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Sex allocation, terminal investment and the effects of environmental constraints on maternal investment in subtropical antechinus

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Cover photo: Female subtropical antechinus named Olive, born in 2012, hand-reared by Daniela Parra-Faundes and released back to the wild on mid-January 2013. This picture was taken three weeks after she was released.

Photo: Daniela Parra-Faundes

Abstract

This thesis addresses questions on sex allocation, life history strategies and costs of reproduction using experimental manipulations of litter sex ratios and field data on ecology and behaviour of the subtropical antechinus (*Antechinus subtropicus*).

In chapter 2, I investigate two major adaptive hypotheses to explain sex ratio bias at birth: the Trivers-Willard Hypothesis (TWH) and the Local Resource Competition Hypothesis (LRCH). I show that sons are more costly to produce than daughters because they have fast growth rates and there are greater survival costs to mothers when they wean more sons. Mothers that naturally produced male-biased litters were slightly heavier than mothers that gave birth to female-biased litters. These results are consistent with the TWH, which states that mothers with more resources to invest benefit by producing high quality competitive sons that will reproduce. However, after increasing the natural bias of litter sex ratios, mothers were able to increase investment to meet demands of rearing more sons than they had naturally produced, without compromising offspring growth. These results are inconsistent with a key prediction of the TWH, that females give birth to the number of sons that they can afford to raise. Also inconsistent with the TWH, male-biased litters grew more quickly after the sex ratio manipulation and, were more likely to survive to weaning. The LRCH predicts that mothers in poor condition should reduce competition from the sex that competes the most, by allocating more to sons than to daughters, as females often remain in their natal home range after weaning. In support of the LRCH, large litter size was associated with slower growth rate in daughters, but not sons. These results differ from previous cross fostering manipulations to test sex allocation in mammals, which have unequivocally supported the TWH.

In chapter 3 I examine changes in reproductive performance and survival with age in females. Senescence and terminal investment are two major models to understand effects of age on reproduction. Reproductive investment and success declines with age if senescence occurs, and investment in young increases near the end of life if terminal investment occurs, improving offspring performance at a cost to mothers. I show that older subtropical antechinuses females are not reproductively senescent. On the contrary, females had a greater investment ability and an overall improvement in reproductive performance with age. Older mothers increased investment in their second litters, and were able to produce high quality, large, fast growing offspring that were also more likely

to survive that the offspring from younger females. However, this greater maternal reduced their own survival. These results provide support for the terminal investment hypothesis (Cockburn 1994, Fisher & Blomberg 2011). Consistent costs of reproduction and terminal investment have often been difficult to demonstrate in wild mammals. My results confirm that marsupials are excellent models to address questions in life history evolution.

In chapter 4 I investigate how rainfall patterns in relation to the reproductive cycle affects demography. I find that reproductive success of subtropical antechinuses is extremely sensitive to changes in environmental conditions, and that both the timing and magnitude of drought are important and may cause severe declines in the whole population. I show that growth, survival and body condition of individuals of this species are driven by rainfall, especially during lactation and weaning. During this study, the pattern of rainfall varied caused by strong effects of La Niña during 2010 and 2011, that ended 14 years of drought. Rainfall peaks varied in relation to the different stages of the reproductive season (mating, pregnancy, lactation and weaning) of subtropical antechinuses at Springbrook National Park.

Overall, low rainfall during lactation reduced maternal condition and investment abilities that were evident by their reduced body mass, offspring growth, weaning success and survival of both mothers and offspring. Younger mothers and male offspring were most affected. In contrast, high rainfall throughout lactation increased maternal investment abilities as they were able to produce high quality, large offspring that were more likely to survive and breed. These high quality offspring also showed a greater investment ability themselves, by producing large, fast growing offspring, suggesting that the resulting increase in quality persisted throughout their lives. Younger mothers were the most favoured by good environmental conditions during lactation as their survival was greatly increased. However, survival of offspring as independent juveniles relies heavily on the predictability and abundance of rainfall during summer at weaning time. Low rainfall at the time when juveniles start to fend for themselves drastically reduced their survival, even if there was high rainfall during lactation. I conclude that growth and survival are determined by fluctuating environmental conditions in this species, in addition to sex allocation and maternal investment which depend on resource availability.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Contributor	Statement of contribution
Daniela Parra Faundes (Candidate)	Wrote the paper (70%)
Diana Fisher	Wrote and edited paper (30%)

Contributions by others to the thesis

Diana Fisher assisted in developing the ideas for this thesis and obtained research funds to support this project. Diana Fisher and Anne Goldizen contributed to discuss results and both helped editing the manuscripts. Simon Blomberg provided statistical advice to analyse data. The candidate performed all field work and data collection, performed the statistical analyses and wrote all the manuscripts. Chapters 2 to 4 were written as papers for publication in peer-reviewed scientific journals, but they have not been sent yet. All co-authors consented to the manuscripts being included in this thesis. Additional contributions can be found in more detail in the acknowledgements section.

Chapter 1: General introduction.

The candidate wrote the manuscript. Diana Fisher and Anne Goldizen commented and help editing the written drafts of the chapter.

Chapter 2: An experimental test of the Trivers Willard and Local Resource Competition hypotheses using three generations of cross fostering in the subtropical antechinus.

Daniela Parra-Faundes (candidate) contributed to the experimental design (70%), performed field work and data collection (100%), wrote the manuscript (100%), performed the statistical analyses (100%), edited the paper (20%). Anne Goldizen helped discussing and interpreting results and edited the manuscript (30%). Diana Fisher provide the funds ti support the project (100%), contributed to the experimental design (30%), helped discussing and interpreting results and edited the manuscripts (50%).

Chapter 3: Age-specific reproductive trade-offs in female subtropical antechinuses.

Daniela Parra-Faundes (candidate) contributed to the experimental design (70%), performed field work and data collection (100%), wrote the manuscript (100%), performed the statistical analyses (100%), edited the paper (20%). Anne Goldizen helped discussing and interpreting results and edited the manuscript (30%). Diana Fisher provide the funds ti support the project (100%), contributed to the experimental design (30%), helped discussing and interpreting results and edited the manuscripts (50%).

Chapter 4: Environmental constraints and their effects on maternal investment in subtropical antechinuses.

Daniela Parra-Faundes (candidate) contributed to the experimental design (70%), performed field work and data collection (100%), wrote the manuscript (100%), performed the statistical analyses (100%), edited the paper (20%). Anne Goldizen helped discussing and interpreting results and edited the manuscript (30%). Diana Fisher provide the funds ti support the project (100%), contributed to the experimental design (30%), helped discussing and interpreting results and edited the manuscripts (50%).

Chapter 5: General Discussion.

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None

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environment and realize that the damage we are causing to the entire ecosystem it would be our doom.

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Thank you.

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Keywords

Antechinus subtropicus, sex allocation, maternal investment, offspring growth, sex ratio, terminal investment, senescence, cross-fostering, climate change.

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List of abbreviation

GLM	Generalized linear model
GLMER	Generalized linear mixed effect model
IUCN	International Union for Conservation of Nature
LMER	Linear mixed effect model
LRCH	Local resource competition hypothesis
TWH	Trivers-Willard hypothesis.

Chapter 1

General Introduction

General Introduction

1. Trade-offs between growth and survival during lactation in mammals

Reproduction is the most demanding task for a female mammal, because energy expenditure reaches a peak during lactation (Loudon & Racey 1987, Bronson 1989, Speakman 2008). Consequently, its success depends on access to enough resources to sustain these high demands, that increase with litter size (Gittleman & Thompson 1988, Kenagy et al. 1989, Fletcher et al. 2013). During this phase, females usually increase their food intake substantially (Randolph et al. 1977, Kenagy et al. 1989, Koenig et al. 1997, Degen et al. 2002). However, there seems to be a physiological limit that prevents mothers from increasing their food intake past a certain limit (Kenagy et al. 1990, Hammond & Diamond 1992, Speakman 2008). Milk production is limited by the capacity of the mammary glands (Speakman 2008). Therefore, pronounced weight loss of mothers during lactation is common as maternal energy stores are converted to milk (Millar 1978, Loudon & Racey 1987, Green et al. 1991, Fisher & Blomberg 2011). In polytocous mammals, the amount of mass loss is generally related to litter size (Kenagy et al. 1990, Sikes 1995, Millesi et al. 1999, Kunkele 2000).

The environment that the mother provides to her offspring modulates their development and responses of the offspring to this are known as maternal effects (Maestripieri & Mateo 2009). Mothers in good body condition can transfer more milk to their offspring and therefore produce larger offspring at weaning (Bernardo 1996, Wauters et al. 1993, Andersen et al. 2000, Ylönen et al. 2004). Milk transfer depends primarily on food availability, the success with which the female has acquired and stored resources, and how efficiently she transfers resources to her offspring (Boydi & McCannt 1989, Kenagy et al. 1990, Clutton-Brock & Godfray 1991, Fairbanks & McGuire 1995). Therefore, reduced food availability and increased competition or interference from group members and/or litter-mates lead to juvenile mortality, low growth rates, delayed maturity and reduced reproductive success (McClure 1987, Festa-Bianchet 1988, Fairbanks & McGuire 1995, McMahon et al. 2000). For example, in marmots and baboons, increased maternal body condition, experience, and dominance rank have positive effects on offspring survival (Allainé 2000, King & Allainé 2002 Altmann & Albert 2005).

Young that are heavier at weaning are more likely to survive and to reproduce successfully than lighter ones (Wauters et al. 1993, Lenihan & Van Viuren 1996, Millesi et al. 1999, Lindström 1999, McMahon et al. 2000, Festa-Bianchet et al. 2000, Clutton-Brock et al. 2001, Bautista et al. 2005, Rödel et al. 2008a,b). Offspring growth in mammals during lactation depends on maternal provisioning, including lactating performance, and other environmental factors such as litter size, as each young's share of milk is reduced with increasing number of littermates (Rödel et al. 2008a, Rutkowska et al. 2011). Sibling competition, either by interference or scramble competition, seems to be common among littermates, and to increase with litter size (Hudson & Trillmich 2008). Under competition, larger/heavier offspring would have a clear advantage over the smaller/lighter ones as they would have improved motor abilities and increased efficiency in energy assimilation (milk conversion into body mass) and be better at maintaining their body temperature (Bautista et al. 2005, Rödel et al. 2008a). For example, in his experimental research with bank voles (*Myodes glareolus*), Koskela (1998) showed that pups from enlarged litters were smaller at weaning than those from smaller litters, and that this effect persisted until the reproductive season. Females that were heavier in winter had a higher probability of reproducing in the next spring. In another study, litter enlargements did not increase the number of weanlings, but significantly decreased weanling's weight and reduced survival and fecundity of mothers (Koivula et al. 2003), showing a high cost of reproduction. In domestic rats and rabbits, pups are smaller when growing in large litters, regardless of maternal size and growth rates (Rödel et al. 2008b). In Antechinuses, low food availability causes slow offspring growth, and offspring that grow more slowly have lower survival (Dickman 1989, Cockburn 1994, Fisher & Cockburn 2006, Parrott et al. 2007, Fisher & Blomberg 2011).

In antechinuses, the energy investment that mothers make during gestation is small, as in all marsupials, since neonates are born at a very early stage of development weighing only ~0.016 g at birth (Marlow 1961). However, investment during lactation is very high, especially during the last month (the final third of the lactation period) (Cockburn 1994), because of the extremely high energy requirements of their large litters (Green et al. 1991). Litters can weight up to five times the weight of the mother at weaning (Cockburn 1994), and as a result, mothers lose on average 21% of their body mass by the end of lactation (Fisher & Blomberg 2011). Increasing litter size has been shown to have a negative effect on the body mass of male and female young yellow-footed antechinuses; young of either sex are smaller in large litters (Coates 1995). Female agile antechinuses with larger litters took longer to wean their offspring than mothers with smaller litters (Cockburn 1992) and generally failed to wean all of their young (Cockburn 1990).

2. The effects of litter sex ratio on maternal care and investment

2.1. Adaptive hypotheses to explain biased sex allocation

Evolutionary theory predicts that parents should invest equally in their progeny regardless of sex (Fisher 1930), however biased sex ratios occur in a wide range of animals including many mammals (Charnov 1982, Hardy 2002, Wild & West 2007). The main reason for this phenomenon is thought to be that the sexes are not equally costly to produce. Sex allocation theory aims to explain why parents invest differentially in sons and daughters based on either differential allocation of resources, or behavioural facilitation (West 2009, Monclús & Blumstein 2012. In mammals, it is likely that multiple maternal and environmental effects contribute to strategies of biased sex allocation (Cockburn et al. 2002, Wild & West 2007). Wild marsupials frequently produce unbalanced offspring sex ratios (Cockburn 1990, Robert & Schwanz 2011).

There are two main hypotheses that aim to explain sex ratio variation in mammals: the Trivers-Willard hypothesis (TWH) and the local resource competition hypothesis (LRCH) (Cockburn et al. 2002, Wild & West 2007). The TWH hypothesis is the one that has received the most support and states that parents in good condition will invest more in the sex with greater fitness returns (Trivers & Willard 1973). In polygynous species, mothers in good condition should invest more in sons, because such mothers are expected to have the ability to produce large, high quality offspring, and having large, competitive sons will give them greater fitness returns than they would obtain by producing high quality daughters (Lee & Moss 1986, Hewison & Gaillard 1999, Cockburn et al. 2002, Cameron 2004, Sheldon & West 2004). In polygynous species, reproductive success of males depends on their size and competitive abilities, and large males will usually outcompete small ones (Meikle et al. 1995, Fisher & Cockburn 2006). However, males are more costly to produce than females as they grow bigger and faster in dimorphic species (Clutton-Brock et al. 1981, Redondo et al. 1992). In contrast, mothers in poor condition, such as those that are thin or small for their age, or young, would benefit by investing more in daughters, as they would be unable to produce large, high quality offspring. Most of their daughters will breed successfully even if they are small because there is reduced or no competition for mates in female mammals and size is less important for reproductive success than it is for males. Daughters are expected to be cheaper to produce because they have slower growth rates compared to males (see below) and they are usually smaller (Clutton-Brock et al. 1981, Lee & Moss 1986, Redondo et al. 1992, Robert et al.

2010).

The LRCH hypothesis states that when one sex remains philopatric and the other disperses, competition for local resources will occur among the philopatric sex. Therefore, mothers should produce more of the dispersing sex (Clark 1978, Greenwood 1980, Silk 1983, Wild & West 2007). In societies with strong female-biased philopatry, as in most mammals, mothers should invest more in sons than in daughters to avoid future competition, especially in high density populations or at sites and times with resource scarcity (Chapman et al. 1989, Komdeur et al. 1995, 1997, Johnson et al. 2001, Cockburn et al. 2002, Isaac et al. 2005). This hypothesis also suggests that under those conditions, mothers should delay the production of the philopatric sex until later in life, when the chances of daughters competing with their mothers are reduced and the mothers' chances of being replaced are higher (Clutton-Brock et al. 1982, Cockburn et al. 2002, Lambin et al. 2001). However, when the philopatric sex provides fitness benefits to the breeders, overproduction of the philopatric sex should be favoured (Cockburn et al. 2002). For example in societies based on dominance rank with matrilineal inheritance of female rank, high ranking females invest more in daughters, whereas low ranking mothers invest more in sons, as they disperse (Simpson & Simpson 1982, Silk 1983, Hiraiwa-Hasegawa 1993, Cockburn et al. 2002). Females are the typical philopatric sex in mammals, and in social species of mammals and other taxa that tend to form strong social bonds with their mother and sisters (Curley & Keverne 2005, Broad et al. 2006, Dunbar & Shultz 2010), but this does not necessarily means that daughters confer fitness benefit to their parents. It is therefore important to understand the costs and benefits experienced by females living in matrilineal groups to better understand sex allocation.

In general, male mammals weigh more than females and grow faster as juveniles (e.g. elephants: Lee & Moss 1986;, seals: Ono & Boness 1996, McMahon et al. 2000; ungulates: Kojola 1993, Birgersson et al. 1998, Hewison & Galliard 1999; and primates: Bercovitch et al. 2000, Johnson 2003). Therefore the energy that mothers need to allocate to raise a son is greater than when raising a daughter. For example, male elephants attempt to suckle more frequent and their suckling bouts are longer than those of females, therefore it is assumed that males have a higher milk intake rate and grow faster than females (Lee & Moss 1986). Low food availability has a strong effect on the survival of male yearlings and mothers have longer interbirth intervals when raising a son (Lee & Moss 1986). In agile antechinuses, sons weigh more even though they are weaned earlier than females (Cockburn 1992), and females are always able to wean some daughters even if the health or quality of the mother is poor (Cockburn 1994). This suggests that males are energetically more expensive to produce than daughters. Moreover, Cockburn (1994) observed that mothers with a high proportion of sons were more likely to die during lactation. This suggests that if the Trivers-Willard effect is operating in antechinuses, mothers in better condition should overproduce sons.

Litter sex ratios sometimes vary with population density (Kruuk et al. 1999, Johnson et al. 2001). For example, dominant female red deer have been described to produce consistently more sons than daughters as male reproductive performance increases with maternal dominance. However, Kruuk et al. (1999) found that this pattern disappear in high population densities. Lambin (1994) studied sex ratio variation in Townsend's voles (*Microtus townsend's*) and observed that when population density was high, litter sex ratios were unbiased. When population density was low, mothers produced female-biased litters. Females born in these female-biased litters formed close associations with their mothers, were more likely to reproduce when the mother was alive (kin facilitation), and produced more female-biased litters. Females born at high population density were less likely to reproduce due to intense competition for space from their female relatives, so competition reduced the benefits of producing philopatric females. In antechinuses, males disperse as soon as they are weaned and females are highly philopatric, so it is very likely that competition for limited resources will occur among females, between the mothers and daughters and among siblings (Chapman et al. 1989). Competition for food resources between group members is one of the main disadvantages of sociality, and can occur through direct interference when individuals directly compete for resources, or when other individuals cause resource depletion (van Schaik 1989). High levels of competition among philopatric females might have a negative effect on many aspects of an individual's survival, sociality, home range and reproductive performance. Dickman (1988) evaluated the effects of interspecific competition and food abundance on litter sex ratios of the agile antechinus in areas where this species coexists with a larger hypothesized competitor species, the dusky antechinus (*A. swainsonii*), using food supplementation. He found that in most locations, the litter sex ratio of agile antechinuses was female-biased, especially where there was an unusually high density of the dusky antechinus. When food was superabundant (either naturally or when supplementary food was provided) or when the intensity of interspecific competition was reduced (when dusky antechinus was removed) pouch-young were biased towards males. This confirmed that competition is a cause of biased sex allocation. Philopatric females not only compete for food resources. For example, common brushtail possums are known to defend access to dens with their philopatric daughters, and when population density is high, especially in areas with high food density, and den availability is reduced, females produced more sons than daughters (Johnson et al. 2001).

2.2. Experimental manipulations of sex ratios

Understanding the vast variety of birth sex ratios in mammals has been difficult due to their complex life histories and even more complex social interactions (Robert et al. 2010, Robert & Schwanz 2011). Furthermore, the TWH and the LRCH are not mutually exclusive and both could be operating in natural population of animals (Robert & Schwanz 2011). An ideal way to test sex allocation hypotheses is to experimentally manipulate offspring sex ratios via cross-fostering, to evaluate if mothers invest more in one sex or the other. This experimental approach permits researchers to disentangle the effects of maternal condition and environmental quality from sex differences in offspring ability to acquire milk on their growth and survival (Koskela et al. 2009, Roberts et al. 2010, Robert & Schwanz 2011). Koskela et al. (2009) conducted a cross-fostering experiment in bank voles in which they manipulated postnatal sex ratio and the quality of the rearing environment by creating litters with only sons or only daughters, with large or small litters (-2 or +2 pups). This species is polygynandrous (multi-male, multi-female mating system) with no or reverse sexual size dimorphism. They observed that daughters grew faster than sons, demonstrating that maternal allocation was biased towards daughter regardless of maternal condition; sons from the enlarged litters were the smallest individuals. Mothers produced more milk for female litters and defended them more intensively. Robert et al. (2010) also performed a cross-fostering experiment in tammar wallabies (*Macropus eugenii*) to test if offspring sex is correlated with maternal investment. The weight of surviving offspring was not influenced by the sex of the offspring originally produced by the mother or the sex of the foster offspring. Females that gave birth to a son had higher investment ability, regardless of the sex of the cross-fostered offspring. This experimental approach shows that the correlation between maternal condition and male-biased sex ratios seen in these mammals does not occur because mothers with more daughters lose condition. Both of these experiments support the Trivers-Willard hypothesis, that maternal condition and ability to allocate energy cause biased sex allocation. Hager and Johnstone (2006) investigated the genetic and phenotypic factors that affect both maternal provisioning and offspring fitness in the house mouse (*Mus musculus*). They conducted a cross-fostering experiment to see if the presence of non-kin litter-mates affected the overall maternal provisioning of the litter. They found that the most important predictor for offspring weight gain was the mother's weight: large mothers had larger offspring, regardless of relatedness. Male-biased litters gained less weight, because males are larger than females and were energetically more costly to produce, and there was increased competition within litters of males.

3. Maternal investment and the costs of reproduction for female mammals

Life history theory predicts a decline in reproduction and survival with age in iteroparous animals (Stearns 1992). The reason for this is that organisms only have limited resources that must be allocated to different essential requirements such as growth, self-maintenance, survival and reproduction (Stearns 1992). Their reproductive success will depend on trade-offs among these, and how these constraints are dealt with (Williams 1966). Therefore, the investment that an individual makes in each reproductive event would reflect the trade-offs between the costs of reproduction (physiological and ecological), offspring quality, their own survival and their future breeding potential (Williams 1966, Clutton-Brock 1984, Speakman 2008).

Investment in current reproduction should also depend on individuals' lifespans. Animals that have a long lifespan should favour their own survival over reproduction, while short-lived ones should favour allocation to reproduction over their own survival (Hamel et al. 2010). Heavy investment in current reproduction typically reduces both the future breeding potential and survival of the mother (Stearns 1992, Speakman 2008). Due to the high energetic requirements of reproduction, particularly during lactation, mammals are an excellent model taxon for studying the costs of reproduction (Hamel et al. 2010).

Senescence and terminal investment are the main hypotheses that attempt to explain age-specific variation in the reproductive performance of organisms (Weladji et al. 2010). The senescence hypothesis states that older females should reduce their investment in reproduction due to progressive deterioration of condition with ageing (Kirkwood & Austad 2000, Selman et al. 2012). The terminal investment hypothesis states that because of the reduction in the possibility of successfully reproducing in the future, older mothers should increase their investment in current reproduction (Williams 1966, Clutton-Brock & Godfray

1991, Stearns 1992), suggesting that older mothers should invest more in reproduction than younger ones. These hypotheses are also not mutually exclusive, despite their opposite predictions (Weladji et al. 2010).

The age of a mother can affect sex allocation because of age-related changes in a female's body condition, experience, or the likelihood of competition between mothers and offspring (Isaac et al. 2005, Martin & Festa-Bianchet 2011). For example, in the bighorn sheep, reproduction frequency and offspring sex ratio of older mothers depends on environmental conditions. In good conditions, old females produce daughters every year, but when conditions were bad, old females produced more sons but not every year (Martin & Festa-Bianchet 2011). Young female reindeer were more likely to produce a son, while older females had shorter gestation times, lighter calves (suggesting that they were in poorer condition) and were more likely to produce a daughter (Holand et al. 2006). In the common brushtail possum, young females produce more sons in their first breeding event and thereafter, their offspring sex ratio is more dependent on their body condition. Mothers in good condition seem to be more likely to produce sons (Isaac et al. 2005). In some species including macropods and antechinuses, older females produce more daughters, and young females that produce daughters suffer poorer reproductive performance, suggesting a negative effect of competition from philopatric daughters on mothers (Stuart-Dick 1987, Cockburn 1992). Cockburn et al. (1985a) measured the litter sex ratios of three species of antechinuses; although there was substantial interpopulation variability with little year-to-year variation, they found different patterns in each species. Litters were female-biased in the brown antechinus *A. stuartii*, close to parity in the yellow-footed antechinus *A. flavipes* and male-biased in the dusky antechinus *A. swainsonii*. They proposed that the extent of bias in litter sex ratios was related to the probability of females breeding twice (degree of female iteroparity). In populations where female semelparity is common and almost all females die after breeding once, females produced female-biased litters, and in populations where mothers have a high probability of breeding twice, as in *A. swainsonii* (Cockburn et al. 1983), females usually produced male-biased litters in their first breeding attempt and female-biased litters in their second breeding season. The authors suggested that as females that breed a second time usually share their nests and home range with their daughters (or at least with one) until the next mating season (Cockburn et al. 1985b), so producing male-biased litters in the first breeding season would reduce long term competition with daughters (Smith 1968, Clutton-Brock et al. 1982). This explanation fits with the local resource competition hypothesis, and assumes that the negative effect of sharing between females would be worse than the negative effect of raising sons on the mother (Cockburn 1992). Fisher and Blomberg (2011) compared the reproductive allocation and fitness of semelparous and iteroparous female brown antechinuses. Litters of semelparous mothers and from the second litter of iteroparous mothers showed a faster growth rates and were more likely to survive than the first litters from iteroparous mothers. Mothers with fast growing offspring significantly reduced their body weight by the end of lactation, and did not survive more than three months after weaning their offspring. This suggests extremely high reproductive costs for mothers that invest heavily in their offspring, compromising their own survival.

4. Environmental effects on maternal investment and sex allocation

Variation in environmental conditions is an important confounding factor affecting the relationship between maternal condition, investment ability and sex allocation in wild population of mammals. In poor environmental conditions, mothers are expected to trade-off between reproduction and their own survival, especially in mammals as reproduction involves extremely high energetic demands during lactation (Speakman 2008). Reduced offspring growth (Festa-Bianchet & Jorgenson 1998) or increasing the production of daughters (Kruuk et al. 1999, Koskela et al. 2004) in poor environmental conditions have been described different species. Furthermore, individual differences in their ability to acquire and allocate resources to different activities and requirements also vary with environmental conditions. This is a major confounding factor affecting maternal investment and sex allocation (Reznick et al. 2000).

Some dasyurids including antechinuses have an extreme life history. They have just a single, extremely short and highly synchronous mating event per year that ends with complete male die-off (obligate male semelparity) leaving a population of pregnant females (Braithwaite & Lee 1979, Oakwood et al. 2001, reviewed in Fisher et al. 2013) (see below). Braithwaite and Lee (1979) suggested that the evolution of such an extreme reproductive strategy was driven by the need of females to secure enough food to sustain the high energetic requirements of lactation and the weaning of large litters combined with long lactation time. Late lactation is the most energetically demanding time for a small female mammal. Lactation in marsupials lasts much longer than in eutherian mammals because young are born after a very brief gestation, and marsupials have a lower metabolic rate than eutherian mammals (Tyndale-Biscoe & Renfree 1987). Lactation in antechinuses lasts for almost 4 months (~14 weeks: 95-110 days; Wood 1970, Cockburn 1992), which is extremely long for a small female mammal that lives for 16-20 months.

Braithwaite and Lee (1979) suggested that antechinuses inhabit highly seasonal, predictable environments such as coastal Australian forests, and that they matched the end of lactation and the time of juvenile independence with the highest annual peak of arthropod abundance (Braithwaite & Lee 1979). They hypothesized that species with semelparous males need to wean young at the time of year when food availability peaks, so they need to mate synchronously and can only raise a single litter in this period of high food availability because of their long lactation time. However, variability in the seasonality of arthropod abundance at different habitats where these dasyurids live has only been explored recently by Fisher et al. (2013). These authors found that the seasonal predictability of arthropod abundance increased with latitude, and that at sites with higher seasonal predictability the duration of the mating season was shorter, males had the lowest post-mating survival, extremely long copulation durations and also the largest testes. In contrast, species of carnivorous marsupials inhabiting tropical areas (where seasonal predictability is reduced) have longer mating seasons and higher post-mating survival of males. These findings support the idea that females synchronize late lactation and weaning to the time of year with the highest abundance of prey by restricting the mating season, causing males to increase their reproductive effort through sperm competition, at the expense of their own survival (Fisher et al. 2013).

These carnivorous marsupials with semelparous life histories, extremely high energetic requirements during lactation and their high dependence on seasonal predictability of insect abundance makes them quite vulnerable to extreme changes of weather conditions. Australia has the highest number of modern extinctions of mammals of any country (22-25 species depending on which islands are counted). Medium-sized mammals in drier mainland climate zones have been worst affected. The main causes are the introductions of exotic predators and competitors, and destruction and fragmentation of habitat. Globally, species with small distributions and specialised habitat requirements are most at risk, which can also vary depending on their life history traits, population density, and overlap with human populated areas.. Interactions between risk factors are also important (Davidson et al. 2009). Although larger-bodied mammals (>3 kg or >5.5 kg, depending on the authors' criteria) are at higher risk of extinction (Cardillo et al. 2005, Davidson et al.

2009), globally, from the extant species of mammals, 25% of them are at risk of extinction, more than 50% are in decline and 15% weigh more than 5.5 kg (Davidson et al. 2009).

Climate change is now considered to be the major threat to biodiversity during the next 100 years (Isaac 2009, Cahill et al. 2013). Vulnerability to climate change is associated with geographical distribution and life history traits, and risk is higher for species restricted to small areas with short generation times (Jiguet et al. 2007, Pearson et al. 2014), especially if climatic changes occur rapidly and include extreme events (Isaac 2009). Disruption of species interactions, in particular decline in food availability, is likely to be a major cause of species declines and extinction due to climate change (Cahill et al. 2013). Subtropical antechinuses have a very small range, extreme life history and extremely high energetic demands of lactation. These traits make them quite vulnerable to climate change. Droughts that reduce food supply can cause severe population decline (Parrott et al. 2007). This is of a special concern because extreme changes in weather conditions are expected to increase in Australia and worldwide, and drought events are likely to increase in frequency and intensity, which can have devastating consequence for small carnivorous marsupials (Rhind & Bradley 2002, Parrott et al. 2007, Recher et al. 2009).

5. Background on *Antechinus subtropicus*, a small carnivorous marsupial

5.1. Taxonomy of the study animal and location of the study

The genus *Antechinus* (Order Dasyuromorphia, family Dasyuridae) was until recently thought to consist of ten species of small marsupials endemic to Australia (Van Dyck 2002). However, during the past three years three new species have been discovered (Baker et al. 2012, 2013, 2014). Populations formerly considered to be the brown antechinus have been divided into four different species based on morphological and genetic evidence in the last 16 years (Dickman et al. 1998, Van Dyck & Crowther 2000, Crowther et al. 2003). The brown antechinus complex (the name given to this group of closely related species) consists of *A. stuartii, A. agilis, A. adustus* and *A. subtropicus,* found in eastern Australia. The brown and agile antechinus (*A. stuartii* and *A. agilis*) are the best studied species of this group (Naylor et al. 2008). These four species of antechinus are closely related to the yellow-footed antechinus (*A. flavipes*) and together are referred as the *stuartii-flavipes* complex. Two of the new species, the buff-footed antechinus (*A. mysticus*) and the silver-headed antechinus (*A. argentus*) are more closely related to *A. flavipes* (Baker et al. 2012, Baker et al. 2013).

This study was carried out on the subtropical antechinus (*Antechinus subtropicus*), which is found only in a small geographic range from south-eastern Queensland (south of Gympie) to north-eastern New South Wales. The species is restricted to high altitude subtropical rainforests where it inhabits areas with dense understorey of tangled vines and fallen, rotten logs and is abundant near Brisbane at Mt Glorious (D'Aguilar Range) and Springbrook National Park (Springbrook Plateau). It is the largest of the brown antechinus complex (Menkhorst & Knight 2001). Wood (1970), Braithwaite (1974) and Braithwaite and Lee (1979) studied *A. subtropicus* (then named *A. stuartii*).

5.2. Nesting and diet

Antechinuses are common, forest dwelling, insectivorous marsupials that inhabit a wide diversity of habitats, from tropical and temperate forest to alpine regions (McAllan et al. 2006). Three species, the dusky antechinus (*A. swainsonii*), the swamp antechinus (*A. minimus*) and the newly described black-tailed antechinus (*A. arktos*: *Baker et al. 2014*) are ground dwelling (not good climbers), have long and strong foreclaws modified for digging, and are adapted to fossorial habits. However, the species belonging to the *stuartii-flavipes* complex are arboreal, with specialised adaptations for climbing such as short, hooked claws (Nowak 1999).

Antechinuses are the most social genus of carnivorous marsupials, nesting communally in tree hollows (the *stuartii-flavipes* complex) or terrestrial nests (*A. minimus*, *A. swainsonii* and most likely *A. arktos*), where they form groups composed of individuals of both sexes (*A. stuartii*: Lazenby-Cohen 1991, *A. flavipes*: Coates 1995, *A. subtropicus*: Fisher et al. 2011; *A. minimus*: Sale et al. 2009, *A. swainsonii*: Cockburn et al. 1985b), although two studies have documented no nest sharing in *A. swainsonii* (Green & Crowley 1989, Sanecki et al. 2006).

These small marsupials (20-40 g) have been described as crepuscular or nocturnal (Wood 1970, Naylor et al. 2008), but yellow-footed antechinuses (*A. flavipes*), dusky antechinuses (*A. swainsonii*) and subtropical antechinuses (*A. subtropicus*) are also active during the day (Coates 1995, Green & Crowley 1989, Fisher et al. 2011, Rojas et al. 2014, personal observations). These solitary foragers occupy stable home ranges (Lazenby-

Cohen & Cockburn 1991) that overlap with each other, as they are not territorial (Fisher et al. 2011). Males' home ranges are larger than those of females (Lazenby-Cohen & Cockburn 1991, Fisher et al. 2011) with males increasing the sizes of their home ranges during the rut (mating season) to overlap with more females, as they are highly promiscuous (Fisher et al. 2011).

These insectivorous marsupials are opportunistic predators consuming predominantly terrestrial invertebrates (Hall 1980, Fox & Archer 1984, Lunney et al. 2001) and the occasional small vertebrate (Fox & Archer 1984, Lunney et al. 2001). Antechinuses consume ~ 60% of their mass in arthropods each day (Nagy et al. 1978) increasing their food consumption in late autumn, presumably to increase their body reserves for reproduction (Green et al. 1991). Males reduce feeding to the minimum during the mating period, if they hunt at all, while females increase food intake to the maximum during late lactation (Green et al. 1991).

5.3. Life history

Species in the genus *Antechinus* have an extremely unusual life history for a mammal; after a brief annual mating season, all males die and the population consists entirely of pregnant females (section 4 above, Braithwaite & Lee 1979, McAllan et al. 2006, Naylor et al. 2008). Male mortality occurs synchronously due to an increased level of stress caused by high levels of testosterone and cortisol, which provokes the collapse of the immune system, causing internal ulceration increase of infections and parasite loads (Bradley et al. 1980, Scott 1987). Males reach maturity at around 10.5 months and live for 11.5 months (Braithwaite & Lee 1979, Naylor et al. 2008). The mating season or rut occurs for ~ 2-3 weeks each year in late winter or early spring on predictable dates for each population (Braithwaite & Lee 1979). The timing of the rut is triggered by the rate of change of photoperiod (McAllan et al. 2006) together with pheromonal cues, on predictable dates that vary with latitude for different populations and species (Naylor et al. 2008). In subtropical antechinuses, the rut occurs in late August at Springbrook, and late September at Mt Glorious.

Females usually live for 16 to 20 months (Lee & Cockburn 1985, Coates 1995) and most (80 – 95%, depending on the population) breed only once (i.e. produce only one litter in their lifetime) (Wood 1970, Fisher & Cockburn 2006, Fisher & Blomberg 2011). The proportion of iteroparous females, those that manage to survive weaning their first litter to reproduce a second time, not only varies in different populations and species, but also seems to be higher in those species with larger body sizes (Cockburn et al. 1983). Females produce only one litter per year of 6-14 young, limited by the number of teats, which depends on the species (Woolley 1966) and on factors that affect habitat productivity and therefore the number of young that mothers can raise, including the geographic location (Cockburn et al. 1983), and the type of habitat (Beckman et al. 2007). Teat number increases with latitude, altitude and in inland populations (Cockburn et al. 1983) and with greater environmental seasonality (Beckman et al. 2007). In the subtropical antechinus the number of teats is eight, so females can wean up to eight young.

Gestation lasts around 28 days, and young antechinuses are born in spring (synchronously within a population, most within a week of one another) at an immature, embryonic stage (~0.016 g). Each neonate fuses to a teat in the pouch at birth and remains attached for 5 - 6 weeks (the pouch-young stage) before detaching (Marlow 1961, Fisher & Blomberg 2009). The nestling stage follows, when the mother leaves the young in a nest woven from leaves, usually in a tree hollow, while she goes out to hunt. She intermittently returns to the nest for a suckling bout. Young are weaned in summer, after a further 7 – 8 weeks (Marlow 1961, Fisher 2005). During the last weeks of lactation, 2-4 weeks before weaning, young commence to emerge from the nest to explore their immediate surroundings and begin to learn to feed themselves, but rush back to the nest when disturbed or when the mother returns from hunting (Coates 1995).

5.4. Social organization and mating system

In late summer, when the young become independent, sons disperse away from their birthplace, whereas females stay at the natal site (philopatry) and continue to share a home range and nest sites with their mother and sisters (matrilineal social organization), as well as unrelated females and males. Strongly male-biased dispersal has been described in detail in the agile antechinus, yellow-footed antechinus, dusky antechinus and brown antechinus, and also occurs in the subtropical antechinus (Cockburn et al. 1985a, Coates 1995, Lazenby-Cohen 1991, Kraaijeveld-Smit et al. 2002, Fisher 2005, Fisher & Blomberg 2011). Inbreeding avoidance and the probability of finding a site with more mating opportunities appear to be the main benefits of dispersal for males (Cockburn et al.

1985b, Fisher 2005). Males disperse more frequently and further away than females, and this effect can be accentuated in low density populations (Fisher 2005). Male dispersal means that mark-recapture studies can only estimate survival and lifetime fitness in females, not males (Fisher et al. 2006).

Trapping and radio-tracking data have shown that dispersing males join a matrilineal group that is unrelated to them, and are replaced in their own natal group by immigrating males from other families. Therefore, it seems that male dispersal does not reduce population density, and therefore does not reduce potential competition for resources (Cockburn et al. 1985b, Fisher 2005). It is thought that the mother exerts tight control over the sex and relatedness composition of groups by enforcing the dispersal of newly weaned sons, because normal patterns of male dispersal and social organization are disrupted if the mother dies prior to weaning time (Cockburn et al. 1985b, Fisher 2005). Because daughters remain in their natal site and keep living with their mother as independent adults, sharing nests and home ranges, there might be fitness benefits for females that live in large matrilineal groups. Nothing is currently known about the potential benefits and costs of extended families in these species.

The social structure of antechinuses is cyclic and changes through the year. Pregnant and lactating females nest solitarily during spring until mid summer (*A. stuartii*: Lazenby-Cohen 1991, *A. flavipes*: Coates 1995). Communal nesting occurs during the part of the year when males are alive, from the time of weaning until the mating season: from late summer until late winter (Fisher et al. 2011). Individuals of both sexes share these communal nests and individuals frequently join and leave temporary nesting groups between foraging bouts, and thus *Antechinus* exhibit a fission-fusion social system characterized by groups of fluid composition (see below). Radio-tracking studies have shown that these species communally nest in tree cavities, and that they change nests often (brown antechinus: Cockburn et al. 1985b, Lazenby-Cohen 1991; agile antechinus: Banks et al. 2005; subtropical and brown antechinus: Fisher et al. 2011; dusky antechinus: Cockburn et al. 1985b). Genetic analysis has shown that nesting groups of agile antechinuses (Banks et al. 2005) and subtropical antechinuses (Fisher et al. 2011) are composed of related females, unrelated females and unrelated males, and females in nests have higher relatedness than would be expected if the population shared nests at random. Because antechinuses regularly switch nests (Cockburn & Lazenby-Cohen 1992), these results suggest that groups of related females might be more likely to move together between nests, but this has not been determined. Lorch (2004) suggested that regularly changing nests might be a strategy to avoid predation and parasites.

Antechinuses of both sexes are highly promiscuous with a mating system of scramble polygyny. Males as well as females always share nests during the mating season (Fisher et al. 2006, Sale et al. 2009, Fisher & Blomberg 2011) and group size is greatest during the winter mating season. Lazenby-Cohen and Cockburn (1988) hypothesized that this is because groups of males congregate at leks (arenas to which females are attracted to choose a mate), but Fisher et al. (2011) showed that thermoregulation is a better explanation for the correlation between group size and time of year, because antechinus groups were larger on colder days, and group size was unrelated to the days until mating. Conservation of heat by huddling is a benefit of grouping in many small animals (Krause & Ruxton 2002). Small mammals can reduce heat loss by reducing the fraction of their surface area that is exposed to colder surroundings when they huddle together (Canals et al. 1989). At low temperatures, small mammals increase their metabolic rate in order to maintain their body temperature, but huddling allows individuals to lower their metabolic rate, reducing their energetic requirements and increasing survival in colder environments (Canals et al. 1989). Huddling also increases the temperature of the surrounding air, if it occurs in a confined space, such as a tree cavity or a burrow (Hayes et al. 1992, Krause & Ruxton 2002). In small mammals that usually forage solitarily such as antechinuses, thermoregulatory benefits can be so crucial for survival in winter that unrelated animals that are usually antagonistic will huddle together in nests between foraging bouts when there is a shortage of relatives (Schradin et al. 2006), and this may be the reason why agile and brown antechinuses were found to share nests at one of the few sites where two species in the *stuartii-flavipes* complex overlap geographically (McNee and Cockburn 1992). Antechinuses exhibit several other physiological and behavioural adaptations to conserve energy: frequent torpor; construction of nests in tree cavities with a small entrance with increased insulation by using leaf litter as nesting material; and spending a large proportion of time in these nests (Geiser 1988, Fisher et al. 2011, Rojas et al. 2014). Fisher et al. (2011) provides one reason for changes in group size in the fission-fusion social system of antechinuses, but does not explain the relatedness structure of groups. The costs and benefits of grouping within matrilines are unknown.

6. Thesis aims

The principal aim of this thesis was to study the life-history trade-offs associated with reproduction in a wild population of subtropical antechinuses. In particular, I evaluated how maternal investment varies in relation to offspring sex (sex allocation) and in relation to age (costs of reproduction). By using a cross-fostering experimental design, I swapped some young between mothers to enhance the natural sex ratios of their litters and evaluated the effects of their performance in terms of growth and survival, in the wild. The data collected during the three years of this field study showed how life-long fitness was affected by this manipulation and interactions with environmental conditions. Results are presented in three data chapters (chapters 2 to 4).

Chapter 2

An experimental test of the Trivers Willard and Local Resource Competition hypotheses using three generations of cross fostering in the subtropical antechinus.

In this chapter I provide support for the LRCH and reject some key predictions of the TWH. I show that in this species, producing sons is effectively more costly than producing daughters. However, at the expense on their own survival, mothers were able to increase their investment and raise more sons than the number naturally produced without compromising their offspring's fast growth rates. In addition, mothers that naturally produced female-biased litters, despite being smaller, were able to produce large and fast growing daughters. I also show that litter size only affected the growth of daughters and not sons.

Chapter 3

Age-specific reproductive trade-offs in female subtropical antechinuses.

In this chapter I provide support for the terminal investment hypothesis. I show that older females have a greater maternal investment ability and improved survival than younger females, despite the fact that they have no chance to breed in a third season. Additionally, their large, fast growing offspring were more likely to survive than the offspring from younger females.

Chapter 4

Environmental constraints and their effects on maternal investment in subtropical antechinuses.

In this chapter I show that subtropical antechinuses are extremely sensitive to changes in environmental conditions. Not only the severity but also the timing of events such as droughts are important. Environmental variability that disrupts the seasonal predictability of insect abundance can have serious effects on many aspects of demography and individual fitness, because antechinuses synchronise their reproductive season to match the end of lactation with an annual peak of arthropod abundance that is highly dependent of rainfall.
I show that low and delayed rainfall severely impair growth, maternal investment ability and survival. Conditions experienced early in life persist into adulthood: high rainfall throughout lactation and weaning maximises growth, survival and reproductive success, and therefore fitness in this short-lived species.

Chapter 5

General Discussion.

This last chapter provides a general overview of the previous chapters presented in this thesis while highlighting the significance of the results presented and areas for future research.

7. Research plan and Methodology

7.1. Study Site

This study was carried out at the Springbrook Plateau section of the Springbrook National Park. This park is part of the Gondwana Rainforests of Australia World Heritage with a subtropical rainforest located at around 100km south of Brisbane in the Gold Coast Hinterland in south-east Queensland, Australia (-28.23ºS, 153.28ºE). This is a montane rainforest with emergent eucalypts located at relatively high altitude (~900 m above see level). The climate is subtropical, with maritime influence due to its proximity to the coast, frequent cloud immersion and fog events caused by the relatively high altitude and the interactions between topography (vertical cliffs) and canopy rainforest with an average rainfall of more than 3000 mm per year.

I established two field study sites where *Antechinus subtropicus* were relatively abundant on the Springbrook plateau between August 2010 and August 2013. The largest site was located adjacent to the QNPWS rangers' barracks and the second one next to Goomoolhara falls. Subtropial antechinus is considered a subtropical rainforest specialist and prefer habitats with dense understorey, vine tangles with fallen and rotten logs on the ground and abundant leaf litter. Two previous ecological studies (undergraduate projects supervised by Diana Fisher, Fisher et al. 2011) were carried out at this site with the same population of antechinuses in 2008 - 2009. At this location, the mating season for this species occurs at the end of August each year.

7.2. General field methods: individual identification and measurements

Animals were trapped using aluminium Elliot traps placed 5-10 m apart at marked locations along a disused walking track and adjacent areas. The location of each trap is permanently marked and labelled with coloured flagging tape. Old location marks, derived from previous studies on this species at this site, were used, as well as new ones. Traps were waterproofed by placing each trap in a plastic bag, open at the entrance, and non-absorbent Dacron fibre (pillow stuffing) was provided for bedding. Traps (N=200) were set before dusk and checked every four to six hours, and I used a mixture of peanut butter and rolled oats and two soaked dog kibbles (dog chow) as bait.

Each captured animal was sexed, weighed and microchipped with a unique passive integrated transponder (Trovan, ID-100, 11 x 2.2 mm or 7 x 1.25 mm) for individual identification. The microchip was injected under the skin between the shoulders (as in Fisher 2005). Determination of age in this species is easy, due to the species life history and population structure (males only live for 11.5 months and second year females can be visibly distinguished from juveniles / first-year females by the presence (post-reproductive or second year females) or the absence (pre-reproductive or first-year females) of teats or pouch development (i.e. teats are not visible in first year females until the breeding season) (Cockburn et al. 1983, Lada et al. 2008. All animals were released after handling at the point of capture. If new individuals were trapped, a small sliver of ear tissue (~2mm diameter) was taken using a sterile biopsy punch and stored in 70% ethanol for possible later genetic analysis. I estimated growth and survival of antechinuses from monthly trap-recapture data (as in Fisher et al. 2006).

7.3. Design of the cross-fostering experiment to manipulate the number of females in family groups

I created matrilines of subtropical antechinuses with different numbers of females through cross-fostering (swapping some young between litters). Lactating females were captured from the field site during the second and third week of October (at this location, females give birth at the end of September) and brought into temporary captivity for up to seven weeks. At the time of capture, young were around two to three weeks old and strictly attached to the teat (permanently). The whole idea was to capture the mothers with their entire litter. Mothers were maintained individually (as they nest alone at this stage) in 30 litres plastic containers (45 X 35 X 20 cm, of clear polyurethane) with wire mesh lids. Each container had a wooden nest-box (22 cm^3 with a 3 cm diameter entrance hole) and a mouse running wheel for exercise. Wood shavings, shredded paper and leaf litter were provided as bedding (as in Fisher 2005, Fisher & Blomberg 2009). Water was provided *ad libitum* in an inverted drip bottle. A mixture of beef and kangaroo mince, supplemented with calcium powder, Pentavite drops and dry dog food was given once a day (~40g), supplemented with live insects such as mealworms and crickets (~5 of each). Animals were kept in a well-lit building at the Queensland National Parks and Wildlife Services' barracks (a cottage for environment agency staff, contractors, volunteers and researchers) at Springbrook in 2010 and 2012, adjacent to the study area. In 2011 they were kept in an isolated and air conditioned animal room with natural light at the University of Queensland.

Young voluntarily drop the tea when they are around five to six weeks old and the mother starts leaving them in the nest, instead of carrying them around constantly (end of pouch phase and the beginning of the nestling phase). At this age, young are sufficiently developed to be sexed and individually marked. Each offspring were sexed by visually inspecting their genital area: males are easily distinguishable by the presence of testicles while in females their mammary glands, although very undeveloped (looked like little dots in a circle arrange), were also visible especially with the use of a magnifier. To permanently identify individual young, one to two toe buds were removed, with tiny eye surgery scissors (~3mm blade) under a magnifier (Fisher & Blomberg 2009). The removal of the tiny (< 1 mm) toe-bud tips was quick and causes very little or no bleeding; as it done at a very immature stage, the perception of pain and memory was unlikely to be a problem (actually, young do not even react to the clipping). As a result, the marked toe develops without a nail and was slightly shorter than the others. Thumbs (toe number one) on front and back feet were not clipped, because this toe has no nail. A standardised marking scheme was used and sex of young was part of the code, so each mark can be used twice. Clipping two toes from the same foot was avoided. Fisher and Blomberg (2009) documented that toe-bud clipping in the brown antechinus has no harmful effect on growth, survival and recapture probability, either in captivity or in the wild. Toe bud clipping was performed on late pouch young/early nestlings, when they were still blind and naked at around 5-6 weeks old.

Once all young had been sexed and individually marked (~40 days old), some of them were cross-fostered between mothers to experimentally create litters with skewed sex ratios. Some litters then had a high proportion of females, and the others a high proportion of males. To be able to manipulate the sex ratio and achieve a high bias towards one sex or the other without changing the litter size, cross-fostered pups must be of opposite sex. For example, if the cross-fostering was done between two mothers with 8 pups each, but one of them has 5 daughters and 3 sons and the second mother has 3 daughters and 5 sons, to create two litters with a high sex ratio skew from these two families, 2 males from the first litter were swapped with 2 females of the second litter. The first litter will end up with 7 females and 1 male (litter highly skewed towards females), whereas the second one will end up with 1 female and 7 males (highly skewed towards males).

No more than two pups were cross-fostered from any one litter. This means that each litter contained both pups that were related and unrelated to the mother, so I could determine effects of relatedness in addition to litter sex ratio on maternal allocation, individual growth, behaviour and survival.

Once all families were settled, and it was certain that all young were suckling and gaining weight, all families were released back into the wild in their nest-boxes (~75 days old). Each nest-box, with the mother and her litter, was strapped to a tree at the place where the female was originally captured. This timeframe and soft release method have been successfully used before in similar studies with antechinuses (Fisher 2005, Fisher et al. 2006). In those previous studies, more than half of the families kept using their nest-boxes throughout lactation and the rest moved to nearby trees. These results showed that antechinuses were able to settle back into the wild almost immediately with high survival and normal home ranges. Because only newly weaned individuals disperse (once a year), the females' original home ranges were vacant when they returned to them after captivity.

Survival and growth were monitored using nest-box checking and monthly trapping. Nesting group compositions and sizes were recorded. The following year, the females that were cross-fostered as juveniles, together with new individuals in the population, were trapped when they had young attached to teats in the pouch, to evaluate breeding success and their litter sex ratios. The same experimental design was repeated again in 2011 and 2012, except that in the latter, the cross-fostering experiment was not performed as females were in extremely bad condition (see Chapter 3).

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Chapter 2

An experimental test of the Trivers Willard and Local Resource Competition hypotheses using three cohorts of cross fostering in the subtropical antechinus

An experimental test of the Trivers Willard and Local Resource Competition hypotheses using three generations of cross fostering in the subtropical antechinus

Abstract

In sexually dimorphic mammals, sons are often energetically more expensive to produce than daughters because they grow bigger and faster. The Trivers-Willard Hypothesis (TWH) predicts that in species with size-based male competition for mates and high variance in male mating success, mothers in good condition should give birth to more sons because such mothers are able to invest in offspring growth, whereas mothers in poor condition cannot produce competitive sons and should produce more daughters that will breed anyway. In contrast, the Local Resource Competition Hypothesis (LRCH) predicts that mothers in poor condition should reduce competition between female relatives for dwindling resources by producing more sons, as females often remain in their natal home range after weaning (philopatry). Experimental tests of these hypotheses are rare and have not been carried out previously on wild mammals. I experimentally manipulated offspring sex ratio in subtropical antechinuses (*Antechinus subtropicus*) to disentangle these multiple effects on sex allocation. I created families with either enhanced female- or male- bias by cross-fostering young between litters, to test assumptions and predictions of the TWH and the LRCH. The results support aspects of both hypotheses in antechinus. In agreement with the TWH, mothers that naturally produced male-biased litters were slightly heavier than mothers that naturally produced female-biased litters. However, in disagreement with the TWH, mothers rearing male-biased litters were able to increase allocation of resources to offspring and rear more sons than they had naturally produced, after cross fostering, without compromising offspring growth. Consistent with the LRCH, male-biased litters grew more quickly after increasing their sex ratio bias and not more slowly as expected according to TWH. Mothers raising more sons were also more likely to wean their larger and fast growing offspring than mothers with female-biased litters, by compromising maternal survival. Mothers apparently reduced investment in offspring in favour of their own survival when large female-biased litters were imposed on them, consistent with LRCH. These results differ from previous cross fostering manipulations to test sex allocation, which have unequivocally supported the TWH.

Key words: sex allocation, sex ratio, cross-fostering, maternal investment, Trivers-Willard hypothesis, Local Resource Competition, offspring growth, subtropical antechinus.

Introduction

According to sex allocation theory, differential allocation occurs when parents produce more offspring of one sex or the other, or invest differentially in sons and daughters in terms of parental care, through resource allocation or other behaviours favouring one sex over the other (Armitage 1987, Monclús & Blumstein 2012). Fisher's theory (1930) states that if males and females are equally costly to produce, then parents should invest equally in them. However, when one sex is more costly to produce than the other, the cheaper sex should be favoured (West 2009). Despite chromosomal sex determination in mammals, which ought to result in equal proportions of males and females through random segregation of chromosomes at meiosis (West 2009), biased offspring sex ratios are common (Cockburn et al. 2002, Wild & West 2007, Robert & Schwanz 2011).

The Trivers-Willard hypothesis (TWH) (Trivers & Willard 1973) has been one of the most supported hypotheses to explain sex ratio variation in mammals (Hewison & Gaillard 1999, Cockburn et al. 2002, Cameron 2004, Sheldon & West 2004, Wild & West 2007). Most mammals are polygynous, and male reproductive success depends on size; large and strong males will thus outcompete small, weak ones (Meikle et al. 1995, Fisher & Cockburn 2006). Males of sexually size dimorphic species are costly to produce, as they usually grow bigger and faster than females (Clutton-Brock et al. 1981, Redondo et al. 1992). Therefore, mothers with the ability to produce high quality offspring (i.e. large, heavier mothers) should invest more in sons, because this will give them greater fitness returns than producing high quality daughters. Mothers in poor condition (e.g. thin, small for their age or young) that cannot invest heavily in their offspring will benefit most by producing females, as most daughters will breed, rather than producing small sons that may not reproduce. Female mammals typically experience less reproductive competition and are expected to be cheaper to produce as they reach smaller sizes than males and usually at a slower growth rate (Robert et al. 2010). The TWH operates at the level of the individual mother, but may produce biased sex ratios at the population level (Charnov 1982, Frank 1987). Variation in sex ratios has been reported in populations of mammals at different times, and also in different populations of the same species (Cockburn et al. 2002, Cameron 2004).

Unlike correlative studies of maternal body condition or studies that manipulate food availability, cross-fostering experiments that alter offspring sex ratio at an early stage of development in birds and mammals can directly test the investment capacity of parents. If the Trivers-Willard effect is operating and parents produce the sex ratios according to their (current) investment abilities, parents that originally produced male-biased sex ratios are expected to produce heavier and fast growing offspring, but those that gave birth to female-biased sex ratios should not be able to do so (Bowers et al. 2013). There have been only a handful of such experiments, and only two using mammals, which both supported the TWH (Bowers et al. 2013, Robert et al. 2010, Rutkowska et al. 2011). Mothers that naturally produced male-biased sex ratios were able to raise heavier, fast growing offspring and were more successful at weaning their young (their own and foster young) than mothers that originally gave birth to female-biased sex ratios.

The other major hypothesis to explain primary sex ratio biases in non-cooperatively breeding mammals is the Local Resource Competition Hypothesis (LRCH) (Silk 1983, Clark 1978, Chapman et al. 1989 a,b, Wild & West 2007, Silk & Brown 2008), which proposes that the fitness advantage gained from a male versus female-biased birth sex ratio depends on which sex disperses, and the effects of intraspecific competition on the philopatric sex (Greenwood 1980, Pusey 1987). The LRCH states that when resources are scarce or there is a high-density population, mothers should invest more in the dispersing sex in order to avoid or reduce the cost of future competition for local resources with their kin and between their offspring, and they should delay the production of the philopatric sex until later in life (Clutton-Brock et al. 1982, Cockburn et al. 2002, Lambin et al. 2001, Isaac et al. 2005). Female mammals are typically philopatric and often form long term social bonds with their mother and sisters (Curley & Keverne 2005, Broad et al. 2006, Dunbar & Shultz 2010), while males disperse more frequently and over longer distances, especially in promiscuous and polygynous species (Greenwood 1980, Dobson 1982, Pusey 1987).

Originally, the LRCH was proposed to operate solely at the population level (i.e. birth sex ratios should track population density: mothers should produce more sons in dense populations). It is now thought to apply differentially to individual mothers (Silk 1983, Isaac et al. 2005, Wild & West 2007). When resources are limited and mothers are in poor condition, producing philopatric daughters will increase the competition among them (kin) for the already scarce resources. Thus, if the LRCH is operating, such mothers would benefit more by investing greatly in their dispersing sons.

Both hypotheses, the TWH and LRCH, are based in the assumption that an individual's survival and reproductive success rely ultimately on resource availability. The TWH emphasizes the importance of resource availability during the period of parental investment (gestation and lactation in mammals; i.e. pre-weaning), whereas the LRCH highlights the importance of post-weaning resources. Therefore, if resource abundance during lactation is correlated with its post-weaning abundance, concurrent operation of both hypotheses is highly likely, and this has restricted our understanding of mammalian sex allocation in natural populations (Schwanz & Robert 2014; see also Kruuk et al. 1999, Isaac et al. 2005, Robert & Schwanz 2011).

Although the TWH and LRCH are not mutually exclusive, they often make opposite predictions of how individual mothers should invest in each sex: in polygynous species with males larger than females and male-biased dispersal, females with poor capacity to invest in offspring in their earlier breeding seasons should overproduce sons under the LRCH (to avoid competition with them, as sons leave), and overproduce daughters under the TWH (as they lack enough energy to produce competitive sons). In marsupials, biased offspring sex ratios are common (Cockburn 1990, Isaac et al. 2005), and both hypotheses have found support (Robert & Schwanz 2011, Schwanz & Robert 2014). In antechinus species with a higher occurrence of female iteroparity, which are thus more likely to experience competition with their philopatric daughters, mothers tend to produce more dispersing sons, supporting LRCH (Cockburn et al. 1985). In contrast, Dickman (1988) experimentally varied the level of competition by removing interspecific competition (a sympatric larger species) and by providing access to supplementary food. In his study, agile antechinus mothers produced more daughters in the presence of the larger dusky antechinus and produced more sons when the competition was reduced by experimentally removing the dusky antechinus, providing support for TWH (Robert & Schwanz 2011). Experiments that test maternal capacity to invest by manipulating offspring sex ratio are needed to better understand sex allocation in marsupials.

I conducted such an experiment using a small marsupial, the subtropical antechinus, *Antechinus subtropicus* (Crowther et al. 2003), which produces only one litter of up to 8 young a year (Cockburn et al. 1985). Young are born at an embryonic stage, and remain attached to a teat in the pouch for 5 weeks (Marlow 1961), enabling experimental manipulations of litter sex ratios at a very early stage of development, and evaluation of potential costs, their timing, and trade-offs involved in rearing sex-biased litters (Robert & Schwanz 2011). Some populations of antechinus produce strongly sex-biased litters (Cockburn et al. 1985, Dickman 1988, Davison & Ward 1998). This bias is generated before birth, not by selective infanticide or sex-specific failure to attach to a teat (Davison & Ward 1998). Antechinus females usually have a lifespan of 16-20 months and almost never breed a third time (Fisher & Blomberg 2011). In some species, populations in which almost all females die after breeding once produce female-biased litters, but in populations where females often breed twice (i.e. live for two years), females often produce male-biased litters in their first breeding season and female-biased litters in their second, consistent with the LRCH. Cockburn et al. (1985b) interpreted this as strong support for LRCH: females that will breed a second time face competition from daughters from the first litter but will not survive to face competition from a second litter. However, consistent with the TWH, several lines of evidence have shown that sons are more expensive to raise than daughters. For example in a study of agile antechinuses *(A. agilis),* sons weighed more and were weaned earlier than daughters (Cockburn 1992), mothers with male-biased litters were less likely to survive lactation, mothers in poor condition were able to wean at least some daughters but no sons (Cockburn 1994), and older females that tended to produce female-biased litters were senescent and in poor condition (Cockburn 1992). Mothers always lose weight in late lactation, and in the brown antechinus, those with faster growing litters lose a substantial amount of weight and do not survive more than three months after weaning their offspring (Fisher & Blomberg 2011), indicating an extremely high cost of reproduction and a trade-off between maternal survival and offspring quality.

The aim of the present study was to test the following key assumptions and predictions of the TWH and LRCH by examining natural patterns of sex ratios and conducting an experimental manipulation of litter sex ratios in *Antechinus subtropicus*, by either increasing, decreasing or maintain their natural bias. The TWH is based on two main assumptions: that sons are more expensive to raise than daughters and that offspring growth depends on maternal investment ability. The TWH predicts that mothers that are in good condition have the capacity to produce high quality offspring and thus should invest more in their sons than in their daughters. While mothers that do not have the capacity to produce high quality offspring, such a small, young or mothers in poor condition, should invest more in their daughters as they will not be able to produce competitive sons (small males usually not successful at breeding). This implies that heavier mothers should produce more sons than lighter ones. Therefore, if this is true and this mechanism is operating, I expect the following to occur:

1) If sons are more expensive to produce than daughters, then:

1.1) Sons should grow bigger (weigh more at the same age) and faster (have higher growth rates) than daughters.

2) If offspring growth depends on maternal energy reserves, then:

2.1) Offspring mass should be correlated with maternal mass, especially in sons.

2.2) Lighter and younger mothers should (A) naturally produce female-biased litters and (B) be less likely to wean their offspring than heavier or older mothers that are presumed to have a greater investment ability.

3) If sons are more expensive to produce than daughters and the number of sons that a mother produce depends on her current investment abilities, after sex ratio manipulation, mothers that reared more sons than they naturally produced would not be able to keep up with demand, and should (A) have slower growing offspring, especially sons, (B) lose more mass during lactation, (C) be less likely to wean their offspring successfully and (D) have reduced survival relative to mothers whose natural sex ratios bias were either decreased or maintained.

If the LRCH applies rather than the TWH, I expect to find that light and young mothers have male-biased natural birth sex ratios, while, heavier and older mothers should produce female-biased litters. Moreover, if females respond by trading off current investment against their own future survival, then young mothers rearing female-biased litters should reduce their investment and have slower offspring growth to increase their chances of survival to breed again, while also reducing competition with their philopatric daughters. Increasing female bias is not expected to increase offspring growth, as this would increase competitiveness of daughters, which would compete with these mothers, at the expense of maternal survival (Fisher & Cockburn 2011). Rigorous experimental tests of either the TWH or LRCH are rare (Robert & Schwanz 2011). No such manipulation involving cross-fostering has been done on mammals in the wild previously.

Methods

Sites, trapping and husbandry

In October 2010, 2011 and 2012, I trapped mothers with pouch young that were 2-3 weeks old (see general methods, Chapter one). Second-year females were visually distinguished from first-year females early in the year (prior to the breeding season) by differences in body mass and by the presence of teats and pouch development, as teats are not visible in first year females until the breeding season (Cockburn et al. 1983, Lada et al. 2007, Fisher & Blomberg 2011). Of the 56 breeding females included in this study, 42 were first-year mothers and fourteen were second-years; 28 raised female-biased litters, and 28 raised male-biased litters after cross-fostering. Only four females were trapped in their both breeding events, and one of them only bred on her second attempt (empty pouch in her first breeding season). Each family (the mother and her litter) were kept in captivity for a maximum time of seven weeks, during mid-lactation, before returning them back to the wild at the point of capture. During the captivity time, each offspring was sexed and individually marked (~40 days old) before performing the cross-fostering experiment. Please refer to the methodology section in Chapter 1 for more details.

Experimental design

A total of 351 young were included in this study, 167 females and 184 males, of 56 mothers (12 in 2010, 22 in 2011 and 22 in 2012). 256 young (117 females and 139 males) had a first-year mother, while only 95 young (47 females and 48 males) had a second-year mother. When young were around 59-62 days old, some litters were manipulated by swapping (cross-fostering) up to two young per litter between mothers without changing litter size to experimentally manipulate litter sex ratios. I aimed to create litters with either one or two males or one or two females, with the remainder of the litter the opposite sex. A total of 24 (12 in 2010, 12 in 2011 and 0 in 2012) litters were manipulated and 32 were not manipulated (0 in 2010, 10 in 2011 and 22 in 2012). A total of 14 litters had their natural sex ratio bias exaggerated, 3 litters experienced a reduction of their natural sex ratio bias and 7 litters had their natural sex ratios maintained with litter manipulation (Table S12). Mothers were assigned to each treatment randomly and paired up with mothers with offspring of similar size. A total of 159 young grew up in manipulated litters, 98 of them in litters which their natural sex ratio bias was increased (42 were female-biased and 56 were male-biased), 12 in litters whose natural sex ratio bias were decreased (4 were female-biased and 8 male-biased) and 49 in litters whose their natural sex ratios were maintained (13 were female-biased and 36 were male-biased). 192 young grew up in un-manipulated litters. Totals of 108 and 59 young females were raised in either female- or male-biased litters, respectively. A total of 132 and 52 males were raised in either male- or female-biased litters (see Table S12). Overall, 323 young (151 females and 172 males), were raised by their natural mother and 28 (13 females and 15 males) were cross-fostered and raised by an adoptive mother. Cross-fostering was not performed in 2012. I monitored body mass, and foot and crown-rump length measurements of the mother and their offspring, respectively, every three-five days during captivity. All mothers that were given foster young readily accepted them.

One week after cross-fostering, when young were ~75 days old, families were released back into the wild in their nest-boxes by strapping the box to a tree at the place where the female was originally captured (as in Fisher 2005, Fisher et al. 2006a,b). The survival and growth of mothers and their young were monitored via checking nest-boxes and trapping sessions performed every month for 3-5 nights until the following breeding season. As most male antechinuses disperse away from their birthplace once weaned, their fate is often uncertain. A total of 119 male young were never seen or trapped again after they were released back to the wild and were considered to be dispersed individuals with unknown fates. Therefore, young males were only considered for weaning survival analyses (i.e. survival to soon after weaning as independent young) and were not considered in the breeding survival analyses of individuals (i.e. survival until the following breeding season; see below).

Data analyses

All statistical analyses were conducted in R Studio (v. 0.98.501 R Development Core Team, 2013) and their results summarised in Tables S1 to S11. I used separate linear mixed effects models to assess the effects of litter sex ratio manipulation on individual growth, in mothers (Tables S4-S6) and offspring (Tables S1-S3), during the time they were in captivity, when young were between 40 to 70 days old. I used the R function ‘lmer’ to perform these analyses using individual ID and the time the measurements were performed (as offspring age) because measurements were not carried out at exactly the same time across the years. In particular, I used ‘(offspring age | mother ID)’ on body mass analyses for mothers and ‘(offspring age | young ID) + (age | donor mother ID) + (age | recipient mother ID)’ on body mass analyses for offspring. These sets of random factors allow intercept (different starting points) and slope variation (different growth rates) in the growth curves. I also used unpaired t-tests (no repeated measures) to compare differences between means (see text below).

I used the R functions ‘glm’ and ‘glmer’ in package lme4 for dichotomous variables to analyse the survival of mothers and offspring, respectively, as there were no repeated measures for mothers and maternal ID had to be controlled in offspring survival analyses. I evaluated the potential effects of litter sex ratio manipulation on maternal weaning success (if the mother successfully weaned their offspring or not, Table S7), maternal weaning survival (if the mother survived lactation and was seen alive within the next two months following weaning, Table S8), maternal breeding survival (if the mother survived to breed again to the following mating season, Table S9), offspring weaning survival (if the young survived after weaning as independent individuals and was seen alive within the next two months after weaning, Tables S10) and the breeding survival for female offspring (males disperse away from their birthplace, so their breeding survival is unknown, Table S11). I also used contingency tables to evaluate if there were any differences in the survival of individuals while controlling for one factor at a time using χ^2 or Fisher test.

I tested the potential effects of the experimental manipulation on individual growth (body mass) using three different approaches. First, I treated litter sex ratio as a dichotomous variable: I compared animals with male-biased final litter sex ratios to those with female-biased litter sex ratios. Second, I tested if there was a significant effect of the type of manipulation on growth, i.e. if there was an effect of having the natural sex ratio bias either increased, decreased or maintained as natural (litter sex ratio change). Third, I tested if there was an effect of the proportion of males per litter on growth. For maternal weaning success and survival, I used the same three approaches. I only report the litter sex ratio change results, as there were no differences between approaches (see Tables S7 and S8, respectively). For offspring survival, I report the results from the models using final litter sex ratio (Table S10a and S11a for weaning and breeding survival, respectively) and litter sex ratio change (Table S10b and S11b for weaning and breeding survival, respectively). In the offspring survival models using the final litter sex ratio, I also included the interaction between litter sex ratio and maternal age class (Table 11a).

I defined a litter as male-biased if the proportion of males in the litters was higher than 0.5. I treated the following as fixed factors in the models with body mass: offspring age, maternal foot length (as a measurement of skeletal size), litter size and maternal age class (first- or a second-year female). To assess if there was any sex difference in growth and between treatments, I also included the interactions between offspring sex and age, sex ratio change and age and sex ratio change and sex in the models with body weight (see Tables S2b and S2c). I could include the variable of ‘year’ only in the models that tested the effect of the proportion of males (Tables S3 for offspring mass and Table S8 for maternal mass) because in 2012 the experimental manipulation was not performed. I treated litter size as a continuous variable. When the effect of litter size was significant, I repeated the analyses treating litter size as a categorical variable, instead of a continuous variable to clarify its effects (see Table S2b and S2c).

Results

The success of the cross-fostering experiment

The cross-fostering experiment did not hinder offspring growth or survival. The mean body mass of natural offspring was not significantly different from the mean mass of cross-fostered offspring, and the effect of type of manipulation was not significant in any of the models (*type of offspring*: $t<0.53$, $p>0.53$. See Tables S1a, S2a and S3). The proportions of adopted and natural offspring that survived to weaning were not significantly different (*type of offspring*: $z=-1.37$, $p=0.18$; see Table S10b). From 351 offspring, 323 were raised by their original mother and 28 by an adoptive one; 64% (N=207) of the natural offspring and 71% (N=20) of the adopted ones survived to weaning.

The analyses that investigated final litter sex ratio were evaluating the effects of producing (for mothers) or growing (for the young) in either a female- or a male-biased litter, regardless of whether effects were due to natural or manipulated conditions. The analyses that investigated sex ratio change directly evaluated the effects of the experimental manipulation. Overall, the statistical results appeared to be consistent across the different models, expect for the effect of litter size that showed some variation (see below). Maternal growth and survival were not affected by the proportion of sons, regardless of whether or not their litters were manipulated. Male offspring grew bigger and faster than female offspring, and did better in male-biased litters. Under those conditions, sons were more likely to be weaned than daughters. Female offspring grew bigger and faster than their brothers in female-biased litters, and this was also supported by the models that evaluated the effects of litter sex ratio change. Increasing the natural sex ratio bias of litters increased offspring growth while decreasing the bias reduced offspring growth rates.

Female offspring increased their chances of survival with a second-year mother (See below).

1) Are sons more costly to produce than daughters? Do sons grow bigger and faster than daughters?

My results were consistent with the premise of the TWH that sons grow bigger and faster than daughters, but these effects were weak. Male offspring grew slightly faster than females (Table 1) and this was consistent across models (*sex*: t>2.71, p<0.01; *sex* x *age*: t=2.71, p=0.0098; Figure 1, Table S1 to S3). Mean body mass of males (mean ± SE, 2.59 ± 0.047g; N=184) was not significantly different from that of females (2.53 ± 0.044g; N=167) at the age of 49-51 days old (t=0.92, df=349, p=0.36) or when young were 60-63 days old (t=0.16, df=161, p=0.87). Females at that age weighed on average 5.16 ± 0.12g (N=70) while male offspring had a mean body mass of 5.13 ± 0.18 g (N=93). However, sons were significantly heavier than daughters once weaned (t=11.79, df=226, p<0.0001). The mean body mass of daughters was 18.45 ± 0.19 g, and sons weighed 22.92 ± 0.34 g around a month after weaning (data were collected within two months after release, when juveniles were ~100-120 days old). This suggests that although sexual size dimorphism in offspring failed to occur during my study (at 35 and 70 days old), sons did later grow bigger than daughters. Such sexual size dimorphism should have been noticeable at the end of lactation, closer to weaning.

2.1) Is offspring growth rate correlated with maternal mass, especially growth of sons?

Offspring mass was strongly associated with maternal mass (*maternal body mass*: t=15.6, p<0.0001; Figure 2; Table S2c). Lighter mothers had lighter offspring, supporting the TWH premise. Moreover, second-year mothers were significantly heavier than first-year mothers (*maternal age class*: t>5.33, p<0.0001; Figure 3A, Table S4-S6) and had heavier offspring (t>5.98, p<00001; Figure 3B, Tables S1-S5). However, this strong association between the mass of mothers and their offspring was similar in sons and daughters (*maternal mass* x *offspring sex*: t=-0.95, p=0.35. See Figure 2, Table S1b).

When I used the proportion of males per litter and year to analyse the effects on offspring mass, the significant effect of maternal age class disappeared (maternal age class was significantly associated with offspring mass when year was not included, as in the other models. See Table S3). The offspring from second-year females were heavier than the offspring from first-year mothers in 2010 and 2012, but not in 2011 (Figure 3C). For a deeper analysis of the effects of maternal age class and year on individuals growth and survival, please refer to Chapters 3 and 4.

2.2.A) Does a mother's body size predict the sex ratio bias of her young?

Inconsistent with the TWH premise that mothers produce offspring with sex ratios according to their investment ability, mean maternal mass did not significantly differ according to the natural sex ratio of their litters (*original sex ratio*: t=1.7, p=0.097; Table S4B). However, mothers that naturally produced male-biased litters were slightly heavier (33.88 ± 0.98 g, N=29, 52% of the mothers) than mothers that naturally produced female-biased litters (31.10 ± 0.93 g, N=23, 41% of the mothers; t=2.013, df=50, p=0.049), when young were ~45 days old (based on unpaired t-test).

2.2.B) Did maternal mass affect the probability of weaning young?

According to the TWH, small / thin mothers should be less likely to wean their offspring than large / heavier ones, as they have reduced investment ability. I did not find evidence to support this prediction. Mothers that were able to wean their offspring were not significantly heavier than the ones that failed to wean them (33.40 ± 0.71 g (N=40) for mothers that weaned their offspring successfully and 31.29 ± 1.73 g (N=16) for mothers that did not wean their young (t=1.35, df=53, p=0.18).

None of the factors, including sex ratio change, maternal and offspring mass, maternal age class or litter size, had a significant effect on maternal weaning success (Table S7). However, second-year mothers apparently had a greater capacity to rear their offspring compared to first-year mothers. Forty of 56 mothers in this study (71.4%) weaned their offspring. Ninety three percent of the second-year mothers weaned their young, only one second year mother died along with her young before she weaned them. Sixty-four percent of the first-year mothers weaned their young (χ^2=2.92, df=1, Fisher test: p=0.047).

Litter size had a slight effect on maternal weaning success (t=-1.77, p=0.076; Table S7). Mothers that weaned their young had a mean litter size of seven young while mothers that failed to wean their offspring had eight young on average (Figure 11A). Although the mixed effect models did not support this, mothers that weaned their offspring had litter sizes of 5.5 ± 0.5 and 7.1 ± 0.2 young in female- and male-biased litters, respectively (F=5.17, p=0.003; based on unpaired t-test, Figure 11B).

3. A. Is the sex ratio of litters related to growth rate of offspring?

At first glance, it seemed that offspring growth rates did not conform to expectations of the TWH in the analysis of final litter sex ratio. Final litter sex ratio refers to whether offspring grew up in either a female- or a male-biased litter, regardless of whether or not their natural litter sex ratio was manipulated (Table S1a, Figure 5A). Male-biased litters had slightly higher offspring growth rates than female-biased litters (*final litter sex ratio* x *offspring* age: t=-6.60, p<0.0001), more consistent with predictions of LRCH than the TWH. At 49-51 days old, young from male-biased litters were heavier than young from female-biased litters, weighing on average 2.63 ± 0.047 g (N=191) and 2.49 ± 0.045 g (N=160), respectively (t=2.16, df=349, p=0.032), regardless of their sex (*offspring sex* x final litters *sex ratio*: t=-0.02, p=0.094).

Offspring not only grew bigger in male-biased litters but they also grew faster (final litter *sex ratio* x offspring *age*: t=6.68, F=3.86, p<0.0001, Table S1), however, this effect depended on offspring sex (*offspring sex*: t=3.48, p=0.0012). In female-biased litters, female young grew bigger and faster than males, while the opposite was observed in male-biased litters, in which males grew bigger and faster (Figure 6). When young were 49-51 days old, the mean body mass of male offspring was 2.51±0.08g (N=52) and 2.63±0.06 (N=132) in female- and male-biased litters, respectively. Female offspring at that age weighed on average 2.48±0.05g (N=108) and 2.63±0.08g (N=59) in female- and male-biased litters, respectively, but none of these values were significantly different from each other (F=1.56, p=0.2). However, at 65 days old, the difference in mean body mass increased and was marginally significant (F=0.83, p=0.072): daughters weighed 5.22 ± 0.15 g and 5.05 ± 0.23 g in female- and male-biased litters respectively, and sons weighed 4.67 ± 0.23 g in female-biased litters and 5.20 ± 0.15 g in male-biased litters.

Surprisingly, increasing the natural sex ratio bias of litters increased offspring growth, while decreasing the natural bias reduced their growth rates compared to litters with natural sex ratio bias (*sex ratio* change: t>4.8, p<0.0001; *sex ratio* change *x* offspring age: t<-2.13, p<0.04; Figure 5B. See Tables S2b and S2c). Mean offspring mass was higher in litters that had their sex ratio bias increased, and lower in those that had the bias reduced, in comparison with natural litters (Figure 7A). However, this effect depended on offspring sex (Figure 7B) sons were more negatively affected by decreasing the natural sex ratio bias of their litters than daughters (*sex ratio change* x *offspring sex*: t<-2.1, p<0.04. See Table S2c).

Litter size appeared to have a significant effect on offspring mass, but this effect was not consistent across the different models (see Tables S1 to S3). When I evaluated its effect as a categorical factor, the only significant difference in offspring mass was between litter sizes of one and seven young (Figure 8. See Table S2c). However, 64% of mothers had a litter of 7-8 young and only one female produced a single young (Figure 4). Offspring mass was not only positively affected by the number of litter mates but also by male proportion in their litters (*male* proportion: t=6.24, p<0.0001; Figure 9, Table S3).

3. B. Did mothers rearing more sons lose more mass during lactation than mothers with more daughters?

This prediction was not upheld during mid-lactation (when their offspring were 45 to 75 days old). Maternal mass was not affected by any factor other than maternal age class and year (Tables S4 to S6).

3. C. Did male biased litter sex ratio reduce the probability of weaning young?

As sons are more costly to produce than daughters (above), when I increased natural sex bias, mothers rearing male-biased litters should have been less likely to wean their young. This prediction of the TWH was also not supported. There were no significant effects on maternal weaning success (Table S7). Mothers had the same probability of successfully weaning their young, regardless of whether they were rearing more sons or more daughters (t=-0.26, p=0.80).

3. D. Do mothers have reduced survival when rearing more sons?

Considering that in antechinuses most mothers die soon after weaning their young and only a few of them manage to survive to reproduce again, maternal survival after weaning their offspring may be a good indicator of maternal condition and their investment ability in this species. The significant effect of maternal foot length (as a measure of skeletal size) on weaning survival supports this. Mothers that weaned their offspring had longer feet than mothers that were not able to wean their young, and died along with them during lactation (*maternal foot length*: z=2.34, p=0.02; Figure 10 and Table S8).

Thirty four of the 56 mothers in the study (61%) died within two months of weaning their offspring. Overall, half of the mothers that reared female-biased litters survived lactation (14 of 28), while only 29% of the mothers with male-biased litters did (8 of 28; χ^2=1.87, p=0.17). This effect was not significant according to the mixed effect models that evaluated the effects of litter sex ratio manipulation on maternal weaning survival, (*sex ratio change*: z=-0.28, p=0.78; Table S8). The same occurred with maternal breeding survival (*sex ratio change*: z=-0.33, p=0.74; Table S9), although the proportion of mothers that survived to breed again was slightly higher for mothers that reared female-biased litters compared mothers with male-biased litters (χ^2=3.28, p=0.07) with a with 39% (N=11) and 14% (N=4), respectively.

Litter size appeared to have a significant effect in both maternal weaning and breeding survival (Table S8 and S9). Having large litters reduced maternal survival. Mothers that were seen alive after weaning their young had a mean litter size of seven young (Figure 12A), while mother that survive to breed again had a mean litter size of six young (Figure 12B).

Did sex ratio manipulation affected offspring survival?

When I analysed offspring survival based on litter sex ratio change, I found no significant effects, except that litter size apparently affected female breeding survival (see below and Table S10b and S11b).

However, interesting results appeared when I evaluated the effects of final litter sex ratio (if offspring grew up in either a female- or a male-biased litter, Tables S10a and S11a).

Offspring survival was not compromised in male-biased litters as they were more likely to survive to wean than young from female-biased families (*final litter sex ratio*: z=3.33, p=0.002) (Table S10a). Sixty eight percent of the offspring from male-biased litters (N=130) survived to wean, and 61% of female-biased offspring (N=97). However, litter sex ratio by itself did not affect their survival to breed (final litter *sex ratio*: z=-0.89, p=0.38; Table S11a). Regardless of their litter sex ratios, only 15% of the female offspring survived to breed (N=25 from a total of 167; 18 were from female-biased litters and 7 from male-biased litters; χ^2=0.17, df=1, Fisher test: p=0.69).

Offspring from female-biased litters were more likely to survive weaning if they had a second-year mother (100%) than a first-year one (48%). Young from male-biased litters had the same probability to be weaned if they were raised by a first- or a second-year mother (68%; *final litter sex ratio* x *maternal age class*: z=-2.77, p=0.009; Table S10a). This effect on offspring survival was maintained until the following breeding season: female offspring that had a second-year mother were more likely to survive to breed than female offspring with a first-year mother (*maternal age class*: z=2.44, p=0.02), especially if they grew up in female-biased litters (final litter *sex ratio* x *maternal age class*: z=-2.11, p=0.041). While 23% of all the offspring that had a second-year mother survived to reproduce, only a 12% of the offspring of first-year mothers reproduced (see Table S11a).

Offspring that survived to weaning had a mean litter size of seven compared to an average litter size of eight for those ones that were not weaned (Figure 12C). This effect was significant in the mixed effects model using final litter sex ratio (t=-2.37, p=0.02, Table S10b) and marginally non-significant in the model using sex ratio change (t=-1.8, p=0.08, Table S10b). In contrast, the effect that litter size had on offspring breeding survival was significant in the model using sex ratio change but not with final litter sex ratio (Table S11a and S11b). Female offspring that survive to breed came from litters that had a mean size of 6 young, while the mean litter size for female offspring that disappear before the breeding season was 7 (Figure 11B).

Discussion

The Trivers-Willard hypothesis states that small or thin mothers benefit by producing more daughters, and this mechanism has been supported in some studies of mammals that have focused on food availability and maternal nutrition. Dickman (1988) found in his study of agile antechinuses that mothers produced male-biased litters when food was abundant, either because food was artificially supplemented or competition was reduced by the removal of a larger competitor from the area (the dusky antechinus). This positive correlation between the nutritional state of mothers and sons' growth and survival has also been reported in other marsupials such as brushtail possums (Isaac et al. 2005), allied rock-wallabies (Delean et al. 2009) and in other mammals including field voles (Koskela et al. 2004). Overall, fast growth rates of male young together with increased nutritional requirements have been associated with greater sensitivity to reduced maternal condition and food restrictions in most mammals (Clutton-Brock et al. 1985; McClure 1981, Labov et al. 1986, Kraus et al. 2013). For example, undernourished hamster mothers produced female-biased litters (Labov et al. 1986)

Experimental manipulation of offspring sex ratio in marsupials offers an opportunity to test the advantages of producing one sex over the other, especially when performed before major maternal investment has occurred through lactation. Studies of sex allocation based on maternal nutrition are less direct tests of maternal allocation, as it has been shown that even with unlimited food supply, maternal investment does not necessarily increase (Speakman & Król 2005), even under greater imposed demand for milk (increased litter size, Johnson et al. 2001). In his review of the costs of reproduction in small mammals, Speakman (2008) found that milk production appears to be limited by the physiological capacity of the mammary glands and tended to be fairly constant across studies that he reviewed. Lactating mothers did not increase food consumption when their litters were artificially increased, but they did when the same experiment was carried out with low ambient temperatures, suggesting that maternal demands were increased by thermoregulation; (Speakman 2008).

Cross-fostering offspring between mothers did not affect growth or survival of young subtropical antechinuses, confirming the suitability of marsupials for offspring sex ratio manipulation. Confounding factors that may affect offspring growth and survival in placental mammals include exposure to sibling hormones during much of development in utero. This complication is avoided in marsupials. Experimental cross fostering allowed me to test maternal investment ability and to separate this from sex differences in offspring ability to acquire milk (Robert & Schwanz 2011, Monclús & Blumstein 2012).

Although my results were mainly inconsistent with a Trivers-Willard mechanism to explain sex allocation, my experiments did support one of its central assumptions, that sons are more expensive to produce than daughters, and this was consistent across all the models used. Sons grew faster than daughters, and mothers weaning more sons were less likely to survive lactation and breed again than mothers rearing more daughters.

Growth of young is consistent with assumptions of the TWH, but might also reflect sibling competition

Although the mean mass of sons and daughters was not different at ~50 or 60 days old, male offspring were heavier at independence (~100-120 days old). In other species of antechinuses, the difference in mass between male and female offspring is evident close to weaning (Cockburn 1992), when mothers also suffer major weight loss associated with the increased demands of lactation (Fisher & Blomberg 2011). In mammals generally, offspring mass at weaning often correlates with mass of adults (Altmann & Alberts 2005) and consistent with assumptions of the TWH, body sizes in males are correlated with reproductive success and survival in antechinuses and other promiscuous and polygynous mammals (Fisher & Cockburn 2006, Clutton-Brock et al. 1981, Roff 1992, Redondo et al. 1992).

Surprisingly, when young in my experiments were ~60 days old, the smallest of all were male offspring, but only when females outnumbered them. Sons growing in female-biased litters had the slowest growth rates, while their sisters and foster sisters were almost as large and as fast-growing as males growing in male-biased litters. Consistent with assumptions of the TWH, this might suggest that female offspring require less energy to grow despite having smaller mothers. Additionally, in spite of the increased investment ability of mothers rearing male-biased litters (not only they were heavier but they were also more likely to wean their offspring), their daughters (i.e. females growing in male-biased litters) did not grow as big or as fast as their sons. This is consistent with the assumption that female young do not require as much energy to grow as males. In many size dimorphic mammals, females are more able to compensate undernourishment or poor growth rates than males once independent (Töigo et al. 1999, Festa-Bianchet et al. 2000, Gendreau et al. 2005).

In mammals, sons are often more susceptible to harsh environmental conditions (especially resource/food scarcity), and exhibit higher mortality rates than daughters (Clutton-Brock et al. 1985, van Schaik & de Visser 1990). The main explanation has been their greater energetic requirements and fast growth rates compared to females. Sexual size dimorphism is sometimes lost when mothers lack food during reproduction. For example, male hamster pups are usually heavier than female pups throughout lactation, but when mothers experience food restriction during pregnancy and lactation, their daughters become heavier than their sons (Labov et al. 1986). The same has been described in guinea pigs (Laurien-Kehnen & Trillmich 2004). Consistent with this, in my study, male offspring being raised by a mother with a female-biased litter (lighter than mothers rearing male-biased litters) seem to be equivalent to male offspring being raised by an undernourished mother with decreased investment ability.

The extremely slow growth rates, along with the small sizes of sons in female-biased litters in my experiments might also be explained by scramble competition among littermates, rather than maternal investment strategy that depends on litter sex ratios. In eutherian mammals (and in marsupials after detachment from the teat), pups compete energetically for their mother’s teats, pushing their way through their siblings until finding one to latch onto. Therefore, usually larger and heavier offspring have a clear advantage (Bautista et al. 2005). Heavier pups not only reach the teat sooner and grasp the nipple more strongly due to their enhanced motor and competitive abilities (Bautista et al. 2005), but also have greater milk intake and improved milk assimilation (i.e. efficiency in milk conversion into body mass) than their lighter littermates (Rödel et al. 2008a). Heavier pups also seem to be better at maintaining their body temperature, not only due to their relative reduced heat loss (larger body sizes lose less heat through their surface), but also they are usually found in central positions in the huddle group (Rödel et al. 2008a).

Offspring growth rate in my experiment depended both on offspring sex and the sex of littermates. Sons grew bigger and faster when maturing in male-biased litters, while daughters grew bigger and faster in female-biased litters. These differences in growth rate depending on litter sex composition might not only be explained by differential investment by mothers in their daughters and sons (Clutton-Brock et al. 1981, Cameron & Linklater 2000, Robert & Braun 2012), but also by sex-specific differences in energy demands that young impose on their mother, especially in highly sex-biased litters. Dependent offspring are active recipients of maternal provisioning, so their sex-specific efficiency and behaviour at provisioning should also be considered in the context of offspring growth (Ono & Boness 1996). Milk provisioning in sea lions pups depends on their size and not their sex: large offspring received more milk, regardless if they were male or female (Ono & Boness 1996). Scramble competition for access to teat/milk among littermates has also been reported in rabbits, and usually bigger pups are the winners (Bautista et al. 2005).

Increased competition among siblings of opposite sex is also consistent with the results obtained from the models that analysed the effects of litter sex ratio change. Increasing bias had a positive effect on offspring growth while reducing the bias negatively affected offspring growth. These results are interesting and would worth exploring further. To my knowledge, this has not been described before.

Monotocous mothers (those that produce one offspring at a time) could differentially allocate resources to one sex or the other by changing milk composition or production according to the sex of their offspring (Robert & Braun 2012), although it is unlikely that polytocous marsupials would be able to allocate differently to sons and daughters concurrently within a litter. In my study, offspring body mass influenced maternal body mass, but not the other way around, suggesting that dependent offspring influence maternal provisioning, imposing a cost on the mother. This explanation is plausible at the nestling stage in my study and might explain why females were negatively affected by increased numbers of males in their female-biased litters and why males were so small when growing in female-biased litters.

Costs to mothers are predominantly inconsistent with the TWH mechanism

Female antechinuses typically continue to grow during most of the lactation period; for example brown and agile antechinus mothers increase their body mass during the first 70 days of lactation and experience substantial weight loss during the last month, when energetic requirements peak (Cockburn 1994, Fisher & Blomberg 2011). Weight gain during early and mid lactation may be a strategy for mothers to better cope with the great demands of late lactation by increasing energy reserves, especially after the imposed demands of having to raise more sons. Studies on other small mammals support this. In rodents, for example, lactating mothers increase their food intake rate when their provisioning demands were increased by both rearing enlarged litters (Kunkele 2000, Rutkowska et al. 2011) and male-biased litters (Rutkowska et al. 2011). Increased food intake rate is a plausible explanation for this phenomenon and, because during the captivity time, females had access to *ad lib* food daily, I was expecting to observe some weight changes. However, in my experiments, maternal body mass did not vary during mid-lactation when young were between 45 to 75 days old, regardless if mothers were rearing more sons or more daughters. This is also inconsistent with the assumption of the

TWH that daughters cost less to produce than sons. If daughters are effectively cheaper to produce, then mothers with more daughters should be able to devote more energy to their own growth than mothers rearing more sons.

Mothers may adopt alternative strategies to cope with the increased demands of lactation. Mothers, for example, could increase the length of lactation under harsh conditions. Guinea pigs extend their lactation by 40% under food restriction treatments (Laurien-Kehnen & Trillmich 2004) and the same has been observed in deer mice (Perrigo 1990). Mothers could also reduce their litter sizes through cannibalism (König 1989, Perrigo 1990). Several authors have suggested that mothers tend to prioritise their self-maintenance over offspring growth (Leon &Woodside 1983, Perrigo 1990, Rogowitz 1996, Festa-Bianchet & Jorgenson 1998), although the opposite have been found in some altricial species (Dobson & Michener 1995, König 1989). I did not find support for any of these possible explanations, although I am not certain that mothers did not extend their lactation periods, as they were back in the wild at this stage.

High maternal body mass in mammals indicates sufficient body reserves to raise young successfully, and has been associated with improved offspring condition at weaning (Bernardo 1996). Offspring mass gain reflects milk transfer and is often related to maternal weight loss, because mothers use stored energy to maintain lactation (Bowen et al. 2001, Speakman 2008). Large/heavier mothers produce large/heavier offspring compared to smaller/lighter mothers (Wauters et al. 1993, Arnbom et al. 1994, Taillon et al. 2012). The opposite is observed when access to food is restricted during reproduction. In golden hamsters, undernourished mothers produced stunted offspring, and sons were the most affected (Labov et al. 1986). Another example is Laurien-Kehnen and Trillmich's (2004) study of guinea pigs. When these mothers experienced food restrictions during reproduction, they did not seem to be affected directly, as they did not lose weight during lactation. However, young of both sexes were small and had slow growth rates, persisting into adulthood. Lim et al. (2014) performed a meta-analysis and found consistent support across taxa for all of this, describing that within a certain species, maternal size is positively correlated with offspring size and the number of young per litter and a negative correlation between offspring size and the number of young per litter when maternal mass was controlled for. Consistent with this general pattern in small mammals, I found support for this TWH assumption that maternal energy reserves are correlated with offspring growth in the subtropical antechinus during mid-lactation. Offspring growth during this period was strongly associated to maternal mass regardless of their sex. Also consistent with this, larger mothers (with longer feet) were more likely to successfully wean their young.

In my experiments, consistent with their better body condition higher initial body mass (at 45 days old) and higher offspring growth rates, antechinus mothers that were rearing male-biased litters were more likely to wean their offspring than mothers rearing female-biased litters, in both natural litters and those were manipulated to have more sons. Under the TWH, heavier mothers have resources to raise more sons, but this result also appears to contradict the TWH because mothers with added foster-sons should not be more capable of increasing investment than mothers with more daughters. However, these mothers were clearly trading-off between offspring survival and their chances of future reproduction, as this greater maternal investment in their male-biased litters reduced maternal survival and thus their chances to breed again (p=0.07). This is consistent with has been previously reported in other species of antechinuses (Cockburn 1994, Fisher & Blomberg 2011). In those studies, mothers that weaned more sons or had fast growing offspring were less likely to breed a second time. Mothers incurred more of the high cost of raising sons, but less of the lower cost of raising daughters, which was born to a greater extent by offspring. In agreement with the assumption that sons are more costly to produce than daughters, mothers rearing male-biased litters were less likely to survive and breed again. In contrast, mothers rearing female-biased litters had poor quality (small) sons, were more likely to survive to breed again, indicating a trade-off between the cost of son's growth and mother's survival.

Support for the TWH prediction that small mothers have reduced investment ability

Mothers that naturally produced female-biased litters were slightly lighter in mass than mothers producing male-biased litters at 45 days old. However, this difference was not significant in any of the mixed effect models. These results are inconsistent with the TWH premise that mothers produce offspring sex ratio according to their investment abilities.

Mothers with female-biased litters were unable to produce high quality sons. Therefore, it seems that maternal condition is only relevant to sons and not to daughters. This is consistent with findings in experiments on agile antechinuses. Mothers produce male-biased litters when supplementary food is provided and when competition for food is reduced by the removal of competitors (Dickman 1988). This positive correlation between maternal condition and sons' growth and survival has also been reported in other mammals (e.g. Koskela et al. 2004, Isaac et al. 2005, Delean et al. 2009). Overall, fast growth rates of male young together with their increased nutritional requirements have been associated with their greater susceptibility to reduced maternal condition and food restriction in most mammals (Clutton-Brock et al. 1985; McClure 1981, Labov et al. 1986, Kraus et al. 2013).

Rejection of the TWH prediction that mothers cannot rear more high quality males than they give birth to

The key prediction of the TWH is that females give birth to more sons if they can provide them with resources to produce large and competitive adults. My data do not support this mechanism in the subtropical antechinus. Mothers rearing male-biased litters were able to increase allocation of resources to more sons than they gave birth to without compromising offspring growth; sons from mothers in litters manipulated to be more male-biased grew faster, rather than more slowly. In addition, mothers rearing female-biased litters produced daughters that were as heavy as males from male-biased litters, which contradicts the key prediction of the TWH that mothers producing male-biased litters naturally have more to invest than mothers with female-biased litters (Robert et al. 2010). Mothers that reared female-biased litters apparently had similar investment abilities to allocate resources into their female offspring than mothers from male-biased litters, in spite of their reduced body mass (although their sons had stunted growth). This is in part opposite to what was expected according to the TWH. In previous studies that have manipulated sex ratio, heavier mothers that naturally produced male-biased sex ratios were the only ones with the ability to raise large offspring, regardless their sex (Bowers et al. 2013, Robert et al. 2010).

Mothers may also been trading-off energy allocation between current reproduction and self-maintenance, depending on the chance of future reproductive events. In antechinuses, as the chances for a second-year to reproduce a third time are nil, first-year mothers may have been restricting their current reproductive investment in favour of their own survival when rearing a female-biased litter in their first breeding attempt. Consistent with this, mothers that reared female-biased litters had higher chances to survive to breed again than mothers that reared male-biased litters (although the p-value was only marginally significant; p=0.07). In this study, as mothers were kept in captivity with access to food *ad libitum*, giving them the opportunity to increase their maternal investment. In particular, mothers seemed to have increased their investment in sons when rearing male-biased litters and in their daughters in female-biased litters, as if they would only increase investment in their sons when rearing a good group of high quality competitive males – sons in female-biased litters were stunted.

Most studies that have addressed sex allocation in mammals have been carried out in species that produce very small litters (one or two young) such as primates and ungulates (Koskela et al. 2009). Very few have been inconsistent with predictions of the TWH (but see Gedir & Michener (2014), who found that in Richardson's squirrels sons are heavier than daughters at birth and at weaning, but litter sex ratios were not related to maternal body mass or to maternal investment ability). In animals that produce litters rather than single offspring, interpretation of the mechanism is complicated by the fact that mothers might not only control their sex ratios but also litter size (Dusek et al. 2011) and these multiple offspring will have to share maternal resources (Carranza & Polo 2012). In European rabbits and laboratory rats, for example, pup growth was reduced by increasing litter size (Rödel et al. 2008b). The TWH states that mothers with fewer resources should wean more of the cheaper sex (Dusek et al. 2011). Subtropical antechinus mothers in my study did not conform to this prediction of the TWH, because mothers with male-biased litters weaned litters of eight on average (the maximum possible), but mothers with female-biased litters weaned litters of six on average. I found that large litters were male-biased, but small litter sizes were nearly always naturally biased toward females. This is opposite to expectations of the TWH, for example Ryan et al. (2012) found that large litters of ground squirrels tended to be female-biased and sons reared in smaller litters of ground squirrels grew larger than sons reared in large litters, while daughter's growth was not affected by litter size.

Support for the Local Resource Competition hypothesis

The LRCH predicts that lighter mothers were more likely to have male-biased natural birth sex ratios as a way to avoid future competition with their philopatric daughters and improve their own chances of survival to breed again. Heavier, older mothers were more likely to invest heavily in their daughters. Female offspring were more likely to survive to breed if their mother was a second-year mother, which seems to indicate that mothers indeed are able to anticipate future competition with their daughters. Older mothers might be willing to incur in the cost of competition with their daughters because of their advanced age, as they will not survive to breed again (the chances for a female antechinus to breed on third occasion is null) and their daughters may be advantaged as they will be large (Cockburn et al. 2002). In my study, mothers rearing female-biased litters were just as able to raise large, high quality offspring as mothers rearing male-biased litters. Their daughters and sons were as heavy and grew almost at the same rate, regardless of the fact the mothers with more daughters were lighter than mothers rearing more sons initially. However, it is important to consider that in this experimental manipulation, the natural bias of litter sex ratios was increased, decreased or maintained and not reversed. Therefore, mothers that reared male-biased litters may have been good quality mothers with greater investment ability and that the access to *ad libitum* food during mid-lactation may have allowed increase maternal investment on these antechinus mothers.

Litter size seemed to have affected daughters' growth and survival more negatively than sons'. This is opposite to expectations of the TWH. If sons are more expensive to produce than daughters, increasing litter size should have affected son's growth more negatively than daughter's growth. In wild boars, also a polytocous species, only small litters were male-biased and large litters tended to be biased towards females (Servanty et al. 2007). The same has been observed in ground squirrels, in which also sons where heavier when growing in small litters compared to large litters (Ryan et al. 2012). In this study, I did not manipulate the size of the litters and the opposite was observed. Female-biased litters were on average composed of seven young while male-biased litters had on average eight. This, along with the fact that female offspring were more likely to survive (both to weaning and to breed) if their mother was a second-year, may suggest that female antechinuses were actively investing less in their daughters, at least in their first breeding attempt - evidence to support LRCH rather than the TWH.

Competition among adult females for resources such as food and nest sites often has important consequences for survival and reproductive performance (Stockley & Bro-Jorgensen 2011). Consistent with the LRCH prediction that females facing fitness costs of competition from daughters postpone investment in daughters until later in life, mothers reduced investment in offspring in favour of their own survival when large female-biased litters were imposed on them. Offspring of these mothers showed reduced growth and survival with increasing litter size. This may be because mothers traded off investment in offspring for their own survival to a second year of reproduction, and offspring with more competition from littermates (in larger litters) and greater energy needs (males) suffered most.

Conclusion

I tested the main hypotheses to explain sex allocation in subtropical antechinuses, by manipulating the sex ratio bias of litters, in order to test the investment ability of mothers. To my knowledge, this is the first field experiment using cross-fostering to study sex allocation in a wild population of a polytocous marsupial. There has been only one experimental manipulation of sex ratio via cross-fostering in a wild birds (flycatchers, Bowers et al. 2013), which supported only the TWH, as parents that originally produced male-biased sex ratios were the only ones to produce high quality, fast growing offspring. This study is the first experimental rejection of key predictions of the TWH, in conjunction with support for the LRCH in a wild mammal. It would be interesting to explore what happens if litter sex ratios are reversed rather than exaggerated, and how that experimental manipulation affects individuals growth and survival.

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Figures and Tables

Table 1. Regression parameters for offspring growth (offspring mass in g per day) in relation to offspring sex, litter sex ratio and maternal age.

	Slope (g/day)	R^2	F	p
Female offspring	0.19±0.005	0.76	1587	<0.0001
Male offspring	0.20±0.005	0.71	1458	<0.0001
Female-biased litters	0.18±0.004	0.79	1721	<0.0001
Male-biased litters	0.20±0.005	0.70	1455	<0.0001
Females in female-biased litters	0.19±0.005	0.80	1338	<0.0001
Females in male-biased litters	0.18±0.010	0.66	308.3	<0.0001
Males in female-biased litters	0.16±0.009	0.72	312.3	<0.0001
Males in male-biased litters	0.20±0.006	0.71	1129	<0.0001
First-year mothers' offspring	0.15±0.004	0.64	1152	<0.0001
Second-year mothers' offspring	0.21±0.005	0.80	1724	<0.0001

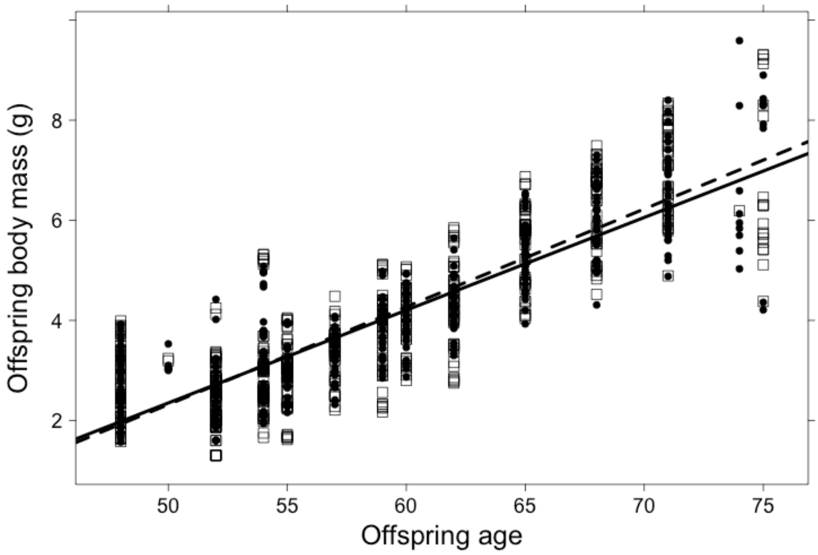


Figure 1. Offspring growth per sex of the young during mid-lactation: females (black dots, continuous line) and males (black open squares, dashed line). See Tables 1, S1 to S3.

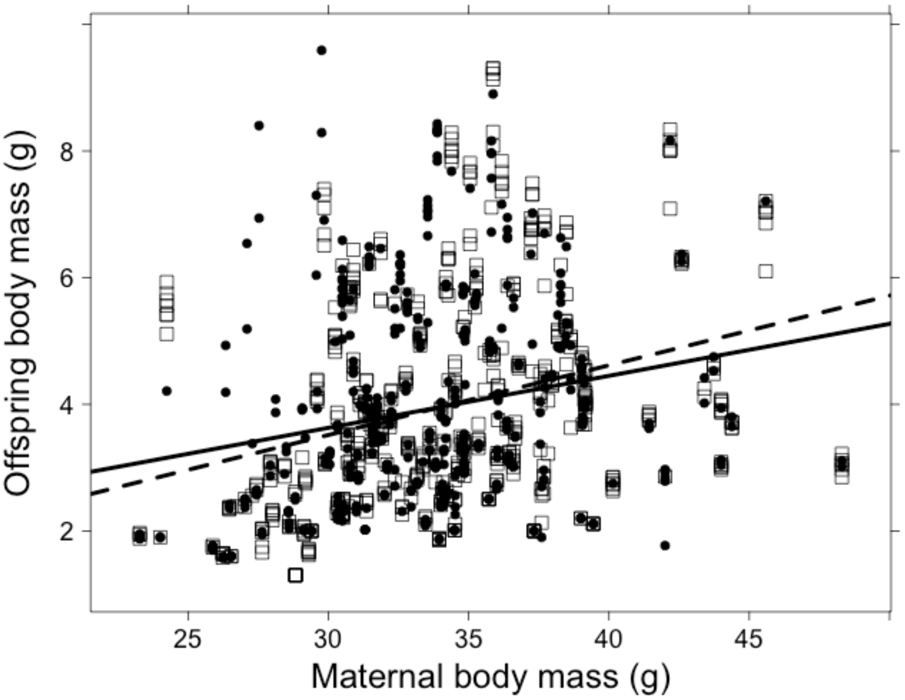


Figure 2. Correlation between offspring body mass and maternal mass per offspring sex: females (black dots, continuous line) and males (black open squares, dashed line) when young were between 45-75 days old (mid-lactation). The effect of maternal mass on offspring body mass was strongly associated in all the models (see Table S1 to S7; t>15, p<0.0001). However, the effect of the interaction between offspring sex and maternal mass was not significant (t=-0.95, p=0.35; Table S1b).

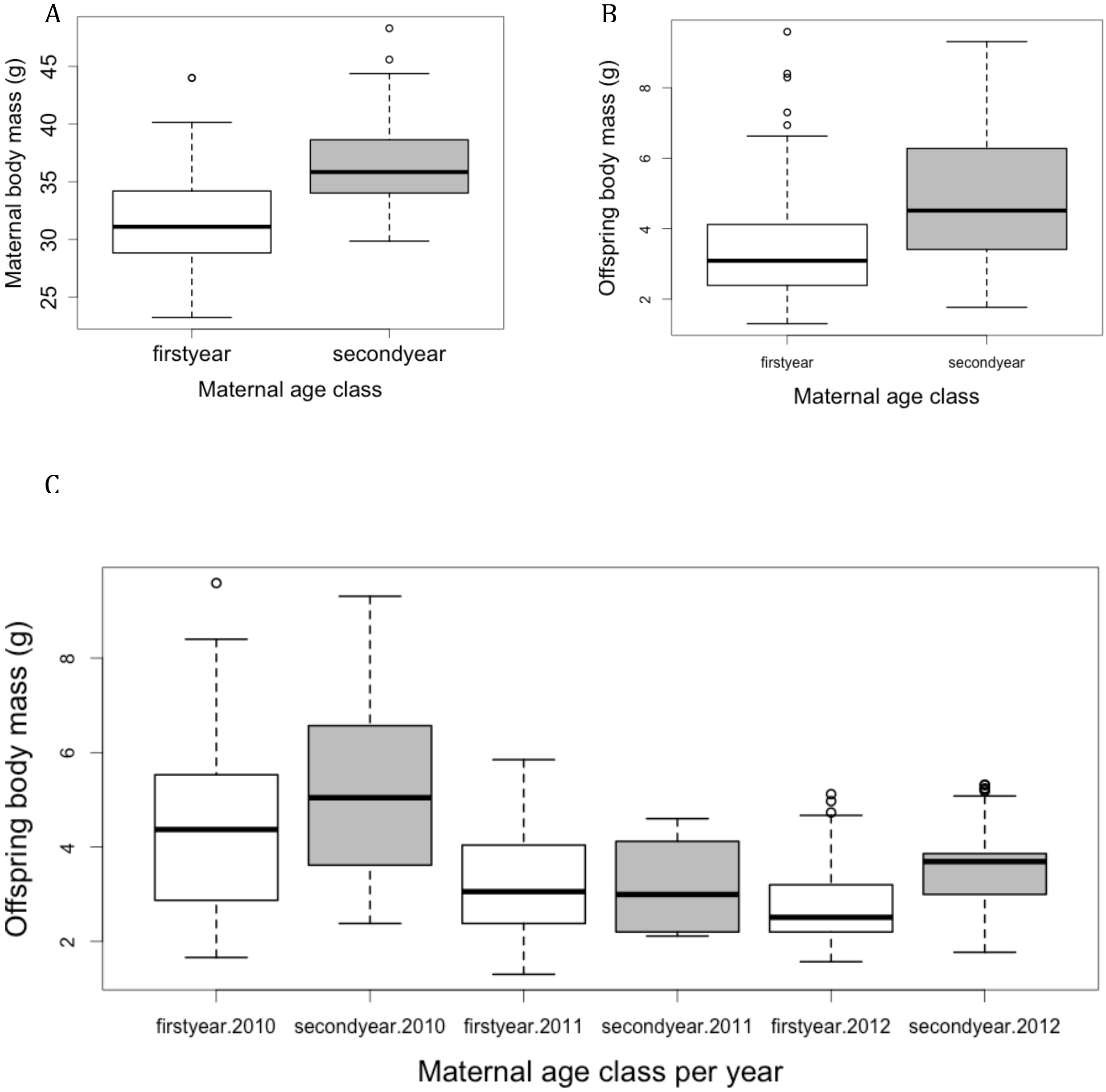


Figure 3. Boxplot showing the effect of maternal age class (first- and second-year females) on (A) maternal mass, (B) offspring mass and (C) its interaction with year on offspring body mass when young were between 45-75 days old (mid-lactation). The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles. See text and Table S6 to S8 for more details in statistical model used.

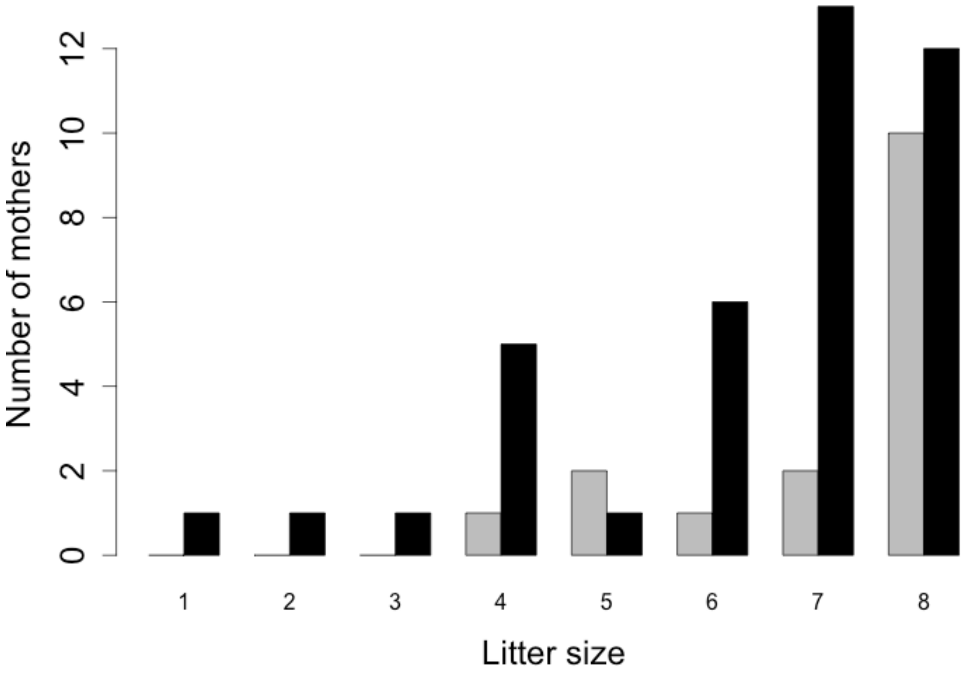


Figure 4. Number of females that were not successful at weaning their young (gray bars) and the ones that did (black bars) per litter size.

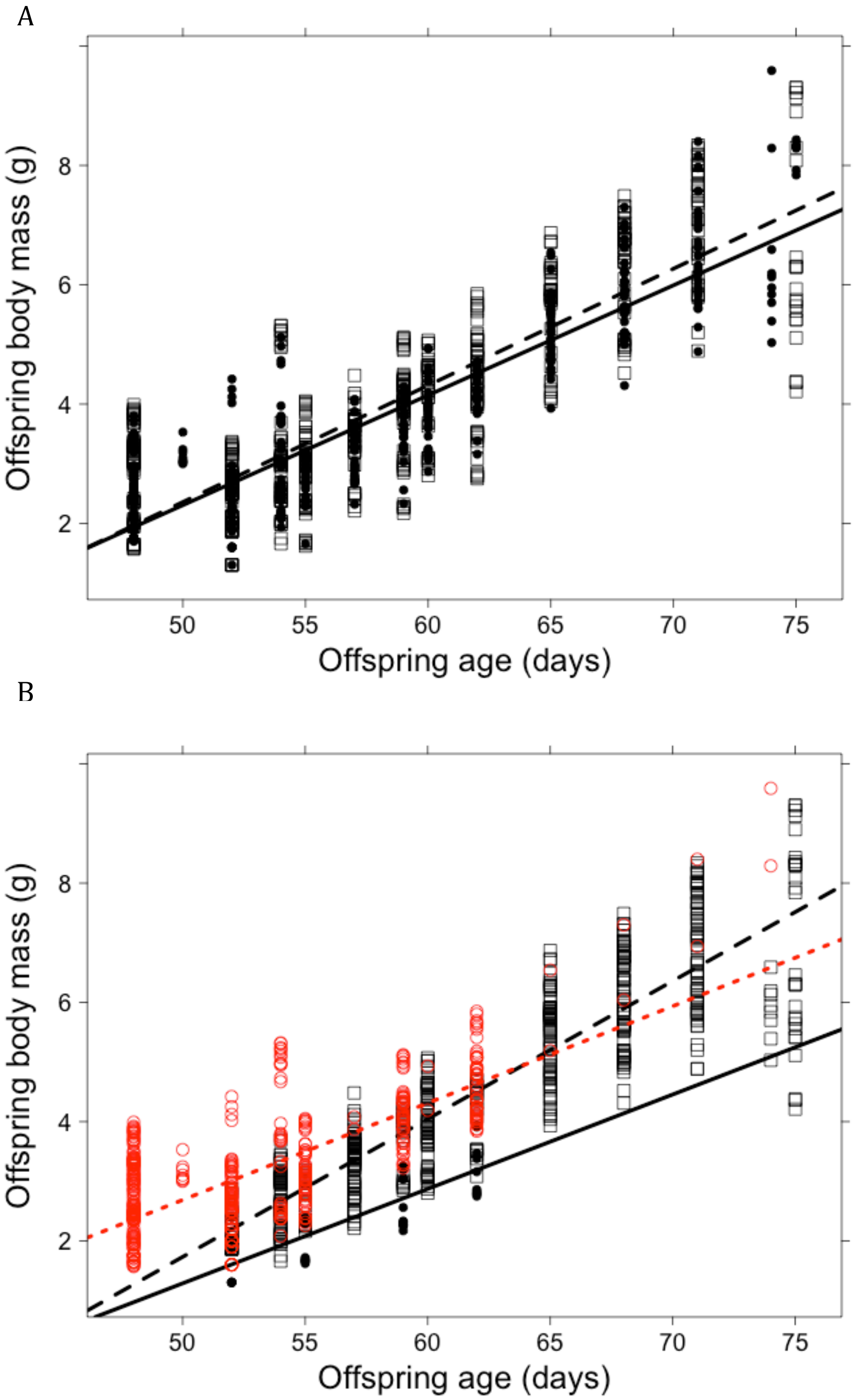


Figure 5. Offspring growth rate during mid-lactation (A) per final litter sex ratio (female-biased litters in black dots with a continuous line and male-biased litters in black open squares with a dashed line) and (B) per litter sex ratio change: decreased natural bias (black filled dots, continuous line), increased bias (black open squares, dashed line) and natural sex ratio bias (red open circles, red dotted line). See Table S2b, S2c.

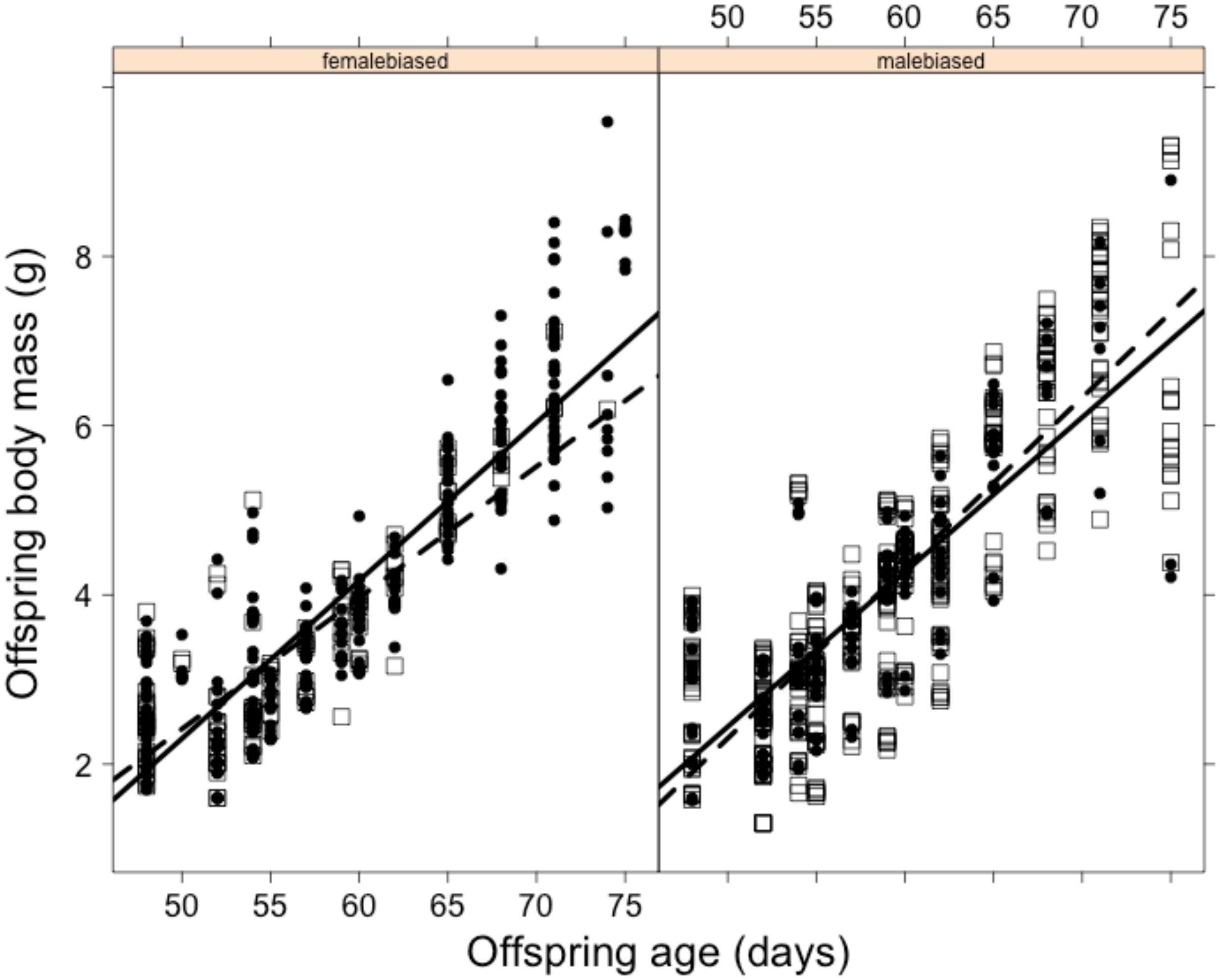
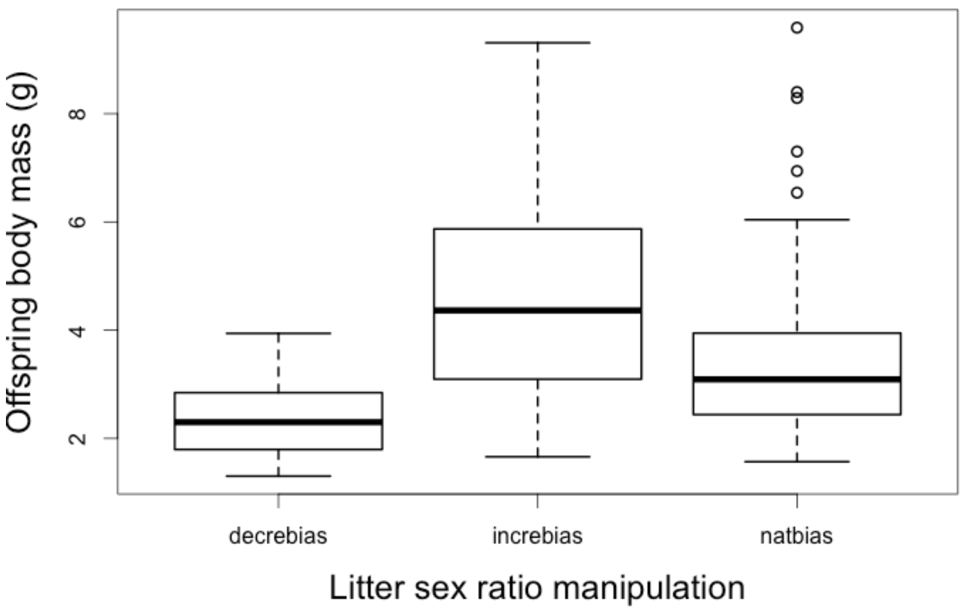


Figure 6. Growth of young during mid-lactation in relation to sex ratio treatment (female-biased litters on left and male-biased litters on right) and sex of the young: females (black dots, continuous line) and males (black open squares, dashed line).

A



B

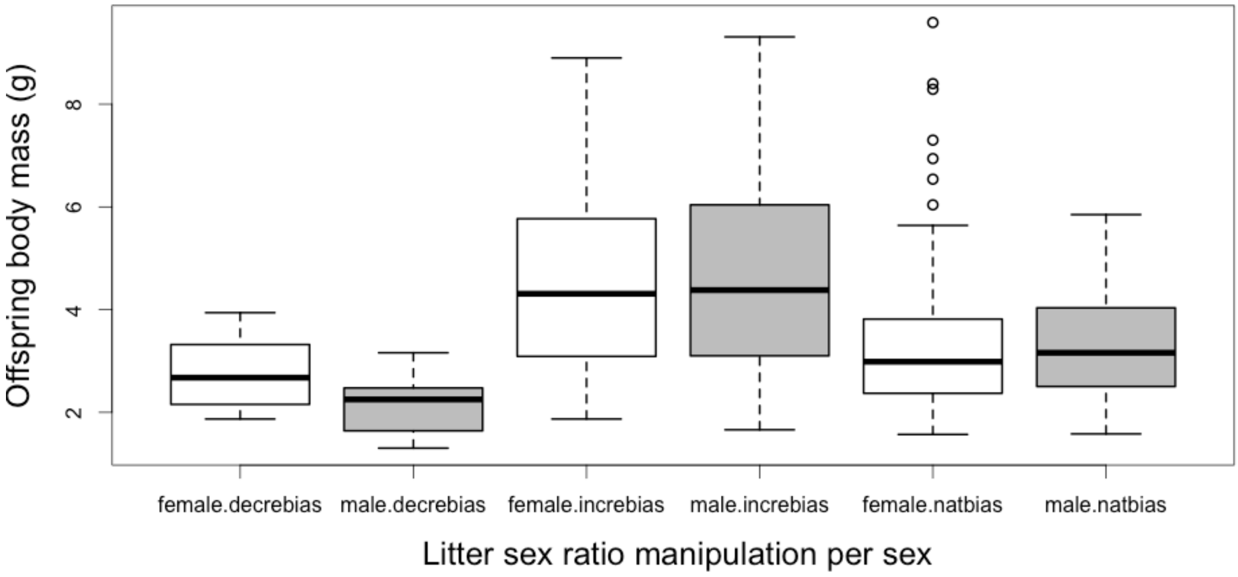


Figure 7. Boxplots showing the effects of (A) litter sex ratio manipulation on offspring body mass (when their natural litter sex ratio was either decreased, increased or maintained as natural) and (B) its interaction with offspring sex (white boxes for females and grey boxes for males) when young were between 45-75 days old (mid-lactation). The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles.

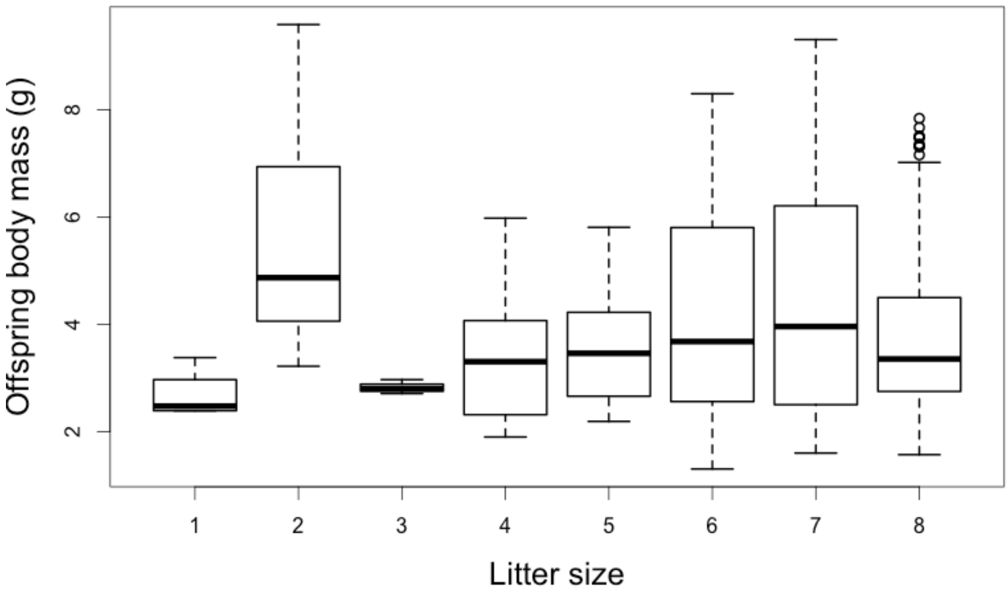


Figure 8. Boxplot showing the effect of litter size on offspring body mass when young were between 45-75 days old (mid-lactation). The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the are outliers represented as open circles.

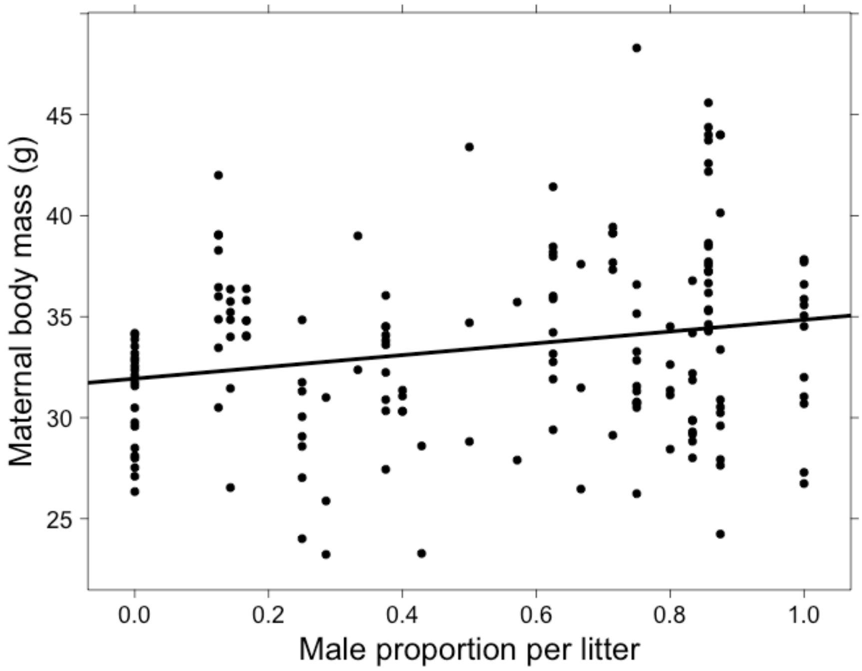


Figure 9. The effect of male proportion per litter on offspring growth when young were between 45-75 days old (mid-lactation).

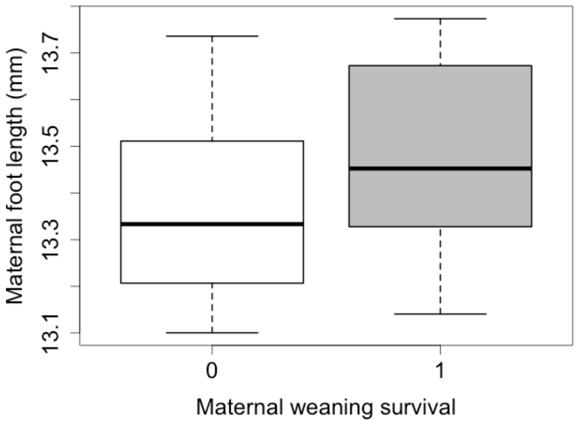


Figure 10. Effects of maternal foot length as a measure of skeletal size and on maternal weaning survival. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the are outliers represented as open circles.

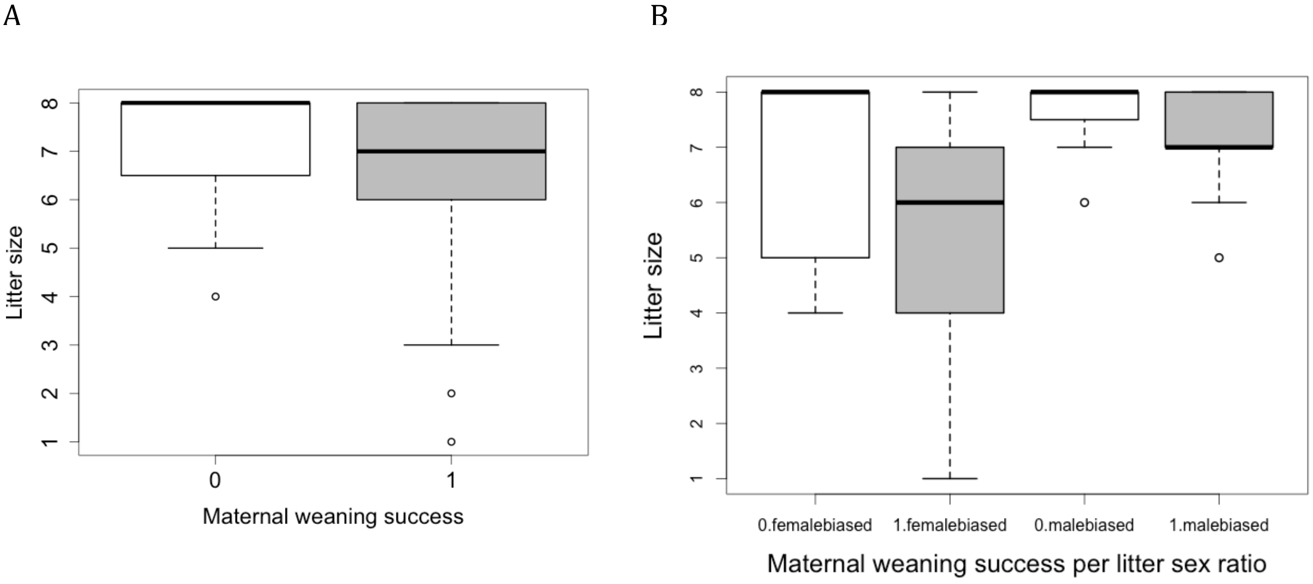


Figure 11. Effect of litter size on (A) maternal weaning success and (B) its interaction with litter sex ratio: white boxes for mothers that were not successful at weaning their young and grey boxes for mothers that weaned their offspring. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles.

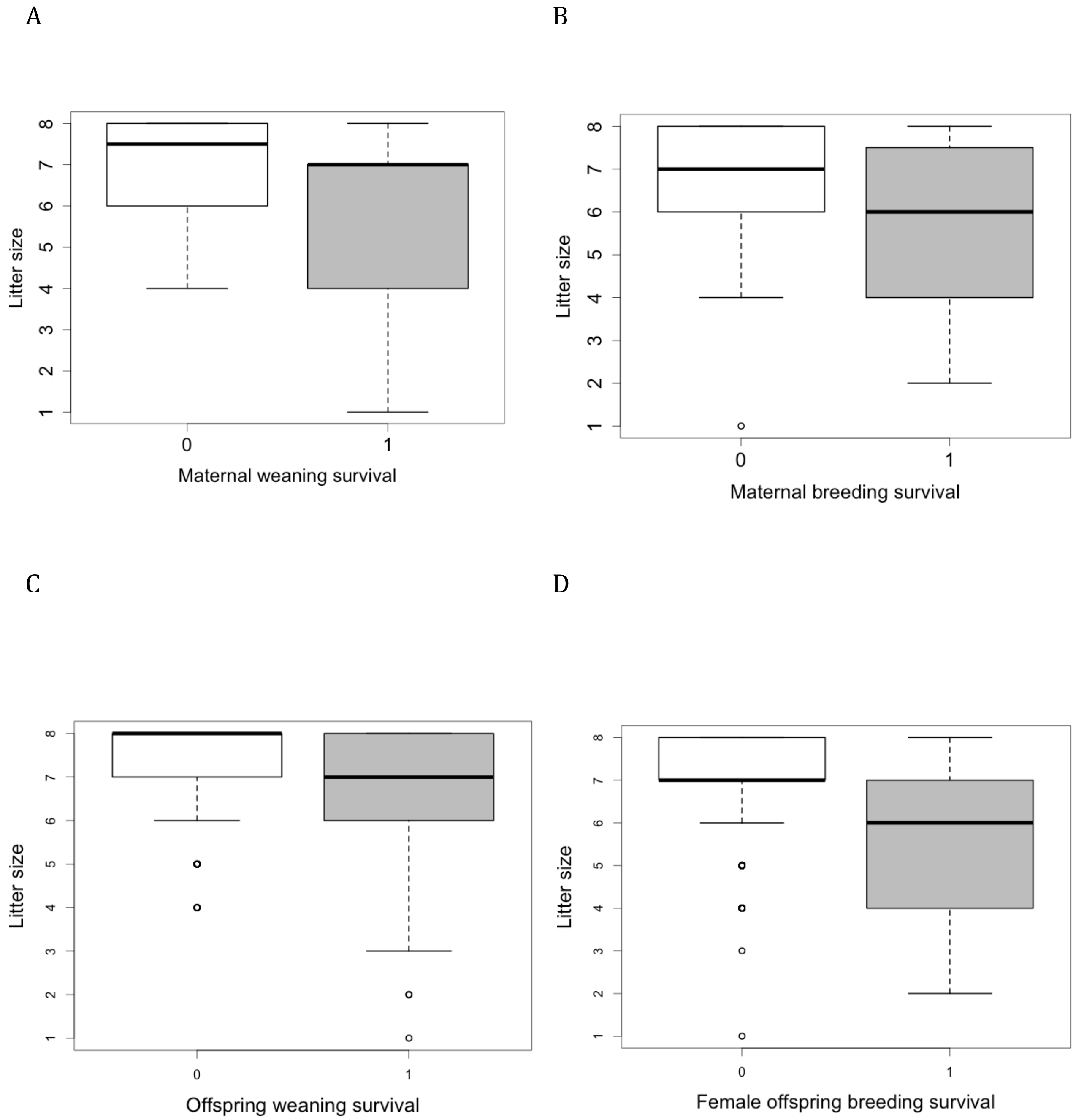


Figure 12. Effects of litter size on (A) maternal weaning, (B) maternal breeding survival, (C) offspring weaning survival and (D) female offspring weaning survival. White boxes for mothers that were not successful at weaning their young and grey boxes for mothers that weaned their offspring. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles.

Supplementary information Chapter 2

Table S1a. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), final litter sex ratio (female- or male-biased litter), maternal age class (if mother was a first- or a second-year female) and litter size. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.66	0.194	-13.69	1.11×10^{-16}
Maternal mass	0.020	0.001	17.24	4.04×10^{-20}
Offspring sex	0.030	0.009	3.48	1.24×10^{-03}
Offspring age	0.052	0.003	16.47	2.01×10^{-19}
Type of offspring	0.001	0.007	0.15	8.79×10^{-01}
Final sex ratio – male-biased	0.511	0.077	6.68	5.19×10^{-08}
Maternal age class	0.271	0.019	14.36	2.24×10^{-17}
Litter size	0.023	0.005	5.09	8.83×10^{-06}
Final sex ratio x offspring age	-0.009	0.001	-6.60	6.89×10^{-08}
Final sex ratio x offspring sex	-0.020	0.011	-1.72	9.38×10^{-02}

AIC: -2217; BIC: -2123, logLik: 1128, deviance: -2331, REMdev: -2255

Table S1b. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), final litter sex ratio (female- or male-biased litter), maternal age class (if mother was a first- or a second-year female) and litter size. To evaluate if there was any sex differences in offspring growth, I included in the model the interaction between offspring sex, age, maternal mass and final litter sex ratio. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.65	0.196	-13.48	1.83×10^{-16}
Maternal mass	0.021	0.001	15.34	2.36×10^{-18}
Offspring sex	-0.057	0.067	-0.85	4.00×10^{-01}
Offspring age	0.052	0.003	16.21	3.49×10^{-19}
Final sex ratio – male-biased	0.579	0.083	7.00	1.71×10^{-05}
Maternal age class	0.272	0.019	14.36	2.25×10^{-17}
Litter size	0.023	0.005	4.88	1.71×10^{-05}
Offspring sex x maternal mass	-0.001	0.001	-0.95	3.51×10^{-01}
Offspring sex x offspring age	0.002	0.001	2.08	4.38×10^{-02}
Final sex ratio x offspring age	-0.009	0.001	-6.92	2.44×10^{-08}
Final sex ratio x offspring sex	-0.019	0.011	-1.65	1.07×10^{-01}

AIC: -2217; BIC: -2123, logLik: 1128, deviance: -2331, REMdev: -2255

Table S2a. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), sex ratio manipulation (decreased, increased or natural bias: if their litter was manipulated by either decreasing, increasing or maintained their natural sex ratio bias, respectively maternal age class (if mother was a first- or a second-year female) and litter size. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.64	0.19	-13.77	9.00×10^{-17}
Maternal mass	0.019	0.001	16.39	2.41×10^{-19}
Offspring sex	0.015	0.006	2.74	9.12×10^{-03}
Type of offspring	0.001	0.008	0.15	8.79×10^{-01}
Offspring age	0.049	0.003	15.23	3.05×10^{-18}
Sex ratio change – Increased bias	0.48	0.045	10.54	4.18×10^{-13}
Sex ratio change – Natural bias	0.47	0.038	12.36	3.05×10^{-15}
Maternal age class	0.134	0.019	7.06	1.56×10^{-08}
Litter size	-0.003	0.005	-0.55	5.85×10^{-01}

AIC: -2338; BIC: -2244, LogLik: 1188, deviance: -2445, REMdev: -2376

Table S2b. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), sex ratio change (SRCH: decreased, increased or natural bias, if their litter was manipulated by either decreasing, increasing or maintained their natural sex ratio bias, respectively), maternal age class (if mother was a first- or a second-year female) and litter size. Three interaction factors between: sex ratio change (SRCH) and offspring age, SRCH and offspring sex and between offspring sex and offspring sex. Random factors for this model were: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-3.519	0.281	-12.52	2.04×10^{-15}
Maternal mass	0.019	0.001	16.27	3.10×10^{-19}
Offspring sex	0.585	0.096	6.07	3.74×10^{-07}
Offspring age	0.064	0.005	12.72	1.23×10^{-15}
SRCH – Increased bias	1.39	0.288	4.82	2.09×10^{-05}
SRCH – Natural bias	1.42	0.270	5.26	5.13×10^{-06}
Maternal age class	0.108	0.018	5.98	5.02×10^{-07}
Litter size	-0.015	0.005	-2.81	7.68×10^{-03}
SRCH-increased bias x age	-0.014	0.005	-2.62	1.25×10^{-02}
SRCH-natural bias x age	-0.015	0.005	-3.06	3.94×10^{-03}
SRCH-increased bias x male	-0.668	0.075	-8.92	4.67×10^{-11}
SRCH-natural bias x male	-0.653	0.075	-8.73	8.39×10^{-11}
Offspring sex x age	0.002	0.001	1.36	1.83×10^{-01}

AIC: -2339; BIC: -2125, LogLik: 1143, deviance: -2387, REMdev: -2285

Table S2c. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), sex ratio change (SRCH: decreased, increased or natural bias, if their litter was manipulated by either decreasing, increasing or maintained their natural sex ratio bias, respectively), maternal age class (if mother was a first- or a second-year female) and litter size as a categorical factor. Three interaction factors between: sex ratio change (SRCH) and offspring age, SRCH and offspring sex and between offspring sex and offspring sex. Random factors for this model were: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-3.07	0.344	-8.90	$4.92\text{x}10^{-11}$
Maternal mass	0.018	0.001	15.60	$1.32\text{x}10^{-18}$
Offspring sex	0.106	0.113	0.94	$3.55\text{x}10^{-01}$
Offspring age	0.059	0.006	10.30	$8.43\text{x}10^{-13}$
SRCH – Increased bias	1.18	0.344	3.41	$1.49\text{x}10^{-03}$
SRCH – Natural bias	1.24	0.288	4.32	$1.02\text{x}10^{-04}$
Maternal age class	0.157	0.021	7.50	$3.84\text{x}10^{-09}$
Litter size N2	0.137	0.105	1.30	$2.01\text{x}10^{-01}$
Litter size N3	-0.018	0.231	-0.08	$9.40\text{x}10^{-07}$
Litter size N4	-0.075	0.081	-0.92	$3.61\text{x}10^{-01}$
Litter size N5	-0.065	0.081	-0.80	$4.27\text{x}10^{-01}$
Litter size N6*	-0.148	0.080	-1.85	$7.16\text{x}10^{-02}$
Litter size N7	-0.248	0.080	-3.12	$3.33\text{x}10^{-03}$
Litter size N8	-0.068	0.080	-0.88	$3.86\text{x}10^{-01}$
SRCH-increased bias x age	-0.013	0.005	-2.13	$3.90\text{x}10^{-02}$
SRCH-natural bias x age	-0.015	0.006	-2.75	$8.81\text{x}10^{-02}$
SRCH-increased bias x male	-0.231	0.101	-2.29	$2.76\text{x}10^{-02}$
SRCH-natural bias x male	-0.213	0.101	-2.10	$4.23\text{x}10^{-02}$
Offspring sex x age	0.002	0.001	2.71	$9.76\text{x}10^{-03}$

AIC: -2323; BIC: -2179, LogLik: 1191, deviance: -2506, REMdev: -2381

Table S3. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: maternal mass (g), offspring sex, type of offspring (natural or adopted offspring), offspring age (days when measurements were performed), final proportion of males per litter, maternal age class (if mother was a first- or a second-year female), litter size and year (2010, 2011, 2012). Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.79	0.154	-18.15	6.47×10^{-21}
Maternal mass	0.020	0.001	17.09	5.56×10^{-20}
Offspring sex	0.018	0.006	3.18	2.85×10^{-03}
Type of offspring	0.003	0.008	0.41	6.83×10^{-01}
Offspring age	0.055	0.003	21.59	1.18×10^{-23}
Male proportion	0.261	0.042	6.24	2.21×10^{-07}
Maternal age class*	0.017	0.029	0.58	5.68×10^{-01}
Litter size	-0.001	0.006	-0.24	8.12×10^{-01}
Year 2011	-0.064	0.025	-2.51	1.61×10^{-02}
Year 2012	0.312	0.038	8.20	4.25×10^{-10}

AIC: -2304; BIC: -2204, logLik: 1172, deviance: -2418, REMdev: -2344

* maternal age class effect is significant if year is not included as factor, just as in the previous two models. The main reason for this to occur is that in 2011 the difference in offspring body mass between first- and second-year females was not observed. See Figure 3.

Table S4a. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: maternal foot length (mm), offspring age (days when measurements were performed), final litter sex ratio (female- or male-biased), maternal age class (if mother was a first- or a second-year female) and litter size. Random factors: (offspring age|mother ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	3.52	1.122	2.88	$6.43x10^{-03}$
Maternal foot length	-0.005	0.092	-0.059	$9.54x10^{-01}$
Offspring age	-0.0006	0.0009	-0.74	$4.65x10^{-01}$
Final litter sex ratio – male-biased	0.054	0.028	1.91	$6.29x10^{-02}$
Maternal age class	0.192	0.031	6.16	$2.82x10^{-07}$
Litter size	-0.002	0.008	-0.25	$8.07x10^{-01}$

AIC: -284; BIC: -252.9, logLik: 152, deviance: -344.4, REMdev: -304

Table S4b. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: maternal foot length (mm), offspring age (days when measurements were performed), original litter sex ratio (f the natural sex ratio of their litters was either female- or male-biased), maternal age class (if mother was a first- or a second-year female) and litter size. Random factors: (offspring age|mother ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	3.40	1.224	2.78	$8.27x10^{-03}$
Maternal foot length	0.026	0.093	0.028	$9.78x10^{-01}$
Offspring age	-0.0006	0.0009	-0.70	$4.86x10^{-01}$
Original sex ratio – male-biased	0.046	0.028	1.70	$9.70x10^{-02}$
Maternal age class	0.190	0.031	6.10	$3.45x10^{-07}$
Litter size	-0.0002	0.008	-0.03	$9.79x10^{-01}$

AIC: -283.2 BIC: -252.1, logLik: 151.6, deviance: -343.6, REMdev: -303.2

Table S5. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: maternal foot length (mm), offspring age (days when measurements were performed), sex ratio manipulation (decreased, increased or natural bias: if their litter was manipulated by either decreasing, increasing or maintained their natural sex ratio bias, respectively), maternal age class (if mother was a first- or a second-year female) and litter size. Random factors: (offspring age|mother ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	2.89	1.34	2.16	3.71×10^{-02}
Maternal foot length	0.032	0.102	0.32	7.52×10^{-01}
Offspring age	0.0005	0.0009	0.54	5.90×10^{-01}
Sex ratio change – Increased bias	0.020	0.079	0.25	8.02×10^{-01}
Sex ratio change – Natural bias	0.098	0.060	1.65	1.06×10^{-01}
Maternal age class	0.181	0.034	5.33	4.17×10^{-06}
Litter size	0.002	0.008	0.25	8.01×10^{-01}

AIC: -284.1; BIC: -249.9, logLik: 153.1, deviance: -350, REMdev: -306.1

Table S6. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: maternal foot length (mm), offspring age (days when measurements were performed), male proportion, maternal age class (if mother was a first- or a second-year female), litter size and year. Random factors: (offspring age|mother ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	1.93	1.28	1.52	1.38×10^{-01}
Maternal foot length	0.098	0.095	1.03	3.08×10^{-01}
Offspring age	0.0002	0.0009	0.22	8.27×10^{-01}
Male proportion	0.044	0.040	1.09	2.81×10^{-01}
Maternal age class	0.240	0.034	7.00	1.87×10^{-08}
Litter size	0.008	0.007	1.07	2.90×10^{-01}
Year-2011	0.136	0.038	3.60	8.67×10^{-04}
Year-2012	0.067	0.040	1.67	1.02×10^{-01}

AIC: -283.3; BIC: -245.9, logLik: 153.6, deviance: -357.1, REMdev: -307.3

Table S7. GLM results for the effects on maternal weaning success (if the mother weaned or not their young) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), sex ratio change (SRCH: for this analysis litters that experienced a decreased in their natural sex ratio bias were not included), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-42.35	30.29	-1.40	0.16
Maternal body mass	0.062	0.094	0.66	0.51
Maternal foot length	3.38	2.269	1.49	0.14
Offspring body mass	-0.35	0.704	-0.49	0.62
SRCH-natural bias	-0.27	1.048	-0.26	0.80
Maternal age class	1.17	1.392	0.84	0.40
Litter size	-0.44	0.249	-1.77	0.076

AIC: 65.98

Table S8. GLM results for the effects on maternal weaning survival (if the mother survive after weaning their offspring) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), sex ratio change (SRCH: for this analysis litters that experienced a decreased in their natural sex ratio bias were not included), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-61.91	27.91	-2.22	0.027
Maternal body mass	0.02	0.083	0.25	0.81
Maternal foot length	5.03	2.15	2.34	0.019
Offspring body mass	-1.03	0.66	-1.56	0.12
SRCH-natural bias	-0.25	0.88	-0.28	0.78
Maternal age class	0.50	1.06	0.47	0.64
Litter size	-0.62	0.23	-2.70	0.007

AIC: 65.98

Table S9. GLM results for the effects on maternal breeding survival (if the mother survive to the following breeding season) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), sex ratio change (SRCH: for this analysis litters that experienced a decreased in their natural sex ratio bias were not included), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-23.51	25.44	-0.92	0.36
Maternal body mass	-0.005	0.086	-0.05	0.96
Maternal foot length	1.85	1.92	0.97	0.34
Offspring body mass	0.22	0.63	0.35	0.73
SRCH-natural bias	-0.31	0.93	-0.33	0.74
Maternal age class	-0.30	1.08	-0.21	0.83
Litter size	-0.39	0.19	-2.00	0.05

AIC: 71.85

Table S10a. GLMER results for the effects on offspring weaning survival (if the young survive for a while after weaning as independent individuals) of the following fixed factors: offspring body mass (g), offspring sex, maternal mass (g), final litter sex ratio (male- or female-biased litter), maternal age class (if mother was a first- or a second-year female)and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	6.15	5.32	1.16	0.25
Offspring body mass	-0.17	1.08	-0.16	0.88
Offspring sex	0.30	0.59	0.52	0.61
Maternal body mass	0.04	0.16	0.27	0.79
Litter sex ratio – male-biased	4.27	1.28	3.33	0.002
Maternal age class*	3.94	2.06	1.91	0.063
Litter size	-1.14	0.48	-2.37	0.023
Sex ratio x maternal age class	-4.78	1.73	-2.77	0.009

AIC: 196.2; BIC: 230.9, logLik: -89.1, deviance: 178.2

Table S10b. GLMER results for the effects on offspring weaning survival (if the young survive for a while after weaning as independent individuals) of the following fixed factors: offspring body mass (g), offspring sex, maternal mass (g), type of offspring (adopted or natural), sex ratio change (SRCH: for this analysis litters that experienced a decreased in their natural sex ratio bias were not included), maternal age class (if mother was a first- or a second-year female)and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	5.17	6.10	0.85	0.40
Offspring body mass	-0.22	1.18	-0.19	0.85
Offspring sex	0.94	0.54	1.72	0.09
Maternal body mass	0.12	0.18	0.66	0.52
Type of offspring	-1.07	0.78	-1.37	0.18
SRCH-natural bias	0.86	1.79	0.48	0.63
Maternal age class	2.52	2.39	1.05	0.30
Litter size	-1.04	0.58	-1.80	0.08

AIC: 205.9; BIC: 240.6, logLik: -93.93, deviance: 187.9

Table S11a. GLMER results for the effects on female offspring breeding survival (if the young survive for a while after weaning as independent individuals) of the following fixed factors: offspring body mass (g), offspring sex, maternal mass (g), type of offspring (adopted or natural), final litter sex ratio (female- or male-biased litter), maternal age class (if mother was a first- or a second-year female), litter size and the interaction between litter sex ratio and maternal age class.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-7.06	4.00	-1.77	0.09
Offspring body mass	-0.03	0.16	-0.19	0.85
Maternal body mass	0.13	0.11	1.16	0.25
Litter sex ratio-male-biased	-1.30	1.46	-0.89	0.38
Maternal age class	4.75	1.94	2.44	0.02
Litter size	-0.39	0.32	-1.25	0.22
Sex ratio x maternal age class	-4.37	2.07	-2.11	0.041

AIC: 277.5; BIC: 311, logLik: -130.7, deviance: 261.5

Table S11b. GLMER results for the effects on female offspring breeding survival (if survived or not to breed) of the following fixed factors: offspring body mass (g), maternal mass (g), sex ratio change (SRCH: for this analysis litters that experienced a decreased in their natural sex ratio bias were not included), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-0.96	2.55	-0.38	0.71
Offspring body mass	-0.005	0.086	-0.05	0.82
Maternal body mass	0.22	0.63	0.35	0.87
SRCH-natural bias	-0.31	0.93	-0.33	0.10
Maternal age class*	-0.30	1.08	-0.21	0.06
Litter size	-0.39	0.19	-2.00	0.006

AIC: 295.4; BIC: 324.7, logLik: -140.7, deviance: 281.4

Table S12. List of all the females captured with their pouch young when they were two to three weeks old in 2010, 2011 and 2012. Mothers and their young were kept in captivity until they voluntarily detach from the teat to be individually sex and marked to then released them back to the wild at the site of capture. Mothers in bold were the ones that were subject to cross-fostering.

Year	Mother	Natural litter sex ratio	Final litter sex ratio	Original litter size	Final litter size	Natural bias	Final bias
2010	**8C58**	3f-5m	0f-6m	8	6	male-biased	male-biased
2010	**6F87**	2f-5m	1f-6m	7	7	male-biased	male-biased
2010	**947E**	2f-5m	1f-6m	7	7	male-biased	male-biased
2010	**D524**	5f-2m	6f-1m	7	7	female-biased	female-biased
2010	**6140**	2f-2m	3f-0m	4	3	unbiased	female-biased
2010	**8032**	2f-6m	1f-7m	8	8	male-biased	male-biased
2010	**8FB0**	6f-1m	7f-0m	7	7	female-biased	female-biased
2010	**7BE5**	4f-3m	5f-2m	7	7	female-biased	female-biased
2010	**8BBD**	5f-3m	7f-1m	8	8	female-biased	female-biased
2010	7953	2f-0m	2f-0m	2	2	female-biased	female-biased
2010	**92F7**	2f-4m	1f-5m	6	6	male-biased	male-biased
2010	**75CA**	2f-5m	1f-6m	7	7	male-biased	male-biased
2011	**B7DF**	4f-4m	5f-3m	8	8	female-biased	female-biased
2011	**C641**	0f-6m	1f-5m	6	6	male-biased	male-biased
2011	8C58	2f-2m	2f-2m	4	4	unbiased	unbiased
2011	**82CE**	4f-0m	3f-1m	4	4	female-biased	female-biased
2011	frf2-rb3	3f-4m	3f-4m	7	7	female-biased	female-biased
2011	BBFF	3f-5m	3f-5m	8	8	male-biased	male-biased
2011	D435	2f-4m	0f-2m	6	2	male-biased	male-biased
2011	**E36F**	3f-5m	3f-5m	8	8	male-biased	male-biased
2011	9887	2f-1m	2f-1m	3	3	female-biased	female-biased
2011	90CA	5f-2m	5f-2m	7	7	female-biased	female-biased
2011	frb4	3f-1m	3f-1m	4	4	female-biased	female-biased
2011	**8339**	3f-4m	2f-5m	7	7	male-biased	male-biased
2011	7BE5	4f-2m	4f-2m	6	6	female-biased	female-biased

2011	**C123**	5f-3m	5f-3m	8	8	female-biased	female-biased
2011	**8691**	0f-1m	1f-0m	1	1	male-biased	female-biased
2011	**7E95**	1f-7m	1f-7m	8	8	male-biased	male-biased
2011	**DE99**	2f-6m	2f-6m	8	8	male-biased	male-biased
2011	frf3-rb4	2f-5m	2f-5m	7	7	male-biased	male-biased
2011	**BBD6**	3f-2m	3f-2m	5	5	female-biased	female-biased
2011	**D899**	3f-5m	3f-5m	8	8	male-biased	male-biased
2011	**A144**	3f-5m	3f-5m	8	8	male-biased	male-biased
2011	**A91E**	1f-4m	1f-4m	5	5	male-biased	male-biased
2011	frf3-rb3	-	-	6	6	undetermined	undetermined
2011	7CEB	-	-	8	8	undetermined	undetermined
2011	6E70	-	-	6	6	undetermined	undetermined
2011	NN1	-	-	7	7	undetermined	undetermined
2011	NN2	-	-	6	6	undetermined	undetermined
2011	7B53	-	-	6	6	undetermined	undetermined
2011	7229	-	-	8	8	undetermined	undetermined
2011	8B71	-	-	7	7	undetermined	undetermined
2012	82CE	3f-5m	3f-5m	8	8	male-biased	male-biased
2012	flb5-rb3	3f-1m	3f-1m	4	4	female-biased	female-biased
2012	flb4-rf2	3f-5m	3f-5m	8	8	male-biased	male-biased
2012	flb4-lf5	-	-	8	8	undetermined	undetermined
2012	flb4-rb3	2f-2m	2f-2m	4	4	unbiased	unbiased
2012	flb3-lb4	-	-	7	7	undetermined	undetermined
2012	flb2-rb3	2f-5m	2f-5m	7	7	male-biased	male-biased
2012	A144	7f-1m	7f-1m	8	8	female-biased	female-biased
2012	3A9D	2f-4m	2f-4m	6	6	male-biased	male-biased
2012	flb3	2f-6m	2f-6m	8	8	male-biased	male-biased
2012	B717	2f-4m	2f-4m	6	6	male-biased	male-biased
2012	NNJ1	2f-6m	2f-6m	8	8	male-biased	male-biased
2012	flb3-rb3	4f-3m	4f-3m	7	7	female-biased	female-biased

2012	frb2	3f-5m	3f-5m	8	8	female-biased	female-biased
2012	44AB	4f-3m	4f-3m	7	7	female-biased	female-biased
2012	frf5	6f-2m	6f-2m	8	8	female-biased	female-biased
2012	20EC	2f-6m	2f-6m	8	8	male-biased	male-biased
2012	flf3	-	-	0	0	-	-
2012	flb2-lf3	6f-2m	6f-2m	8	8	female-biased	female-biased
2012	frf2	2f-6m	2f-6m	8	8	male-biased	male-biased
2012	95F9	5f-2m	5f-2m	7	7	female-biased	female-biased
2012	E056	5f-2m	5f-2m	7	7	female-biased	female-biased
2012	27DA	2f-2m	2f-2m	4	4	unbiased	unbiased
2012	8745	5f-3m	5f-3m	8	8	female-biased	female-biased
2012	46A2	5f-3m	5f-3m	8	8	female-biased	female-biased

Chapter 3

Age-specific reproductive trade-offs in female subtropical antechinuses

Age-specific reproductive trade-offs in female subtropical antechinuses

Abstract

Determining how costs of reproduction vary with age is crucial to understanding life history evolution. Reduced survival or success in future breeding events are the main costs of current reproduction. Such costs are expected to increase with maternal age. Two main non mutually exclusive hypotheses might explain the variation in reproductive effort of individuals as they grow old: the terminal investment hypothesis and the senescence hypothesis. The former predicts that mothers should increase their investment when the chances of breeding again are reduced as they age, and the latter predicts a decrease in maternal reproductive effort due to physiological deterioration with age. In this study, I assessed age-specific trade-offs with reproduction in subtropical antechinus females. Older females increased their maternal investment. Mothers breeding for the second time (in their second year) were able to produce high quality, large offspring that were more likely to survive at the expense of their own survival.

Key words: maternal investment, sex ratio, cross-fostering, terminal investment, senescence, offspring growth, age, subtropical antechinus.

Introduction

The way that individuals allocate limited resources to growth, survival and reproduction is fundamental to their reproductive success (Stearns 1992). Thus, investment in each breeding event results from trade-offs between the high energetic costs of reproduction, offspring quality (size versus number), their own survival and future breeding potential (Williams 1966, Clutton-Brock 1984, Speakman 2008). Due to the extremely high energetic requirements associated with gestation and more importantly with lactation, female mammals are good subjects for studying the costs of reproduction (Hamel et al. 2010).

In female mammals that experience more than one reproductive event in their lifetime (i.e. iteroparous females), current investment in reproduction usually decreases both their future breeding attempts, and survival (Stearns 1992, Speakman 2008). This may be because limited resources such as body fat stores are depleted (Green et al. 1991), or there is a reduced immunological and stress response (Harshman & Zera 2006). The mechanisms may be hormonal regulation interacting with intermediary metabolism that control differential resource allocation, the production of damaging by-products, draining of somatic reserves which might compromise immunological function and decrease protection against stress and toxicity (Harshman & Zera 2006).

There are two main hypotheses to explain the changes in reproductive performance with age in iteroparous females (Weladji et al. 2010): the terminal investment and the senescence hypothesis. Although their predictions are opposite in terms of maternal investment, they are not mutually exclusive and both could operate concurrently in an organism. The terminal investment hypothesis predicts that older mothers should increase their investment in current reproduction when their chances of breeding successfully in the future are reduced or when their mortality risk rises with age (Williams 1966, Clutton-Brock 1991, Stearns 1992). The senescence hypothesis predicts a reduced investment in reproduction in older females due to lack of resources and an overall progressive deterioration of physiological and molecular functions associated with ageing (Kirkwood & Austad 2000, Selman et al. 2012). The duration of lactation and specific life expectancies are thought to determine the timing of such reproductive senescence (Packer et al. 1998).

Several studies with ungulates and pinnipeds have shown that breeding success of females increases as they age (Packer et al. 1998, Coté & Festa-Bianchet 2001) before undergoing reproductive senescence in the last stages of their lives (Kirkwood & Austad 2000). However, most studies have not supported the terminal investment hypothesis, because they have failed to show that improvement in older mothers' breeding success is caused by an increase in their reproductive effort. Instead, in most cases, improved breeding success with maternal age is associated with enhanced parental skills through experience (Cameron et al. 2000, Coté & Festa Bianchet 2001, Weladji et al. 2002, Weladji et al. 2006).

There are many confounding factors that may mask the costs of reproduction and the effects of age on the investment ability of mothers. For example, female mammals may compensate for a decline in reproductive performance as they age by extending the duration of lactation or reducing litter sizes, before reaching complete reproductive cessation at the end of their lives (Packer et al. 1998). Increased body mass, improved access to resources, previous maternal experience (including improved parental skills) or the aquisition of higher social rank with age may also account for increased reproductive success in older mothers (Coté & Festa-Bianchet 2001). Variation in quality of individuals may also conceal the cost of reproduction, as positive correlations between breeding success and survival are also common in female mammals (Clutton-Brock 1984). Maternal investment strongly affects offspring growth and survival during the period of dependency (Mousseau & Fox 1998) and such effects may persist into adulthood (Kerr et al. 2007). However, this is not always the case, and offspring growth, survival and reproduction may be unrelated to maternal allocation (Ylönen et al. 2004). Juveniles may compensate for undernourishment or slow growth rates in their earlier life once they are independent, if they have adequate access to resources (Töigo et al. 1999, Festa-Bianchet et al. 2000, Gendreau et al. 2005).

Most studies on costs of reproduction in mammals have focused on rodents and ungulates (see review in Hamel et al. 2010), and have often yielded ambiguous results due to the many confounding factors affecting life history trade-offs (Nussey et al. 2008). Marsupials are good model species to study the costs of reproduction, as maternal investment occurs mainly through lactation, which is substantially controlled by the mother (Isaac & Johnson 2005, Fisher & Blomberg 2011). Species that have evolved fast life history strategies, such as short-lived mammals, are also particularly appropriate for addressing reproductive costs and trade-offs. Their reproduction is generally less variable compared to their survival than longer-lived animals, suggesting that they maximise their reproductive effort at each breeding attempt. This is expected to favour reproduction at the expense of survival, presumably because they may not have many opportunities to breed again (Hamel et al. 2010).

Antechinuses are small carnivorous marsupials with extreme life history traits (Braithwaite & Lee 1979). Most females breed only once dying soon after weaning their young (ie. most are semelparous) and a minority manage to reproduce a second time, but rarely live for more than two years and never reproduce a third time (Fisher & Blomberg 2011). Therefore, females belong to one of two cohorts: they have either been born in that year or are in their second year of life. The proportion of iteroparous females (second-year females) appears to vary among populations and also species (Cockburn et al. 1983) and to depend on environmental conditions and food abundance (see Chapter 1 for more details).

Previous studies on agile (*Antechinus agilis*; Cockburn 1994) and brown antechinuses (*A. stuartii*; Fisher & Blomberg 2011) have shown opposite results. The first study, on agile antechinuses, found that mothers rearing fast-growing offspring were less likely to survive lactation, suggesting high reproductive costs for such mothers. Cockburn (1994) concluded that second-year mothers were senescent, as they were less likely to survive lactation and produced low quality offspring with low survival rates. In contrast, Fisher and Blomberg (2011), showed that although second-year brown antechinus mothers were less likely to survive lactation and lost much more weight, they produced fast-growing offspring with higher survival rates. This suggests a greater investment ability in older mothers than in younger ones. First-year mothers that produced fast growing offspring were also less likely to survive lactation whereas young mothers with a poorer breeding performance in their first breeding attempt were able to compensate by breeding a second time.

The aim of this chapter is to assess the costs of reproduction in a wild population of subtropical antechinus (*Antechinus subtropicus*) by testing whether the terminal investment hypothesis and/or senescence hypothesis applies to females of this species. The subtropical antechinus is considered to be the largest of the brown antechinus complex, which is composed of four closely related species (in size order from smallest to largest: *A. agilis, A. adustus, A. stuartii*, and *A. subtropicus*); a group also closely related to the yellow-footed antechinus (*A. flavipes*). Agile, brown and yellow-footed antechinuses are the best-studied species of this group (Naylor et al. 2008).

In this chapter, I quantify maternal body mass changes and offspring growth during mid-lactation in captivity and the survival of mothers from this stage until the following mating season, after they were released back to the wild. I compare maternal investment between mothers that were breeding for the first time (first-year mothers) versus mothers breeding for a second time (second-year mothers). The terminal investment hypothesis states that there should be survival or reproductive costs associated with increased investment in current reproduction. Therefore, I expect second-year mothers to have better reproductive performance (large, fast-growing offspring and increased weaning success) than younger mothers, at the expense of maternal growth (older mothers should have greater weight loss during lactation) and survival (older mothers should have a reduced post-weaning survival). In contrast, first-year mothers are expected to have a higher post-weaning survival in their first-breeding attempt and to be more likely to survive to breed again in the following mating season than older mothers (as in brown antechinuses, Fisher & Blomberg 2011). Thus first year mothers should have a reduced breeding performance (smaller, slower growing offspring, and a lower probability of successfully weaning their young), compared to second-year mothers, as a way to maximise their chances of survival. First-year mothers that invest more in their offspring should be less likely to survive lactation than young mothers that are more restrained in their reproductive investment (favouring survival over reproduction). I also expect that offspring from older mothers should be larger and grow faster, have higher survival, and thus should be more likely to reproduce. If the terminal investment hypothesis applies, negative effects of raising a larger litter on maternal growth and survival should be greater in younger mothers.

Methods

Late lactation and weaning in the subtropical antechinus

In this species, the pouch phase lasts for five to six weeks, after which young are no longer attached permanently to a teat, and the nestling phase begins. This final phase of lactation lasts for another seven to eight weeks, during which mothers leave their young in their nest while foraging and come back intermittently for a suckling bout. During the last two weeks of lactation, when young start to explore more outside their nest, is when the highest mortality of young has been recorded in other studies (e.g. Coates 1995). Predation of naive young and other kinds of misadventures occur when young start to explore their surroundings, learn how to forage for themselves, and improve their hunting skills, at the same time as they need to acquire enough energy to support the high energetic demands of growing. Once completely independent, males disperse away from their birthplace and females remain philopatric, sharing home ranges with their mother and sisters (Cockburn et al. 1985, Fisher 2005).

Study site, trapping and husbandry

This field study was carried out at Springbrook National Park using two sites where subtropical antechinuses were relatively abundant. Both sites were at ~900 m above sea level and consist of montane subtropical rainforest with dense understorey, vine tangles with fallen and rotten logs on the ground. A total of 200 Elliot traps were used per night, mainly along a disused track to facilitate inspection. Trap locations, separated by 5-10 m, were permanently marked with flagging tape, and in each spot between one to three traps were placed, depending on the presence of clear runways (Tasker & Dickman 2002). Trapping details are described in the general methods (chapter one).

Seventy six subtropical antechinus females were trapped when their young were two to three weeks old, when the mothers still carry them around constantly (pouch phase). However, only 56 of them were included in this study as the rest died within a few days of being in captivity (12 in 2010, 30 in 2011 and 24 in 2012). I aimed to capture the mothers with their entire litters to determine their natural litter sizes, sex ratios, and to individually mark each one of their offspring, before returning them all back to the wild. Mothers and young were kept in captivity for five to seven weeks (see general methods above for details of husbandry).

During captivity, I measured each animal’s growth (body mass and length) every three to five days. At the beginning, when young were still attached to the teat, mothers were weighed (nearest 0.01 g) with young attached and three pictures were taken with a measuring ruler next to them to calculate the mean crown-rump length for the young and the length of left hind foot of the mother using ImageJ (nearest 0.01 mm) (Rasband 2013). This method was preferred to reduce stress due to excessive manipulation of the animals, after confirming the measurements were the same if using the calipers (data not presented). Once the young detached from the teat, individual measurements of their body mass were taken after young were individually marked. When young were ~62 days of age, a cross-fostering experiment was performed, which consisted in swapping one or two young between some of the mothers in order to study maternal sex allocation (see chapter 1) and benefits of matrilineal social structure. However, the cross-fostering experiment did not affect offspring growth or survival (see chapter 1 and supplementary information). A week after the cross-fostering experiment was performed, when young were ~75 days old, I released the mothers and their young in the wild by strapping their nest-box to a tree at the site of capture. Growth and survival of mothers and their young was determined in the wild, after they were released into their original home ranges two to three weeks before weaning. Around weaning is the period when young antechinuses suffer from the highest mortality rates, according to previous studies. Therefore, it is around this time when differences in survival between treatments are expected to be detected. I monitored this via nest-boxes inspections and trapping sessions performed every month for 3-5 consecutive nights until the following breeding season following the procedures described above. Female antechinuses have stable home ranges with extreme site fidelity (Lazenby-

Cohen & Cockburn 1988, Lazenby-Cohen & Cockburn 1991, Fisher 2005), therefore, detecting and following the fate of females, both mothers and newly weaned young, to monitor their survival and growth is feasible once released back to the wild. In contrast, as males usually disperse away once independent, their fate is uncertain. In this study, 56 mothers with litters were included in the analysis (12 in 2010, 22 in 2011 and 22 in 2012). A total of 256 young (120 females and 136 males) had a first-year mother, while 95 young (47 females and 48 males) had a second-year mother.

Data analyses

I used separate generalized linear mixed effects models (GLMM) to assess the effects of maternal age on growth (in mass) and survival of mothers and offspring. I evaluated the effects of maternal age on weaning success (if the mother successfully weaned their offspring or not), maternal post-weaning survival (if the mother was known to be alive during the first two months after weaning her young) and maternal breeding survival (if the mother survived to breed again the following year, i.e. survived at least six months after young were weaned) and also on offspring growth, offspring weaning survival (if young survived to weaning and were seen alive as independent individuals) and daughter's breeding survival (if there was a difference in their daughters breeding survival. Males disperse once independent, so they fate is uncertain).

Maternal age class (first- or second- year mother), maternal foot length (as a measurement of their skeletal size), offspring age, offspring crown-rump length, offspring sex, litter size (as the number of young per litter can influence maternal investment ability), litter sex ratio treatment (either female- or male-biased), the proportion of males per litter (as a continuous measure of sex ratio bias per litter), manipulation (if natural litter sex ratio was manipulated or kept as natural) and year were treated as fixed factors in the models. I also evaluated the effects of type of offspring (natural or cross-fostered to test whether fostered young were treated differently than natural offspring) on the different models, but in all of them, type of offspring was not significant ($t < 0.05$, $p > 0.9$). Although I performed body mass measurements every three to five days while the animals were in captivity, this was not done at exactly the same age across the three years of this study. Therefore, offspring age as in days when the measurements were done (for both mothers and offspring) along with the identities of mothers and offspring were random factors in the mixed effect models (see Tables S1 to S2). The use of these random factors allowed variation in both intercepts and slopes in the growth curves (as in Chapter 2). I used the R function 'lmer' for body mass analyses of mothers and offspring, while the functions 'glm' and 'glmr' were used for survival analyses of mothers and offspring, respectively (see Chapter 2).

I used different mixed effect models to analyse the effects of individual growth in mass, because the different approaches to classifying litter sex ratio cannot be included at the same time. I used the proportion of males per litter and year together as factors and litter sex ratio (either female- or male-biased litter) and manipulation (if the natural sex ratio of the litter was manipulated or not) together in a separate model. The manipulation was not performed in in 2012, so year can only be included as a factor in the model using male proportion as the measure of sex ratio bias (see Chapter 2). Year effects are not discussed in this current chapter as they are addressed in Chapter 4.

When appropriate, I also used an unpaired t-test (no repeated measures) to compare differences between means, and contingency tables to evaluate differences in survival while controlling for one factor at a time using χ^2 or Fisher test. All statistical analyses were conducted in R Studio (v. 0.98.501 R Development Core Team, 2013) and their results summarised in Tables S1 to S7

Results

If terminal investment is operating, second-year mothers will have better reproductive performance than first-year mothers, at a cost to maternal growth and survival. If so, I predicted that second year mothers should be heavier (a), have large and fast growing offspring (b), have increased weaning success (c) lose more mass during lactation (d), be less likely to survive weaning their offspring (e) and be less likely to survive to the next breeding season (f).

In agreement with the terminal investment hypothesis, older mothers were demonstrated to have a greater investment ability than younger mothers, not only because they were heavier, but they also because they had large, fast growing offspring and were more likely to wean their young than mothers in their first breeding attempt. Unexpectedly, this increased maternal investment of older mothers was not reflected in maternal weight loss during mid-lactation, at least during mid lactation. Also in agreement with the terminal investment hypothesis, first-year females had higher post-weaning survival than second-year mothers, and offspring mass only negatively affected post-weaning survival of older mothers. Accordingly, the higher investment ability of second-year mothers was also reflected on the higher survival rates of their offspring that were more likely to survive to breed than first-year mothers' offspring.

a) Second year mothers were 22% heavier than first year mothers in mid lactation. Second- and first-year mothers weighed on average (mean ± SE) 37.83 ± 1.46 (N = 14) and 31.13 ± 0.59 (N = 42), respectively, when young were 51 days old (*maternal age*: t = 2.45, p = 0.002; see Table S1a).

b) Second-year mothers had larger, heavier offspring (*maternal age class*: t = 6.33, p < 0.0001; Table S2a) and they also grew at a faster rate than offspring from first-year mothers (*maternal age class* x offspring *age*: t = -5.23, p < 0.001; Figure 1; see also Table S2a). When offspring were ~51 days old, their mean body mass was 21% greater if their mother was a second year female: 2.43 ± 0.036 g (N = 256) if their mother was a first-year female and 2.94 ± 0.055 g (N = 95) if their mother was a second-year female (t = 7.51, df = 349, p < 0.0001). Sons grew slightly faster than daughters, regardless the age class of the mother (t > 2.5, p < 0.02. See Tables S2 and Figure 1). There was also a significant effect of sex ratio manipulation affecting offspring body mass (Table S2a), thus I also used a model evaluating the effect of sex ratio change (if the natural sex ratio was increased or maintained. Table S2b). However, its effect was not significant.

c) From 56 mothers studied, 71% (N = 40) weaned their offspring while 29% (N = 16) died along with their young during mid-lactation while in captivity (i.e. before weaning their litter). Second-year mothers were more likely to wean their young than first-year mothers (Fisher test p = 0.047). While 93% of the second-year females weaned their offspring (N = 13 from a total of 14), only 64% of the first-year females were able to wean their litters (N = 27 from a total of 42). Moreover, from the sixteen mothers that died before weaning their young, fifteen of them (94%) were first-year mothers and only one was a second-year female. Surprisingly, the mixed effect model did not find any significant effect other than litter size, which was discussed in Chapter 2 (see Table S3).

d) Regarding maternal weight loss in mid to late lactation for antechinus mothers, my results did not conform with expectations under the terminal investment hypothesis. Maternal body mass did not vary with offspring age, at least during mid-lactation when animals were in captivity, when young were between 45 to 75 days old (Table S1).

However, offspring mass negatively affected the body mass of older mothers. I found strong support for the terminal investment hypothesis in the effects of sex ratio manipulation on individual body mass. Sex ratio manipulation had a positive effect on offspring body mass. Manipulated litters were heavier than natural litters, regardless of the age class of the mother (*manipulation*: t = 3.79, p = 0.0005. Table S2c). Maternal body mass was also positively affected by manipulation, but only in younger mothers. First-year mothers had a higher mean body mass in manipulated litters than unmanipulated litters. In contrast, second-year mothers had a lower mean body mass in manipulated litters than in unmanipulated ones while having the offspring with the highest mean body mass (*manipulation* x *maternal age class*: t = 4.13 p = 0.0002). In agreement with the terminal investment hypothesis, older females used their own body reserves to sustain their offspring growth when their litter sex ratios where increased (Figure 2. Table S1b. See Chapter 2).

e) From the 40 mothers that weaned their young successfully, 55% of them (N = 22) were seen alive within the next two months after weaning their offspring (confirming post-weaning survival), while the rest (N = 18, 45%) died soon after weaning their young. In support of the terminal investment hypothesis, first-year mothers had higher post-weaning survival than older mothers (*maternal age class*: t = 1.98, p = 0.05. Table S4). From those 40 females that weaned their young, fifteen were first-year mothers (68%) and seven (32%) were second-year females. In this study, of the 56 mothers studied, only 14 were second-year mothers and half of them were seen alive after weaning their young.

f) Thirty eight percent of the 15 mothers that weaned their young managed to survive to the following mating season. Eleven were first-year mothers (73%). The four second-year mothers that were seen alive at least during July (mating season occurs in August) represented 30% of the older mothers that weaned their young (none of these females were seen alive after the mating season). However, the model did not find these values to be significant (*maternal age class*: t = 0.96, p = 0.34. Table S5). According to the model I used for maternal breeding survival, the only significant effect was litter sex ratio (t = -2.11, p = 0.04. Table S5). Mothers that raised female-biased litters were more likely to survive to the next breeding season than mothers with male-biased litters. Eleven of 19 mothers that reared a female-biased litter survived to the next breeding season, only four of 21 mothers that reared a male-biased litter did. This result supports the conclusion that producing more sons is costly to the mothers as it reduces their chances of future reproduction (see Chapter 2).

If offspring growth negatively affects maternal survival, then mothers with small offspring or with slow growth rates should be more likely to survive lactation (higher post-weaning survival) and to breed again than mothers with larger, fast growing offspring, especially if the mother is a first-year female. Second-year females were not expected to survive long enough to raise young a third time: no cases of third year females with litters are known in this species or other related species of antechinus. My results support terminal investment in old antechinus mothers. Offspring mass negatively affected post-weaning survival but only in second-year females (*maternal age class* x *offspring mass*: t = -2.03, p = 0.04. Figure 3. Table S4). While first-year mothers that survived for a while after weaning their young had heavier offspring than those young mothers that died soon after the end of lactation, the opposite was observed in second-year mothers. Older mothers that died soon after weaning their young had heavier offspring than those second-year mothers that were still alive within the next two months after weaning their young. This result suggests a high survival cost of producing heavier offspring for older mothers. Offspring mass did not affect maternal breeding survival (t = 1.19, p = 0.23. Table S5).

If terminal investment is operating, I expected that the higher investment in young would mean that offspring from older mothers would be more likely to survive to independence (post-weaning survival) and to reproduce (breeding survival) than offspring from younger mothers. My results match this expectation. While 81% (N = 77 from a total of 95) of young whose mother was a second-year female survived to independence, only a 51% of the offspring from first-year mothers (N = 150 from a total of 256) were seen alive within the next two months after weaning (*maternal age class*: t = 2.20, p = 0.03).

Only 35 individual young managed to survive until the next reproductive event, which is equivalent to only a 10% of the total number of offspring studied. Twenty five of them (71%) were females and only 10 (29%) were males. However, considering that from a total of 164 juvenile females included in this study, only 25 (15.2%) survived long enough to be able to reproduce, the number of male young surviving to breeding age (15% of the 68 with a known fate) appears to be identical to the proportion of females. As males disperse away from their birthplace once independent, I removed them from the breeding survival analyses of offspring, focusing only in the breeding survival of daughters (those ten males had very low re-capture rates and were mainly trapped once or twice sporadically). Daughters from second-year mothers were more likely to survive to reproduce than daughters from first-year mothers (*maternal age*: t = 2.44, p = 0.01). Moreover, female offspring were more likely to survive to breed if they had a second-year mother (*maternal age* x *sex ratio*: t = -2.11, p = 0.04). Fourteen of the 25 daughters that survived to the mating season had a first-year mothers, and eleven were reared by a second-year mother. This is 12% of 117 female young that were raised by a first-year mother, and 23% of 47 female young raised by a second-year mother.

I also expected that litter size should negatively affect younger mothers more than older mothers. This prediction was not upheld. Mothers that were successful in weaning their offspring had a mean litter size of 6.35 ± 0.3 young per litter (median value of seven; N = 40), while the mean litter size for mothers that died during mid-lactation along with their young was 7.13 ± 0.3 young per litter (median value of eight- a saturated pouch; N = 16; *litter size*: t = -2-04, p = 0.04. Table S3). However, this effect did not depend on the mother's age. Because the maximum litter size is eight, this indicates that mothers increased their chance of survival by losing one or two young early in their pouch life.

Litter size did not affect maternal post-weaning or breeding survival (p > 0.1. See Table S4 and S5, respectively).

Discussion

My results do not support the hypothesis that older females are reproductively senescent. As in brown antechinuses (Fisher & Blomberg 2011), but in contrast to the smaller-bodied agile antechinuses (Cockburn 1994), I showed that female subtropical antechinuses have a greater investment ability and an overall improvement in reproductive performance with age. My study supports the terminal investment hypothesis, because females in their second breeding season with no chance to breed successfully again increased their investment, resulting in higher quality offspring. As in the brown antechinus (Fisher & Blomberg 2011), I found evidence that maternal survival costs were closely associated with this increased reproductive investment of older mothers. The increased maternal investment of older breeding females to produce fast-growing large offspring reduced both their maternal mass and post-weaning survival.

Clarifying the costs of reproduction is an essential part of understanding the evolution of life histories. One of the most common costs associated with increased reproductive effort is increased mortality or reduced success in future reproductive events. However, these costs have been difficult to demonstrate in wild populations. A major reason is likely to be the great variability in individual’s abilities to acquire and allocate resources to their different activities and requirements. Furthermore, those abilities will also depend on the circumstances, so a variable environment will also affect resource acquisition performance (Reznick et al. 2000). For example, under certain environmental conditions, some individuals may be better than others at acquiring certain resources or may have access to high quality resources, and hence have more to allocate in all aspects of their life history (Reznick et al. 2000). Subtropical antechinuses are the largest of the four closely related species that form the brown antechinus complex. It may be that their large body size allows them to build more fat reserves when conditions are good and have better endurance to survive longer after lactation, but their relatively great energy requirements might disadvantage larger individuals when food is scarce.

Mothers did not experience any significant weight change during mid-lactation, which is not unexpected as the major weight loss associated with maternal investment in antechinuses is at the end of lactation (Fisher & Blomberg 2011). In other species of antechinus, it has been described that mothers commonly grow during the first 70 days of lactation, regardless of their age (Fisher & Blomberg 2011; Lee et al. 1982). Stress related to captivity may be one explanation for not observing a substantial increase in maternal mass during mid-lactation, especially considering that this species of antechinus seems to be more susceptible to harsh conditions (see Chapter 4).

Second-year mothers were heavier than younger mothers, but their body mass decreased when they were forced to invest more heavily in their offspring by increasing their natural sex ratio bias (see Chapter 2). Sex ratio manipulation, regardless of the age of the mothers, increased mean offspring body mass, and the heaviest young had a second year mother. This suggests that in order to sustain the fast growth rates of their large offspring, older mothers were also using their body reserves. This weight loss suggests that such mothers are allocating their stored fat to their offspring, despite the fact that during this time (captivity) they had access to *ad-libitum* food resources. Depletion of fat reserves, especially during the last stages of lactation is common in females (Green et al. 1991). This is consistent with the physiological costs of reproduction described for small mammals that usually are unable to sustain milk production with current food intake alone (Speakman 2008). In contrast, first-year mothers produced smaller, slow growing offspring and as they were lighter, it seems reasonable to assume that the reason for their slow growing offspring was the lack of sufficient body reserves to support high milk production rates and therefore fast offspring growth rates.

In contrast, first-year females were able to increase their own body mass notwithstanding the increased demands of sex ratio manipulation and still produce heavier offspring than young unmanipuated mothers. This suggests that as in brown antechinus, younger mothers were trading-off between their own growth and current reproduction to favour their own survival over offspring's growth, while older mothers prioritised reproduction over their own growth and self-maintenance. Brown antechinus females in their second breeding attempt can lose up to seven times the weight loss experienced by younger mothers at the end of lactation (Fisher & Blomberg 2011). In my study, animals were released back to the wild at the end of mid-lactation and, in order to let them settle to the new conditions, I did not trap them until around weaning time onwards. Due to great variability in the data obtained and time it takes to trap the target animals once in the wild, it was not possible to evaluate maternal weight loss (this data was mainly used for survival analyses).

If females are in poorer condition or when resources are in short supply, mammals face larger trade-offs between reproduction and their own survival, especially in polytocous mammals with high reproductive demands imposed by lactation (Speakman 2008). Therefore, depending on the chance of future breeding events, mothers should increase their allocation towards their own maintenance (if the chance of breeding again is high) or towards their current reproduction (if the chance is low). In my study, first-year females might have restrained their maternal investment in favour of their own survival, as their chances of breeding another time are higher than for second-year mothers (which never breed successfully a third time). A similar pattern was found in young brown antechinus mothers that reduced investment in their first breeding attempt, and had increased survival enabling them to breed a second time. This compensated for their relatively poor initial breeding performance because lifetime production of offspring was equivalent in mothers that lived for one versus two years (Fisher & Blomberg 2011). These results agree with Cockburn's (1994) suggestion that larger species are more likely to have iteroparous females than smaller ones.

Festa-Bianchet and Jorgenson (1998) also found poor offspring development in ungulates under poor environmental conditions, and noted that it is difficult to determine if reduced offspring growth was caused by maternal restraint or constraint. In my study, this doubt is resolved, as first-year mothers did not take advantage of the increased access to food resources in captivity by increasing offspring growth, they used them to increase their own body reserves when higher demands were imposed on them (sex ratio manipulation). Young mothers may be actively restraining their maternal investment during this time in order to favour their own future survival over their offspring's growth, although late lactation is the most demanding period. In their review of the adaptiveness of maternal effects, Marshall and Uller (2007) argued that it is incorrect to assess the adaptive value of maternal effects based only on outcomes for the offspring. These authors made the observation that maternal effects (i.e. anything that is part of the phenotype of the mother or in her environment that affects the phenotype of their offspring) affect simultaneously both the mother and her offspring, and that ultimately those effects should be more adaptive for the mother than the offspring. This idea would explain why in certain situations maternal effects have a positive effect on the offspring (Bernardo 1996, Mousseau & Fox 1998), but in others they seem to reduce offspring performance (Marshall & Uller 2007). This should be particularly important when mothers expect to have future breeding opportunities and maternal survival is compromised in the current reproductive circumstance, and also when maternal allocation is under control of the mother and not of her offspring, as in marsupials (Isaac & Johnson 2005).

In this chapter, the greater investment ability of older mothers was not only revealed by their heavier, fast growing offspring, but also by their high likelihood of weaning young. First-year mothers were ~30% less likely to wean their young, and often died along with their entire litters before weaning. Moreover, the improved quality of the offspring from second-year mothers was maintained after independence as they were also more likely to survive to the following mating season. These results are consistent with results in female brown antechinuses (Fisher & Blomberg 2011), in which older females produce fast growing offspring with higher survival rates than mothers in their first breeding attempt.

This pattern is opposite to that in agile antechinuses (Cockburn 1994), in which older females appeared to be senescent with poor reproductive performance, and were unable to produce high quality offspring, compared to younger mothers. This might be again due to the difference in size between these two species. The agile antechinus is the smallest species of the complex, while subtropical antechinuses are the largest.

The terminal investment hypothesis implies that there should be survival or reproductive costs associated with the increased maternal investment in older females. My results support this, as younger mothers were more likely to survive the process of weaning their young than older mothers that produced heavier, high quality. I also showed that maternal post-weaning survival of older mothers was negatively affected by offspring body mass, while this was not the case for younger mothers. These results are evidence of the reproductive costs associated with the higher maternal allocation to their second litter in subtropical antechinus mothers of producing high quality offspring, i.e. terminal maternal investment. Although not significant, first-year mothers also had a 16% greater chance of surviving to the next breeding season than older mothers, in agreement with the idea that younger mothers restrict their investment in their first breeding attempt. Females cannot raise a third litter, so they are investing more in their last chance of breeding. The same has been described in brown antechinuses with reduced lactation survival in older mothers associated to their increased maternal allocation (Fisher & Blomberg 2011).

Factors that may have played a role in individual survival include individual quality, variation ability to acquire resources, ability to allocate resources to offspring, and variability in environmental quality, including rainfall (Reznick et al. 2000, Parrot et al. 2007, Descamps et al. 2009). For example, the four second-year mothers that survived, but did not breed, up to a third mating season may have been a subset of high quality individuals (Descamps et al. 2007, Weladji et al. 2008). There were substantial year effects throughout the study (see Chapter four). For example, in 2010 high rainfall was associated with a positive effect on the survival of second-year females. It seems that greater abundance of food with higher rainfall increases the survival of females, especially older ones (Parrot et al. 2007, Chapter four).

Both costs of reproduction and terminal investment are difficult to demonstrate in wild mammals (Fisher & Blomberg 2011). This study confirms that marsupials are suitable models for such investigations of life history evolution because offspring development occurs mainly externally during lactation, under control of the mother (Isaac & Johnson 2005). Second-year females showed greater investment ability, as they produced high quality offspring in their last breeding event. Because of the short lifespan of my model species, variation in reproductive success and survival between individuals in this study ultimately reflect lifetime fitness.

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Figures

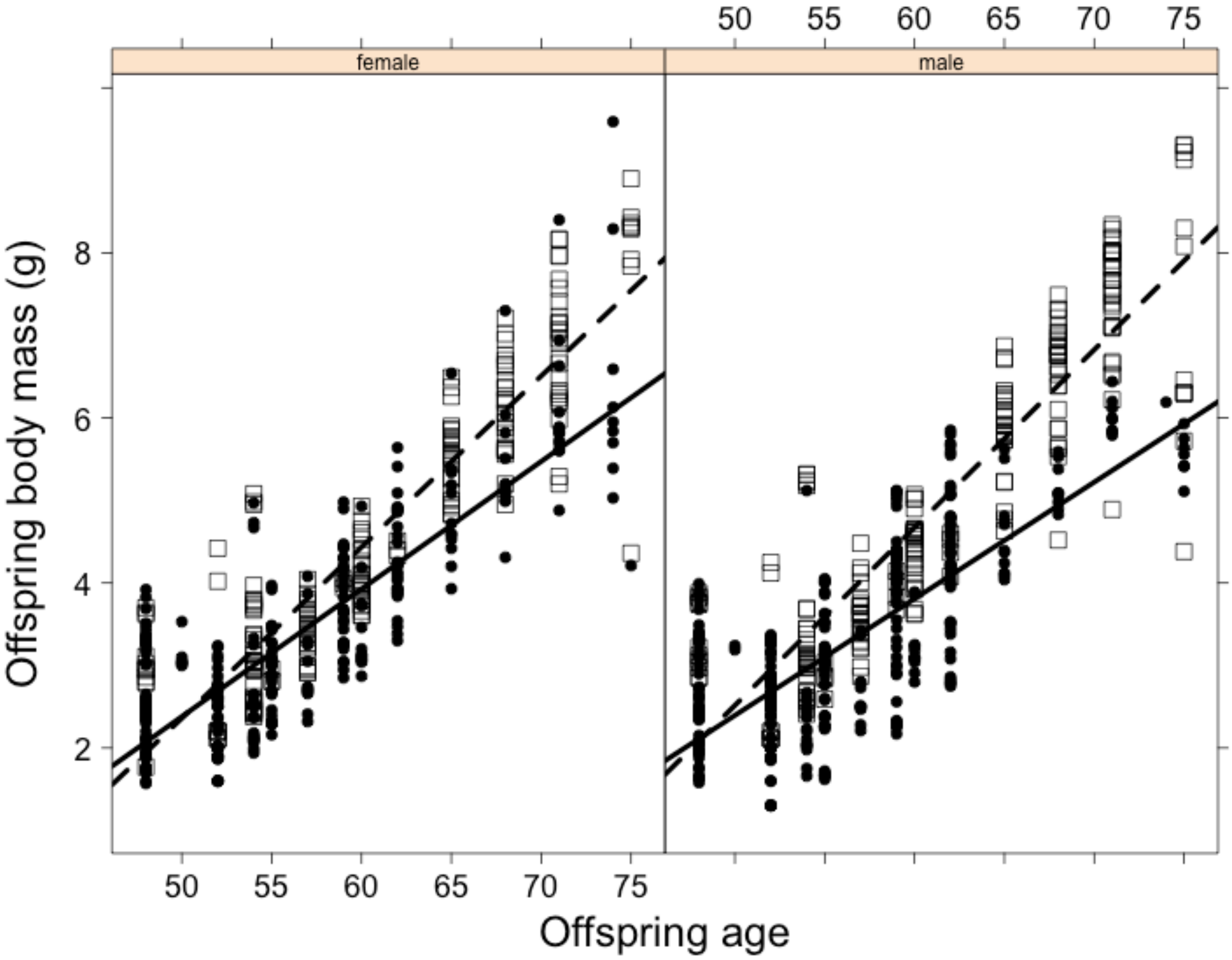
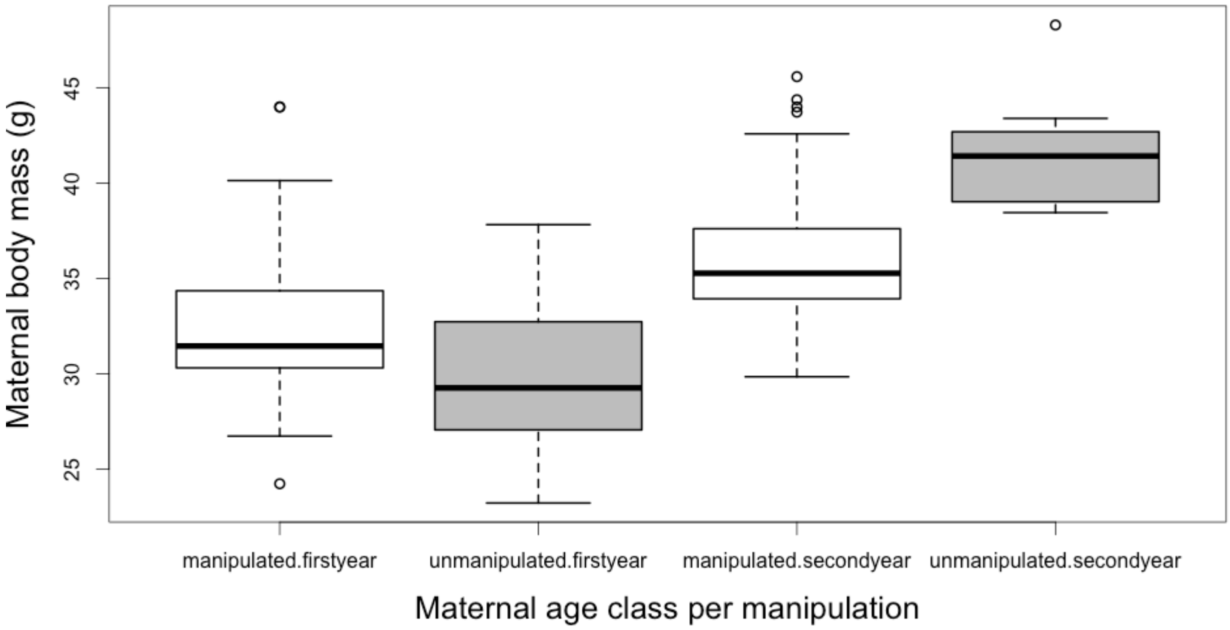


Figure 1. Offspring growth per sex (females on the left and males on the right) and maternal age: offspring of first-year mothers (black filled circles, continuous line) and second-year mothers (black open squares, dashed line). See Table S2 for statistical details.

A



B

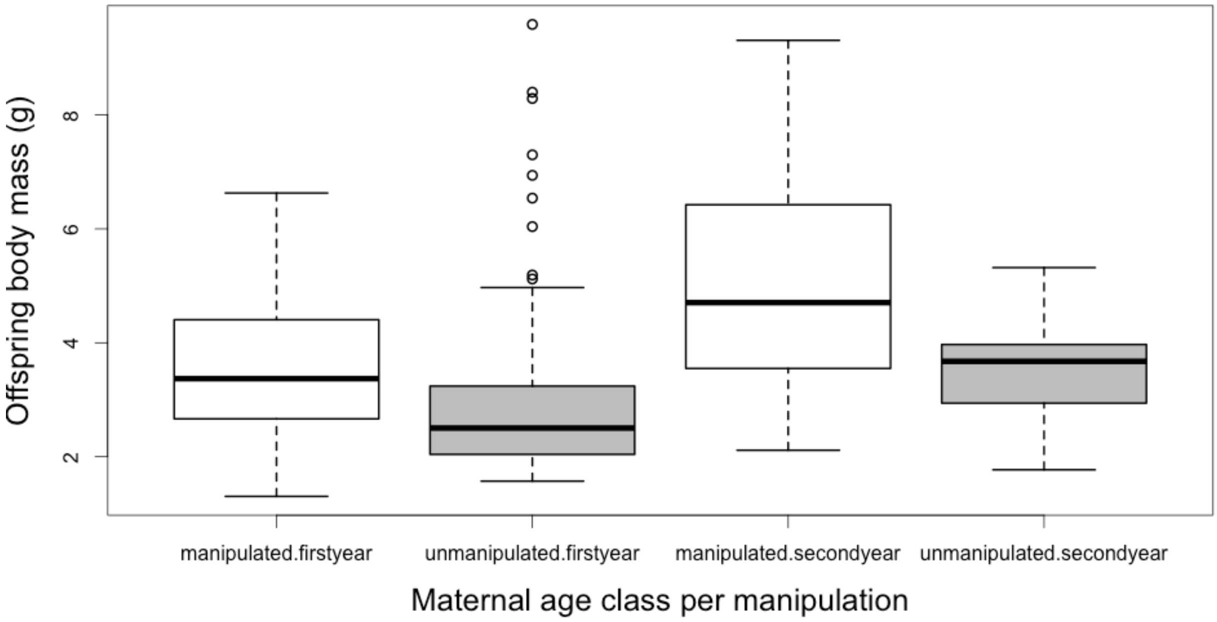


Figure 2. The effect of sex ratio manipulation (white litters were manipulated, grey shaded litters were not manipulated) per maternal age class (first- or second-year mothers) on maternal body mass (A) and offspring body mass (B) when young were between 45-75 days old (mid-lactation). See Tables S1b and S2c, respectively. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles. See text and Table S6 to S8 for more details of statistical model used.

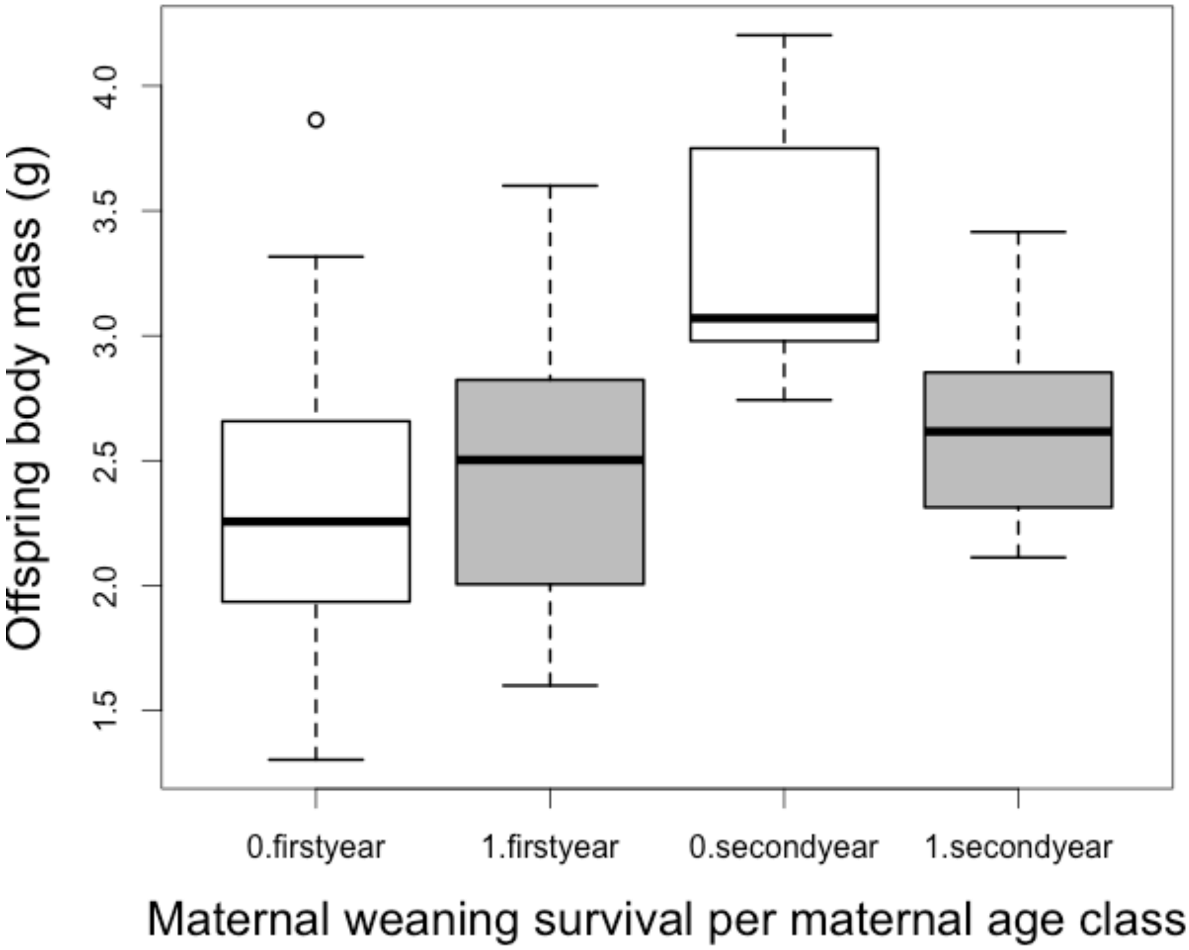


Figure 3. The effect of the interaction between offspring mass during mid-lactation (when young were between 45-75 days old) and maternal age class (first- and second-year mothers) on maternal post-weaning survival. Mothers that died soon after weaning their young are represented in white, while mothers that were seen alive within the next two months after weaning their young are represented in grey. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles. See text and Table S6 to S8 for more details of statistical model. See Table S4.

Supplementary information Chapter 3

Table S1a. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), maternal foot length (mm), maternal age class (if the mother was a first- or a second-year female), litter size (1 to 8), the proportion of males per litter, year (2010, 2011, 2012) along with the interactions between maternal age class with offspring age and litter male proportion. Random factors: (offspring age|mother ID).

Factor	Estimate	Std. Error	t	p
Intercept	1.94	1.28	1.51	0.14
Offspring age	0.0005	0.001	0.40	0.69
Maternal foot length	0.097	0.096	1.02	0.32
Maternal age class	0.27	0.11	2.45	**0.02**
Litter size	0.008	0.008	1.02	0.31
Male proportion	0.04	0.05	0.82	0.42
Year 2011	0.14	0.04	3.50	**0.001**
Year 2012	0.07	0.04	1.63	0.11
Maternal age x offspring age	-0.0006	0.002	-0.34	0.73
Maternal age x male proportion	0.014	0.09	0.14	0.89

AIC: -265.6; BIC: -222.1, logLik: 146.8, deviance: -357.3, REMdev: -293.6

Table S1b. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), maternal foot length (mm), maternal age class (if mother was a first- or a second-year female), litter size (1 to 8), final litter sex ratio (if litter was either female- or male-biased), manipulation (whether the litter was manipulated or not) along with the interactions between maternal age class with offspring age, litter sex ratio and manipulation. Random factors: (offspring age|mother ID).

Factor	Estimate	Std. Error	t	p
Intercept	2.75	1.18	2.32	0.025
Offspring age	-0.0006	0.001	-0.47	0.64
Maternal foot length	0.056	0.09	0.63	0.53
Maternal age class	-0.001	0.13	-0.01	0.99
Litter size	-0.002	0.007	-0.19	0.85
Sex ratio change	0.071	0.036	1.98	0.05
Manipulation	-0.089	0.037	-2.42	0.02
Maternal age x offspring age	0.001	0.002	0.66	0.51
Maternal age x sex ratio	-0.011	0.064	-0.17	0.86
Maternal age x manipulation	0.253	0.061	4.13	0.0002

AIC: -272.3; BIC: -228.8, logLik: 150.2, deviance: -365.2, REMdev: -300.3

Table S2a. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), offspring sex, maternal mass (g), maternal age class (if mother was a first- or a second-year female), litter size, sex ratio change (if the natural sex ratio of litters was increased or maintained) , interactions between maternal age class, offspring age, and sex ratio change. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID). Litters that experienced a decrease in their natural sex ratios were not included in this analysis.

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.33	0.17	-13.55	$1.56\text{x}10^{-16}$
Offspring age	0.05	0.002	20.50	$7.90\text{x}10^{-23}$
Offspring sex	0.01	0.008	2.52	$1.59\text{x}10^{-02}$
Maternal mass	0.02	0.001	14.85	$7.28\text{x}10^{-18}$
Maternal age class	0.52	0.16	3.18	$2.88\text{x}10^{-03}$
Litter size	-0.007	0.006	-1.11	$2.74\text{x}10^{-01}$
Sex ratio change – natural bias	0.008	0.03	0.24	$8.13\text{x}10^{-01}$
Maternal age x offspring age	-0.006	0.002	-2.60	$1.29\text{x}10^{-02}$
Maternal age x sex ratio change	-0.05	0.04	-1.22	$2.31\text{x}10^{-01}$

AIC: -2234; BIC: -2140, logLik: 1136, deviance: -2344, REMdev: -2272

Table S2b. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), offspring sex, maternal mass (g), maternal age class (if mother was a first- or a second-year female), litter size (1 to 8), the proportion of males per litter, year (2010, 2011, 2012) and interactions between maternal age class and offspring age, sex ratio change. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.79	0.16	-17.84	$1.21x10^{-20}$
Offspring age	0.05	0.003	20.88	$4.04x10^{-23}$
Offspring sex	0.02	0.006	3.18	$2.83x10^{-03}$
Maternal mass	0.02	0.001	16.81	$9.92x10^{-20}$
Maternal age class	0.02	0.16	0.15	$8.89x10^{-01}$
Litter size	-0.001	0.006	-0.19	$8.49x10^{-01}$
Male proportion	0.29	0.05	6.27	$1.99x10^{-07}$
Year - 2011	-0.06	0.03	-2.51	$1.61x10^{-02}$
Year - 2012	0.31	0.04	7.17	$1.10x10^{-08}$
Maternal age x offspring age	0.0004	0.003	0.15	$8.82x10^{-02}$
Maternal age x male proportion	-0.05	0.04	-1.34	$1.89x10^{-01}$

AIC: -2296; BIC: -2192, logLik: 1169, deviance: -2419, REMdev: -2338

Table S2c. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), offspring sex, maternal mass (g), maternal age class (if mother was a first- or a second-year female), litter size, litter sex ratio (if litter was either female- or male-biased), manipulation (whether the litter was manipulated or not) and interactions between maternal age class and offspring age, litter sex ratio and manipulation. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-3.07	0.22	-14.13	3.84×10^{-17}
Offspring age	0.05	0.003	17.20	4.44×10^{-20}
Offspring sex	0.02	0.006	3.64	7.67×10^{-04}
Maternal mass	0.02	0.001	15.78	9.04×10^{-19}
Maternal age class	0.97	0.15	6.33	1.63×10^{-07}
Litter size	0.03	0.005	5.07	9.48×10^{-06}
Sex ratio	0.06	0.02	2.80	7.75×10^{-03}
Manipulation	0.26	0.07	3.79	4.97×10^{-04}
Maternal age x offspring age	-0.01	0.002	-5.23	5.64×10^{-06}
Maternal age x sex ratio	-0.04	0.03	-1.58	1.22×10^{-01}
Maternal age x manipulation	-0.23	0.07	-3.07	3.83×10^{-03}

AIC: -2245; BIC: -2140, logLik: 1143, deviance: -2369, REMdev: -2287

Table S3. GLM results for the effects on maternal weaning success (if the mother weaned their young) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), maternal age class (if mother was a first- or a second-year female), litter size (1 to 8) and litter sex ratio. No random factors were used for this model.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-40.81	31.43	-1.30	0.19
Maternal mass	0.02	0.10	0.23	0.82
Maternal foot length	3.32	2.36	1.41	0.16
Offspring mass	-0.26	0.77	-0.34	0.73
Maternal age class	4.02	6.29	0.64	0.52
Litter size	-0.50	0.25	-2.04	0.04
Sex ratio	1.06	0.78	1.36	0.17
Maternal age class x offspring mass	-0.85	2.07	-0.40	0.68

Table S4. GLM results for the effects on maternal post-weaning survival (if the mother was seen alive in the wild within the next two months after weaning her young, when young were 100-120 days old) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), maternal age class (if mother was a first- or a second-year female), litter size (1 to 8), the proportion of males per litter, year (2010, 2011, 2012) and interactions between maternal age class and offspring body mass and maternal foot length. No random factors were used for this model. This analysis excludes all the mothers that died during lactation along with their young in captivity.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-74.68	34.48	-2.17	0.03
Maternal mass	-0.003	0.12	-0.02	0.98
Maternal foot length	5.92	2.64	2.24	0.03
Offspring mass	-0.22	0.86	-0.26	0.80
Maternal age class	9.75	4.92	1.98	0.05
Litter size	-0.47	0.32	-1.46	0.14
Sex ratio	-1.52	0.92	-1.66	0.10
Maternal age x offspring mass	-3.50	1.72	-2.03	0.04

Table S5. GLM results for the effects on maternal breeding survival (if the mother survived after weaning their offspring to the following breeding season) of the following fixed factors: maternal mass (g), maternal foot length (mm), offspring body mass (g), maternal age class (if mother was a first- or a second-year female), litter size (1 to 8), the proportion of males per litter, year (2010, 2011, 2012) and interactions between maternal age class and offspring body mass and maternal foot length. No random factors were used for this model. This analysis excludes all the mothers that died during lactation along with their young in captivity.

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-23.55	29.90	-0.79	0.43
Maternal mass	-0.02	0.11	-0.20	0.84
Maternal foot length	1.70	2.22	0.77	0.44
Offspring mass	0.96	0.80	1.19	0.23
Maternal age class	3.72	3.87	0.96	0.34
Litter size	-0.06	0.24	-0.24	0.81
Sex ratio – male-biased	-1.87	0.89	-2.11	0.04
Maternal age x offspring mass	-1.61	1.39	-1.16	0.25

Table S6. GLMER results for the effects on offspring weaning survival (if the young survive for a while after weaning as independent individuals) of the following fixed factors: offspring body mass (g), offspring sex, maternal mass (g), litter sex ratio (male- or female-biased litter), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	6.15	6.51	0.95	0.35
Offspring body mass	-0.61	1.24	-0.49	0.63
Offspring sex	0.78	0.65	1.19	0.24
Maternal body mass	0.04	0.19	0.21	0.83
Maternal age class	5.57	2.54	2.20	0.03
Litter size	-1.14	0.56	-2.06	0.05
Litter sex ratio – male-biased	4.58	1.55	2.96	0.005
Maternal age class x sex ratio	-5.80	1.99	-2.91	0.006

Table S7. GLMER results for the effects on offspring weaning survival (if survived or not to breed) of the following fixed factors: offspring body mass (g), maternal mass (g), litter sex ratio (male- or female-biased litter), maternal age class (if mother was a first- or a second-year female) and litter size.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-7.06	4.00	-1.77	0.08
Offspring body mass	-0.03	0.16	-0.19	0.85
Maternal body mass	0.13	0.11	1.16	0.25
Maternal age class	4.75	1.94	2.44	0.01
Litter size	-0.39	0.32	-1.25	0.21
Litter sex ratio – male-biased	-1.30	1.46	-0.89	0.37
Maternal age class x sex ratio	-4.37	2.07	-2.11	0.04

AIC: 277.5; BIC: 311, logLik: -130-7, deviance: 261.5

Chapter 4

Environmental constraints and their effects on maternal investment in subtropical antechinuses

Environmental constraints and their effects on maternal investment in subtropical antechinuses

Abstract

Subtropical antechinuses are extremely sensitive to changes in environmental conditions and their effects will depend on the severity and timing of those events. In particular, I show that the population dynamics of these rainforest specialists is strictly driven by rainfall. Their growth and survival depends on the seasonal predictability of arthropod abundance, and the disruption of those annual prey cycles may have dramatic consequences in their abundance and overall population sizes. Because antechinuses synchronise their breeding season to match the end of lactation and weaning with the annual peak of rainfall, their growth, reproductive success and survival greatly depends on the timing those rainfall events. Low rainfall when is needed the most, during lactation and at the time of juvenile independence, significantly affected their growth and their survival and the whole population was drastically reduced. High rainfall during lactation increased both maternal and offspring weaning survival as well as offspring growth, while high rainfall around and after weaning increased their breeding survival. I also show that the conditions experienced during development early in life have profound impacts on their future performance, as those traits acquired persist into adulthood. In this study I present evidence that confirms the high vulnerability of subtropical antechinuses to climate change.

Keywords: Climate change, maternal investment, sex allocation, offspring growth, extinction risk, subtropical antechinus.

Introduction

Mammal habitats around the world have been experiencing changes in weather conditions as a consequence of human-caused global warming (Preston & Jones 2006). Currently, a quarter of all mammal species are at risk of extinction and more than half of all mammal populations are in decline (Ceballos et al. 2005, Davidson et al. 2009). Climate change is considered to be one of the most severe and widespread threats to the survival of species (Walther et al. 2002, Thomas et al. 2004, Isaac 2009, Cahill et al. 2012); however, our understanding of the proximate causes of biodiversity declines due to climate change is very limited (Isaac 2009, Cahill et al. 2012). Extinction risk varies according to the life history of the species and other ecological factors, and is higher for species that have narrower requirements, small or restricted areas of distribution or are adapted to extremely stable environments (Isaac 2009).

With climate change, drought events are likely to increase in frequency and intensity, and catastrophic events such as wild fires and tropical cyclones are also expected to occur more frequently, as well as extraordinary events of intense rainfall or extreme heat waves that may have dramatic consequences for the ecosystem. Such events may have severe effects on natural populations of animals (Welbergen et al. 2008, Recher et al. 2009). It is likely that species with different ecological traits and life histories may be affected, react, recover or adapt differently (Parrott et al. 2007, Jiguet et al. 2007, Recher et al. 2009). Declines in body mass, survival and in some cases overall population size are some of the effects that drought may cause in wild mammals (Lunney 1987, Fisher et al. 2001, Rhind & Bradley 2002, Parrott et al. 2007). Increased droughts are expected, especially during winter in the southern regions of Australia, during spring along the east coast and during autumn in the western regions (CSIRO & BOM 2007). According to several authors, the combined effects of drought, overgrazing, and the introduction of predators such as cats and foxes are responsible for causing shifts in vegetation structure and the decline and extinction of small to medium size mammals in inland Australia during the last two centuries (reviewed by Johnson 2006).

Variation in environmental conditions has been suggested as one reason why evidence supporting sex specific allocation theories can vary between populations, years and species (see Chapter 2), and a reason why costs of reproduction and life-history trade-offs in wild populations of animals are often difficult to observe (see Chapter 3). Individual quality varies in populations of animals, along with their ability to acquire resources. A difference in the ability of individuals to acquire food, high quality nest sites and other resources might be absent in a good year, and it might be exacerbated in a bad one. This effect is thought to be the main reason of why life-history trade-offs are so difficult to demonstrate in wild population of animals (Reznick et al. 2000). Changes in environmental conditions (alternating between benign and hostile conditions) can also explain some components of life history variability in animals, because the same life history response at the same life stage may not always be adaptive (Reznick et al. 2000).

Some carnivorous marsupials, such as antechinuses (*Antechinus* spp.), have evolved extreme life histories in which all males die after the single, extremely short and highly synchronized breeding event that occurs each year (Braithwaite & Lee 1979), during which males mate promiscuously with as many females as possible (Fisher et al 2006). Most females breed only once and die soon after weaning a single litter of young, but a small proportion of females survive to breed a second time in the following mating season (Wood 1970). This proportion is between 8-20% depending on the species; for subtropical antechinuses (*Antechinus subtropicus*) the proportion of second year females in an earlier study was ~17%, (Fisher et al. 2013). Lactation is long in marsupials compared to placental mammals (Tyndale-Biscoe & Renfree 1987) and for antechinuses it lasts for ~4 months; this is particularly long for a small, short lived mammal. Males live for 11 months and females between 16 and 20 months (Braithwaite & Lee 1979, Lee & Cockburn 1985). Species with short life spans are presumed to be favoured in constant, stable environments, and are more likely to be affected by drastic environmental changes (Benton & Grant 1996; Jiguet et al. 2007).

Most species of antechinus inhabit highly seasonal, predictable habitats in the southern regions of Australia. They synchronize their annual short mating season, which is triggered by the rate of change in photoperiod (McAllan et al. 2006) to match the end of lactation and weaning with the annual peak of insect abundance that follows the seasonal pattern of rainfall (Braithwaite & Lee 1979). The mating season occurs on predictable dates that vary with latitude for each population (McAllan et al. 2006). These insectivorous marsupials usually breed in winter, lactate during spring and wean their young at the beginning of summer, when the abundance of arthropods reaches its maximum. Their reproductive success and survival relies on this period of resource abundance (Lee et al. 1982). The energetic requirements for a lactating mammal are the highest during late lactation, especially for predatory species rearing large litters, and at the time of juvenile independence (weaning and few more weeks after that), when juveniles are most vulnerable and inexperienced at hunting. At this time, high abundance of prey should maximise their survival (Speakman 2008, Fisher et al. 2013). Depletion of fat reserves, especially during the last stages of lactation, are common in females (Green et al. 1991).

Their extreme and highly synchronous life history and their great dependence on seasonally predictable resources make antechinuses vulnerable to extreme changes in rainfall, which in some cases may even lead to complete population collapses (Parrott et al. 2007). The overall richness and abundance of arthropods depends greatly on rainfall, reaching a maximum in wet and warm conditions, and declining rapidly in drier conditions regardless of season (Recher et al. 1996, Majer et al. 2003, Recher et al. 2009). Invertebrate communities are highly affected by changes in environmental conditions, and the lack of rainfall during a drought event produces a decline in abundance and richness of species (Strehlow et al. 2002). In dry years, some species of arthropods may even disappear completely (Bell 2006). In addition to making it difficult for individuals to find sufficient food, a shortage of food is also likely to increase intra-and inter-specific competition, and may increase predation risk, because animals must hunt for longer to find enough food in unfavourable conditions and thus are exposed to predators for longer.

If food shortages occurs when a high abundance of food is needed the most (during lactation and weaning), the consequences may be dire for antechinus populations (Parrott et al. 2007). Food availability limits maternal investment ability, so lactating mothers may not be able to sustain lactation, reducing the chances of survival for both the mother and her young (Braithwaite & Lee 1979). When access to supplementary food is provided, mothers increase their body mass and are able to produce large, fast growing offspring (Dickman 1989). In the brush-tail phascogales (*Phascogale tapoatafa*), another semelparous insectivorous marsupial closely related to *Antechinus*, drought caused poor maternal condition, reduced growth of young, reduced body sizes and increased mortality of lactating mothers, delayed male dispersal, increased production of female-biased litters, reduced sexual size dimorphism and increased communal nesting, apparently because huddling conserved energy (Rhind 2002, Rhind & Bradley 2002, Rhind 2003). As a consequence, populations declined severely in all the sites studied and took at least two years to recover in one of them, while in another area studied, phascogales were still absent five years later (Rhind & Bradley 2002). Although it is clear that droughts can seriously impact populations of small dasyurids, authors have called for more studies to better understand the extent of the impacts of drought at the community and ecosystem level, as well as the ability of these species to recover from droughts, especially with the increasing rate of climate change now occurring around the world (Krajewski et al. 2000, Oakwood et al. 2001, Fisher et al. 2013).

It has been suggested that the insectivorous antechinuses are more seriously and more rapidly affected by drier conditions, especially compared to the sympatric omnivorous bush rats (Recher et al. 2009). Recher and colleagues attributed this phenomenon to their diets, as insects decline more rapidly in dry conditions than does vegetation. Parrott et al. (2007) evaluated the effects of a severe drought in three sympatric species of small mammals: two different antechinuses that differ greatly in body size, habits and breeding season (*A. agilis* and *A. swainsonii*) and bush rats that breed all year round (*Rattus fuscipes*). The agile antechinus is the smallest of all the antechinus species, with scansorial and ground dwelling habits, and usually nests in tree hollows, while the dusky antechinus is the largest of the genus, a ground dweller with fossorial habits that consumes soil arthropods. Surprisingly, only the agile antechinus was severely affected by the drought. Drought reduced their body mass, survival (especially of lactating females) and litter size. In contrast, the larger dusky antechinuses and bush rats were not affected at all by the drier conditions in that year (study lasted for three years). These contrasting results highlight the importance of timing as well as severity when considering the effects of climate change on different species. The period of low rainfall, during that particular drought, coincided with pregnancy and lactation in the agile antechinuses, while the breeding season of the dusky antechinuses occurred during a higher period of rainfall, earlier in the year.

The aim of this study was to determine the effects of rainfall pattern during the breeding season of subtropical antechinuses (*A. subtropicus*). In particular, I aimed to evaluate the effects of changes in rainfall during lactation and weaning on maternal investment ability and survival of this rainforest-restricted insectivorous marsupial. Subtropical antechinuses are the largest of the brown antechinus complex and are considered to be abundant within their restricted distribution in south-east Queensland. Data for this study were collected during three consecutive years that showed extreme variation in amount and timing of rainfall in relation to the breeding season of this species. Low rainfall during lactation and weaning is expected to reduce maternal condition and mother’s investment ability and should be reflected in the body mass and survival of both mothers and offspring. In particular, I expected that low rainfall during the months of lactation (October to January) to have strong negative effects on maternal investment ability that should be reflected in the following:

a) Reduced maternal body mass during lactation, but increased weight gain when access to resources was artificially increased during captivity. Younger mothers should be most affected than second-year mothers (in agreement with Chapter 3).

b) Reduced maternal weaning success as mothers should be less likely to wean their offspring, especially small, younger mothers (in agreement with Chapter 3).

c) Reduced offspring growth, with sons being more affected than daughters (in agreement with Chapter 2).

d) Reduced maternal post-weaning survival. Mothers should be less likely to survive lactation (maternal weaning survival) and to breed again (maternal breeding survival).

I also expected that low rainfall at weaning (end of lactation - January) and during the short time afterwards (when offspring start to fend for themselves living as newly independent juveniles) to strongly affect offspring survival:

e) Low rainfall in January-February should reduced offspring survival, both as newly weaned juveniles (offspring weaning survival) and their chances to survive to the following breeding season (offspring breeding survival). Sons should be more affected than daughters (in agreement with Chapter 3).

Methods

Site, study animal and husbandry

This study was carried out at the Springbrook Plateau section of the Springbrook National Park, which is located around 100km south of Brisbane in the Gold Coast Hinterland in south-east Queensland, Australia (-28.23ºS, 153.28ºE) between August 2010 and August 2013. Springbrook is part of the Gondwana Rainforests of Australia World Heritage with a subtropical rainforest located at relatively high altitude (~900 m above see level). The climate is subtropical, with maritime influence due to its proximity to the coast, frequent cloud immersion and fog events caused by the relatively high altitude and the interactions between topography (vertical cliffs) and canopy rainforest. Maritime influence is thought to increase rainfall up to a 40% (Hutley et al. 1997). The site is characterised by warm and wet summers and cool and dry winters with the autumn and spring as intermediates. Due to the relatively high altitude, Springbrook Plateau is consistently around five to ten degrees colder than adjacent lowland, and annual rainfall can reach up to 3000 mm in the higher altitudes, which is around six times higher than the national annual rainfall in Australia (~500 mm). The plateau is the wettest area in mainland Australia outside the wet tropics region of north Queensland, and Tasmania.

The subtropical antechinus (*Antechinus subtropicus*) is a small carnivorous marsupial from the family Dasyuridae, native to south-east Queensland and north-east New South Wales, where it is restricted to small areas of subtropical vine rainforests. This species is the largest of four very closely related species known as the brown-antechinus complex (Menkhorst & Knight 2001), that formerly were considered as a single species with different morphs, but today are recognise as four different species (by ascending order: *A. agilis, A. adustus, A. stuartii, A. subtropicus*; Dickman et al. 1998, Van Dyck & Crowther 2000, Crowther et al. 2003). The best-known species are *A. agilis* and *A. stuartii* (Naylor et al. 2008). These forest dwelling insectivorous marsupials nest communally in tree hollows and other cavities (Lazenby-Cohen & Cockburn 1991) and forage solitary in stable home ranges opportunistically hunting arthropods (Hall 1980) and consuming at least ~60% of their body mass daily (Nagy et al. 1978).

Antechinuses at Springbrook mate in late August, give birth at the end of September, and wean their offspring during early-mid January. January and February are usually the months with the highest rainfall at this location (according to mean patterns over the last three decades, Figure 1B). Females with their pouch young were trapped in mid October each year, when young were around two to three weeks old and strictly attached to the teat, to make sure that mothers were caught with their entire litters. Mothers and their young were kept in temporary captivity (~7 weeks) until the young voluntarily detached from the teat (at 5-6 weeks old) and were able to be sexed and individually marked by toe-bud clipping (Fisher & Blomberg 2009). Mothers were microchipped for individual recognition (Trovan, ID-100 transponder, 11 x 2.2 mm or 7 x 1.25mm). Once all individuals were permanently marked, I swapped some young between litters (the cross-fostering experiment) to manipulate litter sex ratio and assess maternal sex allocation (Chapter 2) and the costs of reproduction (Chapter 3) in this species. Two to three weeks after, mothers and young were released back to the wild at the site of capture. During their time in captivity, mothers were housed individually with their offspring (as they do in the wild) in plastic enclosures (45 x 35 x 20 cm, of clear polyurethane) with wire mesh lids, in which a wooden nest-box lined with leaf litter and shredded paper, an exercise wheel and an inverted drip water bottle was provided (as in Fisher & Blomberg 2009). A mixture of beef and kangaroo mince, wet cat food, soaked dog kibble (~40g in total) was provided daily as well as live insects such as mealworms and crickets (~5 of each). During their time in captivity, growth and survival of mother and young were monitored every three to five days. Once in the wild, I continued to monitor their growth and survival by performing capture-recapture sessions with Elliot trapping, and checking the nest-boxes in which they were released, monitoring them every month until the following mating season.

Animals were trapped using Elliot traps that were baited with a mixture of peanut butter and rolled oats with soaked dog kibbles (dog chow) that were placed along a disused walking track. Traps were waterproofed by covering them with a plastic bag and a handful of Dacron fibre (pillow stuffing material) was provided inside traps as bedding to keep them warm. During each trapping session, I set 200 traps around 3pm and checked them every 4 to 6 hours. Trapping sessions usually lasted around fifteen days in October and three to five days during the other months, except around the mating season (~ end of August) and when females were expected to be giving birth (~ end of September) when I did not trap to avoid interrupting the mating season and to avoid females giving birth in the traps or stress them around that time.

In total, 66 subtropical antechinus mothers were trapped during these three years: 12 in 2010, 30 in 2011 and 24 in 2012. However, data from only 56 were included in the final analyses, because ten of them died soon after being brought to captivity (within a few days): 12 in 2010, 22 in 2011 and 22 in 2012. A total of 351 young were studied, 256 (120 females and 136 males) had a first-year mother and 95 (47 females and 48 males) had a second-year mother (Table 1).

During the three years of this study, one of the strongest La Niña events on record occurred between 2010 and 2011 (Australian Bureau of Meteorology 2012). From spring 2010 to autumn 2011 and also in late 2011, la Niña brought events of heavy rainfall and severe flooding that affected many areas of Australia including south east Queensland, and put an end to a severe drought that had affected Australia for the previous 14 years (since 1996). National rainfall was almost three times above average in September 2010, the wettest September on record; 2010 was the third wettest year and 2010-2011 was the wettest two-year period on record since 1900. In 2012, national rainfall was overall below average, especially in winter and spring, although in summer 2013, the east coast of Queensland and New South Wales experienced heavy rainfall associated with the tropical cyclone Oswald that ended with severe flooding in many areas. Additionally, eastern Australia was severely affected by spring heatwaves in November 2012, when temperatures reached extremely high levels not previously recorded at that time of the year in many areas of Queensland, New south Wales and Victoria. Summer 2012-2013 was the warmest on record, and rainfall was below average for the rest of the year (Australian Bureau of Meteorology 2006, 2010, 2012a,b,c, 2013).

It is very likely that these extreme fluctuations in weather conditions had strong effects on natural populations of animals. I used data from the Australian Bureau of Meteorology to assess the pattern of rainfall during the three years of this study, from August 2010 to August 2013 at Springbrook, in relation to the breeding season of subtropical antechinuses (mating, pregnancy, lactation and weaning) and assessed the potential effects on individual growth, survival and overall condition.

Weather data were obtained from the Australian Bureau of Meteorology - Springbrook Road Station, nº 040607; 28.20ºS, 153.27ºE; 681 m above sea level for rainfall data. Daily temperature data was obtained from Murwillumbah Station (nº 058158; 28.34ºS, 153.38ºE; 8 m above sea level) located at Bray Park, New South Wales, 19.2 km away from Springbrook.

Data Analysis

I used two different approaches to asses year effects on individual body mass. First, I wanted to compare if there was any difference on individuals mass at the same stage (mid-lactation, when young were ~51 days old – no repeated measures) across the three years of this study. To do this, I used a generalized linear model (GLM) for mothers (Table S1a) and a linear mixed effect model (LMER) for their offspring using as a random factor mother ID (Table S2a). Mean offspring body mass (g), maternal foot length (mm), maternal age class (if the mother was a first- or a second-year female), litter sex ratio (either female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) were used as fixed factors for maternal model along with the interactions between year with maternal age class, sex ratio and litter size. The same factors were used for the offspring model except for offspring mass (response variable), which was replaced with maternal body mass, and offspring sex was also added to this model.

I also used two different generalized mixed effect models (GLMM) to assess whether there were any differences between years on individual growth (one for mothers and one for offspring, Table S1b and S2b, respectively) similar to my approach in the previous two chapters of this thesis, using repeated measures for body mass, and individual ID and offspring age at the time when the measurements were performed as random factors. The fixed factors were the same as described above, except for offspring age.

I used contingency tests to evaluate differences across years on maternal weaning success (if the mother successfully weaned her offspring or not) and also on individual survival after weaning (if individuals were seen within the next two months after weaning) and to the following breeding season for both mothers and offspring. Breeding survival, as described in the previous chapter, could also be assessed for female offspring, as males disperse away once weaned. I also complemented these results by using mixed effects models for each one of these response variables (see Tables S3 to S7). All statistical analyses were conducted in R Studio (v. 0.98.501 R Development Core Team, 2013).

Results

I found significant differences in growth and survival of subtropical antechinuses across the three years of this study. These years differed in both the total annual amounts of rainfall (Figure 1A), and, more importantly, they varied in the timing of rainfall peaks in relation to the different stages of the breeding season (Figure 1B). At Springbrook, 2010 was the wettest, receiving 40% above the annual average; 2011 was the driest, although the annual rainfall received was the closest to the average of the last thirty years; and, in 2012 annual rainfall was 15% above average (Figure 1A).

High rainfall occurred during the whole duration of lactation in 2010, especially during the first (October) and third month (December) of lactation, when rainfall was four and three times higher than the average observed during the last 30 years. Rainfall during lactation in 2011 was around average, while in 2012 was below average.

Rainfall at weaning had a different pattern. High rainfall in January – February (weaning and the short time afterwards) was high in 2011 and 2012 (more that two times higher than the average), but not as high in 2010. Even though, rainfall in January 2010 was still 50% above average, in February was 20% below average.

Reduced maternal investment ability in dry years

a) Maternal mass

Overall, mothers were significantly lighter during mid-lactation in 2012, when rainfall was extremelly low throughout the year and especially during the whole breeding season, including lactation. This difference was significant according to the mixed effect model (*year 2012*: t = 2.36, p = 0.02. Table S1b) but not according to the GLM (*year*: t < 1.10, p > 0.28. Table S1a). The mean maternal body mass (mean ± SE) in 2010 (N=12), 2011 (N=22) and 2012 (N=22) was, respectively, 33.21 ± 1.2 g, 33.66 ± 1.0 g and 31.73 ± 1.29 g, when their offspring were ~51 days old.

In agreement with my prediction that younger mothers should be more affected by low rainfall during lactation, in 2012 first-year mothers had the lowest mean mass when young were ~51 days old (mid-lactation), while second-year mothers were the heaviest on average (N=4) than in the previous two years. These results were supported by both the GLM and LMER (*year* x *maternal age*: t > 2.06, p < 0.05. Figure 2B. Table S1a and S1b). Extraordinarily high rainfall during the first-month of lactation in 2010 seem have favoured first-year mothers more than second-year mothers. When young were ~51 days old, the difference in mass between first and second-year mothers was not significant in 2010, but in the following two years the difference in mass between young and old mothers was significant. The difference between first- and second-year mother's mass increased from ~4 g in 2010 to 8 g in 2011 and up to 14 g in 2012. First-year mothers were heaviest in 2011, while second-year mothers were heaviest in 2012 (see Table 2 for details). These results also support the correlation between rainfall and overall condition in antechinuses, as first-year mothers from 2011 and second-year mothers from 2012 were all born in 2010, when offspring growth was the highest observed. This means that improved condition acquired by offspring in 2010 persisted into adulthood (see below).

According to the mixed effect model, mothers only increased their mass during mid-lactation in 2012 during their time in captivity (*year 2012* x *offspring age*: t=-2.25, p = 0.03; Figure 3. Table S1b). This result initially appears to support the argument that in 2012 maternal condition was very low, and females were taking advantage of the access to *ad libitum* food resources offered while in captivity during mid-lactation. However, despite this, half of the mothers died during this time, along with their litters. High mortality among mothers during the time when I kept them in captivity in 2012 forced me to make the decision to release them sooner than planned. Therefore, it is surprising that the model found that mothers in 2012 were actually increasing their mass during mid-lactation, when their time in captivity was shorter than in the previous two years (Figure 3 most data for 2012 is at the left side of the graph), so it is not possible to make the comparision between the three years.

b) Maternal weaning success

Consistent with the idea that low rainfall during lactation reduces weaning succes in antechinuses, mothers were less likely to wean their young in 2012, when rainfall was extremelly low during lactation. The percentage of offspring weaned in 2012 was only 50% compared >80% in the previous two years (χ^2 = 8.19, df = 2, p = 0.017; Fisher test: p = 0.02). However, the mixed effect model found this effect only marginally significant (t = 1.76, p = 0.08. Table S3).

Also consistent with this expectation, first-year mothers and mothers rearing male-biased litters were less likely to wean their young in 2012, especially compared to 2011. The percentages of first-year mothers that weaned their young in 2010, 2011 and 2012 were, respectively, 50%, 84% and 47% (χ^2 = 6.01, df = 2, p = 0.05; Fisher test: p = 0.047), while the respective percentages for second-year mothers were 100%, 100% and 67% (χ^2 = 3.95, df = 2, p = 0.14; Fisher test: p = 0.43). A similar pattern was observed in mothers rearing male-biased litters compared to female-biased litters. The percentage of mothers with male-biased litters that were successful at weaning them in 2010, 2011 and 2012 was 100%, 92% and 40% (χ^2 = 10.31, df = 2, p = 0.006; Fisher test: p = 0.007), while for female-biased litters the percentages were 67%, 80%, 58% (χ^2 = 1.18, df = 2, p = 0.56; Fisher test: p = 0.60), respectively. These results agree with my results in Chapter 2 and 3 that sons are more constly to produce, especially when conditions are not favourable, and that young mothers have a reduced investment ability compared to older mothers.

c) Offspring growth

Offspring grew larger and faster when higher rainfall occurred during lactation. Offspring were heavier in 2010 than in 2011 and 2012 (*year*: t = -4.48, p < 0.0001; Figure 4. Table

S2a). The extraordinary high rainfall during lactation experienced by individuals in 2010 had a positive effect on offspring growth, as the young were heavier and grew faster (see Table 3 to compare growth rates) compared to the next two years during mid-lactation (when they were ~51 days old), and especially compared to 2012, although according to the mixed effect model this effect was only marginally significant (*year* x *offspring age*: t = 1.83, p = 0.07).

Offspring from second-year mothers were overall heavier than offspring from first-year mothers (see Chapter 3), but this difference was not observed in 2011 (Figure 4A). This is because mothers in 2011 were the heaviest (Figure 2A), especially first-year mothers (Figure 2B) and offspring mass was strongly associated to maternal mass (*maternal mass*: t > 4, p < 0.001. See Table S2a and S2b. Also see chapters 2 and 3 of this thesis). These results show that the good condition of the young that were born in 2010 and experienced extraordinarily high rainfall during lactation, persisted into adulthood. In 2011 those individuals were breeding for the first time. These first-year mothers in 2011 demonstrated their increased investment ability by producing high quality offspring, despite the fact that rainfall during lactation in 2011 was around average. Their maternal investment was even superior compared to second-year mothers that year, which are the ones that usually show a greater investment ability (see Chapter 3).

The smallest offspring of all were the ones born in 2012 with first-year mothers, confirming that younger mothers were more affected by low rainfall during lactation. However, offspring from second-year mothers were not as affected (*year 2012* x *maternal age*: t = -1.96, p = 0.057. Figure 4A. See Table S2a) had growth rates similar to the previous years (Table). These results are consistent with the idea that older mothers have higher investment abilities (Chapter 3), thus do better under adverse conditions (low rainfall in 2012) than first year mothers. A similar pattern occurred when comparing offsping mass between female- and male-biased litters (Figure 4B). This also supports the idea that mothers invest more in sons, even when mothers are in poor condition (*year 2012* x *sex ratio –male-biased*: t = 4.24, p < 0.001. Table S2a).

d) Maternal survival

The survival of mothers in captivity (and their young, because if the mother died so did their entire litter) also varied significantly among years. From a total of 67 mothers that were trapped during the three years of this study (12 in 2010, 30 in 2011 and 25 in 2012), 47 survived the period of captivity and 20 died during this time. In 2012 the proportion of females that died in captivity with their entire litters was significantly higher (N=12, 48%) than in the previous two years N=0 in 2010, 0%; N=8 in 2011, 27%; $\chi^2 = 9.18$, df = 2, p = 0.01; Fisher test: p = 0.0068). This confirms the low condition of mothers in 2012, which was an extremely dry year.

The exceptional low rainfall experienced by antechinus mothers during the whole lactation periond in 2012 also reduced their chance to survive the process of weaning their offspring and to be seen alive within the next two months ($\chi^2 = 6.99$, df = 2, p = 0.03; Fisher test: p = 0.03). In 2012, only 18% of mothers survived weaning their young, while in 2010 and 2011, 58% and 50% did (respectively). Their chances plunged to zero if they were second-year mothers or were rearing a male-biased litter ($\chi^2 = 6.36$, df = 2, p = 0.04; Fisher test: p = 0.03). In 2010 and 2011, a second-year mother had at least a 60% chance of surviving after weaning their young and over 40% in male-biased litters. When breeding for the first time or in female-biased litters, mothers did better in 2012. The percentage of first-year mothers that survived weaning in 2012 was ~20%, compared to ~50% in 2011 and 2012. In female-biased litters, ~30% of the mothers survived weaning in 2012 compared to ~60% in the previous years.

According to the GLM model, mothers that had longer feet were more likely to survive the process of weaning their young (t = 2.37, p = 0.02. Figure 5. Table S4). This is consistent with the prediction that larger mothers should have a greater investment ability and thus have a higher post-weaning survival than smaller mothers.

Although the percentage of mothers that survive to breed again varied across the years of study, none of the analyses used in this Chapter to asses year effects were significant (see Table S5). In 2010, 2011 and 2012, respectively, 42%, 27% and 18% of the mothers survived at least until the following mating season. However, it is worth mentioning that high rainfall during lactation (2010) increased maternal breeding survival, especially in first-year mothers. In 2010, 50% of first-year mothers survived to the following breeding season, while in the following two years their chances were 26% in 2011 and 21% in 2012.

e) Offspring survival

The percentage of offspring that were successfully weaned and seen as newly independent juveniles between January and March varied each year in a manner consistent with the idea that high rainfall at weaning favours offspring weaning survival in this species. Those offspring that were heavier and had faster growth rates were more likely to be seen alive as weaned, newly independent, juveniles. In particular, male young, those born in 2010 of a second-year mother, or from a male-biased litter showed higher survival rates. Offspring from a second-year mother had a probability of 93%, 65% and 67% to be weaned in 2010, 2011 and 2012, respectively (χ^2 = 10.88, df = 2, p = 0.004; Fisher test: p = 0.003). If the mother was a first-year female, their chance was 48%, 73% and 47%, respectively (χ^2 = 15.52, df = 2, p = 0.0002; Fisher test: p = 0.0001), higher in 2011. The percentage of offspring from male-biased litters that were successfully weaned in 2010, 2011 and 2012 was, respectively, 88%, 74% and 49% (χ^2 = 19.36, df = 2, p < 0.0001; Fisher test: p < 0.0001). If they came from a female-biased litter, their chance of being weaned in those respective years were 68%, 65% and 51% (χ^2 = 4.12, df = 2, p = 0.13; Fisher test: p = 0.14). The percentage of male offspring weaned in the respective years were 90%, 71% and 49% (χ^2 = 20.22, df = 2, p < 0.0001; Fisher test: p = 0.0001) and for female offspring, 68%, 72% and 52% (χ^2 = 6.29, df = 2, p = 0.043; Fisher test: p = 0.047). These results show that offspring from young mothers were the most affected by the dry conditions of 2012 (mothers with less investment ability; see Chapter 3) and sons were also slightly more affected than females (more expensive to produce; see Chapter 2), consistent with the idea that wetter conditions favour more male offspring. The mixed effect model found that the only significant effect was litter sex ratio (see Table S6).

High rainfall at weaning translated into improved offspring weaning survival. This would explain the reduced offspring weaning survival in 2010 for offspring from first-year mothers caused by the decline in rainfall during January 2011 (weaning time for individuals born in September 2010). These young had a probability of a 48% of surviving to be a newly weaned juvenile, which is 45% less than an offspring from a second-year mother. This result is consistent with the idea that older mothers are able to produce high quality offspring (greater investment ability) compared to younger mothers (see Chapter 3).

High rainfall at weaning does not guarantee offspring survival. Juveniles were not able to compensate for poor condition associated with extremely low rainfall during lactation, and high mortality was observed in 2012, when high rainfall occurred at weaning but not throughout lactation. Female offspring were also more likely to survive to breed in 2011 than in 2010, and especially than in 2012. The percentage of female offspring that survived to breed in 2010, 2011 and 2012 was, respectively, 18%, 24% and 0.06% (χ^2 = 9.05, df = 2, p = 0.011; Fisher test: p = 0.008). Their chance was 56% if their mother was a second-year mother ($\chi^2 = 6.96$, df = 2, p = 0.03; Fisher test: p = 0.04) or 30% if they came from a female-biased litter ($\chi^2 = 6.99$, df = 2, p = 0.003; Fisher test: p = 0.02) in 2011. In 2012, their chance of survival was less than 1% regardless the age of the mother or their litter sex ratio. There was no significant effects according to the mixed effect model (see Table S7).

Discussion

In this study I demonstrate that growth, survival and investment abilities of female subtropical antechinuses are driven by rainfall. Therefore, changes in rainfall patterns can have profound consequences for the entire population, especially when those climatic events disrupt the natural seasonal predictability of the environment (Parrott et al. 2007, Recher et al. 2009). At least average rainfall during early and mid-lactation and high rainfall during late-lactation and at weaning is crucial for high survival of a cohort. High rainfall only at weaning is not sufficient to ensure high offspring survival.

As in red squirrels (Kerr et al. 2007), good body condition of antechinus offspring acquired during unusually favourable conditions during lactation persisted into adulthood. Heavy female offspring born in 2010 continued to be the heaviest in both their first (first-year mothers in 2011) and second breeding attempt (second-year mothers in 2012) and demonstrated high maternal investment abilities in both breeding attempts. They were not only larger and with high probability of survival, but they also produced large, fast growing offspring, were more likely to wean them, and produced high quality offspring that were as large as the offspring from second-year mothers, and also more likely to survive to breed. Therefore, breeding success in antechinuses seems to be related to favourable wet conditions not only at the time of their own development as dependent offspring (when lactating from their mother), but also to the environmental conditions when they are breeding themselves.

My results support the idea that environmental conditions experienced early in life have strong effects on growth, survival and individual reproductive success, and may have strong impacts on population dynamics and life history evolution (Lindström 1999, Descamps et al. 2008). Charmantier and Garant (2005) conducted a meta-analysis to see how changes in environmental conditions affects heritability of different traits, comparing favourable with unfavourable conditions in wild population of animals. They found increased heritability of morphometric traits under favourable conditions. For example, food availability during lactation in red squirrels strongly determines females' reproductive success (Descamps et al. 2008). However, these early environment effects are not always evident, and can be obscured by variable conditions experienced as adults (Descamps et al. 2008). Kerr and colleagues (2007) studied the persistence of maternal traits in wild red squirrel offspring by food supplementation prior and during reproduction, but not at or after weaning. Offspring from food supplemented mothers emerged earlier and were almost three times as likely to survive to the following year (Kerr et al. 2007). Annual fluctuations of environmental quality, in particular of food abundance, significantly affected growth rates in red squirrels, which were higher in times of high food abundance (McAdam & Boutin 2003).

In seasonally predictable environments, mammals often synchronise their breeding so young are weaned at the time when food is abundant. For example, the seed-eating edible dormouse (*Glis glis*) adjusts the timing of its reproduction to the pattern of mast fruiting in beech and oaks trees, and the entire population may skip reproduction in years when seeds of these plants are absent. Because these trees do not produce mast crops two year in a row, these rodents avoid producing offspring straight after a masting year and increase breeding after a year with low fruit production (Schlund et al. 2002). Red squirrels (*Tamiasciurus hudsonicus*) also need reliable resources to sustain reproduction and although these rodents exploit a wide range of resources depending on seasonal availability, they synchronise their reproductive events with the fruiting times of conifers (Fletcher et al. 2013).

Energetic requirements of lactation and weaning large litters are high in antechinuses in relation to their body size (Green et al. 1991; Cockburn et al. 1983), especially in late lactation (Tyndale-Biscoe & Renfree 1987, Russell et al. 1989). They consume ~60% of their body mass each day when not lactating (Nagy et al. 1978, Hall 1980), and as much as their own body mass or more when lactating (Green et al. 1991). High demands of lactation in these insectivorous marsupials have been linked to the evolution of their extreme life history, because they need to match the end of lactation and the time of juvenile independence with the highest annual peak of arthropod abundance to breed successfully (Braithwaite & Lee 1979, Fisher et al. 2013). Seasonal predictability of prey and the high demands of a long lactation are the major forces that cause females to synchronize late lactation and weaning with the highest abundance of prey, and this creates the conditions for intense competition in males (Fisher et al. 2013).

Arthropod abundance depends on climatic factors and varies seasonally and from year to year depending on weather conditions (Strehlow et al. 2002). Patterns of rainfall and the severity of the dry season are the major factors regulating insect abundance across seasons and from year to year (Wolda 1978, Denlinger 1980, Jones 1987, Frith & Frith 1990). Abundance of leaf litter invertebrates appears to also be regulated by environmental factors such as pattern of litter fall, soil moisture and decomposition rates, all of which are also highly related to rainfall patterns (Frith & Frith 1990). Overall, invertebrate abundance and richness of species increases with rainfall and plant flowering during the wet season, especially if the wet season co-incides with warmer temperatures, as in tropical and subtropical zones (Frith & Frith 1990, Recher et al. 2009). Arthropods decline sharply with drier conditions and remain at very low densities during the dry season. This occurs in most invertebrates regardless of niche: leaf litter (Frith & Frith 1990), trunks/bark (Mejer et al. 2003), or canopy (Recher et al. 1996). However, due to year to year variations, this relationship between insect abundance and rainfall may not be straightforward, as it also depends on the interaction between duration and severity of current and previous rainfall events and and the variation between dry and rainy years (Denlinger 1980). In a study of seasonal and annual variation in insect abundance in Kenya, invertebrates reached the largest peak of abundance during a very long, uninterrupted event of heavy rainfall, were strongly suppressed during years with exceptional low rainfall, and were maintained at high densities by unusual rainfall during the dry season (Denlinger 1980). Unexpected rainfall during the dry season increases foliage production and this is thought to increase insect abundance, especially of foliage-feeders (Wolda 1978).

Rainfall in Australia in the period 2010-2012 was highly variable and also varied greatly at Springbrook. These years differed from rainfall patterns in previous years, as there had been more than a decade of drought Australia wide (Bureau of Meteorology 2006, 2010). In my study, the number of females with pouch-young captured in October 2010 (at the beginning of the study) was fewer than half of the number trapped in the following two years (30 lactating females were trapped in both 2011 and 2012 while in 2010, I only caught 12 lactating females). This may suggest that a population recovery during the period of this study followed a decline caused by the extended drought preceding the study (Bureau of Meteorology 2006, 2010, 2012) . Recher et al. (2009) also found that population dynamics of two species of antechinuses (agile and dusky antechinus) closely tracked rainfall, with little lag time. Lada et al. (2007) described an association between low rainfall prior and during the breeding season and low populations in yellow-footed antechinuses. Numbers of another dasyurid, the brush-tailed phascogale, also declined severely and immediately with drought (Rhind & Bradley 2002). Insectivorous marsupials respond quickly to drought because arthropod abundance declines abruptly in dry conditions (Strehlow et al. 2002, Majer et al. 2003, Bell 2006, Lada et al. 2007, Recher et al. 2009).

During the years of this study, the timing of rainfall peaks varied in relation to mating, pregnancy, lactation and weaning of antechinuses. High rainfall during pregnancy and lactation, such as in 2010, benefited mothers by enhancing their investment abilities and survival, and their offspring condition at weaning. Mothers were able to improve their body condition to sustain the high requirements of lactation without reducing survival, especially in young mothers. The weaning success of mothers rearing male-biased litters was more affected by the decline in rainfall during lactation in 2012 than the success of mothers rearing female biased litters. Compared with 2010, there was a 40% drop in success in 2012 in the former and a 10% drop in the latter. Overall, sons appeared to have benefited more than daughters from the favourable conditions during lactation as they had slightly enhanced survival around weaning in 2010 when high rainfall occurred during lactation. These results agree with the conclusions of the sex allocation chapter of this thesis that sons impose greater energy demands on their mother (Chapter 2).

High rainfall during lactation improved maternal survival, especially in second-year mothers. In the first year of my study, two-thirds of lactating females were second-years. This is extremely unusual for antechinuses as usually first-year females are significantly more abundant than second-years. Larger fossorial species such as dusky and swamp antechinuses typically have a greater proportion of iteroparous females than smaller scansorial species in the brown antechinus complex, but past studies have not found more than 40% surviving to breed again (Cockburn et al. 1983).

In iteroparous mammals, younger females must trade-off energy allocation between their own growth and their offspring’s growth. For example, first year female red squirrels had smaller offspring and were less likely to breed again than older mothers (Descamps et al. 2007). The demands of first-time breeding mean that younger mothers are more vulnerable to poor environmental conditions (Descamps et al. 2007). In antechinuses, individuals continue to growth throughout their lives, thus second-year mothers are always heavier than first-year mothers (Fisher & Blomberg 2011). First-year mothers, in order to sustain late lactation and wean a large litter of young (the most demanding part of lactation as their young can weigh up to five times altogether her own body mass; Cockburn 1994, Fisher & Blomberg 2011) need to gain weight during pregnancy and early/mid lactation. I did not observe any changes in body mass during mid-lactation during captivity, but I confirmed that young mothers are more vulnerable to poor environmental conditions than older mothers. First-year females were clearly more negatively affected than second-year mothers by extremely low rainfall during lactation in 2012. Accordingly, first-year mothers benefited most from exceptional wet conditions during pregnancy and lactation in 2010 not only by increasing their investment abilities, but also their breeding survival in a 30%

Under unfavourable conditions of drought at critical stages of lactation, heavier mother subtropical antechinuses had heavier offspring and were more likely to wean them. This is consistent with population studies of many other mammals. Individuals with relatively large body size typically have higher survival, competitive abilities and reproductive success. Heavier mothers have more body reserves, enabling them to produce and transfer more milk to their offspring (Bernardo 1996) so they invest in offspring growth to produce large offspring at weaning (red squirrels: Wauters et al. 1993; bank voles: Ylönen et al. 2004; caribous: Taillon et al. 2012; roe deer: Andersen et al. 2000; elephant seals: Arnbom et al. 1994). The efficiency with which the mother can transfer her body reserves to her young depends on previous and current maternal ability to store and acquire resources, and therefore, ultimately depends on food availability (Boydi & McCannt 1989, Clutton-Brock & Godfray 1991, Fairbanks & McGuire 1995). Reduced food availability is associated with juvenile mortality, low growth rates, delayed maturity and reduced reproductive success (McClure 1987, Festa-Bianchet 1988, Fairbanks & McGuire 1995, McMahon et al. 2000, King & Allinė 2003, Altmann & Alberts 2005). In long-lived, iteroparous mammals, mothers can potentially compensate for poor environmental conditions in later breeding events, but this is not possible in antechinuses. The life history responses to rainfall variation recorded in my study cover the entire reproductive lifespan of individuals in this population, so breeding failures of cohorts and categories of individuals could potentially translate into local extinction of the population, or strong selection on particular life history strategies such as trading off survival in favour of breeding effort as a first year female under high rainfall conditions (Fisher and Blomberg 2011).

My finding that juvenile condition at weaning persists into adulthood and is correlated with survival and reproductive success in later life agrees with several previous studies of mammals (Koskela 1998, Millesi et al. 1999, McMahon et al. 2000, Clutton-Brock et al.

2001, Rödel et al. 2008). My results also agree with a previous experiment with golden hamsters, on the effects of food restriction during reproduction. Undernourished mothers produced stunted offspring and sons were the most affected (Labov et al. 1986). Food restriction during reproduction in guinea pigs also resulted in mothers producing smaller, slow growing offspring and offspring reduced condition persisted into adulthood (Laurien-Kehnen & Trillmich 2004). Van Horne et al. (1997) carried out a study of population dynamics of Townsend's ground squirrels during four years, during which a severe drought affected the study area followed by an extended winter season. This caused a reduction in individual body mass of both young and adults, and an overall decline in population density that persisted at least a year after conditions reverted to normal. Females that managed to survive the harsh conditions produced smaller young and in lower numbers than before the severe weather events. Weather conditions need to be considered on the scale of the subject species' generation time (Van Horne et al 1997). This is more straightforward for antechinuses than for most other mammals (Fisher et al. 2006).

Conclusion

Because of its dense and stable overall population, much of which is in National Parks, the subtropical antechinus is classed by the IUCN as a species of least concern (Burnett & Dickman 2008) and is considered to have low vulnerability to decline (Hagger et al. 2012). However, my study suggests that this status may change in the future as a result of climate change. Climate change is considered to be a major threat to biodiversity during the next 100 years (Isaac 2009, Cahill et al. 2012). Vulnerability to climate change is likely to be determined not only by species geographical distribution but also by life history traits. Species restricted to small areas with short generation times are more vulnerable than similar species with long generation times (Pearson et al. 2014), and especially if those climatic changes occur rapidly and by extreme events (Isaac 2009). Evidence is currently limited, but disruption of food webs, especially declines in food availability for predators are suggested to be important causes of species declines and extinction due to climate change (Cahill et al. 2012). Subtropical antechinuses have a very small geographic range, and their semelparous life history, dependence on predictable prey cycles, montane distribution and extremely high energetic demands of lactation make them vulnerable to climate change. As presented in this chapter, unexpected changes in the patterns of rainfall can cause dramatic consequences for the entire population. According to the Bureau of Meteorology and CSIRO, Australia is expected to show further high climate variability in the following years including warming weather, high fire risk and reduced rainfall, especially during autumn and winter in the eastern regions of Australia (BOM & CSIRO 2014).

The results of this study not only confirm the essential role of dependable rainfall for the survival and overall success of subtropical antechinus, a montane rainforest specialist, but also show the crucial importance of timing of those events. While rainfall during lactation improves weaning survival of both mother and young, high rainfall around the time of independence is essential for high offspring survival to breeding. This study also highlights the importance of the effects of environmental conditions experienced in early life (during development and lactation) as their impacts can persist throughout life, and will determine future performance. I showed that high rainfall during lactation is important for good offspring condition at weaning, which translates into good condition as adults, and in females also translates into greater investment ability in offspring. I also present evidence that confirms the vulnerability of the subtropical antechinus to climate change.

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Figures and Tables

Table 1. Number of individuals, mothers and offspring per maternal age class and sex, used in this study in 2010, 2011 and 2012.

	2010	**2011**	**2012**
Nº mothers - Total	12	22	22
Nº mothers - First-year females	4	19	19
Nº mothers - Second-year females	8	3	3
Nº offspring - Total	77	135	139
Nº offspring - First-year females	23	118	115
Nº offspring - Second-year females	54	17	24
Nº female offspring	38	58	71
Nº male offspring	39	77	68

Table 2. Mean maternal body mass for mothers' age classes when young were ~51 days old (mid-lactation) from 2010 to 2012 at Springbrook National Park.

	2010	**2011**	**2012**
First-year mothers	30.64 ± 1.49 g	32.56 ± 0.90 g	29.81 ± 0.80 g
Second-year mothers	34.50 ± 1.49 g	40.61 ± 1.40 g	43.91 ± 2.20 g

Table 3. Regression parameters for offspring growth (body mass in g per day) per year during mid-lactation.

	Slope (g/day)	R^2	F	p
2010	0.23 ± 0.006	0.78	1737	<0.0001
2011	0.20 ± 0.008	0.60	601.8	<0.0001
2012	0.16 ± 0.002	0.21	48.29	<0.0001

Table 4. Regression parameters for offspring growth (body mass in g per day) during mid-lactation and the effects of maternal age.

	Slope (g/day)	R^2	F	p
2010 – First-year mothers	0.20 ± 0.008	0.80	598.9	<0.0001
2010 – Second-year mothers	0.25 ± 0.006	0.86	2112	<0.0001
2011 – First-year mothers	0.21 ± 0.006	0.61	556.9	<0.0001
2011 – Second-year mothers	0.18 ± 0.026	0.58	50.27	<0.0001
2012 – First-year mothers	0.08 ± 0.027	0.06	9.45	0.0025
2012 – Second-year mothers	0.21 ± 0.032	0.54	43.98	<0.0001

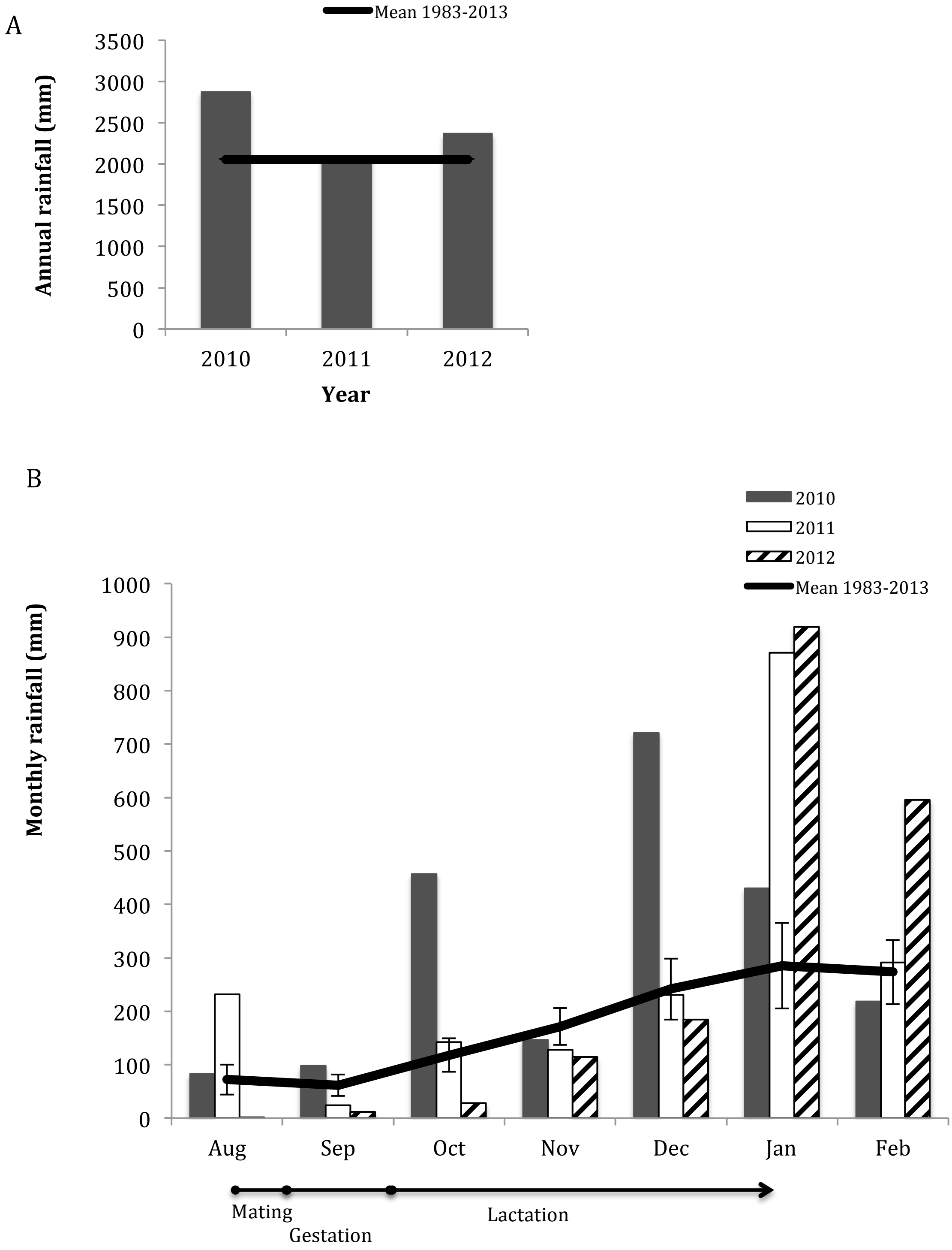


Figure 1. Annual rainfall (A) and monthly rainfall (B) at Springbrook in 2010, 2011 and 2012 in relation to the mean observed during the previous 30 years and in relation to the breeding season for subtropial antechinus. The bars on the mean line Figure B show the 95% confidence intervals. Rainfall data were obtained from the Australian Bureau of Meteorology – Springbrook Road Station (nº 040607; 28.20ºS, 153.27ºE; 681 m above sea level).

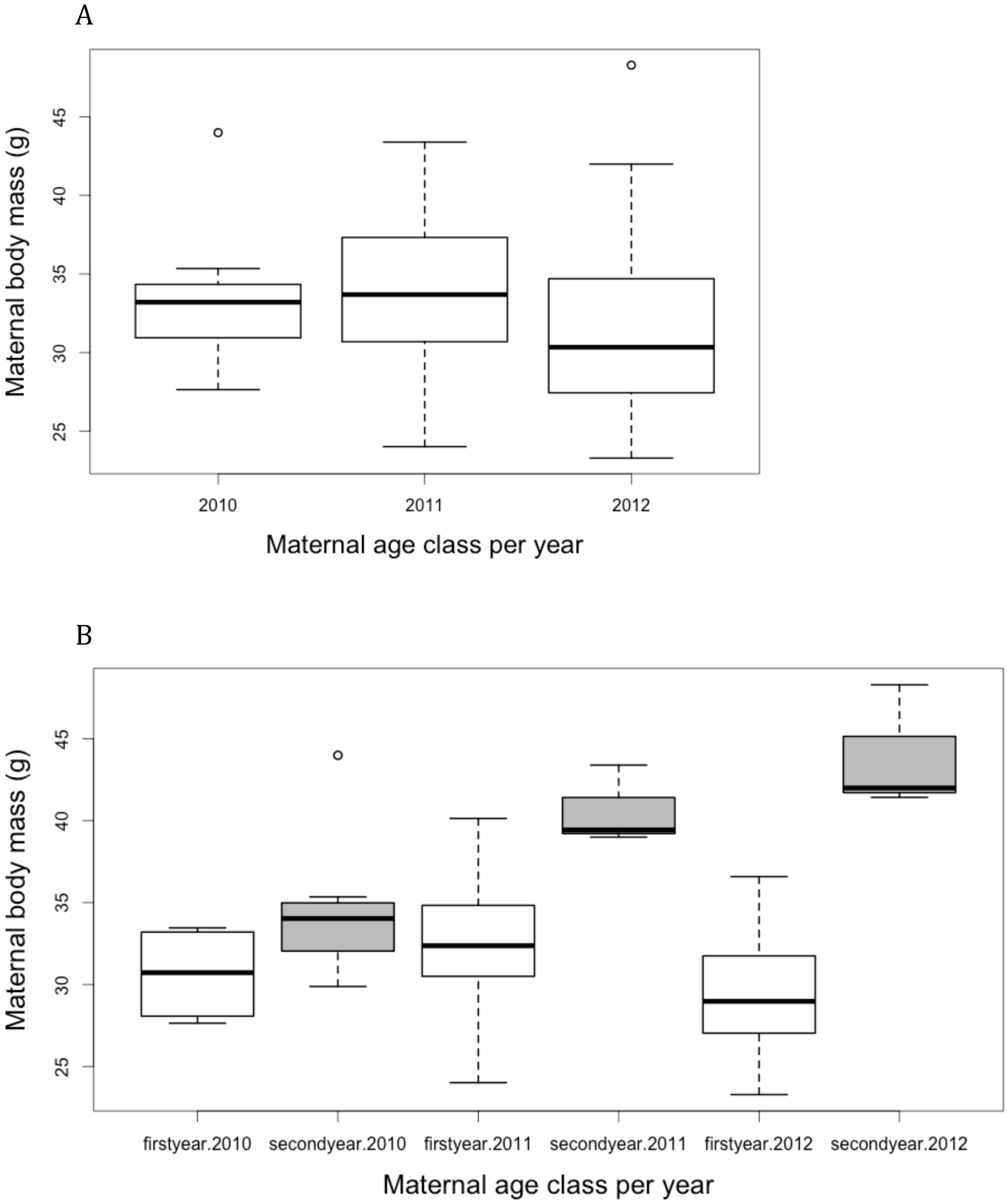


Figure 2. The effect of year on (A) maternal body mass and (B) its interaction with maternal age class (first-year mothers in white and second-year mothers in grey) on maternal body mas when young were ~51 days old (See Table S1a). The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles.

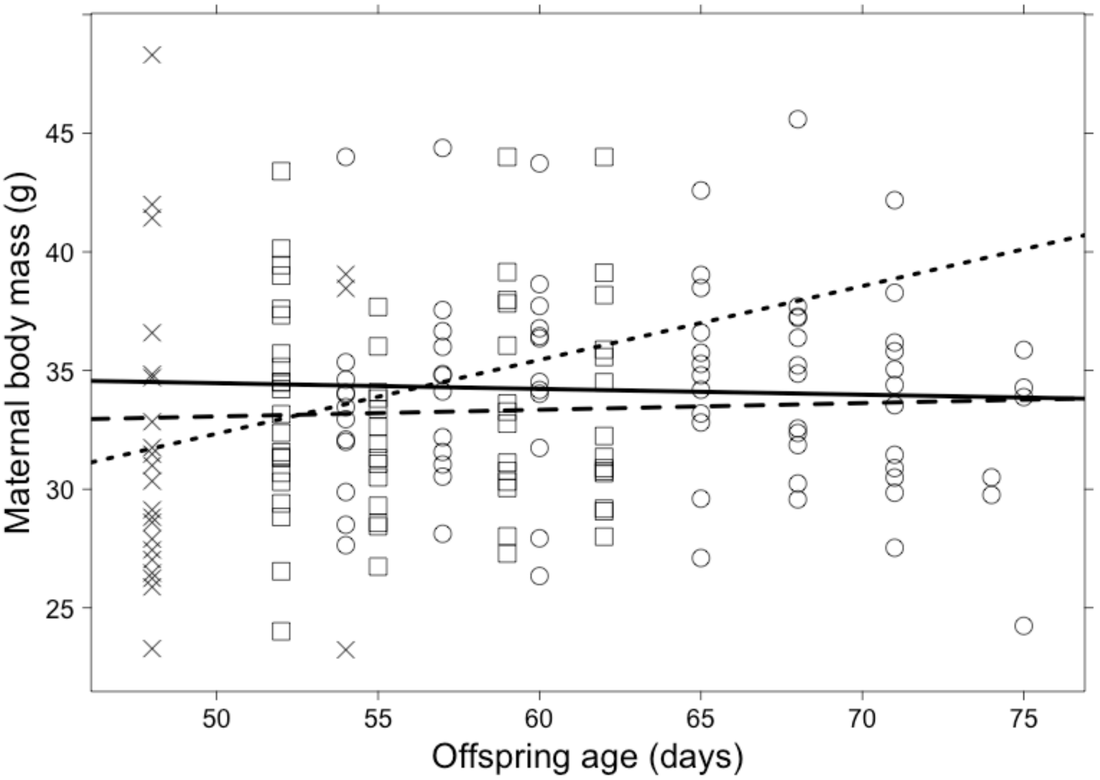
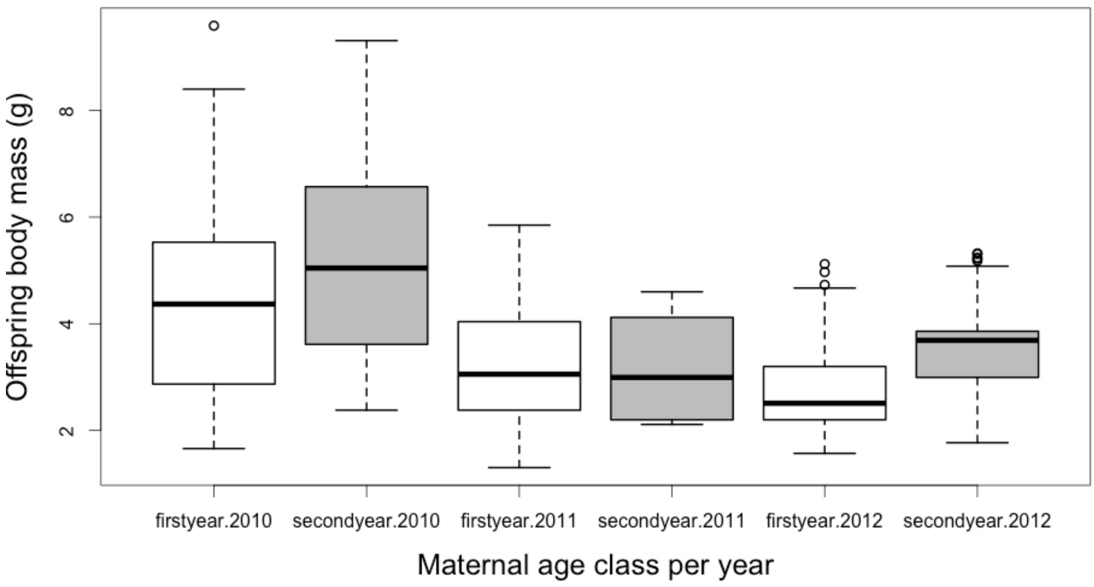


Figure 3. Variation of maternal body mass during mid-lactation, when their offspring went from 45 to 75 days old, per year: 2010 (open squares, continuous line), 2011 (open circles, discontinuous line) and 2012 (x, dotted line). See Table S1b.

A



B

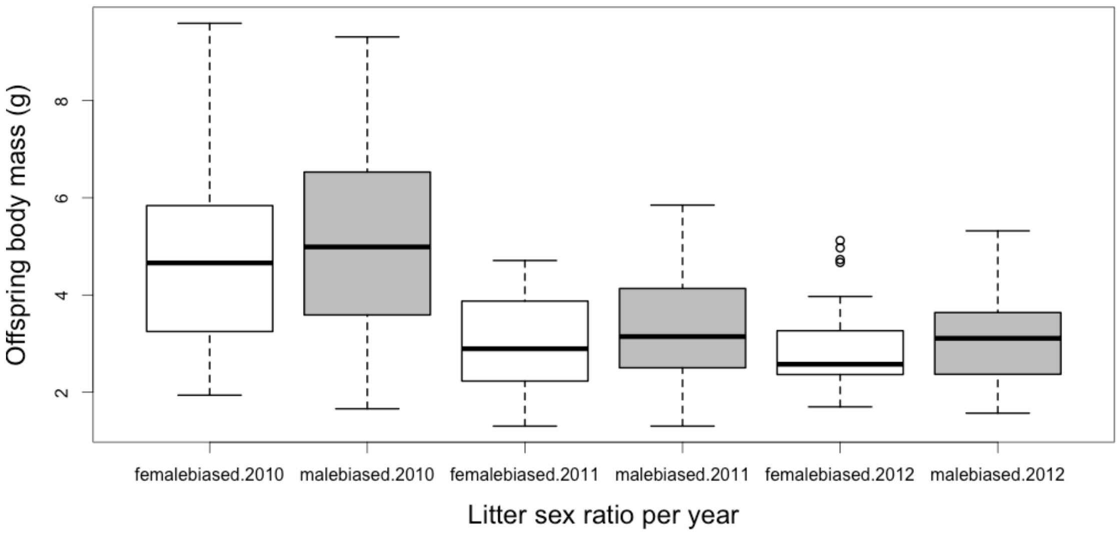


Figure 4. The effect of (A) maternal age class (first-year mothers in white and second-year mothers in grey) and (B) litter sex ratio (female-biased litters in white and male-biased litters in grey) on offspring body mass during mid-lactation (~51 days old) during the three years of study (2010, 2011 and 2012). The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles. See Tables S2a and S2b.

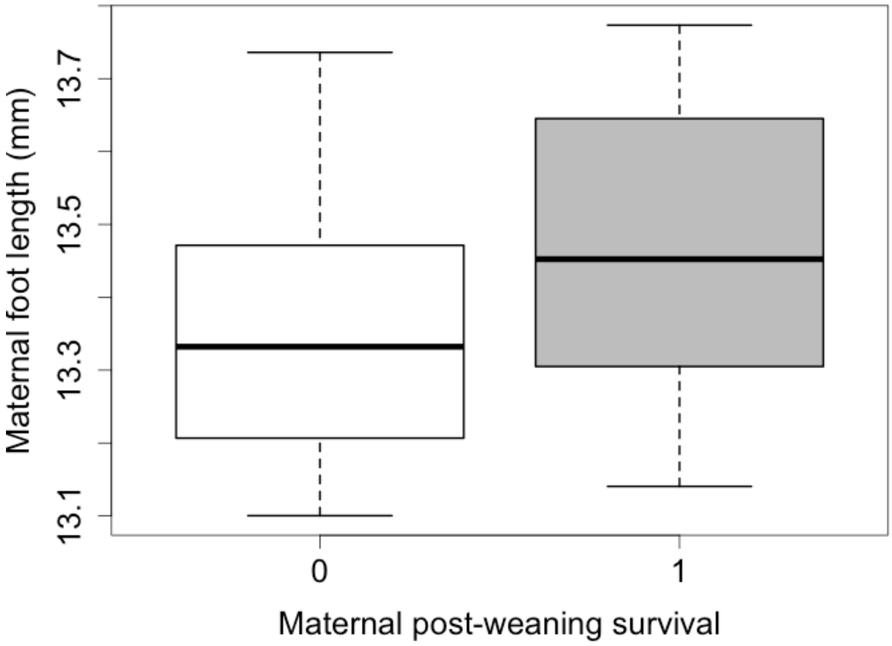


Figure 5. The effect of maternal foot length as a measure of skeletal size on maternal post-weaning survival. Mothers that survived after weaning their young and were seen alive within the next two months after weaning in grey, mothers that died soon after weaning their young in white. The dark horizontal line represents the mean value, the box represents the 25th and 75th percentiles, the whiskers represent the 5th and the 95th percentiles and the outliers are represented as open circles. See Table S4.

Supplementary information Chapter 4

Table S1a. GLM results for the effects on maternal body mass (log(Wt)) of the following fixed factors: mean offspring body mass (g), maternal foot length (mm), maternal age class (if mother was a first- or a second-year female), litter sex ratio (female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class, sex ratio and litter size. No repeated measures.

Factor	Estimate	Std. Error	t	p
Intercept	3.97	1.18	3.37	0.002
Offspring body mass	0.12	0.03	4.38	<0.0001
Maternal foot length	-0.08	0.09	-0.89	0.38
Maternal age class	0.04	0.07	0.63	0.54
Sex ratio	-0.04	0.07	-0.67	0.51
Litter size	0.04	0.02	1.90	0.06
Year 2011	0.16	0.15	1.10	0.28
Year 2012	0.13	0.17	0.77	0.45
Year 2011 x maternal age –second	0.14	0.09	1.57	0.13
Year 2012 x maternal age –second	0.29	0.10	3.00	0.005
Year 2011 x sex ratio –male-biased	0.11	0.08	1.26	0.21
Year 2012 x sex ratio –male-biased	0.05	0.08	0.67	0.51
Year 2011 x litter size	-0.02	0.02	-0.98	0.33
Year 2011 x litter size	-0.04	0.03	-1.32	0.19

AIC: -82.04

Null deviance: 1.26, df = 53

Residual deviance: 0.40, df = 40

Table S1b. LMER results for the effects on maternal body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), maternal age class (if mother was a first- or a second-year female), litter sex ratio (female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class and also with litter sex ratio. Random factors: (offspring age|mother ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	3.30	0.14	23.28	$7.23\text{x}10^{-25}$
Offspring age	0.0001	0.001	0.11	$9.16\text{x}10^{-01}$
Maternal age class	0.11	0.07	1.62	$1.13\text{x}10^{-01}$
Sex ratio	-0.04	0.06	-0.62	$5.41\text{x}10^{-01}$
Litter size	0.02	0.02	1.19	$2.43\text{x}10^{-01}$
Year 2011	0.02	0.20	0.09	$9.28\text{x}10^{-01}$
Year 2012	0.99	0.42	2.36	$2.33\text{x}10^{-02}$
Year 2011 x offspring age	0.001	0.002	0.48	$6.32\text{x}10^{-01}$
Year 2012 x offspring age	-0.02	0.008	-2.25	$2.99\text{x}10^{-02}$
Year 2011 x maternal age –second	0.16	0.08	2.06	$4.58\text{x}10^{-02}$
Year 2012 x maternal age –second	0.25	0.09	2.84	$7.03\text{x}10^{-03}$
Year 2011 x sex ratio –male-biased	0.12	0.08	1.63	$1.11\text{x}10^{-01}$
Year 2012 x sex ratio –male-biased	0.09	0.08	1.17	$2.47\text{x}10^{-01}$
Year 2011 x litter size	-0.02	0.02	-0.81	$4.24\text{x}10^{-01}$
Year 2012 x litter size	-0.04	0.03	-1.31	$1.96\text{x}10^{-01}$

AIC: -244.2; BIC: -185.1, logLik: 141.1, deviance: -374.6, REMLdev: -282.2

Table S2a. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: offspring sex (male or female), maternal body mass (g), maternal age class (first- or second-year mother), litter sex ratio (female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class, sex ratio and litter size. Random factor: (1|mother ID). No repeated measures.

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	0.70	0.37	1.91	$6.31\text{x}10^{-02}$
Offspring sex	0.007	0.01	0.51	$6.12\text{x}10^{-01}$
Maternal body mass	0.03	0.008	4.07	$2.13\text{x}10^{-04}$
Maternal age class	0.16	0.15	1.07	$2.90\text{x}10^{-01}$
Sex ratio	0.009	0.02	0.47	$6.43\text{x}10^{-01}$
Litter size	-0.15	0.03	-5.79	$9.49\text{x}10^{-07}$
Year 2011	-1.18	0.26	-4.48	$6.11\text{x}10^{-05}$
Year 2012	-0.43	0.33	-1.31	$1.98\text{x}10^{-01}$
Year 2011 x maternal age –second	-0.20	0.13	-1.48	$1.47\text{x}10^{-01}$
Year 2012 x maternal age –second	-0.35	0.18	-1.96	$5.73\text{x}10^{-02}$
Year 2011 x sex ratio –male-biased	-0.009	0.03	-0.35	$7.27\text{x}10^{-01}$
Year 2012 x sex ratio –male-biased	0.33	0.08	4.24	$1.27\text{x}10^{-04}$
Year 2011 x sex –male	0.001	0.02	0.09	$9.26\text{x}10^{-01}$
Year 2012 x sex –male	0.01	0.02	0.72	$4.76\text{x}10^{-01}$
Year 2011 x litter size	0.18	0.04	4.36	$8.81\text{x}10^{-05}$
Year 2012 x litter size	0.08	0.05	1.65	$1.06\text{x}10^{-01}$

AIC: -808.4; BIC: -738.9, logLik: 422.2, deviance: -935, REMLdev: -844.4

Table S2b. LMER results for the effects on offspring body mass (log(Wt)) of the following fixed factors: offspring age (days when measurements were performed), offspring sex (female or male), maternal body mass (g), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class, sex ratio and litter size. Random factors: (offspring age|donor mother ID), (offspring age|recipient mother ID), (offspring age|offspring ID).

Factor	**Estimate**	**Std. Error**	**t**	**p**
Intercept	-2.40	0.26	-9.37	$1.21\text{x}10^{-11}$
Offspring age	0.05	0.004	13.51	$1.70\text{x}10^{-16}$
Offspring sex	0.02	0.01	1.38	$1.76\text{x}10^{-01}$
Maternal body mass	0.02	0.001	16.58	$1.62\text{x}10^{-19}$
Maternal age class	0.04	0.04	0.98	$3.32\text{x}10^{-01}$
Sex ratio	-0.05	0.02	-2.96	$5.10\text{x}10^{-03}$
Litter size	0.005	0.01	0.48	$6.36\text{x}10^{-01}$
Year 2011	-0.50	0.31	-1.60	$1.18\text{x}10^{-01}$
Year 2012	0.65	0.45	1.43	$1.61\text{x}10^{-01}$
Year 2011 x offspring age	0.009	0.005	1.83	$7.44\text{x}10^{-02}$
Year 2012 x offspring age	0.004	0.006	0.68	$5.01\text{x}10^{-01}$
Year 2011 x maternal age –second	0.03	0.08	0.33	$7.44\text{x}10^{-01}$
Year 2012 x maternal age –second	0.09	0.11	0.80	$4.31\text{x}10^{-01}$
Year 2011 x sex ratio –male-biased	0.04	0.03	1.45	$1.54\text{x}10^{-01}$
Year 2012 x sex ratio –male-biased	0.55	0.05	11.44	$3.51\text{x}10^{-14}$
Year 2011 x sex –male	-0.003	0.01	-0.24	$8.10\text{x}10^{-01}$
Year 2012 x sex –male	0.005	0.02	0.33	$7.46\text{x}10^{-01}$
Year 2011 x litter size	-0.01	0.01	-0.89	$3.80\text{x}10^{-01}$
Year 2012 x litter size	-0.11	0.05	-2.61	$1.26\text{x}10^{-02}$

AIC: -2368; BIC: -2224, logLik: 1213, deviance: -2555, REMLdev: -2426

Table S3. GLM results for the effects on maternal weaning success (if the mother weaned or not their young) of the following fixed factors: maternal body mass (g), offspring body mass (g), maternal foot length (mm), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8) and year (2010, 2011, 2012) along with the interactions between year and maternal age class and sex ratio.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	$-6.98\text{x}10^{+01}$	$4.77\text{x}10^{+01}$	-1.46	0.14
Maternal body mass	$4.94\text{x}10^{-03}$	$1.51\text{x}10^{-01}$	0.03	0.97
Offspring body mass	$5.18\text{x}10^{-01}$	$9.86\text{x}10^{-01}$	0.53	0.60
Maternal foot length	$5.21\text{x}10^{+00}$	$3.53\text{x}10^{+00}$	1.48	0.14
Maternal age class	$2.03\text{x}10^{+01}$	$2.91\text{x}10^{+03}$	0.007	0.99
Sex ratio	$1.98\text{x}10^{+01}$	$3.20\text{x}10^{+03}$	0.006	0.99
Litter size	$-5.72\text{x}10^{-01}$	$3.58\text{x}10^{-01}$	-1.60	0.11
Year 2011	$3.59\text{x}10^{+00}$	$2.04\text{x}10^{+00}$	1.76	0.08
Year 2012	$2.94\text{x}10^{+00}$	$2.08\text{x}10^{+00}$	1.42	0.16
Year 2011 x maternal age –second	$-2.88\text{x}10^{+00}$	$6.28\text{x}10^{+03}$	0.00	0.99
Year 2012 x maternal age –second	$-2.04\text{x}10^{+01}$	$2.91\text{x}10^{+03}$	-0.007	0.99
Year 2011 x sex ratio –male-biased	$-1.82\text{x}10^{+01}$	$3.20\text{x}10^{+03}$	-0.006	0.99
Year 2012 x sex ratio –male-biased	$-2.01\text{x}10^{+01}$	$3.20\text{x}10^{+03}$	-0.006	0.99

AIC: 64.84

Null deviance: 61.81, df = 53

Residual deviance: 38.84, df = 41

Table S4. GLM results for the effects on maternal post-weaning survival (if the mother was seen alive in the wild within the next two months after weaning her young, when young were 100-120 days old) of the following fixed factors: maternal body mass (g), offspring body mass (g), maternal foot length (mm), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8) and year (2010, 2011, 2012) along with the interactions between year and maternal age class and sex ratio.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-72.69	31.32	-2.32	0.02
Maternal body mass	0.06	0.12	0.50	0.62
Offspring body mass	-0.88	0.92	-0.96	0.34
Maternal foot length	5.65	2.38	2.37	0.02
Maternal age class	1.47	1.60	0.92	0.36
Sex ratio	-1.38	1.48	-0.93	0.35
Litter size	-0.48	0.27	-1.78	0.07
Year 2011	0.29	1.43	0.20	0.84
Year 2012	0.17	1.43	0.12	0.91
Year 2011 x maternal age –second	-1.31	2.20	-0.59	0.55
Year 2012 x maternal age –second	-19.34	2893.65	-0.007	0.99
Year 2011 x sex ratio –male-biased	1.03	1.80	0.58	0.57
Year 2012 x sex ratio –male-biased	-15.93	2022.19	-0.008	0.99

AIC: 76.12

Null deviance: 72.99, df = 53

Residual deviance: 50.12, df = 41

Table S5. GLM results for the effects on maternal breeding survival (if the mother survived after weaning their offspring to the following breeding season) of the following fixed factors: maternal body mass (g), offspring body mass (g), maternal foot length (mm), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8) and year (2010, 2011, 2012) along with the interactions between year and maternal age class and sex ratio.

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-28.38	27.85	-1.02	0.31
Maternal body mass	0.06	0.12	0.47	0.64
Offspring body mass	0.45	0.87	0.52	0.61
Maternal foot length	2.00	2.06	0.97	0.33
Maternal age class	-0.52	1.49	-0.35	0.73
Sex ratio	-0.71	1.38	-0.51	0.61
Litter size	-0.22	0.23	-0.95	0.34
Year 2011	-0.30	1.41	-0.22	0.83
Year 2012	-0.39	1.37	-0.28	0.78
Year 2011 x maternal age –second	-0.61	2.32	-0.26	0.79
Year 2012 x maternal age –second	-16.99	3128.58	-0.005	0.99
Year 2011 x sex ratio –male-biased	-0.44	1.76	-0.25	0.80
Year 2012 x sex ratio –male-biased	-16.99	2105.92	-0.008	0.99

AIC: 76.83

Null deviance: 63.81, df = 53

Residual deviance: 50.83, df = 41

Table S6. GLMER results for the effects on offspring weaning survival (if the young survive for a while after weaning as independent individuals – if seen within the next two months after weaning) of the following fixed factors: offspring body mass (g), offspring sex (female or male), maternal body mass (g), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class and sex ratio. Random factor: (1|mother ID).

Factor	Estimate	Std. Error	z	p
Intercept	3.30	11.66	0.28	0.78
Offspring body mass	-0.63	1.63	-0.39	0.70
Offspring sex	0.17	0.72	0.23	0.82
Maternal body mass	0.31	0.37	0.84	0.41
Maternal age class	4.17	5.16	0.81	0.42
Sex ratio	3.80	1.38	2.76	0.009
Litter size	-1.66	0.96	-1.74	0.09
Year 2011	2.35	4.60	0.51	0.61
Year 2012	1.57	4.89	0.32	0.75
Year 2011 x maternal age –second	-4.99	7.19	-0.69	0.49
Year 2012 x maternal age –second	-3.14	8.47	-0.37	0.71
Year 2011 x sex ratio –male-biased	-2.45	2.12	-1.15	0.26
Year 2012 x sex ratio –male-biased	-5.73	4.03	-1.42	0.16

AIC: 201.9; BIC: 255.9, logLik: -86.94, deviance: 173.9

Table S7. GLMER results for the effects on female offspring breeding survival (if survived or not to breed) of the following fixed factors: offspring body mass (g), offspring sex (female or male), maternal body mass (g), maternal age class (if mother was a first- or a second-year female), litter sex ratio (if litter was either female- or male-biased), litter size (1 to 8), year (2010, 2011, 2012) along with the interactions between year and maternal age class and sex ratio. Random factor: (1|mother ID).

Factor	**Estimate**	**Std. Error**	**z**	**p**
Intercept	-2.40	4.71	-0.51	0.61
Offspring body mass	0.008	0.16	0.05	0.96
Maternal body mass	0.03	0.12	0.22	0.83
Maternal age class	2.21	2.98	0.74	0.46
Sex ratio	-18.33	932.90	-0.02	0.98
Litter size	-0.52	0.32	-1.63	0.11
Year 2011	2.63	2.85	0.92	0.36
Year 2012	-0.77	3.19	-0.24	0.81
Year 2011 x maternal age –second	-1.55	3.22	-0.48	0.63
Year 2012 x maternal age –second	1.98	4.69	0.42	0.68
Year 2011 x sex ratio –male-biased	14.67	932.90	0.02	0.99
Year 2012 x sex ratio –male-biased	18.75	932.91	0.02	0.98

AIC: 281.3; BIC: 335.8, logLik: -127.6, deviance: 255.3

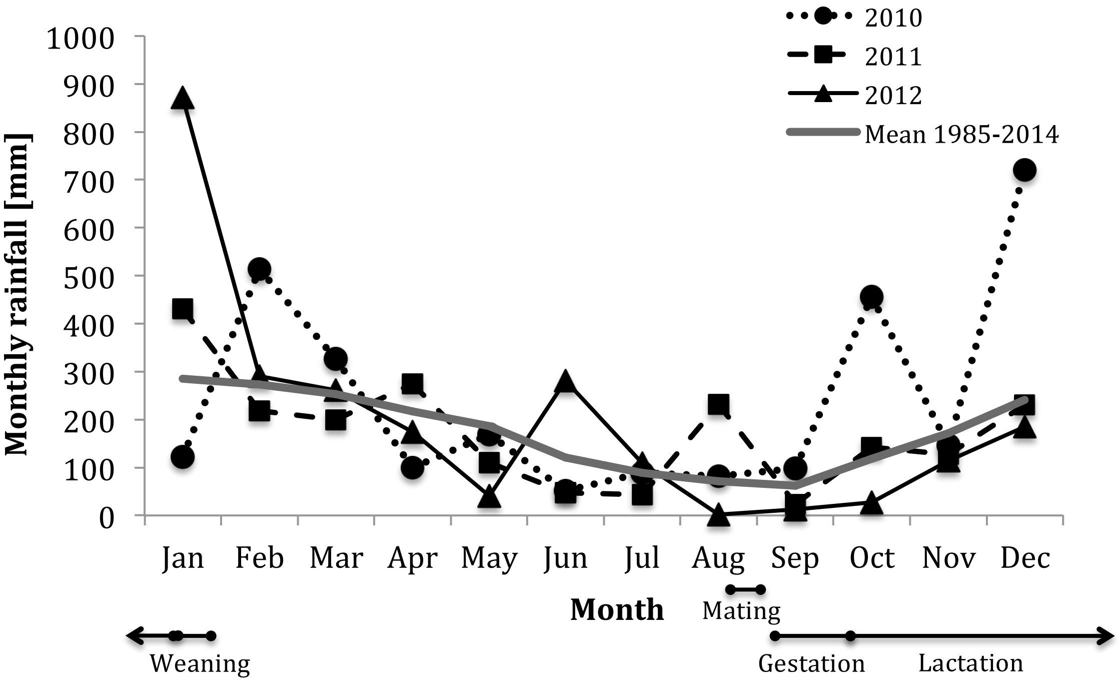


Figure S1. Monthly rainfall during 2010, 2011 and 2012 compared to the mean monthly rainfall observed during the last 29 years (1985 to 2014) at Springbrook, Queensland, Australia. Data obtained from Bureau of Meteorology.

Chapter 5

General Discussion

General Discussion

Evolutionary biologists have been trying to explain the variation in offspring sex ratios in different organisms for decades, and many theories have been proposed (Fisher 1930, Charnov 1982, Hardy 2002, West 2009). Despite chromosomal sex determination in mammals, the production of biased sex ratios is common and the mechanism has proved difficult to understand (Cockburn et al. 2002, Wild & West 2007, Robert & Schwanz 2011). Mammalian complex life histories and their intricate sociality are likely to be the main reasons why it has been so challenging to understand the vast diversity of offspring sex ratios (Cockburn et al. 2002, Wild & West 2007, Robert & Schwanz 2011). Multiple maternal and environmental traits are expected to affect sex allocation strategies of organisms (Cockburn et al. 2002, Robert & Schwanz 2011).

The same kind of problems have occurred in the study of life history trade-offs in wild populations of animals (Reznick et al. 2000). High energetic requirements associated with reproduction in mammals, especially during lactation, make them suitable models to study the costs of reproduction (Hamel et al. 2010). However, trade-offs have often been difficult to demonstrate in wild mammals and many studies have shown ambiguous results (Nussey et al. 2008, Hamel et al. 2010). Many confounding variables may mask detection of the costs of reproduction in the wild, these are likely to be related to differences in maternal and environmental quality. One of the major problems has been the difficulty in identifying the causes of improvement in breeding success of older mothers. In particular, it has been difficult to discern if the increase in the reproductive success of older mothers is due to increasing reproductive effort or simply enhanced maternal skills through experience (Cameron et al. 2000, Coté & Festa Bianchet 2001, Weladji et al. 2002, Weladji et al. 2006).

Variation in environmental quality, individual heterogeneity and the interaction between these two factors may prevent the detection of costs of reproduction and life history trade-offs in wild populations of animals (Van Noordwijk & de Jong 1985, Reznick et al. 2000). When environmental quality is high and food is plentiful, there is little conflict over resource allocation. Conflict occurs in times of food shortage, when trade-offs become evident (Reznick et al. 2000, Nussey et al. 2008). Some individuals may monopolise more resources than others and so face less intense conflicts of energy allocation (Reznick et al. 2000). Moreover, some individuals may perform better under certain environmental conditions and not others, or different individuals may have access to resources of varying quality (Reznick et al. 2000).

Most studies of mammalian life history evolution and sex allocation have been carried out on eutherian mammals, especially on rodents and ungulates. A large proportion of maternal investment occurs during gestation through the placenta and milk production in these taxa. In contrast, in marsupials gestation is extremely short and young are born at an embryonic stage. This suggests that most of the energy transfer from the mother to her young occurs externally during lactation and is under strong maternal control, as female marsupials are able to manipulate milk allocation to their offspring in terms of volume and composition (Tyndale-Biscoe & Renfree 1987). Therefore marsupials are particularly suitable study subjects for studies of sex allocation (Robert & Schwanz 2011) and the costs of reproduction (Isaac & Johnson 2005, Fisher & Blomberg 2011).

My study confirms that antechinuses are informative model species for experimental studies of life history trade-offs and sex allocation in the wild. They are short-lived monoestrus animals, so they are expected to maximise their reproductive effort at each breeding attempt, at the expense of survival (Hamel et al. 2010). They have a simple population structure, they are abundant and easily trapped, and estimation of lifetime fitness in a large number of individuals is possible. There are strong maternal and paternal effects on fitness (Fisher & Cockburn 2006, Fisher & Blomberg 2011). Litter size in antechinuses is large, and limited by the number of teats, so it is uniform in a given population (Cockburn et al. 1985). Sex ratio is discernable at a very immature stage because during the first five to six weeks after birth pouch young are attached to the mother's teat, and by the end of this period the sex of the young can be easily determined (Cockburn et al. 1985). Some populations of antechinuses produce strongly sex-biased litters (Cockburn et al. 1985, Dickman 1988, Davison & Ward 1998) and this bias is generated before birth, not by selective infanticide or sex-specific failure to attach to a teat (Davison & Ward 1998).

The main objective of this three year study was to assess the costs of reproduction and likely adaptive drivers of biased sex allocation in a wild population of subtropical antechinuses. The experimental manipulation of offspring sex ratio allowed me to test maternal investment ability and to separate this from sex differences in offspring ability to acquire milk (Robert & Schwanz 2011, Monclús & Blumstein 2012). The experimental design used during this research also allowed me to evaluate the life-long fitness consequences of maternal investment while accounting for confounding variables of maternal and habitat quality. In addition, the fact that growth and survival of young were unaffected by being swapped between mothers also confirms that manipulation of litter composition is an effective way to study life history trade-offs in wild animals in future research.

There are two main hypotheses to explain sex allocation theory in non-cooperatively breeding mammals: the Trivers-Willard Hypothesis (TWH) and the Local Resource Competition Hypothesis (LRCH) (Chapter 2). The TWH predicts increased investment in sons due to the greater fitness returns associated with producing large, competitive males if the mother has the capacity to do so, but production of more daughters if she does not. The LRCH predicts increased investment in sons regardless of maternal condition, to avoid future competition with philopatric daughters. There have been only a few cross-fostering studies performed previously on other species, and all of them have found support for the TWH. In the second chapter of this thesis, I tested these hypotheses by performing a cross-fostering experiment on subtropical antechinuses and measured their fitness consequences in the wild. My results support some of the assumptions of the TWH, but do not support the predictions. Instead my results support the predictions of the LRCH. In Chapter 2, I showed that although sons are more costly to produce than daughters and heavier females tend to naturally produce male-biased litters (in agreement with the TWH assumptions), smaller mothers with female-biased litters were as able to produce large, fast growing offspring (in agreement with LRCH). Moreover, I showed that after increasing their offspring sex ratio biased, mothers with male-biased litters were able to increase their investment without compromising offspring growth and were more likely to wean them successfully than mothers with female-biased litters, in agreement with LRCH. However, this increased investment reduced their survival as they were less likely to breed again than mothers rearing female-biased litters. In contrast, mothers rearing more daughters decreased investment in their young in favour of their own survival when large litters of daughters were imposed on them, also consistent with LRCH.

In Chapter 3, I tested the two main hypotheses to explain variation in maternal investment in relation to age: the senescence hypothesis and the terminal investment hypothesis. These make opposite predictions. The former predicts decreased investment in older mothers due to deterioration associated with ageing. The latter predicts an increase in female reproductive effort with age, as their chances of breeding again decline. My results support the terminal investment hypothesis. Older females, far from being reproductively senescent, demonstrated greater investment ability compared to younger mothers. Mothers in their second year were heavier, produced large, high quality offspring that grew the fastest and were more likely to survive than the offspring from younger mothers. Additionally these older mothers were more likely to survive until the next reproductive event than younger mothers, even when their chances of breeding successfully for a third time are zero.

In the fourth chapter of this thesis, I evaluated the effects of the environment on maternal investment and showed how important the timing and amount of of rainfall is during their reproductive season. Antechinuses living in seasonal habitats synchronise their mating season with the annual peak of insect abundance so that the end of lactation and weaning occur at times of very high food abundance in high rainfall years. High abundance of prey is required to sustain lactation and increase offspring survival when they are most naïve, inexperienced and have high energetic requirements to sustain growth. During the three years of this study, rainfall patterns varied greatly and so did antechinus performance. High rainfall during lactation increased maternal survival, meaning that mothers were more likely to breed again, and increased offspring growth and survival. I showed that the good condition of these offspring acquired through a beneficial environment during the period of nutritional dependence, persisted into adulthood. Individuals that experienced the best conditions in infancy had large body sizes and exceptionally high maternal investment abilities as adults. They produced litters that grew faster than second-year females of that year. In contrast, low rainfall in young reduced body sizes, later investment ability, survival and reproductive success, and the entire marked population severely declined. The severe effects of low environmental quality confirm predictions of Reznick et al. (2010) that life history trade-offs should only be evident under harsh conditions and low food supply. I also demonstrated that it is essential, when studying wild populations of animals, to consider how long-term weather patterns affect current demography and life history of animals (van Horne et al. 1997). My results suggest that the small geographic range of subtropical antechinuses, their semelparous life history, dependence on predictable prey cycles and extremely high energetic demands of lactation make them vulnerable to climate change.

Future Directions

In this study I exchanged only one or two individual young between mothers during the cross fostering manipulation to increase sex bias of litters. It would be interesting to test if these results persist with a more extreme variation of litter sex ratios. Instead of just enhancing the natural sex ratio produced by a mother, another approach would be to change it completely to the opposite. For example, if a mother produced five daughters and three sons, under the protocol applied in this thesis, this mother would have had either six or seven daughters and two or one sons after manipulation. However, an even more extreme test would be to change her litter sex composition to five sons and three daughters or to six sons and two daughters. This kind of manipulation would test if a mother that naturally produced a female-biased litter has the ability to raise a litter biased towards males or not. My experience with this species suggests that they may be more prone to stress in captivity than some other species of antechinus. Unlike some other studies, they were never seen active out of the nestbox while I was in the room. For more extreme tests, I would recommend to use a more resilient and extroverted species such as the yellow-footed antechinus (*A. flavipes*). In the wild, this species seems to be highly resilient and has adapted to a variety of conditions as it is the most widely distributed species of antechinus, in most states of Australia. This species also persists in very small fragmented patches of remnant vegetation (Marchesan & Carthew 2008). It would also be interesting to test these same questions and experimental approach in a more stable environment in which droughts and floods are absent for a period of years, given the sensitivity of antechinuses to fluctuating environmental conditions and drought that my study has revealed.

Every month during the three years of this field study, I collected data on nesting associations and nest sites, and locations of foraging antechinuses in the wild. Individuals in my study had known family structure and skewed sex ratios as a result of experimental manipulations, so these data can also be used to assess the effects of the number of female relatives sharing a home range on social structure and demography of individual females. As in most mammals (Silk 2007), male antechinuses disperse away from their birth place, but females remain together forming matrilines: groups of related females share nests and home ranges with their mother and sisters (Fisher et al. 2011).

Immediate benefits of group living, such as reduction of predation risk or enhanced foraging efficiency are quite common among social mammals (Krause & Ruxton 2002). However, long-term benefits of sociality in mammals have been difficult to demonstrate, especially in species with complex life histories and long lifespans, and complex, variable social structures (e.g. primates; Silk 2007). However, the limited available evidence (mainly on primates) indicates that improved offspring survival may be a benefit of sociality, which implies that forming social bonds may have positive consequences on individuals' fitness (Silk 2007). As described in chapter 1, subtropical antechinuses form fluid nesting groups composed of related and unrelated females and unrelated males (Fisher et al. 2011), however, nothing is known about the stability or strength of those social associations. By taking advantage of the manipulation of the family structure that I performed on this population, it will be possible to evaluate the benefits of forming social relationships on individuals' lifetime fitness, while accounting for the effects of habitat quality and relatedness. In future work, I will use social network analyses to quantify social associations among related and unrelated females, and evaluate their stability and strength over time. This study will contribute to understanding of social evolution in mammals by determining if there are net fitness benefits of female sociality and matrilines in antechinuses, as there are in long-lived primates (Silk 2007).

Another important aspect that it will be valuable to explore in the future will be the potential effects of relatedness on offspring growth. Because antechinuses are highly promiscuous and a female mates with multiple mates (Fisher et al. 2006), siblings differ in relatedness. Cross-fostered littermates differ further in relatedness. Genetic analyses can be performed using the toe-bud tips removed from the young when they were marked individually, to assess how relatedness affects offspring growth and survival, and to test maternal effects on growth and survival by comparing offspring from the same biological mother raised by different foster mothers.

Another potential avenue of research will be to directly evaluate the effects of habitat quality on the reproductive performance of female antechinuses. In particular, it would be interesting to assess the effects of prey availability (arthropod richness and abundance) and its effects on sex allocation and terminal investment of mothers. Pitfall traps can be used to assess prey availability, and have been shown to be effective for capturing mobile, ground-dwelling invertebrates (Fisher & Dickman 1993 a, b). Also important would be to take concurrent measurements of abiotic factors at the field site such as rainfall, litter depth, density of fallen logs, canopy cover, soil temperature and humidity that can be correlated with arthropod abundance (Wolda 1978, Denlinger 1980, Frith & Frith 1990, Reddy & Venkataiah 1990, Strehlow et al. 2002, Majer et al. 2003). I have already collected data of soil humidity and temperature, canopy cover, tree density and leaf litter depths for each of the home ranges of the studied individuals that I am planning to used in home range analyses. I also have preliminary data for insect abundance using pitfall traps that needs to be analysed, although it is most likely that more data needs to be collected.

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Appendix

Appendix

Book Chapter: Maternal care strategies of marsupials and implications for animal welfare. In: Animal Welfare: Mother and young. Mota-Rojas D, Paranhos M, Alonso-Spilsbury, eds. Elsevier B.V.

Parra-Faundes DV and Fisher DO

This book would actually be published in the first quarter of 2015 and is intended for the Spanish and Latin-American public. The title of the chapter and the book in Spanish are as follow: 'Estrategias de cuidado maternal en marsupiales y sus efectos en el bienestar animal' in 'Bienestar animal: la madre y su cría', respectively.

Marsupials and their mode of maternal care

There are around 350 species of marsupials (i.e. the infraclass Metatheria within the class Mammalia), native to Australia, Papua New Guinea and West Papua, South and Central America (with one species, the Virginia opossum *Didelphis virginiana*, in North America). Marsupial pregnancy is very short (12-46 days; Russel 1982) and metabolic rate is relatively slow in comparison to eutherian mammals, meaning that a long lactation is needed for young to grow large enough for independence, and a large proportion of maternal investment consists of lactation rather than supporting the foetus through a placenta (Lee & Cockburn 1985). The length of the period of maternal care is related to body size (Russel 1982), ranging from 2-4 months in small species to more than a year in larger species. For example, some small carnivorous marsupials spend around half of their lives as nutritionally dependent young (five of the eleven month lifespan of a male antechinus) and in general, lactation in marsupials is ~40% longer than in placental mammals (Hayssen et al 1985).

Birth

Marsupial young are born at a very early stage of development weighing less than 1% of maternal body weight and look similar to an embryo, except that features used by the neonate to locate the teat and survive in the pouch are relatively well developed (Dickman

& Vieira 2006). The newborn must quickly find and latch onto a teat to survive, so the forelimbs (including the hands), mouth and olfactory system are particularly well developed. They grab their mother's fur to propel themselves from the cloaca towards a teat, and once they found it, they seal their mouth around it and remain strictly attached to it for the next few weeks (Russel 1982). Lungs are partially developed at birth, with a variable degree of development between species, which are complemented with partial gas exchange through the thin and moist skin (Russell 1982, Edwards & Deakin 2013). You might think that the strategy of giving birth to such a tiny and apparently helpless embryo would be risky, and in the past some people have assumed that this mode of reproduction is inefficient and inferior to that of eutherian mammals. However, in spite that neonates have an undeveloped immune system (Edwards et al 2012), the birth process and the newborn marsupial are surprisingly resilient, and birth complications are rare. Mothers do not touch or directly help young during or immediately after birth, but hormonal changes at birth trigger mothers to adopt a specific posture, which helps the young to find or be transported to the teat and latch onto it within a few minutes (Russell 1982). Although they are capable of attaching to a teat at birth, marsupial neonates show no evidence of conscious awareness and pain perception through their behaviour (e.g. flinching) or brain activity (with an electroencephalogram) until at least a third to half way through pouch life (Mellor et al 2009).

Pouch life

Pouch life refers to the period when young are attached to a teat. Being in the pouch means that young marsupials are accessible, and this allows mothers control and flexibility through most of the period of development of their young; for example, mothers can jettison an infant, or selectively cull young in a litter or selectively influence milk transfer to different young.

The morphology of the pouch of marsupials varies widely between taxa, notwithstanding, it offers the perfect moist environment for the developing young and it also provides physical and antimicrobial protection (Edwards & Deakin 2013). Not all species have an enclosed pouch with a forward-facing opening like a kangaroo. Koalas and wombats (which are closely related families), bandicoots and some didelphids have a rear-opening pouch. It is sometimes said that burrowing wombats evolved this morphology so that dirt would not get into the pouch during digging, however this does not make sense given that koalas sit upright in trees, and burrowing macropods such as the boodie *Bettongia lesueur* have forward-opening pouches. The only aquatic marsupial, the yapok, can hunt underwater with pouch young because it has an enclosed (rear-opening) pouch with strong musculature that can make a water-tight seal. Kangaroos readily swim in dams and in the sea and also seem able to protect their pouch young using the pouch muscles. Sometimes this is a strategy to evade pursuing dogs; kangaroos are easily capable of drowning dogs that follow them into the water. Females observed swimming have been observed with surviving small pouch young.

Smaller species of marsupials that produce litters (e.g. dasyurids and some didelphids) have only a patch of thickened skin and hairs around the pouch area and may not have a visible pouch when not breeding. Young that are attached to the mother's teats are not enclosed and begin to drag on the ground after a few weeks of growth. Mothers then leave them in a nest or den, returning periodically to suckle them. South and central American opossums including mouse opossums and the larger *Didelphis* often carry young on their backs. Many Australian possums and the koala also do not deposit young in a den, but first carry young on their backs when they outgrow the pouch. Older young that are too big to be carried continue to follow the mother closely until weaning.

The early period of attachment to the teat and prolonged pouch life means that maternal recognition of individual young is poorly developed in marsupials; mothers can assume that the young in their pouch is theirs. Consequently, marsupials do not normally reject young that have been handled by humans or have an unfamiliar scent. Adoption within species and cross-fostering between species are possible.

Marsupial milk not only varies in quantity throughout lactation, but also it changes in composition according to the requirements of the developing young (Krockenberger 2006), and is regulated entirely by the mother (Isaac & Johnson 2005) and not by the offspring as in placental mammals (Delean et al 2009). This is very important when considering to cross-foster or hand raise young, among other things, in order to continue with a normal rate of growth and development: at least, young must be of similar size and age (Taggart et al 2010).

Marsupial reproductive strategies have consequences for animal welfare in:

- Captive breeding and husbandry for conservation and education.

- Wildlife population studies and conservation actions in the wild. These include trapping, disease management, threatened species conservation, and management of overpopulation.
- Urban wildlife management and wildlife rescue.
- Commercial harvest.

In this review we will focus mainly on examples from two major marsupial groups: macropods (mainly ground-dwelling grazing and browsing marsupials), and carnivorous marsupials.

Macropods

Macropods include ~75 species in two families of herbivorous marsupials native to Australia and Papua New Guinea, Macropodidae (the three species of kangaroos, and numerous species of wallabies, wallaroos, pademelons, hare wallabies, rock wallabies, tree kangaroos, dorcopsis and the quokka) and Potoroidae (11 species: small potoroos, bettongs and the musky rat-kangaroo) (Coulson & Eldridge 2010). Except for the musky rat kangaroo which has twins, all macropods have only one young at a time.

Birth in macropods involves the mother sitting hunched on her tail, so that the neonate climbs a short vertical distance between the cloaca and the lip of the pouch (Gemmel et al 2002). When the newborn reaches the pouch, locates a teat by scent and latches on, the tip of the teat swells in its mouth so the young cannot easily be dislodged. Survival of pouch young at this early stage is very high and stress in the mother does not usually result in the loss of the young in most marsupials, except in bandicoots which are prone to cannibalise small pouch young. Female kangaroos with small pouch young can be safely transported (e.g. between zoos), but transport of mothers at later stages of maternal care can result in the death of the young (Jackson 2007).

Cross-fostering is the rearing of young by a surrogate mother. In macropods, cross fostering of threatened species with mothers of more common species has been used by conservation managers to increase reproductive rates of around twenty threatened species in captivity (Taggart et al 2010). Pouch young removal ends the donor mother’s period of lactational inhibition of reproduction, so she will produce another young from her diapausing embryo or she will return to oestrous to produce a new young (Tyndale-Biscoe

& Renfree 1987). This procedure can be performed as early as the beginning of pouch life, when young weighs <1g (Merchant & Sharman 1966, Taggart et al 2005, Taggart et al 2010). Cross-fostering studies have been carried out with six potoroids and thirteen macropodid species with variable success in 30 different crossed trials between species (Taggart et al 2010). Choosing the appropriate surrogate species is crucial in order to ensure the most successful outcome by maximising both the survival of young and female reproductive rate of the endangered species. Foster mothers should be of similar size, with similar pouch life duration and milk composition throughout lactation (Menzies et al 2007), with a pouch young of similar size and age, from a closely related species that is abundant and easy to breed in captivity and that do not occur naturally in the same area to avoid possible recognition problems upon reintroduction of foster-reared animal back to the wild (Taggart et al 2010). The young from the surrogate mother is usually euthanized while still on the teat of its mother (Schultz et el 2006).

In macropods and other marsupials with an enclosed pouch the mother can use the pouch muscles to close the entrance tightly like a drawstring, preventing the young from leaving or entering. Young leave the pouch gradually over weeks (in small wallabies and potoroids) or months (in larger macropodidae), emerging for very short periods at first. Macropod and other joeys at this stage are uncoordinated, easily confused and vulnerable to accidents and predation. Stress causes the mother to relax the pouch muscles, so a large joey that is no longer attached to the teat will fall out when the mother is handled, trapped or chased, and the young can be injured by falling or being trampled in an enclosed space such as a trap or pen. To prevent stress-related ejection and injury of young, researchers and zookeepers tape the pouch entrance shut with masking tape or elastoplast, which is easily removed by the mother when she has calmed down.

People who discover a lone joey in the bush tend to assume that it is orphaned and remove it for hand rearing, however this is not needed in probably most cases. Stress causes female macropods being chased by a predator such as a dingo to jettison the young, which then hides. In the wild the mother will later double back and find the joey if she evades the predator. Like some ungulates, many small to medium sized macropods in dense habitats such as forest and tall grassland have a maternal care strategy which involves young hiding rather than following their mothers when they are old enough to release the teat. For example, infant bridled nailtail wallabies, red-necked wallabies and pademelons spend most of the time hidden under shrubs or swards of grass apart from their mothers, which visit periodically to suckle them (Johnson 1987, Fisher & Goldizen 2001, Fisher *et al.* 2002).

Prolonged acute stress in some species of macropods (e.g. a long chase or struggle at capture, including in the wild after pursuit by predators) can cause capture myopathy (Booth 1999, Peterson 2007, McMahon et al 2013). The symptoms are degeneration and necrosis of skeletal and cardiac muscle caused by lactic acid accumulation. Symptoms of muscle stiffness and twitching, paralysis, twisting of the neck, tachycardia, reluctance to move and inability to lift the head, kidney damage causing red urine, and sometimes kidney or heart failure, appear 1-2 days or up to a month after acute stress. In captivity and wild population studies, this disease can be prevented by quick capture and handling in a cool part of the day. This is particularly important for mothers with pouch young. If handling might to take longer or the species is particularly susceptible (e.g. kangaroos), injecting the animal with diazepam is recommended at capture to relax the muscles. This is likely to affect large pouch young because the pouch muscles will relax, and diazepam reduces macropods' ability to control body temperature. Vitamin E may also help to prevent the condition in captive macropods. Once an animal develops capture myopathy, treatment with diazepam, ice packs, fluids, vitamin E, sodium bicarbonate and corticosteroids can help but will be unsuccessful if muscle necrosis has occurred, and the animal should then be euthanased (Jackson 2007).

Chasing or startling macropods can also cause them to crash into fences which can badly injure mothers and young in the pouch, and to overheat. Breeding enclosures should have no right-angled corners and should not contain obstacles for this reason. Tree kangaroos are exceptions; these arboreal animals need rough-barked branches to climb on. These should be replaced when worn smooth. One early captive breeding program failed to raise any young to weaning before keepers realised that newly emerged young tree kangaroos all slipped off the smooth branches to their deaths on the concrete floor (George 1982).

Most species of macropods (and species in most marsupial families with the exception of wombats) breed successfully in captivity and adapt well, although they are susceptible to several diseases including coccidiosis and fatal bacterial infections of the jaw (lumpy jaw) associated with overcrowding and immunosuppression, and also inappropriate diet. As well as pouch relaxation and dropping young, signs of alarm or stress can include foot-thumping, trembling, licking forearms excessively or flicking their ears or head. Chronic stress causes reduced food intake in captivity which can also affect maternal milk supply, and this response is common to most groups of marsupials (Jackson 2007). Lactation is particularly demanding for koalas because of their low metabolic rate and low food nutrition (Eucalyptus leaves), and lactating koalas are often stressed by handling, whereas non-lactating females are not (Narayan 2013).

Many of the smaller species of macropods and other mid-sized marsupials are threatened because they have declined to tiny natural populations as a result of introduced predators (especially the red fox in Australia) and habitat degradation (Fisher et al 2003, 2007). Macropods are common subjects for captive breeding and reintroductions to reduce the risk of extinction. Reintroductions have often failed, especially when introduced predators were inadequately excluded or controlled at the release site, so there is a perceived conflict between individual welfare and species conservation. Inexperience is thought to be problematic for released individuals. Pople et al. (2001) found some evidence for this, because breeding captive-reared bridled nailtail wallabies in a pen at the reintroduction site and releasing site-bred young improved survival, and wild-born animals survived better than captive-born. Programmes to train marsupials including numbats (Jackson 2007), macropods and quolls to recognise and avoid predators and toxic cane toads appear to have had some success in improving survival. These programmes rely on maternal teaching of young then born in the wild to continue predator avoidance (O’Donnell 2010, Webb et al 2011).

A study of the fate of released hand-reared young possums and relocated adults compared to wild resident individuals found that during the first 100 weeks, introduced ring-tailed possums (both hand-reared and relocated ones) were in a clear disadvantage and their mean survival was around 56% less than resident possums (Augee et al 1996). This disadvantage was mainly caused by the introduction into an unfamiliar territory and not due to lack of learning about predators from the mother, because in the long term the whole population seemed to be suffering the same high rate of predation by non-native predators (from 118 individuals with known fate, only 8 were not eaten by predators and 80% were killed by foxes and cats; Augee et al 1996). Reintroduction is assumed to cause stress, but a study by Lapidge (2005) found evidence of the opposite effect on yellow-footed rock wallabies. Physiological indices of stress and condition improved after release including vitamin E status. The welfare and breeding success of reintroduced individuals was therefore equivalent to wild ones and better than captive wallabies.

There are also welfare issues associated with over-abundance of some larger macropods. Australian rangelands and some urban areas have been altered in the last 150 years to the advantage of grazing kangaroos and wallabies. Forest has been thinned and cleared to create grassland, predators of these large macropods such as dingoes have been removed, and in arid areas drinking water has been provided (for sheep and cattle) (Jonzén et al. 2005). Kangaroos can reach very high densities when pasture is plentiful, then suffer from starvation and disease when it deteriorates in dry times. Lactation declines and large pouch young are first to die in droughts and under the nutritional stress of overcrowding. Mothers can potentially jettison pouch young to improve their own chances of survival, but lack of milk is probably the usual cause of pouch young death. Kangaroo management is controversial because they are considered both as iconic marsupials and pests.

Heavy grazing by artificially dense populations of kangaroos detrimentally affects ecosystems and threatened plants and animals, especially during droughts when small animals rely on ground cover being retained (e.g. Fisher & Goldizen 2001). Commercial harvesting under licences issued by State government agencies is the major form of management in the rangelands. Two to four million kangaroos are harvested annually of a population of 23 to 57 million (McLeod 2010). Animal welfare concerns have been expressed about the potential prolonged suffering of joeys whose mothers are shot. To address this, licences allow only shooting with a high powered rifle that destroys the brain of adults and any large dependent young instantaneously, and a blow to the head that instantly kills small pouch young. These are considered humane method of euthanasia when done by skilled personnel, and commercial shooters must pass a competency test to gain a licence, although there is a possibility that joeys will escape, so harvesting only male kangarooos is the best option for animal welfare (McLeod 2010). McLeod (2010) argued that shooting is best practice for animal welfare in comparison to trapping and barbiturate overdose, which can have negative consequences for large pouch young and dependent young-at-foot (see above). It has been argued that harvesting wild kangaroos for food by shooting is a more humane and environmentally responsible way to obtain meat for human consumption than livestock farming followed by slaughter at abattoirs, because wild kangaroos are less damaging to vegetation than high densities of hard-hoofed livestock, their method of digestion means that they do not produce methane, and they are unaware of their fate (Grigg 2002).

Another idea to promote recovery of arid ecosystems is to remove water points such as dams or exclude macropods from them, because these are only around seven km apart on average throughout the rangelands. Welfare implications of removing water from drought affected animals might appear obvious, but several studies have found no effect on kangaroo density or movements around closed water points, suggesting that the scale of artificial water removal has been insufficient to be effective, and these have concluded that food rather than water is the critical resource. Artificial water can have negative welfare implications. Fukuda and colleagues (2009) recommended that mud around open water points be removed as water dries up during droughts, because kangaroos are often trapped and die.

In urban areas of overcrowding where it is possible to capture or dart a large proportion of females, fertility control is an option to improve animal welfare. Female contraception using implants is most effective and appears to have no welfare implications other than those involved in capture and handling (Coulson & Eldridge 2010).

Urban and rural macropods are frequently hit by vehicles, and joeys orphaned and hand-reared. Young marsupials are accessible for hand-rearing at a very immature stage compared to what is possible in eutherian mammals. Joeys do not develop the ability to thermoregulate until they leave the pouch after several months, and have an undeveloped immune system. These attributes have welfare consequences for hand-reared joeys, which are more problematic the younger they are orphaned. Very small pouch young that are still attached to the teat have their milk intake regulated by the mother, and older ones suck inefficiently if their body temperature is not maintained by a pouch. Rescued joeys need to be warmed before they are artificially fed, and care must be taken not to feed too fast or they can aspirate milk into the lungs and develop pneumonia. The composition of marsupial milk changes through pouch life and special formulas are needed. Hand-reared joeys are prone to gut problems such as twisted bowel especially if incorrectly fed (Jackson 2007).

Carnivorous marsupials

Marsupials that more than half of their diet consist in the consumption of flesh either from vertebrate or invertebrate prey are considered to be carnivorous (Jones et al 2003) and includes the two Australasian orders Dasyuromorphia (the extinct Thylacinidae, the monotypic Myrmecobiidae and ~71 species from Dasyuridae) and Notoryctidae (two species of marsupial moles) and ~66 species of American marsupials from the orders Didelphimorphia (numerous species of opossums and mouse opossums), Paucituberculata (caenolestids or shrew opossums) and the monotypic Microbiotheria (*Dromiciops gliroides*, ‘monito del monte’) (Viera & Astúa de Moraes 2003, Tyndale-Biscoe 2005). All carnivorous marsupials have relatively large litters and most of them have rudimentary pouches (Jones et al 2003).

When young are born, each neonate weighing is less than 0.01g (Dickman & Vieira 2006) they have to propel themselves to the pouch and take hold of a teat. As discussed previously, the morphology of the pouch is well-developed or rudimentary and when present, the position of opening will determine the specific posture the mother adopts when giving birth (and also the maternal care strategy; Russel 1982, Gemmell et al 2002). The Virginian opossum with a forward facing pouch sits on the base of her tail with the tail facing forward and between the hind legs, so then the neonates climb up towards the pouch (Reynolds 1952), similar to what has been described for macropods. Parturition in other American marsupials has not been described in such detail, but it is presumed to be similar to Australian marsupials. For example, female bandicoots (Peramelidae) have a rear-opening pouch, just as the yapok or water opossum (didelphid), and usually lay on their side when giving birth (Gemmel et al 2002). Dasyurids mothers with a rudimentary pouch usually stand on their four feet raising her hips so that the cloaca is in a higher position compared to the hind part of the pouch (mammary area) and lowers her head, sometimes even curling her head under the body. Thus, the neonates move slightly downhill towards the pouch (kowaris: Hutson 1976, quolls: Gemmel et al 2002, Nelson & Gemmel 2003, tasmanian devil: Rose et al 2006, antechinus: Williams & Williams 1982).

Litter size in carnivorous marsupials is limited by the number of teat. Teat number varies across species and some variation can be found even within species (Morton et al 1989). Dasyurid females produce supernumerary young (they give birth to more young than the number of teats) and even though in some cases most of them reach the pouch, the ones that are not able to take hold onto a teat are discarded (Reynolds 1952, Williams & Williams 1982, Morton et al 1998, Gemmel et al 2002, Nelson & Gemmel 2003, Jones et al 2003).

Once the young are attached to the teat, it begins the pouch life. Pouch young must be attached to the teat constantly during the first few weeks of lactation (a third or half of the entire period of lactation) (Tyndale-Biscoe & Renfree 1987). The lips of the young are sealed very tightly around the nipple (Russel 1982, Krockenberger 2006). Therefore, inappropriate removal of the young can be fatal and damage the teat permanently. During this time, young survival is very high (>90%) (Hossler et al 1994, Coates 1995). The pouch provides everything that these developing young need (milk, humidity, warmth and protection), so the main duty of the mother is to keep them clean (Russell 1982).

When young become too big to be dragged around, the mother leaves her litter in a nest or den (i.e. nest phase), while she goes out hunting. Usually by this time, the young are weighing around 50% of their mother's weight, but they are still naked (unfurred), small, unable to thermoregulate and with the eyes closed (Russell 1982). This extreme young vulnerability and the high energetic requirements of lactation exert an intense pressure on the mother that has to keep herself and her young alive (Soderquist 1993).

The mother has to select a 'safe' place to leave her litter away from predators and in insulated nest to minimise heat loss as young at this stage of development are highly ectothermic (Geiser 2003, Edwards & Deakin 2013). Tree cavities, hollow logs, burrows, soil crevices, tussock grass and other protected areas such as artificial nest boxes and human buildings can be used to build up a nest. Animals use these nests to rest during the day (most of the carnivorous marsupials are nocturnal) and between foraging bouts (Marlow 1961, Soderquist 1993, Hossler et al 1994, Tyndale-Biscoe 2005). Most of the carnivorous marsupials are thought to be solitary, but antechinuses (Lazenby-Cohen 1991, Coates 1995, Cockburn et al 1985, Sale et al 2009, Fisher et al 2011) and dunnarts (Morton 1978) can be quite social and share these nests communally except when lactating (lactating females prefer to nest alone) and it seems to be in response to high costs of thermoregulation. Some species build very sophisticated spherical nests by interwoven leaf litter, bark strips and other plant materials with an internal chamber linen with feather, fur and finely teased fibres (*Phascogale tapoatafa*), while others just drag some leaves or grass and place them as a mat with a central depression into a cavity or under a log (like planigales, ningaui, kowari, eastern quolls) (Russel 1982). Lactating mothers devote their time to care for their young, expending a large proportion of their time tiding up the nest, nursing and grooming their young and keeping them warm (Settle & Croft 1982), and short, but frequent, foraging bouts (Lazenby-Cohen 1991, Soderquist 1993), expending >70% of their time in their nests (Lazenby-Cohen 1991). During this time, mothers may actively defend their nest and their young from intruders or when are being handled (Russel 1982, Croft 2003). Mothers will respond to distress calls of their offspring that have been displaced from the pouch or the nest by approaching to them and retrieve them back towards the pouch using her forelimbs (Russell 1982, Settle & Croft 1982).

When the young start opening their eyes and become more active, they will follow the mother and cling to her fur energetically every time she comes back to the nest after a foraging bout, and rapidly will take hold to a teat (Settle & Croft 1982). It seems that mothers picking up their offspring with their mouth is not a common behaviour in carnivorous marsupials, although it has been observed in kowaris and tiger quolls (Russell 1982). When the mother is about to leave the nest, she has to actively make the young to let go the teat, her fur or from her back, by either grooming extensively or shaking her body vigorously (Settle & Croft 1989).

The survival of the young during this nest phase is reduced compare to the pouch phase. Around 50-70% of the young survive to weaning (Cockburn 1994, Hossler et al 1994, Coates 1995, Fisher & Blomber 2011), 30-50% of survive as independent juveniles (sub-adults) and only 10-15% of them survive to successfully breed (Hossler et al 1994, Cockburn 1994, Coates 1995, Fisher et al 2006). Young survival not only depends on maternal provisioning and condition, but also on environmental conditions (Julien-Laferriere & Atramentowicz 1990, Fisher et al 2006). Overall, offspring mortality is greater in young and old mothers and in small, skinny mothers (Julien-Lafarriere & Atramentowicz 1990). When food is scarce, mothers lose a lot of weight and litter failure is quite common under this conditions (Atramentowicz 1992, Coates 1995). Therefore, appropriate timing of lactation with seasonal abundance of resources seems to be fundamental for reproductive success, along with those other maternal behaviours that increase juvenile survival at weaning (Julien-Lafarriere & Atramentowicz 1990, Leiner et al 2008, Delean et al 2009). Hossler and his collegues (1994) found that Virginia opossum mothers showed a great den fidelity towards the end of the lactation period (carnivorous marsupials change the location of their nests constantly as described by Lazenby-Cohen 1991, Hossler et al 1994, Bank et al 2005, Fisher et al 2011) when juvenile mortality reaches its peak (Fisher & Blomberg 2011) and locate them in areas with dense vegetation cover, suggesting a maternal effort to maximise juvenile survival at their time of independence.

Weaning these large litters involves a huge investment for the mother and it compromises her own survival. Their young at the time of independence can weigh as much as three to five times the weigh of the mother (Soderquist 1993, Cockburn 1994, Russell 1982), so this massive maternal investment usually reduces their survival. Most of the carnivore marsupials rarely live to breed in more than one reproductive season, and many breed only once in their lifetime (Jones et al 2003, Lee & Cockburn 1985, Harder 1992, Cockburn 1997).

In antechinuses (Woolley 1966, Wood 1970, and many others) and phascogales (Bradley 1997; reviewed in Lee & Cockburn 1985, Cockburn 1997), individuals reach sexual maturity at 11 months of age and have a single, highly synchronised and short mating season each year (mainly in winter), after which all males die from physiological stress (semelparity; Bradley 1980). Most, if not all, females become pregnant and the majority of them breed only once dying soon after weaning their first litter (semelparous females) of around 6-14 young (living for 16-20 months), while some of them manage to live and breed a second time (iteroparous females; Fisher & Blomberg 2011). In other species, like dibblers (Mills & Bencini 2000) and northern quolls (Oakwood et al 2000), some males survive to breed in a second reproductive event and not all females become pregnant during the breeding season (~65-88%, Begg 1981) producing litters of four to ten young and some of them will even survive to breed in a third year, but not very successfully (Morton et al 1989). In other species of dasyurids like the eastern and tiger quolls, Tasmanian devils and the crest-tailed mulgara (Woolley 1971, Settle 1978, Godsell 1982), females are able to go on a second oestrous if unmated or after loosing prematurely her first litter (litter size of 4-8 young). In species like kultarrs, kowaris and planigales have an extended seasonal breeding season (~6 months, from late winter to mid-summer), females attempt to produce two litters per season. Dunnarts (common, fat-tailed and slender-tailed) reach sexual maturity at about 6 months old, thus breeding in their season of birth may be possible. While others species seem to breed throughout the year like the black-tailed and long-nosed dasyure and common planigales from the Northern Territory (Lee et al 1982, Morton et al 1989)

Although life history strategies in New World marsupials are poorly understood (Leiner et al 2008), most of them are thought to be seasonal polyestrous breeders attempting to produce at least two litters during the breeding season (Lee & Cockburn 1985, Harder 1992). However, species like ‘monito del monte’ (Muñoz-Pedreros et al 2005), Virginia opossum (Hossler et al 1994) and the Brazilian slender opossum (Leiner et al 2008) are seasonal monoestrous breeders producing only one litter per year. In semelparous didelphids, females exhibit a high reproductive investment (large litters ~10 young per litter) like in the Virginian opossum or the Brazilian slender opossum , while didelphids with longer lifespans tend to be iteroparous (more than one litter a year) like *Philander opossum* and *Caluromys philander* and produce small litters (2-4 young per litter) (Leiner et al 2008). Species like *Marmosa incana* (Lorini et al 1994), yellow-sided opossum (*Monodelphis dimidiate*; Pine 1994), short-tailed opossum (*Monodelphis domestica;* Pine et al 1985) and the gracile mouse opossum (*Gracilinanus* microtarsus ; Martins et al 2006) have been described as partially semelparous as some males survive to reproduce a second time. Abundance of food resources appears to have a strong effect on the reproduction of American marsupials (Julien-Laferriere & Atramentowicz 1990).

Mark-recapture and radiotelemetry studies allow the estimation of individuals' survival in the wild. These types of ecological studies are possible as long as the animals can be identified with a permanent marking method. While the use of PIT (passive integrated transponder) tags or microchips is considered to be a good method to mark small mammals (Schooley al 1993, Gibbons & Andrews 2004), to insert a microchip under the very thin and delicate skin of a tiny pouch young is impossible. Toe-bud clipping has been proven to be the only way to safely mark dependent offspring of small marsupials (Fisher & Blomberg 2009). The removal of the the toe-bud (<1mm) is a quick procedure, causes little to no bleeding, and the young does not react much as it is performed at a very immature stage of development (Fisher & Blomberg 2009, Parra personal observation). In order to mark all the dependent offspring in the wild, it has to be done while the young are still permanently attached to the teat. Fisher and Blomberg (2009) evaluated the possible harmful short- and long-term effects of this type of method in brown antechinuses and found that did not affect growth nor survival of the young in captivity or in the wild, proving that it is humane method to permanently mark young marsupials.

Live trapping small to medium size mammals like these carnivorous marsupials can be done by using box or cage live traps such as the enclosed, aluminium ones (Elliot or Sherman traps), the wire meshed ones (Tomahawk traps) or pit fall traps (Catlin et al 1997, Tasker & Dickman 2002, Umetsu et al 2006, Caceres et al 2011) and their effectiveness is variable depending on the species targeted. Accidental death of the trapped animal while in the trap is a constant risk issue that the researcher must to deal with and avoid as much as possible, especially if working with rare species or in long-term studies. Trap-related mortality can be caused by stress while in the trap or during post-capture handling, predation while still in the trap or when the animal is being released, consecutive recaptures (so the animal has not been able to recover from the previous captured), starvation, and extreme environmental conditions (i.e. extreme heat or cold) that a trapped animal is not able to avoid in that situation (Lemckert et al 2006). These risks can be minimised by checking the traps regularly and release the animals as soon as possible, providing some bedding material or a shelter so the animal can nest or refuge when scared, remove extremely dirty traps that are likely to attract predators and check for predators nearby when releasing the animal hopefully at the capture site, provide enough food and offer some fluids when necessary, avoiding extreme weather conditions and providing warm bedding material and protective cover to avoid direct sunlight exposure in cold and hot weather, respectively. It is important to note that juveniles, old or sick individuals are more susceptible to trap-related mortality and that some species are more sturdy than others and cope extremely well under stress and extreme conditions (Lemckert et al 2006). Cockburn (1992) recommended not trapping around the expected date of birth, because neonates could become entangled in bedding material and die. Researchers generally do not trap when mothers first have young in the nest because of the possibility of prolonged separation of nest young from their mothers in traps (trapping when nest young are older is fine because mothers naturally leave them for long periods to encourage development of independence).

Tasmanian devils, the world’s largest of the carnivorous marsupial is facing extinction as a result of a fatal infectious cancer known as Devil Facial Tumour Disease (DFTD) reported in 1996 and that has being spread across the majority range of the species (McCallum et al 2007) causing a decline of up to 80% of the affected wild populations (McCallum & Jones 2006). It mainly affects adults of two to three years of age, generating a shift to a very young age-structured population (Jones et al 2008) as 90% of the older age class has been wipe out (Lachish et al 2009). As a compensatory response, females have started to breed at an early age (precocial breeding or early onset of sexual maturity) that seems to be facilitated by reduced competition due to low population density (Lachish et al 2009). This disease is transmitted through biting mainly during sexual encounters (McCallum et al 2007), and at first appears as small lumps around the mouth that then develop into large deforming tumours around the face and neck, causing the death of the animal within a few months due to starvation (deformed animals are unable to feed), infections and metastases (McCallum 2008). Among the available options to manage a wild infectious disease like this one include isolate healthy individuals together with captive breeding program and culling infected ones (McCallum 2008). Application of euthanasia to all infected individuals of an endangered species has been quite controversial. On one hand, it is perceived as a humane way to end the suffering of a dying animal, but on the other, it was perceived as a cruel measure, especially on those individuals that do not seem to be in a lot of pain, and that may breed. Besides, culling a threatened species will cause a reduction of the population density even more rapidly and it will also prevent to increase our knowledge on the ecology and transmission dynamics of an infectious disease like this one in the wild (Hamede et al 2013). Moreover, Lachish (2009) in her PhD thesis showed that culling all infected individuals not only failed to reduce, but also accelerated both the negative impacts of the disease on the population and on the progression of the disease. Furthermore, excessive culling of individuals may increase disease transmission if it intensifies animal movement due to break down of social structure.

One of the immediate management measures that has being implemented is the establishment of captive and wild “insurance populations” with enough genetic and phenotypic diversity to repopulate once a resistance has evolved, a vaccine has been developed or after the extinction of devil in the wild (Jones et al 2007). These individuals come from wild-sourced individuals kept at zoos or in quarantine, captive-bred devil and orphans from diseased mothers. Should these sick mothers be culled of be left to raise their young? Is one of the many questions that arise within this dreadful drama.

Dasyurids have a long history of being kept in captivity, and even though they have a short lifespan, under appropriate conditions they tend to live for longer (Jackson 2007). Enclosure facilities must consider that carnivorous marsupials are excellent climbers and very fast (especially the small ones), so extra care must be taking into account to avoid escapes. Natural looking enclosures provided with proper soil and leaf litter, hollow logs, branches with leafs/flowers, some rocks and bark, nest boxes and access to natural light or at least heat lamps so they can do some sun basking are fundamental to avoid stress and stereotypic behaviours. Changing the inside of the enclosure constantly, stimulate food searching/handling and changing the feeding pattern regularly seems to be very important too (Jackson 2007). For small species, providing a running wheel helps them to deal with the stress (Phillips et al 2012). Jackson (2007) calculated the minimum size required for enclosures to provide an appropriate housing facility for a pair of captive individuals according to their body sizes, natural mobility (home ranges), social behaviour and mating system. Captive enclosures must be at least large enough to allow breeding, social behaviour and long-term survival, and should be cleaned daily to remove faecal matter and uneaten food. Most dasyurids designate a special place as toilet (i.e. latrine), thus faeces are easily detected. Water bottles and drinking dishes should be cleaned and refilled daily.

Dasyurids are highly promiscuous, therefore multiple paternity is common. Providing the opportunity to mate with multiple males will increase genetic diversity and will favour directly both the young, as they have been described to grow faster than offspring from females that have been mating with a single male (Fisher et al 2006) and the female that will have the opportunity to choose with whom to mate. Some species have been shown to breed and successfully rear their young in captivity such as the striped-faced, Julia-Creek and fat-tailed dunnart (*Sminthopsis macroura, S. douglasi, S. crassicaudata*), yellow-footed, agile and brown antechinus (*Antechinus flavipes, A. Agilis, A. Stuartiiili*), fat-tailed pseudantechinus, little red kaluta, mulgara, kowari, southern dibbler, brush-tailed phascogale, common planigale eastern and western quoll and Tasmanian devil. Some difficult ones are white-footed dunnart, Ningbing false antechinus and kultarr (Jacksoon 2007). It has also been described that breeding success in a captive colony may decline with time over successive generations. Some species breed well for one to two years and on the third year reproductive success declines considerably. There are also detail protocols to hand rear dasyurid marsupials when the mother is lost. These methods have been proven to be quite successful (see Jackson 2007 for further details).

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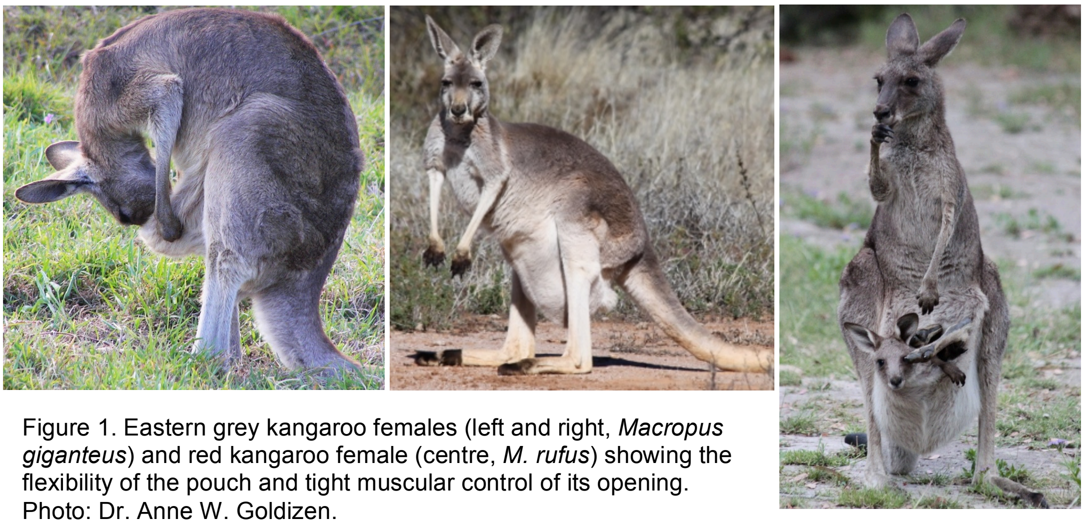

Figure 1. Eastern grey kangaroo females (left and right, *Macropus giganteus*) and red kangaroo female (centre, *M. rufus*) showing the flexibility of the pouch and tight muscular control of its opening.
Photo: Dr. Anne W. Goldizen.

Figure 3. Eastern grey kangaroo mother with joey at foot (left, *Macropus giganteus*) and common brushtail possum carrying her large young on her back (right, *Trichosurus vulpecula*)
Photo: Dr. Anne W. Goldizen.

Figure 3 Brown antechinus mother (*Antechinus subtropicus*) with her eight young exposed.
Photo: Dr. Anne W. Goldizen

Figure 4. Toe-bud clipping procedure to identify small antechinus young (*Antechinus stuartii*).
Photo: Dr. Diana O. Fisher