Effects of Mismatched Pre- and Postnatal Environments on Offspring Sex Ratios in Reintroduced Populations

Arran Lindsay Robinson
May 2020

A thesis submitted in partial fulfilment of the requirements for the degree of:

Master of Science in Environmental Science
School of Earth and Environment
University of Canterbury

Te Whare Wānanga o Waitaha

Effects of Mismatched Pre- and Postnatal Environments on Offspring Sex Ratios in Reintroduced Populations

Arran Lindsay Robinson

Declaration

I hereby declare that this thesis contains no material that has previously been accepted for the award of any other degree at the University of Canterbury or any other institution. To the best of my knowledge, this thesis does not contain any information already published or written by another person, except where acknowledgements are made in the text (i.e., reference material).

Arran Lindsay Robinson 09/05/2020

Statement on authority to access

Table of Contents

List of Figures

Figure Page No.

List of Tables

Acknowledgements

This thesis would not have been possible without the help and support of numerous fantastic people.

Firstly, I would like to thank my primary supervisor, Elissa Cameron. I am extremely grateful for all that you have done for me throughout my studies! I would not have gotten through this project without your support. You were consistently positive and always there to give me great advice whenever I needed.

I would also like to extend my sincere thanks to my secondary supervisor, Daniel Stouffer, who provided great assistance and advice in planning and conducting the analysis for this project.

To my parents, Kaye and Chad: your endless support and guidance throughout my life has been my rock and without it I would never have been able to achieve this feat. And to Chad, I will forever be grateful for all the assistance you have given me in proofreading and formatting this thesis.

I would also like to thank Bridget, with whom I shared an office. Having someone to talk to about the challenges and hurdles I was facing (particularly with data processing) has really helped me get through, and you work ethic and drive was a great motivator for me.

I would like to thank all my friends who have been with me from my first years at university. You have always been there for me throughout all the challenges I have faced, and your support and friendship has not only helped me get through these last years but also made them some of the best times of my life.

This project has been completed in unsettling times amidst the global COVID-19 pandemic. I would like to thank the University of Canterbury and the postgraduate support team for the support they have provided, especially during the Levels 3 and 4 national lockdown.

Abstract

Species reintroductions are becoming increasingly used as a conservation strategy to preserves threatened species and restore natural ecosystems. While species reintroductions can have significant positive impacts for threatened species and ecosystems, a large portion fail, despite our growing understanding of how they should be carried out. A potential contributor to this high rate of failure is offspring sex ratios in wild populations following release. A number of studies have reported unexpected biases in offspring sex ratios of reintroduced animals, and this pattern may be even more prominent than the literature suggests. Skewed sex ratios can slow the growth and recovery of reintroduced populations and subsequently limit the establishment of a viable wild population.

Understanding the mechanisms and drivers of these unexpected sex ratios is an important first step in mitigating the damage they can do to reintroduction programmes. Here I explore the effect that a mismatch between pre- and postnatal environments has on sex allocation in reintroduced animals. Developmental experience in a captive environment can have lifelong effects on an individual's physiology, such that they are unable to mount appropriate physiological responses to the current local conditions once reintroduced into the wild. As sex allocation acts through physiological mechanisms, this may impose constraints on their ability to adjust offspring sex ratios adaptively.

I examined offspring sex ratios in captive and reintroduced populations of three species: Arabian oryx, California condor, and red wolf. Offspring sex ratios of individuals with matched pre- and postnatal environments were compared to those of individuals with mismatched pre- and postnatal environments (i.e., individuals that had been either captured or reintroduced). I then explored the relationships between several predictors of sex allocation (sire (father) and dam (mother) age, sire and dam parity, rainfall around the time of conception, and temperature around the time of conception) and offspring sex ratios, and examined how those relationship were affected by a mismatch between pre- and postnatal environments.

Offspring sex ratios of mismatched Arabian oryx and red wolves did not differ from offspring sex ratios of matched individuals. However, captive born California condors that reproduced in the wild (mismatched environments) produced significantly more male offspring that those born in and reproducing in the wild (matched). A mismatch between pre- and postnatal environments affected the relationships that sex allocation had with rainfall, parental parity, and dam age (only in wild populations) in Arabian oryx. In California condors, the relationships that offspring sex ratios had with parental age, parental parity and temperature were all affected by a mismatch between pre- and postnatal environments. Finally, in red wolves, mismatched environments affected the relationships that offspring sex ratios had with sire age and temperature.

These results support the hypothesis that the mismatched environments associated with reintroduction can impose physiological constraints on the ability of released animals to adjust offspring sex ratios adaptively. This may explain some of the unexpected sex ratio biases observed in reintroduced populations, and indicate that such constraints could be important in the success or failure of species reintroductions.

1 Introduction

1.1 Species Reintroductions

There is a crisis in the current rate of extinctions and number of species under threat of extinction (Stork 2010; Burkhead 2012; Alroy 2015; Thomas and Morris 1994; Loehle and Eschenbach 2012), with human activities accepted as the main cause (Ceballos, et al. 2015; Pimm, et al. 2006; Brashares, et al. 2001). Some reports suggest we are amidst the sixth mass extinction the Earth has seen (Ceballos, et al. 2010; Wake and Vredenburg 2008; Barnosky, et al. 2011). The latest International Union for Conservation of Nature (IUCN) data reports that approximately 26.8% of all species assessed are threatened, ranging from critically endangered to vulnerable, with most species remaining data deficient and therefore unassessed (IUCN 2020). In response to this crisis, new and bold conservation strategies have emerged, including the use of species translocations and reintroductions (Seddon, et al. 2007; Reading, et al. 2002).

Species reintroductions attempt to re-establish wild populations to parts of their former range from which they have become locally extinct (Gusset 2012), while translocations are the movement of species from one area to another with the goal of restocking populations (Bullock, et al. 1996; Seddon, et al. 1999). These strategies are often coupled with captive breeding populations when species are globally threatened (Snyder, et al. 1996). While captive breeding can save the species from total extinction, the capacity for captive population growth is limited. Without the release and re-establishment of a viable wild population, the species will remain functionally extinct (Balmford, et al. 1996). For some time now, reintroductions and translocations have been used as conservation strategies to protect threatened species from extinction and help them recover in the wild (Seddon, et al. 2007).

Species reintroduction programmes take animals from captive facilities and release them into part of their native historic range (Kleiman, et al. 1994). They generally involve multiple releases, and the specific goals vary, from purely to re-establish a wild population of a threatened species once its habitat has been restored (i.e., removal of the threats that drove the population decline in the first place), to providing an ecological function which has been lost, and therefore helping to restore an entire ecosystem (Gibbs, et al. 2008; Griffith, et al. 1989). Wild populations are then intensively monitored and managed to help them grow to a point where they can become stable and independently viable (Seddon, et al. 1999).

1.1.1 The science of reintroduction biology

When species reintroductions were first carried out, they were structured around the management of the species, with little thought given to research (Seddon, et al. 2007). However, during the 1990s this changed, with more focus being put on monitoring and the use of research objectives. This brought about the recognisable field of reintroduction biology (Seddon, et al. 2007). The establishment of this field has highlighted the value of learning from past projects, with specific goals to improve our understanding of how to carry out successful reintroductions (Armstrong and Seddon 2008; Armstrong, et al. 2015).

Despite the establishment of reintroduction biology as a distinct scientific field, it has remained a challenge to define the criteria for success in species reintroductions, and no broadly accepted definition has been found (Seddon 1999). Robert et al. (2015) suggested that, to be successful, a reintroduction programme should produce a self-sufficient, viable population. They then assessed the reliability of using the same criteria as would be used to evaluate a remnant population, such as the IUCN Red List criteria. The IUCN system uses five criteria based around four measures: decline rate, range area, population size, and extinction risk. Species are ranked according to the single criteria that indicated the highest extinction risk level (IUCN 2019). Robert et al. (2015) concluded that, while reintroduced populations could be assessed using the same criteria as remnant populations, rescaling of those criteria is required. This is because, reliable assessment of the success of reintroduction requires that the population has gone through the establishment and growth phases and has reached the regulation phase. Alternatively, Jule et al. (2008) reported that a combination of the following criteria are often agreed upon to indicate success: (1) breeding success in the wild born population, (2) recruitment rates exceeding mortality rates over three years in the wild breeding population, (3) a self-sustaining wild population with at least 500, and (4) establishment of an unsupported and viable wild population.

Despite the difficulty of defining success, species reintroductions have clearly resulted in positive outcomes for threatened species and ecosystems as a whole (Haskins 2015; Smith and Bangs 2009; Kierulff, et al. 2012). A few successful high-profile vertebrates were reintroduced in the 70s and 80s (reintroduction of the Arabian oryx and golden lion tamarin to the Arabian Peninsula and Brazil, respectively; Spalton, et al. 1999; Kleiman et al. 1986), which helped to popularise species reintroductions as a viable conservation strategy (Gusset 2012). Since then, successful reintroductions have been carried out on a range of species from across the globe. In 2008 the IUCN published Global reintroduction perspectives: reintroduction case studies from around the globe. In this they reported on 62 reintroduction case studies from around the world, highlighting the goals, difficulties, lessons learned, and success of the projects. They have since released a further 5 versions, each reporting on new case studies (IUCN 2008; 2010; 2011; 2013; 2016; 2018). There are now 349 case studies presented. Success was ranked as highly successful, successful, partially successful, or failure. Over the years the success rates have been maintained at a fairly high level (21% highly successful, 37% successful, and only 4% fail). However, in the 2011 version, Reading highlighted that past surveys have found that most translocation efforts do fail (IUCN 2011). In 1989, Kleiman (1989) reported that only approximately half of bird reintroductions were successful, and fewer still for mammal reintroductions. Since then, our understanding of how to successfully manage a species reintroduction programme has grown substantially. However, more recent reviews have also presented lower success rates than those presented by the IUCN (Fischer and Lindenmayer 2000; Jule, et al. 2008; Sutton and Lopez 2014). Reading (IUCN 2011) suggests that "people are reluctant to share their failures", which may contribute to the disproportionately high success rates in the IUCN reports. Nevertheless, these reports do highlight a vast number of successful species reintroduction programmes.

In the latest IUCN report, 23 of the 59 cases were ranked as highly successful. These included reintroductions of plants, mammals, birds, reptiles, amphibians, fish, and invertebrates across the globe (IUCN 2018). Some examples of reintroductions that were reported to be highly successful are: reintroduction of Telfair's skink to Gunner's Quoin Island in Mauritius; the North Island kākā reintroduction to a mainland sanctuary in Wellington; the reintroduction of African lions to Akagera National Park in Rwanda; and the reintroduction of the Western barred bandicoot to mainland Australia. It is clear from looking at these reports that reintroductions have had, and will continue to have, a great impact on the protection and recovery of threatened species, across a range of geographic locations and species types (Summarised in; IUCN 2018).

Over the years there have also been a number of unsuccessful reintroduction attempts. In 2009 the brown treecreeper (*Climacteris picumnus*) was reintroduced to part of its former range in New South Wales, Australia. However, due to low survival rates over the first year, the reintroduction failed to meet predetermined criteria for success (Bennett, et al. 2013). Similarly, the reintroduction of captive-bred oribi (*Ourebia ourebi*) in South Africa was deemed unsuccessful; only after 10 years of releases did post-release monitoring begin, which showed that the reintroduced populations were surviving poorly (Grey-Ross, et al. 2009). Captive-bred populations of red wolves were released to two sites, the first in North Carolina and the second in Tennessee (Phillips, et al. 2003; Hedrick and Fredrickson 2008). The Tennessee population struggled and were subsequently transferred back into captivity or to the North Carolina site. While the North Carolina population has fared better, recent reports suggest that if current population trends continue the risk of extinction will be severe (Simonis, et al. 2017).

Over the years, as the field of reintroduction biology has grown, our understanding and practices have improved. As a result, reintroductions have become increasingly successful (Reading, et al. 2013). Nonetheless, a relatively large proportion remain unsuccessful despite our ever-increasing understanding of reintroduction processes (Sutton and Lopez 2014). It is not always obvious what is different between successful and unsuccessful reintroductions. Identifying factors that lead to failed reintroductions can be challenging but is an essential step in increasing success for the future (Armstrong and Seddon 2008).

1.1.2 Why do Reintroductions Fail?

Species reintroductions programmes often incur a large economic cost (Dyar and Wagner 2003; Hayward, et al. 2007). Failed reintroductions are therefore a waste of time and resources, as well as a loss of individuals from threatened species with already limited numbers (Deredec and Courchamp 2007; Macdonald 2009). It is vital that we learn from past failures and identify causal factors that limit success (Cochran-Biederman, et al. 2015). A range of factors may contribute to the success or failure of a reintroduction programme. These include both biological factors, and management/organisational factors (Deredec and Courchamp 2007). Different reintroduction programmes may vary in which aspects prove significant depending on species types, location/country, and the specific goals of the programme (Cochran-Biederman, et al. 2015).

Broadly speaking, reintroduced populations fail when recruitment does not sufficiently exceed loss, limiting growth and preventing the establishment of a viable, self-sustaining population. Several factors have been linked to some of these failures. Habitat quality may play a key role in the success or failure of species reintroduction, as release sites with lower food availability or fewer refuge areas have been linked with reduced survival (Moorhouse, et al. 2009; Bennett et al. 2013). Release sites in which predation is high may also result in failed reintroductions (Hardman, et al. 2016; Moseby, et al. 2015; Moseby, et al. 2011). Wild populations of other species may carry diseases that captive-bred or translocated individuals have little immune resistance to. In such cases, diseases can significantly limit the success of species reintroductions (Viggers, et al. 1993; Ballou 1993). Hunting and poaching is the cause for decline in many threatened species, and in some cases this continues following reintroduction, resulting in the loss of many individuals and leading to programme failure (Spalton et al. 1999; Grey-Ross et al. 2009). These factors, among others, such as inbreeding (Jamieson 2011; Jamieson, et al. 2007), crossbreeding (Hedrick and Fredrickson 2008), and the Allee effect (inverse density dependence; Deredec and Courchamp 2007), can all lead to failures in species reintroduction programmes. Grey-Ross et al. (2009) highlighted the importance of post-release monitoring, which historically has been minimal, to identify factors limiting success and mitigate them going forward. The structure and demography of reintroduced populations can also have an effect on the success of species reintroductions, resulting in more subtle failures in a species ability to persist in the new environment. For example, skewed sex ratios or unnatural age structures in reintroduced populations can limit reproductive output and influence intraspecific interactions to harmful effect (Wedekind 2012; Le Galliard, et al. 2005).

When populations are captive-bred, reintroductions tend to fail more often than when wild-born animals are re-released (Sjöåsen 1996; Bremner-Harrison, et al. 2004; Vickery and Mason 2003). This is partially because of the negative impacts of a number of these factors can be more significant. Captive-bred individuals are more susceptible to starvation, predation, and disease in the wild (Jule, et al. 2008). Additionally, when captive-born animals are reintroduced, birth sex ratios in the population are often skewed unfavourably following release. This pattern may be more prevalent than the literature suggests. For example, in the reintroduced population of Arabian oryx in Oman, the first generation of offspring following release had a male-biased sex ratio (Price 1989). However, this skew has not been published in any scientific journal. Skewed sex ratios following the release of reintroduced animals is rarely considered but can have significant impacts on population recovery.

Sex ratio biases in either direction can reduce the genetically effective population size (population size of an ideal population that loses genetic variability at the observed rate; Caballero 1994) even if the number of individuals is increasing. This can lead to losses of genetic variability and increase the chance of inbreeding depression (Wedekind 2012). Female biased sex ratios may limit the reproductive output in small populations. When few reproductively viable males are present in a population, sperm limitations can greatly reduce female fecundity and drive population collapse (Ginsberg and Milner-Gulland 1994; Sæther, et al. 2003). In many species however, oocyte availability limits population growth. Therefore, male biased sex ratios may be even more harmful in small populations (Wedekind 2002). Male biased sex ratios limit the effective population size (the number of individuals in a population that are able to breed) and reduce population growth (Robertson, et al. 2006; Lens, et al. 1998; Lambertucci, et al. 2013). Male biased sex ratios also increase mate competition and harassment of females. This causes sexual conflict, which can result in injury, mortality, and reduced fecundity in females (Ewen, et al. 2010; Le Galliard, et al. 2005). In species where population growth is limited by oocyte availability, female biased sex ratios may actually be beneficial in order to maximise reproductive output (Wedekind 2002; Wedekind 2012).

Population recovery can be limited by undesirable sex ratios, which in reintroduced populations may make the difference between success and failure.

1.1.3 Sex Ratio Biases in Reintroduced Populations

Skewed sex ratios have been reported in a number of reintroduced species following release (Table 1.1). As reintroduced populations tend to be small and vulnerable to the negative effects associated with sex ratio biases (Wedekind 2012), this could hamper the success of the programme. In some cases, skewed sex ratios are a key factor limiting the growth of reintroduced populations (Milton and Hopkins 2006; Saltz and Rubenstein 1995).

Table 1.1: Skewed sex ratios observed in reintroduced populations following release.

Species	**Reintroduction** **Location**	**Years**	**Observed Sex Ratio**	**Explanation**
Geoffroy's spider monkey (*Ateles geoffroyi*)	Barro Colorado Island, Panama	1959 - 1966	Male biased in the first decade of breeding[1]	Insufficient number of reproducing females for sex ratios to be adaptively adjusted based on dominance hierarchy
Asiatic wild ass (*Equus hemionus*)	Negev Desert, Israel	1982 - 1987	Initially male biased before shifting to female bias[2]	Age structure of the reintroduced population
Pere David's deer (*Elaphurus davidianus*)	Beijing Milu Park and Dafeng Milu Natural Reserve, China	1985 - 1986	Female biased[3]	NA
Black rhinoceros (*Diceros bicornis minor*)	Sam Knott Kudu Reserve, South Africa	1986 - 1997	Female biased[4]	NA
Mountain gazelle (*Gazella gazelle*)	Hawtah Reserve, Saudi Arabia	1991 - 1995	Initially female biased before shifting to male bias[5]	NA
Hihi (*Notiomystis cincta*)	Tiritiri Matangi, New Zealand	1995	Male biased[6]	Sex ratio was density dependent

---

[1] Milton and Hopkins 2006

[2] Saltz and Rubenstein 1995; Saltz 2001

[3] Jiang, et al. 2000

[4] Law, et al. 2014

[5] Dunham 2001

[6] Ewen, et al. 2010

The sex ratios skews in these reintroduced populations were generally unusual and unexpected. In some of the above cases, attempts have been made to explain what caused these skews (Table 1.1). In the reintroduced population of spider monkeys, little explanation was given to the male biased sex ratio in the first generation after release. However, Milton and Hopkins (2006) suggested that the birth sex ratio returning to its expected state was due to the fact that there were finally enough reproductively active females for regular dominance hierarchies to exist. Such dominance hierarchies are thought to be an important driver of sex allocation in this species and their absence may explain the initial unexpected skew (Milton and Hopkins 2006).

Saltz and Rubenstein (1995) suggested that Asiatic wild ass would operate under age-dependent sex allocation, with females in prime reproductive age producing more sons. The observed male biased sex ratios following release was linked to the age structure of the reintroduced population, which had a high proportion of females in the "male-producing age" during this time (Saltz and Rubenstein 1995). However, Saltz has been criticised for overemphasising the importance of maternal age in sex allocation of ungulates (Hewison, et al. 2002), and other studies suggest age has a minimal effect (Cameron 2004).

In the reintroduced population of hihi, Ewen et al. (2010) suggested that birth sex ratios were density dependant. Sex ratios became increasingly male biased when population density increased. However, they did not provide an explanation for that relationship (Ewen, et al. 2010). No explanation was given to explain the skewed sex ratios observed in the other species. For the rhino population, Law et al. (2014) examined the unusual birth sex ratio in relation to birth sequence, maternal identity, year of conception, rainfall, population size, maternal age, and adult sex ratio. They found no significant relationship between birth sex ratio and any of their measured variables (Law, et al. 2014).

Theoretical explanations for sex allocation generally assume equal ability to adaptively adjust offspring sex ratios between individuals. What is rarely considered is that physiological variations between individuals may impose constraint on their ability to adjust offspring sex ratios adaptively (Edwards, et al. 2016a). Variable physiological characteristics, such as stress responsiveness, which are largely determined during development, play a key role in sex allocation (Edwards et al. 2016a). Importantly, when a significant environmental change occurs, such as that experience by reintroduced animals when they are released, their developmental experience and subsequent physiology may limit their ability to respond to environmental influences. This may result in constraints on their ability to adjust sex ratios and explain the unusual sex ratios observed in reintroduced populations (Edwards, et al. 2019).

1.2 Sex Allocation

Sex allocation is the ability of organisms to variably invest in the production of male and female offspring (West 2009). When sex-specific fitness returns vary in response to an external variable, such as current local conditions or ability to invest resources and energy in the production of offspring (Clark 1978; Trivers and Willard 1973), it becomes adaptively beneficial to adjust investment in the sexes accordingly. Sex allocation allows for such a partitioning of resources and helps to maximise fitness returns on investment in offspring (West 2009).

Variation in sex ratios has long been recognised in evolutionary biology. As far back as the 19th century, Darwin explored the evolution of sex allocation, although he did not provide a direct explanation and suggested that the solution should be left for the future (Frank 1990). Since then our understanding has grown considerably, but there is still much to uncover about why and how sex allocation occurs. Empirical support for the theories of sex allocation has been notoriously inconsistent, as observed sex ratios still regularly fail to match predictions (West and Sheldon 2002; Packer, et al. 2000). This is often true in reintroduced populations, where unexpected and unexplained sex ratios have been observed (Saltz 2001; Linklater 2007).

There are several prominent theories of sex allocation and of the physiological mechanisms through which they act. Through developmental limitations to these physiological mechanisms, sex allocation may be constrained (Edwards, et al. 2016a). This may explain some of the unpredicted sex ratios that have been observed; in particular, those in reintroduced populations (Edwards, et al. 2016b; 2019).

1.2.1 Fisher's Principle

Fisher (1930) suggested that variability in fitness returns between investments in the two sexes would lead to variable sex ratios. He explained why this resulted in the sexes usually being produced in equal numbers (or that investment in producing offspring of each sex be equal). If births of males became less common, a newborn male would have more success finding a mate than a newborn female. A genetic disposition to producing more males would be selected for until a sex ratio of 1:1 is reached, at which point the advantage of having males would be lost. The same holds true if the sexes are reversed. Sex ratios on a population level should therefore be maintained at a ratio of 1:1. This process is termed Fisher's Principle.

Fisher's Principle operates under a number of assumptions, which, although Fisher himself did not state explicitly, have been presented in reconstructions of his model (Bull and Charnov 1988). One of these is that variations in factors such as body condition, resource availability, or environmental conditions, affect the fitness of both sexes equally. The breaking of this assumption has led to the development of more recent and detailed explanations for the evolution of adaptive sex allocation (Hardy

1997). Factors such as local environmental conditions and parental ability to invest can differentially affect the fitness returns of producing sons and daughters (Hamilton 1967; Triver and Willard 1973; Clark 1978; Silk 1983). In such cases, it is beneficial to be able to selectively invest in offspring of the sex that is expected to be more successful in passing on its genes, thereby yielding a greater fitness return. This provides the basis for a number of the theoretical explanations for the evolution of sex allocation that have been proposed (Review; Hardy 1997) (Table 1.2).

The most prominent theoretical models have linked to differential investment returns in the presence of sex-biased dispersal patterns (Hamilton 1967; Clark 1978; Gowaty and Lennartz 1985) or differential variability in offspring fitness between the sexes, which varies by reproductive system (Trivers and Willard 1973) (Table 1.2). This can lead to variable sex allocation in response to group structure or local resource availability, and environmental conditions or parental ability to invest in offspring development, respectively.

Table 1.2: Prominent sex allocation hypotheses.

Hypothesis	**Predictor**	**Expected Sex Ratios**	**Source**
Fisharian Sex Allocation	Sex-specific population density	Birth sex ratios should favour the sex that is less common in the population	Fisher, 1930
Local Mate Competition	Local male density relative to number of reproductively active females	As the number of males per female increases, sex ratios of offspring should become increasingly female biased	Hamilton, 1967
Local Resource Competition	Resource competition within the local area	As competition intensity increases, sex ratios should increasingly favour the more dispersing sex	Clark, 1978
Local Resource Enhancement	Sex-specific cooperative behaviour	Sex ratios favour sex that will provide assistance with future reproductive efforts	Gowaty and Lennartz, 1985
Trivers-Willard Hypothesis	Parental ability to invest in offspring	Increased ability to invest in offspring results in sex ratios favouring offspring of the sex that will receive greater fitness benefits from increased condition as adults (usually male)	Trivers and Willard, 1973

1.2.2 Local resource competition (LRC), including Local mate competition (LMC)

The term Local Resource Competition (LRC) was first coined by Clark (1978) to explain sex ratio skews in the primate species *Galago crassicaudatus*. LRC is generally used to refer to competition for resources such as food or nesting sites. In this discussion, the term is used to include Local Mate Competition (LMC), which was theorized by Hamilton (1967) prior to Clark.

Hamilton (1967) explored a case in which the assumption of Fisher that competition for mates is population-wide is broken. Instead he considered mate competition to be localised. His model was constructed under the following parameters: populations consist of isolated patches; offspring born into a particular patch would mate at random within that patch; once inseminated females disperse between patches (Taylor and Bulmer 1980). Males compete for mates amongst themselves within a patch. As a result, the fitness return from producing males depends on the number of males in the patch and the number of females producing offspring. The fitness returns from producing females, however, do not. When more males are expected to be in the population, sex ratios should be female biased (Hardy 1997). LMC can be put plainly as; if competition for mates is likely to be more intensive for one sex than the other, parents should produce more offspring of the less competing sex in order to maximize fitness returns (Clutton-Brock and Iason 1986). LMC is often used to explain female biased sex ratios in invertebrates. Evidence for its influence in vertebrates has been limited and controversial (Hardy 1997).

LRC for environmental resources has been much more prominent in vertebrate species (Silk and Brown 2008; Mari, et al. 2008; Leturque and Rousset 2004). Clark (1987) first suggested it with regards to sex ratios in the primate species *Galago crassicaudatus*, in response to limited local food resources. Males, being more mobile, are less affected by, and do not add to, local competition Thus, fitness returns are diminished by competition when producing females, whereas the fitness returns from producing males are not. When resources are limited and competition is high, mothers should invest more in the production of male offspring. This is reflected by the male biased sex ratios observed for this species, both in the field and in captivity (Clark 1987). LRC may also drive sex allocation when other factors, such as sexual dimorphism, result in differential competition between the sexes (Uller 2006).

While Clark consistently observed a male bias, LRC predicts that sex ratios are variable in response to the availability of a limited resource, and therefore competition intensity (Johnson, et al. 2001). Additionally, the particular resource that drives LRC can vary and may not be initially obvious. Johnson et al. (2001) demonstrated both these points in a study examining sex ratios of the common brushtail possum. In this species female offspring are philopatric (non-dispersing), while male offspring are dispersing. They found a correlation between food availability and offspring sex ratio, not directly, but indirectly through the effects of food availability on population density and per-capita den site availability. Food rich areas led to high population density, which in turn limited availability of dens. Mothers producing female offspring would increase competition with themselves and other daughters, limiting fitness returns. Producing the dispersing male offspring would not. Therefore, when competition was high (low per-capita availability of den sites) offspring sex ratios tended to be male biased. This pattern was variable between different populations depending the availability of den sites. LRC can be driven by a number of limiting resources, including food, territory, nest/den sites, and mating opportunities (Schwanz and Robert 2014; Hjernquist, et al. 2009), and has been shown as the most likely driver of sex ratio skews in a number of species (Gowaty 1993; Hewison and Gaillard 1996; Silk and Brown 2008).

Sex allocation is generally considered to be how a mother (or father) adjusts the sex ratio of her own offspring. With regards to LRC, Silk (1983) highlighted that biased sex ratios may be achieved by interacting with other individuals to reduce the probability that they will rear offspring of a particular sex. This may be achieved by harassment and limiting the resources of non-related females. In doing this, local competition can be reduced for themselves and their relatives.

1.2.3 Local Resource Enhancement (LRE)

Local Resource Enhancement (LRE) theory is in many ways similar to LRC. However, instead of the level of competition varying between the two sexes, the level of assistance/help given to siblings or parents varies between the sexes (Gowaty and Lennartz 1985; Wild 2006). In some species, one sex of offspring is likely to assist with parental care of subsequent broods or litters (Clutton-Brock and Ianson, 1986). Producing offspring of the helpful sex would then improve future reproductive success and increase fitness return on investment. These conditions are expected to lead to a skewed sex ratio in favour of the helpful sex (Gowaty and Lennartz 1985). A number of cases in which sexually divergent cooperation behaviours correlate with biased sex ratios in favour of the cooperative sex have been presented in the literature (McNutt and Silk 2008; Silk & Brown 2008). Levels of cooperation may be variable in response to environmental conditions, making the effects of LRE variable across populations, similar to LRC (Griffin, et al. 2005).

1.2.4 The Trivers-Willard Hypothesis

The Trivers and Willard hypothesis states that if one sex of offspring is disproportionately advantaged by condition, a mother will benefit from adjusting sex ratios in response to the expected condition of her offspring once they reach maturity (Trivers and Willard 1973). In their original paper, Trivers and Willard gave three criteria that should be met for variable offspring sex ratio to be adaptively beneficial under their model: 1) Condition of offspring should be correlated with condition of mother, 2) Condition of offspring once matured should be correlated with condition at the end of parental investment period, and 3) The two sexes should gain differentiating fitness benefits from improved condition. In short, if offspring condition at maturity is dependent on maternal condition, and one sex exhibits more variability in fitness in response to condition, then the mother should selectively adjust her offspring sex ratio based on her own condition.

Trivers and Willard (1973) presented a hypothetical population of ungulates to demonstrate a case where their theory should hold true. In this population, they stated that condition of adults was variable and could be measured. Mothers in good condition would be able to produce offspring that would, at the end of the period of parental investment, be healthier, stronger, and larger than offspring produced by mothers of poor condition. The condition of an individual at the end of the period of parental investment would to some degree be maintained once sexual maturity is reached. In this population, males compete for mating opportunities with females, and strong, dominant males exclude males in poor condition from mating. As females are the limited resource in reproduction, females gain the same mating opportunities regardless of condition. The reproductive success would be highly variable in response to condition for males, but not for females. In this population, a mother in good condition would gain more fitness return on investment in offspring (more grandchildren) by producing sons than daughters. A mother in poor condition, however, would gain more return on investment by producing daughters. This means there is an adaptive benefit to an adult female producing offspring to adjusting offspring sex ratios in accordance with her own condition.

In the original paper (Trivers and Willard 1973), maternal condition is presented as a proxy for the ability of a mother to invest in offspring development. Condition was considered as physical condition, and weight was given as an example of its measure. Ability to invest may however be affected by a number of factors and empirical studies have used a range of indicators to measure condition (Cameron 2004; Sheldon and West 2004).

Maternal physical condition has commonly been used as an indicator of ability to invest in offspring (Trivers and Willard 1973). Females in better physical condition are expected to be able to invest more heavily in offspring. Therefore, they should skew sex ratios of offspring in favour of males. Empirical tests on a number of species have found support for this relationship (Bradbury and Blakey 1998; Kojola and Eloranta 1989; Pike and Petrie 2005; Pike 2005; Kohlmann 1999; Review; Cameron 2004). However, support for this has not been unanimous. Sheldon and West (2004) suggested that physical condition might be a poor index for ability to invest because it does not necessarily correlate with future access to resources, which may be more significant in determining ability to invest over the course of offspring development.

In a more recent study, change in condition around the time of conception was found to be a better predictor for sex ratios than condition itself, as change in condition is more likely to represent future ability to invest (Cameron and Linklater 2007).

An alternative indicator of maternal ability to invest is maternal dominance (Grant 1996). High-ranking or dominant females are expected to have more access to resources, and therefore more ability to invest in offspring. In several studies, high-ranking females were found to produce more sons than low- or mid-ranking females (Clutton-Brock, et al. 1984; Meikle, et al. 1993; Review; Grant 1996). In a meta-analysis conducted by Sheldon & West (2004), when behavioural measures of condition, such as dominance, were used, support for the Trivers-Willard hypothesis was stronger than when physical measures were used.

Maternal age may also influence ability to invest in offspring, leading to sex ratio skews in accordance with the Trivers-Willard hypothesis (Saltz and Kotler 2003). Once females have reached prime age, body condition declines, as they get older. This decline is likely to be accompanied by reduced ability to invest in offspring, therefore, producing fewer sons (Clutton-Brock and Iason 1986). Alternatively, reproductive effort may increase with age as reproductive value (number of future offspring) decreases (Côté and Festa-Bianchet 2001). Therefore, older females are expected to invest more in any single reproduction event leading to the production of more sons. This has been observed in a number of species (Cote and Festa-Bianchet 2001; Weladji, et al. 2003; Blank and Nolan 1983; Thomas, et al. 1989). Despite this support, the relationship between maternal age and sex ratio has come under critique, particularly in mammals (Hewison, et al. 2002). In a meta-analysis, Cameron (2004) found that when age was used as a measure of ability to invest, support for the Trivers-Willard hypothesis was weak.

These are just some of the indicators of ability to invest in offspring development that are prominent in the literature. A number of others have been used, such as maternal parity (Clutton-Brock and Iason 1986) and climate or environmental conditions (Roche, et al. 2006; Berkeley and Linklater 2010), with varying levels of support from empirical studies (Cameron 2004).

1.3 Limitations to Empirical Testing

Sex allocation hypotheses are logically appealing, and strong theoretical reasoning backs the underlying principles. However, empirical testing has produced inconsistent results (Cameron 2004; Sheldon and West 2004). As a result, the hypotheses have been heavily criticised and some authors have suggested that there is no consistent pattern (Frank 1990; Festa-Bianchet 1996; Hewison and Gaillard 1999; Packer, et al. 2000; Kojola 1998). Observed sex ratios often fail to match predictions. Sex ratio skews tend to be smaller than expected (West and Sheldon 2002). Alternatively, altogether unpredicted sex ratios have been observed (Saltz and Rubenstein 1995; Jiang et al. 2000; Ewen et al. 2010).

Some of these inconsistencies can be explained by methodological variations between studies. For example, in empirical studies testing the Trivers-Willard Hypothesis, the type of measure used to indicate ability to invest in offspring, and the timing of measurement significantly affected the likelihood of providing support for the hypothesis. This was shown in two meta-analysis papers (Cameron 2004; Sheldon and West 2004), both of which confirmed the significant inconsistency in results, but found overall support for the Trivers-Willard hypothesis when the appropriate type and timing of measure for ability to invest was used. While the two studies were consistent in their finding that measures of condition should be taken before or close to the time of conception, they found that different measures of ability to invest provided the strongest support for the Trivers-Willard Hypothesis. Sheldon and West (2004), whose study looked at ungulates, found that measures of behavioural dominance were better indicators of ability to invest in offspring than morphological or physiological measures. Cameron (2004), who looked at non-human mammals, found the opposite. This highlights that one universal measure for condition is not the optimal strategy for testing the hypotheses of sex allocation, and that detailed understanding of the focus species is needed to select the best measure in such tests (Douhard 2017).

Another source of inconsistency in empirical support for the hypotheses of sex allocation may be the interaction of multiple drivers, either in support of, or in opposition to, one another. One of the challenges involved in finding empirical evidence to support sex allocation theories is identifying which processes may be involved for any particular species (Cockburn, et al. 2002). By no means are the different drivers of skew incompatible or mutually exclusive from one another. Failure to identify and consider the effects of multiple drivers can lead to predicted sex ratios that do not match observed sex ratios (Moore, et al. 2015).

In primates, Silk and Brown (2008) found that, in accordance with the LRC model, offspring sex ratios could be predicted by the dispersal pattern of that species. That is, when males were the primary disperser, sex ratios would be biased in favour of males to reduce competition for local resources. The reverse was true when females were more likely to disperse. However, in cooperative breeding species, for which males tend to be more active and effective helpers (Emlen, et al. 1986), sex ratios would tend to be biased in favour of males, in accordance with the LRE model. In a species where females disperse and males help in cooperative breeding, LRC would predict female bias while LRE would predict male bias (Silk and Brown 2008). The effects of either process may be dampened as they are acting in opposition to one another.

Similarly, according to the Trivers and Willard hypothesis, good maternal condition should lead to the production of more sons (Trivers and Willard 1973). However, if this is coupled with high maternal rank, which can be inherited in daughters, producing daughters may be advantageous (Sheldon and West 2004).

The strength and direction of offspring sex ratio is dependent on many factors. Making accurate predictions is therefore a difficult task. This requires a full understanding of life-history traits of any subject species. Hence, only a few well- studied species have provided empirical data that can truly support or dispute these hypotheses (Sheldon and West 2004).

A number of papers have highlighted the interactions of these alternative hypotheses and suggested that this may be contributing to the inconsistency or lack of strong empirical evidence (Hiraiwa-Hasegawa 1993; Cockburn, et al. 2002; van Schaik and Hrdy 1991; Moore et al. 2015). Untangling the effect of multiple drivers of sex ratio skews is one of the key challenges that will help us more clearly understand sex allocation.

Another more recently considered explanation for the inconsistency seen in empirical studies is that physiological constraints limit the ability for females to adjust offspring sex ratio adaptively (Edwards, et al. 2016a). The mechanisms of sex allocation act through physiological characteristics that can vary between individuals. This variation can result in different individuals mounting a different physiological response to the current local conditions, and therefore altering offspring sex ratios differently.

1.4 Mechanisms for Adjustment of Offspring Sex Ratio

There are multiple acting mechanisms by which sex allocation is achieved (Navara 2018a; 2018b) (Figure 1.1). Each mechanism may operate independently, or the different mechanisms may be interacting to have additive or opposing effects (Linklater 2007). Adaptive sex allocation mechanisms should allow for external conditions (for example, local resource availability or ability to invest) to be translated into a physiological response, which in turn leads to deviations from 50:50 in the production of males and females. This allows parents to maximise fitness returns based on their own condition and the environmental conditions (Merkling, et al. 2018). Three main physiological mechanisms have been identified for maternal adjustment of offspring sex ratios. These are maternal glucose levels (Cameron 2004), maternal stress levels (Navara 2010), and maternal testosterone levels (Grant 2007).

1.4.1 Developmental sexual dimorphism

A key element in the adjustment of offspring sex ratios is that the mechanisms behind it must be able to distinguish between male and female offspring (Cameron, et al. 2017). To reduce cost to the parents, this should occur as early as possible. Sexual dimorphism has previously been thought to come about after the development of gonads (Arnold 2012). However, recent research has shown that sexual dimorphisms are present in sperm and preimplantation conceptuses (review: Cameron, et al. 2017).

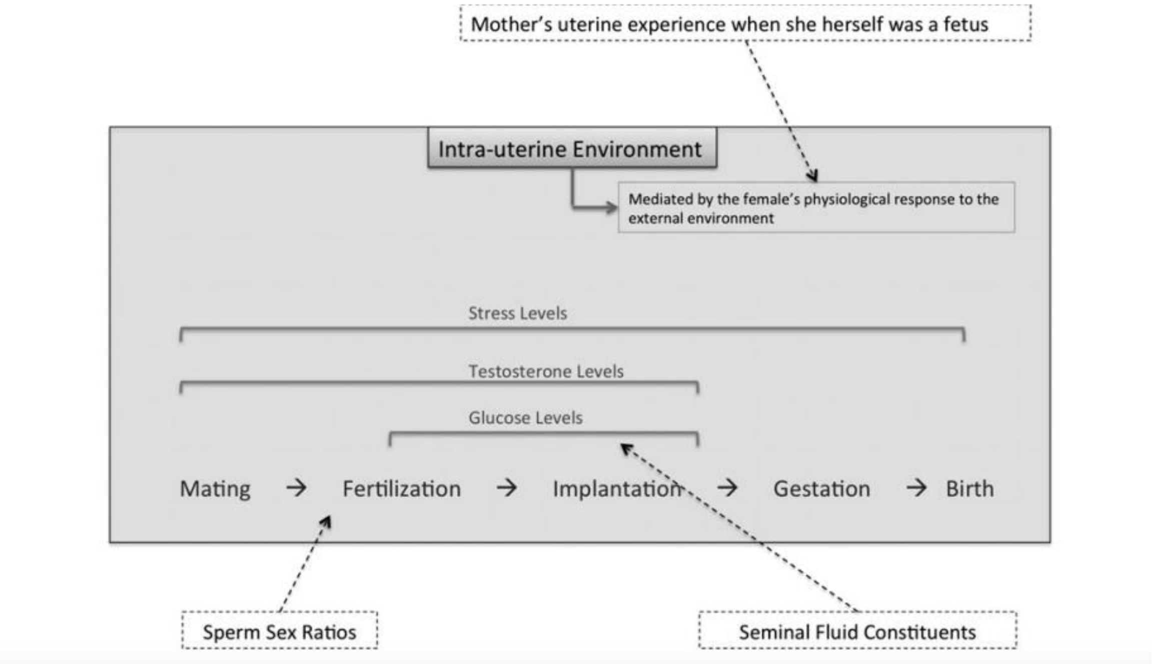

Figure 1.1: Taken, with permission, from Edwards et al. (2016a). "The mechanisms and constraints imposed on maternal sex allocation. Stress, testosterone and glucose levels are hypothesized to influence the offspring sex ratio at different stages from mating to birth through selection or selective-loss of either sex. Dashed boxes show the external constraints acting on maternal sex allocation, and dashed arrows indicate the time during gestation, or the mechanism that these constraints are acting upon."

These differences arise from the differences in sex chromosomes between males and females. In mammals, the Y chromosome is associated with a number of male-specific genes, and the female X chromosome inactivation is not absolute, and does not occur until the blastocyst stage, allowing the expression of both X chromosomes (Berletch, et al. 2011). Therefore, there are a number of differences between males and females during early development that are detectable and sufficient for the mechanisms of sex allocation to act on.

1.4.2 Maternal Glucose

Cameron (2004) proposed that maternal glucose level around the time of conception is a mechanism for sex allocation. This came off the back of some developments in our understanding of the characteristics of conceptuses. Firstly, blastocysts, which develop into embryos, are sexually dimorphic and differentially signal their presence to their mother (Larson, et al. 2001). This may allow for facultative adjustment of sex ratios (Cameron 2004; Larson, et al. 2001). Secondly, glucose influences the development of blastocysts differently for males and females. Increased glucose circulation inhibits female development while enhancing male development (Larson et al. 2001; Gutiérrez-Adán, et al. 2001). In the early stages of development (pre-implantation to early postimplantation) both X chromosomes are active in females (Heard, et al. 1997). This leads to sexual differences in the metabolism of glucose. X-linked proteins facilitate the pentose phosphate pathway for metabolising glucose (Gardner, et al. 2010). This pathway is expressed more in females and is highly efficient, but results in toxic by-products (Edwards, et al. 2016a). When glucose levels are high, the toxic by-products result in lower female survival. However, when glucose levels are low, female survival is enhanced in comparison to male survival (Edwards, et al. 2016a).

This mechanism has been supported through experimental research, and has been extended to suggest that it is the change in glucose levels around the time of conception that has the greatest impact on sex ratio (Cameron and Linklater 2007; Cameron, et al. 2008). Experiments on mice were conducted by supplying dexamethasone, a steroid that inhibits glucose transport and reduces glucose levels in plasma (Burén, et al. 2002), to females around the time of conception. Sex ratios of litters were then measured and compared to control groups. They found the two groups differed significantly in offspring sex ratio, with the sex ratio of the treatment group skewed towards females. More so than maternal glucose levels itself, a change in maternal glucose levels around the time of conception is a stronger predictor of sex ratio (Cameron, et al. 2008). Changes in glucose levels are expected to correlate with changes in condition, which may more accurately indicate the ability to invest in offspring in the future, during pregnancy and beyond (Cameron and Linklater 2007).

Adjustment of offspring sex ratios through maternal glucose levels occurs early on in development (Cameron 2004). This is consistent with both the prediction that sex allocation should occur as early as possible in order to minimise energy and resource waste (Trivers and Willard 1973), and the findings from meta-analyses that suggest measures of condition should be taken around the time of conception (Cameron 2004; Sheldon and West 2004).

1.4.3 Maternal stress levels

A number of studies have shown a strong link between maternal stress levels during the reproductive period and skewed sex ratios. Glucocorticoid levels or environmental stressors have been linked with offspring sex ratios in a range of species, including golden hamsters (Pratt and Lisk 1989), humans (Navara 2010), bridled nailtail wallabies (Moore, et al. 2015), black howler monkeys (Rangel-Negrín, et al. 2018), and white-crowned sparrows (Bonier, et al. 2007). This relationship has been found in both directions depending on the species.

The mechanisms of sex allocation should be most beneficial if active during the early stages of embryonic development, as parental investment in the sex that will provide reduced fitness returns can be minimised (Trivers and Willard 1973). Glucocorticoid measures taken around the time of conception have often been strongly linked with sex ratios. During the early stages of development, gene expression differs between the sexes as X-linked genes are overexpressed in females. This leads to differences in embryonic mortality between the sexes (Rangel-Negrin, et al. 2018).

In humans, males tend to be more susceptible to adverse conditions (Kraemer 2000), including oxidative stress and other changes to the uterine environment associated with maternal stress (Edwards, et al. 2016a). This is consistent with the general observation that environmental stressors lead to a female biased sex ratio in humans (Navara 2010). Similarly, in golden hamsters, social stress during pregnancy resulted in female biased sex ratios (Pratt and Lisk 1989). To explain this, it was suggested that males required more parental investment to survive and have reproductive success as adults. Therefore, male offspring would be more vulnerable to maternal stress during pregnancy if such stress limited ability to invest (Pratt and Lisk 1989). This response is consistent with the Trivers-Willard Hypothesis.

In black howler monkeys, however, increases in glucocorticoids lead to male biased sex ratios. In this species males disperse while females remained in their natal groups (Rangel-Negrín, et al. 2018). Maternal glucocorticoid levels were elevated in fragmented forests with anthropogenic pressures, and high food and reproductive competition. In accordance with the LRC hypothesis, females would benefit from producing a higher proportion of male offspring, as was observed (Rangel-Negrín, et al. 2018).

Although it is not clear if adjustment of offspring sex ratio occurs in direct response to glucocorticoid levels or indirectly through another mechanism (Edwards, et al. 2016b), glucocorticoids can act as an indicator of environmental conditions, which sex allocation can then respond to (Rangel-Negrin, et al. 2018). As glucocorticoids tend to be elevated by environmental stressors, such as high food or mating competition, or natural disasters, they act as a useful physiological indicator of environmental conditions (Navara 2010; Rangel-Negrin, et al. 2018). Therefore, they are well suited as a physiological mechanism by which sex ratio can be adjusted in accordance with those conditions.

1.4.4 Maternal Testosterone

Maternal testosterone levels are the third suggested mechanism that provides a link between environmental and parental conditions, and physiological adjustment of offspring sex ratios (Grant 2007). This mechanism is similar to maternal stress as it falls under the umbrella of the endocrine system, specifically steroid hormones (Navara 2013a; Merkling, et al. 2018). A number of studies have shown a link between maternal testosterone levels and offspring sex ratios, with higher testosterone levels generally leading to the production of more sons. Both experimental manipulation of testosterone levels and studies monitoring natural variations in maternal testosterone have demonstrated this relationship in a range of vertebrate species (Merkling, et al. 2018; Navara 2013a), including chickens (Pinson, et al. 2011), zebra finches (Rutkowska and Cichoń 2006), spotless starling (Veiga, et al. 2004), Japanese quail (Correa, et al. 2011), field voles (Helle, et al. 2008), ibexes (Shargal, et al. 2008), and Barbary macaques (Grant, et al. 2011).

Adjusting sex ratios through the maternal testosterone mechanism is generally considered to be in line with the maternal dominance hypothesis, which can be considered a subset of the Trivers-Willard hypothesis (Sheldon and West 2004). Testosterone levels have been linked to dominance, in that elevated testosterone levels lead to increased dominance behaviours and higher standings in dominance hierarchies (Grant 2007). Increased dominance allows for priority access to resources, and therefore good body condition and high ability to invest in offspring (Ficken, et al. 1990). Therefore, high testosterone levels provide a pathway for ability to invest to be translated into a physiological response that sex ratios can be adjusted in accordance with. Additionally, female testosterone levels vary with changes to cortisol levels (Cumming, et al. 1983; Daly, et al. 2005; Silverin 1998), allowing for variation in sex allocation in response to environmental factors other than dominance levels.

Unlike the other mechanisms, testosterone is expected to occur pre-conception, by altering the accessibility for X- or Y- bearing spermatozoa to the ovum (Grant 2007). This process would allow for testosterone levels to influence offspring sex ratios at the earliest point out of the three mechanisms described. As highlighted by Trives and Willard (1973), such early adjustment should be favoured as it minimises waste of resources invested in offspring. Follicular testosterone levels, which can be highly variable both within and between individuals, can alter the susceptibility of an oocyte to fertilisation by an X or Y bearing spermatozoa (Grant, et al. 2011). This was first observed when examining the follicular testosterone levels and subsequent sex of offspring in a bovine species (Grant and Irwin 2005; Grant, et al. 2008). When follicular fluids contained high levels of testosterone, male embryos were more likely to be produced after fertilisation. As testosterone fluctuates in response to dominance, environmental conditions, and stress, it may act as a pathway for mothers to adjust offspring sex ratios in order to maximise fitness returns (Muehlenbein, et al. 2004).

1.5 Maternal Effects and Mismatched Developmental Experience

A number of physiological processes are involved in the mechanisms (glucose levels, testosterone levels, stress) by which females adjust sex ratios of offspring (Navara 2018a; 2018b). Metabolic rates, glucose resistance, insulin resistance, hypothalamic-pituitary-adrenal (HPA) axis function, hormonal regulation, and stress responsiveness are all physiological characteristics that vary between individuals and can influence glucose levels, testosterone levels, and glucocorticoid levels (Edwards, et al. 2016a). These components of maternal physiology may therefore play a key role in sex allocation, as they vary with the environment, but are also influenced by genetic and developmental factors.

Offspring phenotypes are the product of genotypes and the environment experienced, particularly during development (Bernardo 1996). During gestation (incubation for birds and reptiles), foetuses undergo significant physiological development, including the development of metabolic and hormonal regulation (Edwards, et al. 2016a; Brunton 2010). Therefore, the environment experienced during gestation can influence development and have lifelong effects on offspring physiology.

One way in which gestational experience can vary is through maternal effects. Maternal effects are defined as the effects that a mother's phenotype has on the development of offspring (Edwards, et al. 2019; Mousseau and Fox 1998). Through these maternal effects, the environmental experience of a pregnant female is translated into the gestational environment for her offspring (Gorman and Nager 2004). As a result, an offspring's physiological characteristics are affected by the current local conditions experienced by the mother. Importantly, maternal effects can affect aspects of offspring physiology that directly relate to the proposed mechanisms of sex allocation (Edwards, et al. 2016a) (Figure 1.2).

Environmental factors that affect diet, stress levels, testosterone levels, or general health of a pregnant female can lead to changes to the uterine environment of her offspring, thereby influencing offspring physiology (Gorman and Nager 2004). Maternal hormones are able to cross the placenta in mammals, thereby influencing the environmental conditions in utero (Roussel, et al. 2005). In birds, maternal hormones are deposited in the egg, similarly influencing the environmental conditions for developing offspring (Groothuis, et al. 2005). Therefore, when maternal glucocorticoid or testosterone levels are elevated or depressed, the foetus is exposed to these hormonal changes. Similarly, maternal diet or conditions, such as obesity, can affect offspring development as they lead to suboptimal nutrition in utero (Desai, et al. 2015). These environmental changes during gestation are linked to the development of physiological characteristics, including those relevant to sex allocation (Edwards, et al. 2016a).

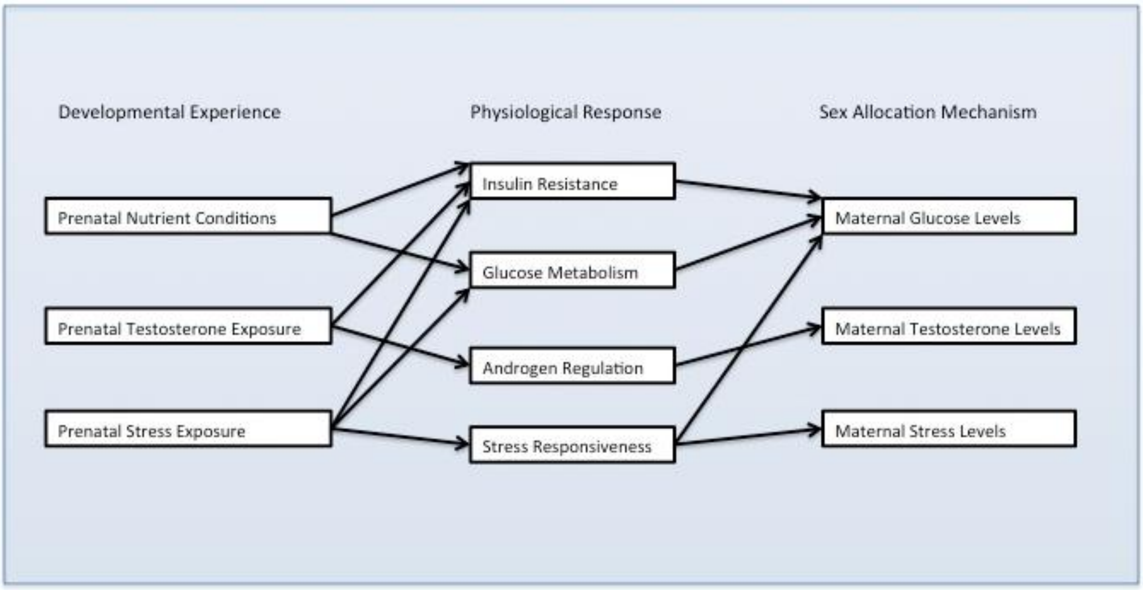

Figure 1.2: Prenatal conditions influence the physiological development of key traits that can affect the mechanisms of sex allocation.

1.5.1 Prenatal Nutrient Conditions

Maternal diet and related conditions such as obesity and emaciation have been linked to a number of changes in offspring physiology (Zambrano, et al. 2006; Obregon 2010; Ravelli, et al. 1999). This includes a suite of metabolic processes, which can influence glucose regulation throughout life. For example, obesity and emaciation have been linked to: glucose intolerance, high cholesterol, insulin resistance, and increased risk of obesity and diabetes (Obregon 2010; Desai, et al. 2015; Lane, et al. 2014a). A number of these responses are maladaptive adaptations the fetus makes under poor nutritional conditions in utero, and in some cases, have shown a direct correlation to maternal diet (Lane, et al. 2014a; Gluckman and Hanson 2004; Shiell, et al. 2000).

These metabolic changes can have lifelong effects on glucose regulation, which may influence sex allocation (Edwards, et al. 2016a). For example, insulin resistance can influence glucose homeostasis and metabolism. Insulin facilitates the absorption of glucose into cells, reducing blood glucose (Wilcox 2005). In states of insulin resistance, the effect of insulin in the body is dampened, which can result in increased levels of glucose circulating in the blood (Wilcox 2005). As glucose levels can play a key role in the physiological mechanisms underpinning sex allocation, insulin resistance may impose constraints on maternal sex allocation. This is consistent with findings that high fructose diets, which are associated with insulin resistance (Saad, et al. 2016; Tappy and Lê 2010), increases the proportion of male offspring born (Gray, et al. 2013). Several other physiological characteristics that are influenced by nutrient conditions during development have also been linked to glucose regulation. Therefore, prenatal nutrient conditions may impose glucose mediated sex allocation (Edwards, et al. 2016a).

1.5.2 Prenatal Testosterone Exposure

The development of a number of physiological characteristics have been linked to prenatal testosterone exposure. Birth weight, infant growth, insulin secretion and resistance, and androgen regulation and sensitivity have all been linked to in utero testosterone exposure (Pfannkuche, et al. 2011; Voegtline, et al. 2013; Eisner, et al. 2000; Bruns, et al. 2004). The metabolic and hormonal changes are associated with the effect that prenatal exposure to excess testosterone has on the development of insulin and androgen receptors in offspring (Eisner, et al. 2000). Importantly, insulin and androgen regulation can influence the mechanisms by which sex allocation is thought to act (glucose and testosterone mechanisms) (Wilcox 2005; Pfannkuche, et al. 2011). These changes can effect circulating glucose concentrations and testosterone levels, and may therefore alter or limit sex allocation. For example, in sheep, a high level of testosterone exposure reduces insulin sensitivity. This results in changes to glucose regulation, and subsequently may constrain maternal sex allocation (Padmanabhan, et al. 2010; Recabarren, et al. 2005). The effects of elevated levels of testosterone exposure during gestation may also affect sex allocation through its influence on testosterone regulation. Male gerbils exposed to high levels of testosterone in utero retain a high level of plasma testosterone throughout life (Clark, et al. 1992). In female rats, exposure to elevated testosterone in utero leads to increased testosterone sensitivity as adults (Ryan and Vandenbergh 2002). These changes can directly influence testosterone mediated sex allocation. Through its effect on both glucose regulation and testosterone regulation in adult offspring, maternal testosterone levels during pregnancy may impose constraints on maternal sex allocation.

1.5.3 Prenatal Stress Exposure

Maternal stress during gestation alters the concentration of glucocorticoids that developing offspring are exposed to, which can lead to a number of physiological changes, including factors associated with sex allocation (Roussel, et al. 2005). The exposure to glucocorticoids prenatally, influences the development of hormone receptors and plays a key role in the development of the HPA axis, an effect that is generally carried into adulthood (Entringer, et al. 2009). For example, prenatal exposure to high levels of stress can lead to the development of an overly sensitive HPA axis and elevated baseline glucocorticoids concentrations in offspring (Brunton 2010; Takahashi and Kalin 1991; Sheriff, et al. 2010). This response is not consistent across all species and studies. However, there is consistency in that prenatal exposure to stress does alter stress responsiveness, probably through its effect on the development of receptors and binding sites of hormones that can either inhibit or increase stress response (Brunton 2010).

The HPA axis mediates stress response and glucocorticoid concentrations, affecting the way in which an individual will respond to local conditions (Smith and Vale 2006). Hence, offspring that develop under different levels of maternal stress during pregnancy may have different stress responses to the same environmental stimuli. As stress is a key factor in the mechanisms of maternal sex allocation, the result may be that some females are constrained in their ability to adaptively adjust offspring sex ratios (Edwards, et al. 2019). Prenatal stress exposure also alters baseline testosterone levels and glucose and insulin metabolism in adults, again, potentially constraining sex allocation (Kaiser and Sachser 2009; Osadchuk, et al. 2000; Seckl 2004; Moss, et al. 2001).

1.5.4 Mechanistic interactions

The three key physiological traits associated with the mechanisms of sex allocation (glucose levels, testosterone levels, and stress) all interact with one another. A change in glucocorticoid levels, for example, can induce changes to both glucose and testosterone levels (Kuo, et al. 2015; Sapolsky 1985). Therefore, if maternal effects affect stress regulation, sex allocation could be constrained by, not only through the stress mechanism, but also through the glucose and testosterone mechanisms.

1.6 Anticipatory Maternal Effects Under Mismatched Prenatal and Postnatal Environments

Maternal effects, particularly under extreme conditions, can often have harmful repercussions on developing offspring. Exposure to poor nutrient conditions or elevated stress and testosterone levels has been linked to a number of physiological disorders in both humans and animals (Entringer, et al. 2011; James 2008, Veiga-Lopez, et al. 2011; Osadchuk, et al 2000; Obregon 2010). However, there is evidence that maternal effects can be an adaptive mechanism by which the physiology of offspring can be ‘programmed’ to optimise survival and reproduction in the current local environment, most notably with regards to maternal stress (Kaiser and Sachser 2009; Mousseau and Fox 1998). Maternal effects that maximise offspring fitness based on the local conditions have been termed anticipatory maternal effects as they anticipate the environment the offspring will be born into (Marshall and Uller 2007). For example, in an environment with high predator density, maternal stress levels are likely to be elevated. Under these conditions during pregnancy, the increased exposure to stress during development can lead to a heightened stress response in offspring. This increases vigilance, a trait that may improve predator avoidance and survival (Meaney 2001; Sheriff, et al. 2009).

Anticipatory maternal effects during development can be beneficial for survival and reproductive success when environmental conditions do not vary between generations (Mousseau and Fox 1998). However, this is not always the case. Environmental conditions do vary, often most severely in response to anthropogenic activities. Maternal effects lead to changes to important physiological and behavioural characteristics that persist throughout life (Gorman and Nager 2004). Therefore, if the environmental conditions during development do not match future environmental conditions, physiological 'programming' may leave offspring with physiological characteristics unsuited to their environment, which can then have detrimental effects (Edwards, et al. 2019).

As an example, Sheriff et al. (2010) found that transgenerational maternal effects resulted in a delay to population recovery following a decline in predator abundance. While these maternal effects allowed for the production of offspring suited to the environment during time of high predation, maternally inherited stress responses hampered the recovery of reproductive rates once predation risk fell. Similarly, artificial stressors acting on pregnant females may result in offspring developing with overly sensitive HPA axes, which can be deleterious in low stress environments (Brunton 2010).

When captive-bred animals are released into the wild during species reintroductions, significant environmental changes can result in physiological development not suited to future environmental conditions (Edwards, et al. 2019). For example, in captive breeding facilities environmental stress may be unnaturally low (or high) (Edwards, et al. 2016a). In such cases, insufficient exposure to stress in utero can result in the development of offspring that are unable to respond to natural stressors in the wild (Brunton 2010). As highlighted earlier, stress response, and its interaction with glucose and testosterone levels are important factors in the mechanisms of sex allocation. If captive-bred animals are unable to respond appropriately to current local conditions in the wild, it may impose constraints on their ability to adjust offspring sex ratios (Edwards, et al. 2016a).

There is already evidence that maternal effects and gestational experience can influence sex allocation in the following generation. In both gerbils and mice, females that develop in close proximity to male siblings are exposed to elevated levels of testosterone (sibling effect). These females produce significantly more male offspring than females gestated adjacent only to other female siblings (Clark and Galef 1995; Vandenbergh and Huggett 1994; Hirlemann, et al. 1990). Similarly, female guinea pigs exposed to synthetic glucocorticoids during gestational development (which alters HPA axis function and has lifelong effects on stress regulation) produced a higher proportion of female offspring (Dunn, et al. 2010). The same effect was observed in laboratory mice. Females that experienced lowered stress environments in utero, through treatment with synthetic glucocorticoids, had female biased offspring sex ratios (Edwards, et al. 2016b). This effect was attributed to altered glucocorticoid concentrations, which subsequently altered glucose levels in treatment females.

Increases in glucocorticoid levels are associated with an induced increase in glucose levels (Goldstein, et al. 1993). In utero treatment with synthetic glucocorticoids resulted in reduced cortisol concentrations during a key phase of the reproductive cycle (luteal phase). The associated decrease in glucose levels during this phase may therefore explain the female biased sex ratios observed (Edwards, et al. 2016b), consistent with findings from a number of studies that high glucose levels lead to male biased sex ratios (Edwards, et al. 2016b; Cameron 2004; Helle, et al. 2008; Larson, et al. 2001).

With regards to changing environments, a mismatch between prenatal and postnatal environments may result in females that are unable to respond to the current local conditions and impose constraints on maternal sex allocation (Edwards, et al. 2019). Edwards et al. (2019) examined this by manipulating pre- and postnatal environments of laboratory mice using treatments with synthetic glucocorticoids. They found that when the in utero environment matched the environment during reproduction later in life, litter sex ratios were dependant on maternal condition, in line with the Trivers-Willard hypothesis. However, when the environment during reproduction did not match the prenatal environment the effect of maternal condition was obscured. Females whose pre and postnatal environments were mismatched were unable to adjust offspring sex ratios in accordance with maternal condition. This supports the hypothesis that under changing environments, maternal effects impose physiological constrains that inhibit maternal sex allocation (Edwards, et al. 2019).

During species reintroductions, animals born in captivity are released to reproduce in the wild (Gusset 2012). This imposes a significant environmental mismatch between development and reproduction as adults. In captivity, social groups are managed, food and water are readily available, and threats and external stressors are minimised (Sterling, et al. 1994; Bassett and Buchanan-Smith 2007; Price and Stoinski 2007). Release into the wild is likely to be accompanied by changes to diet and nutrition, stress levels, and testosterone levels (Sterling, et al. 1994; Price and Stoinsky 2007; Morgan and Tromborg 2007; Van der Weyde, et al. 2016; Dierenfeld 1997). Through maternal effects, the environmental conditions are experienced in utero by developing offspring (Gorman and Nager 2004). This experience can affect the physiological development of captive born animals. As a result, lifelong physiological characteristics may then inhibit their ability to respond to current local conditions in the wild (Edwards, et al. 2016a). Physiological differences between captive-bred and wild animals have previously been reported (Cabezas, et al. 2013; Champagnon, et al. 2012). Hormonal regulation and metabolic function of captive born animals are expected to be unsuited to the environment following release, resulting in changes to glucose levels, testosterone levels, and glucocorticoid concentrations (Edwards, et al. 2016a). These factors all have key roles in the physiological mechanisms of maternal sex allocation (Navara 2018a; 2018b). As a result, sex allocation may be constrained, as the interaction between the physiological mechanisms and the factors that influence the sex-specific fitness of offspring (for example, maternal condition or local resource availability) is inhibited. If the animals released in species reintroductions are constrained in their ability to adjust offspring sex ratio due to physiological 'programming' in a mismatched environment, it may explain some of the unexpected sex ratio biases observed in reintroduced populations (Saltz 2001; Linklater 2007).

2 Methods

My thesis is made up of three studies (Chapters 3-5), each of which examines the effect of mismatched pre- and postnatal environments on sex ratios in a different reintroduced population. The first looks at the Arabian oryx and its reintroduction to the Middle East, the second is on the reintroduction of the California condor to the United States and Mexico, and the third is on the reintroduction of red wolves in the United States. Each chapter has been written in manuscript style and can be read independently. As a result, there is some necessary repetition. This section presents details of the general methods and data used across the three studies.

2.1 Experimental Design

In my research, I am examining the effect that a mismatch between the prenatal environment (experienced during development) and the postnatal environment (experienced while reproducing) has on offspring sex ratios. Specifically, in species reintroductions, where the significant change in environment experienced by released animals creates such a mismatch. Similar to previous studies that have looked at this effect experimentally by manipulating the gestational environment in laboratory mice (Edwards, et al. 2016b; Edwards, et al. 2019), I compare offspring sex ratios of individuals from two treatment groups. My two treatment groups are:

1. Individuals with that developed under prenatal conditions that matched the postnatal conditions in which they reporduce (matched group.) This group includes individuals that have been conceived and born in the same environment type that they reproduce in (i.e., both captive or both wild).
2. Individuals with a mismatch between the prenatal environment in which they developed and the postnatal environment in which the reproduced (mismatched group). This group includes animals that have been conceived and born in a captive environment and then are transferred to the wild where they reproduce. There was also a mismatch when the reverse of this action occurs (i.e., born in the wild then taken into captivity).

I then examine how sex ratios respond to various other predictors to see if that response is affected by mismatch.

2.1.1 Focus Species

To conduct this study, I needed to identify focus species that met a set of requirements. Firstly, each species must have been the subject of a significant reintroduction project, with a sufficient number of births that fall into each treatment type for statistical analysis. This means there must be a number of individuals born in both captivity and the wild, and transferred between the two location types before reproducing. The second requirement was that the species must have a historic studbook, or equivalent data set, that outlines birth location, date of birth, dam (mother), sire (father), and any transfers between locations for each individual.

Many of the cases in which sex ratios following reintroduction have been observed to skew in an unpredictable way have been in mammals (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Jiang, et al. 2000; Law, et al. 2014; Dunham 2001). Previous research in which mismatched gestational experience was found to influence sex allocation had also looked at mammals (Edwards, et al. 2016b; 2019). Mammals were therefore to be included as at least one of the focus species. I was also interested in seeing whether any observed effect would be consistent across different species types, with different mating systems (in which sex allocation is expected to operate differently; Sidorovich, et al. 2007) and differences in sex determination strategies. This meant the inclusion of at least one bird species, as females are the heterogametic sex and therefore are the sole determinants of offspring sex ratios through chromosone and hormonal influences (Smith 2007; Navara 2013b), and at least one monogamous species. Despite the difference in sex determination between mammals and birds, sex allocation mechanisms in both groups are closely linked to physiological characteristics that are influenced by maternal effects and environmental conditions during development (Navara 2013a), making the theory behind this research relevant.

I identified 15 potential focus species (11 mammals and 4 birds) in which captive bred populations were reintroduced to the wild. A number of these were ruled out for various reasons (unable to access data sets, incomplete data, reintroductions did not involve enough individuals released to fully wild locations). I was then left with three species: Arabian oryx (*Oryx leucoryx)*, California condor (*Gymnogyps californianus)*, and the red wolf (*Canis rufus*).

2.2 Reintroductions

2.2.1 Arabian Oryx

The Arabian oryx historically ranged across much of the Arabian Peninsula (Islam, et al. 2011). By 1900, wild populations had significantly declined, and throughout most of the 20th century, the hunting and capture of wild oryx continued to drive this decline until 1972 when the last wild herd was eliminated (Ostrowski, et al. 1998). Fortunately, there were still a number of animals kept in captivity, some of which had been captured specifically to establish captive breeding populations. Over the late 60s and 70s captive breeding of Arabian oryx was highly successful in a number of locations across the world and, in 1982, the first reintroduction took place in Oman (Price 1989). With additional releases, this population initially grew well. However, drought and continued poaching led to the decline of the population and, in 1998, the remaining individuals were rescued back into captivity (Spalton, Lawerence et al. 1999). Despite this set back, Arabian oryx were subsequently reintroduced to a number of sites across the Middle East, and there are now an estimated 1220 Arabian oryx living in the wild (IUCN SSC Antelope Specialist Group 2017).

2.2.2 California Condor

The historic range of the California condor covered a vast area of North America (BirdLife International 2018). However, even before anthropogenic impacts, their range had shrunk to just parts of the American Southwest and Baja California, Mexico. Following European settlement, the remaining populations experienced further dramatic declines (BirdLife International 2018). This was caused by hunting, habitat degradation, and most significantly, lead poisoning from ingesting fragments of lead bullets in carcases (Finkelstein, et al. 2012). By 1982 there were only 22 California condors remaining. In an effort to save the species, all wild condors were captured to establish a captive breeding population. The captive population grew and, in 1992, they were reintroduced back into their former range in California (Walters, et al. 2010). This new wild population has performed well and subsequent reintroductions to Arizona, Utah, and Baja California have followed (Walters, et al. 2010). There are now approximately 310 California condors living in the wild (BirdLife International 2018).

2.2.3 Red Wolf

The red wolf historically ranged throughout eastern USA (Phillips 2018). Wild populations experienced dramatic declines as a result of hunting, habitat loss, and crossbreeding with coyotes (Phillips 2018). In 1973, a programme was launched to capture the remaining wild red wolves and establish a captive breeding population. Four hundred animals were captured from 1973 to 1980, after which they were declared to be extinct in the wild. Of those 400, only 17 were purebred red wolves, three of which were unable to breed. This left just 14 individuals to make up the entire breeding population (Hedrick and Fredrickson 2008). Despite this small number, captive breeding was successful and in 1986 they were released to North Carolina. This was followed by releases to a number of coastal islands in Florida, Mississippi, and South Carolina, as well as a release to a national park in Tennessee (Simonis, et al. 2017). The North Carolina reintroduction has shown the greatest success. However, recent reports suggest that if current population trends continue the risk of extinction will be severe (Simonis, et al. 2017). The Tennessee population was unable to establish and has since been removed. Wild populations of red wolves continue to struggle as hunting pressures, and competition and crossbreeding with coyotes continues to limit recovery (Phillips 2018).

2.3 Studbooks and Data Processing

For each species, data was taken from the relevant historic studbook, which recorded details on all known individuals in the species over the time of the reintroduction. The details included are animal ID, sex, date of birth, location of birth, sire, dam, and the date and location of any transfers for each individual. Using Microsoft Excel and R. Studio, with the packages 'tidyverse' (Wickham 2019), 'gdata' (Warnes, et al. 2017), 'lubridate' (Spinu, et al. 2020), 'dplyr' (François, et al. 2020), and 'measurements' (Birk 2019), the data from these studbooks was converted from PDF to data tables and organised into a workable format.

2.3.1 Treatment Groups

For each species, the individuals were divided into two treatment groups: 'matched' and 'mismatched'. The matched group consisted of individuals that reproduced in the same environment type as the one they were concieved and developed in (i.e., wild and wild, or captive and captive). The mismatched group was of individuals that reproduced in a different location type to the one they were conceived and developed in (i.e., wild and captive, or vice versa). As a number of dams were transferred while pregnant, birth location could not be used in place of conception location. Conception date was calculated by subtracting the average gestation or incubation time for each species (Arabian oryx – 240 days, California condor – 57 days, red wolf – 60 days) from the date of birth. Conception location was then worked out as the location of an individual's dam at the time of conception according to transfer records.

Using the location glossaries presented in the studbooks, each location was identified and classified as captive or wild. Captive locations included zoos, safari parks, animal sanctuaries, wildlife reserves, breeding centres, pre-release facilities, and private collections. In these location types, space was limited, food and water was readily available, shelter was provided, and herd structure, reproduction, and movements were controlled (Peng, et al. 2007; Sterling, et al. 1994; Boue, et al. 2000; Asa, et al. 2010; Morgan and Tromborg 2007). As these species are highly vulnerable, even fully wild populations are closely monitored and their threats managed (Sankar, et al. 2010; Sutherland, et al. 2010; Miller, et al. 2013). However, there are clear and significant differences between the environmental condition in captivity and the wild. Each individual was assigned the appropriate location type for his or her conception location. They could then be grouped as either matched or mismatched according to the location type they were conceived in and the location type their offspring were conceived in.

2.3.2 Other factors related to sex ratio

A number of variables that may directly influence sex allocation, or that are likely to be an indicator for another factor that influences sex allocation, were identified. These were limited to what could be calculated using the data available in the studbooks and a few other sources. While it would have been better to have, for example, a direct measure of body condition for each dam (Pike and Petrie 2005), that data was simply not available. These variables are all in relation to the experience of an individual's sire and dam around the time of conception. They are as follows: sire age, dam age, sire's parity, dam's parity, rainfall around the time of conception, and average temperature around the time of conception.

2.3.3 Parental Age

Parental age has been linked to sex ratio skews in a number of species (Côté and Festa-Bianchet 2001; Ferrer, et al. 2009; Huck, et al. 1988; Saino, et al. 2002). In some species of mammals, females begin breeding before reaching full adult body weight. After a period of peak condition, body condition then declines as they reach the later stages of life (Clutton-Brock and Iason 1986). In such species, offspring sex ratios may change with maternal age, decreasing (fewer males) with either young or old mothers. However, this pattern is not consistent across all species. In some, body condition can be at its peak for the first reproductive event, or increased breeding experience and subsequent decline in reproductive value (the potential number of future female offspring) can encourage increased investment in offspring for older mothers (Clutton-Brock and Iason 1986).

Parental age has also been linked with offspring sex ratios in birds. In Spanish imperial eagles, if either member in a breeding pair had non-adult plumage, offspring sex ratios were significantly male biased (Ferrer, et al. 2009). Similarly, yearling males produced a higher proportion of male offspring than adult males in a population of red-capped robins, despite being in poorer condition (Dowling and Mulder 2006). While it was not exactly clear how parental age might influence sex ratios in the focus species of this study, it seems likely that a relationship may exist. Therefore, parental age was included as one of the factors to examine. Here the effect of parental age on offspring sex ratio was examined for the two treatment groups, individuals with matched or mismatched pre- and postnatal environments. Parental age was measured as the difference, in years, between the conception date of an individual and the birth date of their sire and dam.

2.3.4 Parental Parity

Trivers and Willard (1973) argued that increased parity (the number of previous offspring had by a parent) would lead to a decline in the investment ability of parents, thereby affecting offspring sex ratio. However, as highlighted in reviews by Clutton-Brock and Iason (1986) and Cameron (2004), evidence to support this is limited when lifetime parity is considered. When this relationship was examined, not as lifetime parity, but parity in just the previous year, significant variations in sex ratios have been observed (Clutton-Brock and Iason 1986; Rutberg 1986). Here, parental reproduction success in the year preceding conception was examined for its effect on offspring sex ratio and how that effect varied when pre- and postnatal environments are mismatched. Reproductive success was measured as either having produced offspring in the previous year or not. This was determined using conception dates, and sire and dam ID from the historic studbook of each species.

2.3.5 Environmental Conditions

The third variable that sex ratio is likely to vary in response to, was environmental/climatic conditions around the time of conception. Here I used the average daily temperature (°C) around the time of conception and average rainfall (mm) around the time of conception.

Temperature and rainfall can influence sex ratios in mammals and birds, most likely via indirect pathways. Roche et al. (2006) found that both average air temperature and evaporation rates around the time of conception influenced secondary sex ratios (sex ratio at birth) in dairy cows. They stated that the physiological mechanisms underpinning this effect were unclear. However, as the feed supply of these cattle was highly dependent on climate conditions, they suggested that the effect on sex ratio might be caused by the change in food availability, which has previously been shown to affect sex ratios (Cameron 2004; Roche et al. 2006). Similarly, Catalano et al. (2007) found that in humans born in Scandinavia, sex ratio varied in response to ambient temperature during gestation. Pregnancies during colder months yielded fewer male offspring. In their study area, cold temperatures induced stress in pregnant women via a number of mechanisms, which in turn led to disproportionate rates of abortion of male fetuses (Catalano et al. 2007).

Sex ratio has also been found to vary in response rainfall around the time of conception. Berkeley and Linklater (2010) showed that in a South African population of black rhinoceros, conceptions during times of high rainfall led to an increased offspring sex ratio (more males). They stated that rainfall strongly influenced range condition and resource availability for the population, which was attributed as the cause of sex ratio variation. In arid environments, such as the African savannas that are home to this rhino population, rainfall is expected to affect sex ratios as found by Berkeley and Linklater (2010). However, when resource availability is less dependent on rainfall, the pattern may vary. Kruuk et al. (1999) found that increased rainfall led to a reduced proportion of males born in a population of red deer. Increased rainfall was associated with harsh winter conditions and stress on pregnant females, which led to the loss of a disproportionate number of male fetuses.

Rainfall and temperature around the time of conception was measured as the average daily temperature in degrees Celsius, and the average monthly rainfall in millimetres, for the country and month of conception. Data was downloaded from historic climate datasets available online. The locations presented in the studbook were grouped by country. For each country included in the studbooks (other than the USA), monthly rainfall and temperature was accessed from the World Bank Group historic climate dataset (https://climateknowledgeportal.worldbank.org/). The USA was deemed too large of an area with too much climate variation between locations to use data at the country level. Therefore, each USA location was additionally grouped by state.

Climate data for each USA state was gathered using climate reports provided by the Iowa State University (https://mesonet.agron.iastate.edu/climodat/). For each state, monthly rainfall and temperature details were taken as the average from ten randomly selected climate stations within that state.

Each individual animal from the studbooks was then matched up with monthly rainfall and temperature details by the month, year, and country or state of its conception.

2.4 Statistical Analysis

Data were analysed using the program R. Studio. The packages ‘lme4’ (Bates, et al. 2020) and ‘dfoptim’ (Varadhan and Borchers 2018) were used for analysis, and the packages ‘ggplot2’ (Wickham, et al. 2020), ‘ggefects’ (Lüdecke and Aust 2020), and ‘jtools’ (Long 2020) were used for graphing. All results are interpreted using either 90% or 95% confidence intervals.

The effect of mismatched gestational experience on sex ratio itself was explored using generalized linear mixed effect models with binomial error. Models with a number of different biologically relevant fixed and random effects were compared using Akaike information criterion (AIC) to find the best-fit model. The variables included as fixed and random effects were dam ID, sire ID, dam age, sire age, dam parity in the previous year, sire parity in the previous year, conception location, conception location type (captive or wild), rainfall around the time of conception, and temperature around the time of conception.

The effect that the various other factors (parent age, parental parity, and climate conditions) had on sex ratio was examined similarly, using generalised linear mixed effect models. Again, a number of biologically relevant fixed and random effects were included, and the model was gradually simplified until the most parsimonious combination, based on the AIC, was identified. Each factor was examined, first for all individuals in each species, then independently for mismatched individuals and matched individuals. An additional model was used to examine the effect of each of the selected factors and mismatch simultaneously, including their interactive effect. Specific details for each model are presented in the relevant species manuscripts.

3 Arabian oryx

3.1 Introduction

Sex allocation theory predicts that offspring sex ratios should be variable in order to maximise transgenerational fitness when benefits of parental investment have different effects on male and female offspring (West 2009). Offspring fitness varies in response to a number of environmental factors, and this variation is often sex specific (Clark 1978; Trivers and Willard 1973). As a result, reproductive efforts should favour the production of offspring of the sex that will yield the greatest fitness returns depending on current local conditions. A number of hypotheses have been presented to explain the adaptive significance of variation in sex ratios. Some of the most prominent and well-established hypotheses include: the local resource competition hypothesis (Clark 1978), the local resource enhancement hypothesis (Gowaty and Lennartz 1985), and the Trivers-Willard hypothesis (Trivers and Willard 1973). Factors like local resource availability and parental ability to invest in offspring development (Trivers-Willard hypothesis) are expected to affect the fitness of male and female offspring differently in certain species. Parents should therefore adjust offspring sex ratios in accordance with such factors (West 2009).

The most prominent theories of adaptive sex allocation are logically appealing and have been supported by numerous empirical studies (Review; Clutton-Brock and Iason 1986). However, support has been plagued with inconsistencies (Cameron 2004), and observations have often failed to match predictions. Observed sex ratio biases tend to be weaker than theoretically predicted (West and Sheldon 2002). Alternatively, altogether unpredicted sex ratio biases have also been observed (Weatherhead, et al. 1998; Doutrelant, et al. 2004). This is often the case in species reintroductions. In a number of species, reintroduced populations have exhibited unexpected sex ratio biases, which have been explained either poorly or not at all, and may have limited the success of these reintroduction programmes (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Jiang, et al. 2000; Law, et al. 2014; Dunham 2001; Ewen, et al. 2010).

Several aspects of sex allocation may explain some of the inconsistencies in results. Methodological inconsistencies between studies have almost certainly caused some of the variation in support for sex allocation (Cameron 2004; Sheldon and West 2004). The interaction of multiple drivers of sex allocation may also have contributed to the inconsistency of empirical support. Observed sex ratios are unlikely to match predictions that are based on a single hypothesis when sex allocation is being driven by multiple factors (Hiraiwa-Hasegawa 1993; Cockburn, et al. 2002; van Schaik and Hrdy 1991; Moore, et al. 2015). However, there is still a significant amount of inconsistency in results from studies testing the hypotheses of sex allocation, both between and within species (Edwards, et al 2016b; West 2009).

More recently, physiological constraints have been considered as a source of inconsistency in support for adaptive sex allocation hypotheses (Hiraiwa-Hasegawa 1993; Cockburn, et al. 2002; van Schaik and Hrdy 1991; Moore et al. 2015). Sex allocation, whatever the adaptive significance, must be implemented by physiological mechanisms that translate current local conditions into sex-biased investment in offspring (Krackow 1995). As physiology is variable between individuals, some individuals may respond to environmental conditions differently to others, and therefore adjust offspring sex ratios differently (Edwards, et al. 2016a). Three well established physiological mechanisms for maternal sex allocation in mammals have been proposed (Chapter 1). They are, maternal glucose levels (Cameron 2004), maternal stress levels (Navara 2010), and maternal testosterone levels (Grant 2007).

Each of these mechanisms is mediated by physiological characteristics that can vary between individuals within a species. It is generally considered that all individuals should have an equal aptitude for sex allocation (Edwards, et al. 2016a). However, physiological characteristics can alter the way an individual responds to its current local conditions, potentially constraining sex allocation. (Edwards, et al. 2016a) Hypothalamic–pituitary–adrenal (HPA) axis function, glucose and insulin metabolism/resistance, and baseline testosterone levels are all variable between individuals (Mousseau and Fox 1998). At the same time, they all affect the interaction between current local conditions and the physiological responses in glucose levels, testosterone levels, and/or glucocorticoid levels, which mediate sex allocation (Edwards, et al. 2016a).

Offspring phenotypes are the product of both its genotype and its environment (Bernardo 1996; Edwards, et al. 2016). One of the greatest sources of non-genetic physiological variation is the environment experienced in utero, while metabolic and hormonal regulation is developing (Bernardo 1996; Lane, et al. 2014b). In utero conditions are largely controlled by maternal effects, through which the environmental experience of a pregnant female is expressed in the prenatal environment (Gorman and Nager 2004). Maternal effects can have significant lifelong effects on offspring physiology (Edwards, et al. 2016a). The evolutionary basis for this is to allow offspring to develop in an environment that matches the expected postnatal environment so that physiological characteristics can be 'programmed' accordingly (Mousseau and Fox 1998). However, when there is a mismatch between pre- and postnatal environments, they may develop physiological characteristics that leave them unsuited to respond to current local conditions as adults (Innes-Gold, et al. 2019). This may impose constraints on their ability to adjust offspring sex ratios adaptively and in accordance with sex allocation hypotheses (Edwards, et al. 2016a). There is already evidence that prenatal environment can affect sex ratios in the following generation (Clark and Galef 1995; Edwards, et al. 2016b), including support for pre- and postnatal mismatch constraining sex allocation in response to factors that are predicted to drive sex ratios (Edwards, et al. 2019).

Species reintroductions inadvertently create these conditions, where pre- and postnatal environment is mismatched. Captive born animals develop in an environment which may have low stress, high quality nutritional diets, and unnatural social groups (Peng, et al. 2007; Sterling, et al. 1994; Boue, et al. 2000; Asa, et al. 2010; Morgan and Tromborg 2007). During species reintroductions, such captive born animals are released into the wild. Their physiological characteristics may subsequently be unsuited to the environmental conditions in the wild (Edwards, et al. 2019). For example, in captivity, pregnant females may be exposed to unnaturally low levels of stress, altering the prenatal environment for her offspring (Edwards, et al. 2016a; Brunton 2010). Without prenatal exposure to stress, offspring can develop with low stress responsiveness (Takahashi and Kalin 1991; Sheriff, et al. 2010). Animals born in captivity then released into the wild during species reintroduction may therefore be unable to respond appropriately to natural stressors (Edwards, et al. 2016a). In reintroduced species that adjust offspring sex ratios in response to stress, sex allocation would then be constrained by physiological limitations. This may explain some of the unexpected sex ratio biases that have been observed in reintroduced populations (Edwards, et al. 2019).

Here I examine the effects of mismatched pre- and postnatal environments on sex allocation in the reintroduced populations of Arabian oryx (*Oryx leucoryx*). Mismatch occurs when captive born animals are released and successfully reproduce in the wild, and also when wild born animals are captured and reproduce in captivity. Sex allocation theories suggest that sex ratios should vary in response to a number of factors. I test the relationship between sex ratios and three additional variables (rainfall, parental age, and parental parity in the previous year) that may influence sex allocation (Berkeley and Linklater 2010; Clutton-Brock and Iason 1986; Rutberg 1986; Ferrer, et al. 2009; Dowling and Mulder 2006), and explore the interaction between these variables and environmental mismatch in Arabian oryx. I predict a mismatch between pre- and postnatal environments will impose constraints on maternal ability to adjust offspring sex ratios. However, it is difficult to predict whether it would lead to the production of more sons or daughters. Additionally, I do not have details of population sex ratios for Arabian oryx. Therefore, my proposed hypotheses are:

1. Mismatch between pre- and postnatal environments in reintroduced animals will result in offspring sex ratios that differ, not from parity (i.e., a 1:1 male to female sex ratio), but from offspring sex ratios of animals whose pre- and postnatal environment is matched.

2. Offspring sex ratios will vary in response to climate, parental age, and parental parity in the previous year, but this effect will be obscured when pre- and postnatal environments are mismatched.

In captive populations, the predictors of sex allocation I have used (parent age, parent parity, and rainfall) may have a dampened effect on offspring sex ratios. As food and resources are provided for the animals, rainfall, for example, is less likely to influence ability to invest in offspring, and subsequently sex ratios. Additionally, mismatch in opposite directions (i.e., captive to wild, and wild to captive) may have an inverse effect on sex allocation, obscuring overall results. Therefore, as well as conducting my analysis on the full Arabian oryx dataset, I also repeated it, looking at just wild populations. Wild populations are made up of individuals born in the wild (matched environments), as well as individuals born in captivity that have been released to the wild (mismatched environments). In this group, there is no anthropogenic control of the environment, which may dampen sex allocation, and mismatch is only the release of captive born animals into the wild.

3.2 Methods

3.2.1 Study species

The Arabian oryx (*Oryx leucoryx*) is a medium-sized antelope from the family bovidae (Price 1989). They live in social herds of, on average, about 10 individuals. These herds establish straightforward dominance hierarchies that involve all adults of both sexes (Price 1989). Sexual dimorphisms between male and female exist in Arabian oryx but are very slight. Males are on average larger than females (Price 1989). They are polygynous breeders, as dominant males mate with multiple females in a single breeding season. Historically, they ranged across much of the Arabian Peninsula (Islam, et al. 2011), but over the 19th and 20th centuries, wild populations significantly declined. In 1972 they became extinct in the wild, and only captive individuals in zoos, captive breeding facilities, and private collections, sustained the species (Ostrowski, et al. 1998).

Following their extinction in the wild, captive populations have grown and Arabian oryx have since been reintroduced to several locations across the Middle East (IUCN SSC Antelope Specialist Group 2017). The first reintroduction was in 1982 with the release of 10 animals to the Arabian Oryx Sanctuary in Oman (Price 1989), and most recently, 22 animals were released to the Wadi Rum Protected Area in Jordan in 2009. The various reintroduced populations have had mixed success but, overall, the species has grown well. There are now approximately 1220 Arabian oryx living in the wild across the Arabian Peninsula (IUCN SSC Antelope Specialist Group 2017).

3.2.2 Data

The data sets used for this study were taken from the historic listings section of the International Studbook for Arabian Oryx, published by Marwell Wildlife (Goodwin 2013). In this, animal ID, gender, date of birth, birth location, dam, sire, and transfers were recorded for each known individual in captivity and in closely monitored wild populations. Using this, I was able to identify individuals that had a mismatched pre- and postnatal environment, and the sex ratios of their offspring. I also determined the age of sires and dams at the time they conceived offspring, whether they had reproduced in the year prior to conception, and rainfall during the months leading up to conception. Rainfall was recorded as monthly precipitation levels (mm) at the conception location. For individuals conceived in the USA, this was measured at the state level and data was taken from Iowa State University climate reports (https://mesonet.agron.iastate.edu/climodat/). For individuals born in all other countries, it was measured at the country level, using rainfall data was taken from the historic climate datasets of the World Bank Group (https://climateknowledgeportal.worldbank.org/).

3.3 Statistical Analysis

All statistical analysis was done in R.Studio Version 1.1.463. The packages used are outlined in chapter x. Results are presented using 90% confidence intervals.

3.3.1 Offspring Sex Ratios in Environmentally Matched and Mismatched Arabian Oryx

Generalised linear mixed effect models with binomial error were used to determine whether offspring sex ratios of individuals with mismatched pre- and postnatal environments differed from those with matched environments. A number of different models with biologically relevant fixed and random effects were compared on the Akaike information criterion (AIC) to find the model of best fit. The most complex model included dam age, sire age, and rainfall around the time of conception as fixed effects, and dam ID, sire ID, conception location, conception location type, dam reproductive productivity in the year prior to conception, and sire reproductive productivity in the year prior to conception as random effects. The model was gradually simplified, and the most parsimonious model was identified, which included just the effect of mismatch and sire ID as a random effect.

3.3.2 Parental Age

In ungulates, parental age, particularly maternal age, has been linked to offspring sex ratios (Saltz and Kotler 2003; Côté and Festa-Bianchet 2001). Briefly, young and old females are often in poorer reproductive condition and are less able to invest in offspring than females in prime age. As a result, young and old females produce female biased offspring sex ratios, while mid-aged females produce male biased offspring sex ratios (Clutton-Brock and Iason 1986). Therefore, Arabian oryx may be expected to adjust offspring sex ratios in accordance with parental age around the time of conception. In testing this, I used generalised linear models with binomial error, first to assess the effects of dam age and sire age on offspring sex ratios for all Arabian oryx, regardless of mismatch. I then examined the effect of these variables on each treatment group, Arabian oryx with matched and mismatched environments, individually. Finally, I used generalised linear mixed effect models to investigate the effects of parental age and treatment group together. The most parsimonious model included just parental age (either dam age or sire age), treatment, their interactive effect, and sire ID as a random effect.

3.3.3 Parental Parity

Parental parity is taken as reproductive productivity in the year prior to conception. Previous studies on the effects of parental parity on offspring sex ratios have found that parity is most likely to have a significant effect when measured in this way (Clutton-Brock and Iason 1986; Rutberg 1986). The effect of parental parity was analysed in the same way as parental age, using generalised linear models and generalised linear mixed effect models with binomial error. The effect of parity was first examined on the population as a whole, then on each treatment group (matched and mismatched environments) individually. Finally, using mixed effect models, the effects were examined simultaneously, including the interaction between the variables and sire ID as a random effect.

3.3.4 Climate Conditions

In the arid environments of the Arabian Peninsula, vegetation growth is likely to be limited by water availability. Therefore, rainfall should directly affect vegetation growth, and subsequently resource availability and ability to invest in offspring for Arabian oryx (Berkeley and Linklater 2010). Hence, it is likely that rainfall around the time of conception has an effect on offspring sex ratios. However, as the effects of rainfall act through vegetation growth, there may be a lag before changes in the amount of rainfall result in changes in ability to invest in offspring.

Prior to my analysis, I had no indication as to what the extent of this lag would be. Consequently, when examining the effect of rainfall on offspring sex ratio, I considered a range of time frames. I used generalised linear models with binomial error to examine how offspring sex ratios respond to the level of rainfall as:

1. the average rainfall during the month of conception
2. the average rainfall in the two months leading up to conception
3. the average rainfall in the three months leading up to conception
4. the average rainfall in the six months leading up to conception.

The most significant effect was found when using the average rainfall in the three months leading up to conception, and this value is therefore used in the following models and will henceforth be referred to as rainfall.

Generalised linear models with binomial distribution were then used to investigate the effects of rainfall on offspring sex ratios in mismatched and matched treatment groups individually. As for the other variables, I then used generalised linear mixed effect models with binomial distribution to examine the effects of rainfall and treatment together, along with their interactive effect and sire ID as a random effect.

3.3.5 Wild Populations

The factors used here (parental age, parental parity, and climate) are all linked to sex allocation through their expected effect on ability to invest in offspring. In captivity, resources such as food and water are provided for the animals. As a result, ability to invest in offspring should be less dependent on these variables, as access to surplus resources may mask their effect. To combat this, I have repeated the analysis above, examining just the wild populations of Arabian oryx. In this, mismatch between pre- and postnatal environments is created only when captive born individuals are released into the wild, and not when wild born animals are captured.

3.4 Results

3.4.1 Full data set

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in both wild and captive populations combined are presented in Table 3.1.

Offspring sex ratios of environmentally mismatched Arabian oryx did not differ from offspring sex ratios of environmentally matched Arabian oryx. Both matched and mismatched treatment groups had offspring sex ratios that were close to parity (0.506 and 0.507 respectively).

Table 3.1: Model outputs for the effects of environmental mismatch, sire age, dam age, sire parity, dam parity, rainfall, and their interaction effects on offspring sex ratios in all populations of Arabian oryx.

Model/Test	Estimate	Std. error	*z* value	p- value
Mismatch vs Match	-0.016	0.129	-0.123	NS[1]
Sire Age	-0.005	0.011	-0.500	NS
Sire Age Matched Environments	-0.004	0.011	-0.323	NS
Sire Age Mismatched Environments	0.065	0.074	0.870	NS
Sire Age Mismatch Interaction	0.062	0.077	0.807	NS
Dam Age	-0.018	0.010	-1.730	0.084 .
Dam Age Matched Environments	-0.012	0.011	-1.137	NS
Dam Age Mismatched Environments	-0.040	0.036	-1.093	NS
Dam Age Mismatch Interaction	-0.028	0.045	-0.626	NS
Sire Parity	-0.039	0.070	-0.562	NS
Sire Parity Matched Environments	-0.037	0.075	-0.494	NS
Sire Parity Mismatched Environments	0.142	0.242	0.586	NS
Sire Parity Mismatch Interaction	0.184	0.258	0.714	NS
Dam Parity	0.034	0.065	0.522	NS
Dam Parity Matched Environments	0.066	0.068	0.965	NS
Dam Parity Mismatched Environments	-0.112	0.229	-0.490	NS
Dam Parity Mismatch Interaction	-0.191	0.270	-0.709	NS
Rainfall	0.002	0.001	2.622	0.009 *
Rainfall Matched Environments	0.002	0.001	2.619	0.009 *
Rainfall Mismatched Environments	-0.002	0.004	-0.422	NS
Rainfall Mismatch Interaction	-0.004	0.004	-0.915	NS

---

[1] Not Significant

. Significant to the 90% confidence interval ($p < 0.1$)

* Significant to the 95% confidence interval ($p < 0.05$)

Sire age did not affect sex ratio in the population as a whole, nor in either of the treatment groups. There was also no interaction effect between sire age and treatment. However, when viewing the interaction between sire age and mismatch graphically, there appears to be a slight trend when environments are mismatched, with increased sire age resulting in a higher proportion of male offspring, but not when environments are matched (Figure 3.1). Dam age had a slight effect on sex ratio in Arabian oryx, with older females producing fewer male offspring. This effect was not observed when either treatment group was examined individually, and there was no interaction effect between treatment group and dam age on sex ratios (Figure 3.1).

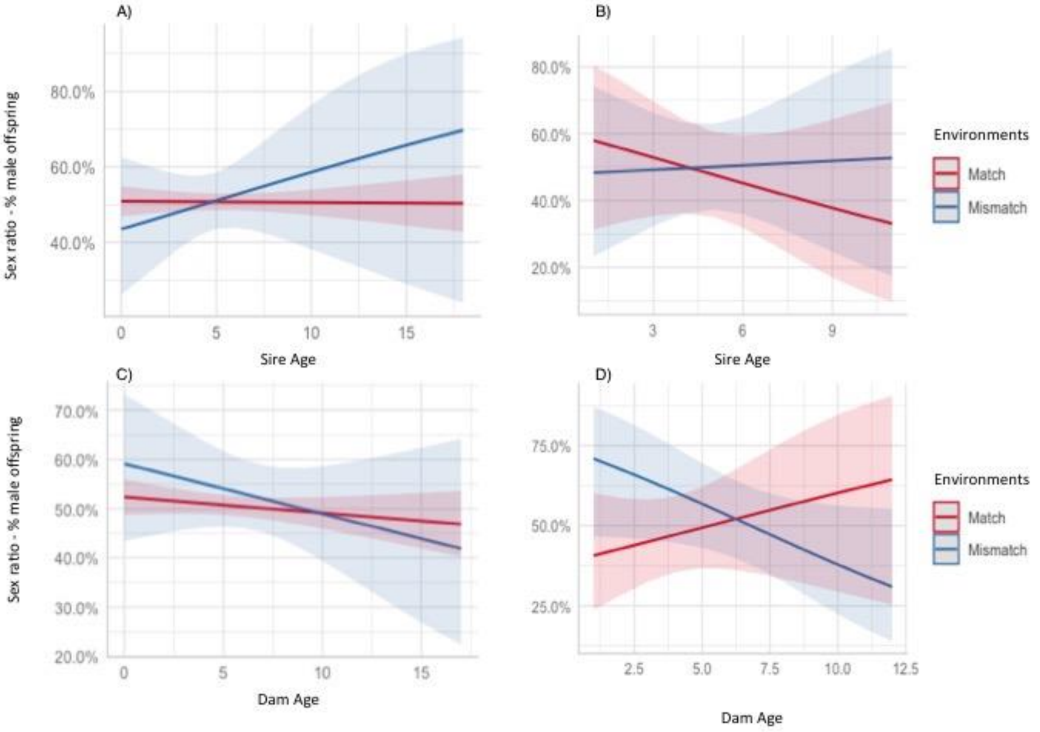

Figure 3.1: The effect of parental age at time of conception on offspring sex ratios in matched and mismatched pre- and postnatal environments. A) Sire age in captive and wild populations. B) Sire age in just wild populations. C) Dam age in captive and wild populations. D) Dam age in just wild populations.

Neither sire nor dam parity had any effect on offspring sex ratio in any of the examined groups and there was no interaction effect. However, there was a greater disparity between the sex ratios of offspring born to Arabian oryx that had reproduced in the previous year and those that had not when environments were mismatched (Figure 3.2). This was true for both sire parity and dam parity, but the direction of the effect was opposite. When dams had reproduced in the previous year, sex ratios were lower (fewer males were produced), and when sires had reproduced in the previous year, sex ratios were higher (more males were produced).

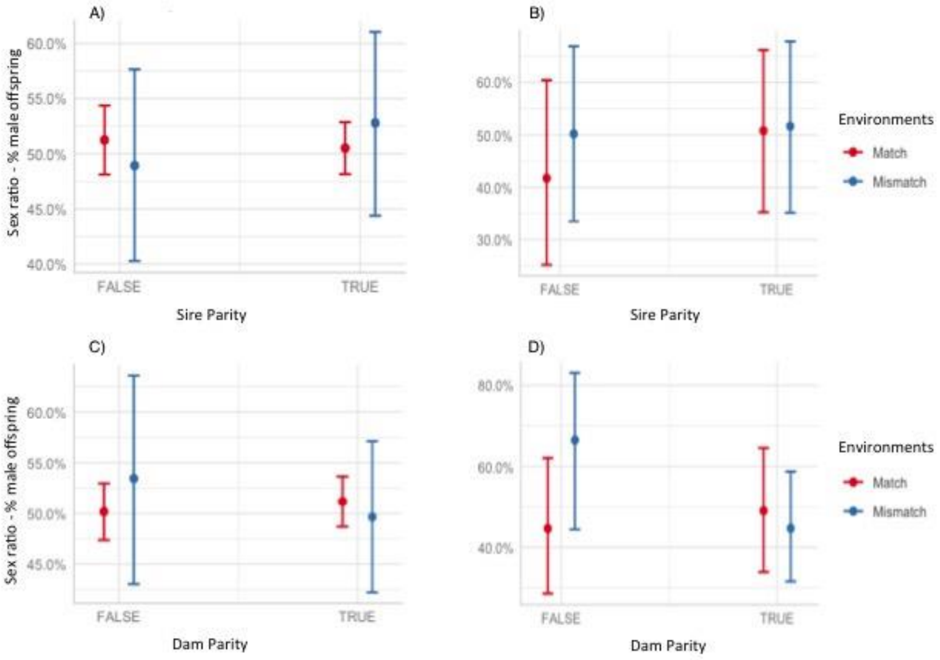

Figure 3.2: The effect of parental parity on offspring sex ratios in matched and mismatched pre- and postnatal environments. A) Sire parity in captive and wild populations. B) Sire parity in just wild populations. C) Dam parity in captive and wild populations. D) Dam parity in just wild populations. Parity – True means parents had reproductive success in the year prior to conception.

Rainfall leading up to conception had a significant effect on sex ratio when looking at populations as a whole. As rainfall increased, sex ratios became increasingly male biased. The same effect was observed in the matched treatment group. However, in the mismatched group, rainfall had no effect on sex ratio. Although there was no significant relationship between rainfall and sex ratio when environments were mismatched, there was a slight trend in the opposite direction to the relationship when environments were matched (Figure 3.3). Despite this, there was no significant interaction effect between treatment and rainfall.

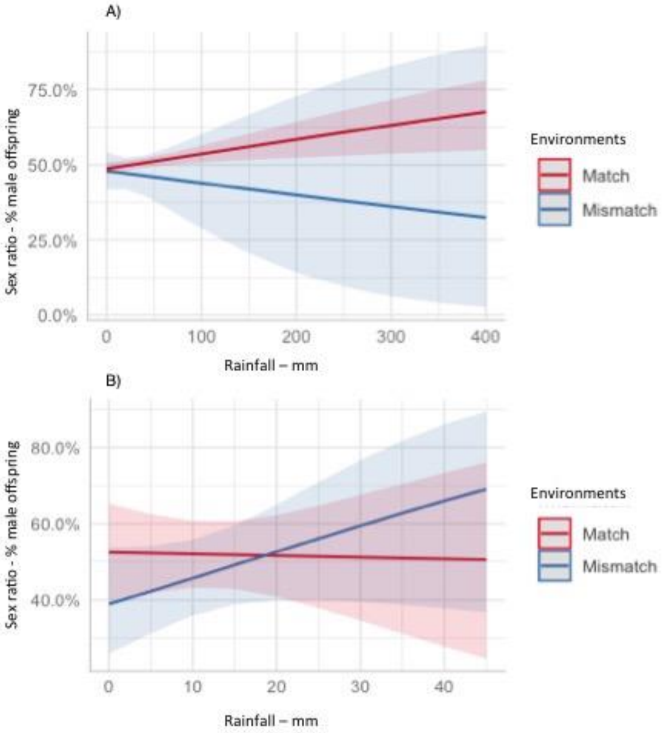

Figure 3.3: Effect of average monthly rainfall (mm) over three months leading up to conception on offspring sex ratios of Arabian oryx with matched and mismatched pre- and postnatal environments, in A) captive and wild populations together, and B) just wild populations.

3.4.2 Wild Populations

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in wild populations alone are presented in Table 3.2.

Similar to the full data set, mismatch treatment had no effect on offspring sex ratios in just wild populations. Individuals that experienced a mismatch between pre- and postnatal environments did not produce offspring sex ratios that differ from those of individuals with matched environments (0.493 and 0.504 respectively).

Also in line with analysis for the full data set, in wild populations, sire age had no effect on offspring sex ratios, regardless of treatment. Dam age, however, did affect offspring sex ratio in wild populations, but only in the mismatched treatment group. Following environmental mismatch, sex ratios become increasingly female biased as dam age increases (Figure 3.1). This effect was not observed in either the matched group, or the full wild population. There was also a slight interaction effect between treatment group and dam age on sex ratios.

Table 3.2: Model outputs for the effects of environmental mismatch, sire age, dam age, sire parity, dam parity, rainfall, and their interaction effects on offspring sex ratios in wild populations of Arabian oryx.

Model/Test	Estimate	Std. error	z value	p- value
Mismatch vs Match	0.123	0.320	0.385	NS[1]
Sire Age	0.002	0.078	0.026	NS
Sire Age Matched Environments	-0.041	0.105	-0.389	NS
Sire Age Mismatched Environments	0.061	0.119	0.510	NS
Sire Age Mismatch Interaction	0.120	0.167	0.719	NS
Dam Age	-0.073	0.046	-1.588	NS
Dam Age Matched Environments	0.039	0.080	0.496	NS
Dam Age Mismatched Environments	-0.163	0.071	-2.303	0.021 *
Dam Age Mismatch Interaction	-0.242	0.133	-1.827	0.068 .
Sire Parity	0.277	0.321	0.865	NS
Sire Parity Matched Environments	0.409	0.465	0.880	NS
Sire Parity Mismatched Environments	0.174	0.446	0.391	NS
Sire Parity Mismatch Interaction	-0.308	0.681	-0.452	NS
Dam Parity	-0.226	0.269	-0.842	NS
Dam Parity Matched Environments	0.223	0.353	0.631	NS
Dam Parity Mismatched Environments	-0.874	0.455	-1.923	0.055 .
Dam Parity Mismatch Interaction	-1.075	0.705	-1.524	NS
Rainfall	0.010	0.013	0.803	NS
Rainfall Matched Environments	-0.002	0.017	-0.103	NS
Rainfall Mismatched Environments	0.028	0.020	1.417	NS
Rainfall Mismatch Interaction	0.030	0.026	1.142	NS

---

[1] Not Significant

. Significant to the 90% confidence interval ($p < 0.1$)

* Significant to the 95% confidence interval ($p < 0.05$)

Similar to sire age, sire parity had no effect on offspring sex ratios in any treatment group within wild populations. Dam parity also had no effect on sex ratios in when looking at the entire pool of wild Arabian oryx, nor in the matched treatment group. However, when environments were mismatched, there was a slight correlation between dam parity and offspring sex ratio with females that had not reproduced in the previous year giving birth to a higher proportion of male offspring than females that had done so (Figure 3.2). Despite different effects between the two treatment groups, there was no interaction effect.

In wild populations of Arabian oryx there was no effect of rainfall on offspring sex ratios. This was consistent between treatment groups, as well as when they were combined, and there was no interaction between treatment and rainfall. However, rainfall around the time of conception had a visible effect on offspring sex ratios when pre- and postnatal environments were mismatched. This effect was obscured when pre- and postnatal environments were matched (Figure 3.3).

3.5 Discussion

With the transfer of Arabian oryx between captivity and the wild in order to establish captive breeding populations and reintroduce them to their former native range, the prenatal environment experienced during development is mismatched from their postnatal environment when reproducing as adults. This mismatch, however, did not result in population wide offspring sex ratios that differ from those with matched environments. Mismatches created by transfers in opposite directions (captive to wild, and wild to captive) may have had an opposite effect on offspring sex ratios. These opposing effects may have masked the overall relationship when analysed together. However, analysis on just wild populations of Arabian oryx accounts for this, as mismatch occurs only when captive born individuals are released into the wild. In the wild populations individuals that had experienced a mismatch between pre- and postnatal environments did not have an offspring sex ratio that differed from those with matched environments. This supports the rejection of hypothesis 1, which states that offspring sex ratio should differ between the groups.

Mismatched environments did influence the relationship between offspring sex ratios and several factors that were expected to influence sex allocation in this species. While this study only provided weak statistical support for this effect, mismatched environments do appear to affect sex allocation in Arabian oryx in response to parental parity and rainfall in the months leading up to conception. This suggests that the lifelong physiological development controlled by maternal effects may constrain sex allocation in this reintroduced population when pre- and postnatal environments are mismatched.

There is no evidence that maternal effects when pre- and postnatal environments were mismatched imposed constraints on sex allocation in response to parental age when captive and wild populations were analysed together. Sire age did not have a statistically significant effect on offspring sex ratios in Arabian oryx. This was the case, regardless of mismatch. However, this was not unexpected, as unlike dam age, sire age has not been liked with offspring sex ratios in ungulates (Saltz and Kotler 2003). While there was no observable relationship between sire age and offspring sex ratio when environments were matched, when environments were mismatched, there appeared to be a slight, non-significant trend (Figure 3.1). Despite this, there was not sufficient evidence to suggest that the effect of sire age of offspring sex ratio was constrained by mismatched pre- and postnatal environments.

Dam age was a significant predictor of offspring sex ratio when both matched and mismatched treatment groups were combined. However, neither group on its own showed this relationship. As dam age increased, offspring sex ratios tended towards female bias (Figure 3.1). Body condition and ability to invest in offspring are expected to decline with age, predicting a female biased sex ratio, as was observed (Clutton-Brock and Iason 1986; Saltz and Kotler 2003). While this relationship was not significant in either treatment group, it appeared to be slightly stronger when environment was mismatched. However, there was no evidence to suggest that mismatched environments altered the way in which Arabian oryx adjust offspring sex ration in response to dam age.

In the combined captive and wild populations, when environments were mismatched, there appeared to be a relationship (although not statistically significant) between dam parity (reproductive success in the prior year) and offspring sex ratio, which was not present when environments were matched (Figure 3.2). Reproduction is costly and females that have recently reproduced may have less resources and energy available to invest in offspring development (Clutton-Brock and Iason 1986). Therefore, when females reproduce in consecutive years, the sex of the later offspring is more likely to be female, in accordance with the Trivers-Willard Hypothesis (Martin and Festa-Bianchet 2011). This was the observed pattern when environments were mismatched. It is not clear why this relationship was detectable when environments were mismatched but not when they were matched. It may be that a consequence of maternal effect on physiology left females overly sensitive to the effects of parity when postnatal environment was mismatched, such that it was expressed more strongly than is adaptively beneficial.

Sire parity expressed a similar pattern to dam parity, in that a slight relationship was observable when environments were mismatched, but not when they were matched. However, the effect of sire parity on sex ratios was opposite to the expected relationship (Figure 3.2). Males that had successfully reproduced in the previous year were more likely to have male offspring. Although this result was unexpected, it is not entirely surprising. Reproducing in the previous year is less likely to have an impact on ability to invest in new offspring for male Arabian oryx, as they invet less in each reproduction. Past reproductive success has even been linked with an increase to offspring sex ratio in favour of males in some mammal species (Douhard, et al. 2016). Again, it is unclear why this pattern would be expressed more when pre- and postnatal environments are mismatched.

In the combined captive and wild populations when pre- and postnatal environments were matched, rainfall over the three months leading up to conception was a significant predictor of offspring sex ratios in Arabian oryx. As vegetation growth is dependent on rainfall, higher levels of rainfall over this time should be coupled with increased resource availability for investment in offspring (Berkeley and Linklater 2010). Therefore, in accordance with the Trivers-Willard hypothesis, offspring sex ratios are expected to favour the production of males as rainfall increases (Trivers and Willard 1973). This was the observed relationship when pre- and postnatal environments were matched. However, when environments were mismatched, this relationship was obscured, and no significant effect was present. The general trend, visible in Figure 3.3, was in fact the reverse of the predicted outcome. The interaction between rainfall leading up to conception and mismatched environments provides the most compelling evidence that sex allocation may be constrained by maternal effects when environments change. Under these conditions, reintroduced Arabian oryx failed to adjust offspring sex ratios in an adaptive manner in response to changes in rainfall (Berkeley and Linklater 2010).

A number of the results from analysis of just wild populations did not match results from captive and wild populations together. However, they did provided support for the hypothesis that a mismatch between pre- and postnatal environments can influence the relationships between predictors of sex allocation and offspring sex ratios. In the wild populations, rainfall did not affect offspring sex ratios when pre- and postnatal environments matched. When environments were mismatched, there was a non-significant, but observable trend, with increased rainfall leading to the production of more sons (Figure 3.3). This was the expected response in accordance with the Trivers-Willard hypothesis (Berkeley and Linklater 2010). Similarly, mismatch between pre- and postnatal environments in wild populations affected the relationship of sire age, dam age, and dam parity with offspring sex ratio (Figures 3.1 & 3.2). The prenatal experience of captive born oryx may have resulted in the development of physiological traits not suited to the wild environment. As a result, rainfall, parental age, and dam parity affect the mechanisms of sex allocation differently to individuals born in the wild, thereby constraining sex allocation.

It is not clear why these patterns emerged, or why analysis on wild populations did not match analysis on captive and wild populations together. The purpose of this study is not to explain how different variables affect offspring sex ratios in Arabian oryx, but to examine how those relationships are influenced by a mismatch between pre- and postnatal environments. The results presented here suggest that maternal effects in an environment mismatched from the environment experienced as adults may impose physiological constraints on the ability to adjust sex ratio in reintroduced populations of Arabian oryx. However, they do not provide strong empirical evidence that this is the case. This may be partially due to the structure of this research. As the factors included here were merely indicators of ability to invest in offspring based on the proxy data that was available to me, results were never likely to yield strong support. Parental parity and parental age, while both can predict offspring sex ratios in some species, are not the always the best indicators of ability to invest (Cameron 2004).

Rainfall around the time of conception showed the strongest relationship with sex allocation. However, this was also not the most accurate measure of ability to invest in offspring (Cameron 2004). I was only able to measure rainfall at the countywide scale, which is relatively low resolution. This measure fails to detect variations within a group or population of Arabian oryx, which may be considerable. Despite these shortcomings, results presented here do suggest that sex allocation in reintroduced populations of Arabian oryx may be constrained by the lifelong physiological impacts of maternal effects in a mismatched environment. I recommend that this study act as a stepping-stone for future research, which takes an experimental approach and uses a direct indicator for ability to invest at the individual level within a reintroduced population.

4 California Condor

4.1 Introduction

Sex allocation is the selective investment in offspring of one sex over the other in order to maximise fitness returns (West 2009). When sex-specific fitness returns vary in response to the current local conditions (e.g., local resources or ability to invest in offspring; Clark 1978; Trivers and Willard 1973), parents will gain a differing fitness return on investment in sons verses daughters (West 2009). Offspring sex can be one of the most important phenotypes in predicting fitness (Navara 2018c). Sex allocation has been reported in a wide range of species types (Clutton-Brock and Iason 1986), including birds (Komdeur and Pen 2002). Prominent theoretical explanations for sex allocation in birds include the local resource competition model (Gowaty 1993), local resource enhancement model (Gowaty and Lennartz 1985), and the Trivers-Willard hypothesis (Trivers and Willard 1973). These hypotheses are logically appealing and have been supported with empirical evidence (Review; Clutton-Brock and Iason 1986). However, results from empirical studies have also been inconsistent and compelling evidence is scarce (Komdeur and Pen 2002; Navara 2018d). Additionally, the vast diversity of life histories between different bird species creates the need to examine sex allocation at the species level (Navara 2018d).

When considering the complexity of sex allocation in birds, it is not surprising that observed sex ratios at times fail to match predictions (Navara 2018d). This is often the case in reintroduced populations of both mammals and birds, where unexpected sex ratios have been reported following release (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Ewen et al. 2010). In species reintroductions, animals are released into part of their former native range in order to re-establish a wild population (Gusset 2012). Species reintroductions have resulted in some great successes in the management of threatened species (IUCN 2018). However, a large portion of attempts still fail (Fischer and Lindenmayer 2000; Jule, et al. 2008; Sutton and Lopez 2014). Skewed sex ratios following release can damage and slow the growth of reintroduced populations (Wedekind 2012; Ginsberg and Milner-Gulland 1994; Sæther, et al. 2003), and may contribute to the low success rate for these programmes. However, in most reports of skewed sex ratios in reintroduced populations, little or no explanation is provided (Jiang, et al. 2000; Law, et al. 2014; Dunham 2001; Ewen, et al. 2010 Chapter 1.1). It may be that the mismatch between the two environments (captive and wild) experienced by reintroduced animals, imposes physiological constraints on their ability to adjust offspring sex ratios (Edwards, et al. 2019).

Significant physiological development occurs in utero. The environmental conditions experienced during this time can impact that development and may be one of the greatest sources of non-genetic physiological variation (Bernardo 1996; Lane, et al.

2014b). This environment is largely controlled by maternal effects, through which the conditions experienced by a pregnant female are expressed to the fetus (Gorman and Nager 2004). In birds, the window in which maternal effects can directly influence physiological development is limited, as the egg is separated after oviposition (Groothuis and Schwabl 2007). However, maternal deposition of hormones in the yolk will continue to have an effect up until hatching (Navara 2013a). Anticipatory maternal effects allow offspring to develop in an environment that matches the expected postnatal environment so that physiological characteristics can be 'programmed' accordingly (Mousseau and Fox 1998). Maternal effects can have significant and lifelong impacts on several physiological characteristics, including those used in sex allocation (Edwards, et al. 2016a).

Sex allocation is carried out through physiological mechanisms (Navara 2013b). As physiology is variable between individuals (Edwards, et al. 2016a), some individuals may respond to environmental conditions differently to others, and therefore adjust offspring sex ratios differently. In birds, maternal hormones are the most likely physiological mechanism by which sex allocation occurs (Navara 2013b). Maternal steroid hormones vary in response to environmental and maternal conditions, which have been linked to offspring sex ratios (Goerlich-Jansson, et al. 2013). Increased testosterone levels lead to male biased sex ratios, while increased corticosterone lead to female biased sex ratios (Navara 2013a; 2013b; Goerlic-Jansson, et al. 2013). While it remains unclear if these steroid hormones represent direct mediators of sex allocation, it is certainly likely that they are involved (Navara 2013b). Both testosterone and glucocorticoid concentration are mediated by physiological characteristics that are dependent on prenatal experience (Edwards, et al. 2016a). When there is a mismatch between pre- and postnatal environments, as occurs when captive-bred populations are reintroduced into the wild, animals may develop physiological characteristics that are unsuited to respond to current local conditions as adults (Edwards, et al. 2016a). This may impose constraints on their ability to adjust offspring sex ratios adaptively and in accordance with theoretical hypotheses of sex allocation (Edwards, et al. 2019). There is already evidence that prenatal environment can effect sex ratios in the following generation (Clark and Galef 1995; Edwards, et al. 2016b), including support for pre- and postnatal mismatch constraining sex allocation in response to factors that are predicted to drive sex ratios (Edwards, et al. 2019).

Here I examine the effects of mismatched pre- and postnatal environments on sex allocation in the reintroduced populations of California condor (*Gymnogyps californianus*). Mismatch occurs when captive-born animals are released and successfully reproduce in the wild, and also when wild-born animals are captured and reproduce in captivity. Theoretical explanations suggest that sex ratios should vary in response to a number of factors. I test the relationship between sex ratios and four additional variables (parental age, parental parity in the previous year, rainfall, and temperature) that may influence sex allocation (Berkeley and Linklater 2010; Clutton-Brock and Iason 1986; Rutberg 1986; Ferrer, et al. 2009; Dowling and Mulder 2006), and explore the interaction between these variables and environmental mismatch in California condors. I predict a mismatch between pre- and postnatal environments will impose constraints on maternal ability to adjust offspring sex ratios. However, it is difficult to predict whether it would lead to the production of more sons or daughters. Additionally, I do not have details of population sex ratios for California condors. Therefore, my proposed hypotheses are:

1. Mismatch between pre- and postnatal environments in reintroduced animals will result in offspring sex ratios that differ, not from parity (i.e., a 1:1 male to female sex ratio), but from offspring sex ratios of animals whose pre- and postnatal environment is matched.
2. Offspring sex ratios will vary in response to climate, parental age, and parental parity in the previous year, but this effect will be obscured when pre- and postnatal environment is mismatched.

The effect of the four factors measured here that I expect to influence sex allocation (parental age, parental parity, rainfall, and temperature), may be masked by supplementary resources and control of the environment in captivity. Additionally, mismatch in opposite directions (i.e., captive to wild, and wild to captive) may have an inverse effect on sex allocation, obscuring overall results. Therefore, as well as conducting my analysis on the full California condor dataset, I also repeated it, looking at just wild individuals. In this group, there is no anthropogenic control of the environment, which may dampen sex allocation, and mismatch is only the release of captive born animals into the wild.

4.2 Methods

4.2.1 Study Species

The California condor (*Gymnogyps californianus*) is a large land bird native to North America, from the family Cathartidae (Synder and Synder 2000). California condors are monogamous breeders and have slight sexual dimorphism between the sexes, with males being on average slightly larger than females (Synder and Synder 2000). They reach sexual maturity around the age of 5 or 6 years, at which point they mate for life in a single pair. Breeding pairs produce just one offspring per clutch and provide an extensive amount of parental care (Synder and Synder 2000).

The historic range of the California condor covered a vast area of North America (BirdLife International 2018). Even before anthropogenic impacts, however, their range had shrunk to just parts of the American Southwest and Baja California, Mexico. Following European settlement, the remaining populations experienced further dramatic declines (BirdLife International 2018). By 1987, all remaining California condors had been captured to establish a captive breeding population. The captive population grew and, in 1992, they were reintroduced back into their former range in California (Walters, et al. 2010). This new wild population has performed well, and subsequent reintroductions to Arizona, Utah, and Baja California have followed. There are now approximately 310 California condors living in the wild (BirdLife International 2018).

4.2.2 Data

Data on California condor reintroductions was taken from the historic listings section of the California Condor North American Studbook, published by San Diego Zoo Global (Mace 2014). In this, animal ID, gender, date of birth, birth location, dam, sire, and transfers were recorded for each known individual in captivity and in closely monitored wild populations. Using this, I was able to identify individuals that had a mismatched pre- and postnatal environment, and the sex ratios of their offspring. I also determined the age of sires and dams at the time they conceived offspring, whether they had reproduced in the year prior to conception, and climate details around the time of conception. Climate details included the average monthly rainfall around the time of conception (mm), and the average daily temperature in the month of conception. For individuals conceived in the USA, this was measured at the state level and data was taken from Iowa State University climate reports (https://mesonet.agron.iastate.edu/climodat/). For individuals born outside the United States (Mexico), rainfall was measured at the country level and data was taken from the historic climate datasets of the World Bank Group (https://climateknowledgeportal.worldbank.org/).

4.3 Statistical Analysis

All statistical analysis was done in R.Studio Version 1.1.463. The packages used have been outlined in Chapter 2. Results are presented using 90% confidence intervals.

4.3.1 Offspring Sex Ratios in Environmentally Matched and Mismatched California Condor

Generalised linear mixed effect models with binomial error were used to determine whether offspring sex ratios of individuals with mismatched pre- and postnatal environments differed from those with matched environments. A number of different models with biologically relevant fixed and random effects were compared on the Akaike information criterion (AIC) to find the model of best fit. The most complex model included dam age, sire age, rainfall around the time of conception, and temperature around the time of conception as fixed effects, and dam ID, sire ID, conception location, conception location type, dam reproductive productivity in the year prior to conception, and sire reproductive productivity in the year prior to conception as random effects The model was gradually simplified and the most parsimonious model was identified, which included no random effects. Therefore, a generalised linear model with binomial distribution but no mixed effects was used. The final model included just the effect of mismatch and sire age as a fixed effect.

4.3.2 Parental age

Offspring sex ratios have been linked to parental age and ability to invest, which may be expected to vary with parental age in monogamous birds (Magrath, et al. 2002; Addison and Kitaysky 2008; Ferrer, et al. 2009). Both maternal and paternal age can influence offspring sex ratios. Therefore, parental age may be related to offspring sex ratios in California condors. I used generalised linear models with binomial error to independently assess the effects of dam age and sire age on offspring sex ratios in California condor. This was first done for all individuals regardless of mismatch. I then examined the effect these variables had in each treatment group, matched and mismatched, individually. Finally, I examined the effects of parental age and treatment group together using models that included parental age (either dam age or sire age), treatment, and their interactive effect.

4.3.3 Parental Parity

In this study, parental parity is taken as reproductive productivity in the year prior to conception. Similar to parental age, ability to invest in offspring development may be affected by parity (Clutton-Brock & Iason 1986; Rutberg 1986). Pairs that successfully reproduced in the prior year would have less capacity to invest in offspring due to the costly nature of their extensive parental care (Synder and Synder 2000). As such, parental parity (both maternal and paternal) may influence offspring sex ratio in California condors. The same methods were used to analyse the effect of parental parity as parental age. Generalised linear models with binomial error were used to assess the effects of maternal and paternal parity on offspring sex ratios. This was first done for the full dataset of California condors, regardless of mismatch. Matched and mismatched treatment groups were then tested independently. Finally, the effects of environmental mismatch and parental parity were tested simultaneously to assess the interaction effect between the two variables.

4.3.4 Climate Conditions

Climate factors can affect offspring sex ratios in a number of ways. Both temperature and rainfall can influence resource availability and, subsequently, ability to invest in offspring (Roche, et al. 2006; Berkeley and Linklater 2010). Extreme climate conditions can also induce stress in breeding animals, thereby potentially affecting sex allocation (Catalano, et al. 2007; Kruuk, et al. 1999). In this study, rainfall (mm) around the time of conception and temperature (°C) around the time of conception were included as factors that may influence sex allocation in California condors. I examined the effects of these two variables on offspring sex ratios using generalised linear models with binomial error. As for the previous variables, the effect of temperature and rainfall was first examined for all California condor individuals, then for each treatment group independently. Finally, the effects of environmental mismatch and climate variables were examined together using models that included either temperature or rainfall, along with mismatch treatment and the interactive effect of the two factors.

4.3.5 Wild Populations

The effects of climate and parental age and parity may be somewhat masked in captive populations. As the environment is controlled and resources are readily available, these variables are not likely to affect ability to invest in offspring as heavily as they would in the wild. To combat this, I have repeated the analysis above, examining just the wild populations of California condor. Wild populations are made up of individuals born in the wild (matched environments), as well as individuals born in captivity that have been released to the wild (mismatched environments) In this, mismatched pre- and postnatal environments are created only when captive-born individuals are released into the wild, and not when wild-born animals are captured.

4.4 Results

4.4.1 Full data set

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in both wild and captive populations combined are presented in Table 4.1.

California condors that experienced a mismatch between pre- and postnatal environment did not produce offspring sex ratios that differ from those of individuals with matched environments (Figure 4.1 ‘A’).

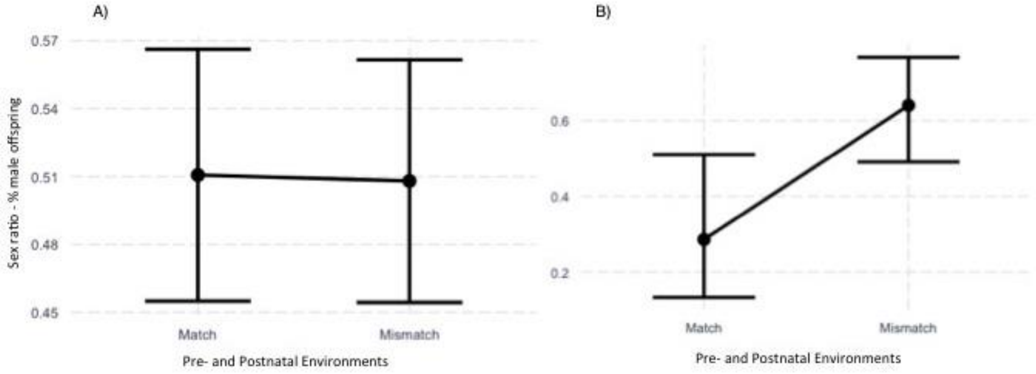

Figure 4.1: The effect of mismatch between pre- and postnatal environments on offspring sex ratios. When both captive and wild populations were analysed together (A), mismatched environments had no effect on offspring sex ratios. However, in the wild populations alone (B), California condors that had experienced a mismatch between pre- and postnatal environment produced a higher proportion of sons than those that had not experienced a mismatch.

Table 4.1: Model outputs for the effects of environmental mismatch, sire age, dam age, sire parity, dam parity, rainfall, temperature and their interaction effects on offspring sex ratios in captive and wild populations of California condors.

Model/Test	Estimate	Std. error	*z* value	p- value
Mismatch vs Match	-0.011	0.158	-0.067	NS[1]
Sire Age	-0.009	0.010	-0.856	NS
Sire Age Matched Environments	0.011	0.015	0.732	NS
Sire Age Mismatched Environments	-0.026	0.014	-1.841	0.066 .
Sire Age Mismatch Interaction	-0.037	0.020	-1.799	0.072 .
Dam Age	-0.003	0.014	-0.203	NS
Dam Age Matched Environments	0.034	0.026	1.292	NS
Dam Age Mismatched Environments	-0.018	0.017	-1.060	NS
Dam Age Mismatch Interaction	-0.052	0.031	-1.661	0.097 .
Sire Parity	-0.138	0.173	-0.795	NS
Sire Parity Matched Environments	-0.148	0.247	-0.598	NS
Sire Parity Mismatched Environments	-0.136	0.244	-0.559	NS
Sire Parity Mismatch Interaction	0.011	0.347	0.033	NS
Dam Parity	-0.134	0.172	-0.777	NS
Dam Parity Matched Environments	-0.229	0.244	-0.940	NS
Dam Parity Mismatched Environments	-0.046	0.243	-0.191	NS
Dam Parity Mismatch Interaction	0.183	0.344	0.532	NS
Rainfall	-0.001	0.001	-0.509	NS
Rainfall Matched Environments	-0.001	0.002	-0.761	NS
Rainfall Mismatched Environments	0.000	0.001	-0.051	NS
Rainfall Mismatch Interaction	0.001	0.002	0.604	NS
Temperature	-0.015	0.017	-0.882	NS
Temperature Matched Environments	-0.032	0.022	-1.416	NS
Temperature Mismatched Environments	0.012	0.029	0.434	NS
Temperature Mismatch Interaction	0.044	0.036	1.216	NS

---

[1] Not Significant

. Significant to the 90% confidence interval ($p < 0.1$)

Sire age had no effect on offspring sex ratios in either the full dataset, or the matched group. However, when pre- and postnatal environment was mismatched sire age had a significant effect on sex ratios with older males producing a higher proportion of female offspring. There was also a slightly significant interaction effect between sire age and environmental mismatch (Figure 4.2).

Dam age also had no effect on sex ratios for the population as a whole, and this relationship was consistent for each of the treatment groups, matched and mismatched, when tested independently. There was, however, a significant interactive effect between dam age and mismatch. When pre- and postnatal environments were matched, as dam age increased offspring sex ratio tended towards a male bias. When environments were mismatched, the trend was reversed (Figure 4.2).

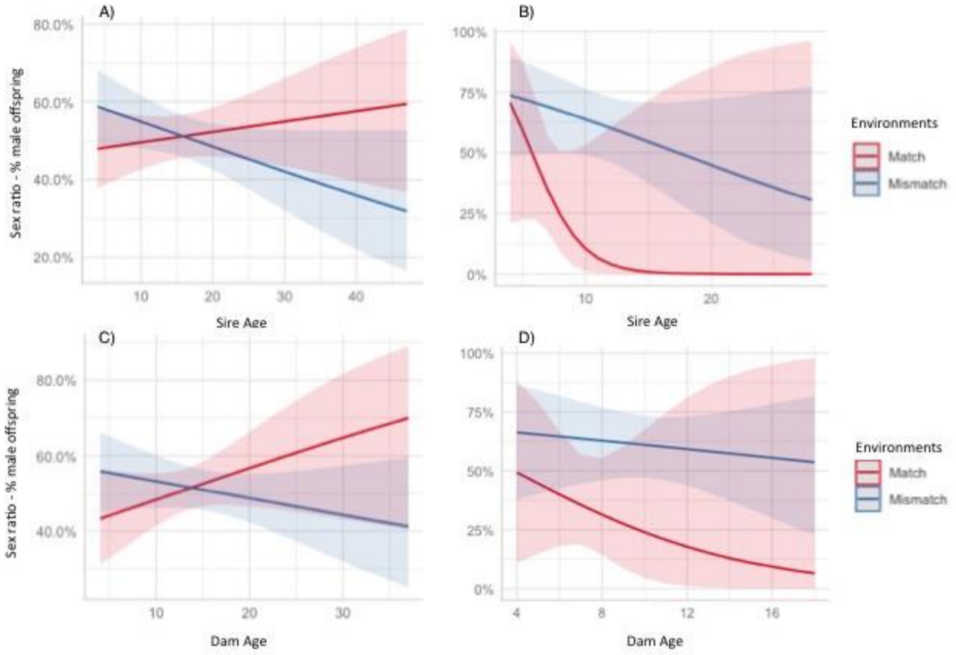

Figure 4.2: The effect of parental age at time of conception on offspring sex ratios in matched and mismatched pre- and postnatal environments. A) Sire age in captive and wild populations. B) Sire age in just wild populations. C) Dam age in captive and wild populations. D) Dam age in just wild populations.

Neither sire nor dam parity had any effect on offspring sex ratio regardless of treatment group and there was no interaction effect.

The same was found for both rainfall and temperature, with neither variable effecting sex ratios in any treatment group and no significant interaction effect. While no significant relationship was present, when graphing the effect of temperature on sex ratios in the matched and mismatched groups, there was an observable trend. As temperature increased, offspring sex ratios favoured females when environments were matched, but males when environments were mismatched (Figure 4.3).

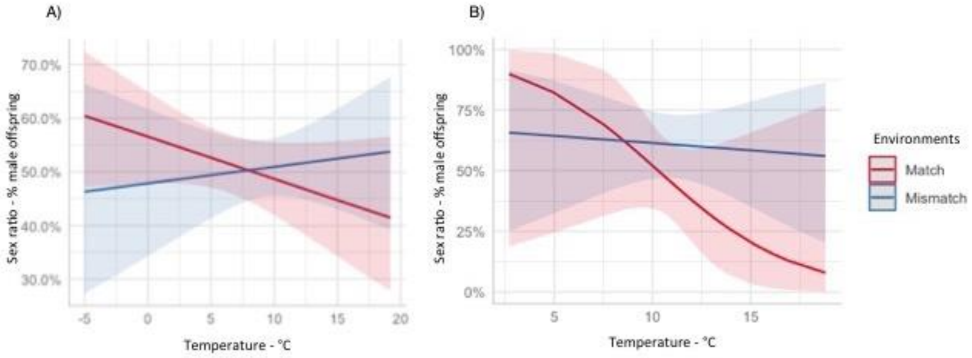

Figure 4.3: Effects of temperature (°C) at the time of conception on offspring sex ratios under matched and mismatched pre- and postnatal environments, in A) captive and wild populations together, and B) just wild populations.

4.4.2 Wild Populations

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in wild populations alone are presented in Table 4.2.

Table 4.2: Model outputs for the effects of environmental mismatch, sire age, dam age, sire parity, dam parity, rainfall, temperature, and their interaction effects on offspring sex ratios in wild populations of California condors.

Model/Test	Estimate	Std. error	*z* value	p- value
Mismatch vs Match	1.492	0.601	2.483	0.013 *
Sire Age	-0.021	0.051	-0.401	NS[1]
Sire Age Matched Environments	-0.505	0.350	-1.443	NS
Sire Age Mismatched Environments	-0.077	0.062	-1.253	NS
Sire Age Mismatch Interaction	0.428	0.355	1.203	NS
Dam Age	0.024	0.071	0.342	NS
Dam Age Matched Environments	-0.188	0.301	-0.624	NS
Dam Age Mismatched Environments	-0.038	0.083	-0.458	NS
Dam Age Mismatch Interaction	0.150	0.312	0.480	NS
Sire Parity	-1.642	0.523	-3.138	0.002 *
Sire Parity Matched Environments	-2.485	1.041	-2.387	0.017 *
Sire Parity Mismatched Environments	-1.022	0.644	-1.586	NS
Sire Parity Mismatch Interaction	1.463	1.224	1.195	NS
Dam Parity	-1.692	0.538	-3.145	0.002 *
Dam Parity Matched Environments	-2.485	1.041	-2.387	0.017 *
Dam Parity Mismatched Environments	-1.030	0.677	-1.521	NS
Dam Parity Mismatch Interaction	1.455	1.242	1.172	NS
Rainfall	0.002	0.003	0.734	NS
Rainfall Matched Environments	0.002	0.004	0.377	NS
Rainfall Mismatched Environments	0.006	0.006	0.989	NS
Rainfall Mismatch Interaction	0.004	0.007	0.584	NS
Temperature	-0.066	0.089	-0.743	NS
Temperature Matched Environments	-0.288	0.228	-1.263	NS
Temperature Mismatched Environments	-0.025	0.100	-0.251	NS
Temperature Mismatch Interaction	0.263	0.249	1.056	NS

---

[1] Not Significant

* Significant to the 95% confidence interval ($p < 0.05$)

Unlike in the full dataset, when examining just wild populations, mismatched environments had a significant effect on offspring sex ratios. Individuals born in captivity and released during reintroduction, thereby experiencing a mismatch between pre- and postnatal environments produced a higher proportion of males when reproducing in the wild (Figure 4.1).

In wild populations parental age, both dam and sire, had no effect on offspring sex ratio in any of the groups tested. There was also no interaction effect between parental age and treatment. However, parent age had a noticeable effect on offspring sex ratios when the relationship was graphed. This effect was dampened when pre- and postnatal environments were mismatched (Figure 4.2)

Sire parity affected the offspring sex ratios in wild populations when looking at both treatment groups together, with fathers that had successfully reproduced in the year prior to conception producing a smaller proportion of male offspring. This effect was consistent in the matched environment group. However, when environment was mismatched, there was no significant effect (Figure 4.4). Despite sire parity having a different effect on sex ratio between the groups, there was no significant interaction effect of sire parity and treatment. The same results were true for dam parity.

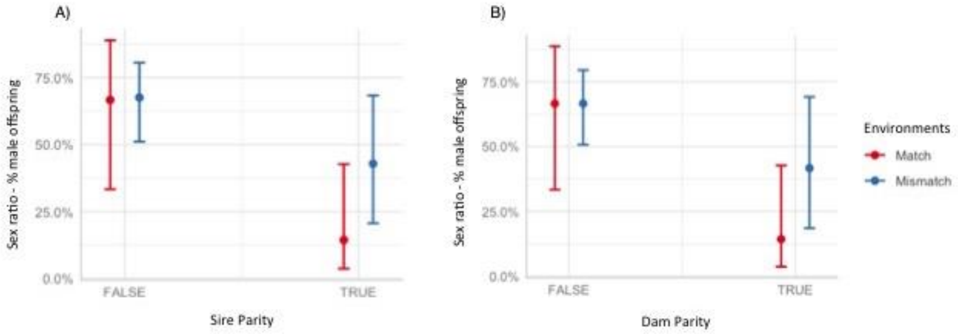

Figure 4.4: The effect of parental parity on offspring sex ratios in matched and mismatched pre- and postnatal environments. A) Sire parity in just wild populations. B) Dam parity in just wild populations. Parents were more likely to produce female offspring if they had successfully reproduced in the previous year, especially when pre- and postnatal environments were matched.

Similar to the full dataset, rainfall and temperature had no effect on offspring sex ratios in any treatment group, and there was no significant interaction effect between climate details and treatment. However, temperature around the time of conception had a visible effect on offspring sex ratios when the relationship was graphed. This effect was obscured when pre- and postnatal environments were mismatched (Figure 4.3).

4.5 Discussion

The establishment of a captive population and re-establishment of wild populations involved in the reintroduction of California condor has involved the transfer of many animals between two highly different environments, captive, and wild. When individuals were conceived and born in one environment type, then transferred to the other, a mismatch between pre- and postnatal conditions occurs. However, when mismatches created by transfers in both directions (i.e. captive to wild, and wild to captive) were examined together, their effect did not result in offspring sex ratios different from those of individuals with matched environments (Figure 4.1). Because release and capture are associated with opposite changes in environments, their effect on offspring sex ratios may also be opposite. Therefore, the overall effect when examining both transfer types together may be obscured.

The effect of mismatch on sex ratios in just wild populations supports this. In just wild populations, mismatch only occurs when captive born animals are released to the wild. I found that, in wild populations, California condors that had experienced a mismatch in pre- and postnatal environments produced a higher proportion of male offspring than those with matched environments (Figure 4.1). This result provides support for hypothesis 1, that the change in environment created by reintroduction can affect offspring sex ratios in California condor.

Of the predictors tested against sex ratios here, only rainfall around the time of conception had no significant effect, and no visible trend throughout the analysis. There was a significant interaction effect between mismatched environments and both sire age and dam age on offspring sex ratios. Additionally, while there were no significant relationships between parental age and sex ratios in the wild populations, there are some visible trends, which were affected by mismatch (Figure 4.2). Both sire and dam parity had a significant effect on offspring sex ratios in the wild populations (Figure 4.4), but not when the wild and captive populations were analysed together. Temperature around the time of conception also had no significant effect on sex ratios. However, there was a visible trend when graphing this relationship, and that trend was influenced by environmental mismatch (Figure 4.3).

Dam and sire age are closely linked in California condors as pairs mate for life in a monogamous pair (Synder and Synder 2000). Therefore, it is not surprising that the relationship between sire and sex ratios, and the relationship between dam age and sex ratios were similar. If sex ratios change in response to dam age, the same trend would be observed with sire age, as a pair ages together. As females are the heterogametic sex in birds, it is likely that dam age is the causal effect and sire age just correlates (Smith 2007; Navara 2013b). However, there is some evidence that sire age affects offspring sex ratios in birds (Ferrer, et al. 2009; Dowling and Mulder 2006), as well as that females adjust offspring sex ratios in response to mate condition (Ellegren, et al. 1996). Therefore, the effect of sire age being causal cannot be ruled out.

Here I found no significant effect of parent age on offspring sex ratios when the matched and mismatched groups were together. However, when individuals had a mismatch between pre- and postnatal environments, a significant relationship was present. There was also a significant interaction effect between parental age and mismatched environments on offspring sex ratios. When environments were matched, as parental age increased, sex allocation favoured the production of sons. When environments were mismatched, the reverse was true (Figure 4.2). In the wild populations alone, no interaction effect between parental age and mismatch on offspring sex ratios was present. However, when these relationships were graphed, both sire and dam age appeared to affect offspring sex ratios more when environments were matched than when they were mismatched (Figure 4.2).

Previous studies have reported a relationship between parental age and offspring sex ratios (Ferrer, et al. 2009; Dowling and Mulder 2006), but an explanation for this pattern is not clear. What is important here is that when environments were mismatched, the relationship between offspring sex ratios and parental age changed. This suggests that development under different environmental conditions from those in which animals reproduces, may influence the interaction between parental age and the mechanisms of sex allocation, thereby constraining sex allocation.

As for parental age, the mating system of California condors creates a strong link between sire parity and dam parity. As a result, their effects here were again similar. When captive and wild populations were put together in the species wide analysis, parent parity did not affect offspring sex ratios. However, in the wild populations alone there was an effect present. In captivity, animals are supplied with an abundance of resources, potentially limiting the effect of past reproductive efforts on ability to invest in offspring. Additionally, in captivity, egg removal was carried out to encourage extra breeding (Synder and Synder 2000). Therefore, a number of the cases where parents had reproduced in consecutive years may not have involved the extensive parental care that is seen in the wild. As a result, ability to invest in future offspring would not have been affected.

In the wild population, successful reproduction in the year prior to conception resulted in a smaller proportion of offspring being male. As reproductive efforts and parental care incur a large energetic cost, ability to invest in future offspring may be limited for some time. (Clutton-Brock and Iason 1986) Therefore, this result is consistent with the Trivers-Willard hypothesis (Martin and Festa-Bianchet 2011). However, California condors do not meet the assumptions of the Trivers-Willard model, as they are monogamous breeders and the Trivers-Willard hypothesis operates in polygynous species (Trivers and Willard 1973). A definitive explanation for this relationship is therefore unclear. When pre- and postnatal environments were mismatched by release from captivity into the wild, the effect of parent parity on offspring sex ratios was dampened. This suggests that the effects of anticipatory maternal effects on physiological development in a mismatched environment imposes constraint on the ability of wild California condors to adjust offspring sex ratios in accordance with sire and dam parity in the previous year.

Temperature around the time of conception had no significant effect on offspring sex ratios in California condors, regardless of mismatch treatment. This was consistent across both the analysis of captive and wild populations together, and the analysis of wild populations alone. However, temperature appears to have a slight effect on offspring sex ratios when pre- and postnatal environments are matched, with fewer male offspring being born as temperatures increase (Figure 4.3). Individuals with physiological traits that are not suited to their current environments as a result of anticipatory maternal effects under mismatched conditions may fail to mount natural responses to changes in temperature, which in turn alter the way they adjust offspring sex ratios and resulting in the trends observed here.

These results provide support for hypothesis 2, that a mismatch between pre- and postnatal environments imposes physiological constraints to the ability of California condors to adjust offspring sex ratios. Mismatched environments created by the capture and release of California condors altered the relationship of offspring sex ratios with parental age, parental parity, and temperature around the time of conception. While the results presented here do support this hypothesis, they do not provide conclusive empirical evidence and many of the observed effects had only weak statistical support. This is possibly due to the structure of this study and the limited data available to me from past species reintroductions.

Cameron (2004) highlighted the flaw in analysing sex allocation retrospectively with datasets collected for alternative purposes. The predictors of sex allocation used were restricted to the available data, and may not be the most important factors for sex allocation in California condor. Additionally, the climate conditions used here were measured at relatively low resolution (state-wide). Variation that occurs within and between populations in a single state would not be detected.

Repeating this study with an ongoing species reintroduction and using well-established predictors of sex allocation at the individual level may yield more conclusive results. An understanding of the specific differences between captive and wild locations involved in that reintroduction programme would also be beneficial for predicting and explaining observations.

Despite the shortcomings of this study, it does provide evidence that sex allocation is constrained by physiological development in a mismatched environment. Verifying this relationship could have significant implications for conservation. This could be taken into consideration in future reintroduction programmes, and efforts can be made to mitigate its effect. Preventing sex ratio skews in reintroduced populations may improve the success rate of these programmes and may aid in the preservation of threatened species.

5 Red Wolf

5.1 Introduction

In the current global extinction crisis (Stork 2010; Burkhead 2012; Alroy 2015), bold conservation strategies have been used in order to preserve threatened species in the environment. One such strategy is the use of species reintroductions and translocations (Seddon, et al. 2007; Reading, et al. 2002). Species reintroductions are used to re-establish wild populations of threatened species to part of their former native range from which they have been lost (Gusset 2012). While there have been many great successes in the management of threatened species by using this strategy, reintroductions still often fail (Fischer and Lindenmayer 2000; Jule, et al. 2008; Sutton and Lopez 2014; Chapter 1.1). One such failure is the reintroduction of red wolves (Canis rufus) in North America, which has been deemed largely unsuccessful (Simonis, et al. 2017). Success rates are particularly low when reintroduced populations are made up of captive born individuals (Sjöåsen 1996; Bremner-Harrison, et al. 2004; Vickery and Mason 2003). In the wild, captive-born animals are more susceptible to starvation, predation, and diseases than wild born animals (Jule, et al. 2008). Another factor that may contribute to the low success rate in reintroductions of captive-born animals is the offspring sex ratio in first generation following release (Capter 1.1). Sex ratio biases have been reported in a number of reintroduced populations following release (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Jiang, et al. 2000; Law, et al. 2014; Dunham 2001; Ewen, et al. 2010), and this pattern may be even more prominent than the literature suggests (Chapter 1.1). Such sex ratio biases can have a number of harmful effects that can damage and slow the growth of the population (Wedekind 2012; Ginsberg and Milner-Gulland 1994; Sæther, et al. 2003), potentially resulting in failure of the programme.

Many species have demonstrated the ability to adjust offspring sex ratios in order to maximise fitness returns on reproductive investment (Clutton-Brock and Iason 1986). This process is termed sex allocation and is adaptively beneficial when sex-specific offspring fitness is variable (West 2009). Several sex allocation hypotheses are prominent in the literature, which predict adjustment of offspring sex ratios in response to a particular variable (Clark 1978; Trivers and Willard 1973; Gowaty and Lennartz 1985). For example, The Trivers-Willard hypothesis predicts that in polygynous species, mothers in good condition (high level of ability to invest in offspring) will produce offspring in good condition, and males will gain a greater fitness boost from being in good condition than females. Therefore, when mothers themselves are in good condition, they should favour the production of sons (Trivers and Willard 1973). Red wolves are monogamous breeder and therefore may not fit the parameters of this model (Sparkman, et al. 2017). However, sex allocation also occurs in monogamous species (Sidorovich, et al. 2007) and may still be affected by physiological constraints derived from anticipatory maternal effects. Other hypotheses relate to various factors that may influence the fitness returns on investment in the two sexes unequally, including: resource competition (Silk 1983), sex-specific dispersal patterns (Clark 1978), sex-specific cooperative behaviours (Gowaty and Lennartz 1985), and different cost associated with the production of sons and daughter (Pratt and Lisk 1989).

While these sex allocation hypotheses are logically appealing, empirical evidence to support them has been notoriously inconsistent, particularly in mammals (Cameron 2004; Sheldon and West 2004). Methodological inconsistencies between studies (Cameron 2004; Sheldon and West 2004) and interacting drivers of sex allocation (Hiraiwa-Hasegawa 1993; Cockburn, et al. 2002; van Schaik and Hrdy 1991; Moore, et al. 2015) have been highlighted as sources of some of this inconsistency. However, a relatively large degree of variability still exists between results of different studies (Edwards, et al 2016b, West 2009), and observed sex ratios fail to match predictions. This is generally the case when reintroduced populations exhibit skewed sex ratios following release (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Jiang, et al. 2000; Law, et al. 2014; Dunham 2001; Ewen, et al. 2010). Many of these skews are either poorly explained or not explained at all (Chapter 1.1). As sex allocation must act through physiological mechanisms (Edwards, et al. 2016a), abnormalities in certain physiological characteristics for some individuals can influence or constrain sex allocation and may explain some of the inconsistencies in results and unexpected sex ratio skews that have been observed (Edwards, et al. 2016a). Importantly, in reintroduced populations, the transfer of captive born individuals to breed in the wild is a potential source for such physiological abnormalities to come about (Edwards, et al. 2019).

When captive-born animals are released into the wild during species reintroductions, there is a mismatch between the prenatal environment, in which a significant portion of physiological development occurs (Bernardo 1996; Lane, et al. 2014b), and postnatal environment after release. Through anticipatory maternal effects, captive-born animals may develop permanent physiological characteristics that are suited to that environment (Edwards, et al.2016a). For example, in captivity, animals may be exposed to unnaturally low environmental stress. When a pregnant female experiences this, maternal effects express that experience to the developing fetus (Edwards, et al. 2016a; Brunton 2010). As a result, the fetus will develop in a prenatal environment with unnaturally low glucocorticoid concentrations. This prenatal experience can have a number of effects on physiological characteristics (Edwards, et al. 2016a), one of which may be the development of a dampened stress response (Takahashi and Kalin 1991; Sheriff, et al. 2010). A dampened stress response may be appropriate in the captive environment, as environmental stressors are unnaturally low. However, when the postnatal environment does not match the prenatal environment, as would occur in species reintroductions, it may leave animals unable to mount an appropriate stress response in the wild (Edwards, et al. 2016a). Maternal effects can influence a number of physiological characteristics, such that when pre- and postnatal environments are mismatched, animals are unable to respond appropriately to the current local conditions (Edwards, et al. 2016a). These include changes to glucose metabolism and regulation, androgen regulation, and stress responsiveness (Lane, et al. 2014a; Gluckman and Hanson 2004; Shiell, et al. 2000; Pfannkuche, et al. 2011; Voegtline, et al. 2013; Brunton 2010; Takahashi and Kalin 1991), all of which are key processes in the proposed physiological mechanisms used in mammalian sex allocation (Cameron 2004; Navara 2010; Grant 2007).

To summarise, when pre- and postnatal environments are mismatched (as occurs in species reintroductions), animals develop in conditions that lead to physiological characteristics that are unsuited to their postnatal environment. This may change the way in which glucose and testosterone is regulated, and effect stress responsiveness. As these factors are key to the physiological mechanisms used in sex allocation, it may alter the way in which animals adjust offspring sex ratios. Therefore, the reintroduction of captive-born animals and the associated mismatch between pre- and postnatal environments may impose physiological constraints on an individuals ability to adaptively adjust offspring sex ratios, and may explain the unexpected skews observed in reintroduced populations.

Sex ratios are difficult to predict in monogamous species as they often fail to match the assumptions of sex allocation hypotheses (Sidorovich, et al. 2007). However, physiological mechanisms may still be present, allowing for anticipatory maternal effects in a mismatched environment to influence offspring sex ratios. Testing this effect in a monogamous species such as the red wolf allows for assessment of the range of species types that should be considered when attempting to minimise unexpected sex ratio skews in reintroduced populations.

Here I examine the effects of mismatched pre- and postnatal environments on sex allocation in the reintroduced populations of red wolves (*Canis rufus*). Mismatch occurs when captive born animals are released and successfully reproduce in the wild, and also when wild born animals are captured and reproduce in captivity. Theoretical hypotheses suggest that sex ratios should vary in response to a number of factors. I test the relationship between sex ratios and three additional variables (parental age, rainfall, and temperature) that may influence sex allocation (Berkeley and Linklater 2010; Clutton-Brock and Iason 1986; Rutberg 1986; Ferrer, et al. 2009; Dowling and Mulder 2006), and explore the interaction between these variables and environmental mismatch. I predict a mismatch between pre- and postnatal environments will impose constraints on maternal ability to adjust offspring sex ratios. However, it is difficult to predict whether it would lead to the production of more sons or daughters, especially in monogamous species such as the red wolf. Additionally, I do not have details on natural population sex ratios in red wolves. Therefore, my proposed hypotheses are:

1. Mismatch between pre- and postnatal environments in reintroduced animals will result in offspring sex ratios that differ, not from parity (i.e., a 1:1 male to female sex ratio), but from offspring sex ratios of animals whose pre- and postnatal environment is matched, and,
2. Offspring sex ratios will vary in response to climate, parental age, and parental parity in the previous year, but this effect will be obscured when pre- and postnatal environments are mismatched.

The four predictors of sex allocation (sire age, dam age, rainfall, and temperature), may have a dampened effect on offspring sex ratios in captivity as supplementary resources and food is provided to the animals. Additionally, mismatch in opposite directions (i.e., captive to wild, and wild to captive) may have an inverse effect on sex allocation, obscuring overall results. Therefore, as well as conducting my analysis on the full red wolf dataset, I also repeated it, looking at just wild populations of red wolves. In this group, there is no anthropogenic control of the environment, which may dampen effects on sex allocation, and mismatch only occurs when captive born animals are released into the wild

5.2 Methods

5.2.1 Study Species

The red wolf (*Canis rufus*) is a critically endangered canine species, native to North America (Phillips 2018). They are social animals, forming packs around a single monogamous breeding pair and their offspring from a number of years (Sparkman, et al. 2011). Breeding occurs once a year between January and March, and both males and females participate in parental care, often with the assistance of older offspring in the pack (Sparkman, et al. 2011). Males and females are sexually dimorphic in that males are slightly larger than females (Waddell, 2014).

The red wolf is one of the most endangered wolf species in the world (Phillips 2018). Historically, they ranged throughout the south-eastern USA, but wild populations experienced dramatic declines due to hunting, habitat loss, and crossbreeding with coyotes (Phillips 2018). During the 1970s the remaining wild red wolves were captured, leaving the species extinct in the wild. Fourteen individuals became the source of a captive breeding population. Despite this small number the population grew and in 1986 they were reintroduced to North Carolina (Hedrick and Fredrickson 2008). This was followed by releases to a number of coastal islands in Florida, Mississippi, and South Carolina, as well as a release to a national park in Tennessee. The Tennessee population, however, was unable to establish and has since been removed (Simonis, et al. 2017). Wild populations of red wolves continue to struggle as hunting pressures, and competition and crossbreeding with coyotes continues to limit recovery (Phillips 2018).

5.2.2 Data

Data on red wolf reintroductions was taken from the historic listings section of the Red Wolf International Studbook, published by Point Defiance Zoo & Aquarium (Waddell 2014). In this, animal ID, gender, date of birth, birth location, dam, sire, and transfers were recorded for each known individual in captivity and in closely monitored wild populations. Using this, I was able to identify individuals that had a mismatch between their pre- and postnatal environments, and the sex ratios of their offspring. I also determined the age of sires and dams at the time they conceived offspring, and climate details around the time of conception. Climate details included the average monthly rainfall around the time of conception (mm), and the average daily temperature in the month of conception. This was measured at the state level and data was taken from Iowa State University climate reports (https://mesonet.agron.iastate.edu/climodat/).

5.3 Statistical Analysis

All statistical analysis was done in R.Studio Version 1.1.463. (packages used listed in the methods chapter). Results are presented using 95% confidence intervals.

5.3.1 Offspring Sex Ratios in Environmentally Matched and Mismatched Red Wolves

Generalised linear mixed effect models with binomial error were used to determine whether offspring sex ratios of individuals with mismatched pre- and postnatal environments differed from those with matched environments. A number of different models with biologically relevant fixed and random effects were compared on the Akaike information criterion (AIC) to find the model of best fit. The most complex model included dam age, sire age, rainfall around the time of conception, and temperature around the time of conception as fixed effects, and dam ID, sire ID, conception location, and conception location type as random effects. The model was gradually simplified and the most parsimonious model was identified, which included no random effect. Therefore, a generalised linear model with binomial distribution but no mixed effect was used. The final model included just the effect of mismatch, and sire and dam age as fixed effects.

5.3.2 Parental age

In a number of mammalian species, parental age has been linked to offspring sex ratios (Côté and Festa-Bianchet 2001; Clutton-Brock and Iason 1986). Ability or disposition to invest in offspring can vary with age (Clutton-Brock and Iason 1986). As a result, offspring sex ratios may vary accordingly. Without species-specific insight, it is not clear exactly how sex ratios should respond to different parental ages in red wolves.

However, it is likely that an effect is present. Therefore, I examined the effect of dam and sire age on offspring sex ratios independently using generalised linear models with binomial error. I first tested this relationship across the entire red wolf dataset, regardless of environmental mismatch. I then separated the matched and mismatched treatment groups and examined this relationship in each independently. Finally, I examined the effects of parental age and treatment group together using models that included parental age (either dam age or sire age), treatment, and their interactive effect.

5.3.3 Climate Conditions

Climate factors can affect offspring sex ratios in a number of ways. Both temperature and rainfall can influence resource availability, and subsequently, ability to invest in offspring (Roche, et al. 2006; Berkeley and Linklater 2010). Additionally, extreme climate conditions can also induce stress in breeding animals, thereby potentially affecting sex allocation (Catalano, et al. 2007; Kruuk, et al. 1999). In this study, rainfall (mm) around the time of conception and temperature (°C) around the time of conception were included as factors that may influence sex allocation in red wolves. I examined the effects of these two variables on offspring sex ratios using generalised linear models with binomial error. As for parental age, the effect of temperature and rainfall was first examined for all red wolf individuals, then for each treatment group independently. Finally, the effects of environmental mismatch and climate variables were examined together using models that included either temperature or rainfall, along with mismatch treatment and the interactive effect of the two factors.

5.3.4 Wild Populations

The effects of parental age and climate may be somewhat masked in captive populations. As the environment is controlled and resources are readily available, these variables are not likely to effect ability to invest in offspring as heavily as they would in the wild. To combat this, I have repeated the analysis above, examining just the wild populations of red wolves. Wild populations are made up of individuals born in the wild (matched environments), as well as individuals born in captivity that have been released to the wild (mismatched environments). In this, mismatched pre- and postnatal environments are created only when captive born individuals are released into the wild, and not when wild born animals are captured.

5.4 Results

5.4.1 Full data set

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in both wild and captive populations combined are presented in Table 5.1.

Table 5.1: Model outputs for the effects of environmental mismatch, sire age, dam age, rainfall, temperature and their interaction effects on offspring sex ratios in captive and wild populations of red wolves.

Model/Test	Estimate	Std. error	*z* value	p- value
Mismatch vs Match	-0.082	0.162	-0.507	NS[1]
Sire Age	-0.036	0.021	-1.747	0.081 .
Sire Age Matched Environments	-0.042	0.022	-1.897	0.058 .
Sire Age Mismatched Environments	-0.004	0.060	-0.069	NS
Sire Age Mismatch Interaction	0.038	0.064	0.594	NS
Dam Age	-0.028	0.024	-1.154	NS
Dam Age Matched Environments	-0.027	0.025	-1.067	NS
Dam Age Mismatched Environments	-0.009	0.083	-0.105	NS
Dam Age Mismatch Interaction	0.018	0.087	0.207	NS
Rainfall	-0.001	0.001	-1.211	NS
Rainfall Matched Environments	-0.001	0.001	-1.047	NS
Rainfall Mismatched Environments	-0.002	0.004	-0.483	NS
Rainfall Mismatch Interaction	-0.001	0.004	-0.164	NS
Temperature	0.022	0.013	1.730	0.084 .
Temperature Matched Environments	0.015	0.014	1.122	NS
Temperature Mismatched Environments	0.081	0.040	2.024	0.043 *
Temperature Mismatch Interaction	0.065	0.042	1.545	NS

---

[1] Not Significant

. Significant to the 90% confidence interval ($p < 0.1$)

* Significant to the 95% confidence interval ($p < 0.05$)

Red wolves that experienced a mismatch between pre- and postnatal environment did not produce offspring sex ratios that differ from those of individuals with matched environments.

When examining the effects of sire age, I found a slight relationship with offspring sex ratio. This effect was consistent within the matched environments group. In these, as sire age increased, sex ratios tended towards female bias. However, when environment was mismatched there was no effect (Figure 5.1). Despite this, there was no significant interaction effect between sire age and mismatch.

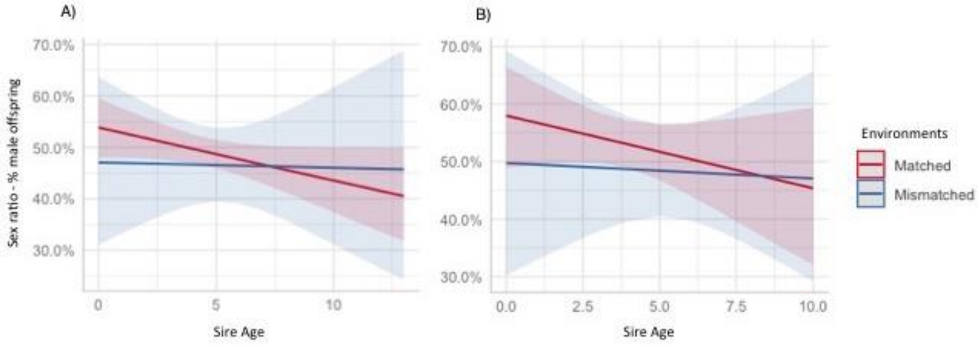

Figure 5.1: Effects of sire age (years) at time of conception on offspring sex ratios under matched and mismatched pre- and postnatal environments, in A) all populations together, and B) just wild populations.

Unlike sire age, dam age had no effect on offspring sex ratio regardless of treatment group and there was no interaction between dam age and treatment.

When considering the effects of climate factors, I found that rainfall had no effect on offspring sex ratio in any of the treatment groups. However, there was a significant relationship between temperature and sex ratios. This relationship was also present for the environmental mismatch treatment group, with higher temperatures around the time of conception yielding a higher proportion of male offspring. When pre- and postnatal environments were matched, temperature did not affect offspring sex ratios (Figure 5.2). There was, however, no significant interaction effect between temperature and mismatch.

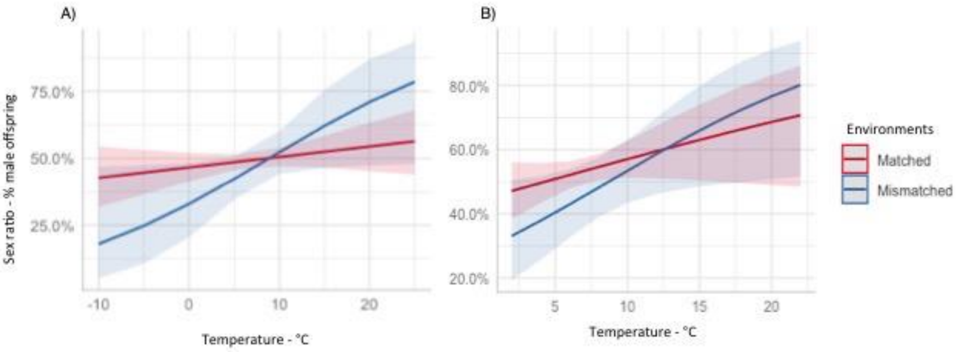

Figure 5.2: Effects of temperature (°C) at the time of conception on offspring sex ratios under matched and mismatched pre- and postnatal environments, in A) all populations together, and B) just wild populations.

5.4.2 Wild Populations

The results of the statistical analysis on the effects of the various predictors on offspring sex ratios in wild populations alone are presented in Table 5.2.

Table 5.2: Model outputs for the effects of environmental mismatch, sire age, dam age, rainfall, temperature and their interaction effects on offspring sex ratios in just wild populations of red wolves.

Model/Test	Estimate	Std. error	*z* value	p- value
Mismatch vs Match	-0.132	0.192	-0.689	NS[1]
Sire Age	-0.045	0.036	-1.261	NS
Sire Age Matched Environments	-0.054	0.044	-1.214	NS
Sire Age Mismatched Environments	-0.011	0.074	-0.145	NS
Sire Age Mismatch Interaction	0.043	0.086	0.499	NS
Dam Age	0.004	0.037	0.119	NS
Dam Age Matched Environments	0.003	0.041	0.073	NS
Dam Age Mismatched Environments	0.043	0.093	0.469	NS
Dam Age Mismatch Interaction	0.040	0.101	0.398	NS
Rainfall	0.001	0.002	0.569	NS
Rainfall Matched Environments	0.001	0.002	0.452	NS
Rainfall Mismatched Environments	0.001	0.005	0.124	NS
Rainfall Mismatch Interaction	0.000	0.005	-0.058	NS
Temperature	0.055	0.024	2.247	0.025 *
Temperature Matched Environments	0.050	0.032	1.555	NS
Temperature Mismatched Environments	0.105	0.049	2.164	0.030 *
Temperature Mismatch Interaction	0.055	0.058	0.949	NS

---

[1] Not Significant

* Significant to the 95% confidence interval ($p < 0.05$)

Similar to the full data set, mismatch treatment had no effect on offspring sex ratios in just wild populations. Individuals that experienced a mismatch between pre- and postnatal environments did not produce offspring sex ratios that differ from those of individuals with matched environments.

In wild populations, neither sire nor dam age had a significant effect on offspring sex ratios regardless of treatment group, and there was no interactive effect between parent age and treatment. However, when graphed, a trend was visible in the interaction between mismatch and sire age (Figure 5.1). Similar to results when both wild and captive populations were considered, when environments were matched, increased sire age resulted in a greater proportion of female offspring. This trend was not present when environments were mismatched.

As for the captive and wild populations combined analysis, rainfall had no effect on offspring sex ratios in wild red wolves.

When pre- and postnatal environments were mismatched, sex ratios responded to temperature, becoming male biased when temperature increases. This relationship was also seen when examining the effect of temperature on both treatment groups combined in wild populations (Figure 5.2). However, when there was no environmental mismatch, temperature did not affect offspring sex ratio (Figure 5.2).

5.5 Discussion

The transfer of red wolves between captive and wild environments involved in captive breeding and reintroduction creates a mismatch between prenatal condition, under which animals develop physiologically, and postnatal conditions, under which they reproduce. This mismatch may impose constraints on their ability to respond to the current local conditions and to adjust offspring sex ratios accordingly. These constraints could explain some of the unexpected sex ratios observed in reintroduced populations following release.

In the captive and reintroduced populations of red wolves, environmentally mismatched individuals did not produce offspring with sex ratios different from those with matched pre- and postnatal environments. When captive and wild populations are examined together, both release and capture create a mismatch. These two transfer types lead to mismatches in opposite directions. In other words, the environmental changes experienced by a captured individual, are opposite to those experienced by a released individual. These opposite mismatches may have had opposite effects on offspring sex ratios, resulting in the overall effect being obscured. However, this does not appear to be the case here. I conducted the same analysis for just wild populations of red wolves. In this, mismatched environments occurred only when captive born individuals were released to the wild. In just wild populations, mismatch between pre- and postnatal environments did not alter offspring sex ratios. This result leads to the rejection of hypothesis 1.

While mismatched pre- and postnatal environments did not affect population sex ratios as a whole, it did appear to influence the relationship between some predictors of sex ratios and sex ratios. Maternal age had no effect on offspring sex ratios in these populations of red wolves. Similarly, rainfall around the time of conception had no effect. However, both sire age and temperature around the time of conception influenced offspring sex ratios, and this relationship was altered by a mismatch between pre- and postnatal environments.

When all populations of red wolves (captive and wild) were analysed together, sire age had a significant effect on offspring sex ratios (Table 5.1). As sire age increased, offspring sex ratios tended towards female bias (Figure 5.1). It may be that as males age, their condition declines. As wolves live in packs led by an alpha male and female, a decline in condition to the alpha male may be coupled with a pack wide decline in resource availability and condition. This would result in decreased ability to invest in offspring and, in accordance with the Trivers-Willard hypothesis (Trivers and Willard 1973), a female biased offspring sex ratio. However, as red wolves are monogamous breeders their life history traits do not match the assumptions of the Trivers-Willard model (Sidorovich, et al. 2007). Additionally, previous studies on offspring sex ratios in wolves do not support this relationship (Mech 1975; Packard and Mech 19880). Therefore, the explanation for this relationship is still unclear.

In this study, the effect of environmental mismatch on the relationship between sire age and sex ratio is of more important than the relationship itself. When pre- and postnatal environments were matched, sire age had a significant effect on offspring sex ratio. However, when environments were mismatched, this relationship was not present (Figure 5.1). This result is in line with hypothesis 2. In just wild populations, the relationship between sire age and sex ratios was not significant. However, when graphed, the relationship, and the effect of mismatched environments on this relationship appears the same as when captive and wild populations were analysed together (Figure 5.1).

Development in an environment that does not match the environment experienced as reproducing adults may have resulted in physiological traits that are unsuited to respond to local conditions. Therefore, mismatched individuals translate the effects of sire age to the mechanisms of sex allocation differently to matched individuals. As a result, they are unable to adjust offspring sex ratio in response to sire age.

Temperature around the time of conception also had a significant effect on offspring sex ratios (Tables 5.2). As temperature increased, offspring sex ratios tended towards male bias. Again, this relationship is difficult to explain. Extreme cold temperatures may create a stressful environment, and resource availability may increase as temperature increases. If so, this relationship would be in line with the Trivers-Willard hypothesis. However, as red wolves do not fit the assumption of this model (Sidorovich, et al. 2007), and previous research has suggested that wolf offspring sex ratios skew towards males under stress (McGinley 1984), the explanation for this relationship remains unclear.

When pre- and postnatal environments were mismatched, the same relationship between temperature and sex ratio was observed (Figure 5.2). However, when environments were matched, temperature did not have a significant effect on offspring sex ratio. This was consistent for the analysis of captive and wild populations together, and just wild population. This result is not in line with hypothesis 2, as the relationship was present in mismatched individuals but not matched individuals. However, it does support the theory that mismatch between pre- and postnatal environments can alter the way in which animals adjust offspring sex ratios in response to predictors. The prenatal conditions experienced by mismatched individuals may have resulted in the development of physiological traits that led to an exaggerated relationship between temperature and sex ratios following transfer between captivity and the wild.

The adaptive significance of the relationship between sex ratios and sire age and temperature was not clear. However, the purpose of this study was not to explain these effects, but to examine the effect of mismatched environments on those relationships. Results presented here support the hypothesis that mismatch between pre- and postnatal environments associated with species reintroductions can alter the relationship between sex ratios and their predictors. This may be through physiological constraints to the mechanisms of sex allocation, imposed by anticipatory maternal effects under changing environmental conditions (Edwards, et al. 2016a). While the results presented here support this hypothesis, they do not provide conclusive empirical evidence. The predictors of sex allocation used were restricted to the available data, and may not be the most important factors for sex allocation in red wolves. Cameron (2004) highlighted the flaw in analysing sex allocation retrospectively with datasets collected for alternative purposes. Additionally, the climate conditions used here were measured at relatively low resolution (state-wide). This nullifies variations that may occur between and within populations in a single state. It would be beneficial to repeat this study using well-established predictors of sex ratio, measured at the individual level.

Despite the shortcomings of this study, it does provide evidence that sex allocation is constrained by physiological development in a mismatched environment. Verifying this relationship could have significant implications for conservation. This could be taken into consideration in future reintroduction programmes, and efforts can be made to mitigate its effect. Preventing sex ratio skews in reintroduced populations may improve the success rate of these programmes and may aid in the preservation of threatened species.

6 General Conclusions, Conservation Implications and Future Research

6.1 General Conclusions

Species reintroductions are becoming increasingly used as a conservation strategy to preserves threatened species and restore natural ecosystems (Seddon, et al. 2007). However, despite our growing understanding of how to carry out these programmes, a large portion still fail, and often for unknown reasons (Fischer and Lindenmayer 2000; Jule, et al. 2008; Sutton and Lopez 2014). A common trend following release is that reintroduced populations produce offspring with unexpected sex ratios (Milton and Hopkins 2006; Saltz and Rubenstein 1995; Jiang, et al. 2000; Law, et al. 2014; Dunham 2001; Ewen, et al. 2010). Skewed sex ratios can limit population growth and recovery (Wedekind 2012; Ginsberg and Milner-Gulland 1994; Sæther, et al. 2003), and may contribute to the high rate of failure in these programmes. This study provides the first step in testing, and supporting, the hypothesis that the mismatch between prenatal and postnatal environments experienced by reintroduced animals imposes physiological constraints that limit their ability to adjust offspring sex ratios adaptively.

Sex allocation operates through physiological mechanisms that translate current local conditions into the selective production of sons or daughters (Cameron 2004; Navara 2010; Grant 2007). However, as physiology is variable, some individuals may respond to the local condition differently to others, and subsequently adjust offspring sex ratio differently (Edwards, et al. 2016a). One of the greatest non-genetic sources of this variation is the environmental condition experienced during development, particularly in utero (Bernardo 1996; Lane, et al. 2014b). When animals develop under conditions that differ from their environment as adults, their developmental experience can influence physiological characteristics (Edwards, et al. 2016a). This can result in an inability to mount appropriate physiological responses to the current local conditions, which can in turn influence the mechanisms of sex allocation, thereby limiting their ability to adjust offspring sex ratios adaptively (Edwards, et al. 2016a).

In this study, I show that the mismatch between pre- and postnatal environments experienced when captive born animals are reintroduced to the wild alters the relationship between offspring sex ratios and several predictors of sex allocation in three species, the Arabian oryx (Chapter 3), the California condor (Chapter 4), and the red wolf (Chapter 5). In Arabian oryx and red wolves, offspring sex ratios did not differ between individuals with mismatched environments and those with matched environments. However, in California condors, captive born individuals that reproduced in the wild (mismatched environments) produced a significantly greater proportion of male offspring than wild born individuals (matched environments).

Additionally, in each species, a mismatch between pre- and postnatal environments changed the relationship between offspring sex ratios and at least one other predictor of sex allocation, despite these predictors being relatively loose proxies for more well-established predictors like body condition or ability to invest (Trivers and Willard 1973).

In Arabian oryx, the relationships that offspring sex ratios had with rainfall, parental parity in the previous year, and dam age (only in wild populations) were affected by mismatch. In California condors, the relationships that offspring sex ratios had with temperature around the time of conception, parental parity and parental age were all affected by mismatch. In red wolves, mismatch had an effect on the relationships that offspring sex ratios had with temperature around the time of conception, and sire age. Which variable would interact with mismatched environments was not predictable. However, in each species, the predicted response that some factors would interact with mismatch in their effect on offspring sex ratios was observed. These results suggest that the developmental experience of mismatched individuals resulted in physiological differences that altered the way in which they respond to local conditions, thereby constraining sex allocation.

Each of these species has unique life history traits that showcase the diversity of species types that may be constrained in their abiliity to adjust offspring sex ratios by anticipatory maternal effects in a mismatched environment (Price 1989; Synder and Synder 2000; Sparkman, et al. 2011). Arabian oryx are polygynous ungulates, which are expected to carry out condition-dependent sex allocation. California condors and red wolves on the other hand, are monogamous breeders and are less likely to conform to the hypotheses of sex allocation. Additionally, in California condors, females are the heterogametic sex and therefore are the sole determinants of offspring sex ratios through chromosomes, as well as through hormonal influences (Smith 2007; Navara 2013b). Despite these differences, mismatched pre- and postnatal environments in all three species influenced the relationship between sex ratios and various predictors. However, in each species, different predictors interacted with mismatched environments in their effect on offspring sex ratios. This highlights the importance in understanding species-specific details about sex allocation processes and mechanisms.

6.2 Conservation Implications

In order to minimise the occurrence and impacts of skewed sex ratios in reintroduced populations, we first need to understand the mechanisms driving these trends. This study provides the first step to understanding how the mismatch between pre- and postnatal environments experienced by reintroduced animals can contribute to the unexpected sex ratio skews that have been observed. While further research is required, these results can aid in making predictions of, and controlling offspring sex ratios in, reintroduced populations. In doing so, management of populations and mitigation of undesirable sex ratios can be achieved more easily.

Species reintroductions generally involve multiple releases of captive-born animals to the wild. If, in the initial releases, offspring sex ratios are unexpectedly skewed, actions can be taken to correct that trend in the future. Captive breeding facilities could take greater measures to recreate conditions that match the wild environment. Releases could take a more gradual approach to the transition from captivity to the wild. Alternatively, sex ratios of future release groups could be adjusted to account for future skews that are expected to occur.

As well as conservation implications, this study could have implications for future research testing the hypotheses of sex allocation (Edwards, et al 2019). It is generally assumed that all individuals within a species have an equal aptitude for adjustment of offspring sex ratios (Edwards, et al. 2016a). However, I show here that, when physiological development occurs under mismatched conditions, sex allocation can be constrained. This is consistent with findings from Edwards et al. (2019). Physiological constraints caused by mismatched environments may have contributed to the inconsistency in support for the hypotheses of sex allocation, and should be considered before making conclusions in the future.

6.3 Future Research Directions

While this work does provide support for the hypothesis that mismatched environments impose physiological constraints on sex allocation in reintroduced populations, further research is required to fully understand this effect and the extent to which it operates. This study examined past reintroduction events, and therefore it was limited by the availability of data. As a result, more relevant and, perhaps, precise predictors of sex allocation were unobtainable (for example, body score index or dominance rank; Pike and Petrie 2005; Grant 1996). Future work should be conducted on an ongoing species reintroduction programme. Ideally, sex allocation would be well understood in the focus species, with recognised and supported predictors available. This study could then be repeated, looking at the effect of mismatched environments on the relationship between sex allocation and a more direct predictor of offspring sex ratios, measured at the individual level. Additionally, a more detailed understanding of the specific differences in environmental conditions between captivity and the wild could help the management of sex ratios in future reintroduced populations.

7 References

Alroy, J. (2015). Current extinction rates of reptiles and amphibians. *Proceedings of the National Academy of Sciences of the United States of America, 112* (42), 13003-13008.

Armstrong, D. P., & Seddon, P. J. (2008). Directions in reintroduction biology. *Trends in Ecology and Evolution, 23* (1), 20-25.

Armstrong, D. P., Moro, D., Hayward, M. W., & Seddon, P. J. (2015). Introduction: the development of reintroduction biology in New Zealand and Australia. In D. P. Armstrong, M. W. Hayward, D. Moro, & P. J. Seddon (Eds.), *Advances in Reintroduction Biology of Australian and New Zealand Fauna* (pp. 1-6).

Arnold, A. P. (2012). The end of gonad-centric sex determination in mammals. *Trends in Genetics, 28* (2), 55-61.

Asa, C. S., Traylor-Holzer, K., & Lacy, R. C. (2010). Can conservation-breeding programmes be improved by incorporating mate choice? *International Zoo Yearbook, 45* (1), 203-212.

Ballou, J. D. (1993). Assessing the Risks of Infectious Diseases in Captive Breeding and Reintroduction Programs. *Journal of Zoo and Wildlife Medicine, 24* (3), 327-335.

Balmford, A., Mace, G. M., & Leader-Williams, N. (1996). Designing the Ark: Setting Priorities for Captive Breeding. *Conservation Biology, 10* (3), 719-727.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O., Swartz, B., Quental, T. B., et al. (2011). Has the Earth's sixth mass extinction already arrived? *Nature, 471*, 51-57.

Bassett, L., & Buchanan-Smith, H. M. (2007). Effects of predictability on the welfare of captive animals. *Applied Animal Behaviour Science, 102*, 223-245.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H., Singmann, H., et al. (2020). lme4: Linear Mixed-Effects Models using 'Eigen' and S4. *R package version 1.1-23*, CRAN.

Bennett, V. A., Doerr, A. A., Doerr, E. D., Manning, A. D., Lindenmayer, D. B., & Yoon, H.-J. (2013). Causes of reintroduction failure of the brown treecreeper: Implications for ecosystem restoration. *Austral Ecology*, *38* (6), 700-712.

Berkeley, E. V., & Linklater, W. L. (2010). Annual and Seasonal Rainfall May Influence Progeny Sex Ratio in the Black Rhinoceros. *South African Journal of Wildlife Research*, *40* (1), 53–57.

Berletch, J. B., Yang, F., Carrel, L., & Disteche, C. M. (2011). Genes that escape from X inactivation. *Human Genetics*, *130* (2), 237–245.

Bernardo, J. (1996). Maternal Effects in Animal Ecology. *American Zoologist*, *36* (2), 83–105.

BirdLife International. (2018). *Gymnogyps californianus. The IUCN Red List of Threatened Species.* IUCN.

Birk, M. A. (2019). measurements: Tools for Units of Measurement. *R package version 1.4.0*, CRAN.

Blank, J. L., & Nolan, V. (1983). Offspring Sex Ratio in Red-Winged Blackbirds is Dependent on Maternal Age. *Proceedings of the National Academy of Sciences of the United States of America*, *80* (19), 6141-6145.

Bonier, F., Martin, P. R., & Wingfield, J. C. (2007). Maternal corticosteroids influence primary offspring sex ratio in a free-ranging passerine bird. *Behavioral Ecology*, *18* (6), 1045–1050.

Boue, F., Delhomme, A., & Chaffaux, S. (2000). Reproductive management of silver foxes (Vulpes vulpes) in captivity. *Theriogenology*, *53* (9), 1717-1728.

Bradbury, R. B., & Blakey, J. K. (1998). Diet, Maternal Condition, and Offspring Sex Ratio in the Zebra Finch, Poephila guttata. *Proceedings: Biological Sciences*, *265* (1399), 895-899.

Brashares, J. S., Arcese, P., & Sam, M. K. (2001). Human demography and reserve size predict wildlife extinction in West Africa. *Proceedings of the Royal Society B: Biological Sciences*, *268*, 2473–2478.

Bremner-Harrison, S., Prodohl, P. A., & Elwood, R. W. (2004). Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (Vulpes velox). *Animal Conservation forum*, *7* (3), 313-320.

Bruns, C. M., Baum, S. T., Colman, R. J., Eisner, J. R., Kemnitz, J. W., Weindruch, R., et al. (2004). Insulin Resistance and Impaired Insulin Secretion in Prenatally Androgenized Male Rhesus Monkeys. *The Journal of Clinical Endocrinology & Metabolism*, *89* (12), 6218–6223.

Brunton, P. J. (2010). Resetting the Dynamic Range of Hypothalamic-Pituitary-Adrenal Axis Stress Responses Through Pregnancy. *Journal of Neuroendocrinology*, *22* (11), 1198-1213.

Bull, J. J., & Charnov, E. (1988). How fundamental are Fisherian sex ratios? *Oxford Surveys in Evolutionary Eiology*, *5*, 96-135.

Bullock, J. M., Hodder, K. H., Manchester, S. J., & Stevenson, M. J. (1996). *Review of information, policy and legislation on species translocation.* Joint Nature Conservation Committee.

Burén, J., Liu, H. X., Jensen, J., & Eriksson, J. W. (2002). Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *European Journal of Endocrinology*, *146* (3), 419–429.

Burkhead, N. M. (2012). Extinction Rates in North American Freshwater Fishes, 1900–2010. *BioScience*, *62* (9), 798–808.

Caballero, A. (1994). Developments in the prediction of effective population size. *Heredity*, *73*, 657–679.

Cabezas, S., Carrete, M., Tella, J. L., Marchant, T. A., & Bortolotti, G. R. (2013). Differences in acute stress responses between wild-caught and captive-bred birds: a physiological mechanism contributing to current avian invasions? *Biological Invasions*, *15*, 521–527.

Cameron, E. Z. (2004). Facultative adjustment of mammalian sex ratios in support of the Trivers–Willard hypothesis: evidence for a mechanism. *Proceedings of the Royal Society B: Biological Sciences*, *271*, 1723–1728.

Cameron, E. Z., & Linklater, W. L. (2007). Extreme sex ratio variation in relation to change in condition around conception. *Biology Letters*, *3*, 395–397.

Cameron, E. Z., Edwards, A. M., & Parsley, L. M. (2017). Developmental sexual dimorphism and the evolution of mechanisms for adjustment of sex ratios in mammals. *Annals of the New York Academy of Sciences*, *1389* (1), 147-163.

Cameron, E. Z., Lemons, P. R., Bateman, P. W., & Bennett, N. C. (2008). Experimental alteration of litter sex ratios in a mammal. *Proceedings: Biological Sciences*, *275* (1632), 323-327.

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., & Palmer, T. M. (2015). Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances*, *1* (5), e1400253.

Ceballos, G., García, A., & Ehrlich, P. R. (2010). The Sixth Extinction Crisis Loss of Animal Populations and Species. *Journal of Cosmology*, *8*, 1821-1831.

Champagnon, J., Guillemain, M., Elmberg, J., Massez, G., Cavallo, F., & Gauthier-Clerc, M. (2012). Low survival after release into the wild: assessing "the burden of captivity" on Mallard physiology and behaviour. *European Journal of Wildlife Research*, *58*, 255–267.

Clark, A. B. (1978). Sex Ratio and Local Resource Competition in a Prosimian Primate. *Science*, *201* (4351), 163-165.

Clark, M. M., & Galef, B. G. (1995). A gerbil dam's fetal intrauterine position affects the sex ratios of litters she gestates. *Physiology & Behavior*, *57* (2), 297-299.

Clark, M. M., vom Saal, F. S., & Galef, B. G. (1992). Intrauterine positions and testosterone levels of adult male gerbils are correlated. *Physiology & Behavior*, *51* (5), 957-960.

Clutton-Brock, T. H., & Iason, G. R. (1986). Sex Ratio Variation in Mammals. *The Quarterly Review of Biology*, *61* (3), 339-374.

Clutton-Brock, T. H., Albon, S. D., & Guinness, F. E. (1984). Maternal dominance, breeding success and birth sex ratios in red deer. *Nature*, *308*, 358–360.

Cochran-Biederman, J. L., Wyman, K. E., French, W. E., & Loppnow, G. L. (2015). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, *29* (1), 175-186.

Cockburn, A., Legge, S., & Double, M. C. (2002). Sex ratios inbirds and mammals: can the hypotheses be disentangled? In I. C. Hardy (Ed.), *Sex Ratios: Concepts and Research Methods* (pp. 266-286). Cambridge: Cambridge University Press.

Correa, S. M., Horan, C. M., Johnson, P. A., & Adkins-Regan, E. (2011). Copulatory behaviors and body condition predict post-mating female hormone concentrations, fertilization success, and primary sex ratios in Japanese quail. *Hormones and Behavior*, *59* (4), 556-564.

Cumming, D. C., Quigley, M. E., & Yen, S. S. (1983). Acute Suppression of Circulating Testosterone Levels by Cortisol in Men. *The Journal of Clinical Endocrinology & Metabolism*, *57* (3), 671–673.

Côté, S. D., & Festa-Bianchet, M. (2001). Offspring Sex Ratio in Relation to Maternal Age and Social Rank in Mountain Goats (Oreamnos americanus). *Behavioral Ecology and Sociobiology*, *49* (4), 260-265.

Daly, W., Seegers, C. A., Rubin, D. A., Dobridge, J. D., & Hackney, A. C. (2005). Relationship between stress hormones and testosterone with prolonged endurance exercise. *European Journal of Applied Physiology*, *93*, 375–380.

Deredec, A., & Courchamp, F. (2007). Importance of the Allee effect for reintroduction. *Ecoscience*, *14* (4), 440-451.

Desai, M., Jellyman, J. K., & Ross, M. G. (2015). Epigenomics, gestational programming and risk of metabolic syndrome. *International Journal of Obesity*, *39*, 633–641.

Dierenfeld, E. S. (1997). Captive wild animal nutrition: a historical perspective. *Proceedings of the Nutrition Society*, *56* (3), 989-999.

Douhard, M. (2017). Offspring sex ratio in mammals and the Trivers-Willard hypothesis: In pursuit of unambiguous evidence. *Bioessays*, *39* (9), 1700043.

Doutrelant, C., Covas, R., Caizergues, A., & du Plessis, M. (2004). Unexpected sex ratio adjustment in a colonial cooperative bird: pairs with helpers produce more of the helping sex whereas pairs without helpers do not. *Behavioral Ecology and Sociobiology*, *56*, 149-154.

Dowling, D. K., & Mulder, R. A. (2006). Combined influence of maternal and paternal quality on sex allocation in red-capped robins. *Journal of Evolutionary Biology*, *19* (2), 440-449.

Dunham, K. M. (2001). Status of a reintroduced population of mountain gazelles Gazella gazella in central Arabia: management lessons from an aridland reintroduction. *Oryx*, *35* (2), 111-118.

Dunn, E., Kapoor, A., Leen, J., & Matthews, S. G. (2010). Prenatal synthetic glucocorticoid exposure alters hypothalamic–pituitary–adrenal regulation and pregnancy outcomes in mature female guinea pigs. *The Journal of Physiology, 588* (5), 887-899.

Dyar, J. A., & Wagner, J. (2003). Uncertainty and species recovery program design. *Journal of Environmental Economics and Management*, *42* (2), 505-522.

Edwards, A. M., Cameron, E. Z., & Wapstra, E. (2016a). Are there physiological constraints on maternal ability to adjust sex ratios in mammals? *Journal of Zoology*, *299* (1), 1-9.

Edwards, A. M., Cameron, E. Z., Wapstra, E., & McEvoy, J. (2019). Maternal effects obscure condition-dependent sex allocation in changing environments. *Royal Society Open Science*, *6*, 181885.

Edwards, A. M., Cameron, E. Z., Wapstra, E., Ferguson-Smith, M. A., Horton, S. R., & Thomasson, K. (2016b). Gestational experience alters sex allocation in the subsequent generation. *Royal Society Open Science*, *3*, 160210.

Eisner, J. R., Dumesic, D. A., Kemnitz, J. W., & Abbott, D. H. (2000). Timing of Prenatal Androgen Excess Determines Differential Impairment in Insulin Secretion and Action in Adult Female Rhesus Monkeys. *he Journal of Clinical Endocrinology & Metabolism*, *85* (3), 1206–1210.

Ellegren, H., Gustafsson, L., & Sheldon, B. C. (1996). Sex Ratio Adjustment in Relation to Paternal Attractiveness in a Wild Bird Population. *Proceedings of the National Academy of Sciences of the United States of America*, *93* (21), 11723-11728.

Emlen, S. T., Emlen, J. M., & Levin, S. A. (1986). Sex-Ratio Selection in Species with Helpers-At-The-Nest. *The American Naturalist*, *127* (1), 1-8.

Entringer, S., Epel, E. S., Kumsta, R., Lin, J., Hellhammer, D. H., Blackburn, E. H., et al. (2011). Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *108* (33), E513–E518.

Entringer, S., Kumsta, R., Hellhammer, D. H., Wadhwa, P. D., & Wüst, S. (2009). Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Hormones and Behavior*, *55* (2), 292-298.

Ewen, J. G., Thorogood, R., & Armstrong, D. P. (2010). Demographic consequences of adult sex ratio in a reintroduced hihi population. *Journal of Animal Ecology*, *80* (2), 448-455.

Ferrer, M., Newton, I., & Pandolfi, M. (2009). Small Populations and Offspring Sex-Ratio Deviations in Eagles. *Conservation Biology*, *23* (4), 1017-1025.

Festa-Bianchet, M. (1996). Offspring sex ratio studies of mammals: Does publication depend upon the quality of the research or the direction of the results? *Écoscience*, *3* (1), 42-44.

Ficken, M. S., Weise, C. M., & Popp, J. W. (1990). Dominance Rank and Resource Access in Winter Flocks of Black-Capped Chickadees. *The Wilson Bulletin*, *102* (4), 623-633.

Finkelstein, M. E., Doak, D. F., George, D., Burnet, J., Brandt, J., Church, M., et al. (2012). Lead poisoning and the deceptive recovery of the critically endangered California condor. *Proceedings of the National Academy of Sciences of the United States of America*, *109* (28), 11449-11454.

Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological Conservation*, *96* (1), 1-11.

Fisher, R. A. (1930). *The genetical theory of natural selection.* Oxford: Clarendon Press.

François, R., Henry, L., & Müller, K. (2020). dplyr: A Grammar of Data Manipulation. *R package version 0.8.5*, CRAN.

Frank, S. A. (1990). Sex Allocation Theory for Birds and Mammals. *Annual Review of Ecology and Systematics*, *21*, 13-55.

Gardner, D. K., Larman, M. G., & Thouas, G. A. (2010). Sex-related physiology of the preimplantation embryo. *Molecular Human Reproduction*, *16* (8), 539–547.

Gibbs, J. P., Marquez, C., & Sterling, E. J. (2008). The Role of Endangered Species Reintroduction in Ecosystem Restoration: Tortoise–Cactus Interactions on Española Island, Galápagos. *Restoration Ecology*, *16* (1), 88-93.

Ginsberg, J. R., & Milner-Gulland, E. J. (1994). Sex-Biased Harvesting and Population Dynamics in Ungulates: Implications for Conservation and Sustainable Use. *Conservation Biology*, *8* (1), 157-166.

Gluckman, P. D., & Hanson, M. A. (2004). The developmental origins of the metabolic syndrome. *Trends in Endocrinology and Metabolism*, *15* (4), 183-187.

Goerlich-Jansson, V. C., Müller, M. S., & Groothuis, T. G. (2013). Manipulation of Primary Sex Ratio in Birds: Lessons from the Homing Pigeon (Columba livia domestica). *Integrative and Comparative Biology*, *53* (6), 902-912.

Goldstein, R. E., Wasserman, D. H., McGuinness, O. P., Lacy, D. B., Cherrington, A. D., & Abumrad, N. N. (1993). Effects of chronic elevation in plasma cortisol on hepatic carbohydrate metabolism. *The American Journal of Physiology Endocrinology and Metabolism*, *264* (1), E119-E127.

Goodwin, I. (2013). *International studbook for Arabian Oryx, Oryx leucoryx, 2013 Edition.* Marwell Wildlife.

Gorman, H. E., & Nager, R. G. (2004). Prenatal developmental conditions have long–term effects on offspring fecundity. *Proceedings: Biological Sciences*, *271* (1551), 1923-1928.

Gowaty, P. A. (1993). Differential Dispersal, Local Resource Competition, and Sex Ratio Variation in Birds. *The American Naturalist*, *141* (2), 263-280.

Gowaty, P. A., & Lennartz, M. R. (1985). Sex Ratios of Nestling and Fledgling Red-Cockaded Woodpeckers (Picoides borealis) Favor Males. *The American Naturalist*, *126* (3), 347-353.

Grant, V. J. (2007). Could maternal testosterone levels govern mammalian sex ratio deviations? *Journal of Theoretical Biology*, *246* (4), 708-719.

Grant, V. J. (1996). Sex determination and the maternal dominance hypothesis. *Human Reproduction*, *11* (11), 2371–2375.

Grant, V. J., & Irwin, R. J. (2005). Follicular fluid steroid levels and subsequent sex of bovine embryos. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *303A* (12), 1120-1125.

Grant, V. J., Irwin, R. J., Standley, N. T., Shelling, A. N., & Chamley, L. W. (2008). Sex of Bovine Embryos May Be Related to Mothers' Preovulatory Follicular Testosterone. *Biology of Reproduction*, *78* (5), 812–815.

Grant, V. J., Konečná, M., Sonnweber, R.-S., Irwin, R. J., & Wallner, B. (2011). Macaque mothers' preconception testosterone levels relate to dominance and to sex of offspring. *Animal Behaviour*, *82* (4), 893-899.

Gray, C., Long, S., Green, C., Gardiner, S. M., Craigon, J., & Gardner, D. S. (2013). Maternal Fructose and/or Salt Intake and Reproductive Outcome in the Rat: Effects on Growth, Fertility, Sex Ratio, and Birth Order. *Biology of Reproduction*, *89* (3), 51, 1-8.

Grey-Ross, R., Downs, C. T., & Kirkman, K. (2009). Reintroduction Failure of Captive-Bred Oribi (Ourebia ourebi). *African Journal of Wildlife Research*, *39* (1), 34-38.

Griffin, A. S., Sheldon, B. C., & West, S. A. (2005). Cooperative Breeders Adjust Offspring Sex Ratios to Produce Helpful Helpers. *The American Naturalist*, *166* (5), 628-632.

Griffith, B., Michael Scott, J., Carpenter, J. W., & Reed, C. (1989). Translocation as a Species Conservation Tool: Status and Strategy. *Science*, *245* (4917), 477-480.

Groothuis, T. G., & Schwabl, H. (2007). Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? *Philosophical Transactions of the Royal Society B*, *363* (1497), 1647–1661.

Groothuis, T. G., Müller, W., Engelhardt, N. V., Carere, C., & Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neuroscience & Biobehavioral Reviews*, *29* (2), 329-352.

Gusset, M. (2012). Species reintroduction. In R. K. Craig, J. C. Nagle, B. Pardy, O. J. Schmitz, & W. K. Smith (Eds.), *Berkshire Encyclopedia of Sustainability, Volume 5: Ecosystem Management and Sustainability* (pp. 371-376). Berkshire Publishing.

Gutiérrez-Adán, A., Granados, J., Pintado, B., & De La Fuente, J. (2001). Influence of glucose on the sex ratio of bovine IVM/IVF embryos cultured in vitro. *Reproduction, Fertility and Development*, *13* (6), 361-365.

Hamilton, W. D. (1967). Extraordinary Sex Ratios. *Science*, *156* (3774), 477-488.

Hardman, B., Moro, D., & Calver, M. (2016). Direct evidence implicates feral cat predation as the primary cause of failure of a mammal reintroduction programme. *Ecological Management and Restoration*, *17* (2), 152-158.

Hardy, I. C. (1997). Possible factors influencing vertebrate sex ratios: an introductory overview. *Applied Animal Behaviour Science*, *51*, 217-241.

Haskins, K. E. (2015). Alternative perspectives on reintroduction success. *Animal Conservation*, *18* (5), 409-410.

Hayward, M. W., Adendorff, J., O'Brien, J., Sholto-Douglas, A., Bissett, C., Moolman, L. C., et al. (2007). Practical Considerations for the Reintroduction of Large, Terrestrial, Mammalian Predators Based on Reintroductions to South Africa's Eastern Cape Province. *The Open Conservation Biology Journal*, *1*, 1-11.

Heard, E., Clerc, P., & Avner, P. (1997). X-chromosome inactivation in mammals. *Annual Review of Genetics*, *31*, 571-610.

Hedrick, P. W., & Fredrickson, R. J. (2008). Captive breeding and the reintroduction of Mexican and red wolves. *Molecular Ecology*, *17* (1), 344-350.

Helle, S., Laaksonen, T., Adamsson, A., Paranko, J., & Huitu, O. (2008). Female field voles with high testosterone and glucose levels produce male-biased litters. *Animal Behaviour*, *75* (3), 1031-1039.

Hewison, A. J., & Gaillard, J. M. (1996). Birth-sex ratios and local resource competition in roe deer, Capreolus capreolus. *Behavioral Ecology*, *7* (4), 461–464.

Hewison, A. J., & Gaillard, J.-M. (1999). Successful sons or advantaged daughters? The Trivers–Willard model and sex-biased maternal investment in ungulates. *Trends in Ecology and Evolution*, *14* (6), 229-234.

Hewison, A. J., Gaillard, J.-M., Blanchard, P., & Festa-Bianchet, M. (2002). Maternal Age Is Not a Predominant Determinant of Progeny Sex Ratio Variation in Ungulates. *Oikos*, *98* (2), 334-339.

Hiraiwa-Hasegawa, M. (1993). Skewed birth sex ratios in primates: Should high-ranking mothers have daughters or sons? *Trends in Ecology and Evolution*, *8* (11), 395-400.

Hirlemann, S., Spetz, J.-F., Haug, M., Brain, P. F., & Parmigiani, S. (1990). Prior intra-uterine position of lactating mice: Effects on attackability by adult resident females. *Italian Journal of Zoology*, *57* (1), 67-71.

Hjernquist, M. B., Thuman Hjernquist, K. A., Forsman, J. T., & Gustafsson, J. (2009). Sex allocation in response to local resource competition over breeding territories. *Behavioral Ecology*, *20* (2), 335–339.

Huck, U. W., Pratt, N. C., Labov, J. B., & Lisk, R. D. (1988). Effects of age and parity on litter size and offspring sex ratio in golden hamsters (Mesocricetus auratus). *Journal of Reproduction and Fertility*, *83* (1), 209-214.

Innes-Gold, A. A., Zuczek, N. Y., & Touchon, J. C. (2019). Right phenotype, wrong place: predator-induced plasticity is costly in a mismatched environment. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20192347.

Islam, M. Z., Ismail, K., & Boug, A. (2011). Restoration of the endangered Arabian Oryx Oryx leucoryx, Pallas 1766 in Saudi Arabia lessons learnt from the twenty years of re-introduction in arid fenced and unfenced protected areas. *Zoology in the Middle East*, *54* (3), 125-140.

IUCN. (2010). *Global Re-introduction Perspectives: 2010 Additional case-studies from around the globe.* (P. S. Soorae, Ed.)

IUCN. (2011). *Global Re-introduction Perspectives: 2011 More case studies from around the globe.* (P. S. Soorae, Ed.)

IUCN. (2013). *Global Re-introduction Perspectives: 2013 Further case-studies from around the globe.* (P. S. Soorae, Ed.)

IUCN. (2016). *Global Re-introduction Perspectives: 2016 Case-studies from around the globe.* (P. S. Soorae, Ed.)

IUCN. (2018). *Global Re-introduction Perspectives: 2018 Case studies from around the globe.* (P. S. Soorae, Ed.)

IUCN. (2008). *Global Re-introduction Perspectives: Re-introduction case-studies from around the globe.* (P. S. Soorae, Ed.)

IUCN SSC Antelope Specialist Group. (2017). *The IUCN Red List of Threatened Species e.T15569A50191626.*

IUCN Standards and Petitions Committee. (2019). *Guidelines for Using the IUCN Red List Categories and Criteria. Version 14.* IUCN.

James, W. H. (2008). Further evidence that some male-based neurodevelopmental disorders are associated with high intrauterine testosterone concentrations. *Developmental Medicine & Child Neurology*, *50* (1), 15-18.

Jamieson, I. G. (2011). Founder Effects, Inbreeding, and Loss of Genetic Diversity in Four Avian Reintroduction Programs. *Conservation Biology*, *25* (1), 115-123.

Jamieson, I. G., Tracy, L. N., Fletcher, D., & Armstrong, D. P. (2007). Moderate inbreeding depression in a reintroduced population of North Island robins. *Animal Conservation*, *10* (1), 95-102.

Jiang, Z., Yu, C., Feng, Z., Zhang, L., Xia, J., Ding, Y., et al. (2000). Reintroduction and Recovery of Père David's Deer in China. *Wildlife Society Bulletin*, *28* (3), 681-687.

Johnson, C. N., Clinchy, M., Taylor, A. C., Krebs, C. J., Jarman, P. J., Payne, A., et al. (2001). Adjustment of Offspring Sex Ratios in Relation to the Availability of Resources for Philopatric Offspring in the Common Brushtail Possum. *Proceedings: Biological Sciences*, *268* (1480), 2001-2005.

Jule, K. R., Leaver, L. A., & Lea, S. E. (2008). The effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological Conservation*, *14* (2), 355-363.

Kaiser, S., & Sachser, N. (2009). Effects of Prenatal Social Stress on Offspring Development: Pathology or Adaptation? *Current Directions in Psychological Science*, *18* (2), 118-121.

Kierulff, M. C., Ruiz-Miranda, C. R., Procópio de Oliveira, P., Beck, B. B., Martins, A., Dietz, J. M., et al. (2012). The Golden lion tamarin Leontopithecus rosalia: a conservation success story. *International Zoo Yearbook*, *46* (1), 36-45.

Kleiman, D. G. (1989). Reintroduction of Captive Mammals for Conservation. *BioScience*, *39* (3), 152-161.

Kleiman, D. G., Beck, B. B., Dietz, J. M., Dietz, L.-A., Ballou, J. D., & Coimbra-Filho, A. F. (1986). Conservation Program for the Golden Lion Tamarin: Captive Research and Management, Ecological Studies, Educational Strategies, and Reintroduction. In K. Benirschke (Ed.), *Primates* (pp. 959-979).

Kleiman, D. G., Price, M. R., & Beck, B. B. (1994). Criteria for reintroductions. In P. J. Olney, G. M. Mace, & A. T. Feistner (Eds.), *Creative Conservation* (pp. 287-303).

Kohlmann, S. G. (1999). Adaptive Fetal Sex Allocation in Elk: Evidence and Implications. *The Journal of Wildlife Management*, *63* (4), 1109-1117.

Kojola, I., & Eloranta, E. (1989). Influences of Maternal Body Weight, Age, and Parity on Sex Ratio in Semidomesticated Reindeer (Rangifer T. tarandus). *Evolution*, *43* (6), 1331-1336.

Komdeur, J., & Pen, I. (2002). Adaptive Sex Allocation in Birds: The Complexities of Linking Theory and Practice. *Philosophical Transactions: Biological Sciences*, *357* (1419), 373-380.

Krackow, S. (1995). Potential mechanisms for sex ratio adjustment in mammals and birds. *Biological Reviews of the Cambridge Philosophical Society*, *70* (2), 225-241.

Kraemer, S. (2000). The fragile male. *BMJ*, *321*, 1609.

Kuo, T., McQueen, A., Chen, T.-C., & Wang, J.-C. (2015). Regulation of Glucose Homeostasis by Glucocorticoids. In J.-C. Wang, & C. Harris (Eds.), *Glucocorticoid Signaling. Advances in Experimental Medicine and Biology* (Vol. 872, pp. 99-126). New York, NY: Springer.

Lüdecke, D., & Aust, F. (2020). ggeffects: Create Tidy Data Frames of Marginal Effects for 'ggplot' from Model Outputs. *R package version 0.14.3*, CRAN.

Lambertucci, S. A., Carrete, M., Speziale, K. L., Hiraldo, F., & Donázar, J. A. (2013). Population Sex Ratios: Another Consideration in the Reintroduction – Reinforcement Debate? *PLoS One*, *8* (9), e75821.

Lane, M., McPherson, N. O., Fullston, T., Spillane, M., Sandeman, L., Kang, W. X., et al. (2014b). Oxidative Stress in Mouse Sperm Impairs Embryo Development, Fetal Growth and Alters Adiposity and Glucose Regulation in Female Offspring. *PLoS One*, *9* (7), e100832.

Lane, M., Robker, R. L., & Robertson, S. A. (2014a). Parenting from before conception. *Science*, *345* (6198), 756-760.

Larson, M. A., Kimura, K., Kubisch, H. M., & Roberts, R. M. (2001). Sexual Dimorphism among Bovine Embryos in Their Ability to Make the Transition to Expanded Blastocyst and in the Expression of the Signaling Molecule IFN-τ. *Proceedings of the National Academy of Sciences of the United States of America*, *98* (17), 9677-9682.

Law, P. R., Fike, B., & Lent, P. C. (2014). Birth sex in an expanding black rhinoceros (Diceros bicornis minor) population. *Journal of Mammalogy*, *95* (2), 349–356.

Le Galliard, J.-F., Fitze, P. S., Ferrière, R., & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences of the United States of America*, *102* (50), 18231-18236.

Lens, L., Galbusera, P., Brooks, T., Waiyaki, E., & Schenck, T. (1998). Highly skewed sex ratios in the critically endangered Taita thrush as revealed by CHD genes. *Biodiversity and Conservation*, *7* (7), 869-873.

Leturque, H., & Rousset, F. (2004). Intersexual Competition as an Explanation for Sex-Ratio and Dispersal Biases in Polygynous Species. *Evolution*, *58* (11), 2398-2408.

Linklater, W. L. (2007). Translocation reverses birth sex ratio bias depending on its timing during gestation: evidence for the action of two sex-allocation mechanisms. *Reproduction, Fertility and Development*, *19* (7), 831-839.

Loehle, C., & Eschenbach, W. (2012). Historical bird and terrestrial mammal extinction rates and causes. *Diversity and Distributions*, *18* (1), 84-91.

Long, J. A. (2020). jtools: Analysis and Presentation of Social Scientific Data. *R package version 2.0.5*, CRAN.

Macdonald, D. W. (2009). Lessons Learnt and Plans Laid: Seven Awkward Questions for the Future of Reintroductions. In M. W. Hayward, & M. J. Somers (Eds.), *Reintroduction of Top-Order Predators* (pp. 411-448).

Mace, M. (2014). *California Condor North American Studbook: Gymnogyps californianus.* San Diego Zoo Global.

Mari, L., Gatto, M., & Casagrandi, R. (2008). Local resource competition and the skewness of the sex ratio: A demographic model. *Mathematical Biosciences and Engineering*, *5* (4), 813-830.

Marshall, D. J., & Uller, T. (2007). When Is a Maternal Effect Adaptive? *Oikos*, *116* (12), 1957-1963.

Martin, J. G., & Festa-Bianchet, M. (2011). Sex ratio bias and reproductive strategies: What sex to produce when? *Ecology*, *92* (2), 411-449.

McGinley, M. A. (1984). The Adaptive Value of Male-Biased Sex Ratios Among Stressed Animals. *The American Naturalist*, *124* (4), 597-599.

McNutt, J. W., & Silk, J. B. (2008). Pup production, sex ratios, and survivorship in African wild dogs, Lycaon pictus. *Behavioral Ecology and Sociobiology*, *62* (7), 1061-1067.

Meaney, M. J. (2001). Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. *Annual Review of Neuroscience*, *24*, 1161-1192.

Mech, L. D. (1975). Disproportionate Sex Ratios of Wolf Pups. *The Journal of Wildlife Management*, *39* (4), 737-740.

Meikle, D. B., Drickamer, L. C., Vessey, S. H., Rosenthal, T. L., & Fitzgerald, K. S. (1993). Maternal dominance rank and secondary sex ratio in domestic swine. *Animal Behaviour*, *46* (1), 79-85.

Merkling, T., Nakagawa, S., Lagisz, M., & Schwanz, L. E. (2018). Maternal Testosterone and Offspring Sex-Ratio in Birds and Mammals: A Meta-Analysis. *Evolutionary Biology*, *45* (1), 96–104.

Miller, S. M., Bissett, C., Burger, A., Courtenay, B., Dickerson, T., Druce, D. J., et al. (2013). Management of Reintroduced Lions in Small, Fenced Reserves in South Africa: An Assessment and Guidelines. *African Journal of Wildlife Research*, *43* (2), 38-154.

Milton, K., & Hopkins, M. E. (2006). Growth of a Reintroduced Spider Monkey (Ateles geoffroyi) Population on Barro Colorado Island, Panama. In A. Estrada, P. A. Garber, M. S. Pavelka, & L. Luecke (Eds.), *New Perspectives in the Study of Mesoamerican Primates: Concluding Comments and Conservation Priorities* (pp. 417-435).

Moore, E. P., Hayward, M., & Robert, K. A. (2015). High density, maternal condition, and stress are associated with male-biased sex allocation in a marsupial. *Journal of Mammalogy*, *96* (6), 1203–1213.

Moorhouse, T. P., Gelling, M., & Macdonald, D. W. (2009). Effects of habitat quality upon reintroduction success in water voles: Evidence from a replicated experiment. *Biological Conservation*, *142* (1), 53-60.

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, *102*, 262-302.

Moseby, K. E., Read, J. L., Paton, D., Copley, P., Hill, B. M., & Crisp, H. A. (2011). Predation determines the outcome of 10 reintroduction attempts in arid South Australia. *Biological Conservation*, *144* (12), 2863–2872.

Moseby, K., Carthey, A., & Schroeder, T. (2015). The influence of predators and prey naivety on reintroduction success: current and future directions. In D. Armstrong, M. Hayward, D. Moro, & P. J. Seddon (Eds.), *Advances in Reintroduction Biology of Australian and New Zealand Fauna* (pp. 29-42).

Moss, T. J., Sloboda, D. M., Gurrin, L. C., Harding, R., Challis, J. R., & Newnham, J. P. (2001). Programming effects in sheep of prenatal growth restriction and glucocorticoid exposure. *American Journal of physiology. Regulatory, Integrative and Comparative Physiology*, *281* (3), R960-70.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology and Evolution*, *13* (10), 403-407.

Muehlenbein, M. P., Watts, D. P., & Whitten, P. L. (2004). Dominance rank and fecal testosterone levels in adult male Chimpanzees (Pan troglodytes schweinfurthii) at Ngogo, Kibale National Park, Uganda. *American Journal of Primatology*, *64* (1), 71-82.

Navara, K. J. (2013a). Hormone-Mediated Adjustment of Sex Ratio in Vertebrates. *Integrative and Comparative Biology*, *53* (6), 877–887.

Navara, K. J. (2018c). Introduction to Vertebrate Sex Ratio Adjustment. In *Choosing Sexes. Fascinating Life Sciences* (pp. 1-11). Springer, Cham.

Navara, K. J. (2018b). Potential Mechanisms of Sex Ratio Adjustment in Birds. In *Choosing Sexes. Fascinating Life Sciences* (pp. 99-121). Springer, Cham.

Navara, K. J. (2018a). Potential Mechanisms of Sex Ratio Adjustment in Humans and Nonhuman Mammals. In *Choosing Sexes. Fascinating Life Sciences* (pp. 55-70). Springer, Cham.

Navara, K. J. (2010). Programming of offspring sex ratios by maternal stress in humans: assessment of physiological mechanisms using a comparative approach. *Journal of Comparative Physiology B*, *180*, 785–796.

Navara, K. J. (2018d). The Bees Do It, but What About the Birds? Evidence for Sex Ratio Adjustment in Birds. In *Choosing Sexes. Fascinating Life Sciences* (pp. 71-97). Springer, Cham.

Navara, K. J. (2013b). The Role of Steroid Hormones in the Adjustment of Primary Sex Ratio in Birds: Compiling the Pieces of the Puzzle. *Integrative and Comparative Biology*, *53* (6), 923–937.

Obregon, M.-J. (2010). Maternal Obesity Results in Offspring Prone to Metabolic Syndrome. *Endocrinology*, *151* (8), 3475–3476.

Osadchuk, L. V., Braastad, B. O., Huhtaniemi, I., & Bakken, M. (2000). Alterations of the pituitary - gonadal axis in the neonatal blue fox (Alopex lagopus) exposed to prenatal handling stress. *Reproduction, Fertility and Development*, *12* (4), 119-126.

Ostrowski, S., Bedin, E., Lenain, D. M., & Abuzinada, A. H. (1998). Ten years of Arabian oryx conservation breeding in Saudi Arabia – achievements and regional perspectives. *Oryx*, *32* (3), 209-222.

Packard, J. M., & Mech, L. D. (19880). Population Regulation in Wolves. In M. N. Cohen, R. S. Malpass, & H. G. Klein (Eds.), *Biosocial Mechanisms of Population Regulation* (pp. 135-150). Yale University Press.

Packer, C., Collins, D. A., & Eberly, L. E. (2000). Problems with primate sex ratios. *Philosophical Transactions of the Royal Society B*, *355*, 1627–1635.

Padmanabhan, V., Veiga-Lopez, A., Abbott, D. H., Recabarren, S. E., & Herkimer, C. (2010). Developmental Programming: Impact of Prenatal Testosterone Excess and Postnatal Weight Gain on Insulin Sensitivity Index and Transfer of Traits to Offspring of Overweight Females. *Endocrinology*, *151* (2), 595–605.

Peng, J., Jiang, Z., Qin, G., Huang, Q., Li, Y., Jiao, Z., et al. (2007). Impact of activity space on the reproductive behaviour of giant panda (Ailuropoda melanoleuca) in captivity. *Applied Animal Behaviour Science*, *104*, 151-161.

Pfannkuche, K. A., Gahr, M., Weites, I. M., Riedstra, B., Wolf, C., & Groothuis, T. G. (2011). Examining a pathway for hormone mediated maternal effects – Yolk testosterone affects androgen receptor expression and endogenous testosterone production in young chicks (Gallus gallus domesticus). *General and Comparative Endocrinology*, *172* (3), 487-493.

Phillips, M. K. (2018). *Canis rufus. The IUCN Red List of Threatened Species.* IUCN.

Phillips, M. K., Henry, V. G., & Kelly, B. T. (2003). Restoration of the Red Wolf. In L. D. Mech, & L. Boitani (Eds.), *Wolves: behavior, ecology, and conservation* (pp. 272-288). Chicago & London: University of Chicago Press.

Pike, T. W. (2005). Sex ratio manipulation in response to maternal condition in pigeons: evidence for pre-ovulatory follicle selection. *Behavioral Ecology and Sociobiology*, *58* (4), 407-413.

Pike, T. W., & Petrie, M. (2005). Maternal body condition and plasma hormones affect offspring sex ratio in peafowl. *Animal Behaviour*, *70* (4), 745-751.

Pimm, S., Raven, P., Peterson, A., Şekercioğlu, Ç. H., & Ehrlich, P. R. (2006). Human impacts on the rates of recent, present, and future bird extinctions. *Proceedings* *of the National Academy of Sciences of the United States of America*, *103* (29), 10941-10946.

Pinson, S. E., Wilson, J. L., & Navara, K. J. (2011). Elevated testosterone during meiotic segregation stimulates laying hens to produce more sons than daughters. *General and Comparative Endocrinology*, *174* (2), 195-201.

Pratt, N. C., & Lisk, R. D. (1989). Effects of social stress during early pregnancy on litter size and sex ratio in the golden hamster (Mesocricetus auratus). *Journal of Reproduction and Fertility*, *87* (2), 763-769.

Price, E. E., & Stoinski, T. S. (2007). Group size: Determinants in the wild and implications for the captive housing of wild mammals in zoos. *Applied Animal Behaviour Science*, *103*, 255-264.

Price, M. R. (1989). *Animal Re-introductions: the Arabian Oryx in Oman.* (G. Caughley, S. K. Eltringham, J. Harwood, D. Pimentel, & M. P. Sissenwine, Eds.)

Rangel-Negrín, A., Coyohua-Fuentes, A., Canales-Espinosa, D., Chavira-Ramírez, D. R., & Dias, P. A. (2018). Maternal glucocorticoid levels affect sex allocation in black howler monkeys. *Journal of Zoology*, *304* (2), 124-131.

Ravelli, A. C., van der Meulen, J. H., Osmond, C., Barker, D. J., & Bleker, O. P. (1999). Obesity at the age of 50 y in men and women exposed to famine prenatally. *The American Journal of Clinical Nutrition*, *70* (5), 811–816.

Reading, R. P., Clark, T. W., & Kellert, S. R. (2002). Towards an Endangered Species Reintroduction Paradigm. *Endangered Species Update*, *19* (4), 142-146.

Reading, R. P., Miller, B., & Shepherdson, D. (2013). The Value of Enrichment to Reintroduction Success. *Zoo Biology*, *32* (3), 332-341.

Recabarren, S. E., Padmanabhan, V., Codner, E., Lobos, A., Durán, C., Vidal, M., et al. (2005). Postnatal developmental consequences of altered insulin sensitivity in female sheep treated prenatally with testosterone. *American Journal of physiology. Endocrinology and Metabolism*, *289* (5), E801-E806.

Robert, A., Colas, B., Guigon, I., Kerbiriou, C., Mihoub, J.-B., Saint-Jalme, M., et al. (2015). Defining reintroduction success using IUCN criteria for threatened species: a demographic assessment. *Animal Conservation*, *18* (5), 397-406.

Robertson, B. C., Elliott, G. P., Eason, D. K., Clout, M. N., & Gemmell, N. J. (2006). Sex allocation theory aids species conservation. *Biology Letters*, *2* (2), 229–231.

Roche, J. R., Lee, J. M., & Berry, D. P. (2006). Climatic Factors and Secondary Sex Ratio in Dairy Cows. *Journal of Dairy Science*, *89* (8), 3221-3227.

Roussel, S., Boissy, A., Montigny, D., Hemsworth, P. H., & Duvaux-Ponter, C. (2005). Gender-specific effects of prenatal stress on emotional reactivity and stress physiology of goat kids. *Hormones and Behavior*, *47* (3), 256-266.

Rutberg, A. T. (1986). Lactation and Fetal Sex Ratios in American Bison. *The American Naturalist*, *127* (1), 89-94.

Rutkowska, J., & Cichoń, M. (2006). Maternal testosterone affects the primary sex ratio and offspring survival in zebra finches. *Animal Behaviour*, *71* (6), 1283-1288.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neuroscience & Biobehavioral Reviews*, *26* (6), 665-678.

Saad, A. F., Dickerson, J., Kechichian, T. B., Yin, H., Gamble, P., Salazar, A., et al. (2016). High-fructose diet in pregnancy leads to fetal programming of hypertension, insulin resistance, and obesity in adult offspring. *American Journal of Obstetrics and Gynecology*, *215* (3), 378.e1-378.e6.

Saino, N., Ambrosini, R., Martinelli, R., Calza, S., Møller, A. P., & Pilastro, A. (2002). Blackwell Science, Ltd Offspring sexual dimorphism and sex-allocation in relation to parental age and paternal ornamentation in the barn swallow. *Molecular Ecology*, *11* (8), 1533-1544.

Saltz, D. (2001). Progeny Sex Ratio Variation in Ungulates: Maternal Age Meets Environmental Perturbation of Demography. *Oikos*, *94* (2), 377-384.

Saltz, D., & Kotler, B. P. (2003). Maternal Age Is a Predominant Determinant of Progeny Sex Ratio Variation in Ungulates: A Reply to Hewison et al. *Oikos*, *101* (3), 646-648.

Saltz, D., & Rubenstein, D. I. (1995). Population Dynamics of a Reintroduced Asiatic Wild Ass (Equus Hemionus) Herd. *Ecological Applications*, *5* (2), 327-335.

Sankar, K., Qureshi, Q., Nigam, P., Malik, P. K., Sinha, P. R., Mehrotra, R. N., et al. (2010). Monitoring of Reintroduced Tigers in Sariska Tiger Reserve, Western India: Preliminary Findings on Home Range, Prey Selection and Food Habits. *Tropical Conservation Science*, *3* (3), 301-318.

Sapolsky, R. M. (1985). Stress-Induced Suppression of Testicular Function in the Wild Baboon: Role of Glucocorticoids. *Endocrinology*, *116* (6), 2273–2278.

Schwanz, L. E., & Robert, K. A. (2014). Proximate and ultimate explanations of mammalian sex allocation in a marsupial model. *Behavioral Ecology and Sociobiology*, *68* (7), 1085-1096.

Seckl, J. R. (2004). Prenatal glucocorticoids and long-term programming. *European Journal of Endocrinology*, *151* (3), U49-62.

Seddon, P. J. (1999). Persistence without intervention: assessing success in wildlife reintroductions. *Trends in Ecology & Evolution*, *14* (12), 503.

Seddon, P. J., Armstrong, D. P., & Maloney, R. F. (2007). Developing the Science of Reintroduction Biology. *Conservation Biology*, *21* (2), 303-312.

Seddon, P. J., Martin, R. B., Trent, S., Barrio Froján, C. R., & Volger, A. P. (1999). Reintroductions, introductions, and the importance of post-release monitoring: lessons from Zanzibar. *Oryx*, *33* (2), 89-97.

Sæther, B.-E., Solberg, E. J., & Heim, M. (2003). Effects of Altering Sex Ratio Structure on the Demography of an Isolated Moose Population. *The Journal of Wildlife Management*, *67* (3), 455-466.

Shargal, D., Shore, L., Roteri, N., Terkel, A., Zorovsky, Y., Shemesh, M., et al. (2008). Fecal testosterone is elevated in high ranking female ibexes (Capra nubiana) and associated with increased aggression and a preponderance of male offspring. *Theriogenology*, *69* (6), 673-680.

Sheldon, B. C., & West, S. A. (2004). Maternal Dominance, Maternal Condition, and Offspring Sex Ratio in Ungulate Mammals. *The American Naturalist*, *163* (1), 40-54.

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010). The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk. *Ecology*, *91* (10), 2983-2994.

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2009). The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology*, *78* (6), 1249-1258.

Shiell, A. W., Campbell, D. M., Hall, M. H., & Barker, D. J. (2000). Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later. *BJOG : an International Journal of Obstetrics and Gynaecology*, *107* (7), 890-895.

Sidorovich, V. E., Stolyarov, V. P., Vorobei, N. N., Ivanova, N. V., & Jędrzejewska, B. (2007). Litter size, sex ratio, and age structure of gray wolves, Canis lupus, in relation to population fluctuations in northern Belarus. *Canadian Journal of Zoology*, *85* (2), 295-300.

Silk, J. B. (1983). Local Resource Competition and Facultative Adjustment of Sex Ratios in Relation to Competitive Abilities. *The American Naturalist*, *121* (1), 56-66.

Silk, J. B., & Brown, G. R. (2008). Local resource competition and local resource enhancement shape primate birth sex ratios. *Proceedings: Biological Sciences*, *275* (1644), 1761-1765.

Silverin, B. (1998). Behavioural and hormonal responses of the pied flycatcher to environmental stressors. *Animal Behaviour*, *55* (6), 1411-1420.

Simonis, J. L., Harrison, R. B., Long, S. T., Rabon, D. R., Waddell, W. T., & Faust, L. J. (2017). Managed movement increases metapopulation viability of the endangered red wolf. *Wildlife Management*, *82* (3), 573-582.

Sjöåsen, T. (1996). Survivorship of captive-bred and wild-caught reintroduced European otters Lutra lutra in Sweden. *Biological Conservation*, *76* (2), 161-165.

Smith, C. A. (2007). Sex Determination in Birds: HINTs from the W Sex Chromosome? *Sexual Development*, *1*, 279–285.

Smith, D. W., & Bangs, E. E. (2009). Reintroduction of Wolves to Yellowstone National Park: History, Values and Ecosystem Restoration. In M. W. Hayward, & M. J. Somers (Eds.), *Reintroduction of Top-Order Predators* (pp. 92-125).

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8* (4), 383–395.

Snyder, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., et al. (1996). Limitations of Captive Breeding in Endangered Species Recovery. *Conservation Biology*, *10* (2), 338-348.

Spalton, J. A., Lawerence, M. W., & Brend, S. A. (1999). Arabian oryx reintroduction in Oman: successes and setbacks. *Oryx*, *33* (2), 168-175.

Spalton, J. A., Lawrence, M. W., & Brend, S. A. (1999). Arabian oryx reintroduction in Oman: successes and setbacks. *Oryx*, *33* (2), 168-175.

Sparkman, A. M., Adams, J., Beyer, A., Steury, T. D., Waits, L., & Murray, D. L. (2011). Helper effects on pup lifetime fitness in the cooperatively breeding red wolf (Canis rufus). *Proceedings: Biological Sciences*, *278* (1710), 1381-1389.

Sparkman, A. M., Blois, M., Adams, J., Waits, L., Miller, D. A., & Murray, D. L. (2017). Evidence for sex-specific reproductive senescence in monogamous cooperatively breeding red wolves. *Behavioral Ecology and Sociobiology*, *71*, 6.

Spinu, V., Grolemund, G., Wickham, H., Lyttle, I., Constigan, I., Law, J., et al. (2020). lubridate: Make Dealing with Dates a Little Easier. *R package version 1.7.8*, CRAN.

Sterling, E. J., Dierenfeld, E. S., Ashbourne, C. J., & Feistner, A. T. (1994). Dietary intake, food composition and nutrient intake in wild and captive populations of Daubentonia madagascariensis. *Folia Primatologica*, *62*, 115–124.

Stork, N. E. (2010). Re-assessing current extinction rates. *Biodiversity and Conservation*, *19*, 357–371.

Sutherland, W. J., Armstrong, D., Butchart, S. H., Earnhardt, J. M., Ewen, J., Jamieson, I., et al. (2010). Standards for documenting and monitoring bird reintroduction projects. *Conservation Letters*, *3* (4), 229-235.

Sutton, A. E., & Lopez, R. (2014). Findings from a survey of wildlife reintroduction practitioners. *F1000 Research*, *3* (29).

Synder, N., & Synder, H. (2000). *The California Condor: A Saga of Natural History & Conservation.* London: Acadenic Press.

Takahashi, L. K., & Kalin, N. H. (1991). Early developmental and temporal characteristics of stress-induced secretion of pituitary-adrenal hormones in prenatally stressed rat pups. *Brain Research*, *558* (1), 75-78.

Tappy, L., & Lê, K.-A. (2010). Metabolic Effects of Fructose and the Worldwide Increase in Obesity. *Physiological Reviews*, *90* (1), 23-46.

Taylor, P. D., & Bulmer, M. G. (1980). Local mate competition and the sex ratio. *Journal of Theoretical Biology*, *86* (3), 409-419.

Thomas, D. C., Barry, S. J., & Kiliaan, H. P. (1989). Fetal Sex Ratios in Caribou: Maternal Age and Condition Effects. *The Journal of Wildlife Management*, *53* (4), 885-890.

Thomas, J. A., & Morris, M. G. (1994). Patterns, mechanisms and rates of extinction among invertebrates in the United Kingdom. *Philosophical Transactions of the Royal Society B*, *344*, 47-54.

Trivers, R. L., & Willard, D. E. (1973). Natural Selection of Parental Ability to Vary the Sex Ratio of Offspring. *Science*, *179* (4068), 90-92.

Uller, T. (2006). Sex-specific sibling interactions and offspring fitness in vertebrates: patterns and implications for maternal sex ratios. *Biological Reviews*, *81* (2), 207-217.

Van der Weyde, L. K., Martin, G. B., & Paris, M. C. (2016). Monitoring stress in captive and free-ranging African wild dogs (Lycaon pictus) using faecal glucocorticoid metabolites. *General and Comparative Endocrinology*, *226*, 50-55.

van Schaik, C. P., & Hrdy, S. B. (1991). Intensity of Local Resource Competition Shapes the Relationship between Maternal Rank and Sex Ratios at Birth in Cercopithecine Primates. *The American Naturalist*, *138* (6), 1555-1562.

Vandenbergh, J. G., & Huggett, C. L. (1994). Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. *Proceedings of the National Academy of Sciences of the United States of America*, *91* (23), 11055-11059.

Varadhan, R., & Borchers, H. W. (2018). dfoptim: Derivative-Free Optimization. *R package version 2018.2-1*, CRAN.

Veiga, J. P., Viñuela, J., Cordero, P. J., Aparicio, J. M., & Polo, V. (2004). Experimentally increased testosterone affects social rank and primary sex ratio in the spotless starling. *Hormones and Behavior*, *46* (1), 47-53.

Veiga-Lopez, A., Steckler, T. L., Abbott, D. H., Welch, K. B., MohanKumar, P. S., Phillips, D. J., et al. (2011). Developmental Programming: Impact of Excess Prenatal Testosterone on Intrauterine Fetal Endocrine Milieu and Growth in Sheep. *Biology of Reproduction*, *84* (1), 87–96.

Vickery, S. S., & Mason, G. J. (2003). Behavioral Persistence in Captive Bears: Implications for Reintroduction. *Ursus*, *14* (1), 35-43.

Viggers, K. L., Lindenmayer, D. B., & Spratt, D. M. (1993). The Importance of Disease in Reintroduction Programmes. *Wildlife Research*, *20* (5), 687 - 698.

Voegtline, K. M., Costigan, K. A., Kivlighan, K. T., Henderson, J. L., & DiPietro, J. A. (2013). Sex-specific associations of maternal prenatal testosterone levels with birth weight and weight gain in infancy. *Journal of Developmental Origins of Health and Disease*, *4* (4), 280–284.

Waddell, W. (2014). *Red Wolf Canis rufus International Studbook.* Point Defiance Zoo & Aquarium.

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 11466-11473.

Walters, J. R., Derrickson, S. R., Derrickson, D. M., Haig, S. M., Marzluff, J. M., & Wunderle, J. M. (2010). Status of the California Condor (Gymnogyps californianus) and Efforts to Achieve Its Recovery. *The Auk*, *127* (4), 969–1001.

Warnes, G. R., Bolker, B., Gorjanc, G., Grothendieck, G., Korosec, A., Lumley, T., et al. (2017). gdata: Various R Programming Tools for Data Manipulation. *R package version 2.18.0,* CRAN.

Weatherhead, P. J., Brown, G. P., Prosser, M. R., & Kissner, K. J. (1998). Variation in offspring sex ratios in the northern water snake (Nerodia sipedon). *Canadian Journal of Zoology*, *76* (12), 2200-2206.

Wedekind, C. (2012). Managing Population Sex Ratios in Conservation Practice: How and Why? In A. Povilitis (Ed.), *Topics in conservation biology* (pp. 81-96).

Wedekind, C. (2002). Manipulating sex ratios for conservation: short-term risks and long-term benefits. *Animal Conservation*, *5*, 13–20.

Weladji, R. B., Holand, Ø., Yoccoz, N. G., & Lenvik, D. (2003). Maternal age and offspring sex ratio variation in reindeer (Rangifer tarandus). *Annales Zoologici Fennici*, *40* (4), 357-363.

West, S. A., & Sheldon, B. C. (2002). Constraints in the Evolution of Sex Ratio Adjustment. *Science*, *295* (5560), 1685-1688.

West, S. (2009). *Sex Allocation.* Princeton: Princeton University Press.

Wickham, H. (2019). Easily Install and Load the 'Tidyverse'. *R package version 1.3.0*, CRAN.

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., et al. (2020). ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. *R package version 3.3.0*, CRAN.

Wilcox, G. (2005). Insulin and Insulin Resistance. *The Clinical Biochemist Reviews*, *26* (2), 19–39.

Wild, G. (2006). Sex Ratios When Helpers Stay at the Nest. *Evolution*, *60* (10), 2012-2022.

Zambrano, E., Bautista, C. J., Deás, M., Martínez-Samayoa, P. M., González-Zamorano, M., Ledesma, H., et al. (2006). A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. *The Journal of Physiology*, *571* (1), 221-230.