# The effect of habitat fragmentation and population isolation on the genetic diversity, reproductive status and population viability of the southern hairy-nosed wombat (*Lasiorhinus latifrons*) in South Australia



**Elisa Sparrow**
Bachelor of Applied Science (Exercise and Sport Science)
Bachelor of Science (Honours)

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School of Earth and Environmental Sciences
The University of Adelaide
Adelaide, SA 5000

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## Abstract

A range of negative consequences associated with habitat fragmentation and population isolation have been demonstrated in a variety of animal species. Such consequences include inbreeding, reduced genetic diversity, increased mortality of young, reduced fecundity and compromised male fertility. Since the time of European settlement, many populations of the southern hairy-nosed wombat (*Lasiorhinus latifrons*) have become fragmented and isolated throughout South Australia, particularly on the Yorke Peninsula. This study aimed to examine the impact of habitat fragmentation and population isolation on the genetic diversity, seminal quality and reproductive success of the southern hairy-nosed wombat. The results showed there were very few wombats remaining on the Yorke Peninsula, with a total of 643 individuals estimated within 24 colonies all of which were geographically isolated by cleared agricultural land. Of these 24 colonies, 21 were estimated to have < 20 animals. Southern hairy-nosed wombats from two of these small isolated Yorke Peninsula colonies, namely Urania and Kulpara, were found to be genetically differentiated from one another, suggesting minimal current migration between these two colonies. These wombats were also genetically differentiated from wombats within the large population at Swan Reach, which is part of a continuous population in the Murraylands. High mean observed heterozygosity values were found in wombats from Urania and Kulpara (0.69 and 0.74 respectively), and these values did not differ significantly from that of the Swan Reach population (0.71). Allelic diversity was slightly lower in the colonies on the Yorke Peninsula; but this was not statistically significant from the population in the Murraylands. Inbreeding was not detected in any population. Despite this, wombats from the Yorke Peninsula were found to be smaller in body morphology and have larger testes. These animals also had significantly lower ejaculate volumes, with greater numbers of sperm morphological abnormalities in the ejaculate. A trend for lower sperm concentration and sperm motility in wombats from the Yorke Peninsula was also observed. The lower seminal quality in Yorke Peninsula male wombats was not reflected in a reduction in the reproductive success of the population, with all three populations examined exhibiting a similar number of females with pouch young, and in late lactation. There was, however, an unequal sex ratio within the Urania population, with a lower number of adult females in the population during the breeding season. The results from this study suggest that colonies of southern hairy-nosed wombats on the Yorke Peninsula are in danger of localised extinction if not appropriately managed and conserved. Future study directions and possible management techniques are discussed in order to minimise localised extinctions and maintain the viability of southern hairy-nosed wombats on the Yorke Peninsula and throughout South Australia.

## **<u>Declaration</u>**

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Elisa Sparrow

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## **Chapter 1: General Introduction**

This introduction outlines the ecology involved in population fragmentation and the general ecology of the southern hairy-nosed wombat, so that, in subsequent chapters, the potential risks associated with population fragmentation in this species of wombat can be investigated and discussed.

## **1.1. Risks associated with habitat fragmentation**

### *1.1.1. Population isolation*

Land clearance causes fragmentation of habitat, where large continuous patches of habitat become subdivided into smaller areas. This practice not only divides the natural vegetation into isolated patches of habitat, but also may result in populations of any resident animal species becoming fragmented (Primack 1998). The relationship between the level of habitat fragmentation, and fragmentation of an animal population is often unknown (Caizergues *et al.* 2003). Fragmented populations of animals may be considered to be similar to island populations, with isolated patches of vegetation and animal communities surrounded by a highly modified landscape (Saunders *et al.* 1991; Primack 1998). The extent of isolation (i.e. partial or complete) depends on numerous factors, such as distance between the patches, level of connectivity between the remnant patches, the species-specific dispersal patterns, species mobility, the degree of modification of the surrounding landscape, and the suitability of the surrounding habitat for animal movement (Saunders *et al.* 1991; Bowne *et al.* 1999). For example, in bush crickets (*Metrioptera roeseli*), poor dispersal was associated with an absence of migration into the surrounding landscape (Berggren *et al.* 2002). Lower dispersal rates can result in the complete isolation of fragmented populations with similar population dynamics to that of an island population. In small mammal species, such as cotton rats (*Sigmodon hispidus*)*,* prairie voles (*Microtus ochrogaster*), and deer mice (*Peromyscus maniculatus*), dispersal is difficult in a fragmented landscape as individuals have to pass through unsuitable habitat (and across relatively large distances) in order to disperse (Diffendorfer *et al.* 1995). These species will therefore tend to disperse less frequently than those in a continuous habitat, due to the high energetic costs and risks associated with movement across the modified landscape (Diffendorfer *et al.* 1995).

In mammalian species, both breeding and natal dispersal is generally male biased (Greenwood 1980), with dispersal in many species being an important mechanism for inbreeding avoidance (Wolff 1993). However, lack of dispersal does not necessarily mean inbreeding will result, with some species displaying kin recognition and hence avoidance of

inbreeding (Pusey and Wolf 1996). In small populations, although inbreeding avoidance may be present, species may still exhibit the genetic and reproductive disadvantages associated with a small effective population size (i.e. the number of individuals that can contribute genes to the next generation) (Taylor 2003). These potential genetic and reproductive problems will be described in detail in the subsequent sections below.

Genetic diversity can be measured by numerous methods, however the most common are heterozygosity (i.e. the proportion of individuals that have two different alleles at a loci), and allelic diversity (i.e. the number of alleles present at a particular loci within the population) (Allendorf and Luikart 2007). Dispersal patterns, and hence gene flow, can be influenced by fragmentation and the extent of isolation, which together can impact population genetic structure (Frankham *et al.* 2002). For example, an island population of grey wolves (*Canis lupus*) was found to have lower genetic variability compared to mainland populations of the same species (Wayne *et al.* 1991). The same is seen in island populations of the black-footed rock-wallaby (*Petrogale lateralis*), and koalas (*Phascolarctos cinereus*), where genetic diversity is extremely low compared to the mainland populations (Eldridge *et al.* 1999; Cristescu *et al.* 2009). This phenomenon is not restricted to isolated or island populations. For example, populations of the Eurasian red squirrel (*Sciurus vulgaris*) that are small, and partly isolated, have lower genetic diversity due to reduced immigration (Trizio *et al.* 2005). Similarly, black grouse (*Tetrao tetrix* L.), which are normally good dispersers, have restricted gene flow following habitat fragmentation (Caizergues *et al.* 2003).

*1.1.2. Inbreeding and reduced genetic variation*

Inbreeding occurs when related individuals mate and produce offspring with decreased allelic variation in the subsequent generation, and hence increased homozygosity (Falconer 1981; Frankham 1995; Eldridge *et al.* 1999; Keller and Waller 2002; Taylor 2003).Within populations, inbreeding can result from population isolation, bottlenecks, or founding by a small number of individuals (Sherwin and Murray 1990; Houlden *et al.* 1996). It is a significant problem in small isolated, and island populations, where there is restricted mate choice, reduced gene flow and a low effective population size (Frankham 1995; Keller and Waller 2002).

Inbreeding increases the risk of exposing deleterious, recessive alleles and furthermore, may increase the percentage of individuals within a population that are homozygous for alleles identical by descent. This often results in inbreeding depression, which can reduce the fitness of a population by alterations to the reproductive and physiological traits of an individual (Frankham *et al.* 2002; Keller and Waller 2002; Taylor

2003). Inbreeding depression has been associated with an increase in the rate of embryo, newborn and juvenile mortality (Johansson and Rendel 1968; Keller and Waller 2002; Taylor 2003). For example, small litters and poor juvenile survival of Isle Royale grey wolves (*Canis lupus*) were found to be associated with high levels of inbreeding (Wayne *et al.* 1991). Reduced litter size and high juvenile mortality may ultimately play a role in the decline of population size. The impact of inbreeding depression is not always observable, however, as its presence within the population is not always reflected by population size (Frankham *et al.* 2002).

*1.1.3. Inbreeding depression and fertility*

Inbreeding depression may influence the survival rates of adults and young, as well as other factors such as fecundity (including fertility and pregnancy rates), mating performance, and parental care (Ryan *et al.* 2003). For example, in a study on dogs, pregnancy rates were found to be significantly lower in inbred groups compared to outbred individuals, with outbred dogs producing at least one more puppy, on average, in each litter (Wildt *et al.* 1982). Similarly, for inbred cows that were mated with related, inbred bulls, the number of pregnancies was lower (36.8%) compared with cows mated with unrelated bulls (65.7%) (Johansson and Rendel 1968). In an isolated lion (*Panthera leo)* population in Tanzania, no immigration has occurred in the past 25 years and within this time, the annual reproductive rate has decreased, which is thought to be related to the 10% decrease in heterozygosity that has occurred (Packer *et al.* 1991).

Two species of felids with very low genetic variation are the cheetah (*Acinonyx jubatus*) and the clouded leopard (*Neofelis nebulosa*) (Newman *et al.* 1985). Comparisons of these two species with those of other felids with higher genetic diversity, has revealed that the cheetah and the clouded leopard have significantly lower sperm motility and lower sperm concentration (sperm number/ml of ejaculate). In addition, sperm morphology also showed great variability (i.e. pleomorphic sperm), with a high number of morphological abnormalities in both the sperm head and the flagellum, suggesting an association between decreased seminal quality and low genetic variation (Wildt *et al.* 1983; Wildt *et al.* 1986). Similar results have also been found when comparing three different populations of lion (*Panthera leo*) in Serengeti National Park, Ngorongoro Crater (Africa) and Sakkarbaug Zoo (India) (Wildt *et al.* 1987a). The Serengeti National Park population spans a large area, with a large number of individuals present, and had the greatest genetic variation. In contrast, the lions at Ngorongoro Crater, that stem from only 6 – 15 founding individuals, were considered isolated. The Sakkarbaug Zoo lions, that have suffered a large population contraction, have also been found

to be highly genetically monomorphic, and, subsequently, had the lowest genetic variation of the three populations. When ejaculate characteristics were compared between the three populations, the lions from the Sakkarbaug Zoo had a lower mean ejaculate volume, sperm motility rating and fewer motile sperm per ml of ejaculate (Wildt *et al.* 1987a). In addition, this population was found to have the greatest percentage of pleomorphic sperm (i.e. a range of different structural forms) with the highest number of sperm abnormalities present in the head and tail (flagellum). The population with the highest level of heterozygosity, the Serengeti population, had the least number of pleomorphic sperm and the lowest incidence of abnormal sperm morphologies (Wildt *et al.* 1987a). The Sakkarbaug lion population also had blood taken at three intervals, with results showing these lions to have a lower mean testosterone concentration, as well as the poorer seminal quality, compared to the Serengeti population (Wildt *et al.* 1987a). This can have an effect on the development of the male sex characters, accessory glands and sexual behaviour (Hickman *et al.* 2001), as well as possibly impairing spermatogenesis. From these results it has been concluded that, in lions, a bottleneck event and the subsequent reduction in genetic variation was associated with reduced seminal quality and lowered testosterone concentration (Wildt *et al.* 1987a).

A decrease in seminal quality could result in reduced male fertilising capacity within a population. The latter has been revealed in humans, where seminal quality has been shown as an important parameter in fertility and pregnancy. A study by Donnelly *et al.* (1998), on both males and females, has found a significant correlation between fertilisation rate, sperm motility and the percentage of sperm with abnormal head morphology. Males associated with lower fertilisation rates typically produced sperm of poorer motility, with a higher proportion of abnormal sperm types (Donnelly *et al.* 1998). In felids, sperm with the lowest success rate of penetration of the egg were those with abnormal morphology (Howard *et al.* 1991). Semen can, therefore, be considered high quality and more likely to have a greater success rate in fertilisation if there is a low prevalence of abnormal sperm morphologies and good sperm motility. In eutherian mammals, abnormal sperm morphology can take on a range of different forms; for example a coiled or bent flagellum, an abnormal mid-piece or acrosome, a broken neck or an undersized or oversized nucleus (Wildt *et al.* 1987a).

The association between inbreeding and seminal quality has not only been examined in felids, but also in other species such as dogs, mice and gazelles. In general, similar results have been observed (Wildt *et al.* 1982; Gomendio *et al.* 2000; Margulis and Walsh 2002), supporting the hypothesis that inbred strains of animals, in general, are less fertile than outbred strains (Johansson and Rendel 1968; Beatty 1970; Wildt *et al.* 1982; Wildt *et al.* 1983; Wildt *et al.* 1986; Wildt *et al.* 1987a; Wildt *et al.* 1988; Donnelly *et al.* 1998).

*1.1.4. Increased local extinctions*

The association between inbreeding, low genetic variation, and population reproductive status (eg decreased fertility, low juvenile survival rate, sperm abnormalities) can increase the probability of local extinctions. This association has been demonstrated in butterflies (*Melitaea cinxia*) where high levels of homozygosity were found to be associated with an increased risk of extinction, due to lower larval survival, reduced adult life span and decreased egg hatching rates (Saccheri *et al.* 1998). In addition to a reduction in reproductive fitness, inbreeding depression may reduce the chance of an individual adapting successfully to an environmental or physiological change (Keller and Waller 2002; Taylor 2003), thereby decreasing population viability and heightening the risk of extinction (Johansson and Rendel 1968; O'Brien *et al.* 1985; Keller *et al.* 1994; Frankham 1995; Keller and Waller 2002). Increased extinction risks associated with inbreeding have been suggested from studies on song sparrows (*Melospiza melodia*) (Keller *et al.* 1994) and cheetahs (*Acinonyx jubatus*) (O'Brien *et al.* 1985). Findings have shown that sparrows with the highest genetic variation were the survivors of two major population crashes (Keller *et al.* 1994), and cheetahs, that have a genotype that is similar to that of highly inbred laboratory and domestic animals (Wildt *et al.* 1983; O'Brien *et al.* 1985; Wildt *et al.* 1987b), were found to be extremely vulnerable to a feline immunological virus (O'Brien *et al.* 1985). Research has shown a remarkably large number of alleles at loci to be associated with disease resistance, therefore, if genetic variation is reduced (particularly in the form of allelic diversity), the population is more susceptible to health problems and/or disease, and thus potentially an increased risk of extinction (Allendorf and Luikart 2007). It has been suggested, therefore, that for populations to survive changes in environmental conditions it is important to have genetic variation (Frankham 1995). These observations have been made in animals from both captive and wild populations, indicating that problems associated with inbreeding are present in both.

Studies on two marsupial species in Australia, *Antechinus agilis* (Banks *et al.* 2005) and *Lasiorhinus latifrons* (southern hairy-nosed wombat) (Walker *et al.* 2008a; Walker *et al.* 2008b), have shown that habitat fragmentation and the associated population isolation alters the breeding behaviour of the isolated populations, when compared to continuous populations of the same species. In both species, dispersal was restricted in the sex that typically dispersed, which led to altered kin relationships (Banks *et al.* 2005; Walker *et al.* 2008a; Walker *et al.* 2008b). Long term population isolation under these circumstances can result in a decrease in the viability of these populations (Walker *et al.* 2008a; Walker *et al.* 2008b).

An increased risk of local extinctions associated with habitat and population fragmentation is not restricted to just those variables associated with genetic diversity and

inbreeding. For instance, natural disasters, such as fire or drought, may result in remnant animal populations perishing, or undergoing marked fluctuations in breeding and recruitment rates. Re-colonising these populations can be difficult if the surrounding habitat is unsuitable for dispersal, or the suitable habitat patches too distant from one another to enable adequate dispersal (Merriam 1991). In addition, entering the inhospitable surrounding habitat can be detrimental for some species as a lack of shelter may make them easy prey for predators, vehicles, and hunters (Hussey *et al.* 1989). The inability to re-establish diminishing or perished populations may therefore increase the risk of local extinctions for these populations (Henle *et al.* 2004). This extinction risk following a catastrophic event would be exacerbated further if inbreeding depression was also present and therefore population reproductive success was reduced (Frankham *et al.* 2002).

There is considerable research suggesting genetic factors contribute to problems associated with fragmentation, however, factors such as natural disasters and the species ecology (e.g. degree of human impact, population size, and distance between populations) should also be considered.

*1.1.5. Habitat fragmentation in South Australia*

Throughout much of Australia, land has been cleared for agricultural activity. In South Australia, only one fifth of the land has sufficient rainfall for cropping pastures, and by 1985, 80% of this land had been cleared. Throughout the 1980s, land in South Australia was cleared at a rate of approximately 28, 800 hectares per year for various agricultural practices, such as livestock grazing, cropping, and pine plantations (particularly in high rainfall areas of the southeast). The consequence of this is that South Australia now has the smallest area of woodland and forest estate of any state in Australia. On the Yorke Peninsula there is only approximately 10% of the original native vegetation remaining (SA Department for Environment and Heritage).

The following section will examine the general ecology and physiology of the southern hairy-nosed wombat, and then discuss the potential risks associated with habitat fragmentation for this species.

**1.2. Life history and biology of the wombat**

*1.2.1. Phylogeny and morphology*

Within the marsupial family Vombatidae, there are three extant species of wombat, *Lasiorhinus krefftii* (northern hairy-nosed wombat), *Lasiorhinus latifrons* (southern hairy-nosed wombat) and *Vombatus ursinus* (common wombat) (Wells 1989, 1995; St John 1998). All species are endemic to Australia (Strahan 1995).

Southern hairy-nosed wombats, the focus of this study, are large marsupials, with adult weights ranging from 19 to 38 kg (Wells 1989; Taggart and Temple-Smith 2008). These wombats are physically distinguished from common wombats by their furry muzzles, soft coats, and long, bare ears (St John and Saunders 1989). Common wombats on the other hand, have bare noses, coarse coats, and short furred ears. The two hairy-nosed species can also be differentiated physically, with the northern hairy-nosed wombats being larger, with body lengths up to 1.3 metres, and with larger, squarer muzzles, compared to the southern hairy-nosed wombat which reaches a maximum of 1 metre in length (Johnson and Gordon 1995) (Figure 1).

NOTE:
This figure is included on page 13
of the print copy of the thesis held in
the University of Adelaide Library.

**Figure 1:** Differing external morphological features of the three extant species of wombats make each species noticeably distinct (adapted from Eastwood 2003)

*1.2.2. Distribution*

The southern hairy-nosed wombat is distributed across the semi-arid plains of South Australia, west of the Murray River, and south-eastern Western Australia. Anecdotal reports, evidence of abandoned burrows, and fossil records, suggest that prior to European settlement, the population spread from the Murraylands in the east, through the Mount Lofty Ranges to

the Yorke Peninsula, Eyre Peninsula, Gawler Ranges and Nullarbor Plain. However, whether there was a continuous distribution (as shown in Figure 2) is unknown, and would have been dependant on the suitability of the habitat (Aitken 1971; Alpers *et al.* 1998; St John 1998). Since European settlement, southern hairy-nosed wombat distribution has become restricted, and population numbers have decreased (Aitken 1971). While southern hairy-nosed wombats are still considered abundant in a several areas, land clearance has meant that the species now has a limited distribution (Johnson 1989; Alpers *et al.* 1998), with some populations small and isolated (Figure 2). As a whole, the southern hairy-nosed wombat is classified as a low risk species (IUCN listing), and is not listed as threatened species through the EPBC Act. However, only the Far West Coast Nullarbor population is considered as being secure, with the others deemed vulnerable, and the Yorke Peninsula population considered endangered (Walker 2004).
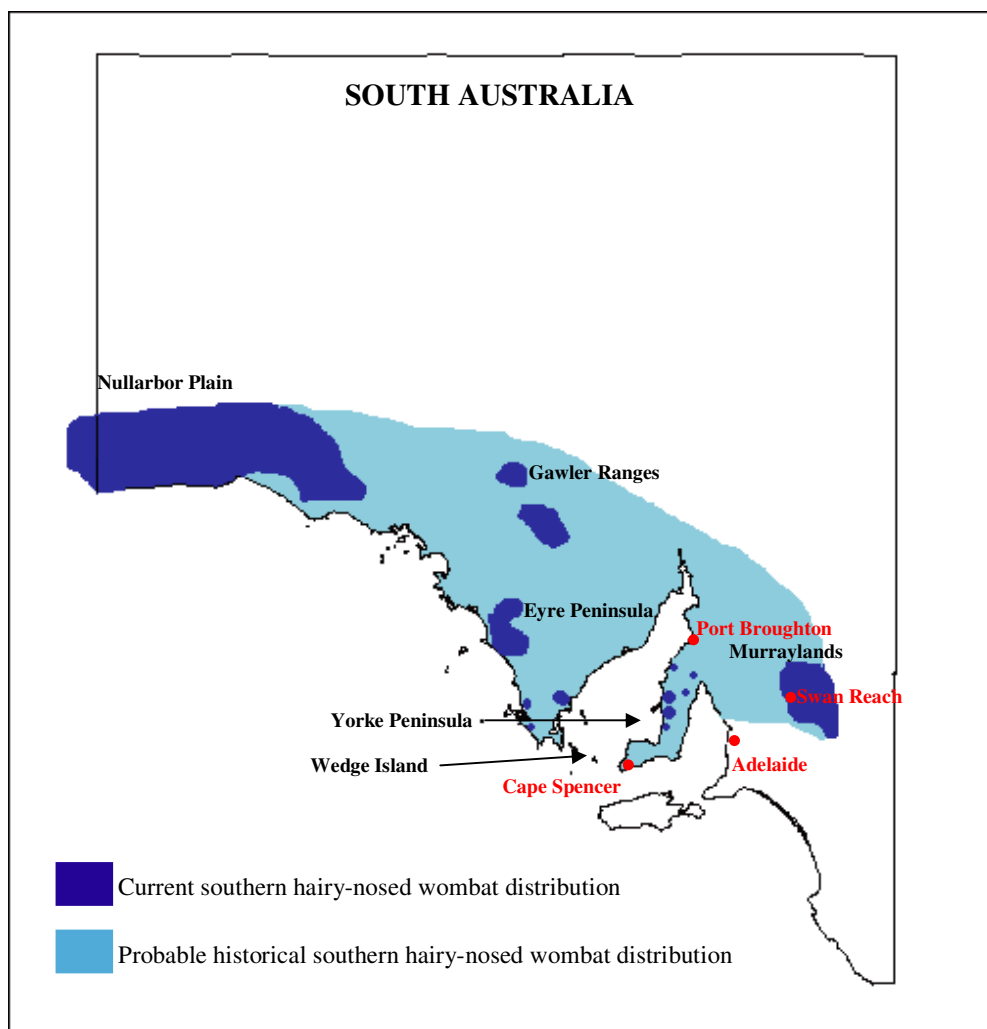


**Figure 2:** Map showing the extant and probable historical distribution of the southern hairy-nosed wombat in South Australia

*1.2.3. Burrow use*

Wombats are the largest extant herbivorous, burrowing mammal (Johnson 1998). Burrows provide protection from predators (including humans), as well as a stable environment (temperature and humidity) within the animals' thermal neutral zone (Shimmin *et al.* 2002). They are vital for their survival in a semi-arid landscape. Wombats can survive in extreme climatic conditions which can vary with temperatures ranging from hot summer days in excess of 40 °C, to freezing winter nights below -2 °C. This they achieve by spending the majority of their time in burrows where the temperature and humidity are relatively stable (< 1 °C change on a daily basis) (Wells 1989; Shimmin *et al.* 2002; Finlayson *et al.* 2005). Burrows are usually excavated in areas that take advantage of a natural weaknesses in the soil substrate to minimise the energetic cost of digging (Shimmin *et al.* 2002), and where there is support for the burrow structure, for example, either under trees/shrubs with roots that stabilise soil, or under limestone calcrete shelves (Mallett and Cooke 1986; Triggs 1996; Horsup 1999).

*1.2.4. Ranging behaviour and dispersal*

Southern hairy-nosed wombats do not travel long distances from their burrows, and can inhabit up to 11 warrens within their home range, depending upon warren size (Wells 1989; Finlayson *et al.* 2005). Radio tracking studies have shown home ranges vary between 1.3 and 4.8 ha in size (Finlayson *et al.* 2005; Taggart and Temple-Smith 2008), whereas genetic analysis on wombat hair samples, has indicated a maximum home range of up to 7.82 ha (Walker *et al.* 2006). It has been observed that home ranges between individual animals may overlap (Taylor 2003; Finlayson *et al.* 2005), and related males often share burrows (Walker *et al.* 2008b).

Dispersal, as indicated by genetic analysis, is adult female biased in southern hairy-nosed wombats (Walker *et al.* 2008b). In northern hairy-nosed wombats, little evidence of juvenile female dispersal was observed, and males of any age rarely dispersed (Johnson and Crossman 1991); an unusual finding, as in most mammal species it is predominantly juvenile males that disperse (Greenwood 1980; Dobson 1982). Dispersal in southern hairy-nosed wombats was considered most likely to occur once juveniles were fully weaned (Walker *et al.* 2008b), although because little is known about how long the young remain with their mother, the timing of dispersal is uncertain. To explain the unusual adult female biased dispersal, Walker (2008b) hypothesised that adult wombats were perhaps more likely to survive the process of finding, or constructing, a new burrow than young animals, as digging a new burrow is energetically expensive, and other adult wombats can prevent juveniles/sub-adults

from entering previously used burrows (Johnson and Crossman 1991; Banks *et al.* 2002; Walker 2004).

*1.2.5. Water requirements*

To conserve water, southern hairy-nosed wombats have evolved certain behavioural strategies, obtaining the majority of their water requirements from ingested food (Wells and Green 1998). In their semi-arid environment, burrows are important for water conservation. In summer, burrow temperature is up to 15 ℃ cooler, and relative humidity significantly higher than ambient conditions, thereby reducing respiratory and evaporative water loss (Shimmin *et al.* 2002). Wombats are nocturnal and emerge to feed at night when conditions are most favourable, generally in the hours just before dawn in summer, or early evening in winter, when the humidity is similar between the inside and outside of the burrow (Wells 1989). The small home ranges, and generally sedentary lifestyle of the wombat, also contribute to the little water expenditure (Wells and Green 1998; Shimmin *et al.* 2002).

Southern hairy-nosed wombats also have evolved a range of physiological strategies to cope with low water availability including: the production of low volume, highly concentrated urine, extremely dry faeces, low standard metabolic rate and long periods of inactivity (Wells and Green 1998; Shimmin *et al.* 2002).

*1.2.6. Nutrition*

Wombats are herbivorous animals that feed predominantly on native grasses (e.g. *Stipa*, *Poa* species), as well as fresh shoots and bulbs when available (St John and Saunders 1989; Wells 1989). They also readily eat introduced grasses and sedges (Mallett and Cooke 1986; Buchan and Goldney 1998; Hume and Barboza 1998). If these food sources are not present, especially in drought, they will dig up grass and plants roots and eat bulbs (St John and Saunders 1989; Wells 1989).

*1.2.7. Reproduction*

Southern hairy-nosed wombats are seasonal breeders, with the breeding season occurring between late July and December, depending on effective rainfall (Gaughwin *et al.* 1998; Hamilton *et al.* 2000; Taggart and Temple-Smith 2008). This time of year often coincides with regular and reliable rainfall in South Australia, and results in the growth of grasses, providing ample feed to enhance female body condition and help facilitate successful reproduction and nursing of pouch young. This is also the period during which recently weaned young gain the body condition necessary for survival across their first summer (Wells

1989; Gaughwin *et al.* 1998; Taggart and Temple-Smith 2008). As the breeding season approaches, the reproductive condition of adult male southern hairy-nosed wombats changes. These changes include an increase in peri-cloacal gland width, which contributes fluid to ejaculation, semen volume, sperm motility, prostate gland weight (Taggart *et al.* 2005), and androgen production (Hamilton *et al.* 2000). Female southern hairy-nosed wombats are thought to be monogamous (Taggart *et al.* 1998b; Taggart and Temple-Smith 2008), whilst males are believed to be polygynous (Walker *et al.* 2008b). Signs of inbreeding avoidance and kin recognition have been observed in numerous southern hairy-nosed wombat populations across South Australia, with closely related individuals of the same-sex associating with one another less than unrelated animals. This was more evident in the small isolated population at Kulpara, on the Yorke Peninsula, where inbreeding potential was increased (Walker *et al.* 2008a).

Gestation in this species lasts for 21 – 22 days, with the majority of young born between mid-August and October (Wells 1989; Taggart and Temple-Smith 2008). Joeys leave the pouch at approx 8 – 9 months of age, and are fully weaned by 12 – 13 months (Taggart *et al.* 2007). Southern hairy-nosed wombats reach sexual maturity at approximately three years of age (Taggart and Temple-Smith 2008).

Female southern hairy-nosed wombats are polyestrus, and can have up to three oestrous cycles every breeding season, with each cycle lasting approximately 36 days (Finlayson *et al.* 2007). The reproductive rate of this species is low, with females giving birth to one young at a time (Tyndale-Biscoe and Renfree 1987; Gaughwin *et al.* 1998; Finlayson *et al.* 2007), and generally only producing a maximum of two young every three years (Taggart and Temple-Smith 2008).

### 1.2.8. Human impact

Since the time of European settlement, factors such as the introduction of pest species (e.g. rabbits, cats, foxes), land clearing, human persecution (culling of wombats by farmers), disease (mange), and competition from cattle and sheep for resources, especially during drought, have resulted in a decline of southern hairy-nosed wombat numbers, and resulted in population fragmentation and isolation on a large scale. As a consequence, small populations of wombats are at risk of further major decline or extinction (James 1977; Aitken 1983; St John and Saunders 1989; Horsup 1998; St John 1998). Likewise, studies by Taylor *et al.* (1994) have found very low genetic variation in the northern hairy-nosed wombats due to inbreeding following population isolation. A skewed sex ratio in northern hairy-nosed wombats, favouring males has also been observed (2.25 : 1) (Banks *et al.* 2003), which further

reduces the potential for population recovery due to a reduction in the number of available breeding females (Horsup 1999; Wedekind 2002).

### 1.2.9. Genetic diversity

As a result of reduced abundance, and the fragmentation and isolation of southern hairy-nosed wombat populations, it is possible that, like in the northern hairy-nosed wombat, several populations may already be, or could soon become, significantly inbred. However, previous studies have not found any evidence of inbreeding in the majority of southern hairy-nosed wombat populations (Alpers *et al.* 1998; Alpers and Sherwin 1999; Walker 2004). The exceptions are Urania (Yorke Peninsula) and Nundroo (Nullarbor), where a significant homozygote excess was recorded (Alpers and Sherwin 1999), suggesting possible local inbreeding. These previous genetic studies on southern hairy-nosed wombats will be discussed further in Chapter 3. Whilst the southern hairy-nosed wombat is not currently regarded as a threatened species, the Yorke Peninsula population is classified as endangered (St John and Saunders 1989), and care must be taken in management of the whole species, in particular, the Yorke Peninsula population, to avoid major loss of genetic diversity and population decline like that which has occurred in its sister taxa, the northern hairy-nosed wombat.

**1.3. Summary and aims**

The literature documented above indicates that fragmentation and isolation of populations can increase the risk of local species extinctions. Isolated populations have a reduced rate of migration between habitat patches which increase the potential for inbreeding within a population. In eutherian mammals, low genetic variation has been associated with a reduction in male reproductive capacity and population viability associated with inbreeding. The known reproductive parameters that are potentially negatively affected in mammal species as a result of inbreeding include seminal quality (sperm morphology, sperm motility, sperm concentration, and ejaculate volume), breeding success and juvenile mortality. The only prior research that has examined the association between inbreeding and reproduction in a marsupial species, was conducted on the koala, the sister taxon of wombats. The bulk of research in this field has been carried out on eutherian mammal species such as felids.

Due to the modified landscape throughout South Australia, a number of southern hairy-nosed wombat populations have become fragmented and are genetically isolated and hence may be regarded at risk. This could be due to a number of reasons, such as land clearing, or the construction of barriers, both of which may reduce animal migration. Inhabitants of these populations may therefore be susceptible to reduced dispersal opportunities, inbreeding and an associated decrease in both reproductive fitness of the males, and population viability (breeding success and juvenile mortality).

The aims of this project on the southern hairy-nosed wombat are to:

- Determine the location and size of remnant wombat colonies on the Yorke Peninsula
- Compare and contrast various morphological characteristics of wombats from the Yorke Peninsula and the Murraylands populations
- Examine and compare the genetic diversity of wombat populations on Yorke Peninsula and in the Murraylands
- Compare and contrast various components of seminal quality (ejaculate volume, sperm motility and sperm concentration) between wombats from small isolated populations and those from a large population
- Compare and contrast sperm morphology (head and tail shape, sperm component lengths) between wombats from small isolated populations, and those from a large population

- Compare and contrast the reproductive status (pouch young presence and late-lactation in adult females, number of sub-adults and juveniles in population) of wombats between small isolated populations, and those from a large population
- Determine the implications of population fragmentation and isolation, and suggest possible management strategies (and further studies) to conserve this species and current levels of genetic diversity.

**Chapter 2: Demographics of southern hairy-nosed wombats from the Yorke Peninsula and Swan Reach (Murraylands) populations**

## 2.1. Introduction

### 2.1.1. Southern hairy-nosed wombats in South Australia

The southern hairy-nosed wombat occurs across southern South Australia in five mainland populations, including the Nullarbor Plain, the Gawler Ranges, Eyre Peninsula, Yorke Peninsula, and the Murraylands, as well as Wedge Island, an island population in the Spencer Gulf (Chapter 1, section 1.2.2., Figure 2).

Most information on southern hairy-nosed wombats has come from research conducted on the large population located in the Murraylands, northeast of Adelaide. Data includes studies on many aspects of southern hairy-nosed wombat ecology and physiology, reproductive biology, home-range, burrow structure, growth and development, genetic structure, and much more (Gaughwin 1981; Alpers *et al.* 1998; Gaughwin *et al.* 1998; Taggart *et al.* 1998b; Alpers and Sherwin 1999; Hamilton *et al.* 2000; Shimmin *et al.* 2002; Taggart *et al.* 2003; Finlayson *et al.* 2005; Taggart *et al.* 2005; Walker *et al.* 2006; Finlayson *et al.* 2007; Taggart *et al.* 2007; Walker *et al.* 2007; Walker *et al.* 2008a; Walker *et al.* 2008b). In contrast, the ecology of southern hairy-nosed wombats occurring on the Yorke Peninsula has not been examined extensively.

### 2.1.2. Population fragmentation and isolation in southern hairy-nosed wombats

The potential negative impact of population fragmentation and isolation on the long term viability of species was discussed earlier (Chapter 1, section 1.1.). Some of the consequences of population isolation, such as inbreeding, reduced genetic variation, reduced migration, and lowered fertility rates, can increase the risk of local species extinction (Frankham 1995; Frankham *et al.* 2002; Keller and Waller 2002; Frankham *et al.* 2004; Allendorf and Luikart 2007). Southern hairy-nosed wombats are considered endangered on the Yorke Peninsula (St John and Saunders 1989) with colonies fragmented and geographically isolated. These wombats have also been found to have the highest number of unique alleles within any southern hairy-nosed wombat population, which suggests that this population has been isolated for a longer period of time than other populations (Walker *et al.* 2008a). Across South Australia, numerous populations of southern hairy-nosed wombats have previously been screened for genetic signatures of isolation (Walker *et al.* 2008a). In southern hairy-nosed wombats females are typically the dispersers, therefore, in a population where migration is present males are more likely to have been born into the population,

whereas females are more likely to have migrated into it. Consequently, females are less likely to be related to one another. The colony at Kulpara, on the northern part of the Yorke Peninsula, appeared to have restricted female dispersal as there were high levels of female relatedness, indicative of females with similar genetic structure living in close proximity to one another (Walker *et al.* 2008a). This apparent lack of recent immigration, as well as a small population size, and ecological isolation, suggest both geographical and genetic isolation of the Kulpara population (Walker 2004; Walker *et al.* 2008a). Whilst the population at Kulpara is geographically and genetically isolated, some evidence of inbreeding avoidance was evident (Walker *et al.* 2008a).

Without immigration one would expect levels of inbreeding in the Kulpara wombat population to increase. This is displayed in a range of studies that have observed an association between small population size and inbreeding (Frankham *et al.* 2002). Nevertheless, geographic isolation of a population/colony is not necessarily reflected in its genetic structure, hence, the demography of a population should also be considered (Gaines *et al.* 1997). For example, in a study of the cotton rat (*Sigmodon hispidus*), a reduction in genetic diversity was detected in isolated populations, however the isolated population still survived for over 200 years (Gaines *et al.* 1997). Similar results have also been observed in island populations of the black-footed rock-wallaby, where populations have been isolated for greater than 1600 generations, have an extremely small effective population size, and exceptionally low heterozygosity values, however these populations still survive (Eldridge *et al.* 1999). Genetic factors alone are not sufficient to cause extinction, with demographic responses to fragmentation, isolation and loss of habitat also playing an important part (Gaines *et al.* 1997). Both these factors should therefore be examined when considering the risk of extinction.

### 2.1.3. Aims

Before examining the more detailed aspects of southern hairy-nosed wombat population fragmentation and isolation on the Yorke Peninsula, such as genetic diversity and reproductive success, it is important to gather more data on the demography of these colonies. At present, knowledge about the demography of these colonies, and how they compare to the larger, continuous population in the Murraylands, is limited. Current colony distribution and size estimates of southern hairy-nosed wombats on the Yorke Peninsula are also needed, as little data on these parameters have been collected in this area in recent times.

Therefore, the specific aims of this chapter are to:

1. Detail the location and size of southern hairy nosed wombat colonies on the Yorke Peninsula

2. Determine any morphological differences between southern hairy-nosed wombats from the Yorke Peninsula and Murraylands populations

## 2.2. Methods

### 2.2.1. Study populations

Murraylands: The Murraylands region covers approximately 4,120,000 hectares, and consists of mallee scrub, arid zone shrublands, floodplains, valleys, and wetlands (Department for Environment and Heritage: South Australia). The wombat study site within this region was located at Swan Reach, approximately 200 kilometres northeast of Adelaide (Figure 2). Research was conducted on Kooloola Station, a 6800 hectare station near Swan Reach, which is predominantly grazing land, with patches of remnant scrub. Estimates of approximately 4000 wombats occur at this site (David Taggart, personal communication). The Kooloola Station wombats form part of the continuous population of wombats in the Murraylands, whose estimated total size is between 10,000 and 100,000 (St John and Saunders 1989).

Yorke Peninsula: The Yorke Peninsula is located west of Adelaide, across the Gulf of St Vincent, and spans approximately 210 kms from Cape Spencer in the south, to Port Broughton in the north, with an area of around 420,000 hectares (Figure 2). The region is predominantly comprised of open paddocks used for grazing and cropping. Estimates of the number of southern hairy-nosed wombats on the peninsula 20 years ago were of approximately 100 animals at Kulpara, at the top of the peninsula, and 100 animals at Urania, on the central Yorke Peninsula (St John and Saunders 1989). As little is known about the distribution of southern hairy-nosed wombats on the Yorke Peninsula, background information on colony whereabouts needed to be collected before field work could commence. For this, a survey of local Department for Environment and Heritage rangers and ecologists, as well as residents, and previous researchers, was conducted by phone, and contact made with local farmers who had reported wombats on, or in the vicinity of, their property. Fifteen days were subsequently spent on the Yorke Peninsula examining wombat activity at these locations. During these field trips, considerable time was also spent visiting local businesses and farms in and around every major town on the Yorke Peninsula, to discuss with residents wombat sightings, and possible population locations. All sealed roads, as well as many unsealed roads were traversed, with regular stops at farm houses to discuss possible southern hairy-nosed wombat locations. Once exact locations were established field trips were organised to capture and process animals at these sites to gather population statistics.

*2.2.2. Estimating population size on the Yorke Peninsula*

After wombat populations were located on the Yorke Peninsula, population size estimates were made using the following equation: 0.43 wombats/per active burrow (Tiver 1981). This estimate is based on capture/mark/recapture research conducted on southern hairy-nosed wombats in the Murraylands from eight warrens (1 to 43 burrows in each warren) (Tiver 1981). An active burrow was determined by examining the area surrounding the burrow and the burrow entrance. Sighting of a wombat in the burrow, an unblocked burrow entrance (i.e. absence of spider webs, plant matter, soil build up, etc), and the presence of fresh scratchings, footprints and scats within a close vicinity, indicated an active burrow.

*2.2.3 Animal capture*

Field work was conducted between 2004 and 2008, throughout all times of the year, and animals were captured and examined on site (University of Adelaide Animal Ethics Permit #S-020-2005, Department for Environment and Heritage Animal Ethics Permit #16/2005, Department for Environment and Heritage Permit to Undertake Scientific Research Permit #S24981). No wombats infested with the pathogenic mite *Sarcoptes scabiei* were used in the study.

Every night in the field, a group of three to six field workers went out to capture wombats from dusk until about 5am (if required). Animals were located using a spot light from the back of a vehicle, except on the occasions where the scrub was inaccessible by vehicle. In these instances, animals were located by spotlight on foot. Upon detection, animals were stunned by a rifle shot approximately 15 cm above their head (Taggart *et al.* 2003). Attempts were then made to capture the animal using large, custom made, hoop nets (Figure 3a)(Taggart *et al.* 2003). Captured wombats were placed in hessian sacks and secured on the back of the vehicle (Figure 3b and Figure 3c). The location of capture was recorded by GPS, so animals could be returned to the capture site the following day. Wombats were then transported back to a central field station for processing.

**Figure 3:** The procedure for catching southern hairy-nosed wombats; (a) wombats are stunned, and then have a large hoop net thrown over them, (b) the wombat is transferred to a hessian sack and (c) the wombat is securely tied to the utility vehicle for transport to the field station (photos taken by Elisa Sparrow).

### 2.2.4. Anaesthesia and data collection

Whilst still in the hessian sack, animals were anaesthetised via an intramuscular injection of Zoletil (3 mg/kg, Vibrac Australia Pty Ltd). Once anaesthetised, wombats were removed from the hessian sack and a variety of morphological measurements collected using callipers, and a 1.5 m measuring tape. Measurements of head length, head width, neck girth, chest girth, body length, ear length, tibia length, pes (paw) length, and eye to ear distance were recorded for each animal captured. In addition, male wombats had their testes width, testes length, testes depth, scrotal width, and accessory gland length and width measured. All animals were also weighed, and body condition subjectively ranked out of five (categorised as below):

1 = very poor condition: vertebral processes, pelvic and shoulder girdles
    obviously protruding (by sight and touch), dull fur easily plucked from
    rump, very high external parasite load

2 = poor condition: vertebral processes, pelvic and shoulder girdles
    protruding (by touch), dull fur, elevated external parasite load

3 = average condition: average cover across vertebral processes, pelvic and
    shoulder girdles, shiny fur, moderate external parasite load

4 = good condition: good cover across vertebral processes, pelvic and
    shoulder girdles (not protruding), shiny fur, low external parasite load

5 = excellent condition: excellent cover across vertebral processes, pelvic
    and shoulder girdles (not protruding/difficult to detect), shiny fur, little,
    or no, external parasite load

For analysis, wombats were categorised as adult, sub-adult or juvenile based upon head length and head width in males, or pouch condition in females. In male wombats, a head length of greater than 180 mm was considered adult (Taggart *et al.* 2007). A head length of less than 180 mm, but greater than 160 mm was considered sub-adult, whilst males with head widths less than 160 mm were considered juvenile. In female wombats, an individual's age class was estimated by examining the pouch. For this, a dry pouch with no depth was classified as a juvenile, whereas a shallow and dry pouch was considered a sub-adult. All deep pouch conditions were classified as an adult wombat (more information on pouch condition in Chapter 6) (Tyrell 2001). No animals affected by mange were included in the results of this study.

*2.2.5. Statistical analysis*

All accumulated data was placed in a Microsoft Excel worksheet. SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analysis. To compare the morphology measurements between the populations, the data was tested for normality, and when the requirements were met, ANOVA models were fitted to the data. When there were only two populations for comparison the data was analysed using independent samples t-tests. Where the test of the null hypothesis was statistically significant ($p < 0.05$) post-hoc tests were performed to determine the direction of difference.

The data collected from animals at Point Pearce were combined for analysis with the animals from Urania, due to the close proximity of these sites. Data from Kulpara and Urania were then analysed separately, and then the data from all animals captured on the Yorke

Peninsula (including Lake Fowler and Kainton Corner) were analysed together, and compared to animals captured at Swan Reach.

## 2.3. Results

*2.3.1. Distribution of southern hairy-nosed wombats on the Yorke Peninsula*

Southern hairy-nosed wombats were found in 25 small, isolated colonies scattered across the Yorke Peninsula, from Wallaroo in the north, to Lake Fowler in the south. No wombat colonies were found west of Lake Fowler on the "foot" of the peninsula (Figure 4). A total of 2523 wombat burrows were observed on the Yorke Peninsula. Of these, 1618 were classified as active. This suggests that approximately 696 wombats remain on the Yorke Peninsula (Table 1). Of the 25 southern hairy-nosed wombat colonies discovered on the Yorke Peninsula, only three of these colonies were estimated to have wombat numbers of over about 100, and these were at Urania, Kulpara and Point Pearce (Figure 5 & Table 1). Of the remaining 22 colonies, 19 had wombat numbers estimated to be less than 10 animals.

**Figure 4:** The distribution of southern hairy-nosed wombats on the Yorke Peninsula in South Australia, as shown by the red markings. The red circles indicate the locations of the study sites

*2.3.2. Yorke Peninsula wombat colonies*

<u>Kulpara</u>: The Kulpara wombat colony was located approximately 6 km northwest of Kulpara, on the northern end of Yorke Peninsula. The animals in this colony were spread across three agricultural properties. This land consisted of cropping and grazing country, with scattered, small patches of remnant vegetation. 340 active burrows were observed at this site indicating approximately 146 wombats may reside there (Figure 5 & Table 1).

<u>Kainton Corner</u>: The Kainton Corner colony was located approximately 10 - 15 km south of Kulpara. This site consisted of two privately owned grazing properties, within 2 km of one another, with small patches of remnant vegetation. Only 13 active wombat burrows were detected at this site. From the number of active burrows, it is estimated this colony has six animals (Figure 5 & Table 1).

<u>Point Pearce</u>: The Point Pearce wombat colony was located approximately 15 km southwest of Maitland on the central Yorke Peninsula. This aboriginal land is predominantly cropping land, with very little native vegetation. Three-hundred and sixty-seven active wombat burrows were observed at Point Pearce, suggesting that approximately 135 animals resided at this site (Figure 5 & Table 1).

<u>Urania</u>: The Urania wombat colony was located approximately 5 km southwest of Urania, 20 km south of Maitland on the central Yorke Peninsula. This land is currently used for cropping and grazing only. Wombats occurred across approximately 600 hectares, with the colony consisting of 633 active burrows. An estimate of 272 animals was recorded for this site (Figure 5 & Table 1).

<u>Brentwood</u>: The Brentwood colony was located approximately 2 km southwest of Brentwood on the south western Yorke Peninsula. This area is predominantly grazing land. Four active wombat burrows, were found at this location, suggesting only two animals remain here (Figure 5 & Table 1).

<u>Weaver's Lagoon</u>: The Weaver's Lagoon colony was located approximately 10 km southwest of Stansbury, on the southern Yorke Peninsula. This government owned land, was dominated by a salt lake and a little native vegetation, surrounded by cropping land. At this site there were 17 active wombat burrows detected with estimates of approximately seven animals resident at this location (Figure 5 & Table 1).

<u>Lake Fowler</u>: The Lake Fowler wombat colony was located approximately 10 km west of Edithburgh, on the southern Yorke Peninsula. The land surrounding the lake is cropping and grazing, with very little remnant vegetation. The Lake Fowler colony consisted of only 25 active burrows, with an estimated 11 wombats residing in this colony (Figure 5 & Table 1).

Wallaroo: The Wallaroo wombat colony was located near the coastline, approximately 35 km west of the Kulpara site, on the north western region of the Yorke Peninsula. The area consists of coastal sand dunes, as well as land used for agricultural and leisure purposes. There was 124 active burrows discovered, suggesting approximately 53 animals in the colony (Figure 5 & Table 1).

Other colonies: Another 148 active burrows were found scattered in other small colonies spanning the Yorke Peninsula. This observation suggested an additional 64 wombats occurred on the Yorke Peninsula in small, isolated colonies (Figure 5 & Table 1).
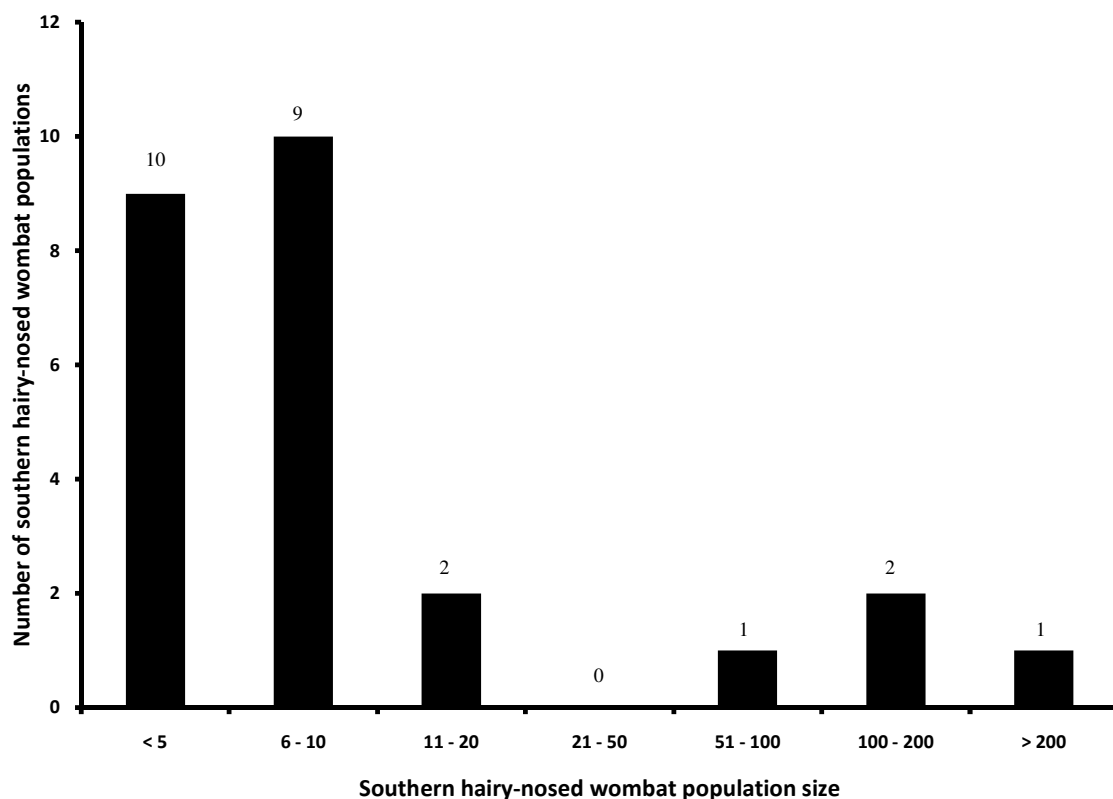


**Figure 5:** The number of southern hairy-nosed wombat colonies on the Yorke Peninsula and their estimated size

**Table 1:** The total number of southern hairy-nosed wombat burrows seen, the number of active burrows and the approximate number of wombats (Tiver 1981) on the Yorke Peninsula

NOTE:
This table is included on page 33
of the print copy of the thesis held in
the University of Adelaide Library.

**\*** Indicates all other wombat colonies on the Yorke Peninsula whose location is not specified

*2.3.3. Southern hairy-nosed wombat population statistics for the Yorke Peninsula and Swan Reach*

On the Yorke Peninsula attempts were made to capture animals at Kulpara, Kainton corner, Point Pearce, Urania, Brentwood, Weavers Lagoon and Lake Fowler. Due to time constraints, and distance between populations, attempts to capture wombats were not made at the smaller sites shown in Figure 5. The majority of field effort was spent at Kulpara and Urania, due to the larger population sizes to increase the probability of capturing animals at these sites (Table 2).

**Table 2**: The number of spotlight/capture nights spent, the number of southern hairy-nosed wombats seen, and caught at each site, average number of animals seen per night, density of animals, male to female ratio and capture success for southern hairy-nosed wombats at each site on the Yorke Peninsula and at Swan Reach. Note: Multiple sites were visited on some nights

| | Yorke Peninsula | | | | | | | | Swan Reach |
|---|---|---|---|---|---|---|---|---|---|
| | *Kulpara* | *Kainton Corner* | *Point Pearce* | *Urania* | *Brentwood* | *Weavers Lagoon* | *Lake Fowler* | *All\** | |
| **Capture nights** | 32 | 4 | 2 | 15 | 3 | 4 | 4 | 64 | 54 |
| **Animals seen** | 675 | 3 | 44 | 421 | 1 | 0 | 5 | 1149 | 2134 |
| **Animals caught** | 88 | 2 | 18 | 111 | 0 | 0 | 1 | 220 | 327 |
| **Average number seen per night** | 21 | <1 | 9 | 28 | <1 | 0 | 1 | 18 | 40 |
| **Density of animals (per km)** | 1.4 | - | 1.9[#] | 1.9[#] | - | - | - | 1.4 | 1.3 |
| **Male: Female ratio** | 1:1 | - | 1.3:1[#] | 1.3:1[#] | - | - | - | 1.1:1 | 0.8:1 |
| **Capture success (%)** | 13 | 67 | 41 | 26 | 0 | 0 | 20 | 19 | 6 |

\* Urania and Kulpara data combined, with the addition of other captured animals on the Yorke Peninsula
[#]Urania and Point Pearce data was combined to obtain these values

Yorke Peninsula: A total of 1149 southern hairy-nosed wombats were seen over 64 nights on the Yorke Peninsula. On average 18 wombats were seen each night. At the Urania colony an average of 28 animals were seen per night. The density of animals at Urania (including Point Pearce) was the greatest seen across any site examined, with 1.9 animals seen per kilometre travelled. The average density of wombats across the known colonies on the Yorke Peninsula was 1.4 animals seen per kilometre travelled. A total of 220 animals were captured on the Yorke Peninsula. The male to female sex ratio was 1.1:1, with 53 % of wombats captured males and 47 % females (Table 2).

Swan Reach: A total of 2134 southern hairy-nosed wombats were seen over 54 nights at Swan Reach in the Murraylands. On average 40 wombats were seen each night; the greatest average number of animals seen at any of the colonies studied. The density of animals at Swan Reach, however, was the lowest of all the colonies examined, with an average of 1.3 animals seen every kilometre travelled. In total, 327 wombats were captured at Swan Reach. The male to female sex ratio was 0.8:1, the lowest of all study sites, with 45 % of captured animals males, and 55 % females (Table 2).

*2.3.4. Morphological measurements of adult southern hairy-nosed wombats*

The mean values (± standard deviation) obtained for the morphological variables of adult southern hairy-nosed wombats are indicated in Table 3.

**Table 3:** Mean morphological measurements of adult southern hairy-nosed wombats from three different study sites (± standard deviation)

| | Swan Reach: | | Yorke Peninsula: | | | | | |
| | | | Urania | | Kulpara | | All* | |
| *Morphology measurement:* | N | Mean | N | Mean | N | Mean | N | Mean |
|---|---|---|---|---|---|---|---|---|
| Head length (mm) | 174 | 185.5 ± 6.3 | 29 | 186.1 ± 6.6 | 41 | 184.5 ± 6.0 | 72 | 185.2 ± 6.3 |
| Head width (mm) | 202 | 134.5 ± 5.2$^a$ | 50 | 129.7 ± 7.8$^b$ | 42 | 130.5 ± 5.2$^b$ | 94 | 130.1 ± 6.8$^{\wedge}$ |
| Neck girth (cm) | 204 | 46.9 ± 5.0$^a$ | 60 | 44.9 ± 4.0$^b$ | 42 | 46.3 ± 4.1$^{a,b}$ | 104 | 45.5 ± 4.1$^{\wedge}$ |
| Chest girth (cm) | 205 | 66.0 ± 3.8$^a$ | 60 | 63.9 ± 4.6$^b$ | 42 | 63.4 ± 4.8$^b$ | 104 | 63.7 ± 4.7$^{\wedge}$ |
| Body length (cm) | 205 | 102.9 ± 4.0$^a$ | 60 | 100.3 ± 3.6$^b$ | 42 | 99.8 ± 4.3$^b$ | 104 | 100.0 ± 3.9$^{\wedge}$ |
| Ear length (mm) | 144 | 88.1 ± 4.7$^a$ | 53 | 91.9 ± 6.7$^b$ | 42 | 92.2 ± 4.5$^b$ | 97 | 92.1 ± 5.5$^{\wedge}$ |
| Tibia length (mm) | 146 | 136.0 ± 7.4$^a$ | 51 | 138.4 ± 5.1$^b$ | 41 | 135.1 ± 6.1$^a$ | 94 | 136.9 ± 5.8 |
| Pes length (mm) | 205 | 94.0 ± 3.9$^a$ | 55 | 91.9 ± 3.5$^b$ | 42 | 88.6 ± 5.0$^c$ | 99 | 90.4 ± 4.6$^{\wedge}$ |
| Eye to ear length (mm) | 132 | 96.9 ± 5.8$^a$ | 33 | 93.8 ± 6.0$^{a,b}$ | 42 | 92.9 ± 5.1$^b$ | 77 | 93.2 ± 5.4$^{\wedge}$ |
| Tail length (mm) | 203 | 39.2 ± 5.8 | 53 | 38.5 ± 5.7 | 42 | 39.5 ± 6.4 | 97 | 39.0 ± 5.9 |
| Weight (kg) | 200 | 24.0 ± 2.4 | 60 | 23.5 ± 2.5 | 42 | 23.2 ± 1.9 | 104 | 23.3 ± 2.3 |
| Condition (score out of 5) | 200 | 3.3 ± 0.7 | 56 | 3.5 ± 0.9 | 41 | 3.6 ± 0.8 | 99 | 3.5 ± 0.8 |

**\*** Urania and Kulpara data combined, plus additional wombats captured at other sites on the Yorke Peninsula

[a,b,c] Differing superscript letters indicate a statistically significant difference in the variables between Swan Reach, Urania and Kulpara ($p < 0.05$), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference ($p \geq 0.05$)

[^] Statistically significant difference in the variables between Swan Reach and the Yorke Peninsula populations ($p < 0.05$)

In adult southern hairy-nosed wombats, no statistically significant differences were found between the populations in mean head length, tail length, weight and condition score ($p \geq 0.05$)

Adult wombats from the Swan Reach population, however, had a significantly larger mean neck girth than Urania animals, and a significantly larger mean eye to ear distance than Kulpara animals ($p < 0.05$). Mean pes length was significantly different between all three populations, with Swan Reach animals having the largest mean pes length and Kulpara animals the smallest ($p < 0.05$). The Swan Reach animals also had significantly larger head width, chest girth and body length, than animals from both Urania and Kulpara ($p < 0.05$). Mean ear length however, was smaller in wombats captured at Swan Reach than those from Urania and Kulpara ($p < 0.05$) (Table 3).

When data from the Urania and Kulpara colonies were pooled and compared to the Swan Reach population, it was found adult wombats from the Swan Reach population were significantly larger than wombats from Yorke Peninsula. The morphological characteristics which showed a significant difference included, mean head width, neck girth, chest girth, body length, pes length, and eye to ear distance ($p < 0.05$). Ear length, however, was significantly smaller in animals from Swan Reach compared to animals from the Yorke Peninsula ($p < 0.05$) (Table 3).

*2.3.5. Morphological measurements of sub-adult southern hairy-nosed wombats*

The mean values (± standard deviation) obtained for sub-adult southern hairy-nosed wombats morphological variables are indicated in Table 4.

**Table 4:** Mean morphological measurements of sub-adult southern hairy-nosed wombats from three different study sites (± standard deviation)

| *Morphology measurement:* | Swan Reach: | | Yorke Peninsula: | | | | | |
| | | | *Urania* | | *Kulpara* | | *All** | |
| | N | Mean | N | Mean | N | Mean | N | Mean |
|---|---|---|---|---|---|---|---|---|
| *Head length (mm)* | 29 | 170.0 ± 7.9 | 1 | 178.0 | 20 | 173.3 ± 7.3 | 22 | 173.4 ± 7.1 |
| *Head width (mm)* | 30 | 124.1 ± 5.4$^a$ | 9 | 117.0 ± 5.7$^b$ | 20 | 122.9 ± 4.5$^a$ | 30 | 121.4 ± 5.7$^\wedge$ |
| *Neck girth (cm)* | 30 | 44.4 ± 4.1$^a$ | 10 | 38.5 ± 1.8$^b$ | 20 | 44.6 ± 3.0$^a$ | 31 | 42.5 ± 3.9$^\wedge$ |
| *Chest girth (cm)* | 30 | 60.7 ± 4.2$^a$ | 10 | 55.8 ± 4.1$^b$ | 20 | 60.8 ± 3.8$^a$ | 31 | 59.2 ± 4.5$^\wedge$ |
| *Body length (cm)* | 30 | 94.5 ± 6.6 | 10 | 93.4 ± 5.9 | 20 | 93.6 ± 4.5 | 31 | 93.5 ± 4.8 |
| *Ear length (mm)* | 23 | 84.5 ± 3.5$^a$ | 9 | 84.4 ± 8.1$^b$ | 20 | 88.7 ± 2.9$^b$ | 30 | 87.6 ± 5.4 |
| *Tibia length (mm)* | 23 | 125.0 ± 7.9 | 9 | 126.5 ± 8.6 | 20 | 128.0 ± 4.7 | 30 | 127.8 ± 6.1 |
| *Pes length (mm)* | 30 | 90.6 ± 3.6$^a$ | 9 | 88.6 ± 5.4$^{a,b}$ | 20 | 85.5 ± 3.7$^b$ | 30 | 86.8 ± 4.6$^\wedge$ |
| *Eye to ear length (mm)* | 19 | 89.0 ± 7.9 | 2 | 77.9 ± 5.7 | 20 | 87.0 ± 7.4 | 23 | 86.0 ± 7.5 |
| *Tail length (mm)* | 30 | 39.1 ± 3.9$^a$ | 9 | 42.2 ± 3.1$^b$ | 20 | 37.3 ± 5.6$^{a,b}$ | 30 | 38.7 ± 5.3 |
| *Weight (kg)* | 30 | 17.9 ± 2.6 | 10 | 17.4 ± 3.6 | 20 | 19.0 ± 2.0 | 31 | 18.5 ± 2.6 |
| *Condition (score out of 5)* | 30 | 3.4 ± 0.6$^a$ | 10 | 3.4 ± 1.0$^b$ | 20 | 4.1 ± 0.7$^b$ | 31 | 3.8 ± 0.8 |

**\*** Urania and Kulpara data combined, plus additional wombats captured at other sites on the Yorke Peninsula
[a,b,c] Differing superscript letters indicate a statistically significant difference in the variables between Swan Reach, Urania and Kulpara ($p < 0.05$), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference ($p \geq 0.05$)
[^] Statistically significant difference in the variables between Swan Reach and the Yorke Peninsula populations ($p < 0.05$)

In sub-adult southern hairy-nosed wombats, no significant difference was found between the populations in mean head length, body length, tibia length, eye to ear distance, and body weight ($p \geq 0.05$) (Table 4).

Mean head width, neck girth, and chest girth were significantly smaller in sub-adult wombats from Urania compared to those from both Swan Reach and Kulpara ($p < 0.05$). Ear length was significantly smaller in Swan Reach animals compared to those from Kulpara and Urania ($p < 0.05$). Tail length was also smaller in Swan Reach animals when compared to Urania animals ($p < 0.05$). However, Swan Reach animals had a longer pes than animals from both Kulpara and Urania ($p < 0.05$) (Table 4).

As seen in adult wombats, when data from the Urania and Kulpara colonies were pooled, it was found sub-adult wombats from the Swan Reach population were significantly

larger than Yorke Peninsula sub-adults ($p < 0.05$). Significantly different morphological features included mean head width, neck girth, chest girth, and pes length (Table 4).

*2.3.6. Morphological measurements of juvenile southern hairy-nosed wombats*

The mean values (± standard deviation) obtained for juvenile southern hairy-nosed wombat body morphology variables are indicated in Table 5.

**Table 5:** Mean morphological measurements of juvenile southern hairy-nosed wombats from three different study sites (± standard deviation)

| | | | Yorke Peninsula: | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | **Swan Reach:** | | **Urania** | | **Kulpara** | | **All\*** | |
| **Morphology measurement:** | N | Mean | N | Mean | N | Mean | N | Mean |
| Head length (mm) | 12 | 149.6 ± 11.55 | 1 | 148.7 | 20 | 149.2 ± 10.9 | 21 | 149.1 ± 10.7 |
| Head width (mm) | 12 | 109.2 ± 8.2 | 1 | 114.6 | 20 | 104.3 ± 8.1 | 21 | 104.8 ± 8.2 |
| Neck girth (cm) | 11 | 38.2 ± 3.8 | 1 | 36.3 | 20 | 38.1 ± 2.8 | 21 | 38.0 ± 2.7 |
| Chest girth (cm) | 11 | 52.1 ± 4.3 | 1 | 44.8 | 19 | 49.0 ± 5.2 | 20 | 48.8 ± 5.1 |
| Body length (cm) | 11 | 82.6 ± 5.7 | 1 | 78.9 | 20 | 77.0 ± 6.9 | 21 | 77.1 ± 6.7 |
| Ear length (mm) | 11 | 79.2 ± 4.1[a] | 1 | 80.7[a,b] | 20 | 74.8 ± 4.8[b] | 21 | 75.1 ± 4.8 |
| Tibia length (mm) | 11 | 116.6 ± 7.0 | 1 | 114.4 | 19 | 109.8 ± 7.2 | 20 | 110.0 ± 7.0 |
| Pes length (mm) | 12 | 83.4 ± 6.3[a] | 1 | 75.5[a,b] | 20 | 77.4 ± 5.4[b] | 21 | 77.3 ± 5.3[^] |
| Eye to ear length (mm) | 11 | 83.7 ± 5.8[a] | 1 | 74.5[a,b] | 19 | 74.4 ± 6.8[b] | 20 | 74.4 ± 6.6[^] |
| Tail length (mm) | 12 | 34.4 ± 5.8 | 1 | 31.0 | 20 | 32.6 ± 6.1 | 21 | 32.5 ± 6.0 |
| Weight (kg) | 12 | 11.6 ± 3.1 | 1 | 10.0 | 20 | 10.0 ± 2.2 | 21 | 10.0 ± 2.1 |
| Condition (score out of 5) | 12 | 3.6 ± 0.7 | 1 | 3.0 | 20 | 4.0 ± 0.6 | 21 | 4.0 ± 0.7 |

**\*** Urania and Kulpara data combined, plus additional wombats captured at other sites on the Yorke Peninsula
[a,b,c] Differing superscript letters indicate a statistically significant difference in the variables between Swan Reach, Urania and Kulpara (p < 0.05), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference (p ≥ 0.05)
[^] Statistically significant difference in the variables between Swan Reach and the Yorke Peninsula populations (p < 0.05)

In juvenile southern hairy-nosed wombats, no statistically significant difference was found between the populations in mean head length, head width, neck girth, chest girth, body length, tibia length, tail length, and weight (p ≥ 0.05). Juvenile wombats from the Swan Reach population, however, were significantly larger than Kulpara animals in mean ear length, pes length, and eye to ear distance (p < 0.05) (Table 5).

When data from the Urania and Kulpara populations were pooled, it was found that juvenile wombats from Swan Reach were significantly larger than Yorke Peninsula animals in mean pes length, and eye to ear distance (p < 0.05) (Table 5).

*2.3.7. Morphological measurements of the testes and accessory glands of the southern hairy-nosed wombat*

Mean morphological measurements (± standard deviation) of the testes of male southern hairy-nosed wombats from breeding (Table 6) and non-breeding seasons (Table 7) are presented.

2.3.7.1 Male testes and accessory gland measurements during the breeding season

Mean reproductive gland measurements collected for the adult male southern hairy-nosed wombats during the breeding season are indicated in Table 6.

**Table 6:** Mean testes and accessory gland morphological measurements of adult male southern hairy-nosed wombat from three different study sites (± standard deviation). Animals were captured during the breeding season

| BREEDING SEASON | Swan Reach: | | Yorke Peninsula: | | | | | |
| | | | *Urania* | | *Kulpara* | | *All\** | |
| *Morphology measurement:* | N | Mean | N | Mean | N | Mean | N | Mean |
|---|---|---|---|---|---|---|---|---|
| *Testes width (mm)* | 57 | 22.6 ± 2.6$^a$ | 13 | 24.3 ± 3.3$^b$ | 20 | 25.3 ± 2.8$^b$ | 26 | 24.2 ± 2.7 $^\wedge$ |
| *Testes length (mm)* | 57 | 31.8 ± 3.9 | 13 | 33.8 ± 3.3 | 20 | 33.1 ± 2.7 | 26 | 33.0 ± 3.1 |
| *Testes depth (mm)* | 56 | 22.2 ± 3.4$^a$ | 13 | 26.9 ± 4.3$^b$ | 20 | 24.4 ± 3.6$^b$ | 26 | 24.8 ± 4.3$^\wedge$ |
| *Scrotal width (mm)* | 57 | 51.8 ± 6.8$^a$ | 13 | 59.6 ± 8.0$^b$ | 20 | 59.7 ± 6.3$^b$ | 26 | 58.4 ±7.1$^\wedge$ |
| *Glans length (mm)* | 51 | 46.1 ± 5.5 | 13 | 46.2 ± 4.2 | 20 | 43.6 ± 8.5 | 25 | 42.2 ± 10.0 |
| *Accessory gland length (mm)* | 57 | 69.5 ± 9.7 | 13 | 70.5 ± 8.2 | 20 | 67.0 ± 9.3 | 22 | 67.4 ± 9.8 |
| *Accessory gland width (mm)* | 57 | 67.8 ± 11.4 | 13 | 68.3 ± 3.3 | 20 | 62.4 ± 7.6 | 22 | 63.9 ± 10.7 |

**\*** Urania and Kulpara data combined, plus additional wombats captured at other sites on the Yorke Peninsula

[a,b,c] Differing superscript letters indicate a statistically significant difference in the variables between Swan Reach, Urania and Kulpara ($p < 0.05$), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference ($p \geq 0.05$)

$^\wedge$ Statistically significant difference in the variables between Swan Reach and the Yorke Peninsula populations ($p < 0.05$)

During the breeding season, no statistically significant difference was found between mean testes length, glans length, and accessory gland length and width from southern hairy-nosed wombats at Swan Reach, Urania or Kulpara ($p \geq 0.05$). Adult male wombats from the Swan Reach population however, had significantly smaller mean testes width, testes depth, and scrotal width than males from either Urania and Kulpara, or when the data from these latter colonies were pooled ($p < 0.05$) (Table 6).

2.3.7.2 Male testes and accessory gland measurements during the non-breeding season

Mean reproductive gland measurements collected for the adult male southern hairy-nosed wombats during the non-breeding season are indicated in Table 7. No data was collected from male southern hairy-nosed wombats at Kulpara during the non-breeding season.

**Table 7:** Mean testes and accessory gland morphological measurements of adult male southern hairy-nosed wombats from two different study sites (± standard deviation). Animals were captured during the non-breeding season

| NON-BREEDING SEASON | Swan Reach: | | Urania: | |
|---|---|---|---|---|
| *Morphology measurement:* | N | Mean | N | Mean |
| *Testes width (mm)* | 36 | 20.5 ± 2.3^ | 9 | 24.0 ± 3.6 |
| *Testes length (mm)* | 36 | 30.7 ± 3.7^ | 9 | 35.0 ± 3.3 |
| *Testes depth (mm)* | 17 | 20.8 ± 3.5^ | 8 | 25.3 ± 2.2 |
| *Scrotal width (mm)* | 35 | 47.4 ± 6.0^ | 9 | 60.2 ± 6.1 |
| *Glans length (mm)* | 0 | - | 8 | 45.0 ± 3.8 |
| *Accessory gland length (mm)* | 30 | 58.7 ± 7.3^ | 8 | 68.5 ± 6.5 |
| *Accessory gland width (mm)* | 31 | 51.0 ± 8.6^ | 8 | 64.2 ± 9.0 |

^ Statistically significant difference in the variables between Swan Reach and Urania ($p < 0.05$)

Male southern hairy-nosed wombats captured from Swan Reach during the non-breeding season had significantly smaller mean testes width, testes length, testes depth, scrotal width, accessory gland length, and accessory gland width than male wombats captured from Urania, on the Yorke Peninsula, during the non-breeding season ($p < 0.05$) (Table 7).

**2.4. Discussion**

There are many negative consequences associated with low genetic diversity for small and isolated populations, which can result in an increased risk of local species extinctions (as discussed in Chapter 1, section 1.1) (Falconer 1981; Ralls and Ballou 1986; Sherwin and Murray 1990; Frankham 1995; Eldridge *et al.* 1999; Keller and Waller 2002). However, genetic structure is not necessarily the only parameter that needs to be considered when determining the likelihood of extinction of fragmented and isolated populations, as ecological factors are also important (Gaines *et al.* 1997). Previous studies on the southern hairy-nosed wombats on the Yorke Peninsula found the colonies to be scattered along the peninsula in small, fragmented, and isolated patches (Johnson 1989; Alpers *et al.* 1998). In the current study, a total of 696 southern hairy-nosed wombats were estimated to reside within 25 colonies on the Yorke Peninsula. Of these 25 colonies, 21 were thought to contain less than 20 animals. Some of these sites, such as those at Weavers Lagoon, Brentwood and Lake Fowler are highly geographically isolated from the other colonies, with at least 20 km of cleared land separating them from other groups. Southern hairy-nosed wombats have relatively small home ranges (Finlayson *et al.* 2005; Walker *et al.* 2006); therefore it may be unlikely that individuals would traverse such a distance (Chapter 1.2.4.). The extremely small sizes of colonies on the Yorke Peninsula would suggest a high level of inbreeding, as avoiding kin would be practically impossible without dispersal. As well as the potential effects of inbreeding, there are possibilities that a natural disaster, such as fire, disease or drought could eradicate these small and isolated colonies, or animals could die as a result of hunting, road accidents, or other human related causes. The combination of these factors may contribute to a higher probability of local colony extinctions for southern hairy-nosed wombats on the Yorke Peninsula.

Research into conservation of fragmented and isolated populations is often based on the theory of island biogeography. Animals are confined to their "island" of remnant vegetation, surrounded by a "sea" of modified and inhospitable habitat (Millien 2006; Prugh *et al.* 2008). Changes in response to habitat fragmentation are thought to be similar to those that occur when species are isolated on island habitats (Millien 2006). In vertebrates, a pattern has been observed for species residing on islands; a tendency for gigantism for small species, and dwarfism for large species; a phenomenon that has been termed the' island rule' (Whittaker and Fernandez-Palacios 2007). In this study, body morphology of the three largest southern hairy-nosed wombat colonies on the Yorke Peninsula (population estimates > 100; Urania, Pt Pearce and Kulpara), were compared with that of the large continuous population at Swan Reach. Data indicate that adult wombats from the isolated populations on the Yorke

Peninsula (and to a lesser extent sub-adults and juveniles), were significantly smaller in body size (e.g. head width, neck and chest girth, body length, pes length, and eye to ear length) than those individuals from Swan Reach. These observations are consistent with island rule theory that proposes that large species tend to dwarf when isolated (Whittaker and Fernandez-Palacios 2007), examples of which are the Nile crocodile (*Crocodylus niloticus*), where animals residing in isolated populations are approximately half the body length than the same species residing in a large population (Shine *et al.* 2001). It is hypothesised that the smaller animal size on an island may evolve to reduce their energy requirements due to the limited resources available (Meiri *et al.* 2008a). The southern hairy-nosed wombats on the Yorke Peninsula have only small patches of remnant habitat in which to reside as burrows in cropping paddocks are often destroyed by land owners. Wombats predominantly feed in close proximity to their burrows (Wells 1989), although they have been observed to graze in surrounding crops on the Yorke Peninsula. This ability to feed in non-native habitat surrounding remnant vegetation patches suggests that the southern hairy-nosed wombats on the Yorke Peninsula are not as limited in resources as true island animals might be, although feed within crops is likely to be highly seasonal.

Species isolated on islands are not the only ones to experience morphological changes with time. Following a population bottleneck associated with a volcanic event, a species of caenolestoid marsupial (*Acolestis owenii*), as well as an octodontoid rodent species (*Spaniomys* spp), have been found to have reductions in dental dimensions, suggesting a reduction in head size which was believed to have resulted from a combination of genetic and environmental effects (Anderson *et al.* 1995). Similar results were also seen in black and white ruffled lemurs (*Varecia variegata*), where animals from a small population were found to be significantly smaller in 6 out of 16 body measurements, including body mass and body length when compared to those from a large population (Baden *et al.* 2008). These results reflect those found in this study, where the adult wombats from the small colonies on the Yorke Peninsula were significantly smaller in 6 of 12 body morphological measurements compared to those of the larger population at Swan Reach.

Body mass did not differ between the colonies in this study, as it did in the lemurs, this is not surprising as body mass can vary substantially in southern hairy-nosed wombats with rainfall and pasture growth, and probably reflects regional variations in rainfall patterns and pasture growth (Gaughwin *et al.* 1998; Taggart *et al.* 2005) (Table 8). Body length, however, did vary, with Yorke Peninsula wombats being significantly smaller than Swan Reach animals. Baden *et al.* (2008) believed the size difference in the lemurs was due to a high density relative to other sites, and restricted emigration opportunities hence a heightened

resource competition in isolated populations. The results of the present study show little difference in the wombat densities per km travelled between the study sites. However, Walker *et al.* (2008b) found the wombat density at Kulpara to be more than double the density in the Murraylands population at Brookfield Conservation Park (approximately 50 km north of Swan Reach). The method used by Walker to assess density involved non-invasive sampling, using hair tapes set up over burrows. Since wombats can be difficult to observe at night, the hair taping method is possibly a more reliable method for predicting population density. In addition, the majority of the field work at Kulpara involved searching for animals on foot, which restricted vision, compared to Swan Reach where all work was conducted from the back of a vehicle, and the view of the landscape, and therefore wombats, was less obstructed. Hence, the density of wombats within the Kulpara colony may have been underestimated.

Dispersal for wombats between colonies on the Yorke Peninsula would be difficult considering the large distances between extant colony sites. The colonies closest to the Urania wombat colony are approximately 40 km away, and are extremely small. The closest wombat colonies to Kulpara are over 20 km away, and also extremely small. As observed in lemurs (Baden *et al.* 2008) increased animal density and minimal migration could also cause resource competition for the wombats on the Yorke Peninsula. However, food and water availability are likely to be higher for animals residing on the Yorke Peninsula compared to that available for wombats from Swan Reach. Unlike the Swan Reach animals, the Yorke Peninsula wombats have access to crops, as well as a much higher average rainfall (Table 8), suggesting potentially increased food availability.

**Table 8:** Climate records for the three sites examined in this study. Data obtained from the Federal Government: Australian Bureau of Meteorology

NOTE:
This table is included on page 44
of the print copy of the thesis held in
the University of Adelaide Library.

The data also indicates that, whilst the Yorke Peninsula animals are significantly smaller in some morphological measurements (including body length), there is no significant difference in their body mass. As mentioned previously, southern hairy-nosed wombat body mass can differ with varying rainfall patterns, and Yorke Peninsula has a greater annual

rainfall than Swan Reach (Table 8). This suggests that the Yorke Peninsula wombats may be better fed, although the body condition score does not reflect this finding. Condition scores, however, are extremely subjective, and do not take into account body fat alone. Presence of mange, and parasite loads, loss of fur and general physical appearance are also represented. Observational data suggested that wombats on the Yorke Peninsula have a greater incidence of mange and parasitic load than those at Swan Reach, which may confound the results.

The southern hairy-nosed wombats on the Yorke Peninsula, whilst smaller in selected body morphological parameters, were found to have greater testis width and depth, as well as a greater scrotal diameter, than Swan Reach animals. During the non-breeding season, animals at Urania had larger testes than those in Swan Reach in all measurements except glans length. These data support previous studies which hypothesise that reproductive output of larger animals is increased on island populations (Meiri *et al.* 2008b). In other animals, such as primates, ejaculate volume, number of motile sperm, and the sperm concentration per ejaculate increase with an increase in testes size (Moller 1988). Therefore, the greater testes dimensions of wombats on the Yorke Peninsula could possibly mean these individuals have better seminal quality, compared to the Swan Reach animals. In koalas however, it has been found that smaller, more inbred populations, have a significantly lower proportion of seminiferous epithelial tissue, when compared to large outbred populations (Montgomery 2002). Interestingly, in koalas, as populations become more inbred, the proportion of interstitial tissue to seminiferous tubule tissue increased (Montgomery 2002). The seminiferous epithelium in mammals contains germ cells undergoing meiosis and haploid cell differentiation into spermatozoa, as well as supporting cells, the Sertoli cells, whereas interstitial tissue between the seminiferous tubules contains connective tissue and Leydig cells that secrete testosterone together with blood vessels and lymphatics (Johnson and Everitt 2000). Therefore, it is possible that wombats from Kulpara and Urania may have larger testes due to an increase in the abundance of interstitial tissue, rather than that of the seminiferous epithelium, with the consequence that, even though the testes are larger in animals in these populations, the tissue in which spermatogenesis occurs may not be greater. Hence, having smaller testes may therefore not disadvantage the wombats from Swan Reach. In other species smaller testes size did not result in a reduction in sperm concentration in gazelles (Cassinello *et al.* 1998) or decreased breeding success in oldfield mice (Margulis and Walsh 2002). In the present study, seminal quality and reproductive success of the three southern hairy-nosed wombat colonies will be examined in detail in subsequent chapters.

## 2.5. Summary

In summary, the southern hairy-nosed wombat populations on the Yorke Peninsula have been shown to be extremely small and geographically isolated. When compared to a large population of southern hairy-nosed wombats at Swan Reach, it is unknown whether the smaller body size, and the greater testes size, in small colonies of wombats residing on the Yorke Peninsula, is due to either genetic and/or environmental factors, resource limitation, a combination of all three, or yet other factors. Data suggest that body morphology may be influenced by population size, although the present study cannot explain the processes behind the differences in morphology between the different colonies of southern hairy-nosed wombats examined, since only basic demographical comparisons between the two populations were determined. To do this, further studies would need to be conducted of additional wombat populations in South Australia.

**Chapter 3: The impact of population fragmentation and isolation on the genetic diversity of three southern hairy-nosed wombat populations in South Australia**

## 3.1. Introduction

*3.1.1. Genetic diversity in isolated populations of Eutherian mammals*

Habitat fragmentation and associated isolation can have a negative impact on the genetic diversity of a population, and can lead to reduced population viability, and an increased risk of extinction, due to factors such as inbreeding depression (Frankham *et al.* 2002).

In European ground squirrels (*Spermophilus citellus*) (Hulova and Sedlacek 2008), and Asian black bears (*Ursus thibetanus*) (Ohnishi *et al.* 2007), habitat fragmentation has lead to isolation of some populations, with these populations showing reduced genetic variation and high levels of inbreeding. This has also been demonstrated in yellow necked mice (*Apodemus flavicollis*) and bank voles (*Myodes lareolus*) (Kozakiewicz *et al.* 2009), where all measures of genetic diversity (mean number of alleles, mean allelic richness, observed and expected heterozygosity) were shown to be higher in mainland populations, compared to island populations (Kozakiewicz *et al.* 2009).

*3.1.2. Genetic structure in hairy-nosed wombats*

Microsatellite analysis of the critically endangered northern hairy-nosed wombat has indicated that the single remaining population of this species in Epping Forest, QLD, has very low genetic variation, with low values of allelic diversity and heterozygosity (observed heterozygosity of 0.28 for 16 loci), when compared to their closest relative, the southern hairy-nosed wombat (Taylor *et al.* 1994). Studies by Alpers *et al.* (1998), Alpers and Sherwin (1999) and Walker (2004), revealed that there was a relatively high degree of genetic variation and no significant inbreeding within numerous southern hairy-nosed wombat populations in South Australia, with the possible exception of Urania (Yorke Peninsula) and Nundroo (Nullarbor Plain), where a significant homozygote excess was observed and a degree of local inbreeding suggested (Alpers and Sherwin 1999). In both Alpers papers, mean observed heterozygosity levels ranged from 0.53 to 0.82. The large population at Swan Reach had a high observed heterozygosity value of 0.74 (Alpers *et al.* 1998). The lowest two values were found on Wedge Island (0.52) and Urania (0.56) (Alpers and Sherwin 1999). The population of wombats from Kulpara had an observed heterozygosity of 0.61 (Alpers and Sherwin 1999), and was found to be the most genetically distinct southern hairy-nosed wombat population studied, suggesting that this population had been isolated the longest

(Walker *et al.* 2008a). As previously mentioned, island and other isolated populations are more likely to be subjected to inbreeding and an associated loss of heterozygosity and allelic variation than are large continuous populations (Frankham 1995; Keller and Waller 2002).

*3.1.3. Potential for inbreeding in Vombatidae*

Mills and Smouse (1994) suggest that those species with low reproductive rates are at a greater risk of extinction. This is because parameters associated with reproductive fitness are generally the most sensitive to inbreeding depression (Frankham *et al.* 2002). This scenario applies to all three species of wombat, which have low reproductive rates. In addition, with their relatively small home ranges, and sedentary lifestyle, there is an increased potential for inbreeding, associated with lack of animal movement.

*3.1.4. Aims*

Previous studies have examined the genetic diversity of southern hairy-nosed wombat populations in the Murraylands, and on the Yorke Peninsula, of South Australia (Alpers *et al.* 1998; Alpers and Sherwin 1999; Walker 2004). The current study re-examined the same variables within these populations for the maintenance of consistency across the data set, i.e. the animals that were used for the collection of genetic data were those same individuals used in the following chapters for examination of seminal quality. This enabled any associations between these different variables to be examined.

In addition, the results from Chapter 2 indicated that the animals from the two Yorke Peninsula populations (Kulpara and Urania) were significantly smaller in body size, and larger in testes size than wombats from the larger Murraylands population examined near Swan Reach. The genetic diversity of those wombats captured in Chapter 2 was examined to determine if any correlated genetic difference was present, as previous studies have linked a reduction in mammal head size to a combination of genetic and environmental factors (Anderson *et al.* 1995).

The aim of this chapter was to determine the levels of genetic diversity, and genetic differentiation in three populations of southern hairy-nosed wombats in South Australia. One large continuous outbred population and two smaller, geographically isolated populations are compared to determine any associations between population fragmentation and isolation, and differences in genetic variation.

## 3.2. Methods

### 3.2.1. Study populations

The southern hairy-nosed wombat populations at Kulpara, Urania and Swan Reach (Chapter 2, section 2.2.1.) were compared in this study. Samples were also collected from wombats residing at the Fowlers Lake colony, on the southern Yorke Peninsula (Chapter 2, section 2.3.2.)

### 3.2.2. Animal capture and data collection

Each wombat captured (as described in Chapter 2, section 2.2.3.) had its ear sterilised and an ear biopsy taken using a leather punch. Only adult wombats (based on classifications outlined in Chapter 2, section 2.2.4.) were used in the genetic analysis.

As only one wombat was captured at the Fowlers Lake colony, hair samples from the colony were collected to increase sample size for genetic testing. Hair samples were collected by suspending double sided tape across the entrance of the burrow. Tape was secured in place by two plastic garden stakes on each side of the burrow. Tapes were examined the following day and any hair present removed and placed in an envelope. Hairs that were clumped together were assumed to be from the same animal.

### 3.2.3. Genetic analysis

Ear biopsies were stored in 100% ethanol in an Eppendorf tube until required for analysis, and hair samples were frozen at -80ºC. DNA was extracted from both the ear biopsies and hair samples using the Gentra DNA extraction kit, following the protocol recommended by the manufacturer.

### 3.2.4. Microsatellite analysis

Twelve microsatellite loci (L12, Lk34, Lkr109, Lla54CA, Lk09, Lla16CA, Lla67CA, Lla71CA, Lkr107, Lla68CA, Lk27, and Lk26) previously developed for wombats (Beheregaray *et al.* 2000) were used. Polymerase chain reaction (PCR) amplifications were carried out in 10μl reaction volumes containing approximately 10 ng of DNA, 0.04 μl (0.2 units) of HotMaster taq DNA polymerase (5 Prime Pty Ltd), 200 nM of each primer, 200 μM of dNTP, and 1x HotMaster reaction buffer. Cycling started at 94 ºC for two minutes, then, depending on which primer was used, was followed by one of three different "touchdown" PCR settings (30 cycles at 94 ºC/15s, annealing/30s, 72 ºC/45s) as previously described (Beheregaray *et al.* 2000), with a final incubation at 72 ºC for two minutes. Lkr107, L12, Lla54CA and Lkr109 used touchdown 62 - 55 (in the annealing process the temperature

decreased 62 – 61 – 59 – 57 – 55 ºC), Lk07 used touchdown 65 - 60 (in the annealing process the temperature decreased one degree per cycle), and Lk34, Lla68CA, Lla71CA, Lla16CA, Lla67CA, Lk27 and Lk26 used touchdown 55 - 47 (in the annealing process the temperature decreased two degrees per cycle) (Beheregaray *et al.* 2000). However, four of these loci (Lkr107, Lla68CA, Lk27, Lk26) did not PCR-amplify reliably (i.e. they showed a high frequency of non-amplifications) and were excluded from further analyses. Therefore, eight loci were used in the final analysis.

The computer program ARLEQUIN version 3.0 (Excoffier *et al.* 2005) was used to determine compliance of the three southern hairy-nosed wombat populations to Hardy-Weinberg expectations, and to calculate the expected heterozygosity value ($H_E$), and the observed heterozygosity value ($H_O$). An exact test of Hardy-Weinberg equilibrium, locus by locus, was performed for all three populations (number of steps in Markov chain = 1000000, and number of dememorization steps = 100000). Pairwise $F_{ST}$ values were also calculated in ARLEQUIN, to the significance level of $\alpha = 0.05$, using 110 permutations. GENEPOP version 3.4 (Raymond and Rousset 1995) was used to determine whether there was linkage disequilibrium between pairs of loci in each population, with each pair of loci compared and 100 batches and 1000 iterations per batch run. Data was adjusted using strict Bonferroni corrections for multiple comparisons (Rice 1989), with an initial significance level of $\alpha = 0.05$. Mean number of alleles per locus and mean $F_{IS}$ between the populations was also calculated through GENEPOP, with the $F_{IS}$ values computed as in Weir and Cockerham (1984) Mean allelic richness was calculated using FSTAT version 2.9.3.2, and the significance values ($\alpha = 0.05$) between the populations of allelic richness, $F_{IS}$ and $H_O$ determined using 1000 permutations (Goudet 2002).

STRUCTURE version 2.2 (Pritchard *et al.* 2000) was used to infer population structure and cluster individuals in populations based on their genotypic data, without any *a priori* information on the populations. The parameter set used an admixture model, with 10,000 iterations, after a 10,000 iteration burn-in period. Five runs of models K = 1 to K = 10 were used to infer the number of genetic populations (K). The highest ΔK score was calculated using the program Structure Harvester (*http://taylor0.biology.ucla.edu/struct_harvest/*) and the best fit model selected based on the specifications by Evanno *et al.* (2005).

**3.3. Results**

Ear biopsies were taken from 64 adult southern hairy-nosed wombats from Kulpara, 86 adult wombats from Urania, and 175 adult wombats from Swan Reach.

In addition to the above analyses, samples (one ear biopsy and four hair samples) were collected from five southern hairy-nosed wombats from Lake Fowler on the southern Yorke Peninsula. However, due to the amplification of only three loci, and the extremely small sample size, these results were not included in the final analyses.

*3.3.1. Hardy-Weinberg Equilibrium and linkage disequilibrium*

ARLEQUIN and GENEPOP analyses showed that genotype frequencies for all eight microsatellite loci from each of the study populations at Swan Reach, Urania and Kulpara did not differ significantly from the Hardy-Weinberg equilibrium frequencies (Table 9, Table 10, and Table 11), and there was no evidence of linkage disequilibrium (initial $\alpha = 0.05$, strict Bonferroni correction).

**Table 9:** Observed ($H_o$) and expected heterozygosity ($H_E$) values for eight loci in adult southern hairy-nosed wombats from the Swan Reach population (N = 175)

| Locus | $H_o$ | $H_E$ | p-value | Standard error |
|:---:|:---:|:---:|:---:|:---:|
| L12 | 0.73 | 0.74 | 0.21 | 0.001 |
| Lk34 | 0.58 | 0.57 | 0.68 | 0.001 |
| Lkr109 | 0.65 | 0.65 | 0.63 | 0.001 |
| Lla54CA | 0.84 | 0.82 | 0.16 | 0.001 |
| Lk09 | 0.69 | 0.70 | 0.32 | 0.001 |
| Lla16CA | 0.64 | 0.66 | 0.04 | 0.000 |
| Lla67CA | 0.78 | 0.84 | 0.25 | 0.001 |
| Lla71CA | 0.76 | 0.73 | 0.23 | 0.002 |

**Table 10:** Observed ($H_o$) and expected heterozygosity ($H_E$) values for eight loci in adult southern hairy-nosed wombats from the Urania population (N = 86)

| Locus | $H_o$ | $H_E$ | p-value | Standard error |
|---|---|---|---|---|
| L12 | 0.81 | 0.79 | 0.17 | 0.001 |
| Lk34 | 0.59 | 0.65 | 0.21 | 0.001 |
| Lkr109 | 0.83 | 0.80 | 0.49 | 0.001 |
| Lla54CA | 0.72 | 0.79 | 0.31 | 0.001 |
| Lk09 | 0.58 | 0.68 | 0.05 | 0.001 |
| Lla16CA | 0.62 | 0.68 | 0.33 | 0.001 |
| Lla67CA | 0.63 | 0.71 | 0.04 | 0.000 |
| Lla71CA | 0.74 | 0.74 | 0.52 | 0.001 |

**Table 11:** Observed ($H_o$) and expected heterozygosity ($H_E$) values for eight loci in adult southern hairy-nosed wombats from the Kulpara population (N = 64)

| Locus | $H_o$ | $H_E$ | p-value | Standard error |
|---|---|---|---|---|
| L12 | 0.83 | 0.81 | 0.38 | 0.001 |
| Lk34 | 0.63 | 0.69 | 0.16 | 0.001 |
| Lkr109 | 0.72 | 0.66 | 0.69 | 0.001 |
| Lla54CA | 0.72 | 0.80 | 0.10 | 0.001 |
| Lk09 | 0.64 | 0.56 | 0.35 | 0.001 |
| Lla16CA | 0.81 | 0.76 | 0.23 | 0.001 |
| Lla67CA | 0.78 | 0.76 | 0.57 | 0.001 |
| Lla71CA | 0.78 | 0.70 | 0.45 | 0.001 |

*3.3.2. Genetic diversity*

The mean measures of genetic diversity for three southern hairy-nosed wombat populations in South Australia, using the eight polymorphic loci are outlined in Table 12. Both mean observed and expected heterozygosity is high within each population (range 0.69 to 0.74), and there is very little difference in heterozygosity between the Swan Reach, Urania and Kulpara populations. No significant difference was found between the populations in $H_O$ (p ≥ 0.05). The mean number of alleles per locus was highest at Swan Reach (6.88) and lowest at Kulpara (5.13). However, as the sample size was substantially larger at Swan Reach, allelic richness was also calculated as this measure takes into account sample size and accounts for non-detection of alleles. The results from examination of mean allelic richness also show Swan Reach to have the greatest number of alleles per locus (6.0), and Kulpara the

smallest (5.1) (Table 12),  however this difference was not statistically significant (FSTAT version 1.2; $p \geq 0.05$). $F_{IS}$ measures the deficit of heterozygotes observed relative to the expected Hardy-Weinberg equilibrium. The results show slightly positive values for Swan Reach (0.006) and Urania (0.0058), and a negative value for Kulpara (-0.035). No significant difference was found between these populations (Table 12; $p \geq 0.05$). $F_{IS}$ values for these three populations are all close to zero and the ± standard deviations all include zero in their range. This indicates that individuals are mating at random and there is no evidence for inbreeding at the level of the individual within populations (Table 12).

**Table 12**: Mean measures of genetic diversity using eight loci for three southern hairy-nosed wombat populations in South Australia (± standard deviation)

| | N | Mean heterozygosity[a] | | Mean number of alleles per locus[b] | Mean allelic richness[c] | Mean $F_{IS}$[b] |
|---|---|---|---|---|---|---|
| | | $H_E$ | $H_O$ | | | |
| **Swan Reach** | 175 | 0.71 ± 0.09 | 0.71 ± 0.08 | 6.88 ± 2.5 | 6.0 ± 2.0 | 0.006 ± 0.04 |
| **Urania** | 86 | 0.73 ± 0.09 | 0.69 ± 0.1 | 5.75 ± 1.5 | 5.6 ± 1.3 | 0.058 ± 0.07 |
| **Kulpara** | 64 | 0.71 ± 0.08 | 0.74 ± 0.07 | 5.13 ± 1.6 | 5.1 ± 1.6 | -0.035 ± 0.09 |

[a] Calculated using ARELEQUIN version 3.0
[b] Calculated using GENEPOP version  3.4
[c] Calculated using FSTAT version 2.9.3.2

*3.3.3. Genetic differentiation*

The $F_{ST}$ value measures genetic differentiation between sampled populations, at Swan Reach, Urania and Kulpara. The $F_{ST}$ values for all three populations were high. All $F_{ST}$ values were shown to be significantly different from one another, indicating considerable genetic differentiation between the populations (Table 13).

**Table 13:** Genetic differentiation (pairwise $F_{ST}$) between southern hairy-nosed wombats at the three sampled populations in South Australia

| $F_{ST}$ | Kulpara | Swan Reach |
|---|---|---|
| Swan Reach | 0.18* | |
| Urania | 0.15* | 0.18* |

* Statistically significant difference between the populations

The program STRUCTURE was used to independently test whether there was genetic differentiation among populations of the southern hairy-nosed wombats without *a priori* assumptions of population structure. Specifications by Evanno *et al.* (2005) suggest the best estimate for number of clusters is the highest ΔK value (the second order rate of change of the likelihood function with respect to K). The results from this analysis suggest that the true number of clusters (K), based on the best fit model, was K = 2, however K = 3 also showed a very high ΔK value (Table 14, Figure 6).

**Table 14:** Genetic clustering analysis of all adult southern hairy-nosed wombats from this study using the computer program STRUCTURE. K = number of sub-populations, Ln P(D) and Var Ln P(D) = posterior probability of the data for a given and variance for each model, and ΔK = the second order rate of change of the likelihood function with respect to K

| K | Ln P(D) | Var Ln P(D) | ΔK |
|---|---------|-------------|-----|
| 1 | -9722.36 | 3248 | - |
| 2 | -8093.96 | 80.96 | 941.15* |
| 3 | -7487.04 | 117.78 | 403.71 |
| 4 | -7437.04 | 215.22 | 2.96 |
| 5 | -7402.82 | 308.10 | 2.72 |
| 6 | -7392.94 | 396.06 | 12.40 |
| 7 | -7515.30 | 499.54 | 1.08 |
| 8 | -7413.56 | 620.82 | 4.38 |
| 9 | -7406.40 | 689.76 | 2.74 |
| 10 | -7490.68 | 890.00 | - |

* Best fit model for K based on specifications by Evanno *et al.* (2005)
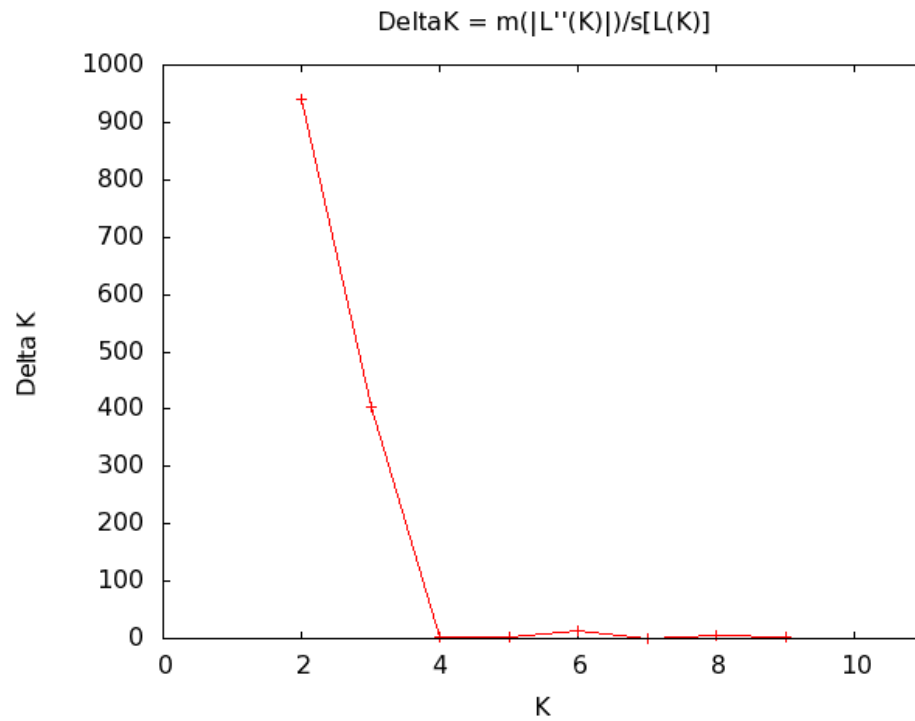
**Figure 6:** The estimated number of populations (K) plotted against ΔK using Structure Harvester. The highest ΔK (ΔK = 941.15), at K = 2 suggests this is the best fit model, based on Evanno *et al.* (2005)

Based on the assumption that K = 2, the summary bar plot below shows the estimated membership coefficients for each individual, in each cluster (Q), with each individual represented by a single line, divided into K (= 2) coloured segments. In general, the animals from Urania and Kulpara show high Q values (>0.95), supporting their membership of a York Peninsula population that is distinct from the population at Swan Reach (Figure 7).
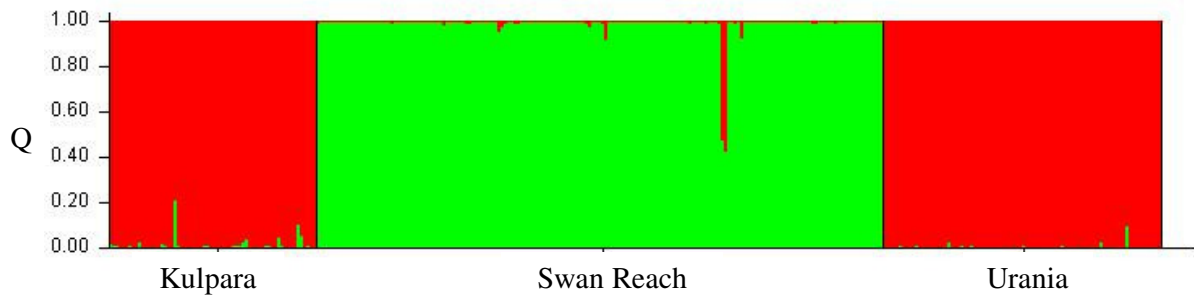


**Figure 7:** Estimates of Q (estimated membership coefficients for each individual in each cluster) for southern hairy-nosed wombats, with each individual represented by a single line, divided into coloured segments based on the estimated number of populations (K) = 2. The x-axis value represents the original population ID used in the STRUCTURE program

Whilst the STRUCTURE best fit model for southern hairy-nosed wombats was K = 2 (Yorke Peninsula and Swan Reach), all three populations (Kulpara, Urania and Swan Reach) show significant pairwise $F_{ST}$ values (p < 0.05) (Table 13). STRUCTURE analyses show that the majority of individuals clustered with other wombats from their respective population of origin (Figure 8 and Figure 9), with a number of notable exceptions that appear to have mixed ancestry within the Urania population (five individuals out of 86, or 6 %) and Swan Reach population (two individuals out of 175, or ~1 %) with high Q values for membership of the Kulpara population. These results support the conclusion that each of the three populations is genetically distinct, with limited migration between them.
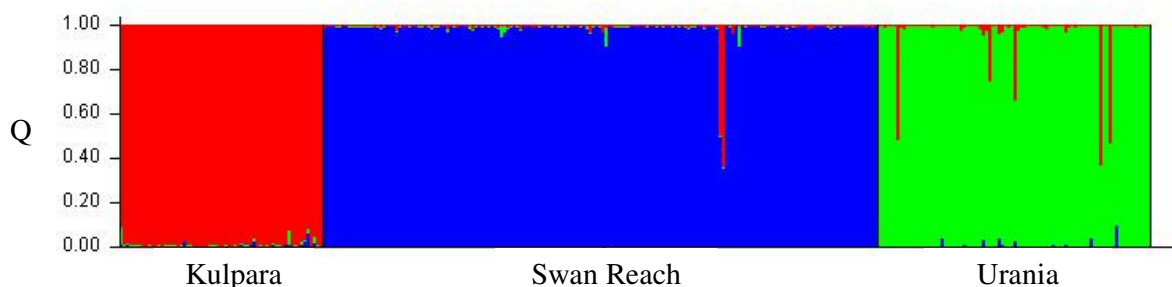


**Figure 8:** Estimates of Q (estimated membership coefficients for each individual in each cluster) for southern hairy-nosed wombats, with each individual represented by a single line, divided into coloured segments based on the estimated number of populations (K) = 3. The x-axis value represents the original population ID used in the STRUCTURE program
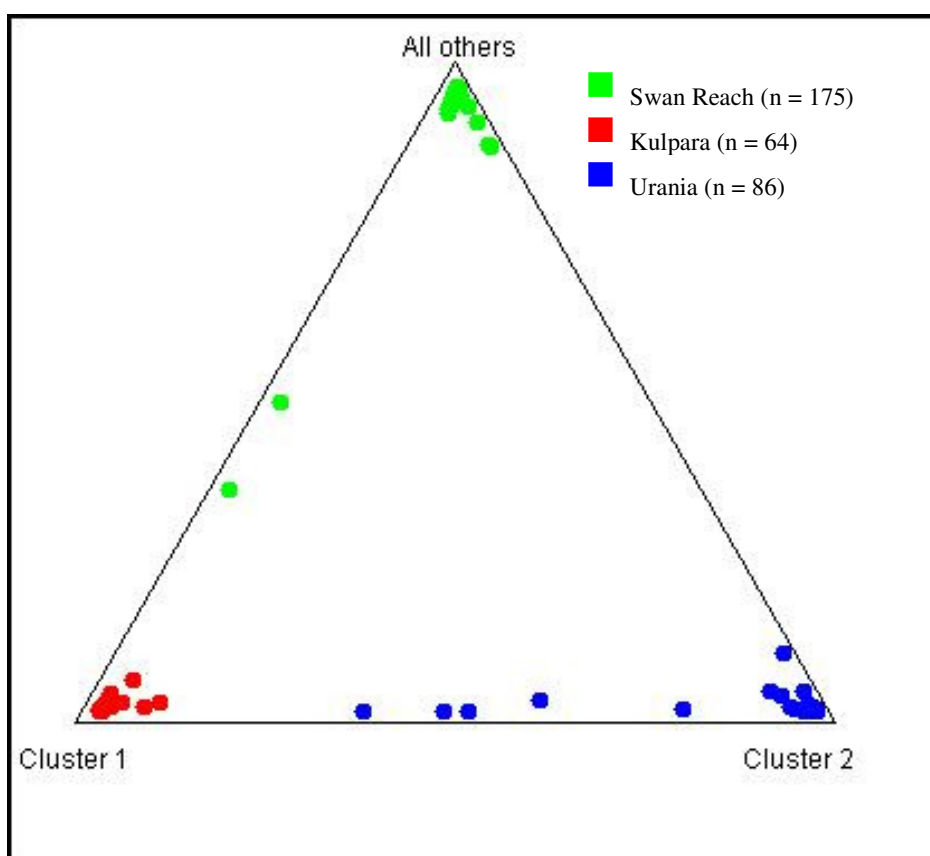
**Figure 9:** Triangle plot of Q, clustering individual southern hairy-nosed wombats based on their genotype. The dots represent each wombat, and the colour represents the population of origin

### 3.4. Discussion

Habitat fragmentation and the associated population isolation can negatively impact genetic diversity and population viability (Frankham *et al.* 2002). Small and isolated populations often have reduced genetic diversity (heterozygosity and allelic diversity) when compared to a large continuous population. This has been observed in a wide range of animals, from eutherian mammals such as wolves (Wayne *et al.* 1991), Asian black bears (Ohnishi *et al.* 2007), mice and voles (Kozakiewicz *et al.* 2009) and squirrels (Trizio *et al.* 2005; Hulova and Sedlacek 2008), to marsupials such as possums (Hansen *et al.* 2009) and brush-tail rock wallabies (Hazlitt *et al.* 2006), and even in amphibians, such as frogs (Johansson *et al.* 2007). High inbreeding values were also detected in many of these studies. The likelihood of extinction in populations with these characteristics can be elevated through inbreeding depression, which reduces the ability to adapt to physiological and/or environmental change (Frankham *et al.* 2002).

In Chapter 2, it was shown that southern hairy-nosed wombat populations on the Yorke Peninsula have become highly fragmented since European settlement, and are now restricted to a limited number of very small and physically isolated populations. Frankham *et al.* (2002) states that "inbreeding is inevitable in small populations", and examined the relationship between population size and expected heterozygosity by reviewing research on a variety of animal and plant species (N=23). It was observed that in 22 of the species there was a positive correlation between heterozygosity and population size. This is in contrast to results from the current study, where a correlation between population and size and heterozygosity was not found for the two small populations of southern hairy-nosed wombats on the Yorke Peninsula (Urania and Kulpara) when examining the observed and expected heterozygosities. Both heterozygosity values were high, and similar to each other, as well as being comparable to the heterozygosity values at the large continuous population at Swan Reach. However, there were a slightly greater number of alleles per locus, and higher allelic richness in the wombats from the Swan Reach population compared to Urania and Kulpara, although this was not statistically significant. These results contrast to those reported in other genetic studies of animal species (reviewed in Chapter 1 and this chapter), where heterozygosity values for small populations are reduced due to the effects of inbreeding (Primack 1998; Frankham *et al.* 2002, 2004; Allendorf and Luikart 2007). However, similar results to that found for the southern hairy-nosed wombats have also been found in a range of other animal species. Research on mountain pygmy possums (*Barramys parvus*) (Mitrovski *et al.* 2007), white footed mice (*Peromyscus leucopus*) (Mossman and Waser 2001), forest skinks (*Gnypetoscincus queenslandiae*) (Sumner *et al.* 2004), copper redhorse (*Moxostoma hubbsi*)

(Lippe *et al.* 2006), orangatuns (*Pongo pygmaeus*) (Goossens *et al.* 2005), howler monkeys (*Alouatta palliate*) (Milton *et al.* 2009), and ornate box turtles (*Terrapene ornate*) (Kuo and Janzen 2004), all observed high heterozygosity values in small, and isolated populations. Some of the suggested reasons for this phenomenon were: (1) there was still some gene flow between the populations, i.e. the surrounding habitat matrix does not deter migration between fragmented populations (Mitrovski *et al.* 2007), (2) habitat fragmentation is only recent (Goossens *et al.* 2005; Mitrovski *et al.* 2007; Milton *et al.* 2009) and (3) not enough generations have passed for heterozygosity and/or allelic diversity to decrease (Mossman and Waser 2001). Point three is thought to be especially true for those animals that have a long life span, like the copper redhorse, which have a life span of approx 30 years, with a late maturation age of 10 years (Lippe *et al.* 2006), or the ornate box turtle, with a life span of between 25 and 50 years (Kuo and Janzen 2004).

In the current study, genetic differentiation results from $F_{ST}$ analyses, where all three populations (Swan Reach, Urania and Kulpara) were significantly genetically different, contrasted with results from STRUCTURE analysis, where Evanno (2005) specifications suggested only two genetically different populations (Swan Reach and Yorke Peninsula). However, examination of different plots (bar and triangle) in STRUCTURE for K = 3, show that the majority of individuals tend to group into their population of origin. In addition, the $\Delta$K value for K = 3 was also much higher than all the other possible clusters (apart from K = 2). As the STRUCTURE program is less sensitive to subtle population structure than $F_{ST}$ values (Pritchard *et al.* 2000) (particularly with only eight loci), only small amounts of recent migration may account for these contrasting results. A number of individuals with mixed ancestry in the Urania population may have resulted from a small amount of migration of animals between Urania and Kulpara (currently a distance of approximately 75 km). However, it is uncertain whether these migration events pre- or post- date the fragmentation of these populations. Taken overall, these findings suggest that the three populations are genetically differentiated and there is limited migration between them. Previous research on wombats at these three locations has also provided evidence for three genetically distinct populations (Alpers *et al.* 1998; Walker 2004).

In Chapter 2, it was found that southern hairy-nosed wombats on the Yorke Peninsula were significantly smaller in body morphometric measurements, and significantly larger in testis measurements than animals from Swan Reach. It may therefore be possible that this genetic differentiation between the two regions may account for these morphological differences; however, more study would need to be conducted to examine this phenomenon

and determine the mechanisms causing the morphological differences between the populations.

The apparent limited migration between these small populations of southern hairy-nosed wombats on the Yorke Peninsula, may explain why no difference was observed in heterozygosity levels between these populations and the large Swan Reach population. Alpers and Sherwin (1999) proposed that animals migrate between populations up to 75 km apart, and Walker (2004) proposed that there is some gene flow between populations on the Yorke Peninsula; however, this was between regions close to one another (i.e. animals in colonies on the coast migrate to other colonies on the coast, rather than to inland colonies). Alpers *et al.* (1998) suggested there was little gene flow between Kulpara and Urania, which is consistent with Walker's (2004) suggestions, because Urania is coastal and Kulpara inland. The results from the current study correspond with this theory; suggesting that there appears to be some migration between Urania and Kulpara, but it is either minimal or historical. According to Frankham *et al.* (2002), it only takes one migrant to prevent allele fixation, and this is independent of population size, as migration is proportionally much higher in smaller populations.

Whilst gene flow may be a valid reason for explaining the results of this study, Walker *et al.* (2008a; 2008b) also found the normal pattern of female dispersal was inhibited and some evidence for inbreeding avoidance in southern hairy-nosed wombats at Kulpara. Inbreeding avoidance may explain the low $F_{IS}$ values observed in this study at Kulpara. Inbreeding would be expected in a small and geographically isolated population with little migration, such as Kulpara, however, the current study showed no evidence of inbreeding within the population. Inbreeding avoidance could also account for the high heterozygosity values. However, if these populations remain small, and there is no gene flow, given sufficient time, individuals will become more related and therefore increase the chance of mating with relatives. Increased mating between closely related individuals eventually leads to a reduction in the number of heterozygous individuals within the population, and hence a reduction in genetic diversity. When there is no gene flow, the level of inbreeding depends on effective population size (Frankham *et al.* 2002). The Kulpara population is extremely small with < 200 animals and Urania (combined with Pt Pearce) has approximately 500 animals. As a general rule, any population with an effective population less than 500 animals in the long term is at a risk of genetic drift fixing alleles more rapidly (Frankham *et al.* 2002). Both these southern hairy-nosed wombat populations, as well as all the others on the Yorke Peninsula, fall below this value and are therefore possibly at risk in the future of a further reduction in

genetic diversity, unless the current migration and inbreeding avoidance levels are sufficient to maintain diversity.

Another possible explanation for the high heterozygosity values at Urania and Kulpara might be that not enough generations of animals have been born since the population became isolated for the effects of isolation on genetic diversity to take effect. For example, based on modelling, a mammal species with a generation time of 10 years, and an effective population of around 50 in 1900, would still retain 90% of its heterozygosity today (Amos and Balmford 2001). Wombats have long life spans (over 15 years), a slow reproductive rate of only one young per year, and reach sexual maturity at approximately three years of age (Taggart and Temple-Smith 2008). Therefore, the generation time for the southern hairy-nosed wombat may be too long for any influence on heterozygosity to be observed considering the populations on the Yorke Peninsula have only been isolated for between 60 - 100 years. Hence, the full effects of habitat fragmentation may yet to be realised and may not be noticeable for many generations (Keyghobadi 2007).

Whilst heterozygosity was high in the Yorke Peninsula populations of southern hairy-nosed wombats, the number of alleles per locus and allelic richness was slightly less than the large population at Swan Reach. These results are comparable to those found in skinks, where a slight decrease in allelic diversity, but not expected heterozygosity, of the isolated populations was reported (Sumner *et al.* 2004). Heterozygosity is less sensitive than allelic diversity to increased rates of genetic drift over the short term. This might be expected in recently bottlenecked populations (Nei *et al.* 1975). It is unknown whether recent bottlenecks have occurred in the wombat populations at Kulpara and Urania, and therefore it cannot be hypothesised whether a bottleneck event may have influenced the allelic diversity in these populations.

No inbreeding in southern hairy-nosed wombats was observed in this study, with $F_{IS}$ values of 0.058 and -0.035 for Urania and Kulpara respectively, and both values not being significantly different from zero. Walker (2004) also found no evidence of inbreeding within any of the sampled populations. However, in the report by Alpers and Sherwin (1999), there was a homozygote excess reported at Urania, with a possibility of some local inbreeding suggested. In addition, the mean observed heterozygosity of animals from Urania and Kulpara were lower than the expected heterozygosity. According to Frankham *et al.* (2004), inbreeding coefficient (F) can be deduced by using the equation $F = 1 - H_O/H_E$. If this equation is used on the data from Alpers and Sherwin (1999), then the respective inbreeding coefficients for Urania and Kulpara are 0.26 and 0.22 which is representative of partial

inbreeding (Frankham *et al.* 2004). However, caution should be taken when assessing these results as the sample size for the two populations was low (< 20 wombats).

### 3.5. Summary

In the fragmented, small and geographically isolated populations of southern hairy-nosed wombats on the Yorke Peninsula no statistically significant difference was found in the observed and expected heterozygosity values when compared to the large and continuous population of wombats at Swan Reach. There was however, a slight decrease in allelic diversity in the Yorke Peninsula populations. Three reasons may account for this result (1) gene flow is present between the populations, (2) relatives may avoid breeding with one another (inbreeding avoidance), and (3) the generation time for wombats is too long for any observable genetic differences at present.

Despite the small population size of wombats on the Yorke Peninsula, no discernable inbreeding was detected. Even though no difference in heterozygosity was found in these populations, they must be managed appropriately for two reasons (1) they are all genetically distinct, and (2) the populations are extremely small and it is unlikely that effective migration is occurring, therefore they are likely to be susceptible to a reduction in genetic diversity and inbreeding depression in the future. There is already some evidence of reduced genetic diversity based on the lower allelic diversity in this study. The northern hairy-nosed wombat has only one single population remaining, consisting of approximately 83 individual, and in this population, the average number of alleles per locus is 2.0, and the expected and observed heterozygosity values are 0.32 and 0.33 respectively (Taylor *et al.* 1994). This could be the future for southern hairy-nosed wombats in the small and isolated populations on the Yorke Peninsula if they are not monitored and managed appropriately.

## Chapter 4: Comparisons of semen quality in southern hairy-nosed wombat populations in the Murraylands and on the Yorke Peninsula

### 4.1. Introduction

*4.1.1. Impact of inbreeding on seminal quality in vertebrates*

There are currently no published studies in marsupials examining the association between population size, genetic diversity, inbreeding and reproductive parameters (such as seminal quality and reproductive success). Southern hairy-nosed wombats are not endangered but the fragmented and isolated nature of some of the colonies of this species, as well as the small number of animals in some of the colonies, would suggest that some colonies of this species might be prone to inbreeding depression and loss of genetic diversity.

The effect of inbreeding on semen quality and fertility, and hence reproductive success, has been studied extensively in vertebrates (Wildt *et al.* 1982; Wildt *et al.* 1983; Wildt *et al.* 1987a; Gomendio *et al.* 2000; Frankham *et al.* 2002; Asa *et al.* 2007; Gomendio *et al.* 2007). Inbreeding within a population has the potential to decrease the reproductive success of the population, and hence, eventually increase the risk of extinction (as detailed in Chapters 1.1.3 and 1.1.4). High levels of inbreeding have been associated with reduced sperm motility in Mexican grey wolves (*Canis lupus baileyi*) (Asa *et al.* 2007), gazelles (Gomendio *et al.* 2000; Roldan *et al.* 2006) and various species of felids (Wildt *et al.* 1983; Wildt *et al.* 1987a; Gomendio *et al.* 2000). In both dogs and house mice, sperm concentration (sperm count per ejaculate) has been shown to be higher in individuals that exhibit greater heterozygosity (Wildt *et al.* 1982; Margulis and Walsh 2002), Semen quality can be assessed by determining various seminal characteristics such as ejaculate volume, sperm concentration, sperm motility and the percentage of morphologically normal sperm. These characteristics reflect the potential for successful male reproduction, as sperm concentration and ejaculate volume generally appear to be good indicators of male fertility, e.g. ganders (*Anser cygnoides*) (Liu *et al.* 2008), and red deer (*Cervus elaphus hispanicus*) (Gomendio *et al.* 2007).

*4.1.2. Aims*

The aims of this chapter were to:

- Compare and contrast the semen quality (ejaculate volume, sperm motility, sperm concentration) of male southern hairy-nosed wombats from two small isolated populations on the Yorke Peninsula (Urania and Kulpara), and the large population at Swan Reach in the Murraylands

- Determine if there is any association between genetic diversity (heterozygosity and allelic diversity) and semen quality

### 4.2. Methods

Testosterone levels of male southern hairy-nosed wombats were examined during a pilot study (Kulpara N = 8, Urania N = 19, Swan Reach N = 25) (Sparrow, unpublished results). No significant differences were found between any of the populations; hence testosterone levels were not measured as part of this project.

#### 4.2.1. Animal capture and processing

Data was collected from southern hairy-nosed wombats at three colonies; the two small, isolated colonies on the Yorke Peninsula (Kulpara and Urania) (Chapter 2, Figure 4), and one large colony at Swan Reach, which is part of the Murraylands population (Chapter 2, Figure 2). See Chapter 2 (sections 2.2.1. and 2.3.1) for more detail of these three colonies, and sections 2.2.3 and 2.2.4., for capture and processing details.

#### 4.2.2. Electro-ejaculation

Previously it had been found that of 12 southern hairy-nosed wombat semen samples collected outside the breeding season, 11 (92%) were aspermic (Taggart *et al.* 1998b), with seasonal differences being recorded in semen volume, sperm concentration, and sperm motility (Taggart *et al.* 2005). In the present study, which was conducted between 2004 and 2008, male southern hairy-nosed wombats were captured and electro-ejaculated within the recognised breeding season for this species, between the months of July and December.

Adult male wombats were anaesthetised with Zoletil (refer Chapter 2.2.4), and anaesthesia maintained with isofluorane (2.5 %) in oxygen (1 L/min) for the duration of the electro-ejaculation procedure (Figure 10a). At commencement, the penis was everted from the prepubertal sack, cleaned, and the glans length measured. The tip of the penis was then placed into a 50 ml plastic container. A lubricated (KY Jelly) electro-ejaculation probe was then inserted approximately 12 cm into the rectum of the wombat, and semen collected using a 50 Hz stepped up sin wave electro-ejaculation unit (Figure 10b). Three series of ten electrical stimuli (40 mA, 50 mA, 60 mA) were generated, each stimulus lasting 3-5 seconds, and replicated twice per animal in the same session (Taggart *et al.* 1996; Taggart *et al.* 1998b).
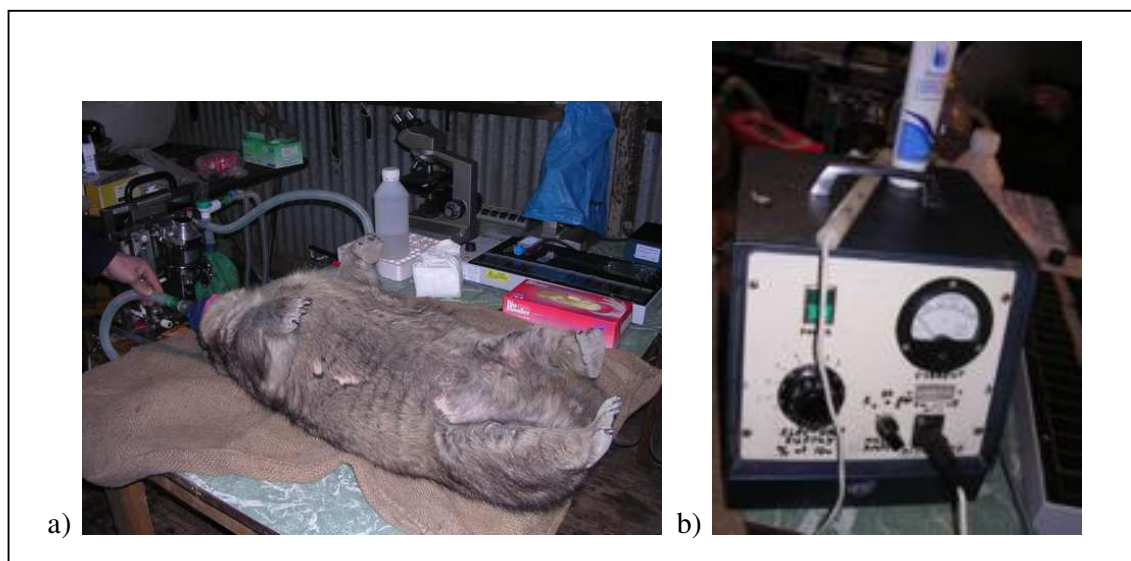
**Figure 10:** (a) A male southern hairy-nosed wombat under anaesthesia induced by isofluorane and (b) the sin wave electro-ejaculation unit, probe and lubricant used for the electro-ejaculation procedure (photos taken by Elisa Sparrow).

*4.2.3. Semen analysis*

The volume of semen samples collected was recorded and the presence or absence of sperm determined using an Olympus phase contrast light microscope (magnification x 200). If sperm were present, a full semen analysis was then undertaken.

Sperm motility: For motility analysis, semen was diluted (to approximately $1 \times 10^6$ sperm/ml) with pre-warmed Medium 199 (Sigma Aldrich Company, LTD), to which 4 % foetal calf serum and 450 mg/100 ml glucose had been added. A drop of semen was placed on a pre-warmed slide and observed using a phase contrast or differential interference microscope, with warming stage attached. For motility determination > 150 sperm were counted for each wombat ejaculate. In each field of view, under the microscope, the number of sperm in each of five motility categories was recorded. The five motility categories were designated as follows:

1 = no movement

2 = movement with no forward progression (or in circles)

3 = slow, forward, progression

4 = moderate, forward, progression

5 = rapid, forward, progression

A motility index for the sample was calculated, based on these motility scores, using the following formula: (% sperm in category 1 x 0) + (% sperm in category 2 x 1) + (% sperm in category 3 x 2) + (% sperm in category 4 x 3) + (% sperm in category 5 x 4) (Taggart *et al.* 1998b).

Sperm concentration: The number of sperm per ml of the ejaculate was determined using a haemocytometer and calculated using the following formula:

- Number of sperm (five large squares of a haemocytometer) X 10,000 X dilution factor

This value was then multiplied by the ejaculate volume to obtain the total sperm number. Aliquots of semen samples were then fixed in 10 % neutral buffered formalin so that sperm morphology could be subsequently determined (see Chapter 5).

### 4.2.4. Statistical analysis

All data was placed in a Microsoft Excel worksheet. SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical analysis. To compare semen characteristics in adult male wombats between the three locations (Swan Reach, Kulpara and Urania), data was tested for normality. When the requirements were met, a one-way ANOVA model was fitted to the data. If the assumption of normality was not supported, a Kruskal-Wallis test was performed. Where the test of the null hypothesis was statistically significant ($p < 0.05$) post-hoc tests were carried out to determine the direction of difference. Since in chapter 3, (section 3.3 and section 3.4), it was determined that Swan Reach, Urania and Kulpara populations were genetically distinct from one another, each of these sites was analysed separately.

Wombat head width and length are indicators of the size of the individual, i.e. a bigger head width or head length suggests a larger animal. Body weight can also be a representation of the condition of the individual animal. To determine any associations between these measured parameters (head length, head width, weight) and semen characteristics, the data from all populations was pooled. When data was not normally distributed Spearman's correlation coefficient was used. The relationship between wombat head length, head width, weight and the binary outcome spermic/aspermic was investigated using log binomial regression models.

**4.3 Results**

*4.3.1. Semen analysis*

　　Of the 50 male southern hairy-nosed wombats electro-ejaculated from the Swan Reach population (Murraylands), spermatozoa were present in 20 (40 %) of them. At Urania (Yorke Peninsula), 7 out of 21 males that were electro-ejaculated contained sperm, giving a success rate of 33 %, whereas at Kulpara (Yorke Peninsula), of the 24 males electro-ejaculated, 18 ejaculates contained spermatozoa; a success rate of 75 % (Table 15). Sperm motility was analysed in the field, however, due to the amount of time required to conduct all procedures for an individual male wombat (e.g. anaesthesia, morphological measurements, electro-ejaculation, and semen analysis), sperm concentration could not always be analysed on site. On these occasions, semen was fixed and sperm concentration was analysed back at the university laboratory. Due to the time lapse, some semen samples coagulated, and therefore sperm concentration could not be accurately estimated. Hence the sample sizes for sperm concentration may be lower than the other variables, particularly at Swan Reach where more animals were electro-ejaculated.

**Table 15:** Number of male southern hairy-nosed wombats electro-ejaculated, the number of males with sperm in their semen, and the percentage success rate in the three populations

|  | **Swan Reach** | **Urania** | **Kulpara** |
|---|---|---|---|
| **Number of males electro-ejaculated** | 50 | 21 | 24 |
| **Number of males with sperm in semen*** | 20 | 7 | 18 |
| **Success of electro-ejaculation (%)** | 40 | 33 | 75 |

* does not include those with very dilute semen (i.e. those semen samples with very few sperm present)

4.3.1.1. Ejaculate volume

The mean ejaculate volume for spermic male southern hairy-nosed wombats was greatest at Swan Reach (7.2 ± 0.5 ml) and Kulpara (7.2 ± 0.8 ml). The ejaculate volume was significantly lower at Urania (3.6 ± 0.8 ml) ($p < 0.05$) (Table 16 and Figure 11).

**Table 16:** Mean ejaculate volume (± standard deviation) for spermic male southern hairy-nosed wombats from three populations in South Australia (N = sample size)

|  | N | Ejaculate volume (ml) |
| --- | --- | --- |
| **Swan Reach** | 18 | 7.2 ± 0.5 |
| **Urania** | 7 | 3.6 ± 0.8* |
| **Kulpara** | 13 | 7.2 ± 0.8 |

* Significantly different from other two values ($p < 0.05$)
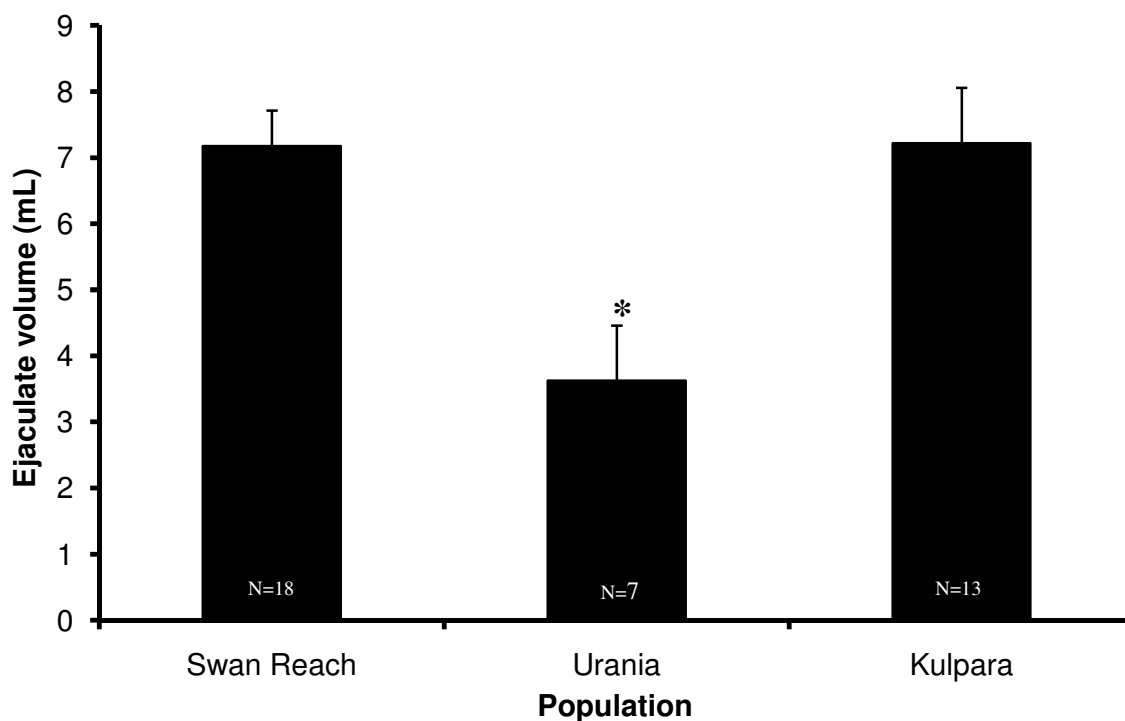


**Figure 11:** Mean (± standard deviation) ejaculate volume for spermic male southern hairy-nosed wombats from one Murraylands population (Swan Reach) and two Yorke Peninsula populations (Urania and Kulpara). Sample size (N) indicated on graph
* Significantly different ($p < 0.05$)

4.3.1.2. Sperm motility

There was no significant difference seen in mean motility index between Swan Reach, Urania and Kulpara (263.4 ± 9.9, 242.7 ± 18.7, 237.2 ± 21.1 respectively) (p ≥ 0.05) (Table 17 and Figure 12). However, whilst not statistically significant, a trend can be observed with wombats from the smaller populations on the Yorke Peninsula (Urania and Kulpara) having a lower motility index compared to those from the Swan Reach population (Table 17 and Figure 12).

**Table 17:** Mean motility index (± standard deviation) for the populations of male southern hairy-nosed wombats (N = sample size)

|  | N | Motility index |
|---|---|---|
| **Swan Reach** | 16 | 263.4 ± 9.9 |
| **Urania** | 7 | 242.7 ± 18.7 |
| **Kulpara** | 12 | 237.3 ± 21.1 |
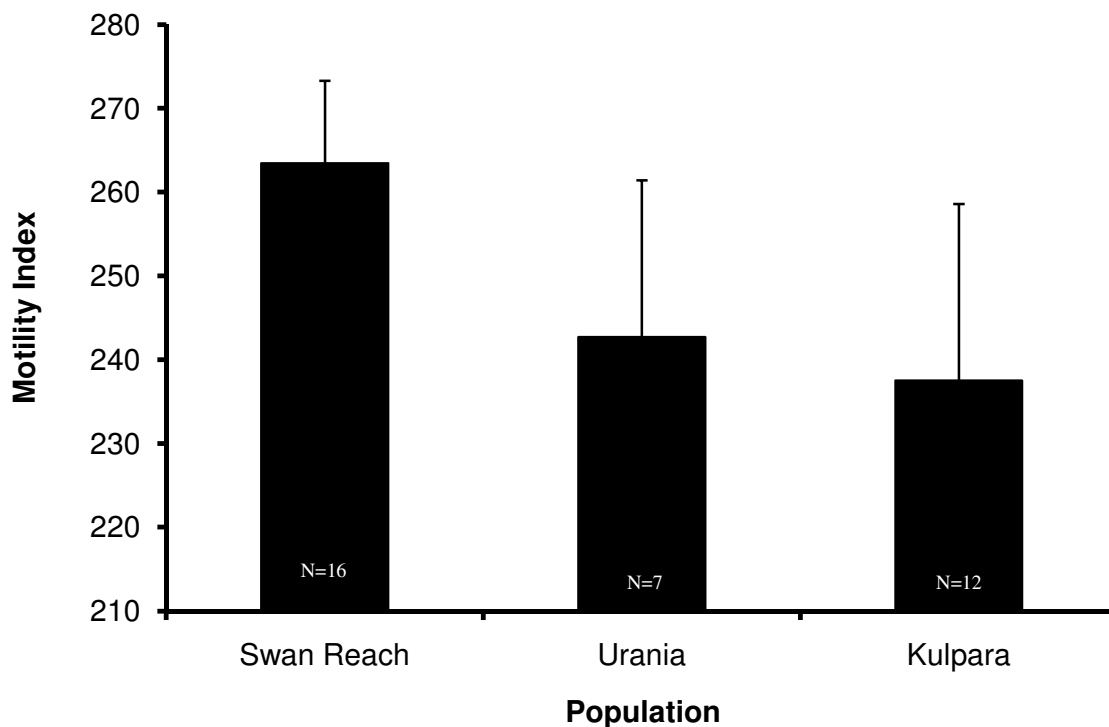


**Figure 12:** Mean (±standard deviation) motility index in male southern hairy-nosed wombat ejaculates for populations in the Murraylands (Swan Reach) and on the Yorke Peninsula (Urania and Kulpara). Sample size (N) indicated on graph

<u>4.3.1.3 Sperm concentration</u>

There were  no significant differences in the mean total sperm number per ejaculate between Swan Reach, Urania and Kulpara ($119.5 \times 10^6 \pm 31.0 \times 10^6$, $37.3 \times 10^6 \pm 16.9 \times 10^6$, and $62.2 \times 10^6 \pm 30.9 \times 10^6$ respectively) ($p \geq 0.05$) (Table 18 and Figure 13). There were also no significant differences in sperm number per ml (Swan Reach = $15.8 \times 10^6 \pm 4.2 \times 10^6$, Urania = $9.7 \times 10^6 \pm 4.5 \times 10^6$ and Kulpara = $9.0 \times 10^6 \pm 4.6 \times 10^6$ respectively) ($p \geq 0.05$) between the three populations studied (Table 18 and Figure 13). However, whilst not statistically significant, a trend can be observed with wombats from the smaller populations on the Yorke Peninsula having a lower average total sperm number, and sperm number/ml when compared to those from the large Swan Reach population (Table 18 and Figure 13).

**Table 18:** Mean (± standard deviation) total sperm number per ejaculate, and mean (± standard deviation) sperm number per ml of semen, for male southern hairy-nosed wombats from the three populations (N= sample size)

|  | N | Total sperm number | Sperm number/ml |
|---|---|---|---|
| **Swan Reach** | 9 | $119.5 \times 10^6 \pm 31.0 \times 10^6$ | $15.8 \times 10^6 \pm 4.2 \times 10^6$ |
| **Urania** | 7 | $37.3 \times 10^6 \pm 16.9 \times 10^6$ | $9.7 \times 10^6 \pm 4.5 \times 10^6$ |
| **Kulpara** | 10 | $62.2 \times 10^6 \pm 30.9 \times 10^6$ | $9.0 \times 10^6 \pm 4.6 \times 10^6$ |

(a)



(b)
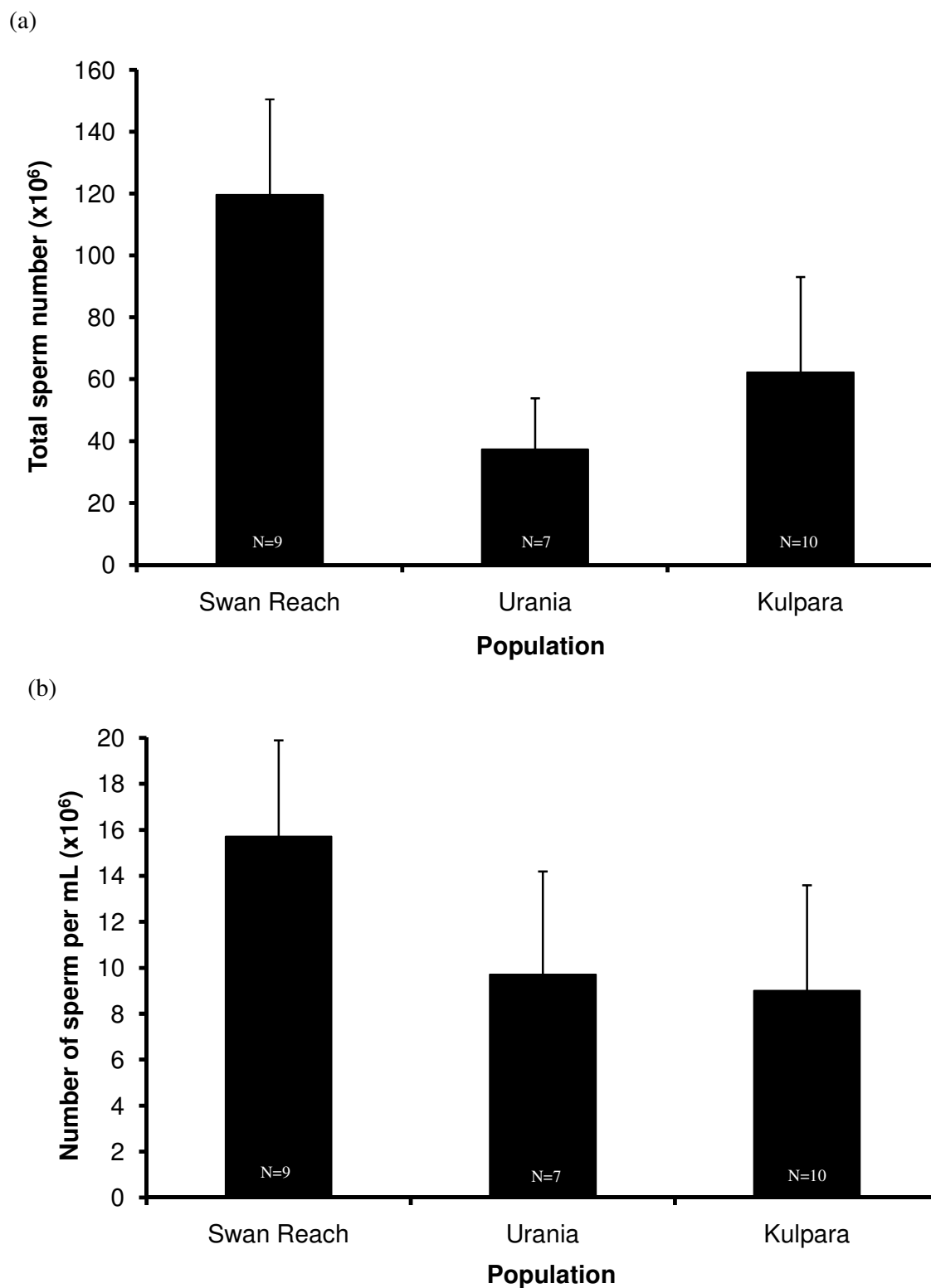


**Figure 13:** (a) Mean (± standard deviation) total sperm number (x10$^6$), and (b) mean (± standard deviation) number of sperm per ml (x10$^6$) in male southern hairy-nosed wombat ejaculates for populations in the Murraylands (Swan Reach) and on the Yorke Peninsula (Urania and Kulpara). Sample size (N) indicated on graph

*4.3.2. Associations between body size and semen quality*

There were significant positive correlations between wombat head width and ejaculate volume, as well as wombat head length and the presence of sperm in collected semen. These findings suggest that larger male southern hairy-nosed wombats (based on head width and length) were more likely to produce spermic ejaculates, and ejaculates of larger volume (Table 19).

**Table 19:** Pooled data from all adult male southern hairy-nosed wombats used in this study and the relationship between semen characteristics and head length, head width, and weight (top = correlation coefficient, bottom = p-value)

| | **Head length** | **Head width** | **Weight** |
|---|---|---|---|
| **Spermic semen** | 0.0302 0.0445* | 0.0212 0.2545 | 0.0538 0.2543 |
| **Ejaculate volume** | 0.06824 0.6882 | 0.32596 0.0429* | 0.07874 0.6337 |
| **Number of motile sperm** | -0.10532 0.6086 | 0.10643 0.6048 | 0.16732 0.4139 |
| **Motility index** | 0.18651 0.2834 | 0.18241 0.2870 | 0.30554 0.0700 |
| **Total sperm number** | -0.0475 0.8177 | 0.2169 0.2871 | 0.1392 0.4975 |
| **Sperm per ml** | -0.10532 0.6086 | 0.10643 0.6048 | 0.16732 0.4139 |

* Statistically significant ($p < 0.05$)

**4.4 Discussion**

Research on species of felids, gazelles, and wolves has shown that inbreeding, and a low genetic diversity (heterozygosity and allelic diversity), is negatively associated with optimal functioning of the male reproductive system. In particular, characteristics such as seminal quality, total ejaculate volume, percentage of motile sperm, and sperm concentration have all been found to be reduced in small inbred populations compared to outbred groups (Wildt *et al.* 1983; Wildt *et al.* 1987a; Cassinello *et al.* 1998; Asa *et al.* 2007).

In the present study it has been found that male southern hairy-nosed wombats captured at Swan Reach and Kulpara had, in general, larger seminal ejaculate volumes than males captured at Urania. However, microsatellite analyses of the three populations (Chapter 3, section 3.3, Table 12) suggested little difference in the mean observed and expected heterozygosity values between the three populations. Mean allelic diversity was not significantly different between the populations, although it was slightly lower at Urania than at Swan Reach, but higher than in the Kulpara population. Whilst the data did not suggest any inbreeding, a previous study (Alpers and Sherwin 1999) suggested possible inbreeding at Urania. The sample size for this current study, however, was much larger than the earlier investigation and therefore the earlier difference suggested may have been a chance event.

The results from this current study, therefore, suggest that there is no association between the genetic diversity and ejaculate volume in the three populations of southern hairy-nosed wombats at Swan Reach, Urania and Kulpara. Whilst there was a significantly smaller mean ejaculate volume in the wombats at Urania, there is little difference in the heterozygosity values of the populations. Also, whilst the allelic diversity was lowest at Kulpara, the mean ejaculate volume for animals in this population was not significantly different from that observed in the Swan Reach wombats, where the highest allelic diversity was found to occur. This differs from conclusions on a study of lions where less genetically diverse populations were found to have a lower ejaculate volume (Wildt *et al.* 1987a), although this latter study used allozyme loci, rather than microsatellite analysis, to determine genetic heterozygosity.

The data collected in the present study suggest two contradictory conclusions. Head width, which presumably relates to body size, significantly positively correlated with ejaculate volume, therefore predicting that the wombats on Yorke Peninsula, based on their smaller head width, should have, on average, lower ejaculate volumes than the Swan Reach animals. However, the wombats in the Yorke Peninsula populations had significantly larger testes, which suggest these animals should have a higher ejaculate volume than those at Swan Reach (Moller 1988). Nevertheless, as discussed in Chapter 2.4, greater size of the testes may

relate to a greater volume of interstitial tissue rather than seminferous epithelium, so a greater ejaculate volume is not necessarily indicated by greater testes mass. Whether these two conflicting observations counteract each other is not known, Swan Reach and Kulpara wombats on average did not differ in the volume of ejaculate produced. Perhaps some unknown external factor is the reason for the reduced ejaculate volume in southern hairy-nosed wombats at Urania.

This study found no significant difference in sperm motility between the wombat populations examined. This result differs from studies on felids, where sperm motility (percent motile, sperm per ejaculate, and total number of motile sperm per ejaculate) was significantly lower in populations with reduced genetic diversity (Wildt *et al.* 1987a). Likewise when inbred cheetahs were compared with genetically diverse domestic cats, sperm motility was again significantly lower (Wildt *et al.* 1983). Similarly, in wolves (Asa *et al.* 2007), gazelles (Gomendio *et al.* 2000), and Shetland ponies (van Eldik *et al.* 2006) an inverse correlation existed, where high inbreeding coefficients were associated with lower percentages of motile sperm per average ejaculate. The present observations showing no significant difference in sperm motility between the three populations of southern hairy-nosed wombats may be due to the similar heterozygosity of the populations and an absence of apparent inbreeding. Differences between the extent of allelic variation and inbreeding observed within species may also explain differences observed in seminal quality between studies. For example, in gazelles, only in the species with high levels of inbreeding was there a negative correlation between inbreeding coefficient and seminal quality with intermediate and low levels of inbreeding showing no relationship with seminal quality (Gomendio *et al.* 2000). Interestingly, trends in the motility index data equate with that seen in allelic diversity, with Swan Reach animals displaying the greatest sperm motility index and allelic diversity and wombats from Kulpara showing the lowest.

Similarly, in the present study, no significant difference was found in sperm concentration between the three southern hairy-nosed wombat populations in this study. The sperm number per/ml however, was markedly higher in animals from Swan Reach, compared to those from Urania and Kulpara. Furthermore, the total number of sperm per ejaculate in the Swan Reach animals was, on average, nearly twice as high compared to the animals at Kulpara, even though these populations had very similar ejaculate volumes. Whilst these relationships were not statistically significant, a trend in the data is evident with southern hairy-nosed wombats from the smaller, isolated populations having a lower sperm concentration than those from the large Swan Reach population. The direction of these trends (i.e. Swan Reach with the greatest sperm concentration and Urania and Kulpara with lower

concentrations), correlate well with trends observed in allelic diversity between the populations (i.e. Swan Reach had the greatest allelic diversity). In koalas (Montgomery 2002) and lions (Wildt *et al.* 1987a) similar associations were found to occur where the concentration of sperm in the ejaculate decreased proportionally with that of genetic diversity, even though no statistically significant differences were found to occur. Fertility trials on geese (Liu *et al.* 2008) and red deer (Malo *et al.* 2005; Gomendio *et al.* 2007) have suggested that a higher sperm concentration reflects greater fertility. In contrast, research on oldfield mice has shown that, whilst inbreeding is associated with a decline in testicular sperm count, there is no association between inbreeding and decreased reproductive performance. Nevertheless, these findings suggest that the sperm count is likely to continue to decline as inbreeding increases. At some point a threshold may occur where fertility becomes compromised (Margulis and Walsh 2002).

Head length, which may be representative of a larger male wombat, was found to correlate positively with an increased chance of sperm presence in an ejaculate; however, as no difference in head length was observed across the three populations examined, it is reasonable to assume that each population is as likely as one another to produce sperm in an ejaculate.

In the current study the significantly lower mean ejaculate volume for the Urania animals does not appear to be associated with lowered genetic diversity. In addition, no positive associations were detected between genetic diversity, sperm concentration, and sperm motility. The high genetic diversity of the small and isolated populations on the Yorke Peninsula was unexpected as inbreeding, and hence a reduced genetic diversity, could be expected to have occurred in the small populations (Balloux *et al.* 2004). The maintenance of high heterozygosity in Urania and Kulpara may be the result of gene flow through migration as some evidence exists that there is limited migration (Chapter 3, section 3.4). Another likely explanation is inbreeding avoidance within the population (Walker 2004; Walker *et al.* 2008a), or there may not have been sufficient generations of isolation for negative influences of low genetic diversity and inbreeding to take effect, particularly as southern hairy-nosed wombats are long lived, have a slow reproductive rate and do not reach maturity until three years of age (Taggart and Temple-Smith 2008).

## 4.5. Summary

Whilst there is no evidence that inbreeding depression is impacting on sperm motility, sperm concentration and ejaculate volume at the present time, continued observation and further studies of the Yorke Peninsula populations are warranted due to the lower ejaculate volume at Urania, the very small population sizes, the slightly lower allelic diversity, the apparent demographic isolation, and the trend for smaller sperm concentration at both Urania and Kulpara. It is possible the sample size may have been too small for demonstration of a statistically significant difference. Future studies are required to increase sample sizes, and must take into account possible differences in environmental conditions between the populations, such as (1) nutritional and water capacity of available feed, and (2) chemical use on agricultural properties. Restricted water intake has been shown to impair spermatogenic activity and reduce sperm counts in deer mice (Nelson 1993), and ejaculate volume and sperm concentration in rabbits (Marai *et al.* 2002). Experiments on male rodents and humans have shown that some commonly used chemicals in pesticides can impair spermatogenesis (Ong *et al.* 2002; Fisher 2004) and reduce semen quality (Swan *et al.* 2003). Nutrition is also an important factor as some nutrient deficiencies may negatively influence spermatogenesis in humans (Wong *et al.* 2003).

The populations of southern hairy-nosed wombats on the Yorke Peninsula are considered vulnerable (St John and Saunders 1989). With continued population isolation, a reduction in ejaculate volume and sperm concentration could well occur in association with inbreeding and thus in the long term reduce fertility and breeding success of this population to such an extent that the viability of these colonies/populations is compromised.

**Chapter 5: A comparison of sperm morphology in southern hairy-nosed wombats in South Australia**

## 5.1. Introduction

### 5.1.1. Basic mammalian spermatozoon structure

A mammalian spermatozoon has two main components, the head and the tail (or flagellum). The head contains a nucleus with the male haploid genome, and the acrosome, which is required in sperm-egg binding and penetration (Fawcett 1970; Bedford and Kim 1993; Gomendio *et al.* 2006) . The shape of both the sperm head and the acrosome is generally species specific and highly conserved, and has been used as a taxonomic tool in both eutherian mammals and marsupials (Setchell 1982; Temple-Smith 1987). The tail is divided into three parts, the mid-piece, the principal piece, and the end piece. The mid-piece contains a mitochondrial helix and is responsible for energy production to facilitate motility; whilst the principal and end pieces comprise the bulk of the mechanical components necessary for generating the flagellar beat required to propel the sperm forward (Setchell 1982; Gomendio *et al.* 2007).

### 5.1.2. Marsupial spermatozoon structure

In most marsupials, except wombats and koalas, the sperm flagellum (tail) inserts midway along the ventral surface of the sperm head (nucleus), and throughout most of spermatogenesis lies perpendicular to the head. This contrasts to the situation in eutherian mammals where the sperm head lies parallel to the long axis of the sperm tail. In most marsupials, during epididymal maturation of the spermatozoon, the sperm head then rotates 90° until it is parallel with the long axis of the tail. As a result the mature marsupial spermatozoon differs from that of eutherian mammals in the positioning of the acrosome; which in marsupials is located on the dorsal surface of the sperm head. The acrosome of most marsupial sperm therefore does not form a cap over the anterior region of sperm head, as it does in eutherian mammals (Hughes 1965; Setchell 1977; Temple-Smith 1987; Tyndale-Biscoe and Renfree 1987; Mate and Rodger 1996; Lin *et al.* 1997; Ricci 1997).

### 5.1.3. The structure of koala and wombat spermatozoa

The size of the various components of marsupial spermatozoon examined in Phalangeridae (brushtail possums), Peramelidae (bandicoots), Dasyuridae (dasyurids), Petauridae (gliders and possums), Phascolarctidae (koalas and wombats), Tarsipedidae (honey possums), and Macropodidae (kangaroos and wallabies) vary significantly (Hughes 1965; Cummins and Woodall 1985). Total sperm length in marsupials varies from 356 µm in the

honey possum (*Tarsipes rostratus*; Tarsipedidae), the largest of all mammal spermatozoa, to approximately 80 µm in Phascolactidae spermatozoa, which are the smallest of all known marsupial sperm. Dasyurids also have very large spermatozoa (approx 250 µm) (Cummins and Woodall 1985). Species within the Dasyuridae and Tarsipedidae families have the longest sperm head, mid-piece and tail lengths. In comparison, species within the Phascolarctidae have the smallest flagellum length of all the families examined (Hughes 1965). Sperm head length within the Phalangeridae and Peramelidae families are the smallest of all species examined (Hughes 1965). Mid-piece length was shortest in species from the Phalangeridae (Hughes 1965). In addition to differences in the length of various sperm components between marsupial families, marsupial sperm morphology between the different groups also varies widely (Hughes 1965; Cummins and Woodall 1985), however, none more so than sperm from species within the family Phascolarctidae (koalas and wombats) (Hughes 1965; Temple-Smith 1987; Temple-Smith and Taggart 1990; Wildt *et al.* 1991). This marsupial group has a range of pleomorphic sperm morphologies within an average ejaculate (Temple-Smith and Taggart 1990; Breed *et al.* 2001). The head (nuclear) shape of spermatozoa in this family differs from those of all other marsupials, with the most common type of sperm found in an average ejaculate having a hooked, or strongly curved, nucleus (Harding *et al.* 1987; Temple-Smith and Taggart 1990; Wildt *et al.* 1991; Montgomery 2002). Sperm head-tail alignment in koala and wombat sperm also differs from other marsupials, with the tail of the sperm attaching to the posterior region of the sperm head. Likewise, the "comma-shaped" acrosome located on the ventral side of the nucleus, within a nuclear concavity, differs markedly from the dorsal nuclear position of the acrosome in most other marsupials (Hughes 1965; Breed 1994; Ricci 1997; Breed *et al.* 2001).

A pilot study of southern hairy-nosed wombat semen samples, obtained during the breeding season, established seven pleomorphic sperm head morphologies within the average male southern hairy-nosed wombat ejaculate (Sparrow, unpublished results). The most common sperm head shape observed in an average wombat or koala ejaculate is a hooked sperm head (Harding *et al.* 1987; Temple-Smith and Taggart 1990; Wildt *et al.* 1991; Montgomery 2002).

### 5.1.4. Impact of inbreeding on sperm morphology in eutherian mammals and marsupials

Numerous studies on felids have found an association between higher numbers of abnormal sperm morphologies within populations that have low genetic variability. These abnormalities include a coiled flagellum, bent mid-piece, bent flagellum, bent flagellum tip, protoplasmic droplet, or a micro- or macro-cephalic defect (i.e. excessively small or large

sperm head) (Wildt *et al.* 1983; Wildt *et al.* 1986). Similar results have been found in Mexican gray wolves (*Canis lupus baileyi*), wild rabbits (*Oryctolagus cuniculus*), koalas, and Shetland pony stallions, where there was a significant negative correlation between inbreeding coefficient and the percentage of normal sperm morphologies in the ejaculate (Montgomery 2002; Gage *et al.* 2006; van Eldik *et al.* 2006; Asa *et al.* 2007). The main abnormalities seen in wolves were detached heads and coiled tails. Sperm motility was also negatively correlated with inbreeding coefficient in wolves. The poor semen quality observed in the inbred wolves was significantly correlated with low reproductive success, suggesting fertility was compromised (Asa *et al.* 2007).

Both sperm head and tail morphology is extremely important for fertility, with tail structure playing a role in determining the direction and speed of sperm movement, and head morphology, in particular acrosome location, being critical for egg coat penetration, as the acrosomal enzymes are essential to digesting a passage through the zona pellucida (ova membrane) (Breed 1994; Asa *et al.* 2007; Gomendio *et al.* 2007). In addition, the structure of both the sperm head and tail is crucial to sperm swimming velocity (Gomendio *et al.* 2007). Research has shown high proportions of morphologically normal sperm to be a good indicator of fertilisation ability in ganders (*Anser cygnoides*), and red deer (*Cervus elaphus hispanicus*) (Malo *et al.* 2005; Liu *et al.* 2008).

*5.1.5. Aims*

Research conducted on both eutherian mammals and marsupials (outlined in Chapter 1 and current chapter), has indicated an association between low genetic diversity and increased abnormalities in sperm morphology. The structure of the sperm is important for the fertility, and hence the long term viability, of that population. Southern hairy-nosed wombats are not endangered, however, the small population size and fragmented and isolated nature of their distribution in some populations within South Australia, potentially makes these populations susceptible to inbreeding, and as a consequence, the occurrence of low genetic diversity, and associated morphological and biological abnormalities. Whilst inbreeding was not observed in the three populations examined in previous chapters (Swan Reach, Urania and Kulpara), a pilot study with limited individuals did suggest differences in the proportion of sperm head types between the populations. Therefore, the aim of this chapter is to determine if there was a relationship between genetic diversity, population size and the sperm structure (specifically, sperm head morphology, sperm tail morphology and lengths of sperm components) from small isolated, and large, continuous populations of southern hairy-nosed wombats in South Australia.

## 5.2. Methods

### 5.2.1. Study populations

Southern hairy-nosed wombats from the small, isolated populations at Kulpara and Urania on the Yorke Peninsula, and from the large, continuous population at Swan Reach in the Murraylands (Chapter 2, section 2.2.1.), were examined.

### 5.2.2. Animal capture, processing and semen collection

Wombats were captured and processed as outlined in Chapter 2 (sections 2.2.3. and 2.2.4). Semen samples were collected from captured male southern hairy-nosed wombats as described in Chapter 4 (section 4.2.2.). Sperm collected from these animals was used in the present analysis.

### 5.2.3. Semen analysis

The fixed semen samples (Chapter 4, section 4.2.3.) were transported back to the University of Adelaide where morphological analysis was undertaken. Sperm samples were mixed thoroughly using a glass pipette and an aliquot taken and spread on multiple glass slides. Slides were then cover-slipped and the sperm sample was examined using an Olympus Nomaski microscope (magnification x 268).

Sperm measurements: Measurements of the head, mid-piece and tail of 20 sperm from each animal were recorded using the program NIS Elements BR 2.30. The head length was measured from the distal end of the head to the caudal region, at the implantation fossa where the tail attaches to the head. The mid-piece length was measured from the leading edge of the sperm tail to the end of the mid-piece fibre network. The principal piece was measured from the end of the mid-piece to the tip of the tail. The total sperm tail length was the sum of the mid-piece and principal piece. Photos of sperm were taken using a digital camera attached to the Nomaski microscope.

Sperm head morphology: All sperm present on each slide, for which a clear image could be obtained, were examined and categorised, based on head morphology. Each sperm was categorised into one of the seven types identified (Figures 14 to 20). A total of 200 sperm from each animal were counted and allocated to their appropriate category. The different sperm types were identified based on various morphological characteristics. Tail shape was irrelevant in this categorisation. The various types that were categorised are as follows:

Type 1 – Sperm with a hooked shaped head (Figure 14):

- Curved or hook shaped head
- Bend in the middle of the head
- Distal portion of head is markedly curved and pointing caudally, or back towards the head
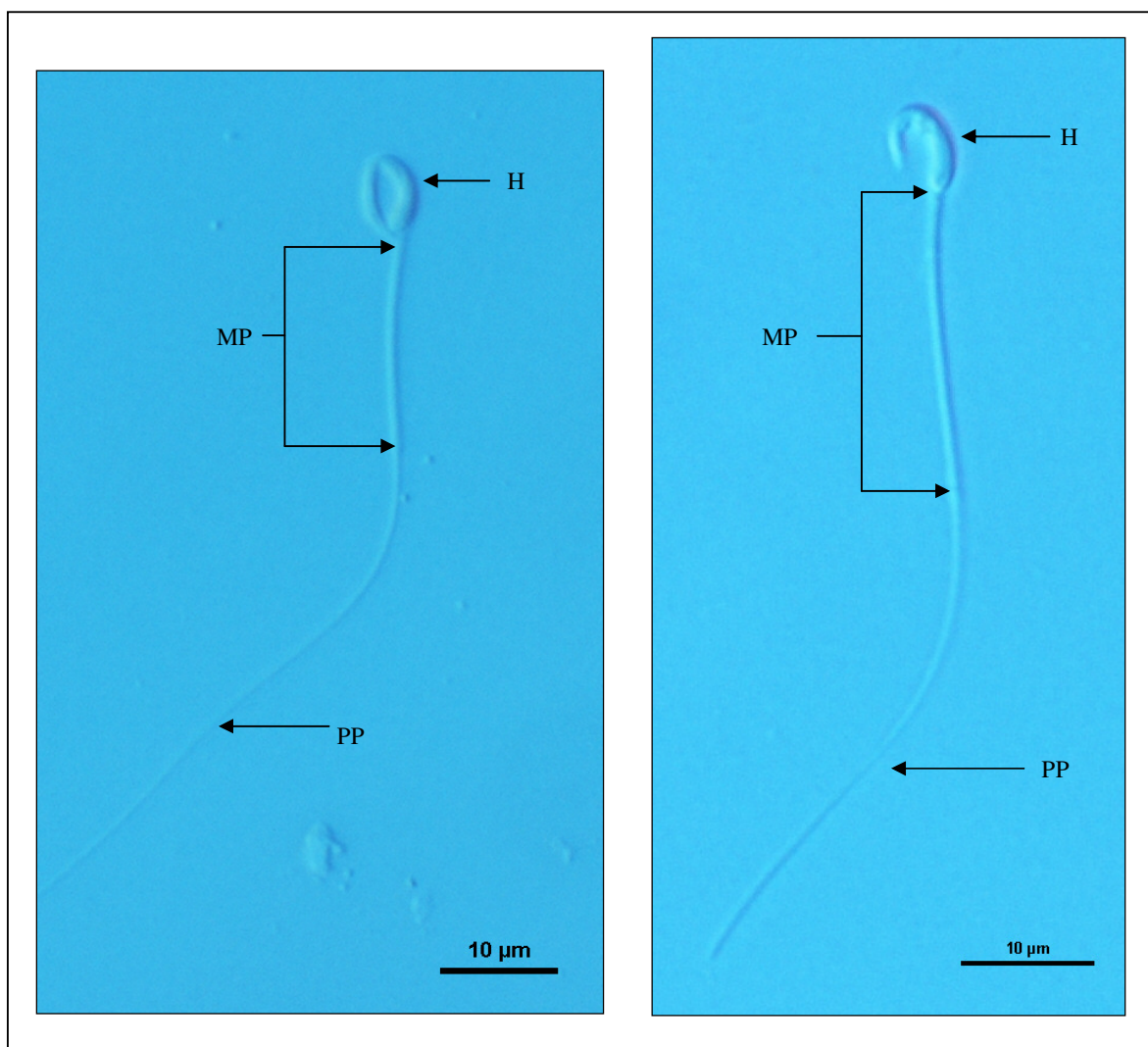


**Figure 14:** Common hooked head sperm shapes (Type 1), in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 2 – Sperm with a broken neck (Figure 15):

- Head deflected back at an angle greater than 90° to the flagellum

- Broken neck

- Nuclear shape irrelevant
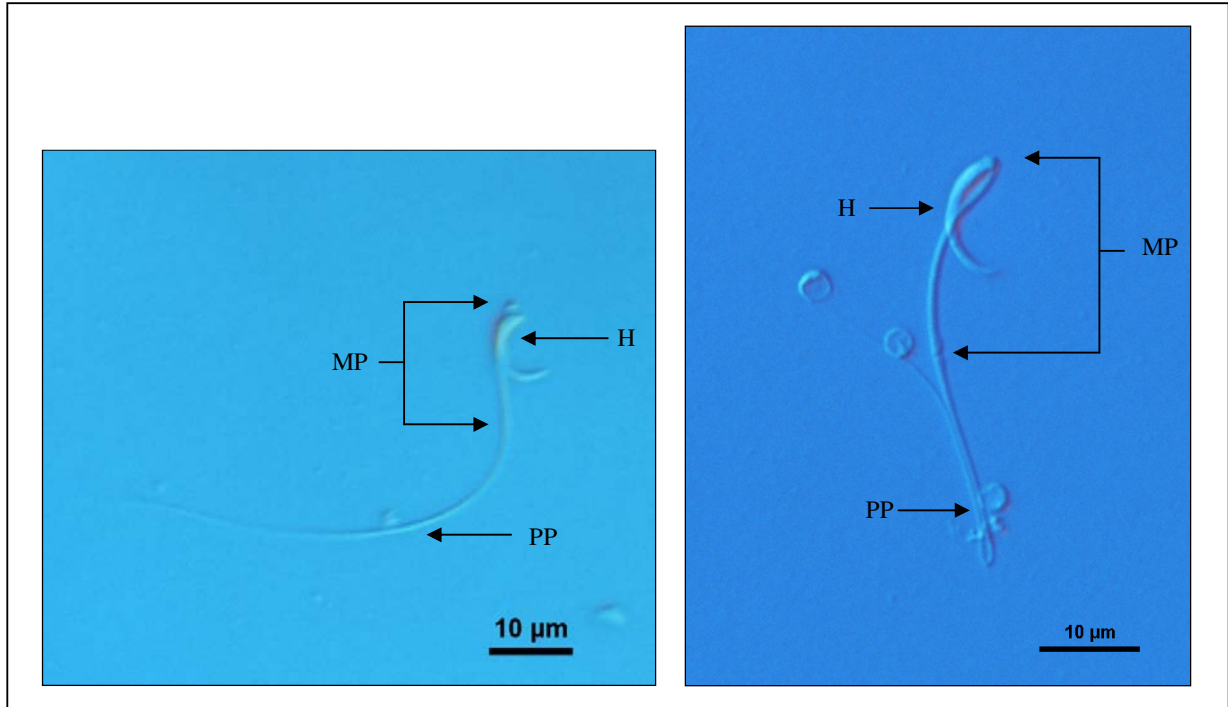


**Figure 15:** Type 2 sperm head shapes in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 3 – Sperm with a distal hook in the head (Figure 16):

- Distal region of head deflected

- Mid-region of head straight, or, if slightly curved, distal end has a hook, rather than just a slight bend
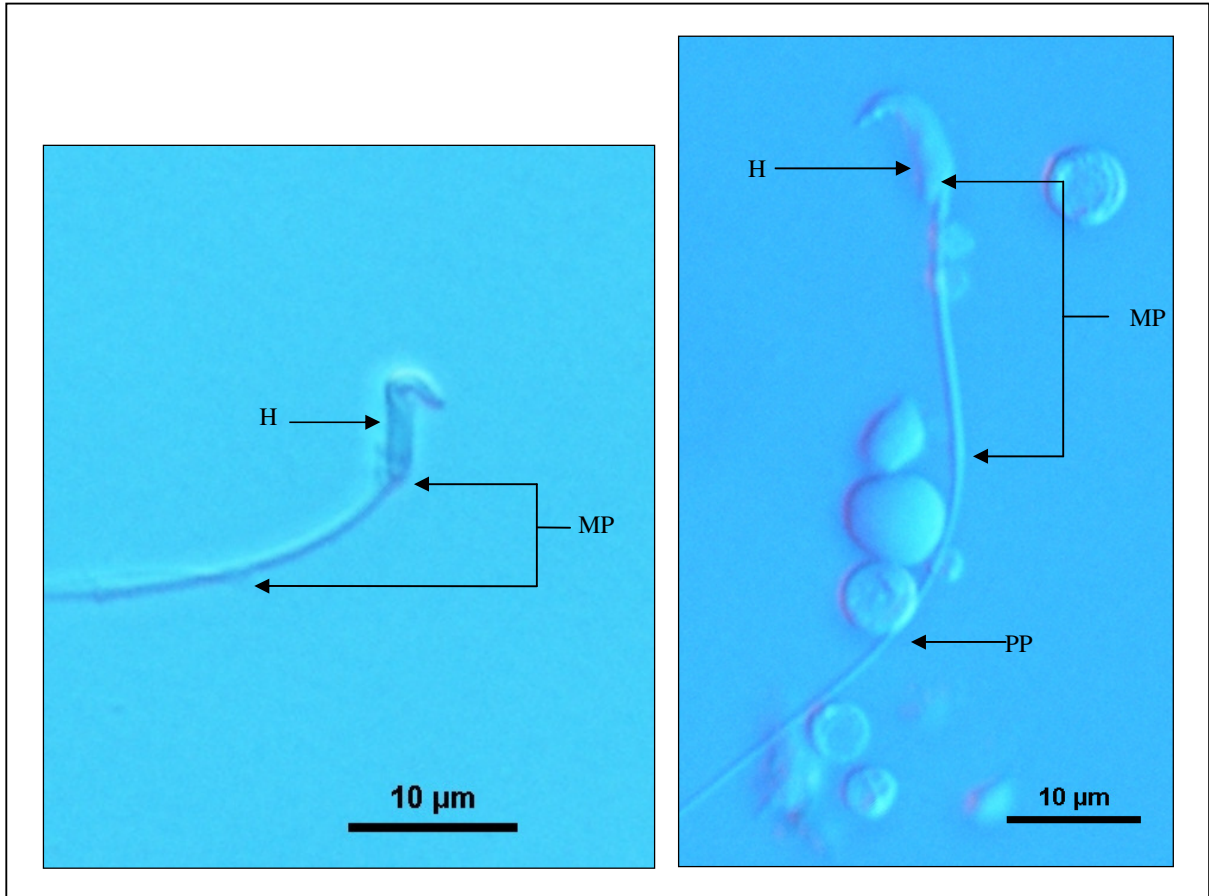


**Figure 16:** Type 3 sperm head shapes in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 4 – Sperm with a proximal bend in head (Figure 17):

- Deflection proximal region of head

- No deflection in distal or mid region of head
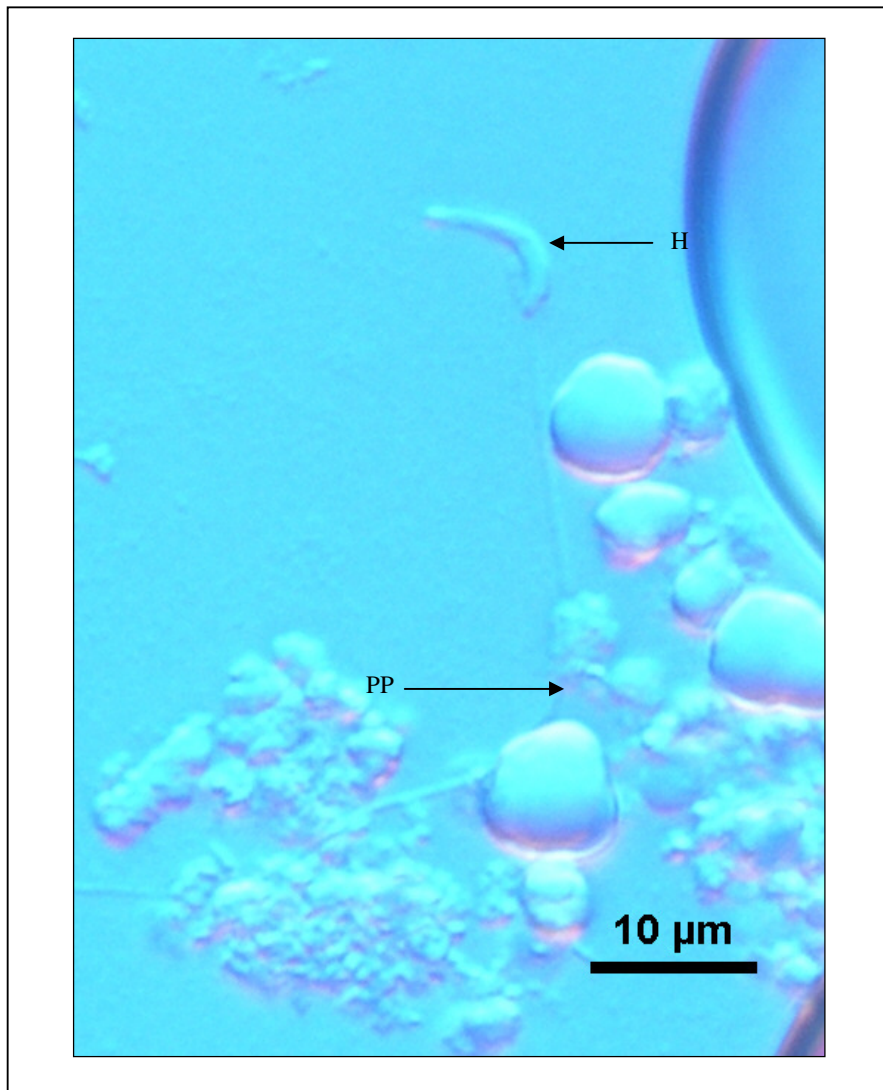


**Figure 17:** Type 4 sperm head shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, PP = principal piece

Type 5 – Sperm with a slight curvature in the middle of the head (Figure 18):

- Slight curvature in middle of head

- Head does not deflect back the same direction as the flagellum
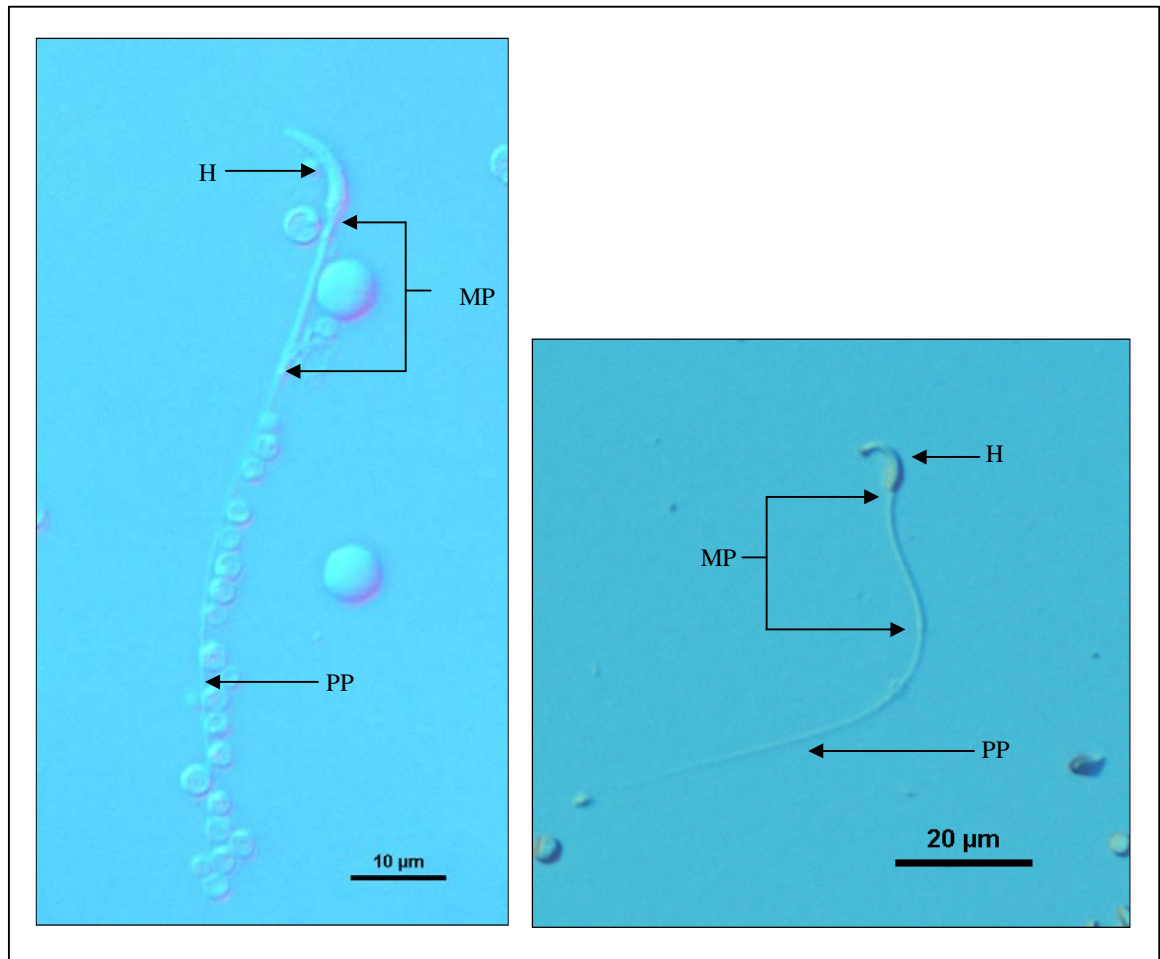


**Figure 18:** Type 5 sperm head shapes in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 6 – Sperm with a "straight" head (Figure 19):

- Head lacks marked curvature
- Head may be S-shaped with apical deflection parallel to the flagellum
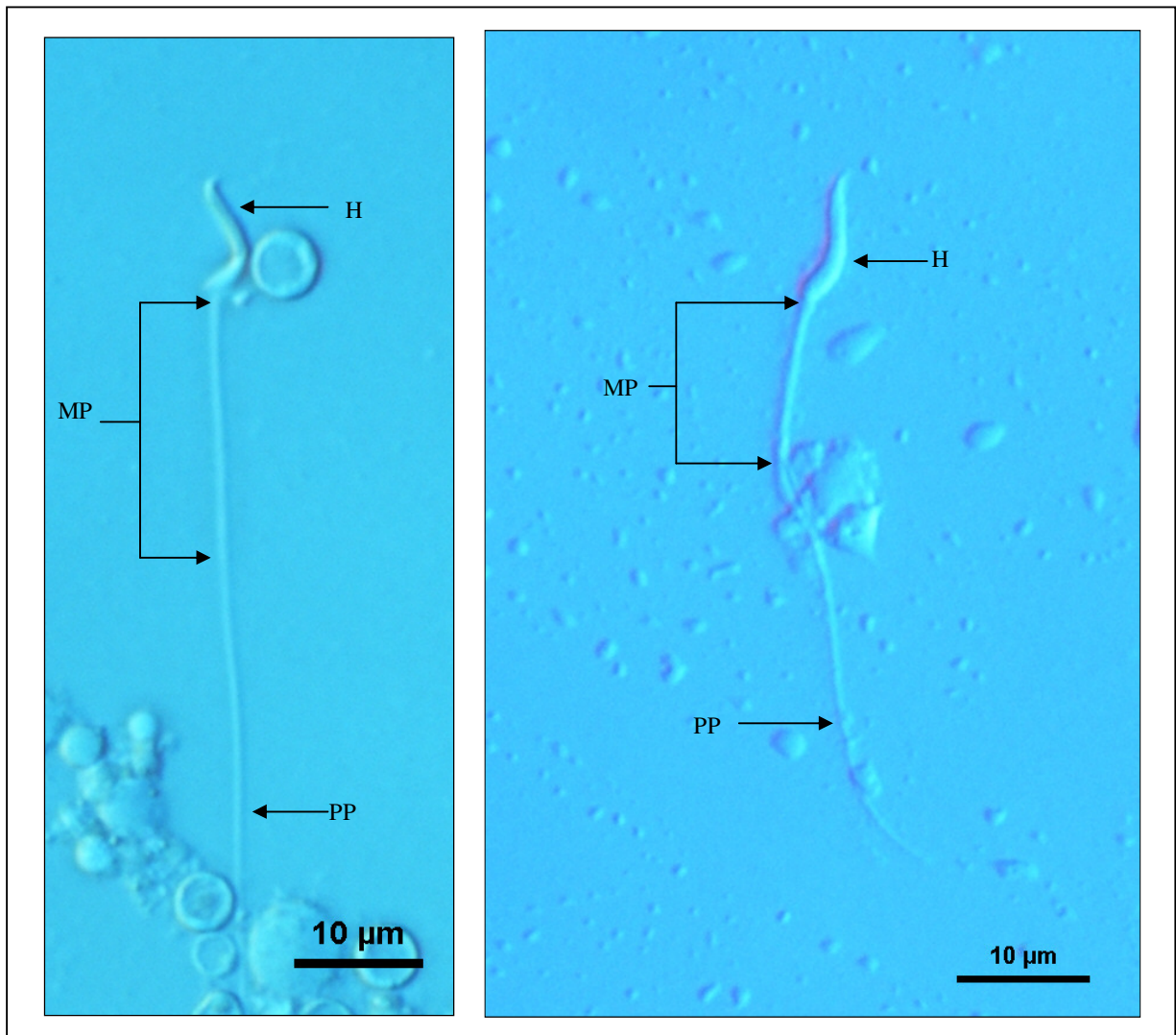


**Figure 19:** Type 6 sperm head shapes in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 7 – Sperm lacking a head (Figure 20):

- Sperm tail has no head attached
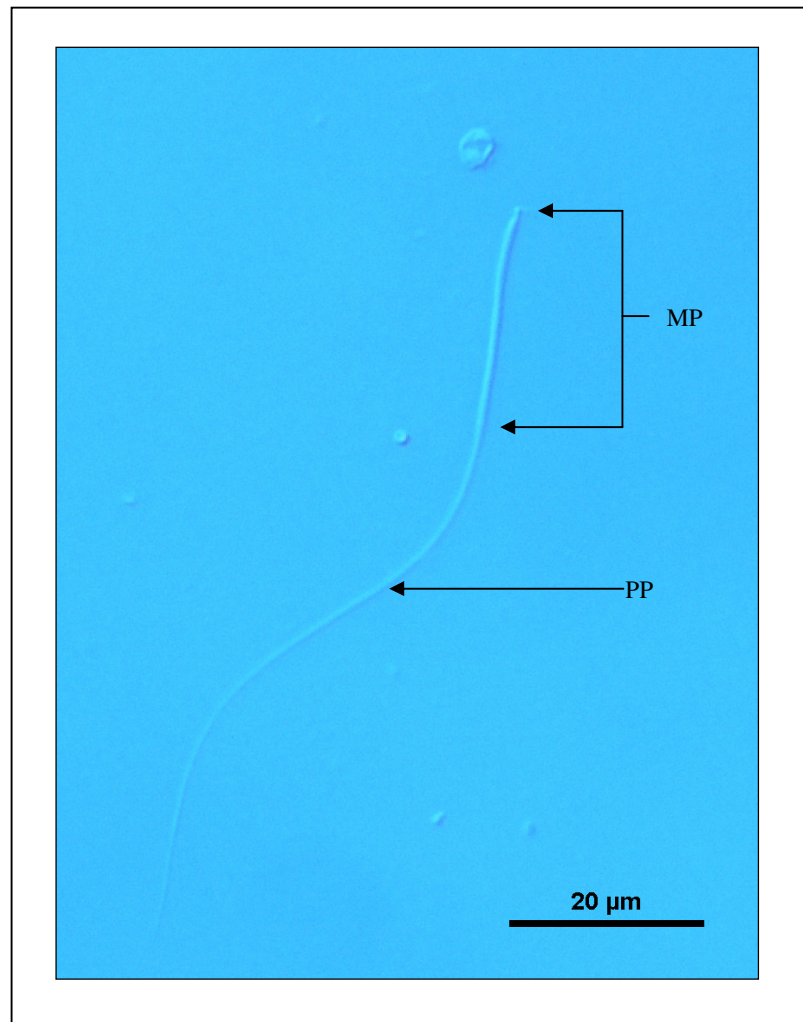


**Figure 20:** Type 7 sperm head shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). MP = Mid-piece, PP = principal piece

Sperm tail morphology: All sperm present on each slide, for which a clear image could be obtained, were then examined and categorised, based on tail morphology, into one of seven sperm tail types (Figures 21 to 27). A total of 20 samples from each wombat were categorised. The head shape was irrelevant in this categorisation. Categories were defined as follows:

Type 1 – Straight tail (Figure 21)

- Straight tail
- Slight bend in tail (< 45º)
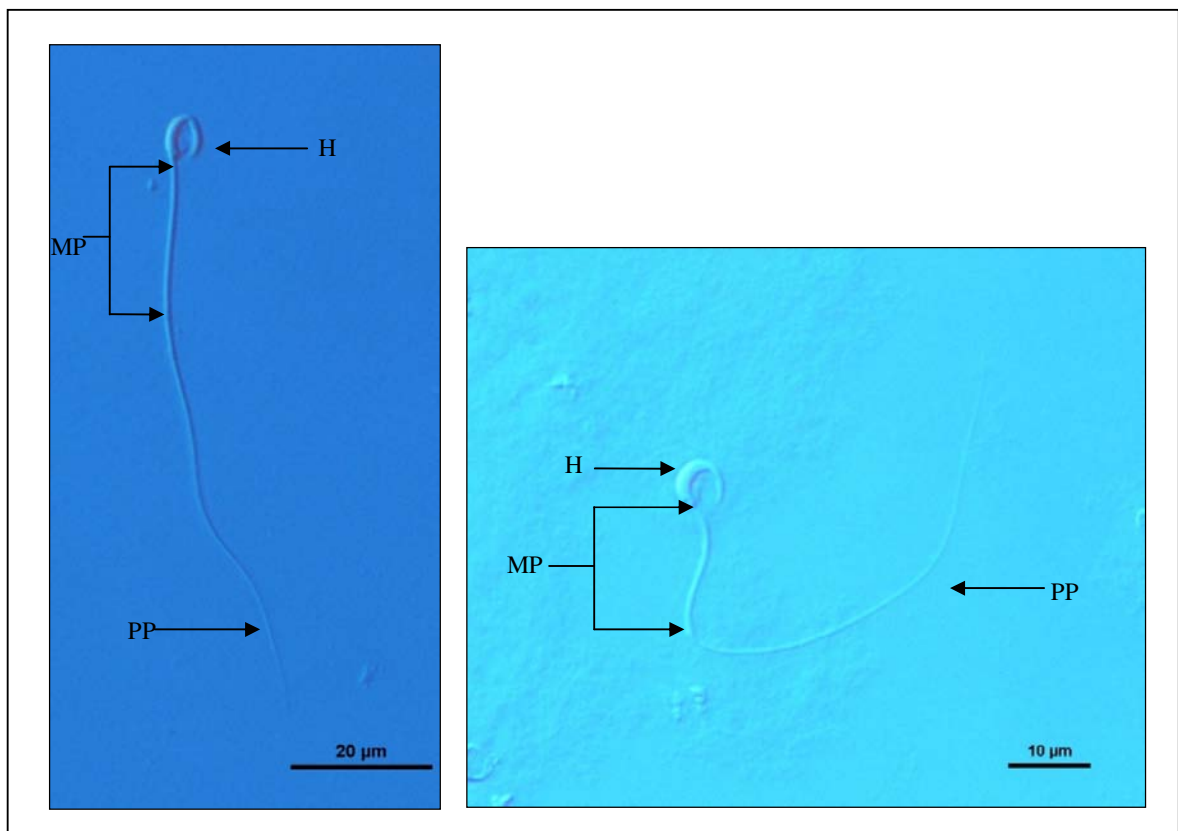


**Figure 21:** Type 1 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 2 – Sperm with a bent mid-piece (Figure 22):

- Bend occurs in mid-piece and causes head to deflect back caudally
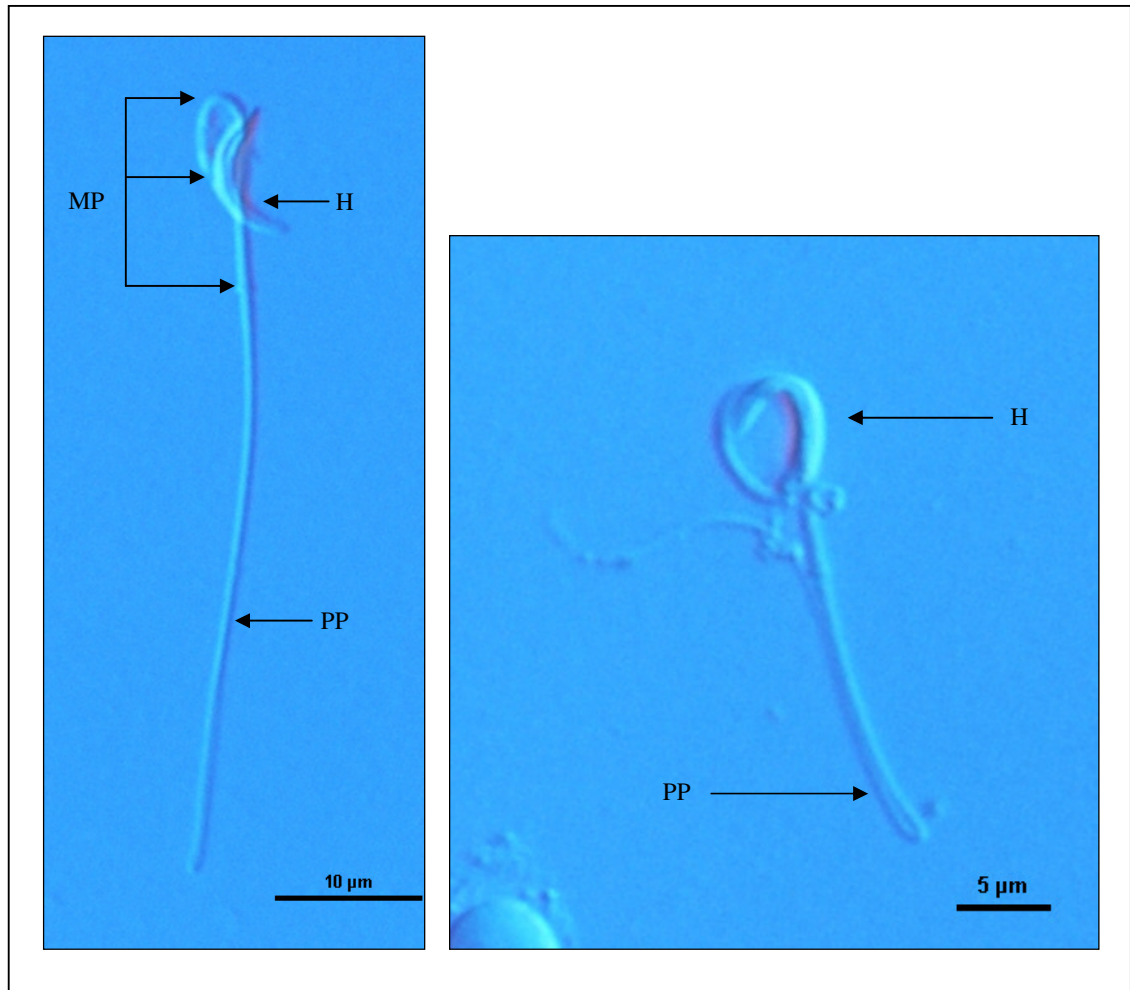


**Figure 22:** Type 2 sperm tail shapes in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 3 – Tail bend (Figure 23)

- Bend in tail > 45º

- Tail does not make contact with any other part of the flagellum
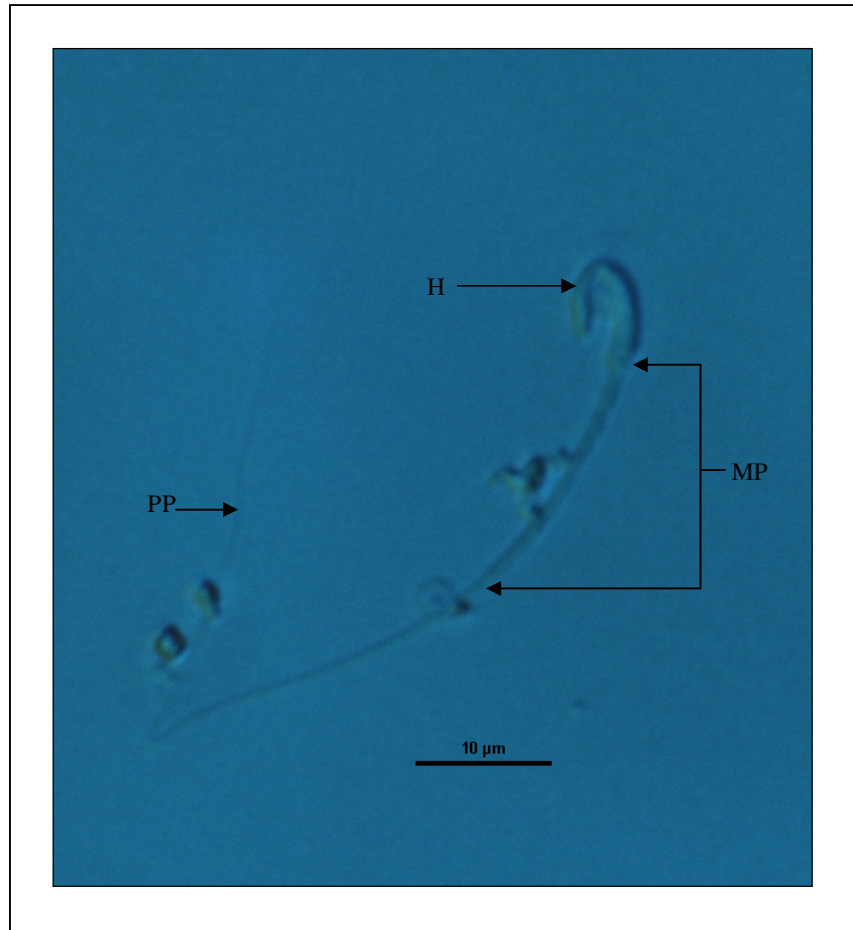


**Figure 23:** Type 3 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 4 – Tail kinked (Figure 24)

- Tail bends > 45° and contact is made at another point on the flagellum
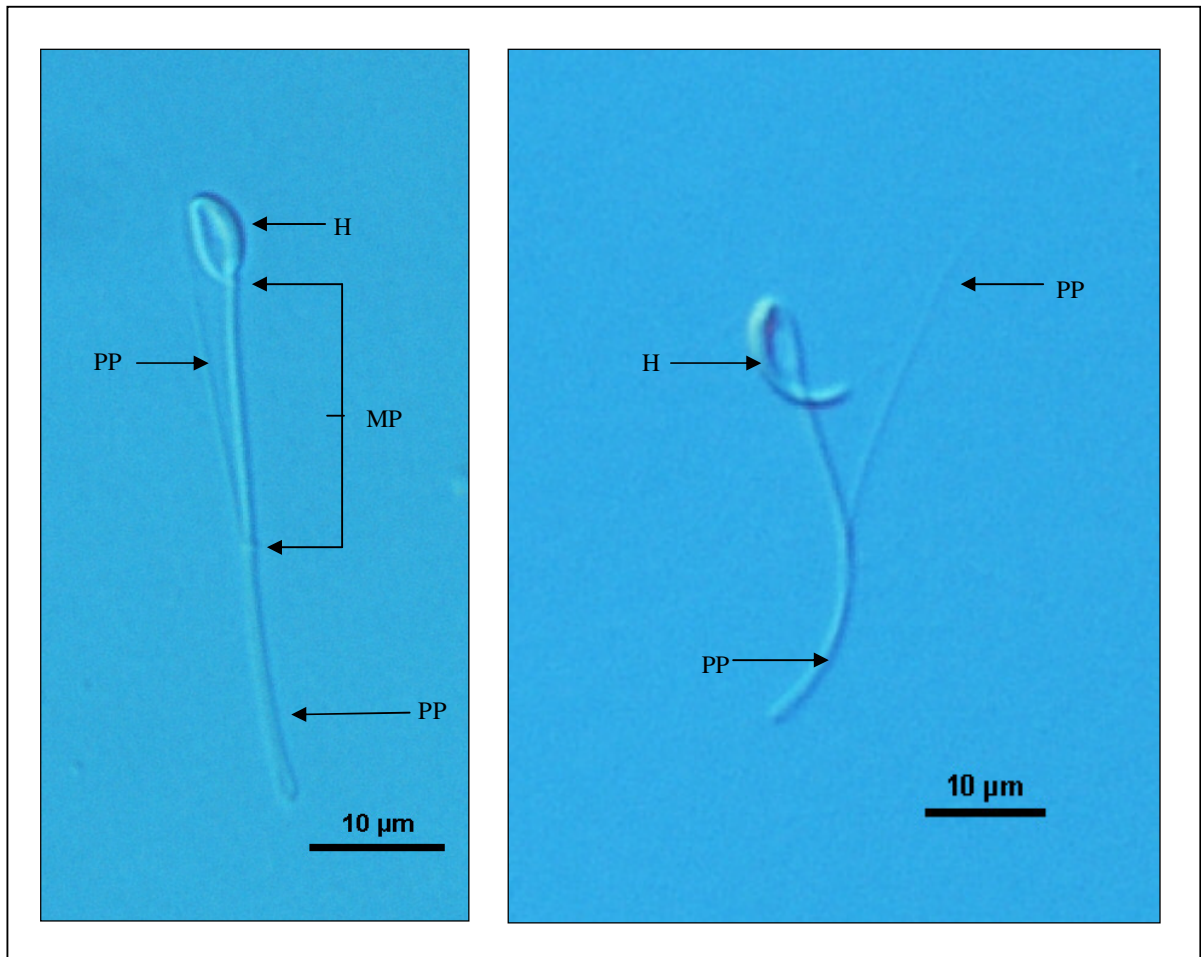


**Figure 24:** Type 4 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 5 – Distal kink (Figure 25)

- Bend/kink in last ¼ of tail only
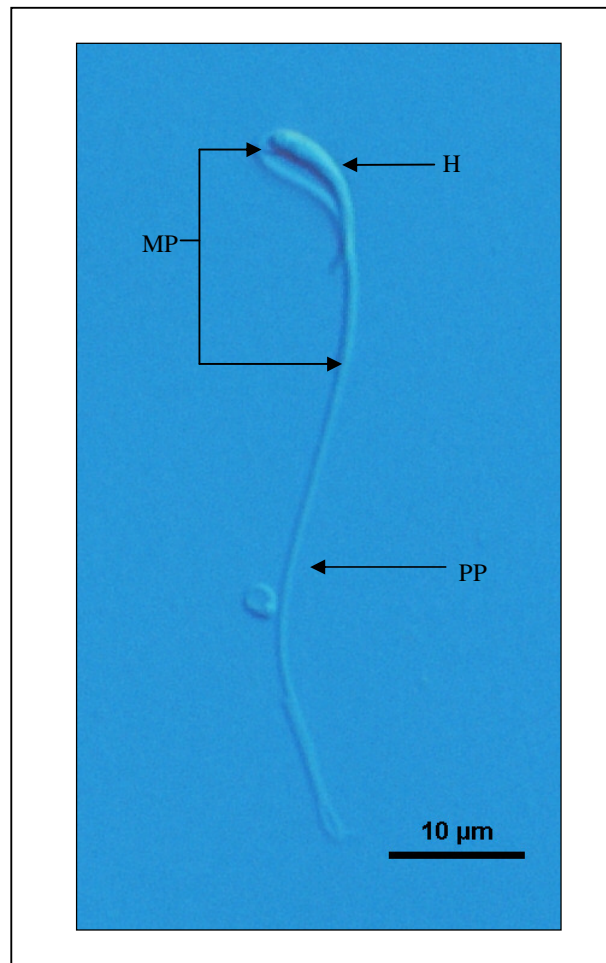


**Figure 25:** Type 5 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 6 – Coil (Figure 26)

- Tail makes contact with other parts of the flagellum more than once
- Tail is coiled
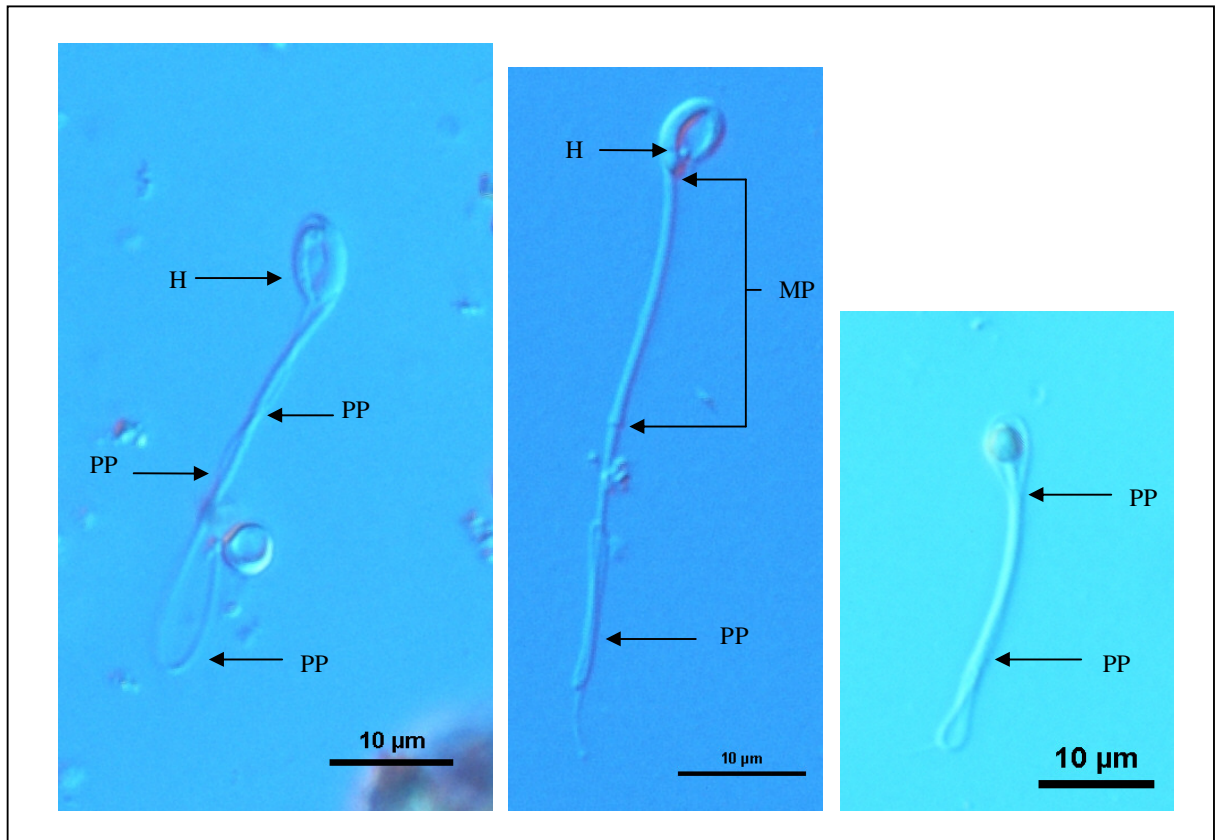


**Figure 26:** Type 6 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). H = head, MP = Mid-piece, PP = principal piece

Type 7 – Miscellaneous sperm shapes (Figure 27):

- Other sperm types that do not fit the above categories, e.g. two tail sperm
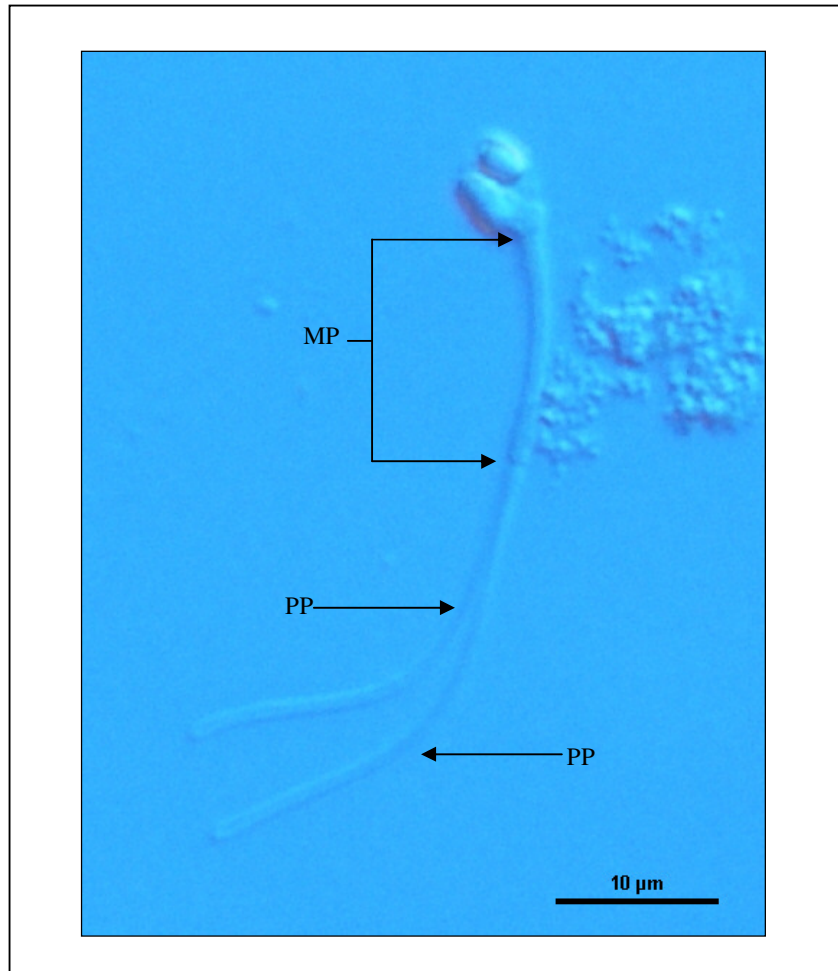


**Figure 27:** Type 7 sperm tail shape in southern hairy-nosed wombat ejaculates, using Nomaski microscope (40x). MP = Mid-piece, PP = principal piece

*5.2.4. Statistical analysis*

Statistical analysis of the accumulated data was performed using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA). To compare sperm morphological measurements between the populations, data were tested for normality, and when the requirements were met, ANOVA models were fitted to the data. When normality was not met, data were analysed using the non-parametric Kruskal-Wallis test. Where the test of the null hypothesis was statistically significant ($p < 0.05$) post-hoc Wilcoxon tests were performed to determine the direction of difference.

Southern hairy-nosed wombats from Swan Reach, Urania and Kulpara were shown to be genetically differentiated to one another (Chapter 3, section 3.3; Table 13, Figure 8 and Figure 9). As a consequence, each of these sites was analysed as a separate population, rather than combining the Urania and Kulpara colonies to examine the Yorke Peninsula as a whole.

Southern hairy-nosed wombat head length and head width were used as an indicator of individual body size, body weight was used to estimate individual condition, and accessory gland length and width were used as indicators of androgen levels and reproductive activity (Taggart *et al.* 2005). To determine any possible relationships between these variables and sperm morphology, the data from each were pooled from all the populations. If data were normally distributed, correlations were established using Pearson's correlation coefficients. However, if data did not appear to be normally distributed, correlations were investigated using Spearman's correlation coefficients.

For the purpose of this study, sperm with normal head morphology was classified as those sperm displaying no, or only slight variation from the common hook shape head, and with no obvious obstructions to the acrosome (head morphotypes 1, 3, 4, 5 & 6). Types 2 and 7 head morphologies were considered abnormal as the sperm head was either missing altogether, or pointing back caudally in the direction of the tail, suggesting that these sperm were not functional. Normal tail morphologies were classified as those which were straight (type 1). The other sperm tail morphotypes (types 2, 3, 4, 5, 6 & 7) all had varying degrees of kinks and bent mid-pieces, and were subsequently considered abnormal.

## 5.3. Results

### 5.3.1. Sperm morphological components

The mean length of each sperm component found in southern hairy-nosed wombat ejaculates at populations at Swan Reach, Urania and Kulpara are detailed in Table 20. The mean sperm head length did not differ significantly between populations ($p \geq 0.05$), and ranged from 11.4 µm at Kulpara, to 11.8 µm at Swan Reach, and 11.8 µm at Urania (Table 20). Likewise, no significant difference was observed between the mean sperm head, mid piece, principal piece or total sperm tail length in the three populations examined ($p \geq 0.05$) (Table 20). Mean mid piece length ranged from 18.2 µm at Urania, to 19.1 µm at Kulpara. Mean principal piece ranged from 55.5 µm at Urania, to 56.8 µm at Swan Reach. Mean total tail length ranged from 73.7 µm at Urania, to 75.5 µm at Kulpara (Table 20).

**Table 20:** Average length (± standard deviation) of each sperm component found in adult male southern hairy-nosed wombat ejaculates from three different populations in South Australia. N = number of animals examined, 20 sperm assessed per animal

| | Swan Reach: | | Urania: | | Kulpara: | |
|---|---|---|---|---|---|---|
| *Sperm component:* | **N** | **Mean** | **N** | **Mean** | **N** | **Mean** |
| *Head piece (µm)* | 17 | 11.8 ± 1.0 | 5 | 11.8 ± 1.3 | 16 | 11.4 ± 1.3 |
| *Mid piece (µm)* | 17 | 18.3 ± 0.9 | 5 | 18.2 ± 1.0 | 16 | 19.1 ± 1.1 |
| *Principal piece (µm)* | 17 | 56.8 ± 3.2 | 5 | 55.5 ± 1.9 | 16 | 56.4 ± 2.1 |
| *Total tail length (µm)* | 17 | 75.0 ± 3.1 | 5 | 73.7 ± 2.6 | 16 | 75.5 ± 2.3 |

*5.3.2. Sperm head morphology*

The mean percentage of each sperm head morphology type found in southern hairy-nosed wombat ejaculates at populations at Swan Reach, Urania and Kulpara, is outlined in Table 21 and Figure 28. Type 1 sperm (hooked head) was the most common sperm head morphology observed in all populations, with a mean of 40.3 ± 20.1 % type 1 sperm per ejaculate in wombats from Swan Reach, 34.8 ± 10.4 % in wombats from Urania, and 28.3 ± 16.0 % in wombats from Kulpara. No significant difference was seen between the populations in any sperm head morphology type (p ≥ 0.05). However, whilst not significant, a trend was observed in the percentage of type 1 sperm between the populations, and the percentage of normal sperm types (types 1, 3, 4, 5 & 6), with a reduced percentage of these morphotypes in an average ejaculate from wombats at Urania and Kulpara, when compared to those collected from wombats from the Murraylands population at Swan Reach (Table 21 and Figure 28).

**Table 21:** Average proportion (± standard deviation) of each sperm head morphology found in southern hairy-nosed wombat ejaculates collected from different wombat populations in South Australia. N = number of animals examined, 200 sperm per animal

| | **Swan Reach:** | | **Urania:** | | **Kulpara:** | |
|---|---|---|---|---|---|---|
| *Sperm head morphology:* | **N** | **Mean** | **N** | **Mean** | **N** | **Mean** |
| *Type 1(%)* | 20 | 40.3 ± 20.1 | 6 | 34.8 ± 10.4 | 18 | 28.3 ± 16.0 |
| *Type 2(%)* | 20 | 25.5 ± 11.0 | 6 | 25.9 ± 6.8 | 18 | 25.1 ± 9.0 |
| *Type 3(%)* | 20 | 4.2 ± 3.8 | 6 | 5.7 ± 1.8 | 18 | 4.1 ± 3.1 |
| *Type 4(%)* | 20 | 1.1 ± 0.9 | 6 | 1.3 ± 2.1 | 18 | 1.2 ± 1.5 |
| *Type 5(%)* | 20 | 7.7 ± 5.4 | 6 | 9.4 ± 4.7 | 18 | 9.0 ± 6.9 |
| *Type 6(%)* | 20 | 2.5 ± 2.8 | 6 | 3.5 ± 3.0 | 18 | 3.6 ± 2.7 |
| *Type 7(%)* | 20 | 6.3 ± 5.6 | 6 | 4.5 ± 2.7 | 18 | 5.3 ± 5.6 |
| *Type 1, 3, 4, 5 & 6 combined (%) ^* | 20 | 55.8 ± 20.7 | 6 | 54.6 ± 9.5 | 18 | 46.2 ± 18.0 |

^ Normal sperm head morphology - as outlined in Methods section
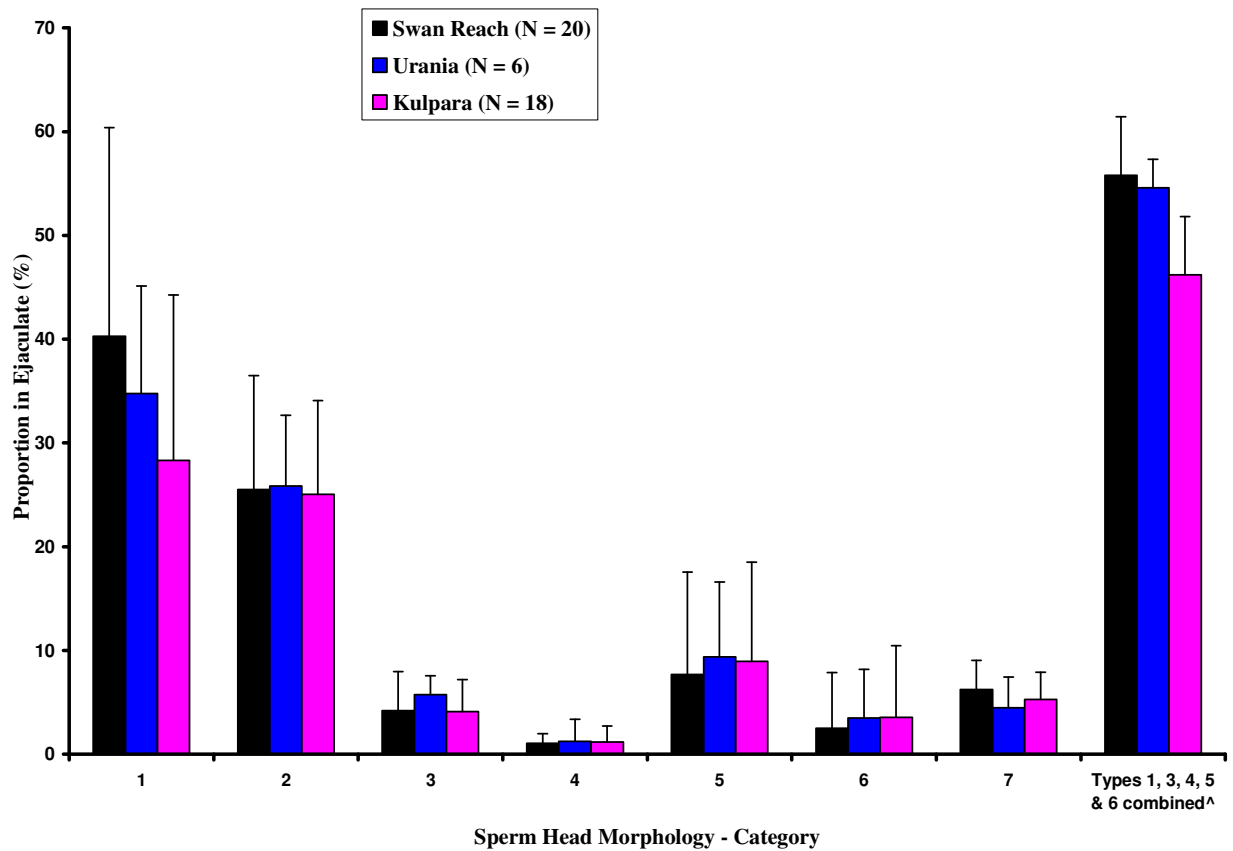
**Figure 28:** Mean percentage (± standard deviation) of each sperm head morphology type found in southern hairy-nosed wombat ejaculates in three different populations in South Australia.
Sample size (N) - indicated
^ Normal sperm head morphology – as classified in the Methods section

*5.3.3. Sperm tail morphology*

The mean proportion of each sperm tail morphology type found in southern hairy-nosed wombat ejaculates at populations at Swan Reach, Urania and Kulpara are outlined in Table 22 and Figure 29. Type 1 sperm tail morphology (straight) was the most common seen in all three populations, with an average of 82.4 ± 16.2 % at Swan Reach, 61.0 ± 32.1 % at Urania, and 75.6 ± 26.6 % at Kulpara, however there was no significant difference between the populations ($p \geq 0.05$). There was a significantly greater mean percentage of type 2 (bent mid-piece) and type 7 (miscellaneous abnormalities) sperm tail morphology in ejaculates from wombats at Kulpara (21.1 ± 9.5 % and 2.4 ± 4.8 % respectively) when compared to wombats from Swan Reach (12.2 ± 9.8 % and 0.2 ± 0.5 % respectively) and Urania (14.8 ± 7.2 % and 0.3 ± 0.4 % respectively) ($p < 0.05$). No significant difference was seen in the mean percentage of sperm tail morphology types 3, 4, 5 and 6 present in wombat ejaculates from Swan Reach, Urania, and Kulpara ($p \geq 0.05$). A trend toward fewer abnormal sperm tail morphologies (type 2, 3, 4, 5, 6, & 7 combined) in the Murraylands population at Swan Reach was apparent (Table 22 and Figure 29).

**Table 22:** Average proportion (± standard deviation) of each sperm tail morphology type found in southern hairy-nosed wombat ejaculates at different populations in South Australia. N = number of animals examined, 20 sperm assessed per animal (Type 1,3,4,5 & 6), and 200 sperm assessed per animal (Type 2 & 7)

| | **Swan Reach:** | | **Urania:** | | **Kulpara:** | |
|---|---|---|---|---|---|---|
| *Sperm tail morphology:* | **N** | **Mean** | **N** | **Mean** | **N** | **Mean** |
| *Type 1 (%)* | 17 | 82.4 ± 16.2 | 5 | 61.0 ± 32.1 | 16 | 75.6 ± 26.6 |
| *Type 2 (%)* | 20 | 12.2 ± 9.8[a] | 6 | 14.8 ± 7.2[a] | 18 | 21.1 ± 9.5[b] |
| *Type 3 (%)* | 17 | 0.6 ± 1.7 | 5 | 1.0 ± 2.2 | 16 | 1.2 ± 2.8 |
| *Type 4 (%)* | 17 | 11.8 ± 12.7 | 5 | 22.0 ± 21.4 | 16 | 15.3 ± 19.8 |
| *Type 5 (%)* | 17 | 2.6 ± 4.7 | 5 | 7.0 ± 8.4 | 16 | 4.4 ± 7.0 |
| *Type 6 (%)* | 17 | 2.6 ± 3.6 | 5 | 9.0 ± 10.2 | 16 | 3.5 ± 5.5 |
| *Type 7 (%)* | 20 | 0.2 ± 0.5[a] | 6 | 0.3 ± 0.4[a] | 18 | 2.4 ± 4.8[b] |
| *Type 2, 3, 4, 5, 6 & 7 combined (%)* ^ | 17 | 28.82 ± 18.4 | 5 | 54.05 ± 27.0 | 16 | 46.56 ± 32.8 |

[a,b] Differing superscript letters indicate a statistically significant difference in sperm tail morphology between Swan Reach, Urania and Kulpara ($p < 0.05$), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference ($p \geq 0.05$)
^ Abnormal sperm tail morphology – as outlined in Methods section
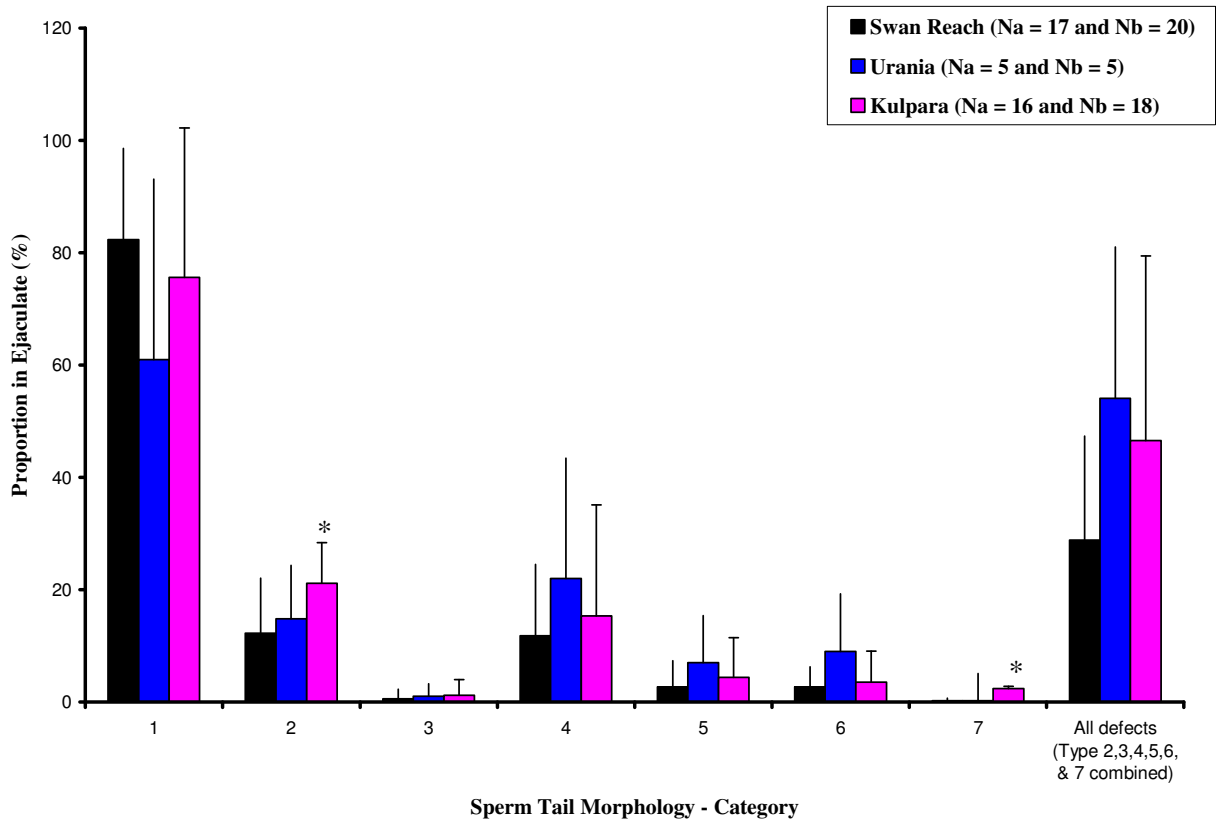
**Figure 29:** Mean percentage (± standard deviation) of each sperm tail morphology type found in southern hairy-nosed wombat ejaculates at different populations in South Australia.
N - indicated (Na = sample size for types 1, 3, 4, 5, 6 & all defects, and Nb = sample size for types 2 & 7)
*Statistically significant difference (p < 0.05)

*5.3.4. Correlations between body size and sperm morphology*

As the most common sperm head shape seen in all three populations of southern hairy-nosed wombats was the hook head (type 1), those sperm heads with only slight variation from type 1, and no obstruction (or absence) of the acrosome (i.e. types 3, 4, 5 and 6) were classified as normal for the purposes of this analysis. There was a significant positive correlation observed between normal sperm head morphology and weight, suggesting that as weight of a male southern hairy-nosed wombat increases, so does the proportion of normal sperm head morphologies within an ejaculate (Table 23). There was also a significant negative correlation between tail morphology type 2 (bent mid-piece) and head width and body weight, suggesting that as weight and head size decreased the proportion of tail morphotypes two in the ejaculate increased (Table 23).

**Table 23:** Pooled data from all adult male southern hairy-nosed wombats previously used in this study and their relationship between sperm morphology and head length, head width, and weight (top value = correlation coefficient, bottom = p-value)

| Sperm morphology: | Body Morphology: | | | Accessory glands: | |
|---|---|---|---|---|---|
| | **Head length** | **Head width** | **Weight** | **Length** | **Width** |
| **Head piece** | -0.12115 0.5018 | 0.07581 0.6651 | 0.16741 0.3364 | -0.15890 0.3619 | -0.00189 0.9914 |
| **Mid-piece** | -0.19696 0.2719 | -0.17167 0.3241 | -0.14153 0.4174 | -0.03073 0.8609 | 0.04236 0.8091 |
| **Tail piece** | -0.00520 0.9771 | -0.05079 0.7720 | 0.07444 0.6708 | -0.12067 0.4899 | -0.16443 0.3452 |
| **Principle piece** | 0.07591 0.6746 | 0.01872 0.9150 | 0.14032 0.4214 | -0.11437 0.5130 | -0.18879 0.2774 |
| **Normal head morphology** | 0.09294 0.5736 | 0.21985 0.1672 | 0.40235* 0.0091 | 0.25801 0.1034 | 0.20493 0.1987 |
| **Tail morphology 1** | -0.12019 0.5053 | -0.11535 0.5094 | 0.09423 0.5903 | 0.06506 0.7104 | 0.08172 0.6407 |
| **Tail morphology 2** | -0.14342 0.3837 | -0.32938* 0.0355 | -0.52308* 0.0004 | -0.36914 0.0175 | -0.26326 0.0963 |
| **Tail morphology 3** | 0.02469 0.8915 | 0.07772 0.6572 | -0.09504 0.5871 | -0.06014 0.7315 | 0.01027 0.9533 |
| **Tail morphology 4** | 0.09155 0.6124 | 0.01618 0.9265 | 0.02427 0.8899 | -0.02210 0.8997 | -0.09610 0.5829 |
| **Tail morphology 5** | 0.05231 0.7725 | 0.02399 0.8912 | 0.02049 0.9070 | 0.00179 0.9918 | 0.02560 0.8839 |
| **Tail morphology 6** | 0.14493 0.4210 | 0.11489 0.5111 | -0.08828 0.6141 | 0.03943 0.8221 | -0.01333 0.9394 |
| **Tail morphology 7** | -0.05481 0.7403 | -0.06724 0.6762 | -0.26030 0.1002 | -0.12224 0.4464 | -0.08464 0.5988 |

* Statistically significant (p < 0.05)

**5.4. Discussion**

 Species within the sub-order Vombatiformes (wombats and koalas) differ from other marsupials, by exhibiting a variety of different sperm head shapes within a normal ejaculate. Similar results have been seen in *Notomys alexis*, the spinifex hopping mouse, where a range of pleomorphic sperm head shapes have been observed in an average ejaculate (Bauer and Breed 2006). This high variability of sperm head morphology in the spinifex hopping mouse has been hypothesised as being due to low levels of inter-male sperm competition (Bauer and Breed 2006). Female southern hairy-nosed wombats are thought to be monogamous (Taggart and Temple-Smith 2008), and show a lack of sexual dimorphism. Males have a low relative testes mass, sperm number and sperm tail length compared to body mass. All these factors suggest that sperm competition is low in this species (Taggart *et al.* 1998a). It has been suggested that sperm competition is an evolutionary force and selection may influence sperm morphology (Roldan *et al.* 1992). Hence, a possible reason for the range of heterogeneous sperm head shapes in the ejaculate of southern hairy-nosed wombats could also be the reduction, or absence of inter-male sperm competition, which does not result in any strong effective selection for a particular morphological sperm head type.

 Previous studies in both koalas and wombats describe the most common sperm type present within ejaculates as sperm with a hooked, strongly curved, head (Harding *et al.* 1987; Temple-Smith and Taggart 1990; Wildt *et al.* 1991; Montgomery 2002). Similar results were seen in the current study, with the hooked, head shape (type one; Figure 14), being the most common sperm head shape seen in all three populations of southern hairy-nosed wombats examined. Some of the other types of sperm head morphotypes that occur less frequently in the ejaculate are also hooked shaped, but the angle of the sperm head is less marked, and the curvature occurs in locations other than the mid region of the head. Examples of these sperm morphologies include sperm head types 3 (Figure 16), 4 (Figure 17), and 5 (Figure 18). Sperm with "straight" heads were also seen (type 6). Other sperm morphologies, such as those with a broken neck (type 2) and a flagellum with no attached head (type 7), are probably functionally inferior. Type 2 sperm morphology (broken neck) was the second most common head shape observed. The high number of broken neck sperm could possibly be the result of a methodology issue (e.g. neck damaged through use of the pipette, or placement of the cover slip etc). Tail morphology was considered normal for type 1 sperm (straight tail), with all the other morphotypes classified as abnormal (types 2, 3, 4, 5, 6 and 7). In type 2 (bent mid-piece) and type 6 sperm (coiled), the acrosome was obstructed by the tail, and in the other tail morphotypes (types 3, 4, 5 and 7) varying degrees of tail kinks were observed, possibly

limiting sperm movement, suggesting these abnormal sperm tails are also likely to be functionally inferior (like those classified as having an abnormal head morphology).

Previous studies have shown a decrease in genetic diversity to be associated with an increase in sperm abnormalities (both tail and head defects) in the ejaculate of felids, koalas, wolves, ponies and rabbits (Wildt *et al.* 1983; Wildt *et al.* 1986; Wildt *et al.* 1987a; Montgomery 2002; Gage *et al.* 2006; van Eldik *et al.* 2006; Asa *et al.* 2007). In the present study it was observed that the proportion of each sperm head type present in the ejaculate, varied between the three wombat populations, with the largest population at Swan Reach, having the highest percentage of type 1 sperm present in the ejaculate ($40.3 \pm 20.1$ %), and the smallest population, Kulpara, having the lowest ($28.3 \pm 16.0$ %), however, this difference was not statistically significant. A similar trend was also observed when the normal sperm head types (types 1, 3, 4, 5 and 6) were combined for analysis, with Swan Reach having the greatest proportion of normal head morphotypes, and Kulpara the lowest. Whilst these results were not statistically significant, a trend can be observed, where wombats from the smaller isolated populations on the Yorke Peninsula had a lower proportion of the common ("normal") sperm head shapes compared to the large Swan Reach population.

Unlike koalas and wombats, felids do not have a range of varying sperm head morphologies (excluding head size) present in normal ejaculates. In these species, sperm that are considered abnormal are those that have tightly coiled or bent flagella, absent mitochondrial sheaths, abnormal acrosomes, deranged or bent mid-pieces, cytoplasmic droplets, broken necks, and unusually large (macrocephalic), or small (microcephalic) heads (Wildt *et al.* 1983; Wildt *et al.* 1986; Wildt *et al.* 1987a). The current study on sperm in wombats found significant differences in the sperm tail morphology between the samples of animals from the three populations, however, no differences were found in the different sperm component lengths (including head size). Wombats from Kulpara had a significantly higher proportion of sperm with type 2 (bent mid-piece), and type 7 tails (miscellaneous abnormalities), in the ejaculate ($21.1 \pm 9.5$ % and $2.4 \pm 4.8$ %, respectively), compared to wombats from Swan Reach which had the lowest proportion of sperm tail types 2 and 7 ($12.2 \pm 9.8$ % and $0.2 \pm 0.5$ %, respectively). Although not statistically significant, a trend was observed, where animals from the small and geographically isolated populations on the Yorke Peninsula had a greater number of sperm tail abnormalities than wombats from the larger Swan Reach, Murraylands population, which had higher proportions of type 1 (straight) sperm tail morphologies present in the ejaculate.

Previous investigations in koalas and felids have reported a decrease in the proportion of normal sperm per ejaculate, and an increase in the percentage of abnormal sperm, as the

degree of genetic heterogeneity decreases (Wildt *et al.* 1983; Wildt *et al.* 1986; Wildt *et al.* 1987a; Montgomery 2002). Results from Chapter 3 showed there was little difference in heterozygosity values between the three populations of southern hairy-nosed wombats, although there was, however, a slight decrease in the allelic diversity (number of alleles and allelic richness) as population size became smaller, i.e. the largest population at Swan Reach had the greatest allelic diversity, and the smallest population at Kulpara had the least diversity. Two of the abnormal sperm types in the tail; type 2 (bent mid-piece) and type 7 (miscellaneous abnormalities), were in significantly higher proportions per average ejaculate in the Kulpara population, which has the lowest allelic diversity and population size. In addition, whilst again not statistically significant, the proportion of the common sperm head shape (type 1), was highest in wombats from Swan Reach, and lowest in those from Kulpara. Tail morphology was also observed to have a higher number of abnormalities (i.e. kinks and coils) in the smaller more isolated populations of the Yorke Peninsula, when compared to the large Swan Reach population, but again this was not statistically significant. No inbreeding was recorded for southern hairy-nosed wombats in this present study; however there was a slight association between proportion of normal sperm morphologies in an ejaculate, and allelic diversity and population size, with the smaller populations having a decreased allelic diversity, and an increased number of abnormalities in both the sperm tail and head.

An alternative theory developed to explain the association between declining genetic diversity and increasing concentration of abnormal sperm morphologies, proposes that populations with an initially large number of abnormal sperm within an ejaculate, also have an elevated rate of infertility, thereby resulting in reduced reproductive success, and hence causing the genetic diversity to decline further (Slate and Pemberton 2002). The theory is a reversal of the hypothesis that suggests inbreeding increases the frequency of deleterious, recessive alleles and therefore the proportion of abnormal sperm morphologies in the ejaculate (Frankham *et al.* 2002; Keller and Waller 2002). By extension, this theory is applicable then to the population of southern hairy-nosed wombats at Kulpara, which may eventually have an increased loss of genetic diversity due to lower fertility and reduced reproductive success.

Other than reduced genetic diversity, several environmental factors may contribute to the greater proportion of sperm abnormalities in the wombats from the Kulpara population. Analysis of these environmental variables should be included in future research. Chemical use, water intake and nutrition can all contribute to changes in seminal quality, including sperm morphology (Nelson 1993; Marai *et al.* 2002; Ong *et al.* 2002; Swan *et al.* 2003; Wong *et al.* 2003; Fisher 2004). In the Florida panther it has been shown that, regardless of the effects of inbreeding, environmental contaminants negatively influenced the reproductive

success of males (Facemire and Gross 1995). Panthers were exposed to chemicals through consumption of their prey, the racoon. These contaminants were found to disrupt the endocrine system and decrease the production of androgens. As androgens are important in spermatogenesis, the production of sperm was therefore impaired (Facemire and Gross 1995). Future studies should also include monitoring of testosterone levels within the study animals to determine any possible disruptions to the endocrine system.

Age has also been shown to influence semen quality, with ejaculate volume, sperm motility and proportion of normal sperm morphologies decreasing with age in humans (Kidd *et al.* 2001). In lab mice, the percentage of sperm head abnormalities in an ejaculate was largest in both juvenile and older males (Krzanowska 1981; Albert and Roussel 1983). In leopards, sperm concentration decreased in both younger and older animals (de Haas van Dorsser and Strick 2005), whereas in cheetahs, concentration was reduced in only the older animals (Durant 2000). Determining the age of wombats is difficult once they are sexually mature (> 3 years), as traditional techniques such as monitoring wear patterns on teeth is impossible due to their continuously growing molars. Semen collection from known age captive wombats may be a useful way to explore the influence of maturity on semen quality in this group.

The seminal quality of male southern hairy-nosed wombats in this study was sampled only during the breeding season (July – Dec) in an attempt to minimise any differences in sperm morphology due to seasonality. Other parameters associated with reproduction (ejaculate volume, scrotal width, number of sperm per ml, percentage of motile sperm, and accessory gland weights) have been observed to change with the onset of the breeding season (Taggart *et al.* 2005). Sperm morphology was not examined in this latter study, and the effect of seasonality, if any, on the sperm morphotypes of southern hairy-nosed wombats is unknown. In seasonal breeding goats in the Mediterranean it has been shown that there is a higher proportion of abnormal sperm morphotypes during the non-breeding season; however, the number of abnormalities was still within the accepted range for fertility (Roca *et al.* 1992). These are possibilities that should be considered in future research in this area. However, other studies have suggested that sperm development and morphology are under rigorous genetic control (Beatty 1970), and that contribution from non-genetic, biological factors to sperm morphology is trivial (Wildt *et al.* 1983).

Research in wolves and deer has shown a significant relationship between sperm morphology and sperm motility, with a higher number of abnormal sperm (mainly detached heads and coiled tails) correlated with a reduced overall motility within an ejaculate (Asa *et al.* 2007; Gomendio *et al.* 2007). In the present study, no significant differences were found in

the motility rating of the ejaculate of southern hairy-nosed wombats between the populations studied (Chapter 4). This result was not surprising as no difference between populations was observed in sperm tail morphology or sperm component lengths (i.e. head, mid-piece, tail). These two factors are important for sperm motility, as the beating motion of the tail is responsible for forward progression, and the mid-piece for energy production. Despite the higher proportion of sperm tail abnormalities (type 2 and 7) in male wombats from Kulpara, there did not appear to be any affect on the sperm motility rating of the ejaculates from this region. However, in wombats, the integrity and position of the acrosome in these sperm, their structural stability, and their ability to successfully fertilise, is unknown.

In humans, a higher percentage of abnormal sperm morphologies results in reduced fertilisation rates (Johansson and Rendel 1968; Donnelly *et al.* 1998). Likewise, a highly significant relationship between sperm morphology and reproductive success has been established in wolves (Asa *et al.* 2007), and the sperm nuclear shape is used as a predictor of bull fertility (Ostermeier *et al.* 2001). In felids, sperm with the lowest success rate of penetration of the egg were those with abnormal morphology (Howard *et al.* 1991). Conversely, in koalas, low genetic diversity (Montgomery 2002; Cristescu *et al.* 2009), and a decrease in the proportion of normal sperm morphologies in an average ejaculate (Montgomery 2002), has been recorded in isolated populations, nevertheless these populations still appear to have high fertility, with some populations growing rapidly (e.g. Kangaroo Island) (Cristescu *et al.* 2009). The correlation between sperm morphology and fertility in these populations of koalas however, was not directly examined. In southern hairy-nosed wombats, ejaculates containing a higher proportion of sperm with bent mid-pieces (type 1), and other miscellaneous abnormalities (type 7), were found in animals from the small isolated population of Kulpara. Whilst the fertilising capacity of these abnormal sperm morphotypes is not known in wombats, it is considered highly likely that sperm with a bent mid-piece, or other tail or head abnormalities, will not be able to migrate up the female reproductive tract following mating, and/or fuse with an egg at fertilisation. The fertility of male southern hairy-nosed wombats at Kulpara may therefore be reduced compared to those from other populations examined.

It is unknown what percentage of abnormal sperm morphologies in a southern hairy-nosed wombat ejaculate may result in a reduction of fertility. Fertility is compromised when greater than 20 % of sperm per ejaculate are abnormal in bulls (Chenoweth and Ball 1980) and > 40 % in rabbits (Gage *et al.* 2006). In dogs, if the percentage of abnormal sperm morphologies in an ejaculate is greater than 40 %, reproductive success decreases from 61 % to 13 % (Asa *et al.* 2007). In fertile men 20-35 % of sperm have some form of structural

defect (Donnelly *et al.* 1998; Kubo-Irie *et al.* 2004). In the current study all three populations of wombats had greater than 20 % abnormal sperm head morphologies, with type 2 sperm (broken neck) averaging around 25 % of the sperm morphology found in an average ejaculate. Whether this is a structural defect, or a methodology issue associated with processing the semen samples, is unknown. In bulls and humans, the sperm morphologies identified as reducing fertility include those sperm with tail defects. In this study on wombats sperm tail defects accounted for over 20 % of ejaculated sperm. Studies in domestic animals have also shown that it is not just sperm morphology that determines fertilisation success, but also their functional competence (Petrunkina *et al.* 2007), as some morphologically normal sperm may still not be able to fertilise (Mortimer *et al.* 1982). To determine the possible implications of abnormal sperm morphology on reproductive success in the southern hairy-nosed wombat, additional studies using methods such as artificial insemination, or *in vitro* fertilisation, would be required to ascertain at what  point fertility becomes compromised based on their sperm morphology. Male fertility is only half the picture, as female fertility must also be taken into account (Amann 2005).

Male competition for mates also has to be considered when discussing reproductive success (Gomendio *et al.* 2007). In some species of macropods (Johnson 1989; Fisher and Lara 1999), and eutherian mammals such as rodents (e.g. *Cavia apera*) (Adrian *et al.* 2008; Asher *et al.* 2008) and deer (McElligott *et al.* 2001; Charlton *et al.* 2007), it has been shown that males with a larger body size and mass are more likely to sire offspring as a larger body size can influence dominance and victory in male/male competition (McElligott *et al.* 2001), thereby increasing the chance of mating. In red deer and cavies, the selection of larger males is believed to be the result of female choice, because a larger male suggests greater strength and resource availability, which are both of benefit to the mother and young (Charlton *et al.* 2007; Adrian *et al.* 2008; Asher *et al.* 2008). The results from this study on southern hairy-nosed wombats showed a positive relationship between male weight and the proportion of normal sperm head morphotypes (types 1, 3, 4, 5 and 6) present in the ejaculate. Likewise, a negative relationship was observed between sperm tail type 2 (bent mid-piece), and head width and body weight. These results suggest that as male wombat weight and head width increase, the proportion of normal sperm (both in head and tail morphology) in an ejaculate also increases. In addition, the results from Chapter 4 (section 4.3.2.) showed a positive relationship between head width and ejaculate volume. It therefore appears that as body size and weight in male southern hairy-nosed wombats' increases, so does the ejaculate volume, and proportion of normal sperm head types. This could be the result of natural selection due to female choice, or selection for males with increased androgen levels to help facilitate

successful male/male competition, and territory/female defence. These results, however, must be treated with caution, as it is unknown what actually constitutes a "normal" sperm in southern hairy-nosed wombats, the common hooked sperm head (type 1) can be classified as such, however types 3, 4, 5 and 6 may or may not have the same fertilising potential and must be examined in more depth to understand the relationship between sperm head morphology and fertility of male southern hairy-nosed wombats.

An issue in this study, possibly affecting the results, was the small sample size of ejaculates, with sperm present, collected for semen analysis. This wasn't for lack of effort however, as gathering sperm data for this project was labour intensive and required considerable time in the field, with little gain. As described earlier (Chapter 2.3.2.; Table 2), capture success of wombats was not particularly high, and of these animals captured only the adult males could be used for this section of the project. In addition not every electro-ejaculation of an adult male wombat resulted in successful collection of an ejaculate with sperm present (Chapter 4.3.1; Table 15). Sample sizes may therefore have been too small to detect a difference.

## 5.5. Summary

As discussed in detail earlier (Chapter 3, section 3.4), the long generation time of southern hairy-nosed wombats, relative to their time since isolation, may not be sufficient to show much difference in genetic diversity. Generally, reproductive parameters are the first to be affected by inbreeding depression (Frankham *et al.* 2002; Keller and Waller 2002), and inbreeding impacts strongest on sperm morphology (Asa *et al.* 2007) as spermatogenesis is a complex process which requires precise genetic and physiological control (Gage *et al.* 2006). Defects in spermatogenesis are thought to be an early indicator of increased inbreeding within a population (Gomendio *et al.* 2000). Therefore, whilst genetic analyses in the small and isolated populations of southern hairy-nosed wombats on the Yorke Peninsula did not indicate inbreeding, the significant increase in the proportion of abnormal sperm tail types per ejaculate, and the trend towards reduced numbers of sperm with the hook shaped head, increased sperm numbers with tail kinks, and a lower sperm concentration, may be an early warning sign of inbreeding in these populations.

## Chapter 6: Reproductive success in southern hairy-nosed wombat populations in South Australia

### 6.1 Introduction

*6.1.1. Low genetic variation and reproductive success*

Some factors that have been associated with inbreeding include a reduction in fertility, an increase in the mortality rate of embryos, newborns and juveniles (Johansson and Rendel 1968), a reduction in pregnancy rates (Johansson and Rendel 1968; Wildt *et al.* 1982), and a decrease in the annual reproductive rate (Packer *et al.* 1991) (as discussed in Chapter 1). In Iberian red deer (*Cervus elaphus*) it has been shown that lifetime breeding success in males (based on paternity) and females (based on number of calves born in a lifetime) is positively associated with heterozygosity (Slate *et al.* 2000).

Documented reductions in fertility and reproductive success associated with low genetic variation could be due to numerous factors. One such factor is semen quality. In ganders (*Anser cygnoides*) for instance, it has been shown that semen quality (which incorporates ejaculate volume, sperm concentration, and the percentage of live and morphologically normal sperm) is a good indicator of fertility (Liu *et al.* 2007). Research on felids, wolves (*Canis lupus baileyi*), and Iberian red deer have established a significant relationship between the reproductive success and sperm morphology, where a greater proportion of abnormal sperm morphologies in an ejaculate resulted in decreased fertility of the male, and hence reduced reproductive success (Howard *et al.* 1991; Malo *et al.* 2005; Asa *et al.* 2007; Gomendio *et al.* 2007). Sperm morphology has been associated with sperm motility and sperm velocity (Chapter 4.1.3.), and in Iberian red deer it was shown that sperm velocity was also positively correlated with reproductive success (Malo *et al.* 2005; Gomendio *et al.* 2007). Total sperm number can also influence fertility, with insemination of greater numbers of sperm increasing the chance of reproductive success. Reproductive success, however, still depends upon the proportion of normal sperm within the ejaculate, as a strong correlation between high sperm morphological abnormalities, low sperm velocity, and a reduced fertility has been shown in many species, for example red deer (Gomendio *et al.* 2007).

The effect of inbreeding on sex ratio is another way the population reproductive success can be reduced (Frankham 1995). In the highly inbred northern hairy-nosed wombat for example, a skewed sex ratio favouring males has been observed (2 ¼:1) (Banks *et al.* 2003). This reduces the potential for breeding success of the population due to the limited number of females available for breeding (Horsup 1999; Wedekind 2002).

*6.1.2. Aims*

Genetic diversity and semen quality were examined in both the large population of southern hairy-nosed wombats at Swan Reach in the Murraylands, and in two small isolated populations located at Urania and Kulpara, on the Yorke Peninsula (Chapters 3, 4 and 5). Results from these analyses indicate that, whilst there was no indication of inbreeding in the small populations, allelic diversity was slightly lower, and there was a significantly greater proportion of abnormal sperm head morphotypes in an average ejaculate at Kulpara, and a reduced ejaculate volume at Urania, suggesting lower semen quality in male wombats from these populations. As discussed above, semen quality has been linked to fertility; therefore, the aim of this chapter was to determine if there were any differences in reproductive success between the three populations of southern hairy-nosed wombats studied. Population reproductive success was characterised as the proportion of young animals (pouch young, juveniles, and sub-adults) within each population, and the number of females in late lactation (which suggests young recently left the pouch).

## 6.2. Methods

### *6.2.1. Study populations*

Southern hairy-nosed wombats from the small and isolated populations at Kulpara and Urania on the Yorke Peninsula, and from the large population at Swan Reach in the Murraylands (Chapter 2, section 2.2.1.), were examined.

### *6.2.2. Animal capture and processing*

The female wombats that were captured and processed in Chapters 2 and 3 were also used for data collection in this chapter (Chapter 2, section 2.2.2.). Animals were classified as adult, sub-adult or juvenile based on the specifications outlined in Chapter 2 (section 2.2.4).

### *6.2.3. Reproductive condition*

Adult females had their pouch examined whilst under anaesthetic (Figure 30).



**Figure 30:** The pouch of a southern hairy-nosed wombat being examined to determine reproductive condition. In this case a pouch young was present (photo taken by Elisa Sparrow).

Reproductive condition of each female was determined by assessing the state of the pouch and was characterised as follows (Tyrell 2001):

- Non-cycling – dry, pink and dirty

- Cycling – deep, moist and dirty

- Pregnant – very moist, bright to dark red, clean, increased muscle and muscle tension around pouch lip

- Pouch young present – observable young

- Late lactation – elongated teat, expressing milk, enlarged mammary gland

*6.2.4. Statistical analysis*

Female animals with pouches indicating that they were either non-cycling and cycling were recorded, however these were removed from the statistical analysis and the results, as they did not give a clear indication as to the reproductive success of that female. For example, a cycling wombat may not necessarily get pregnant, and likewise an animal listed as non-cycling may eventually come into season and get pregnant. No pregnant females were recorded across this period and so this category was also excluded from the results.

Statistical analysis of the accumulated data was performed using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA). To compare the proportion of adult female wombats, and juveniles and sub-adults (both sexes), as well as those adult females that had pouch young and were in late lactation, between the three populations, log-binomial regression models were fitted to the data.

**6.3. Results**

The percentage of wombats captured that were either adult females, females with pouch young, females in late lactation, juveniles or sub-adults (both sexes) were determined for populations examined during the study period. Details from the entire study, as well as just during the breeding season, or non-breeding season are presented in Table 24 and Figure 31. No significant differences were found between the populations in the proportion of females with pouch young and females in late lactation during any time of year ($p \geq 0.05$). However, throughout the entire study, and also within the breeding season, there were a significantly larger number of juvenile wombats captured within the Kulpara population compared to those captured at both the Urania and Swan Reach populations ($p < 0.05$) (Table 24a, 24b and Figure 31a, 31b). During the breeding season only, there were also a significantly lower proportion of adult females in the population at Urania, when compared to those at the Swan Reach population ($p < 0.05$) (Table 24b and Figure 31b). During the non-breeding season no statistical analysis could be undertaken on females in late lactation or sub-adult wombats due a failure to capture any animals in this cohort during the non-breeding season. There were, however, a significantly larger number of sub-adult wombats captured in the Kulpara population during the non-breeding season compared to those at the Urania and Swan Reach populations ($p < 0.05$) (Table 24c and Figure 31c).

**Table 24:** Percentage of adult females, females with pouch young, females in late lactation, juveniles and sub-adults (both sexes) in the three South Australian southern hairy-nosed wombat populations examined during (a) the entire study (b) the breeding season, and (c) the non-breeding season

(a)

| All seasons:<br>% wombats in population | Swan Reach | | Urania | | Kulpara | |
|---|---|---|---|---|---|---|
| | **N** | *%* | **N** | *%* | **N** | *%* |
| **Adult females** | 179 | 55 | 57 | 44 | 43 | 49 |
| **Females with pouch young** | 38 | 21 | 10 | 18 | 4 | 9 |
| **Females in late lactation** | 48 | 27 | 15 | 26 | 6 | 15 |
| **Juvenile wombats** | 26 | 8$^a$ | 5 | 4$^a$ | 16 | 18$^b$ |
| **Sub-adult wombats** | 71 | 22 | 5 | 25 | 18 | 20 |

(b)

| Breeding season:<br>% wombats in population | Swan Reach | | Urania | | Kulpara | |
|---|---|---|---|---|---|---|
| | **N** | *%* | **N** | *%* | **N** | *%* |
| **Adult females** | 102 | 58$^a$ | 18 | 33$^b$ | 39 | 48$^{a, b}$ |
| **Females with pouch young** | 11 | 11 | 0 | 0 | 3 | 8 |
| **Females in late lactation** | 46 | 45 | 8 | 44 | 10 | 26 |
| **Juvenile wombats** | 16 | 9$^a$ | 1 | 2$^a$ | 16 | 20$^b$ |
| **Sub-adult wombats** | 30 | 17 | 13 | 24 | 14 | 17 |

(c)

| Non-breeding season:<br>% wombats in population | Swan Reach | | Urania | | Kulpara | |
|---|---|---|---|---|---|---|
| | **N** | *%* | **N** | *%* | **N** | *%* |
| **Adult females** | 78 | 51 | 39 | 53 | 4 | 67 |
| **Females with pouch young** | 27 | 35 | 10 | 26 | 1 | 25 |
| **Females in late lactation** | 4 | 4 | 7 | 18 | 0 | 0$^\wedge$ |
| **Juvenile wombats** | 11 | 7 | 4 | 5 | 0 | 0$^\wedge$ |
| **Sub-adult wombats** | 43 | 28$^a$ | 20 | 27$^a$ | 4 | 67$^b$ |

[a,b] Differing superscript letters indicate statistically significant differences in percentage of wombats in the population between Swan Reach, Urania and Kulpara ($p < 0.05$), whereas no superscript letters, or same superscript letters, indicate no statistically significant difference ($p \geq 0.05$)

(a) Entire study:



Legend:
- ■ **Swan Reach**
- ■ **Urania**
- ■ **Kulpara**
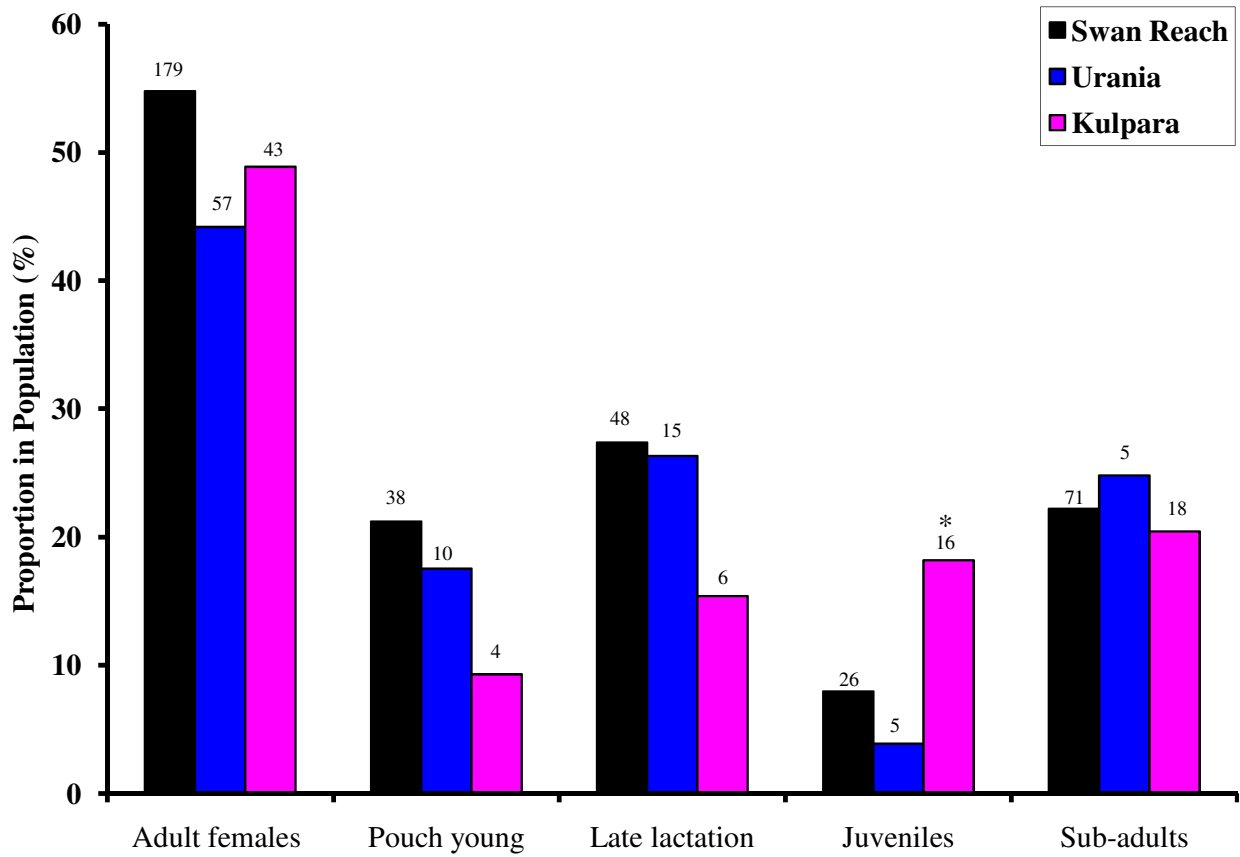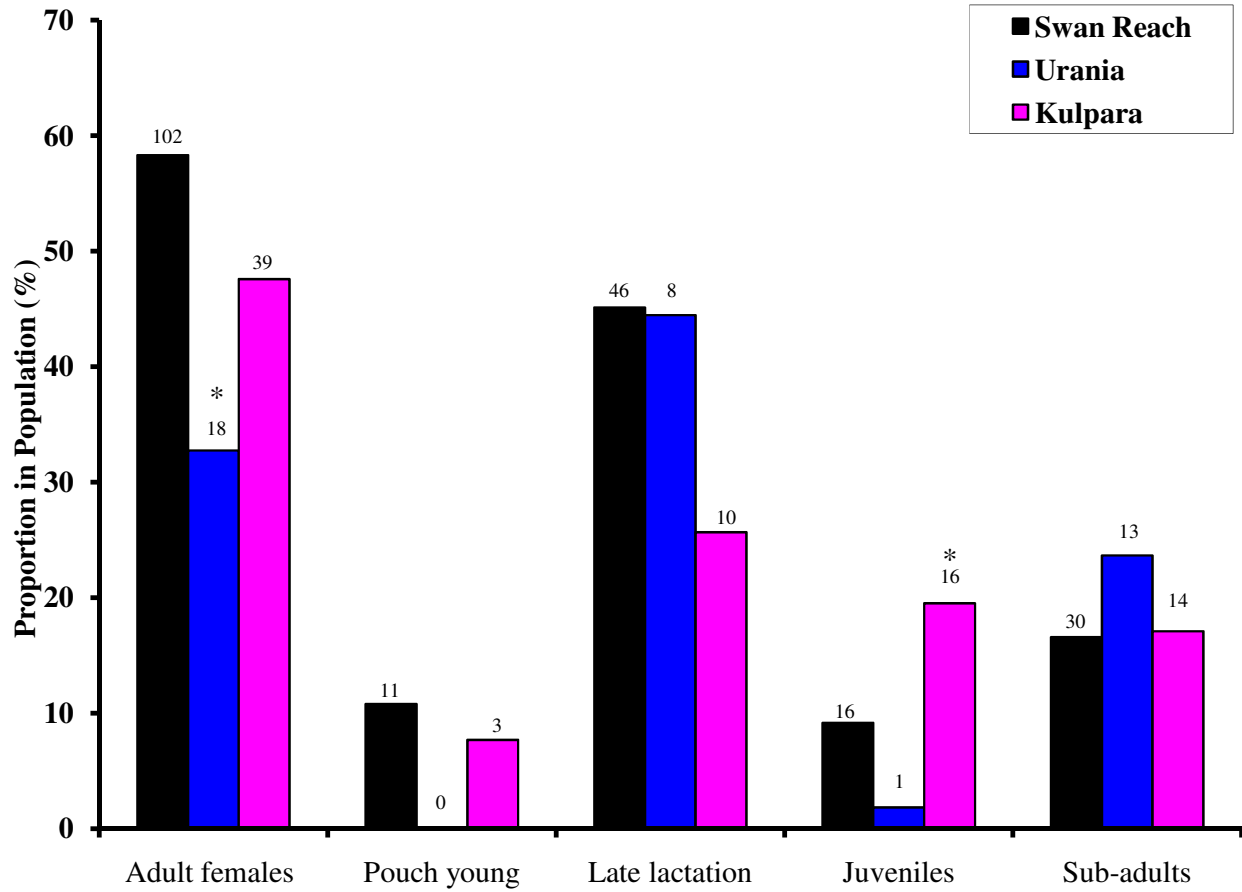
(b) Breeding season:

(c) Non-breeding season:
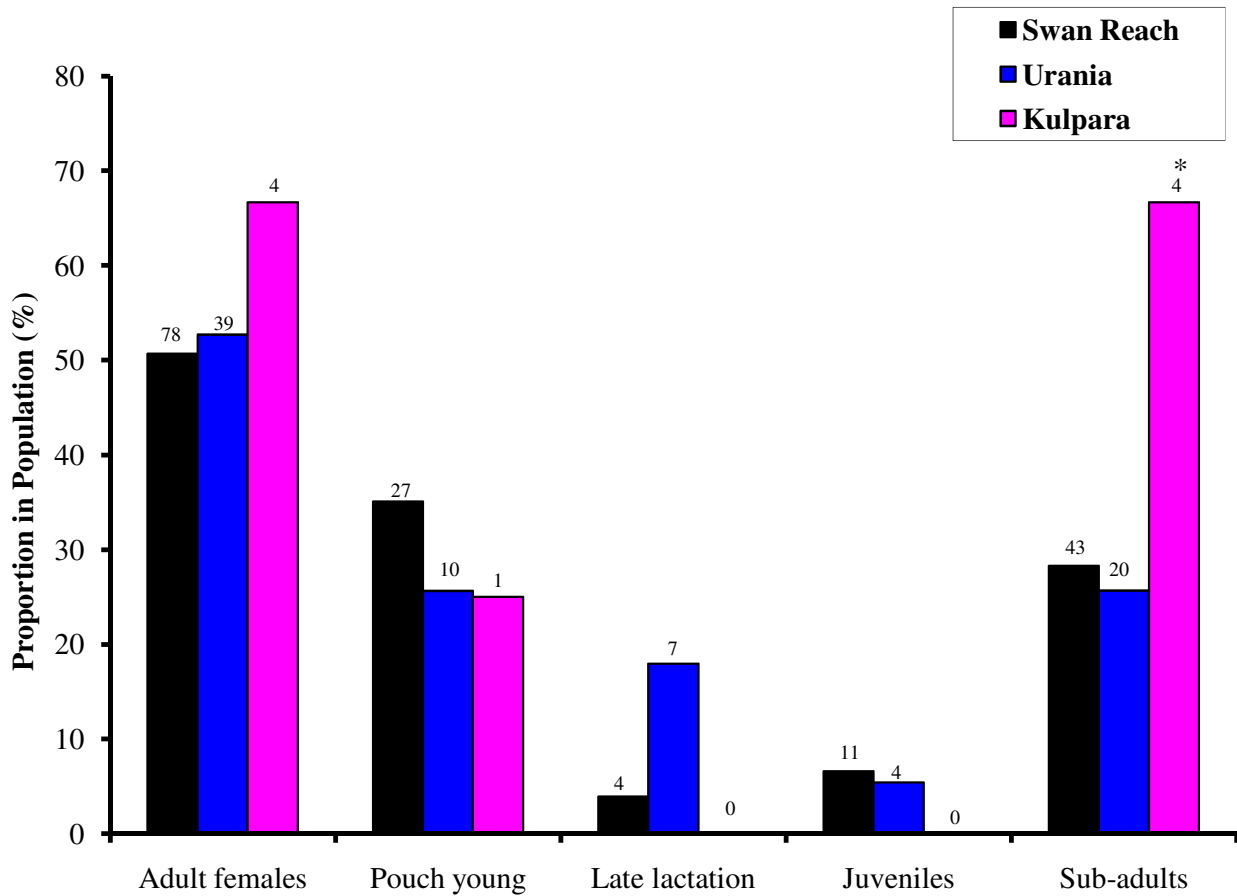


**Figure 31:** Proportion of adult females and those with pouch young, and in late lactation, as well as juveniles and sub-adults of both sexes, recorded in the three populations of southern hairy-nosed wombats examined during (a) the whole study period, (b) the breeding season, and (c) the non-breeding season.

Number of animals observed in each category (N) = indicated above column

* Statistically significant difference (p < 0.05)

## 6.4. Discussion

Successful reproduction is the basis of species survival, therefore fertility of both males and females is extremely important in population fitness and species conservation (Wildt *et al.* 2003). Semen quality, and in particular morphology of the sperm head and tail, has been shown to influence fertility in males, and hence the reproductive success of a population in many mammalian species, including humans, wolves, bulls and felids (Johansson and Rendel 1968; Howard *et al.* 1991; Donnelly *et al.* 1998; Ostermeier *et al.* 2001; Asa *et al.* 2007). Abnormalities in morphology of the sperm tail can reduce sperm velocity, which has been shown to be important in fertilisation success (Gomendio *et al.* 2007). In addition, the position of the acrosome is critical for egg coat penetration and fusion, and the orientation of the mid-piece a key factor in sperm motility (Johnson and Everitt 1980), both of which contribute to overall reproductive success.

Male southern hairy-nosed wombats from Kulpara were observed to have a significantly higher proportion of abnormal sperm morphologies in an average ejaculate, when compared to animals from the Urania and Swan Reach populations (Chapter 5). Hence, it was suggested that the fertility of males in this population may be impaired. Reproductive success in female southern hairy-nosed wombats from all three populations was examined in this chapter to determine if breeding success reflected the proportion of abnormal sperm morphotypes found in ejaculates. No significant differences were seen between Swan Reach, Urania and Kulpara in the proportion of pouch young observed, or the proportion of females in late lactation, at any time of year. However, during the entire sampling period, the wombats from Kulpara had a significantly higher proportion of juvenile animals (18 %), compared to those animals from Urania (4 %) and Swan Reach (8 %). The same result was observed when the populations were compared across the breeding season, with the Kulpara population having a greater proportion of juveniles (20 %), compared to Urania (2 %) and Swan Reach (9 %). During the non-breeding season the Kulpara population also had a significantly higher number of sub-adult animals (67 %), compared to Urania (26 %) and Swan Reach (29 %). Together these data suggest that perhaps younger animals at Kulpara might have better survival rates than those animals at Urania and Swan Reach. Lower juvenile survival rates have been associated with reduced genetic diversity and increased inbreeding (Saccheri *et al.* 1998); however, this does not appear to be the case in the current study. The populations that had a smaller number of juveniles and sub-adults, Urania and Swan Reach, had higher allelic diversity than Kulpara (Table 12), and heterozygosity was similar across the three populations, with no indication of inbreeding. Another variable must therefore be influencing the higher juvenile and sub-adult survivorship found at Kulpara. Food availability is critical to

the survival of young wombats (Wells 1989), and from superficial examination the three southern hairy-nosed wombat populations appear to have different resource availability. The wombats at Kulpara reside predominantly within two small patches of remnant native vegetation, as well as on privately owned land, historically used for grazing, but which currently has only minimal commercial use. Cropping paddocks surround the wombat population, with a few warrens located in these paddocks. The wombats at Swan Reach are located predominantly in heavily grazed paddocks, with little native remnant vegetation. This location also had the lowest rainfall of all three sites over the study period, and a lower average rainfall (Table 8). In addition, whilst data were not recorded on rabbit numbers, qualitative observations throughout the duration of this study suggest that considerably more rabbits occur at the Swan Reach study site than were observed at Urania or Kulpara. As a consequence, increased resource competition is likely. The Urania site has the highest annual rainfall of all three study locations; however the wombats reside in an area that is heavily grazed, with little native vegetation. This suggests that the Kulpara site, where animals have a greater area of remnant native vegetation, less land degradation due to grazing, and reduced competition with livestock and rabbits, probably have greater food availability, contributing to higher survival rates of juvenile and sub-adult wombats. To determine if there is any association between resource availability and survival of young wombats, future studies should examine multiple variables: (1) nutritional status of the animals (scat analysis), (2) flora survey of the area, and (3) assessment of potential resource competition (e.g. rabbits and livestock).

These results also suggest therefore, that the reproductive success of the southern hairy-nosed wombats at Kulpara did not appear to be significantly compromised by higher male sperm abnormalities, with no difference in the percentage of pouch young or late lactation females within the population, and higher numbers of juveniles and sub-adults within the population. This contrasts with data on wolves (Asa *et al.* 2007), and red deer (Gomendio *et al.* 2007), where there was a highly significant association observed between sperm morphology and reproductive success. Both these studies, however, used captive females and artificially inseminated them with sperm to determine specific relationships between sperm morphology and fertilisation success, rather than the indirect method used in this current investigation on wombats. In a wild population of animals, many other factors must be taken into account, such as (1) female choice/male dominance, and (2) female fertility. To some extent, these variables can be controlled for in captive populations. As discussed in Chapter 5 (section 5.4), female choice and male/male competition can play a role in the reproductive success of a population. In many species of marsupials and eutherian

mammals, larger males are more likely to sire a greater proportion of the offspring within the population (Johnson 1989; Fisher and Lara 1999; McElligott *et al.* 2001; Charlton *et al.* 2007; Adrian *et al.* 2008; Asher *et al.* 2008). This result can be either generated through female choice, with females choosing to mate with the larger males, or male/male competition, with the dominant males mating with a greater number of females. In male southern hairy-nosed wombats, results showed that the bigger males (as determined by weight and head width), were more likely to have a larger ejaculate volume and a greater proportion of morphologically normal sperm in the ejaculate (Chapter 4 & 5), thereby possibly increasing seminal quality and the chance of fertilisation. Female southern hairy-nosed wombats are monogamous, and males polygynous (Taggart and Temple-Smith 2008), therefore if the largest males mate with multiple females in small populations such as Kulpara and Urania, reproductive success is still likely to be high, regardless of the increased proportion of sperm abnormalities across the male population. In addition, whilst female southern hairy-nosed wombats are monogamous, it is not known whether they will mate with other males if they do not become pregnant after numerous oestrous cycles.

Male fertility is not the sole contributor to population reproductive success and hence female fertility must also be taken into account. Fertility in females can be compromised due to numerous factors. In populations of wild species, such as water voles (*Arvicola terrestris*), nutritional status can influence female fertility (McEvoy and Robinson 2003), with food deprived female water voles having reduced fecundity, which was believed to be due to the disruption of the hormonal regulation of oestrus (Bazhan *et al.* 1996).

Whilst a greater percentage of sperm abnormalities were present in male southern hairy-nosed wombat ejaculates at Kulpara, the results from this study suggest that at present, the reproductive output has not yet been disadvantaged. Within the Kulpara population, sperm morphology was extremely variable between individual males, suggesting females may be mating with males that had a greater proportion of morphologically normal sperm. Or alternatively, and most likely, the presence of abnormal sperm in an ejaculate does not reduce male fertility and population reproductive success in southern hairy-nosed wombats. Sperm morphology is not the only variable that can effect fertility; problems in sperm metabolic pathways, integrity of proteins on the plasma membrane, regulation of the cellular volume, ability of the sperm to bind to egg coats, and capacitation capability in the female reproductive tract, can all influence the chance of fertilisation (Amann 2005; Petrunkina *et al.* 2007). All these factors make it difficult to determine exactly what influence an increase in morphologically abnormal sperm can have on fertility in male southern hairy-nosed wombats,

and the reproductive success of the population, without further experimentation using captive animals, and possibly artificial insemination, to control these variables discussed above.

In red deer sperm, males with a greater proportion of spermatozoa of normal morphology in their semen were more likely to produce sons, whereas the individuals with a higher proportion of sperm abnormalities were likely to produce female offspring. It was hypothesised that this would decrease the likelihood of male offspring inheriting the high proportion of sperm abnormalities (Gomendio *et al.* 2007). Examination of the male to female sex ratio of juvenile and sub-adult southern hairy-nosed wombats for each population in this study (Appendix 2), revealed that the sex ratio of male to female at Swan Reach was 1 : 1.5, Urania 1 : 1.2, and Kulpara 1 : 1. This result does not appear to correspond with that found in red deer where those males with a greater proportion of normal sperm were more likely to produce male offspring (Gomendio *et al.* 2007). The southern hairy-nosed wombats at Kulpara had a greater number of sperm abnormalities; however, there seems to be no difference in the sex ratio of juvenile and sub-adult wombats within this population, and in fact the wombats from Swan Reach which have the highest proportion of normal sperm produced more female offspring. This suggests that the sex ratio in southern hairy-nosed wombats does not appear to be associated with selection driven by sperm morphology; however, this requires further examination.

During the breeding season, a significantly lower number of adult female southern hairy-nosed wombats were present in the Urania population (33 %), when compared to Swan Reach (58 %). The Kulpara population did not differ significantly from either population, with approximately half the adult wombats in the population being female (48 %). An unequal sex ratio has also been observed in the critically endangered northern hairy-nosed wombat population; however, this is reputedly more extreme than in the southern species, with 2.25 males for every female (Banks *et al.* 2003). The lower number of females in the southern hairy-nosed population at Urania could restrict the population growth potential, as population growth is influenced by the availability of oocytes (i.e. female reproductive potential), rather than the availability of sperm (Wedekind 2002). Restricted population growth can have a detrimental effect on the population by reducing the effective population size, and therefore the number of individuals that can contribute genes to the next generation (Sherwin and Murray 1990; Primack 1998; Frankham *et al.* 2002). A low effective population size can result in a reduction in genetic diversity, thereby increasing the risk of inbreeding depression and ultimately the threat of extinction (Primack 1998; Frankham *et al.* 2002, 2004).

In the agile antechinus (*Antechinus agilis*) habitat fragmentation has been shown to influence the sex ratio of the species. In fragmented colonies a lower number of males were observed compared with those (Banks *et al.* 2005) residing in continuous habitats. In this species males disperse, so it was hypothesised that there was a high degree of mortality associated with dispersal, contributing to the unequal sex ratio (Banks *et al.* 2005). In southern hairy-nosed wombats it has been suggested that adult females disperse (Walker *et al.* 2008b); if similar, and high dispersal mortality occurs in this species, this may also possibly contribute to the lower number of adult females found in the colony at Urania. As colonies on the Yorke Peninsula are separated by large distances, and southern hairy-nosed wombats have no natural predators (excluding humans), some possible reasons for high dispersal mortality might be: (1) an increased risk of culling by farmers, or vehicle accidents, or (2) a lack of shelter (limited number, or absence, of burrows and native vegetation cover), and resource availability (restricted suitable food).

## 6.5. Summary

Male southern hairy-nosed wombats from the Kulpara population, on the Yorke Peninsula, were observed to have a significantly higher number of abnormal sperm morphologies in an average ejaculate when compared to the populations at Swan Reach and Urania. Whilst sperm morphology has been linked to reduced fertility previously, this was not reflected in this study, with no differences in the number of pouch young, or females in late lactation observed between the three populations. However, the number of juvenile and sub-adult wombats captured was higher in the Kulpara population when compared to the Swan Reach and Urania populations, suggesting higher survival rates for young animals, possibly due to greater resource availability.

**Chapter 7: Concluding statement**

**7.1. Introduction**

Land clearance causes fragmentation of remnant habitat, and therefore populations of resident species also become fragmented (Primack 1998). Consequently, animal dispersal is likely to be restricted, resulting in isolation of the population, thereby increasing the chances of inbreeding (Saunders *et al.* 1991; Bowne *et al.* 1999; Berggren *et al.* 2002; Frankham *et al.* 2002; Keller and Waller 2002). Inbreeding is likely to decrease the genetic diversity in a population by increasing the number of homozygous individuals in a population (Falconer 1981; Eldridge *et al.* 1999; Frankham *et al.* 2002; Keller and Waller 2002; Taylor 2003). Many negative implications have been associated with inbreeding; these include an increased rate of embryo, newborn and juvenile mortality (Johansson and Rendel 1968; Keller and Waller 2002; Taylor 2003), reduced fecundity (Packer *et al.* 1991; Ryan *et al.* 2003), reduced seminal quality, and compromised male fertility (Wildt *et al.* 1982; Wildt *et al.* 1987a; Wildt *et al.* 1987b; Cassinello *et al.* 1998; Asa *et al.* 2007; Gomendio *et al.* 2007). As a consequence of these factors, the risk of extinction for species that become fragmented is increased, when compared to those in continuous populations (Johansson and Rendel 1968; O'Brien *et al.* 1985; Keller *et al.* 1994; Frankham 1995; Keller and Waller 2002). Some populations of southern hairy-nosed wombats in South Australia have a limited and fragmented distribution, particularly those on the Yorke Peninsula, where numerous small colonies are geographically isolated. The aim of this study was to examine the impact of habitat fragmentation and population isolation on the genetic diversity, seminal quality and reproductive success of the southern hairy-nosed wombat.

## 7.2. Summary of findings

### 7.2.1. Demography of wombat colonies on the Yorke Peninsula

A total of 696 individual southern hairy-nosed wombats were estimated to reside within 25 remnant colonies on the Yorke Peninsula. Of these colonies, only three consisted of over 100 animals (those at Urania, Point Pearce and Kulpara), with 21 of the colonies estimated to have less than 20 animals residing within the colony. In general, the wombats on the Yorke Peninsula were found to be significantly smaller in body morphological measurements, but yet had larger testes morphology compared to wombats from the large population at Swan Reach in the Murraylands. It was unknown whether these morphological differences observed between the Yorke Peninsula and Murraylands were due to genetic or environmental influences, or a combination of both.

### 7.2.2. Genetic diversity and structure of wombat populations

Microsatellite analyses revealed that the large wombat population at Swan Reach, and the small, and geographically isolated, populations at Urania (including Point Pearce) and Kulpara, had high observed and expected heterozygosity values with no suggestion of inbreeding. Allelic diversity (number of alleles and allelic richness) was highest at Swan Reach and lowest at Kulpara, but no significant differences were observed in any of the measures of genetic diversity (heterozygosity and allelic diversity) between the populations. Migration between populations, a long generation time, and inbreeding avoidance were hypothesised as possible reasons for this finding.

Whilst migration was proposed as a potential cause for the high genetic diversity of the small populations on the Yorke Peninsula, the results from analysis of the genetic structure showed that the wombats from Swan Reach, Urania and Kulpara were all genetically differentiated from one another; this suggests only very minimal or historical migration between the populations.

### 7.2.3. Seminal quality in wombat populations

A significantly lower ejaculate volume was observed in wombats from the Urania population, whereas a greater percentage of abnormal sperm morphologies were seen in ejaculates from Kulpara wombats. The difference in these variables between the populations did not correlate with heterozygosity indices from the respective populations, suggesting no association between heterozygosity and seminal quality in southern hairy-nosed wombats at this time. However, whilst not significant, a trend was observed in the Yorke Peninsula populations, with wombats from these populations having a lower number of normal sperm

head and tail morphologies within ejaculates, reduced sperm motility, reduced sperm concentration, and lower allelic diversity. Significant reductions in seminal quality are associated with reduced fertility of males, and therefore impaired reproductive success within populations (Donnelly *et al.* 1998; Asa *et al.* 2007; Gomendio *et al.* 2007).

*7.2.4. Reproductive success of wombat populations*

Whilst seminal quality was lowest in wombats from Kulpara, this did not appear to influence the reproductive success of the population with no significant differences detected between the three populations in the percentage of females with pouch young, or females in late lactation. There was, however, an unequal sex ratio at Urania, with a significantly lower number of adult females in the population during the breeding season, which can limit the potential for population growth (Wedekind 2002).

A significantly higher number of juvenile and sub-adult wombats were observed at Kulpara, with better available resources within this population proposed as a probable reason for this finding.

**7.3. Implications for the future of southern hairy-nosed wombats on the Yorke Peninsula**

Whilst results from the current study showed high heterozygosity values in the populations of southern hairy-nosed wombats examined on the Yorke Peninsula, the very small colony sizes imply susceptibility to a loss of genetic diversity and probable inbreeding in the future (Frankham *et al.* 2002). In addition to the very small colony size of wombats on the Yorke Peninsula, other findings, such as the geographic isolation of the colonies, lower seminal quality, an unequal sex ratio, and a trend towards lower allelic diversity, suggest that these colonies may be at an increased risk of local extinctions.

As well as the genetic and reproductive variables mentioned above, wombats from small colonies, like those on the Yorke Peninsula, may also be vulnerable to local extinctions as a result of environmental disasters such as fire, drought or the effects of global warming. In addition, the spread of disease can be detrimental to a small population, with infectious diseases such as sarcoptic mange, caused by the pathogenic mite *Sarcoptes scabiei* potentially effecting population numbers, breeding success and sex ratios. Whilst not specifically examined in this study, animals infected with mange were identified from both the Urania and Kulpara populations. Wombats with mange have significantly higher mortality rates than disease free animals (Ruykys *et al.* 2009), therefore further increasing the risk of extinction in small populations.

**7.4. Conservation and management**

To reduce extinction risk in southern hairy-nosed wombat populations on the Yorke Peninsula, a management plan must be implemented in the near future. This plan should address the issues associated with the protection of remnant scrub where the remaining wombats currently reside. Eventually, however, the small sizes of these colonies will likely result in inbreeding. The Urania and Kulpara populations were shown to have high heterozygosity values, whereas the other colonies on the Yorke Peninsula were not examined, so the genetic diversity is unknown at present. To increase the chance of population survival, heterozygosity within the colonies needs to be maintained, or possibly increased, depending on heterozygosity in the unexamined colonies. One possibility to maintain/increase heterozygosity within these populations is to facilitate migration between the populations by increasing connectivity through corridors or stepping stones. Corridors are linear patches of suitable vegetation connecting remnant habitats (Hussey *et al.* 1989; Bennett 1990; Primack 1998), whereas stepping stones are small blocks of hospitable vegetation between remnant patches that decrease the amount of time animals spend in the open (Bennett 1990). Both methods have been shown to increase movement between remnant patches in various animal species (Bennett 1990; Date *et al.* 1991; Haas 1995; Hill 1995; Gilbert *et al.* 1998; Haddad *et al.* 2000; Coffman *et al.* 2001; Tewksbury *et al.* 2002; Varkonyi *et al.* 2003). Both corridors and stepping stones would add positive and negative attributes for wombat colonies on the Yorke Peninsula. Stepping stones may be the most viable solution, as they would require less effort to construct and wombats have been observed to travel through open grazing/cropping paddocks. However, corridors may reduce the chance of wombats encountering humans and vehicles, as well as less exposure to the elements, thereby increasing the chance of survival whilst migrating (Hussey *et al.* 1989). Both the stepping stone and corridor methods have positive effects on farmland, with more vegetation providing shade and shelter for their stock, as well as possibly helping to reduce erosion of top soil. The greatest problem associated with increased connectivity and migration however, is the possibility of increased disease transference, and, in particular, mange.

Another possible management tool is to actively manage the genetic diversity within the isolated southern hairy-nosed wombat populations on the Yorke Peninsula by translocating wombats between populations. This technique of genetic rescue has been effective in the Scandinavian population of grey wolves (*Canis lupis*), where the immigration of one male wolf resulted in the average heterozygosity value for the population going from 0.49 to 0.62, and the population size increasing from only 10 animals to 100, with 10 - 11 breeding packs (Ingvarsson 2002; Vila *et al.* 2003). An increase in genetic diversity following

translocation has also been seen in prairie chickens and adders (*Vipera berusi*) (Westemeier *et al.* 1998; Reynolds *et al.* 1999). As wombats from the Yorke Peninsula show some signs of inbreeding avoidance, the introduction of a new male wombat from a different gene pool may increase the potential for unrelated matings within the population (Ingvarsson 2002), and therefore possibly increase the proportion of females breeding and hence colony size. However, there are some issues that should be considered in the translocation of southern hairy-nosed wombats. Translocated animals must be adapted to the environment in which they are moved so as to avoid outbreeding depression, which can result in reduced juvenile survival and fecundity. The age and condition (e.g. presence of mange, general health) of translocated wombats must also be considered (Tallmon *et al.* 2004). One positive aspect of genetic rescue for wombats, as compared to corridors or stepping stones, is the ability to control the spread of mange and manipulate the sex ratio within the population.

Wildlife managers could consider translocation of southern hairy-nosed wombats from other populations on to the Yorke Peninsula, or the construction of corridors or stepping stones, to maintain/increase the genetic diversity within the smaller, more isolated colonies of wombats on the Yorke Peninsula. These techniques could also be applied to other wombat colonies facing the same problems. Management of the remnant populations in this manner may help ensure that the genetic diversity of the species as a whole does not decline and that male reproductive fitness and overall reproductive success in these small colonies is not impaired, thus giving these wombats the greatest chance possible to survive long term in their existing habitats.

Another issue which must be addressed for the conservation of wombats to be successful on the Yorke Peninsula, and elsewhere, is the attitude of landowners towards this species. Wombats can cause damage to farmland by digging burrows under fences, as well as in cropping paddocks. These excavations often result in damage to expensive farm machinery (i.e. falling into a wombat burrow), as well as costing time and money to landowners. Hence, many landowners have a negative opinion of wombats and would like to see a reduction in numbers, or complete removal, of this species from their properties. Landholders can apply for culling permits in South Australia only if wombat numbers on the property are considered high. Due to the vulnerable status of southern hairy-nosed wombats on the Yorke Peninsula culling permits would not be issued, however non-lethal methods such as translocation, to a more appropriate site, may be an option that appeals to landholders without impacting wombat numbers. Alternatively, the re-vegetation of small patches of habitat specifically for wombat occupancy may also be an option. Observations, from time spent in the field with the current study, suggested that when remnant habitat was available wombats tended to remain

within this area, without venturing too far from the boundaries. Wombats only have a small home-range and will not travel far if food, shelter and potential mates are close by. This idea may be promoted as a compromise between landholders and conservationists, as re-vegetation has positive attributes for both parties. For farmers it will possibly reduce the damage wombats do in their cropping paddocks, as well as increasing available shade for stock and reducing soil erosion. For those working to conserve the species it will potentially promote less illegal culling of wombats, as well as the migration of animals through the stepping stone technique, and is worthy of more focussed research.

## 7.5. Future Studies

Further research needs to be conducted on the genetic diversity and reproductive capacity of the small southern hairy-nosed wombat colonies on the Yorke Peninsula not examined in the current study. This would provide information on their viability and what management tools would be best suited to ensure their survival on a case by case basis. Research into the effects of the proposed management techniques discussed above (i.e. corridors, stepping stones and translocations) should be conducted to determine how they influence wombat behaviour, and also which are the most viable options for the conservation of this species.

Additional studies should also be conducted to further advance our knowledge on the possible implications of small population sizes on southern hairy-nosed wombats. Collecting data from additional populations, such as the largest population of southern hairy-nosed wombats (Nullarbor Plain), would expand these results and provide an excellent basis for comparison. In addition, southern hairy-nosed wombats located on Wedge Island would make a good model population for comparisons on the effects of genetic isolation on this species. This Wedge Island population is completely isolated from other study populations, and was founded from only six individuals (three males and three females) in 1971 (Copley 1994).

Experimental analysis of seminal quality in southern hairy-nosed wombats using artificial insemination could provide insight into the effect of sperm quality on fertilisation potential. In particular, the implications of varying proportions of pleomorphic sperm morphologies in ejaculates, and their effects on fertility could be explained.

Environmental factors should also be studied and considered as potential influences on the seminal quality and reproductive success observed in the Yorke Peninsula population. Future research could examine the chemicals used by landowners on their property, and the potential impact that they may have on seminal quality and reproductive success of southern hairy-nosed wombats that reside on their land. In addition, the nutritional status of wombats (through faecal examination) and water availability, and hydration, of individuals should also be examined to determine any influences these variables may have on the parameters associated with reproductive success.

The current study provided some insight into the genetic diversity, seminal quality and reproductive success of southern hairy-nosed wombats in the Murraylands, and on the Yorke Peninsula. Consideration of conservation measures for this species now, and increased efforts to address gaps in our knowledge of this species, identified in this study, will help ensure the long term survival of this species on the Yorke Peninsula and in other fragmented landscapes.

**Appendices**

**Appendix 1:** Genotypes for each individual southern hairy-nosed wombat at three populations (Kulpara, Swan Reach and Urania) using eight loci

| | | L12 | | Lk34 | | Lkr109 | | Lla54CA | | Lk09 | | Lla16CA | | Lla67CA | | Lla71CA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K1 | KULPARA | 124 | 124 | 213 | 217 | 159 | 173 | 138 | 155 | 161 | 161 | 253 | 263 | 137 | 145 | 169 | 192 |
| K10 | KULPARA | 130 | 130 | 215 | 217 | 159 | 173 | 138 | 151 | 148 | 161 | 255 | 257 | 141 | 151 | 186 | 192 |
| K11 | KULPARA | 124 | 130 | 201 | 217 | 159 | 171 | 138 | 166 | 161 | 163 | 259 | 259 | 141 | 145 | 169 | 186 |
| K12 | KULPARA | 128 | 134 | 201 | 215 | 159 | 173 | 130 | 166 | 161 | 163 | 255 | 259 | 137 | 139 | 186 | 192 |
| K13 | KULPARA | 130 | 134 | 215 | 215 | 173 | 173 | 130 | 138 | 148 | 161 | 255 | 259 | 141 | 141 | 186 | 192 |
| K14 | KULPARA | 128 | 136 | 213 | 215 | 171 | 173 | 130 | 130 | 148 | 163 | 259 | 259 | 139 | 141 | 169 | 192 |
| K15 | KULPARA | 128 | 134 | 215 | 215 | 159 | 171 | 142 | 151 | 148 | 161 | 255 | 259 | 139 | 151 | 186 | 186 |
| K16 | KULPARA | 124 | 126 | 213 | 213 | 159 | 159 | 130 | 130 | 148 | 161 | 255 | 261 | 137 | 141 | 169 | 192 |
| K17 | KULPARA | 126 | 130 | 215 | 215 | 171 | 173 | 130 | 130 | 148 | 161 | 259 | 259 | 141 | 141 | 169 | 186 |
| K18 | KULPARA | 126 | 136 | 213 | 217 | 159 | 171 | 130 | 130 | 161 | 161 | 255 | 259 | 137 | 151 | 186 | 192 |
| K19 | KULPARA | 130 | 134 | 215 | 217 | 171 | 173 | 155 | 166 | 161 | 161 | 257 | 259 | 141 | 141 | 169 | 192 |
| K2 | KULPARA | 128 | 136 | 201 | 213 | 159 | 171 | 130 | 142 | 161 | 161 | 255 | 259 | 135 | 137 | 169 | 169 |
| K21 | KULPARA | 126 | 128 | 201 | 201 | 159 | 159 | 142 | 151 | 148 | 161 | 0 | 0 | 137 | 145 | 186 | 192 |
| K23 | KULPARA | 130 | 134 | 215 | 215 | 159 | 171 | 142 | 166 | 161 | 161 | 255 | 261 | 137 | 139 | 186 | 192 |
| K24 | KULPARA | 124 | 130 | 201 | 215 | 171 | 173 | 130 | 130 | 161 | 161 | 253 | 261 | 141 | 141 | 169 | 192 |
| K25 | KULPARA | 130 | 134 | 215 | 217 | 159 | 171 | 130 | 130 | 161 | 163 | 255 | 259 | 141 | 145 | 186 | 192 |
| K26 | KULPARA | 126 | 126 | 213 | 217 | 159 | 159 | 130 | 166 | 161 | 161 | 255 | 257 | 141 | 151 | 169 | 192 |
| K27 | KULPARA | 130 | 134 | 215 | 217 | 173 | 173 | 138 | 138 | 161 | 161 | 257 | 259 | 141 | 151 | 169 | 192 |
| K28 | KULPARA | 128 | 130 | 213 | 215 | 159 | 173 | 142 | 166 | 148 | 161 | 255 | 259 | 137 | 141 | 169 | 169 |
| K29 | KULPARA | 128 | 134 | 215 | 215 | 159 | 173 | 130 | 142 | 148 | 163 | 255 | 259 | 141 | 151 | 186 | 192 |
| K3 | KULPARA | 126 | 136 | 217 | 217 | 159 | 171 | 130 | 166 | 161 | 161 | 257 | 259 | 139 | 151 | 169 | 186 |
| K30 | KULPARA | 134 | 136 | 201 | 215 | 171 | 173 | 130 | 138 | 161 | 163 | 259 | 259 | 139 | 151 | 169 | 169 |
| K31 | KULPARA | 126 | 128 | 215 | 217 | 159 | 173 | 130 | 166 | 148 | 163 | 255 | 261 | 141 | 145 | 169 | 192 |
| K32 | KULPARA | 124 | 130 | 215 | 215 | 171 | 173 | 166 | 166 | 161 | 161 | 259 | 259 | 141 | 141 | 169 | 192 |
| K34 | KULPARA | 130 | 130 | 201 | 201 | 171 | 171 | 130 | 130 | 148 | 163 | 255 | 257 | 141 | 141 | 188 | 192 |
| K35 | KULPARA | 130 | 134 | 215 | 217 | 171 | 173 | 155 | 166 | 161 | 161 | 257 | 259 | 141 | 141 | 169 | 192 |
| K37 | KULPARA | 134 | 134 | 215 | 215 | 159 | 173 | 142 | 142 | 148 | 161 | 255 | 261 | 137 | 139 | 169 | 186 |
| K38 | KULPARA | 134 | 134 | 213 | 215 | 171 | 173 | 130 | 151 | 161 | 161 | 261 | 261 | 137 | 141 | 169 | 186 |
| K39 | KULPARA | 134 | 136 | 213 | 213 | 159 | 173 | 130 | 138 | 161 | 161 | 255 | 261 | 135 | 141 | 186 | 192 |
| K4 | KULPARA | 130 | 134 | 215 | 215 | 159 | 159 | 142 | 142 | 148 | 161 | 255 | 259 | 137 | 151 | 186 | 186 |
| K42 | KULPARA | 130 | 134 | 201 | 215 | 159 | 171 | 130 | 138 | 161 | 163 | 257 | 257 | 135 | 141 | 192 | 192 |
| K43 | KULPARA | 126 | 126 | 213 | 217 | 159 | 173 | 130 | 142 | 148 | 161 | 255 | 261 | 139 | 141 | 169 | 169 |
| K44 | KULPARA | 126 | 134 | 215 | 215 | 159 | 159 | 155 | 166 | 148 | 161 | 255 | 261 | 141 | 141 | 186 | 188 |
| K45 | KULPARA | 126 | 136 | 201 | 213 | 173 | 173 | 130 | 138 | 148 | 161 | 259 | 261 | 141 | 151 | 169 | 186 |
| K47 | KULPARA | 126 | 134 | 215 | 215 | 159 | 173 | 138 | 155 | 148 | 161 | 255 | 261 | 141 | 141 | 169 | 188 |
| K48 | KULPARA | 134 | 134 | 213 | 215 | 159 | 173 | 138 | 138 | 161 | 161 | 255 | 255 | 135 | 141 | 169 | 192 |
| K49 | KULPARA | 130 | 134 | 201 | 201 | 159 | 173 | 138 | 151 | 161 | 161 | 259 | 259 | 141 | 151 | 169 | 186 |
| K5 | KULPARA | 128 | 128 | 215 | 215 | 171 | 171 | 130 | 138 | 148 | 161 | 257 | 261 | 131 | 137 | 188 | 192 |
| K50 | KULPARA | 124 | 130 | 215 | 217 | 159 | 173 | 130 | 138 | 161 | 161 | 253 | 255 | 135 | 145 | 186 | 192 |
| K53 | KULPARA | 124 | 134 | 215 | 217 | 159 | 159 | 130 | 130 | 161 | 161 | 255 | 259 | 141 | 151 | 169 | 186 |
| K54 | KULPARA | 130 | 134 | 215 | 215 | 159 | 171 | 138 | 155 | 148 | 163 | 257 | 259 | 135 | 137 | 186 | 188 |
| K56 | KULPARA | 134 | 136 | 201 | 201 | 171 | 173 | 130 | 155 | 157 | 161 | 255 | 261 | 141 | 141 | 169 | 192 |
| K59 | KULPARA | 130 | 134 | 215 | 217 | 159 | 173 | 138 | 138 | 161 | 163 | 257 | 259 | 145 | 151 | 186 | 186 |
| K6 | KULPARA | 130 | 130 | 215 | 215 | 171 | 171 | 138 | 166 | 161 | 163 | 259 | 261 | 139 | 141 | 192 | 192 |
| K61 | KULPARA | 128 | 130 | 215 | 217 | 171 | 173 | 130 | 155 | 148 | 163 | 255 | 257 | 139 | 141 | 169 | 192 |
| K62 | KULPARA | 124 | 134 | 201 | 215 | 159 | 171 | 130 | 166 | 148 | 161 | 255 | 257 | 135 | 137 | 169 | 169 |
| K64 | KULPARA | 134 | 134 | 213 | 213 | 159 | 171 | 130 | 151 | 148 | 161 | 259 | 261 | 141 | 141 | 169 | 186 |
| K65 | KULPARA | 126 | 134 | 201 | 215 | 159 | 159 | 130 | 142 | 161 | 161 | 255 | 257 | 135 | 137 | 169 | 192 |
| K66 | KULPARA | 128 | 130 | 201 | 215 | 159 | 159 | 142 | 151 | 148 | 161 | 257 | 259 | 145 | 145 | 169 | 186 |

**Appendix 1:** continued

| K67 | KULPARA | 128 | 134 | 215 | 215 | 171 | 171 | 130 | 151 | 148 | 148 | 255 | 261 | 139 | 151 | 186 | 188 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K69 | KULPARA | 126 | 128 | 201 | 213 | 159 | 173 | 130 | 142 | 148 | 161 | 255 | 259 | 137 | 141 | 169 | 186 |
| K7 | KULPARA | 128 | 130 | 213 | 215 | 159 | 173 | 142 | 166 | 148 | 161 | 255 | 259 | 137 | 141 | 169 | 169 |
| K71 | KULPARA | 130 | 136 | 215 | 217 | 159 | 171 | 130 | 155 | 161 | 161 | 257 | 257 | 135 | 139 | 186 | 192 |
| K72 | KULPARA | 120 | 134 | 213 | 215 | 159 | 171 | 138 | 138 | 148 | 148 | 257 | 259 | 137 | 137 | 169 | 186 |
| K73 | KULPARA | 128 | 130 | 213 | 215 | 159 | 173 | 142 | 166 | 148 | 161 | 255 | 259 | 137 | 141 | 169 | 169 |
| K74 | KULPARA | 128 | 136 | 213 | 215 | 171 | 173 | 130 | 130 | 148 | 163 | 259 | 259 | 139 | 141 | 169 | 192 |
| K75 | KULPARA | 130 | 134 | 215 | 217 | 171 | 173 | 155 | 166 | 161 | 161 | 257 | 259 | 141 | 141 | 169 | 192 |
| K76 | KULPARA | 128 | 134 | 215 | 217 | 159 | 159 | 138 | 155 | 161 | 163 | 255 | 259 | 141 | 145 | 186 | 186 |
| K77 | KULPARA | 124 | 134 | 215 | 217 | 171 | 173 | 138 | 151 | 157 | 161 | 257 | 261 | 135 | 141 | 186 | 192 |
| K78 | KULPARA | 120 | 130 | 213 | 217 | 159 | 159 | 138 | 138 | 148 | 161 | 253 | 257 | 137 | 141 | 192 | 192 |
| K79 | KULPARA | 128 | 136 | 213 | 213 | 173 | 173 | 130 | 166 | 148 | 161 | 259 | 261 | 137 | 141 | 169 | 192 |
| K8 | KULPARA | 120 | 130 | 213 | 215 | 159 | 171 | 138 | 166 | 148 | 161 | 253 | 259 | 137 | 139 | 169 | 192 |
| K81 | KULPARA | 124 | 126 | 215 | 215 | 159 | 173 | 130 | 155 | 148 | 161 | 255 | 255 | 135 | 137 | 186 | 192 |
| K9 | KULPARA | 124 | 130 | 201 | 215 | 159 | 173 | 151 | 151 | 161 | 161 | 255 | 257 | 137 | 141 | 169 | 192 |
| SH1 | URANIA | 0 | 0 | 0 | 0 | 0 | 0 | 138 | 138 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SH2 | URANIA | 126 | 128 | 0 | 0 | 0 | 0 | 138 | 138 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SH3 | URANIA | 126 | 130 | 213 | 217 | 0 | 0 | 138 | 138 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SH4 | URANIA | 120 | 120 | 215 | 215 | 0 | 0 | 144 | 144 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BART | SWAN REACH | 124 | 140 | 209 | 217 | 159 | 159 | 130 | 130 | 159 | 161 | 253 | 259 | 143 | 145 | 184 | 186 |
| SR1 | SWAN REACH | 138 | 140 | 209 | 219 | 159 | 159 | 162 | 170 | 157 | 157 | 257 | 259 | 139 | 145 | 184 | 196 |
| SR10 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 138 | 170 | 157 | 163 | 257 | 259 | 145 | 151 | 184 | 186 |
| SR100 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 159 | 162 | 162 | 157 | 159 | 259 | 259 | 137 | 143 | 184 | 196 |
| SR102 | SWAN REACH | 116 | 138 | 209 | 219 | 159 | 171 | 130 | 170 | 159 | 161 | 257 | 259 | 143 | 143 | 184 | 196 |
| SR103 | SWAN REACH | 138 | 138 | 209 | 217 | 159 | 179 | 151 | 166 | 159 | 159 | 0 | 0 | 145 | 151 | 186 | 196 |
| SR104 | SWAN REACH | 120 | 138 | 219 | 219 | 159 | 171 | 138 | 170 | 157 | 159 | 253 | 259 | 145 | 151 | 196 | 196 |
| SR105 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 171 | 138 | 170 | 157 | 159 | 253 | 259 | 137 | 145 | 186 | 196 |
| SR107 | SWAN REACH | 116 | 116 | 219 | 219 | 159 | 171 | 138 | 138 | 161 | 161 | 257 | 257 | 139 | 143 | 184 | 186 |
| SR108 | SWAN REACH | 138 | 140 | 217 | 219 | 159 | 159 | 130 | 142 | 157 | 157 | 259 | 259 | 139 | 151 | 184 | 188 |
| SR109 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 159 | 138 | 162 | 159 | 163 | 257 | 257 | 149 | 151 | 192 | 196 |
| SR11 | SWAN REACH | 116 | 116 | 217 | 217 | 159 | 179 | 138 | 170 | 157 | 159 | 253 | 259 | 139 | 141 | 184 | 186 |
| SR110 | SWAN REACH | 124 | 138 | 217 | 217 | 171 | 179 | 138 | 151 | 159 | 159 | 259 | 259 | 139 | 145 | 186 | 196 |
| SR111 | SWAN REACH | 140 | 140 | 209 | 217 | 171 | 171 | 142 | 161 | 159 | 161 | 257 | 257 | 139 | 149 | 192 | 196 |
| SR112 | SWAN REACH | 120 | 138 | 209 | 209 | 159 | 159 | 138 | 142 | 157 | 157 | 257 | 257 | 137 | 145 | 192 | 196 |
| SR113 | SWAN REACH | 116 | 138 | 213 | 217 | 159 | 179 | 138 | 162 | 159 | 161 | 259 | 259 | 139 | 151 | 186 | 192 |
| SR114 | SWAN REACH | 124 | 138 | 213 | 217 | 171 | 179 | 138 | 138 | 159 | 161 | 259 | 259 | 139 | 143 | 186 | 196 |
| SR115 | SWAN REACH | 136 | 140 | 213 | 219 | 159 | 179 | 142 | 170 | 159 | 159 | 259 | 259 | 151 | 151 | 192 | 196 |
| SR116 | SWAN REACH | 136 | 138 | 213 | 217 | 159 | 179 | 130 | 138 | 159 | 163 | 253 | 259 | 139 | 151 | 192 | 196 |
| SR117 | SWAN REACH | 138 | 138 | 213 | 217 | 171 | 179 | 166 | 170 | 157 | 159 | 257 | 259 | 139 | 151 | 184 | 196 |
| SR118 | SWAN REACH | 124 | 124 | 213 | 217 | 159 | 179 | 162 | 170 | 159 | 161 | 257 | 259 | 143 | 151 | 196 | 196 |
| SR119 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 159 | 138 | 162 | 159 | 163 | 257 | 257 | 149 | 151 | 192 | 196 |
| SR12 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 179 | 138 | 142 | 157 | 163 | 253 | 259 | 149 | 151 | 186 | 188 |
| SR120 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 173 | 138 | 142 | 157 | 161 | 253 | 253 | 137 | 137 | 184 | 196 |
| SR121 | SWAN REACH | 140 | 142 | 213 | 217 | 159 | 171 | 138 | 162 | 157 | 163 | 253 | 259 | 145 | 149 | 184 | 192 |
| SR122 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 162 | 170 | 157 | 157 | 253 | 257 | 151 | 151 | 196 | 196 |
| SR124 | SWAN REACH | 116 | 138 | 209 | 219 | 159 | 171 | 162 | 162 | 159 | 159 | 257 | 259 | 143 | 145 | 186 | 196 |
| SR125 | SWAN REACH | 138 | 138 | 209 | 217 | 171 | 171 | 138 | 170 | 157 | 161 | 257 | 259 | 145 | 149 | 186 | 196 |
| SR126 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 159 | 138 | 162 | 159 | 163 | 257 | 257 | 149 | 151 | 192 | 196 |
| SR127 | SWAN REACH | 120 | 140 | 217 | 217 | 171 | 179 | 162 | 170 | 157 | 157 | 257 | 259 | 139 | 151 | 184 | 196 |
| SR128 | SWAN REACH | 116 | 138 | 213 | 219 | 159 | 179 | 138 | 170 | 161 | 161 | 257 | 259 | 145 | 149 | 184 | 186 |
| SR129 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 179 | 138 | 170 | 157 | 163 | 257 | 257 | 137 | 151 | 188 | 192 |
| SR13 | SWAN REACH | 138 | 138 | 217 | 219 | 159 | 159 | 130 | 170 | 159 | 159 | 253 | 259 | 139 | 151 | 186 | 196 |
| SR130 | SWAN REACH | 120 | 124 | 213 | 219 | 159 | 179 | 138 | 170 | 157 | 159 | 253 | 259 | 139 | 139 | 192 | 196 |
| SR131 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 179 | 142 | 166 | 157 | 161 | 257 | 259 | 137 | 151 | 186 | 186 |

**Appendix 1:** continued

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR132 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 171 | 170 | 170 | 159 | 161 | 259 | 259 | 137 | 137 | 186 | 196 |
| SR133 | SWAN REACH | 138 | 138 | 213 | 217 | 159 | 159 | 142 | 170 | 159 | 161 | 257 | 259 | 139 | 143 | 186 | 186 |
| SR134 | SWAN REACH | 116 | 116 | 217 | 217 | 159 | 179 | 130 | 138 | 157 | 159 | 257 | 259 | 139 | 139 | 196 | 196 |
| SR135 | SWAN REACH | 138 | 138 | 213 | 217 | 179 | 179 | 170 | 170 | 159 | 163 | 257 | 259 | 149 | 151 | 186 | 186 |
| SR136 | SWAN REACH | 120 | 124 | 213 | 217 | 159 | 159 | 130 | 162 | 159 | 163 | 253 | 259 | 137 | 149 | 186 | 186 |
| SR138 | SWAN REACH | 138 | 138 | 217 | 217 | 171 | 179 | 138 | 142 | 157 | 159 | 253 | 259 | 145 | 151 | 186 | 196 |
| SR139 | SWAN REACH | 116 | 138 | 213 | 217 | 177 | 179 | 161 | 170 | 157 | 157 | 257 | 259 | 143 | 149 | 192 | 192 |
| SR14 | SWAN REACH | 136 | 138 | 217 | 219 | 159 | 159 | 130 | 138 | 157 | 159 | 253 | 257 | 143 | 145 | 186 | 192 |
| SR140 | SWAN REACH | 116 | 138 | 209 | 217 | 171 | 179 | 138 | 170 | 157 | 159 | 257 | 259 | 151 | 151 | 186 | 196 |
| SR141 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 179 | 138 | 162 | 159 | 159 | 253 | 257 | 141 | 151 | 196 | 196 |
| SR142 | SWAN REACH | 138 | 138 | 217 | 219 | 159 | 159 | 130 | 170 | 159 | 159 | 253 | 259 | 139 | 151 | 186 | 196 |
| SR143 | SWAN REACH | 120 | 138 | 213 | 217 | 171 | 179 | 138 | 142 | 159 | 163 | 257 | 259 | 137 | 143 | 186 | 196 |
| SR144 | SWAN REACH | 138 | 140 | 217 | 219 | 173 | 179 | 138 | 138 | 157 | 161 | 257 | 259 | 139 | 143 | 196 | 196 |
| SR145 | SWAN REACH | 120 | 142 | 213 | 213 | 159 | 171 | 170 | 170 | 157 | 159 | 257 | 259 | 139 | 143 | 184 | 196 |
| SR146 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 179 | 138 | 142 | 159 | 161 | 253 | 259 | 145 | 145 | 184 | 196 |
| SR147 | SWAN REACH | 124 | 138 | 217 | 217 | 159 | 179 | 130 | 138 | 157 | 159 | 253 | 253 | 137 | 145 | 184 | 196 |
| SR148 | SWAN REACH | 124 | 138 | 209 | 213 | 171 | 179 | 161 | 162 | 157 | 159 | 257 | 257 | 149 | 151 | 184 | 186 |
| SR149 | SWAN REACH | 138 | 138 | 209 | 217 | 171 | 179 | 138 | 142 | 161 | 163 | 257 | 259 | 139 | 143 | 186 | 196 |
| SR15 | SWAN REACH | 138 | 140 | 213 | 217 | 159 | 179 | 138 | 151 | 157 | 159 | 253 | 257 | 137 | 145 | 196 | 196 |
| SR150 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 159 | 130 | 151 | 159 | 159 | 257 | 259 | 137 | 151 | 196 | 199 |
| SR151 | SWAN REACH | 124 | 140 | 213 | 217 | 159 | 171 | 151 | 170 | 159 | 161 | 257 | 259 | 137 | 143 | 184 | 186 |
| SR152 | SWAN REACH | 140 | 140 | 213 | 217 | 159 | 159 | 138 | 168 | 157 | 159 | 257 | 257 | 139 | 151 | 188 | 196 |
| SR153 | SWAN REACH | 138 | 138 | 217 | 219 | 179 | 179 | 151 | 161 | 157 | 161 | 259 | 267 | 139 | 145 | 196 | 196 |
| SR154 | SWAN REACH | 124 | 140 | 213 | 217 | 171 | 179 | 161 | 170 | 163 | 163 | 257 | 257 | 139 | 145 | 192 | 192 |
| SR155 | SWAN REACH | 124 | 138 | 217 | 217 | 159 | 179 | 138 | 138 | 161 | 161 | 257 | 259 | 139 | 143 | 188 | 196 |
| SR156 | SWAN REACH | 124 | 142 | 209 | 217 | 159 | 179 | 138 | 162 | 157 | 161 | 253 | 257 | 143 | 143 | 186 | 186 |
| SR157 | SWAN REACH | 120 | 138 | 213 | 217 | 159 | 171 | 142 | 157 | 157 | 159 | 257 | 257 | 145 | 151 | 184 | 186 |
| SR158 | SWAN REACH | 116 | 140 | 217 | 217 | 159 | 159 | 166 | 166 | 159 | 163 | 253 | 259 | 139 | 151 | 186 | 186 |
| SR159 | SWAN REACH | 120 | 138 | 217 | 217 | 159 | 171 | 130 | 170 | 157 | 157 | 253 | 257 | 143 | 149 | 184 | 186 |
| SR16 | SWAN REACH | 120 | 140 | 217 | 217 | 179 | 179 | 162 | 170 | 159 | 159 | 259 | 259 | 139 | 139 | 196 | 196 |
| SR160 | SWAN REACH | 140 | 140 | 217 | 219 | 179 | 179 | 138 | 162 | 157 | 161 | 259 | 259 | 145 | 149 | 186 | 196 |
| SR161 | SWAN REACH | 138 | 140 | 213 | 217 | 159 | 179 | 130 | 142 | 161 | 161 | 257 | 259 | 149 | 151 | 184 | 196 |
| SR162 | SWAN REACH | 138 | 140 | 217 | 219 | 159 | 159 | 142 | 170 | 157 | 159 | 257 | 257 | 139 | 143 | 184 | 186 |
| SR163 | SWAN REACH | 138 | 140 | 213 | 217 | 159 | 179 | 138 | 138 | 157 | 161 | 253 | 259 | 139 | 149 | 186 | 196 |
| SR164 | SWAN REACH | 116 | 136 | 217 | 217 | 179 | 179 | 142 | 161 | 159 | 161 | 257 | 259 | 139 | 139 | 186 | 196 |
| SR166 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 138 | 170 | 157 | 163 | 253 | 259 | 149 | 151 | 186 | 186 |
| SR167 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 138 | 151 | 157 | 157 | 259 | 259 | 143 | 145 | 196 | 196 |
| SR168 | SWAN REACH | 124 | 140 | 217 | 217 | 159 | 171 | 142 | 142 | 159 | 161 | 257 | 259 | 145 | 149 | 188 | 196 |
| SR169 | SWAN REACH | 140 | 140 | 217 | 217 | 159 | 179 | 170 | 170 | 157 | 163 | 257 | 259 | 149 | 151 | 186 | 192 |
| SR17 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 159 | 130 | 151 | 159 | 159 | 257 | 259 | 137 | 151 | 196 | 199 |
| SR170 | SWAN REACH | 138 | 140 | 209 | 213 | 171 | 179 | 138 | 170 | 157 | 161 | 253 | 259 | 139 | 145 | 184 | 186 |
| SR171 | SWAN REACH | 138 | 140 | 213 | 219 | 159 | 179 | 138 | 170 | 159 | 161 | 253 | 257 | 151 | 151 | 188 | 196 |
| SR172 | SWAN REACH | 136 | 140 | 213 | 219 | 159 | 179 | 142 | 170 | 159 | 159 | 259 | 259 | 151 | 151 | 192 | 196 |
| SR173 | SWAN REACH | 116 | 138 | 217 | 217 | 171 | 179 | 138 | 142 | 157 | 159 | 257 | 259 | 145 | 145 | 184 | 196 |
| SR174 | SWAN REACH | 138 | 138 | 217 | 219 | 159 | 179 | 138 | 170 | 159 | 159 | 253 | 253 | 145 | 149 | 186 | 196 |
| SR175 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 138 | 170 | 157 | 163 | 253 | 259 | 149 | 151 | 186 | 186 |
| SR176 | SWAN REACH | 116 | 138 | 209 | 217 | 171 | 171 | 138 | 138 | 157 | 163 | 253 | 259 | 139 | 139 | 184 | 186 |
| SR177 | SWAN REACH | 116 | 124 | 209 | 217 | 159 | 179 | 130 | 170 | 157 | 159 | 257 | 257 | 137 | 139 | 196 | 196 |
| SR178 | SWAN REACH | 116 | 120 | 213 | 219 | 159 | 171 | 138 | 142 | 159 | 161 | 253 | 257 | 139 | 139 | 186 | 186 |
| SR179 | SWAN REACH | 120 | 138 | 213 | 217 | 159 | 179 | 130 | 138 | 159 | 163 | 257 | 259 | 139 | 141 | 192 | 196 |
| SR18 | SWAN REACH | 120 | 140 | 213 | 217 | 159 | 159 | 162 | 170 | 159 | 159 | 253 | 253 | 141 | 143 | 188 | 196 |
| SR180 | SWAN REACH | 120 | 140 | 213 | 217 | 159 | 179 | 162 | 170 | 157 | 157 | 257 | 259 | 139 | 143 | 184 | 196 |
| SR181 | SWAN REACH | 136 | 140 | 217 | 217 | 159 | 179 | 138 | 170 | 157 | 161 | 253 | 253 | 139 | 151 | 184 | 186 |
| SR182 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 173 | 138 | 142 | 157 | 161 | 253 | 253 | 137 | 137 | 184 | 196 |

**Appendix 1:** continued

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR183 | SWAN REACH | 120 | 138 | 213 | 217 | 159 | 179 | 153 | 170 | 157 | 163 | 253 | 257 | 141 | 143 | 184 | 186 |
| SR184 | SWAN REACH | 116 | 124 | 213 | 217 | 159 | 171 | 138 | 142 | 157 | 159 | 253 | 257 | 139 | 149 | 188 | 196 |
| SR185 | SWAN REACH | 136 | 140 | 217 | 217 | 159 | 159 | 138 | 170 | 163 | 163 | 257 | 257 | 137 | 149 | 184 | 192 |
| SR186 | SWAN REACH | 138 | 140 | 213 | 217 | 179 | 179 | 138 | 170 | 0 | 0 | 257 | 257 | 141 | 151 | 188 | 196 |
| SR187 | SWAN REACH | 116 | 138 | 213 | 213 | 171 | 179 | 138 | 170 | 157 | 159 | 257 | 257 | 137 | 151 | 196 | 198 |
| SR188 | SWAN REACH | 136 | 138 | 217 | 219 | 159 | 179 | 138 | 138 | 157 | 163 | 253 | 257 | 139 | 149 | 186 | 192 |
| SR189 | SWAN REACH | 120 | 138 | 217 | 217 | 159 | 159 | 142 | 162 | 159 | 163 | 253 | 257 | 143 | 145 | 184 | 186 |
| SR19 | SWAN REACH | 138 | 140 | 209 | 217 | 159 | 171 | 138 | 170 | 159 | 161 | 257 | 257 | 137 | 137 | 184 | 186 |
| SR2 | SWAN REACH | 124 | 140 | 217 | 217 | 159 | 171 | 138 | 162 | 159 | 159 | 257 | 257 | 143 | 143 | 186 | 196 |
| SR20 | SWAN REACH | 124 | 138 | 209 | 217 | 159 | 179 | 138 | 170 | 159 | 161 | 253 | 257 | 137 | 149 | 186 | 196 |
| SR21 | SWAN REACH | 124 | 138 | 217 | 217 | 171 | 179 | 151 | 170 | 157 | 161 | 259 | 259 | 139 | 151 | 196 | 196 |
| SR22 | SWAN REACH | 140 | 140 | 217 | 217 | 171 | 171 | 130 | 162 | 157 | 159 | 253 | 257 | 143 | 145 | 186 | 196 |
| SR23 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 179 | 138 | 138 | 157 | 159 | 253 | 257 | 149 | 151 | 186 | 196 |
| SR24 | SWAN REACH | 138 | 138 | 213 | 217 | 171 | 179 | 151 | 161 | 157 | 161 | 257 | 259 | 137 | 143 | 188 | 196 |
| SR25 | SWAN REACH | 116 | 138 | 217 | 219 | 159 | 179 | 151 | 170 | 157 | 163 | 257 | 257 | 137 | 139 | 184 | 196 |
| SR26 | SWAN REACH | 136 | 140 | 217 | 219 | 159 | 179 | 166 | 170 | 159 | 159 | 257 | 259 | 139 | 151 | 196 | 196 |
| SR27 | SWAN REACH | 138 | 140 | 213 | 217 | 171 | 179 | 142 | 142 | 159 | 159 | 257 | 259 | 137 | 145 | 196 | 196 |
| SR28 | SWAN REACH | 116 | 138 | 209 | 217 | 179 | 179 | 142 | 170 | 159 | 161 | 257 | 257 | 139 | 143 | 196 | 196 |
| SR29 | SWAN REACH | 138 | 138 | 209 | 217 | 159 | 179 | 142 | 166 | 157 | 161 | 257 | 257 | 145 | 151 | 184 | 184 |
| SR3 | SWAN REACH | 124 | 140 | 217 | 217 | 171 | 171 | 130 | 162 | 157 | 159 | 253 | 257 | 143 | 145 | 186 | 196 |
| SR30 | SWAN REACH | 116 | 138 | 213 | 217 | 159 | 179 | 161 | 170 | 157 | 159 | 253 | 253 | 137 | 149 | 184 | 196 |
| SR31 | SWAN REACH | 138 | 138 | 217 | 219 | 159 | 179 | 151 | 162 | 159 | 159 | 259 | 259 | 149 | 151 | 186 | 192 |
| SR32 | SWAN REACH | 120 | 138 | 217 | 217 | 171 | 179 | 138 | 170 | 157 | 159 | 257 | 259 | 137 | 149 | 184 | 196 |
| SR33 | SWAN REACH | 116 | 120 | 217 | 217 | 159 | 159 | 162 | 170 | 159 | 161 | 257 | 259 | 139 | 145 | 184 | 196 |
| SR35 | SWAN REACH | 116 | 138 | 209 | 217 | 159 | 179 | 130 | 170 | 159 | 159 | 253 | 257 | 149 | 151 | 186 | 188 |
| SR37 | SWAN REACH | 124 | 138 | 217 | 217 | 171 | 179 | 151 | 170 | 157 | 161 | 259 | 259 | 139 | 151 | 196 | 196 |
| SR38 | SWAN REACH | 138 | 138 | 209 | 213 | 171 | 171 | 130 | 138 | 161 | 163 | 257 | 257 | 139 | 143 | 186 | 186 |
| SR39 | SWAN REACH | 138 | 138 | 213 | 217 | 159 | 171 | 142 | 142 | 159 | 161 | 257 | 259 | 137 | 139 | 196 | 196 |
| SR4 | SWAN REACH | 120 | 138 | 213 | 217 | 171 | 179 | 142 | 170 | 157 | 161 | 253 | 259 | 145 | 151 | 196 | 196 |
| SR40 | SWAN REACH | 116 | 140 | 213 | 217 | 0 | 0 | 162 | 170 | 159 | 159 | 253 | 257 | 143 | 149 | 184 | 196 |
| SR41 | SWAN REACH | 124 | 140 | 217 | 217 | 179 | 179 | 138 | 162 | 159 | 159 | 257 | 257 | 143 | 143 | 186 | 196 |
| SR42 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 179 | 142 | 162 | 159 | 161 | 257 | 259 | 139 | 141 | 186 | 186 |
| SR43 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 179 | 138 | 170 | 159 | 161 | 257 | 259 | 149 | 149 | 184 | 196 |
| SR44 | SWAN REACH | 138 | 138 | 213 | 219 | 159 | 179 | 138 | 142 | 161 | 163 | 257 | 259 | 149 | 149 | 184 | 196 |
| SR45 | SWAN REACH | 138 | 140 | 217 | 217 | 179 | 179 | 138 | 142 | 157 | 163 | 253 | 259 | 149 | 149 | 186 | 188 |
| SR46 | SWAN REACH | 124 | 138 | 213 | 217 | 159 | 171 | 138 | 142 | 159 | 161 | 259 | 259 | 137 | 151 | 186 | 196 |
| SR47 | SWAN REACH | 116 | 120 | 213 | 217 | 179 | 179 | 142 | 162 | 148 | 161 | 259 | 261 | 137 | 141 | 169 | 192 |
| SR48 | SWAN REACH | 138 | 140 | 213 | 217 | 159 | 179 | 130 | 142 | 148 | 161 | 255 | 255 | 135 | 137 | 186 | 192 |
| SR49 | SWAN REACH | 138 | 138 | 217 | 219 | 171 | 179 | 151 | 162 | 159 | 159 | 253 | 257 | 143 | 149 | 184 | 196 |
| SR5 | SWAN REACH | 138 | 140 | 213 | 213 | 171 | 171 | 161 | 161 | 159 | 161 | 259 | 259 | 151 | 151 | 184 | 199 |
| SR50 | SWAN REACH | 116 | 138 | 209 | 217 | 159 | 159 | 138 | 138 | 159 | 161 | 257 | 259 | 139 | 141 | 186 | 192 |
| SR51 | SWAN REACH | 138 | 138 | 217 | 217 | 159 | 179 | 130 | 142 | 159 | 161 | 257 | 259 | 149 | 149 | 184 | 196 |
| SR52 | SWAN REACH | 120 | 138 | 213 | 217 | 171 | 179 | 153 | 170 | 161 | 163 | 257 | 259 | 149 | 149 | 184 | 196 |
| SR53 | SWAN REACH | 140 | 140 | 217 | 217 | 159 | 159 | 138 | 161 | 157 | 163 | 253 | 259 | 149 | 151 | 186 | 188 |
| SR54 | SWAN REACH | 116 | 116 | 217 | 217 | 159 | 171 | 138 | 170 | 159 | 161 | 259 | 259 | 137 | 151 | 186 | 196 |
| SR55 | SWAN REACH | 140 | 140 | 209 | 217 | 159 | 171 | 138 | 142 | 159 | 163 | 253 | 253 | 139 | 149 | 188 | 196 |
| SR57 | SWAN REACH | 136 | 138 | 217 | 219 | 171 | 179 | 138 | 142 | 157 | 157 | 253 | 257 | 137 | 137 | 184 | 184 |
| SR59 | SWAN REACH | 116 | 138 | 217 | 217 | 171 | 179 | 138 | 161 | 159 | 161 | 257 | 257 | 139 | 149 | 196 | 196 |
| SR6 | SWAN REACH | 138 | 138 | 213 | 217 | 159 | 179 | 166 | 170 | 157 | 159 | 257 | 259 | 139 | 151 | 184 | 196 |
| SR61 | SWAN REACH | 120 | 138 | 213 | 219 | 171 | 171 | 138 | 170 | 157 | 159 | 253 | 257 | 151 | 151 | 184 | 196 |
| SR62 | SWAN REACH | 116 | 136 | 213 | 217 | 171 | 171 | 161 | 170 | 157 | 157 | 253 | 259 | 151 | 151 | 186 | 186 |
| SR63 | SWAN REACH | 116 | 120 | 217 | 217 | 159 | 179 | 138 | 162 | 157 | 159 | 257 | 257 | 143 | 151 | 184 | 184 |
| SR64 | SWAN REACH | 116 | 138 | 213 | 213 | 159 | 179 | 138 | 161 | 157 | 157 | 253 | 257 | 149 | 149 | 184 | 196 |
| SR65 | SWAN REACH | 116 | 138 | 213 | 217 | 159 | 179 | 170 | 170 | 159 | 159 | 257 | 259 | 137 | 139 | 184 | 196 |

**Appendix 1:** continued

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SR66 | SWAN REACH | 116 | 140 | 217 | 217 | 0 | 0 | 0 | 0 | 157 | 157 | 259 | 259 | 139 | 149 | 184 | 188 |
| SR67 | SWAN REACH | 138 | 140 | 209 | 217 | 159 | 171 | 130 | 142 | 159 | 159 | 257 | 259 | 143 | 149 | 186 | 196 |
| SR68 | SWAN REACH | 140 | 140 | 217 | 219 | 179 | 179 | 138 | 162 | 157 | 161 | 259 | 259 | 145 | 149 | 186 | 196 |
| SR69 | SWAN REACH | 138 | 138 | 213 | 217 | 159 | 159 | 142 | 166 | 157 | 157 | 253 | 257 | 141 | 145 | 184 | 196 |
| SR7 | SWAN REACH | 116 | 138 | 213 | 213 | 159 | 179 | 138 | 161 | 157 | 157 | 253 | 257 | 149 | 149 | 184 | 196 |
| SR70 | SWAN REACH | 116 | 116 | 217 | 217 | 159 | 179 | 138 | 170 | 157 | 159 | 253 | 259 | 139 | 141 | 184 | 186 |
| SR71 | SWAN REACH | 116 | 124 | 213 | 217 | 159 | 179 | 138 | 170 | 161 | 163 | 253 | 259 | 149 | 151 | 184 | 192 |
| SR72 | SWAN REACH | 116 | 138 | 213 | 217 | 159 | 179 | 170 | 170 | 159 | 159 | 257 | 259 | 137 | 139 | 184 | 196 |
| SR73 | SWAN REACH | 116 | 142 | 213 | 219 | 159 | 179 | 161 | 170 | 157 | 159 | 257 | 259 | 149 | 149 | 184 | 196 |
| SR76 | SWAN REACH | 116 | 138 | 217 | 219 | 159 | 171 | 142 | 170 | 157 | 159 | 253 | 257 | 139 | 145 | 184 | 186 |
| SR77 | SWAN REACH | 138 | 140 | 217 | 219 | 173 | 179 | 138 | 138 | 157 | 161 | 257 | 259 | 139 | 143 | 196 | 196 |
| SR78 | SWAN REACH | 120 | 138 | 217 | 217 | 159 | 159 | 142 | 142 | 157 | 159 | 257 | 257 | 145 | 151 | 192 | 192 |
| SR79 | SWAN REACH | 116 | 138 | 213 | 217 | 159 | 179 | 159 | 161 | 157 | 159 | 253 | 253 | 137 | 149 | 184 | 196 |
| SR8 | SWAN REACH | 116 | 116 | 209 | 217 | 159 | 159 | 138 | 138 | 157 | 159 | 257 | 259 | 139 | 145 | 196 | 196 |
| SR80 | SWAN REACH | 140 | 140 | 217 | 217 | 179 | 179 | 170 | 170 | 157 | 157 | 253 | 259 | 151 | 151 | 184 | 196 |
| SR81 | SWAN REACH | 116 | 138 | 217 | 217 | 159 | 179 | 138 | 142 | 159 | 159 | 253 | 253 | 151 | 151 | 196 | 196 |
| SR82 | SWAN REACH | 116 | 120 | 217 | 217 | 159 | 179 | 142 | 162 | 159 | 161 | 253 | 253 | 141 | 149 | 184 | 196 |
| SR83 | SWAN REACH | 120 | 124 | 213 | 217 | 159 | 159 | 142 | 162 | 159 | 161 | 257 | 259 | 145 | 145 | 184 | 186 |
| SR84 | SWAN REACH | 138 | 140 | 217 | 217 | 159 | 159 | 130 | 151 | 157 | 159 | 259 | 259 | 151 | 151 | 186 | 199 |
| SR85 | SWAN REACH | 116 | 136 | 209 | 219 | 171 | 179 | 138 | 161 | 157 | 157 | 257 | 257 | 139 | 149 | 186 | 196 |
| SR87 | SWAN REACH | 138 | 140 | 213 | 217 | 159 | 171 | 138 | 170 | 157 | 163 | 253 | 259 | 137 | 151 | 184 | 196 |
| SR88 | SWAN REACH | 140 | 142 | 217 | 217 | 159 | 171 | 162 | 170 | 159 | 159 | 257 | 257 | 143 | 143 | 184 | 196 |
| SR89 | SWAN REACH | 116 | 116 | 213 | 217 | 159 | 179 | 130 | 162 | 157 | 159 | 257 | 259 | 149 | 151 | 186 | 196 |
| SR9 | SWAN REACH | 138 | 138 | 209 | 213 | 159 | 179 | 151 | 161 | 159 | 159 | 257 | 257 | 139 | 143 | 184 | 196 |
| SR90 | SWAN REACH | 116 | 140 | 213 | 217 | 171 | 179 | 162 | 170 | 159 | 159 | 253 | 257 | 143 | 149 | 184 | 196 |
| SR91 | SWAN REACH | 138 | 140 | 213 | 217 | 179 | 179 | 142 | 170 | 159 | 159 | 257 | 259 | 137 | 139 | 196 | 196 |
| SR92 | SWAN REACH | 138 | 142 | 213 | 217 | 179 | 179 | 138 | 170 | 157 | 159 | 257 | 257 | 139 | 149 | 184 | 196 |
| SR94 | SWAN REACH | 124 | 140 | 213 | 217 | 159 | 159 | 138 | 170 | 157 | 157 | 253 | 259 | 137 | 149 | 184 | 196 |
| SR95 | SWAN REACH | 120 | 138 | 217 | 217 | 159 | 159 | 130 | 130 | 157 | 157 | 257 | 259 | 139 | 139 | 184 | 186 |
| SR96 | SWAN REACH | 138 | 140 | 209 | 209 | 159 | 159 | 138 | 162 | 157 | 161 | 253 | 257 | 137 | 143 | 186 | 196 |
| SR97 | SWAN REACH | 140 | 140 | 217 | 217 | 159 | 179 | 142 | 170 | 159 | 161 | 257 | 257 | 139 | 141 | 186 | 186 |
| SR98 | SWAN REACH | 136 | 140 | 217 | 219 | 159 | 179 | 162 | 170 | 157 | 159 | 257 | 259 | 137 | 139 | 186 | 196 |
| Y10 | URANIA | 120 | 134 | 213 | 213 | 173 | 175 | 144 | 144 | 148 | 159 | 259 | 259 | 127 | 127 | 192 | 194 |
| Y12 | URANIA | 120 | 134 | 213 | 213 | 169 | 171 | 144 | 153 | 148 | 163 | 263 | 263 | 127 | 145 | 180 | 186 |
| Y13 | URANIA | 120 | 134 | 213 | 215 | 171 | 173 | 153 | 153 | 148 | 163 | 255 | 255 | 127 | 127 | 186 | 186 |
| Y14 | URANIA | 120 | 124 | 201 | 213 | 159 | 175 | 132 | 168 | 159 | 159 | 259 | 263 | 127 | 145 | 186 | 194 |
| Y15 | URANIA | 120 | 130 | 213 | 215 | 159 | 175 | 138 | 153 | 148 | 159 | 263 | 263 | 121 | 121 | 180 | 186 |
| Y16 | URANIA | 120 | 130 | 213 | 217 | 171 | 175 | 138 | 144 | 148 | 148 | 253 | 259 | 145 | 145 | 180 | 186 |
| Y17 | URANIA | 124 | 128 | 213 | 215 | 171 | 171 | 144 | 168 | 161 | 161 | 255 | 259 | 145 | 145 | 192 | 192 |
| Y18 | URANIA | 130 | 130 | 213 | 215 | 169 | 175 | 138 | 144 | 163 | 163 | 255 | 263 | 121 | 135 | 186 | 186 |
| Y19 | URANIA | 120 | 120 | 213 | 215 | 159 | 171 | 153 | 168 | 159 | 161 | 255 | 263 | 121 | 127 | 180 | 192 |
| Y2 | URANIA | 130 | 130 | 213 | 213 | 173 | 175 | 144 | 168 | 159 | 163 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y20 | URANIA | 128 | 134 | 201 | 217 | 169 | 171 | 144 | 162 | 148 | 159 | 259 | 263 | 145 | 145 | 186 | 194 |
| Y21 | URANIA | 120 | 128 | 213 | 215 | 159 | 171 | 153 | 153 | 148 | 159 | 259 | 263 | 121 | 145 | 186 | 190 |
| Y22 | URANIA | 128 | 134 | 213 | 215 | 169 | 173 | 132 | 162 | 159 | 163 | 259 | 259 | 121 | 121 | 190 | 194 |
| Y23 | URANIA | 126 | 134 | 213 | 213 | 159 | 169 | 144 | 168 | 159 | 159 | 253 | 255 | 127 | 145 | 186 | 186 |
| Y24 | URANIA | 128 | 134 | 213 | 215 | 173 | 173 | 138 | 144 | 148 | 163 | 259 | 263 | 127 | 127 | 186 | 186 |
| Y25 | URANIA | 130 | 130 | 213 | 213 | 173 | 175 | 144 | 168 | 159 | 163 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y26 | URANIA | 120 | 126 | 213 | 213 | 171 | 173 | 138 | 168 | 148 | 159 | 263 | 263 | 121 | 121 | 186 | 190 |
| Y27 | URANIA | 128 | 134 | 215 | 215 | 159 | 159 | 132 | 153 | 148 | 163 | 263 | 263 | 127 | 145 | 180 | 192 |
| Y28 | URANIA | 120 | 120 | 213 | 213 | 159 | 173 | 132 | 153 | 148 | 148 | 255 | 259 | 121 | 127 | 186 | 190 |
| Y29 | URANIA | 120 | 130 | 215 | 215 | 173 | 175 | 144 | 162 | 159 | 163 | 267 | 267 | 121 | 127 | 186 | 186 |
| Y3 | URANIA | 120 | 120 | 217 | 217 | 159 | 175 | 144 | 153 | 159 | 159 | 253 | 259 | 127 | 137 | 186 | 194 |
| Y30 | URANIA | 126 | 128 | 213 | 215 | 159 | 175 | 138 | 153 | 148 | 159 | 255 | 259 | 121 | 121 | 180 | 186 |

**Appendix 1:** continued

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| Y31 | URANIA | 120 | 128 | 213 | 213 | 159 | 175 | 153 | 153 | 148 | 148 | 259 | 267 | 121 | 145 | 186 | 190 |
| Y32 | URANIA | 120 | 124 | 213 | 213 | 171 | 173 | 168 | 168 | 159 | 159 | 259 | 263 | 127 | 145 | 186 | 190 |
| Y33 | URANIA | 120 | 120 | 213 | 217 | 159 | 173 | 162 | 168 | 159 | 159 | 255 | 263 | 127 | 127 | 186 | 194 |
| Y34 | URANIA | 130 | 134 | 213 | 215 | 171 | 173 | 132 | 168 | 159 | 159 | 259 | 259 | 121 | 145 | 186 | 190 |
| Y35 | URANIA | 128 | 130 | 213 | 213 | 171 | 175 | 138 | 144 | 159 | 161 | 255 | 267 | 121 | 145 | 186 | 192 |
| Y36 | URANIA | 120 | 120 | 217 | 217 | 169 | 171 | 138 | 144 | 161 | 163 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y37 | URANIA | 128 | 134 | 215 | 217 | 175 | 175 | 138 | 138 | 148 | 148 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y38 | URANIA | 120 | 126 | 213 | 213 | 169 | 175 | 138 | 153 | 148 | 163 | 259 | 259 | 121 | 121 | 194 | 194 |
| Y39 | URANIA | 120 | 128 | 215 | 217 | 171 | 175 | 144 | 168 | 148 | 159 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y4 | URANIA | 128 | 134 | 201 | 201 | 159 | 159 | 144 | 153 | 159 | 159 | 259 | 267 | 127 | 145 | 186 | 194 |
| Y40 | URANIA | 128 | 134 | 215 | 217 | 171 | 175 | 138 | 144 | 148 | 148 | 259 | 259 | 121 | 135 | 186 | 186 |
| Y41 | URANIA | 120 | 126 | 215 | 215 | 159 | 169 | 138 | 144 | 148 | 159 | 257 | 259 | 121 | 121 | 180 | 192 |
| Y42 | URANIA | 126 | 128 | 217 | 217 | 171 | 175 | 144 | 144 | 163 | 163 | 255 | 259 | 121 | 135 | 186 | 192 |
| Y43 | URANIA | 134 | 134 | 213 | 215 | 159 | 173 | 138 | 153 | 161 | 163 | 259 | 259 | 121 | 137 | 186 | 192 |
| Y44 | URANIA | 130 | 134 | 215 | 217 | 175 | 175 | 138 | 144 | 159 | 163 | 255 | 259 | 121 | 121 | 186 | 192 |
| Y45 | URANIA | 120 | 134 | 213 | 217 | 173 | 175 | 138 | 153 | 159 | 159 | 259 | 259 | 121 | 145 | 180 | 186 |
| Y46 | URANIA | 126 | 130 | 213 | 217 | 159 | 171 | 144 | 168 | 148 | 159 | 259 | 267 | 121 | 139 | 192 | 194 |
| Y47 | URANIA | 120 | 134 | 215 | 217 | 159 | 171 | 138 | 138 | 159 | 161 | 255 | 259 | 121 | 127 | 180 | 186 |
| Y48 | URANIA | 120 | 120 | 201 | 215 | 171 | 173 | 138 | 168 | 159 | 163 | 255 | 267 | 121 | 121 | 180 | 186 |
| Y49 | URANIA | 126 | 128 | 213 | 213 | 171 | 171 | 144 | 153 | 161 | 163 | 259 | 259 | 121 | 121 | 180 | 190 |
| Y5 | URANIA | 120 | 134 | 215 | 215 | 173 | 175 | 153 | 168 | 159 | 163 | 259 | 263 | 121 | 127 | 180 | 186 |
| Y50 | URANIA | 128 | 130 | 215 | 217 | 171 | 175 | 138 | 138 | 148 | 163 | 259 | 259 | 135 | 139 | 186 | 186 |
| Y51 | URANIA | 130 | 134 | 213 | 215 | 173 | 175 | 138 | 138 | 148 | 163 | 259 | 259 | 121 | 135 | 186 | 186 |
| Y52 | URANIA | 120 | 128 | 215 | 215 | 159 | 175 | 138 | 168 | 148 | 159 | 255 | 259 | 121 | 121 | 180 | 192 |
| Y53 | URANIA | 134 | 134 | 213 | 215 | 159 | 173 | 153 | 153 | 148 | 163 | 255 | 259 | 121 | 127 | 180 | 192 |
| Y54 | URANIA | 120 | 134 | 213 | 215 | 173 | 175 | 138 | 138 | 159 | 159 | 255 | 263 | 121 | 127 | 190 | 192 |
| Y55 | URANIA | 120 | 130 | 201 | 213 | 159 | 159 | 153 | 168 | 148 | 159 | 255 | 259 | 127 | 127 | 194 | 194 |
| Y56 | URANIA | 126 | 130 | 213 | 215 | 171 | 175 | 138 | 144 | 159 | 163 | 255 | 259 | 127 | 127 | 186 | 194 |
| Y57 | URANIA | 120 | 134 | 213 | 217 | 159 | 171 | 138 | 144 | 159 | 159 | 259 | 259 | 127 | 145 | 180 | 190 |
| Y58 | URANIA | 120 | 128 | 213 | 215 | 169 | 169 | 138 | 162 | 148 | 159 | 259 | 259 | 121 | 121 | 180 | 194 |
| Y59 | URANIA | 126 | 134 | 215 | 215 | 169 | 169 | 138 | 138 | 148 | 159 | 259 | 267 | 127 | 137 | 180 | 190 |
| Y6 | URANIA | 134 | 134 | 201 | 213 | 169 | 171 | 168 | 168 | 159 | 161 | 255 | 263 | 145 | 145 | 192 | 194 |
| Y60 | URANIA | 130 | 130 | 213 | 213 | 173 | 175 | 144 | 168 | 159 | 163 | 259 | 259 | 121 | 127 | 180 | 186 |
| Y61 | URANIA | 120 | 130 | 213 | 217 | 159 | 173 | 153 | 168 | 159 | 159 | 253 | 259 | 127 | 127 | 186 | 194 |
| Y62 | URANIA | 130 | 130 | 201 | 213 | 159 | 173 | 153 | 153 | 148 | 148 | 255 | 259 | 127 | 127 | 194 | 194 |
| Y63 | URANIA | 120 | 134 | 201 | 213 | 169 | 171 | 132 | 153 | 159 | 159 | 263 | 263 | 145 | 145 | 190 | 194 |
| Y64 | URANIA | 126 | 134 | 213 | 213 | 171 | 173 | 144 | 153 | 148 | 163 | 259 | 267 | 127 | 127 | 186 | 192 |
| Y65 | URANIA | 134 | 136 | 213 | 215 | 175 | 175 | 138 | 153 | 148 | 163 | 255 | 259 | 121 | 145 | 186 | 190 |
| Y66 | URANIA | 130 | 134 | 201 | 215 | 159 | 173 | 138 | 144 | 148 | 159 | 255 | 267 | 127 | 145 | 186 | 186 |
| Y67 | URANIA | 126 | 134 | 213 | 213 | 171 | 173 | 144 | 153 | 148 | 163 | 259 | 267 | 127 | 127 | 186 | 192 |
| Y68 | URANIA | 128 | 134 | 213 | 213 | 159 | 169 | 153 | 153 | 148 | 148 | 255 | 263 | 127 | 137 | 186 | 186 |
| Y69 | URANIA | 126 | 130 | 201 | 213 | 159 | 169 | 144 | 153 | 159 | 163 | 263 | 263 | 121 | 127 | 190 | 192 |
| Y7 | URANIA | 120 | 126 | 213 | 213 | 159 | 171 | 138 | 144 | 148 | 159 | 259 | 263 | 121 | 127 | 190 | 194 |
| Y70 | URANIA | 126 | 134 | 213 | 213 | 173 | 175 | 138 | 168 | 159 | 159 | 263 | 263 | 121 | 137 | 180 | 190 |
| Y71 | URANIA | 120 | 128 | 213 | 213 | 169 | 175 | 138 | 153 | 159 | 159 | 259 | 263 | 127 | 127 | 186 | 190 |
| Y72 | URANIA | 120 | 120 | 217 | 217 | 159 | 175 | 144 | 153 | 159 | 159 | 253 | 259 | 127 | 137 | 186 | 194 |
| Y73 | URANIA | 120 | 124 | 201 | 213 | 159 | 175 | 132 | 168 | 159 | 159 | 259 | 263 | 127 | 145 | 186 | 194 |
| Y74 | URANIA | 120 | 126 | 215 | 217 | 171 | 173 | 144 | 168 | 159 | 159 | 253 | 259 | 121 | 121 | 186 | 194 |
| Y75 | URANIA | 124 | 128 | 213 | 215 | 169 | 171 | 153 | 168 | 161 | 161 | 257 | 259 | 135 | 135 | 192 | 192 |
| Y76 | URANIA | 120 | 126 | 213 | 213 | 169 | 171 | 153 | 153 | 148 | 148 | 255 | 259 | 121 | 145 | 186 | 186 |
| Y77 | URANIA | 120 | 134 | 213 | 215 | 171 | 171 | 153 | 168 | 148 | 148 | 259 | 263 | 121 | 127 | 186 | 186 |
| Y78 | URANIA | 120 | 124 | 213 | 215 | 171 | 173 | 132 | 151 | 148 | 161 | 259 | 261 | 121 | 145 | 186 | 192 |
| Y79 | URANIA | 120 | 134 | 213 | 213 | 173 | 175 | 144 | 144 | 148 | 159 | 259 | 259 | 127 | 127 | 192 | 194 |
| Y8 | URANIA | 120 | 138 | 213 | 217 | 175 | 175 | 144 | 144 | 148 | 148 | 253 | 267 | 127 | 145 | 186 | 186 |

**Appendix 1:** continued

| Y80 | URANIA | 130 | 130 | 213 | 213 | 173 | 175 | 144 | 144 | 148 | 148 | 259 | 267 | 127 | 145 | 180 | 186 |
| Y81 | URANIA | 120 | 134 | 213 | 215 | 169 | 171 | 144 | 153 | 159 | 159 | 259 | 259 | 121 | 127 | 190 | 190 |
| Y82 | URANIA | 120 | 126 | 201 | 213 | 169 | 173 | 153 | 153 | 148 | 163 | 263 | 263 | 121 | 127 | 192 | 192 |
| Y83 | URANIA | 120 | 128 | 201 | 213 | 159 | 159 | 153 | 153 | 159 | 159 | 259 | 259 | 127 | 127 | 190 | 194 |
| Y85 | URANIA | 120 | 128 | 201 | 213 | 159 | 171 | 138 | 168 | 148 | 159 | 259 | 263 | 121 | 127 | 190 | 194 |
| Y86 | URANIA | 120 | 134 | 201 | 215 | 159 | 173 | 132 | 144 | 159 | 161 | 259 | 263 | 121 | 145 | 180 | 194 |
| Y87 | URANIA | 126 | 134 | 201 | 213 | 159 | 169 | 153 | 168 | 159 | 159 | 253 | 263 | 127 | 145 | 186 | 194 |
| Y88 | URANIA | 126 | 134 | 213 | 215 | 173 | 175 | 144 | 144 | 159 | 163 | 259 | 259 | 121 | 127 | 186 | 186 |
| Y89 | URANIA | 120 | 134 | 213 | 213 | 171 | 175 | 132 | 138 | 148 | 159 | 259 | 263 | 127 | 145 | 186 | 190 |
| Y9 | URANIA | 120 | 128 | 213 | 213 | 171 | 171 | 153 | 153 | 148 | 148 | 263 | 263 | 145 | 145 | 186 | 186 |

**Appendix 2:** Number of sub-adult and juvenile southern hairy-nosed wombats caught at the three populations examined

| Number of wombats caught | Swan Reach | Urania | Kulpara |
|---|---|---|---|
| Sub-adult:<br>Female<br>Male | 19<br>11 | 5<br>5 | 8<br>12 |
| Juvenile:<br>Female<br>Male | 6<br>6 | 1<br>0 | 12<br>8 |

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