	camera survey	Annette Cook, Julia Lees	
	Coolawanyah Station	*Dasyurus hallucatus*	tracks	opportunistic	Cindy and Kim Parsons (Coolawanyah Station)	on station roads
2009	Coolawanyah Station	*Dasyurus hallucatus*	sighting	opportunistic	Cindy and Kim Parsons	in the homestead
2007	Coolawanyah Station	*Dasyurus hallucatus*	carcass	opportunistic	Cindy and Kim Parsons	homestead - dog killed one in kennel
2008	Coolawanyah Station	*Dasyurus hallucatus*	sighting	opportunistic	Cindy and Kim Parsons	homestead - raiding chook pen
	Yalleen Station	*Dasyurus hallucatus*	absence record	opportunistic	Julie and Mick Percy (station managers)	Not seen in "last few years"
	Yalleen Station	*Dasyurus hallucatus*	sighting	opportunistic	Julie and Mick Percy (station managers)	"a few years ago" - homestead?
7/12/11	Yalleen Station	*Dasyurus hallucatus*	tracks	opportunistic	Julie and Mick Percy (station managers)	in creek bed

2010	Pardoo Station	*Dasyurus hallucatus*	carcass	opportunistic	Graeme Rogers	Quoll living in tractor, killed when started
1995-2005	Bonnie Downs	*Dasyurus hallucatus*	sighting	opportunistic	Graeme Rogers	Many quolls seen
2011	Pardoo Station	*Macrotis lagotis*	sighting	opportunistic	Graeme Rogers	
2011	Mt Edgar Station	*Dasyurus hallucatus*	Sighting	opportunistic	Rosie Doolie	Had photos of quolls
unsure	Cane river conservation reserve	*Dasyurus hallucatus*	trapped	opportunistic	Paul Shadler (DEC caretaker - Mt Minnie)	relocated to cattle pool
1999	Cane river conservation reserve	*Dasyurus hallucatus*		fauna survey	LANDSCOPE expedition	records at old homestead
Feb-12	South of Cane River Conservation Reserve	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	
2011-2012	West Pilbara Iron Ore project area	*Dasyurus hallucatus*	Sighting	opportunistic	Kimberley Flowerdew (Environmental Offiver, API)	This project occupies an area of Red Hill station east and south of the homestead. Monitoring program is in place – trapping and cameras. Area to be set aside for conservation purposes.
2012	Red Hill Station	*Dasyurus hallucatus*	carcass	opportunistic	Leanne and Digby Crocker (Red Hill Station)	homestead - eaten by olive python
2012	Red Hill Station	*Dasyurus hallucatus*	trapped	opportunistic	Leanne and Digby Crocker (Red Hill Station)	hand raised babies orphaned in homestead ceiling

Mar-12	Red Hill Station	*Dasyurus hallucatus*	Sighting	opportunistic	Leanne and Digby Crocker (Red Hill Station)	homestead ceiling
2002	Red Hill Station	*Trichosurus vulpecula*	Sighting	opportunistic	Leanne and Digby Crocker (Red Hill Station)	in tree near homestead
2011	Sherlock Station	*Dasyurus hallucatus*	carcass	opportunistic	Catherine and Jay Peterson (Sherlock Station)	dead in water tank, north of homestead
Apr-12	Sherlock Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	Sherlock River Hwy crossing
1950s-current	Indee Station	*Dasyurus hallucatus*	Sighting	opportunistic	Colin Brierly (Indee Station)	"always been quolls" in granite outcrops and ridges south of homestead: 50 yrs
1950s-current	Indee Station	*Pseudomys chapmani*	burrows	opportunistic	Colin Brierly (Indee Station)	throughout property
1950s-current	Indee Station	*Dasyurus hallucatus*	Sighting	opportunistic	Colin Brierly (Indee Station)	homestead - old shearing shed
Apr-12	Indee Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	homestead
Apr-12	Indee Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	Granite Red Rocks
Apr-12	Indee Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	Chirt Range
1992-current	Mallina Station	*Dasyurus hallucatus*	Sighting	opportunistic	Sandy and Barry Franklin (Mallina Station)	quolls "always been here": 20 yrs
Apr-12	Mt Edgar Station	*Dasyurus*	Sighting	opportunistic	Brian Devroe	on the property in

		hallucatus				outcrops to east of camp
May-12	De Grey Station	*Dasyurus hallucatus*	absence record	opportunistic	Mark Bettini	
7/05/12	De Grey Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	in rocky area adjacent to neighboring Atlas Iron Pardoo mine
2012	Pardoo Mine	*Dasyurus hallucatus*	carcass		Kerstin Weber (Environmental staff, Atlas Iron)	Regularly killed by haul trucks on mine access road
2012	Pardoo Mine	*Dasyurus hallucatus*	Sighting	opportunistic	Kerstin Weber (Environmental staff, Atlas Iron)	common, and frequently seen near infrastructure
1997	Giralia Station	*Dasyurus hallucatus*	Sighting	opportunistic	Denver Blake (Giralia)	record from fishing camp
2012	Giralia Station	*Dasyurus hallucatus*	absence record	camera survey	Annette Cook, Julia Lees	No suitable habitat present
15/10/12	Cane river conservation reserve	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	Cattle Pool, northern border
18/10/12	Mallina Station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	granite boulder area south of homestead
18/10/12	Croydon outstation	*Dasyurus hallucatus*	Sighting	opportunistic	Tom	Resident in the house at the outstation. When one disappears it is usually replaced by another
24/10/12	Mardie station	*Dasyurus hallucatus*	remote camera	camera survey	Annette Cook, Julia Lees	at pool along Fortescue River tributary
unsure	Mardie station	*Dasyurus*	Sighting	opportunistic	Rob	Fortescue River bridge

		hallucatus				
8/04/13	Indee Station	*Dasyurus hallucatus*	trapped	targeted survey	Annette Cook, Julia Lees	Red rock trapping site; 26 individuals, 140 trap nights
10/04/13	Mt Dove mine	*Dasyurus hallucatus*	trapped	targeted survey	Sarah Thomas, Emma Reid, Zoe Stokes (Environmental staff - Atlas Iron)	Translocated individuals from active mining area. Artificial habitat created onsite, camera monitoring detected no quolls to date.
1/05/13	Millstream-Chichester NP	*Dasyurus hallucatus*	trapped	targeted survey	Neal Brougham, DEC senior ranger	Trapped at python pool: 4 individuals/ 120 trap nights
8/05/13	Karijini NP	*Dasyurus hallucatus*	absence record	camera survey	Annette Cook, Julia Lees	Hamersley Gorge road
18/08/13	Karijini NP	*Dasyurus hallucatus*	remote camera	camera survey	Judy Dunlop, Julia Lees	1 individual at Kalamina Gorge
18/08/13	Karijini NP	*Dasyurus hallucatus*	scat	targeted survey	Judy Dunlop, Julia Lees	Kalamina gorge
16/08/13	Karijini NP	*Dasyurus hallucatus*	scat	targeted survey	Judy Dunlop, Julia Lees	Dales gorge
19/08/13	Karijini NP	*Dasyurus hallucatus*	scat	targeted survey	Judy Dunlop, Julia Lees	Joffre's gorge

Appendix 4: Murdoch University Genetic Analysis

YEAR ONE - FINAL REPORT

Genetic Analysis of Northern Quolls from the Pilbara Region of Western Australia

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August, 2013

Summary

- This study used genetic information to investigate a population genetics of northern quolls, *Dasyurus hallucatus* in the Pilbara region of Western Australia.
- It is the largest study undertaken on a top-end predator in the Pilbara.
- The study greatly increases the amount of genetic material available from the region to over 250 individuals; previously just 19 individuals were available.
- Genetic analyses of nuclear markers from the 32 sampling locations are comparable with our previous studies of individuals from 3 sites from the Pilbara region.
- DNA profiles were examined at 11 nuclear genes (microsatellite) from 253 individuals.
- Northern quolls in the Pilbara showed a similar level of genetic variation compared to the limited sample of quolls sampled from the previous study.
- Measures of genetic diversity of northern quolls from the Pilbara were lower than that recorded on the Kimberly mainland.
- The genetic profiles were consistent with our earlier studies that indicate the Pilbara population is a single genetic cluster, suggesting that dispersal of individuals occurs between localities across the region.
- While genetic diversity is lower than in mainland populations in the Kimberly, quolls retain moderate genetic diversity, and show no evidence of recent or long-term population bottleneck.
- Spatial autocorrelation was used to infer a female-biased dispersal at a local level.

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Background

The Federal Governments EPBC Act policy statement 3.25 ***Environmental Protection and Biodiversity Conservation Act 1999 referral guidelines for the endangered northern quoll,*** **Dasyurus hallucatus***,* stipulates under the section **Targeted Survey** that:

> *In Western Australia, both a reconnaissance and targeted survey should conform with a level 1 assessment and level 2 survey described in the Environment Protection Authority (EPA) Position Statement 3, Guidance Statement 56, and EPA and Department of Environment and Conservation (DEC) Technical Guide - Terrestrial Vertebrate Fauna Surveys for Environmental Impact Assessment*

This stipulation has resulted in all targeted surveys for the species being obligated to obtain tissue material from captured animals and for that material to be lodged by consultants with the Western Australian Museum for later genetic analysis and evaluation. More specifically;

- *In Western Australia, tissue samples (ear clippings) should be collected from all individuals captured and analysed with the aim of increasing genetic knowledge of the northern quoll in Western Australia (ethics clearance is required for this procedure). Tissue samples should be sent to the Western Australian Museum with the following details: Weight, sex, pes (left hindfoot measurement), tail diameter / circumference, crown reproductive condition, presence/absence of bite marks and parasites, locality (GPS coordinate in lat and long), collectors name and date.*

A great many consultants representing a large number of consultancy firms have participated in the provision of northern quoll material from across the Pilbara over the past two years and, although the quantity of material is much diminished in recent months, it is this tissue collection that forms the basis for the current study and report. Our appreciation to the numerous collectors and consultants should be acknowledged at this point as they, in combination with the available funding from offsets programs from BHP Billiton, Atlas Iron Limited and Fortescue Metals Group Ltd, have been fundamental to the success of the project.

Funding to undertake the molecular work was facilitated by the Federal Government's Department of Sustainability, Environment, Water, Population and Communities (SEWPaC) though the Western Australian Government's Department Parks and Wildlife (formerly DEC). We acknowledge these sources of funding for the successful completion of the project. We would also implore that anyone surveying northern quolls that collection of material continue, as there are many areas that are presently under-represented (e.g. south-west Pilbara) in this study.

1. Introduction

The largest surviving predatory marsupial in northern Australia, the northern quoll, *Dasyurus hallucatus*, is reportedly in sharp decline in the eastern and central parts of its range, disappearing from most of its former range in northern Queensland and the Northern Territory (Braithwaite & Griffiths, 1994). This decline has been directly associated with the spread of the introduced poisonous prey species (Estoup et al., 2004), the cane toad, *Rhinella marinus*Linnaeus 1758 (Doughty, 2013). Recent studies in northern Australia have illustrated the problems of assigning causal factors to mammal declines. Braithwaite & Muller (1997) postulated declines in populations of many tropical Australian mammals arise from environmental modification caused by climate change and lowered groundwater levels, while Woinarski et al. (2001) proposed it resulted from a combination of habitat alterations resulting from grazing, changed fire regimes, particularly the shrub layer of tropical savannas, as well as predation from feral cats. There is anecdotal evidence that the demise of tropical mammal populations is also occurring in the Kimberley of Western Australia. The northern quoll remains relatively abundant and widespread in two regions of its western distribution, the Kimberley and Pilbara. Both regions have populations on off-shore islands formed within the past 10,000 years with the rise in sea level after the last glacial maximum and typically housing relictual populations. The Pilbara region would appear to be a last strong hold for quoll populations in Western Australia.

Northern quolls formerly occurred almost continuously (and commonly) across some 5000 km of northern Australia from near Brisbane, Queensland to the Pilbara, Western Australia. Of great significance to the conservation of this species across Australia is the observation that several populations appear to have resisted the catastrophic declines associated with the advent of the cane toad in Queensland (Estoup et al., 2004). Understanding the conservation significance of a species requires knowledge about how genetic diversity is organised and how the population is structure prior to the impact of a threatening process. This is needed because it can inform the likely responses and is therefore important in collating strategies for conservation, restoration or stock management. Understanding population processes is particularly important when a species is faced with exposure to a pest that is known to contribute to localised declines elsewhere. Ultimately, the goals of conservation management and restoration of native species relies on adequate information about the pre-threatened ecology of the species, a consideration that is often not assessed until after impact.

The northern quoll has recently been listed by the Australian Government Department of the Environment and Heritage as a vulnerable taxon. It is a classic example of the impact of an introduced threatening process on an iconic native taxon. Since their introduction in 1935, cane toads have spread from the north-east of Australia to inhabit much of the north, and their arrival in the Kimberly region of Western Australia is well advanced, with toads now found 50 km to the west of Lake Argyle, and moving unabated at ~ 50 km/year (D. Pearson, pers. comm.). As a result of this expansion it is expected that northern quolls will show a range contraction in the Kimberley, and later the Pilbara in Western Australia, that will mirror their decline across the rest of northern Australia.

There appears to be little, if any, ecological support for the categorical statements regarding the decline in the northern quoll in Western Australia made by numerous authors and subsequently paraphrased by various others on government. The only published and extensive studies of Western Australian northern quolls that provide detailed biological information are from Schmitt et al. (1989) and How et al. (2009). There remain a number of conservation concerns that may influence the status of northern quolls including habitat modification (controlled-burning, increased pastoral use, extensions of feral populations of introduced mammals), disease susceptibility (e.g. toxoplasmosis) and the introduction of feral carnivores and poisonous prey such as cane toads.

A detailed recent assessment of populations of the northern quoll throughout Western Australia (How *et al*. 2009) documented their genetic differentiation, particularly focusing on insular populations. They noted that populations in Western Australia occur in two discrete geographic regions, the Kimberley and Pilbara, separated by the arid Great Sandy Desert, with many adjacent continental island populations. Both mitochondrial DNA sequences and nuclear (microsatellite) loci revealed clear differentiation of the Kimberley and Pilbara regions. There was little indication that any population within the distribution of the species had experienced recent declines but there was marked variation in sexual dimorphism suggesting diversity in demographic performance has occurred. The northern quoll populations in Western Australia also differ from those in Queensland and the Northern Territory in demographic parameters (Schmitt *et al.* 1989) and represent the last intact populations in Australia that have not experienced major declines concomitant with the spread of the cane toad.

1. 1 Study aims

The study had three specific aims:

1. To document the diversity and 'genetic importance' of the northern quoll, *Dasyurus hallucatus*, in the Pilbara region;
2. Determine if there are patterns of population structure including phylogeography and regional management units;
3. To investigate if there is a relationship between genetic relatedness and spatial distribution. This could then be used to infer the spatial use by quolls in different regions of the Pilbara.

2. Study Area and Methods (Laboratory and Analyses)

2.1 Project area

To put the present study in some past historical framework, a previous study by How et al. (2009) used three sampling sites for the Pilbara region: Dolphin Island (*n*=10 quolls), Woodstock (*n*=2) and Robe River Valley area (*n*=7).

This study incorporated samples from a much larger area of the Pilbara (see Table 2.1), aided by the considerable contribution of samples associated with consultants to the (mineral) resource sector.

2.2 Material for evaluation

The distribution of the northern quoll in Australia and the locations of populations discussed in this report are presented in Figure 2.1.

This study used a total of 572 tissue samples taken from individual northern quolls from the Pilbara, Kimberley and Northern Territory (Table 2.1). These tissue samples are evaluated in this report with respect to the material already available from the detailed genetic evaluations of northern quolls conducted by How *et al.* in 2009.

2.2.1 Sampling locations

We used tissue from 32 sampling locations for the findings in this report. Four sites had multiple locations (in surrounding areas; Table 2.1). Thirteen sites had more than 10 quoll samples collected (per location) and 12 locations had less than five samples. We were able to add an additional 234 quoll samples to our existing dataset.

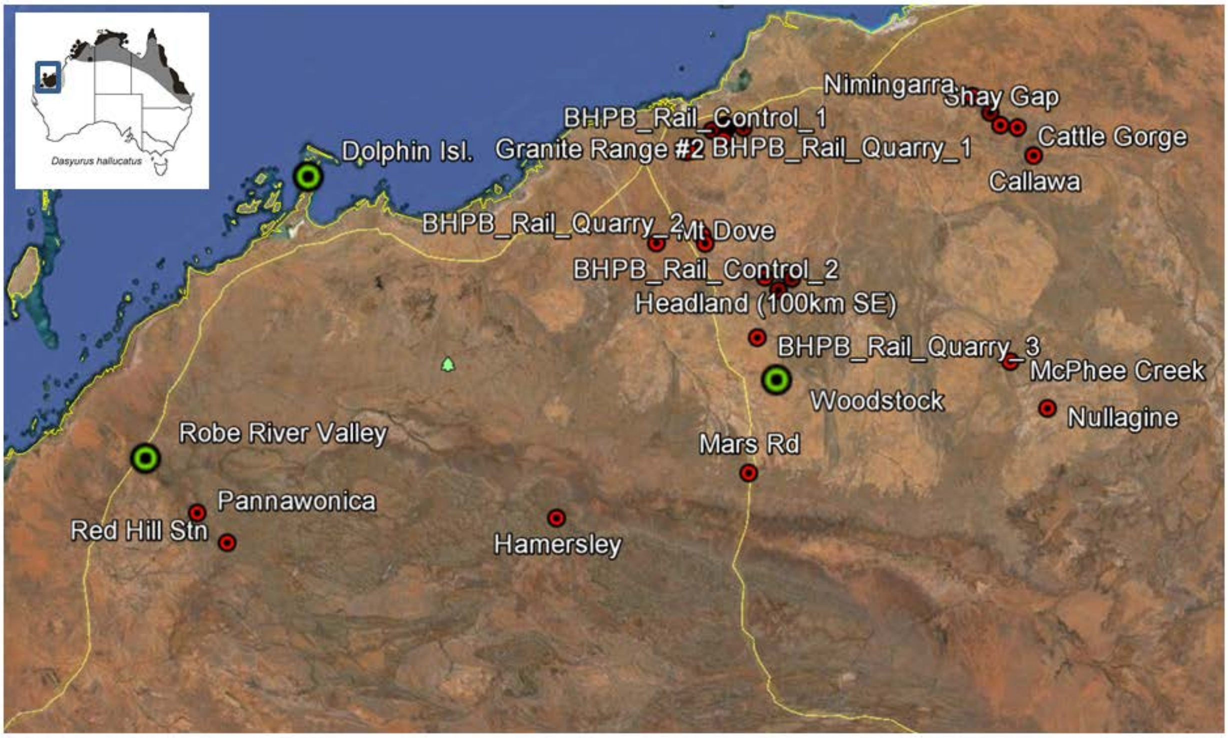

Figure 2.1. General map showing the sampling locations in the Pilbara. The sites marked with a green mark are those originally described in the How et al. (2009) study.

2.3 Molecular Methods

2.3.1 Nuclear microsatellite amplification and analysis

We also amplified 11 microsatellite loci as described in Spencer *et al.* (2007) from 32 sampling locations (Table 2.1). Briefly, PCRs were carried out in a total volume of 30 µl with ~100 ng DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.3 µM of each primer & 1 U *Taq*. Size was determined by co-running a Genescan500 standard (Applied Biosystems, Melbourne). Fragment analysis was carried out on a 3730xl DNA Analyser (ABI systems, Melbourne) and scored with the aid of

GENEMARKER (SoftGenetics). Three control samples from previous studies were run in each PCR run to ensure compatibility between different datasets used in the analysis.

Descriptive statistics and assumptions were calculated using GenAlEx 6.4 (Peakall & Smouse 2006) and HW-QUICKCHECK (Kalinowski 2005). The rarefaction method, as implemented using FSTAT (Goudet 1995), was used to calculate the allelic richness. This method allows a direct comparison between populations because it equalise the sampling effort. We calculated the hierarchical population structure by calculating F_{ST} (measured as θ) and R_{ST} for all pairs of populations using FSTAT (Goudet 1995). Both F_{ST} and R_{ST} values are commonly calculated as measures of genetic divergence when examining population structure (e.g. Hampton et al. 2004).

Evidence of recent population bottlenecks was investigated by testing for a deficiency of heterozygosity using BOTTLENECK (Piry et al. 1999). Due to the relatively small number of polymorphic loci analysed (n=10), a Wilcoxon sign-rank test was estimated. A mixed model of microsatellite mutation was assumed with a single step mutation assumed at 90%, variance of 12, as suggested by Piry et al. (1999) and Hampton et al. (2004).

For the Pannawonica site (with a reasonable sample size of >20 quolls), we investigated their dispersal ability by testing for a relationship between pairwise population genetic measures (Peakall & Smouse 2006) and geographical distance (measured as decimal latitude & longitude) using genetic spatial autocorrelation analysis, performed using the program GenAlEx 6.4 (Peakall & Smouse 2006). The spatial autocorrelation analysis implemented in GenAlEx calculates an autocorrelation coefficient (*r*) for genetic distances (Smouse and Peakall 1999) as a function of geographical distance (km). We used distance classes based on one kilometre classes and generated the 95% confidence intervals around the expectation of no spatial genetic structure using 1000 random permutations. The geographical distance at which the mean *r* value drops below zero has been referred to as the 'neighbourhood size' or 'patch size' (Peakall et al. 2003) and represents the largest spatial scale at which genetic similarity is non-random.

Table 2.1 General sample localities and numbers (*n*) of northern quolls, *Dasyurus hallucatus* used in this study. The locality names can be visualised in Figure 2.1.

Sampling location	Lat (dec.)	Long (dec.)	***Number of samples***
Pilbara			
BHPB Rail sites			
Quarry 1	-20.5283	118.6497	6
Control 1	-20.5209	118.6891	4
Quarry 2	-20.9201	118.6859	17
Control 2	-20.9574	118.6961	5
Quarry 3	-21.3969	118.9050	1
Yarrie sites			
Nimingarra Mine	-20.3940	120.0365	8
Nimingarra control	-20.4739	120.1156	2
Shay Gap	-20.5333	120.1594	2
Cattle Gorge	-20.5497	120.2408	2
Callawa	-20.6814	120.3074	19
Abydos Station	-21.1466	119.0947	39
Headland (100km SE)	-21.1314	118.9661	3
Turner River	-21.1942	119.0259	23
Hamersley	-22.1187	117.8657	1
Mars Rd	-21.9955	118.8069	1
McPhee Creek	-21.5964	120.1114	11
Mt Dove	-20.9357	118.4638	2
Nullagine	-21.8189	120.2742	1
Pannawonica			
Pannawonica 1	-21.9389	116.1297	23
Red Hill Station	-22.0856	116.2608	19
Poondano site			
Poonando Central 1	-20.4555	118.8221	13
Poonando Central 2	-20.4550	118.8324	2
Poonando Central 3	-20.4550	118.8404	5
Poondano West	-20.4544	118.7769	7
Granite Outcrop	-20.4819	118.8301	2
Granite Range 1	-20.4930	118.8252	12
Granite Range 2	-20.5013	118.8259	3
Table Top Hill	-20.4374	118.8439	14
Poondano East	-20.4545	118.9275	5
Robe River Valley	-21.6669	115.9087	10
Woodstock	-21.5888	118.9833	2

Dolphin Island	-20.4833	116.8333	7
Kimberley mainland & islands[1]			
Kimberley	-	-	56
Bigge Island	-14.6021	125.2129	12
Boongaree Island	-15.0881	125.1709	14
Koolan Island	-16.1333	123.7500	200
Northern Territory			
Kakadu, sample 1	-	-	28
Kakadu, sample 2	-	-	36

[1] **The Kimberley sample(s) are pooled from previous and this study, and also include a selection of Kimberley islands (from the single Kimberley population cluster) described in How et al. (2009).**

3. Results

3.1. Molecular Population Genetics: microsatellite

3.1.1 Population structure

All northern quoll samples from the Pilbara clustered in a ‘Pilbara’ population (Fig. 3.1). From the entire sample, 99.7 percent of individuals clustered with their source population (Table 3.1). We detected two mainland populations clusters (Fig. 3.1) in Western Australia, each consisting of a single to 11 geographical sample locations (Fig. 2.1; Table 3.2). The Pilbara appears to form a single, genetically homogenous population cluster. The other cluster from the Kimberley consisted of mainland animals, and populations from islands (described in How et al. 2009). Each coloured (statistically inferred) population will hereafter be referred to as discrete population.

(a)

(b)

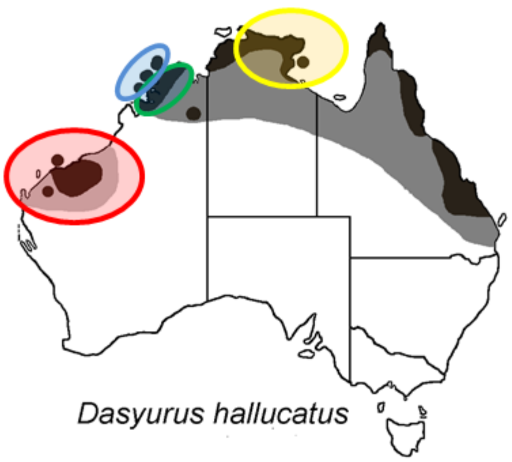

Figure 3.1 Bayesian population structure analysis of the northern quolls, *Dasyurus hallucatus*, to populations, based on 11 nuclear microsatellite loci (a) assuming a population number of K = 4. Individuals are along the x-axis. The y-axis denotes the cumulative posterior probability of an individual's placement in particular population(s). The analysis uncovered (b) four distinct populations (the population sample from Pilbara are in red; the Kimberley mainland in green and adjacent islands in blue and yellow indicating the samples from Kakadu (see Table 2.1).

Table 3.1 The number of quolls, *Dasyurus hallucatus,* which were assigned to their own population (self-population) or clustered with another population (Other pop).

Population	Self-Population	Other Pop	% assigned to another population
Pilbara	234	0	None
Previous study (How et al. 1990)	19	0	None
Bigge Island	13	0	None
Boongaree Island	17	0	None
Kimberley (and islands)	72	2	2.7
Koolan Island	200	0	None
Total (Percentage)	555 (99.7 %)	2 (0.3 %)	

Sampling location	*n*	N_A (*S.D.*)	H_E (*S.D.*)	H_O (*S.D.*)	*F* (S.E.)
A. Pilbara					
BHPB Rail sites					
Rail Quarry 1	6	4.0 (0.5)	0.751 (0.043)	0.727 (0.088)	-0.112 (0.144)
Rail Control 1	4	3.8 (0.4)	0.708 (0.076)	0.727 (0.079)	-0.187 (0.054)
Rail Quarry 2*	17	5.6 (1.0)	0.750 (0.018)	0.702 (0.067)	0.026 (0.091)
Rail Control 2*	5	4.4 (0.4)	0.786 (0.048)	0.782 (0.074)	-0.176 (0.118)
Rail Quarry 3	1	1.7 (0.0)	0.727 (0.141)	0.727 (0.141)	-
Yarrie sites*					
Nimingarra Mine	8	4.8 (0.1)	0.731 (0.025)	0.716 (0.070)	-0.039 (0.089)
Nimingarra control	2	2.5 (0.0)	0.636 (0.084)	0.682 (0.102)	-0.427 (0.093)
Shay Gap	2	2.4 (0.2)	0.636 (0.103)	0.545 (0.125)	-0.141 (0.181)
Cattle Gorge	2	2.7 (0.1)	0.712 (0.087)	0.591 (0.113)	-0.093 (0.149)
Callawa	19	5.6 (0.9)	0.736 (0.030)	0.706 (0.067)	-0.006 (0.096)
Abydos Station*	39	4.9 (2.3)	0.726 (0.030)	0.656 (0.052)	0.078 (0.060)
Headland (100km SE)	3	3.4 (0.2)	0.815 (0.037)	0.712 (0.090)	-0.139 (0.156)
Turner River*	23	6.2 (2.0)	0.705 (0.073)	0.664 (0.085)	0.028 (0.078)
Hamersley	1	1.5 (0.0)	0.545 (0.157)	0.545 (0.157)	-
Mars Rd	1	1.3 (0.1)	0.364 (0.152)	0.364 (0.152)	-
McPhee Creek	11	5.2 (0.8)	0.757 (0.020)	0.660 (0.065)	0.070 (0.079)
Mt Dove	2	2.3 (0.2)	0.697 (0.080)	0.773 (0.104)	-0.573 (0.142)

Nullagine	1	1.6 (0.1)	0.727 (0.141)	0.727 (0.141)	-
Wheatstone/Onslow	1	1.6 (0.1)	0.727 (0.141)	0.727 (0.141)	-
Pannawonica*					
Pannawonica	23	5.5 (1.9)	0.690 (0.072)	0.691 (0.078)	-0.029 (0.051)
Red Hill Station	19	5.5 (1.6)	0.701 (0.072)	0.629 (0.071)	0.076 (0.043)
Poondano site*					
Poondano Central 1	13	5.8 (0.3)	0.764 (0.024)	0.643 (0.055)	0.123 (0.070)
Poondano Central 2	2	2.7 (0.0)	0.727 (0.085)	0.682 (0.122)	-0.24 (0.172)
Poondano Central 3	5	3.9 (0.1)	0.691 (0.033)	0.609 (0.064)	0.003 (0.110)
Poondano West	7	4.7 (0.1)	0.73 (0.066)	0.697 (0.085)	-0.040 (0.086)
Granite Outcrop	2	2.9 (0.1)	0.773 (0.082)	0.682 (0.102)	-0.187 (0.135)
Granite Range 1	12	5.6 (0.3)	0.784 (0.018)	0.738 (0.069)	0.017 (0.090)
Granite Range 2	3	3.0 (0.0)	0.691 (0.049)	0.515 (0.082)	0.112 (0.133)
Table Top Hill	14	6.3 (0.1)	0.753 (0.027)	0.689 (0.044)	0.046 (0.057)
Poondano East	5	3.7 (0.2)	0.664 (0.052)	0.589 (0.061)	-0.029 (0.093)
Robe River Valley	10	5.5 (2.1)	0.726 (0.086)	0.736 (0.182)	
Woodstock	2	1.6 (0.5)	0.636 (0.153)	0.636 (0.145)	
Dolphin Island	7	2.8 (0.6)	0.474 (0.041)	0.390 (0.056)	

Table 3.2 Measures of microsatellite diversity for each sampling location in (a) Pilbara samples, (b) Kimberley and (c) Northern Territory samples. *n*, number of individuals genotypes; N_A, number of alleles; N_E; observed (H_o) and expected (H_E) heterozygosity; F=Fixation index; S.D., Standard deviation; S.E., standard error

Table , continued

B. Kimberley mainland & islands[1]

Kimberley	56	12.1 (2.2)	0.833 (0.024)	0.526 (0.035)	0.357 (0.043)
Bigge Island	13	2.8 (1.1)	2.530 (1.060)	0.345 (0.060)	0.302 (0.039)
Boongaree Island	17	3.3 (1.0)	4.040 (1.010)	0.420 (0.060)	0.372 (0.039)
Koolan Island	200	5.0 (2.2)	0.459 (0.030)	0.423 (0.037)	0.079 (0.052)

C. Northern Territory

Sample site 1	28	7.6 (2.5)	0.718 (0.075)	0.718 (0.093)	-0.012 (0.071)
Sample site 2	36	6.9 (3.1)	0.723 (0.074)	0.661 (0.091)	0.066 (0.084)

In general, there were only small differences in the diversity amongst sampled sites from the Pilbara. Estimates of the allelic diversity (number of alleles) showed the greatest variation, with between two and six alleles/sample site, however this measure is highly influenced by sample size. Genetic diversity, as measured by heterozygosity did not vary to any degree, with most sampling sites displaying about 70% heterozygosity (Table 3.1). The fixation index (*F*) suggests that quolls at any one of the sampling sites showed random mating (i.e. F~0, and +1 highly inbreed). The exception was the reduced diversity found on Dolphin Island, which is not uncommon as many studies have found reduced diversity in the insular island samples, compared with their mainland counterparts.

Table 3.2 Measures of genetic diversity in each of the clusters of northern quolls from the Pilbara, Dolphin Island (in the Pilbara), Kimberley mainland, Kimberley islands (pooled) and Kakadu in the Northern Territory. *n*, number of individuals genotypes; N_A, number of alleles; N_E; observed (H_O) and expected (H_E) heterozygosity; F=Fixation index (values around 0 suggest mating is random, +1 highly inbreed); S.D., Standard deviation; S.E., standard error

Sampling location	***n***	N_A (*SD*)	H_E (*SD*)	H_O (*SD*)	*F*
Pilbara	252	10.36 (1.21)	0.772 (0.018)	0.689 (0.040)	0.101
Dolphin Island (Pilbara)	7	2.80 (0.6)	0.474 (0.041)	0.390 (0.056)	0.287
Kimberley mainland	38	11.09 (0.78)	0.839 (0.022)	0.612 (0.020)	0.254
Kimberley islands	218	8.01 (0.67)	0.514 (0.029)	0.417 (0.0033)	0.186
Kakadu, N.T.	64	7.91 (1.05)	0.731 (0.076)	0.689 (0.040)	0.041

3.1.2 Descriptive statistics and population genetic 'health'

A total of 252 northern quoll samples were successfully scored at 11 highly variable microsatellite loci (Table 3.3). All locations and sample groups were polymorphic at all loci with moderate variation, containing between 4 and 9 alleles per locus (7.20 ± 4.25 SD) with heterozygosity (H_E) ranging from 27 to 90% (mean = 0.74 ± 0.021; Table 3.2).

Table 3.3 Allelic variability for different nuclear markers for the Pilbara samples (n=252 quolls). *n*, number of individuals genotypes; N_A, number of alleles (standardised for sample size); N_E, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Marker name	N_A	N_E	H_O	H_E
3.1.2	3.73	3.01	0.814	0.783
3.3.1	3.77	2.90	0.639	0.670
3.3.2	4.13	3.15	0.703	0.727
4.4.2	4.27	3.22	0.739	0.689
pDG1A1	5.13	3.92	0.810	0.847
pDG1H3	2.93	2.18	0.551	0.552
pDG5G4	3.13	2.55	0.609	0.649
pDG6D5	4.20	2.98	0.734	0.737
pDG7F3	4.03	3.19	0.371	0.606
Sh3o	2.80	1.96	0.390	0.491
Sh6e	3.30	2.59	0.747	0.738

A sample containing more than five quolls in a location will capture 80% of the information (Fig. 3.3) and single individuals are useful for assignment testing. Estimating the number of alleles is known to be sensitive to sampling effects. Measures such as allelic number are more useful with samples of five (or more) quolls (Fig. 3.3), however most measures appear to be relatively robust to sampling effects.

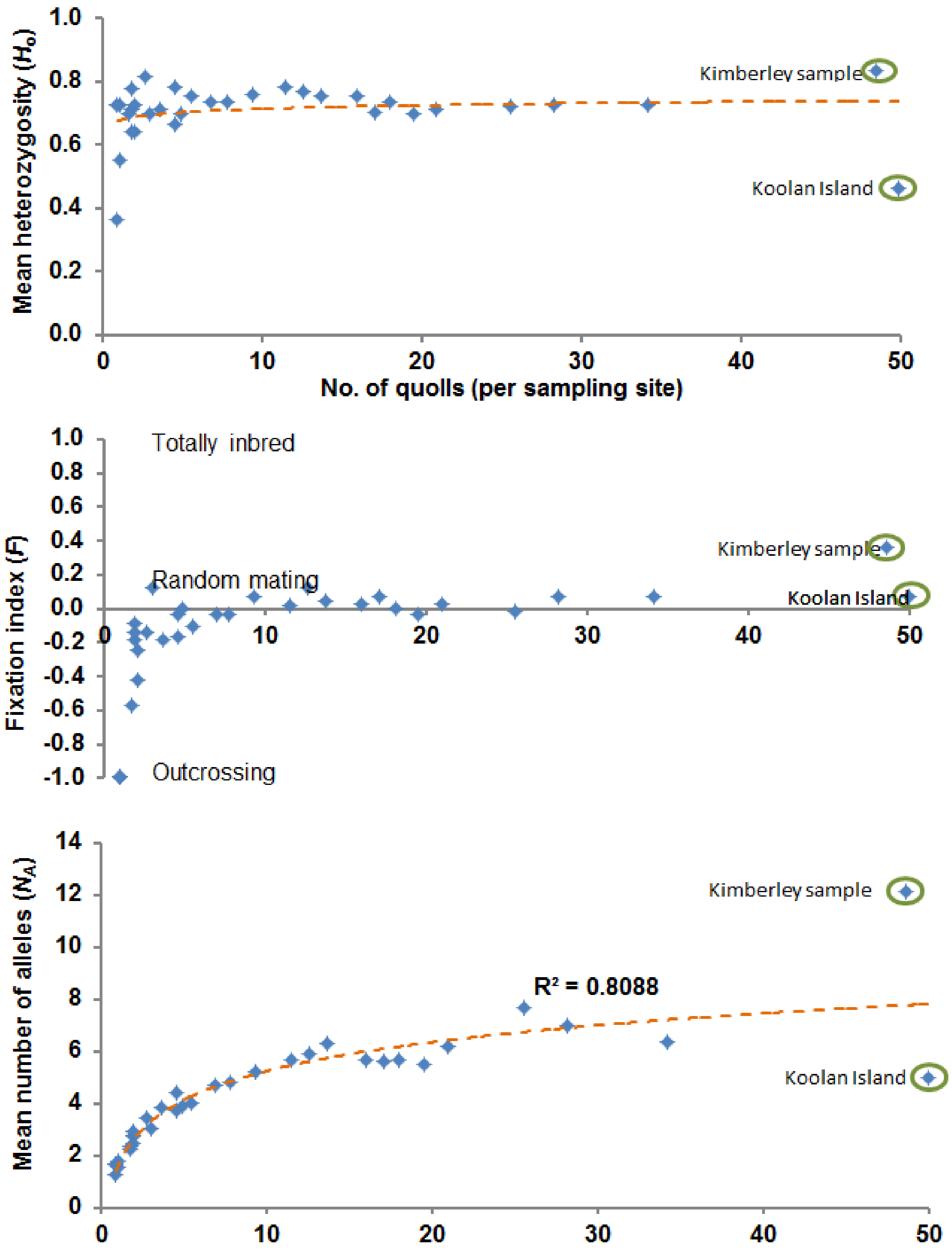

Figure 3.3 Measures of genetic variation, including heterozygosity (H_E, top), fixation index (*F*, middle) and mean number of alleles per locus (N_A, bottom) as a function of sample size.

3.1.3 Detection of recent and long-term bottlenecks

No northern Quoll population showed evidence of either a recent (*P* > 0.20; Table 3.5) or long-term bottlenecks (e.g. allelic diversity, heterozygosity; Table 3.2). Not surprisingly, the populations found on all mainland locations retain higher levels of diversity and no genetic signatures of long-term reduction in diversity and effective population sizes, suggesting that these populations do not appear to have experienced reductions in numbers (i.e. bottlenecks), unlike the findings from small, island populations of quolls.

Table 3.5 The detection of a genetic bottleneck was not found in any of the northern quoll populations.

Inferred population	n	Significance (*P*-value)	Shifted mode	Genetic bottleneck
Pilbara	24.9	0.2065	Normal	No
Kimberley	72.7	0.3501	Normal	No
Koolan Island	200.0	0.2158	Normal	No
Kakadu, N.T.	64.0	0.1844	Normal	No

3.1.4 How unique are the populations? Differentiation among and within populations?

Global (pooled) estimates of F_{ST} (0.277) and R_{ST} (0.333) between the four northern quoll populations identified were similar and indicated low to moderate levels of genetic differentiation amongst these populations. Pair-wise F_{ST} and R_{ST} values (Table 3.6) indicated low levels of differentiation between all pairs of populations (i.e. values ~0.1), except between the Pilbara and Kimberley populations (F_{ST} = 0.03), where genetic differentiation was lower than that found between the island populations.

Table 3.6 Pairwise F_{ST} estimates of population differentiation among four northern quoll populations, based upon the observed genotypes that were estimated from 11 microsatellite loci. Values above 0.1 indicate a high degree of genetic differentiation.

	Kimberley	Kimberley Islands	NT
Kimberley	-		
Kimberley Islands	0.092	-	
NT	0.098	0.208	-
Pilbara	0.033	0.147	0.102

3.1.5 Localised dispersal: Spatial autocorrelation

Spatial structure analysis was limited to an analysis of samples from Panawonica as this population had a reasonable number of adult animals. Only females showed a significant spatial structure (p=0.011, Fig 3.4). Females of northern quolls had a significant (p=0.005) positive mean autocorrelation coefficient value (r) for the first three distance class (i.e. pairs within 15km of distance are significantly more related than random pairs). The r value intercepts zero at around 15km suggesting this is their neighbour size. In support of this, the mean area-span (based on sampling sites) was approximately the same distance.

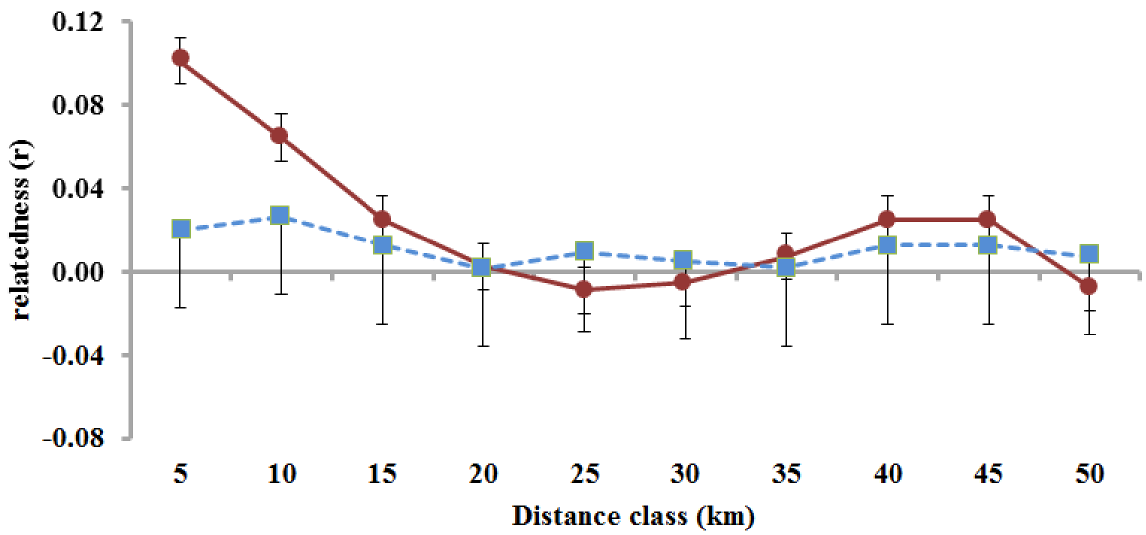

Figure 3.4. Multilocus spatial autocorrelation analyses for northern quolls showing that the relatedness between quolls with increasing distance class (km) in females (red) but not males (blue). Data points are correlation coefficient values (*r*) of the genetic distance between northern quolls

within each 5 km distance class. Standard error bars are not shown for clarity.

4 Discussion

4.1 Summary

The study has provided valuable information on the genetic structure and performance of quolls from the Pilbara region of Western Australia. Using genetic information to investigate the population genetics of northern quolls, *Dasyurus hallucatus*, the study increased the amount of genetic material available from just 19, to over 250 individuals. Genetic analyses of nuclear markers from the 32 sampling locations are comparable with our previous studies of individuals from 3 sites within the Pilbara region. The DNA profiles identified that the northern quolls showed a similar level of genetic variation, compared to the limited sample of quolls sampled from a previous study and they were lower compared with the Kimberly mainland. The genetic profiles identified a single Pilbara genetic cluster and dispersal of individuals occurs between localities in the region. While genetic diversity is lower than that found on the mainland populations in the Kimberly, quolls retain moderate genetic diversity, and show no evidence of recent or long-term population bottlenecks and we infer a female-biased dispersal at a local level.

4.2 How important are the sampling areas in terms of quoll diversity?

Genetically, northern quolls in the Pilbara region are remarkably similar to quolls found at other locations, a finding that is in agreement with recent mitochondrial work on quolls by How et al. 2009 for larger Kimberley populations. Population structure analysis shows that they clearly group well within a ‘Pilbara’ cluster, undifferentiated from animals that have been sampled from the Pilbara previously. It should be noted that the sampling was focused on the north east of the species Pilbara distribution, and it would be of great interest to understand the diversity in its western distribution, where it apparently is at lower densities.

4.3 How unique are quoll genetics in the Pilbara compared with other quoll populations?

In terms of genetic diversity, the population is similar to a number of other studied northern quoll populations, including animals collected from a number of populations (but smaller marker number) from the Northern Territory (H_E 0.135-0.643; Cardoso et al. 2009), the Pilbara and Kimberley (Table 3.2; How et al. 2009). The quolls from the Pilbara represent a substantial increase in sampling effort, but the diversity of the site is not significantly larger than our previous estimates from the Pilbara (Table 3.4; see also How et al. 2009). Overall the results suggest that the population of northern quolls in the Pilbara region have moderate levels of genetic diversity and show no signs of population decline.

4.4 How are the quolls from Pilbara spatially organised and how far can they disperse?

The general detected trend suggests a sex biased dispersal with females showing a stronger philopatric behavior than males, which appear to disperse. However, this is based on a relatively small sample, from a single sampling site (Panawonnica), and clearly need to be explored with a larger dataset at more regional scale(s). The dispersal distance, as defined by a 'neighbour-size' appears to vary, but can be up to 15 kilometers. While we do not have demographic data to examine the movement pattern of quolls, the genetic analysis indicates that quolls are capable of moving widely over the area, and that this information will be enhanced by the increasing amount of radio tracking information on the species in the Pilbara.

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Targeted survey guidelines for the endangered northern quoll, *Dasyurus hallucatus*

The Federal Governments EPBC Act policy statement 3.25 Environmental Protection and Biodiversity Conservation Act 1999)

Targeted survey

A targeted survey is recommended for any proposal occurring within the modelled distribution of the species (see Figures 1-5) where the reconnaissance survey identifies the presence of quolls and / or habitat critical to the survival of northern quoll.

The objective of the targeted survey should be to determine the relative abundance and distribution of northern quolls likely to be impacted by the proposed development. The survey protocol should be designed so that the total population of northern quolls in the impact area can be calculated. A targeted survey should be undertaken pre development and during the months of May, June, July or August (primarily to avoid any disturbance during the reproductive period) and involve a trapping program using preferably wire cage traps or large size Elliot traps.

As a minimum, a targeted survey should consider the following:

- Carefully configure the trapping program to address project impact and non-impact zones so that results are adequate to inform monitoring programs and project siting options.
- Trapping should be concentrated in habitat critical to the survival with some consideration of non-rocky foraging and dispersal habitats.
- In Western Australia traps should be set for seven consecutive nights, unless two or more individuals are caught twice, in which case the traps should be closed after four nights of trapping.
- In the Northern Territory and Queensland, traps should be set for a minimum of three nights with the aim of sampling as many sites possible over the three nights
- Where large Elliott traps are the primary trapping technique, a minimum of four cage traps should be used per trap configuration.
- To be considered effective, traps should be baited with sardines or a bolus mix of oats and peanut butter with honey being optional. Chicken wings and diced bacon are also optional.
- Traps should be rebaited at least every second day (baits should be fresh), cleared within 2-3 hours of sunrise and have adequate shade cover during the day. Consideration should be given to closing traps during the day to eliminate by-catch and potential heat stress issues.
- In Western Australia, tissue samples (ear clippings) should be collected from all individuals captured and analysed with the aim of increasing genetic knowledge of the northern quoll in Western Australia (ethics clearance is required for this procedure). Tissue samples should be sent to the Western Australian Museum with the following details: Weight, sex, pes (left hindfoot measurement), tail diameter / circumference, crown reproductive condition, presence/absence of bite marks and parasites, locality (GPS coordinate in lat and long), collectors name and date.

- o Targeted surveys may be supplemented by one of several non-invasive survey techniques such as latrine searches in habitat critical to the survival, use of motion sensitive cameras and / or hair tubes. These methods should however not be relied upon to demonstrate northern quolls are not present in an area.

Targeted survey effort

- Trapping effort for a targeted survey should be determined by the formula $y = 50x^{0.5}$, where y is the number of trap-nights and x is the area of potential northern quoll habitat in hectares.
- Trapping effort is calculated as the number of traps by the number of nights of trapping (e.g. trap-nights).
- For linear habitat critical to the survival of the species (e.g. gorges, major drainage lines, breakaways less than 100 m wide), 1 trap per 100 linear metres is recommended.

Those wishing to demonstrate the absence of northern quolls in an area, or who are planning trapping programs over large areas of northern quoll habitat (>15 000 ha), should contact the department to discuss survey design and effort. Please send an email to speciespolicy@environment.gov.au.

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