Delaying the Weaning of Captive Rhesus Macaques (*Macaca mulatta*) Destined for Use in Neuroscience Research: An Assessment of the Costs and Benefits

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Degree: PhD (Doctor of Philosophy)

Date Registered: 23/09/2019

Date of Submission: 20/09/2023

Abstract

This thesis aims to assess the costs and benefits of delaying permanent maternal separation (“weaning”) of purpose-bred captive rhesus macaques (*Macaca mulatta*) and to determine if UK guidelines on weaning age should be refined to improve animal welfare. Current UK guidelines advise weaning should occur as late as possible and not before 10-14 months. Whilst weaning earlier than this has negative and long-lasting consequences on behaviour and health; it is unclear if later weaning provides welfare benefits. Perceived costs of increased aggression and decreased productivity are used to justify earlier weaning, despite there being limited data to support this. Delaying weaning is a simple and inexpensive refinement that can potentially improve welfare as well as the validity and reliability of scientific data derived from animals destined for research. As such, this investigation is imperative. Taking advantage of variable weaning ages at the Medical Research Council’s Centre for Macaques, health record data was collated from 2004 – 2022 on injuries, alopecia scores, weights, *Campylobacter* incidence and inter-birth-intervals (IBI). The full retrospective dataset contained observations for 800 macaques (436 females). Additionally, in 2019-2022, 136 monkeys from 4 birth cohorts (59 females) underwent novel object tests close to their 3rd birthday to assess neophobia. Finally, in 2020-2022, video data from breeding groups were collected to assess levels of aggression directed towards older juveniles. The data show that weaning is associated with increased *Campylobacter* infection, and increased alopecia prevalence and severity. However, a later weaning age was found to mitigate these acute effects. The data do not support the perceived costs used to justify early weaning: later weaning was not correlated with decreased productivity and whilst older juveniles in breeding groups were at greater risk of injury than weaned counterparts, overall rates were low. In conclusion, these data support the welfare benefits of delayed weaning.

Acknowledgements

Undertaking this PhD research has granted me privileges I could have only imagined, and I consider myself incredibly fortunate to have had this invaluable opportunity. Working closely with the monkeys at the CFM, witnessing the dedication and compassion of the staff in caring for them, and engaging in countless discussions with brilliant minds in animal behaviour and welfare have been truly inspiring. I am extremely grateful to the NC3Rs for their funding to support this research. There are numerous individuals without whom this PhD research would not have been possible, and I am deeply grateful to all of them.

First and foremost, my heartfelt thanks go to my supervisors, Professor Melissa Bateson, and Dr Claire Witham. Professor Bateson has been an exemplary role model throughout this research, providing unwavering support and encouragement. From the moment I arrived at Newcastle, she made me feel welcome, investing significant time in thoughtful discussions and integrating me into the Newcastle lab environment. Her guidance in rigorous scientific methods has been invaluable, and I can only hope to emulate a fraction of her scientific brilliance.

Dr Claire Witham is truly a genius, a master of many disciplines, and a caring person. Her impact on this project has been immeasurable. Without her guidance, none of this would have been possible. Despite her full-time commitment at the CFM, Claire showed unwavering dedication and understanding during one of the most challenging periods of my life. I owe my successful completion of this PhD to her. And of course, I will always cherish the welcomed distractions of cute monkey videos and images in the office – for which I am equally grateful!

During my time at Newcastle, I was fortunate to be warmly welcomed by colleagues within the CBE. I extend my deepest gratitude to Dr Vivek Nityananda, Professor Lucy Asher, and Professor Tom Smulders for providing me with the opportunity to present my work and offering valuable feedback. Their insights have significantly enhanced most of the chapters in this thesis. I am also indebted to my panel members, Dr Colline Poirier and Professor Chris Petkov, for their invaluable input during annual progress reviews. Their feedback greatly contributed to the structure and scientific rationale behind each chapter. From Newcastle, I also owe thanks to Hannah Cave, Verity Kaiper-Holmes, Isabel Lamb, and Eva Tan for their efforts coding temperament test videos.

I must extend additional thanks to Colline for inviting me to her lab group meetings when I was a lonely newcomer at Newcastle. Participating in those meetings in the early stages of this project proved immensely beneficial for developing good scientific communication and methods. I am also deeply grateful to the rest of Colline's lab group for our insightful discussions during that time – Thank you, Janire Catellano-Bueno, Nathan Kindred, and Sam Groves.

To Josh Reukauf and Giulia Ciminelli, thank you for the invaluable friendship I've shared over these past few years. The bond forged through our collective experiences as PhD researchers has made Newcastle feel like home every time I've visited. Our fruitful discussions have significantly improved the quality of this thesis.

The technicians at the CFM deserve special recognition for their dedication and hard work. Their tireless efforts to ensure the well-being of the macaques have been instrumental in the success of this research. I extend my deepest gratitude to Sara, Faye, Seb, Jim, Char, Georgie, Josh, Kate, Katie, Lou, Matt, Scotty, and Olivia. Without their commitment to the monkeys, this research, and all other research at CFM, would not have been possible.

Thank you to my family, Michelle, Neil, Wil, Chelsey, and El Seano for their support. To Jade and Toby, your unwavering love and support have sustained me throughout my time as a PhD student, and I am deeply grateful. Without you, I wouldn't have had the fortitude to reach this point. To Mila, thank you for being my inspiration during the final stages of this thesis. I already love you and cannot wait to meet you.

I would also like to thank Dr Mark Prescott for his support of this project and providing data used in Chapter 7 for the productivity analysis; Dr Kris Coleman and Dr Ori Pomerantz for welcoming me into their facilities and providing their expertise and unique perspectives on my data – I thoroughly enjoyed my time with them both.

Lastly, I extend my heartfelt gratitude to the monkeys. Having the opportunity to work so closely with these incredible and fascinating animals has been a privilege I will cherish forever. While they continue to be used in research, I want to express my thanks for their contributions. I hope this thesis can, in some way, contribute to supporting them and their well-being.

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Chapter 1. General Thesis Introduction

1.0 Chapter summary

In the UK, rhesus macaques (*Macaca mulatta*) that are used in research must be purpose bred under Schedule 2 of the Animals (Scientific Procedures) Act 1986 (ASPA). Most macaques supplied to UK institutions for use in neuroscience research originate from the Medical Research Council's Centre for Macaques (CFM). At the CFM, macaques are kept in one-male/ multi-female breeding groups and any individuals that are destined to be supplied will eventually be separated from their natal group and placed into same-sex 'weaning' groups. Current guidelines advise that macaques should be kept in their breeding groups for as long as possible, and that permanent maternal separation (henceforth, weaning) should not occur prior to 10-14 months of age. It is a requirement for licensed establishments to continually address principles of the 3Rs (the replacement, refinement, and reduction of animals in research), and the age at which macaques are weaned has been identified as a promising candidate for refinement. There is a lot of evidence that very early weaning or adverse rearing conditions have serious negative and long-lasting consequences on monkeys' behaviour and health. To take a few examples, very early weaning is associated with behavioural disturbances; social disruptions; neophobia; injury prevalence; and alopecia. The aims of this project are to collect behavioural, cognitive, and health data to test the hypothesis that weaning macaques later than current UK guidelines will produce significant welfare benefits for individuals throughout their life from birth to laboratory.

1.1 The use of non-human primates in research

In the UK, the most used species in scientific research in 2021 were mice (54%), fish (15%), birds (14%), and rats (11%), of the 3.06 million procedures (UK Home Office, 2022). Non-human primates (herein, primates) made up around 0.09% of these procedures (UK Home Office, 2022) compared to 0.3% in EU member states (European Commission, 2019) and 9.1% of covered species in the USA (USDA, 2020). The most common primate species used were cynomolgus macaques (*Macaca fascicularis*: 2561 procedures), totalling 91.63% of all procedures carried out on primates. The remaining procedures were carried out on rhesus monkeys (*Macaca mulatta*: 118 procedures) and marmosets and tamarins (116 procedures) (UK Home Office, 2022). Macaques are a particularly popular genus for studies that aim to translate findings to the human population as they share close phylogenetic proximity with humans and share similarities in their physiology and cognition (Kalin & Shelton, 1989). This similarity extends to some cognitive and motor function, brain organisation, and patterns of age-related cognitive and motor decline, rhesus macaques in particular are often considered better models for human neurocognitive and motor aging compared to rodents due to their closer resemblance to humans in these aspects (Workman et al., 2018).

There is currently debate for and against the use of primates in research (for example, see Treue & Lemon, 2023 for arguments for; see Bailey, 2023 for arguments against). Non-human primate models are argued to have made important contributions to biomedical research (Mitchell et al., 2018; Treue & Lemon, 2023). They are considered essential for biological and biomedical research, including drug safety studies before moving to a clinical human population because of their physiological similarity of the organ systems (SCHEER, 2017; Epstein & Vermeire, 2017; Treue & Lemon, 2023). Their need is often justified over alternative animal models to bridge the gap for translational benefits (Careaga et al., 2017). For example, the absence of a dlPFC homolog in rodents, a key area controlling attention and working memory in primates, is a strong argument for the necessity of primate research (Thiele & Bellgrove, 2018).

The similarities between humans and primates raises clear ethical concerns surrounding their use as they meet the criteria for sentience (Bailey, 2023). As primates are behaviourally complex social animals, it can be difficult and costly to meet their complex needs in captivity (Careaga et al., 2017; Dewi & Cline, 2021).

Furthermore, it is argued that the benefits from their use have been overstated with very few areas showing promise when assessed years later. For example, Bailey (2023) analysed papers using primates as a model published in medical journals since 2005 and argued that one out 25000 treatments shows promise 20 years on.

Whether one agrees or disagrees with the use of non-human primates, while they are used, there is a clear goal that both sides must share and that is to enhance their welfare as best as possible. Animal welfare is an ethical imperative in research involving animals. It is widely recognised that primates have the capacity to suffer and experience positive welfare (Truelove et al., 2020). Conducting research in a manner that prioritises animal welfare demonstrates ethical responsibility and respect for the animals involved (Schapiro & Hau, 2023). Furthermore, by ensuring that animals are in a state of good welfare, researchers can obtain more reliable and representative data, increasing the value and applicability of their research (Prescott & Lidster, 2017; Lewis, 2019). Poor welfare conditions, such as stress or pain, can introduce confounding variables and bias into the data, leading to unreliable or misleading results (Hopkins and Latzman 2017; Shivley 2017; Prescott et al., 2022; Schapiro & Hau, 2023). Improved housing conditions (Schapiro et al., 2000; Benton et al., 2013), Positive Reinforcement Training (Schapiro et al., 2001; Laule et al., 2003; Schaprio et al., 2005; Lambeth et al., 2006; Magden et al., 2013), and environmental enrichment (Schapiro et al. 1998; Bayne, 2005; Weed & Raber, 2005; Bayne & Wurbel, 2014) have all shown to be associated with more relevant physiological parameters for biomedical studies. For example, social housing is a clear necessity for the welfare of non-human primates and Schapiro *et al.* (2000) found that socially housed macaques yielded more relevant immunological responses on an immunodeficiency virus vaccine study.

The most common areas that use primates in research are the biomedical sciences including toxicology, infectious diseases, and neuroscience (The Royal Academy of Medical Sciences, 2006). Primates are important species within these areas and have proved to be suitable models of HIV/AIDS, lung disorders, drug efficacy, and more recently COVID-19 (Nelson et al., 2013; Phillips et al., 2014; Castro-Gonzalez et al., 2018; Cardoso-Moreira et al., 2019). Rhesus macaques are routinely used as models for studying normal human development and human diseases because the genes and regulatory networks underlying development are largely conserved across these species (Nelson et al., 2013; Castro-Gonzalez et al., 2018; Cardoso-Moreira et al., 2019). In neuroscience in particular, primates have provided crucial insights into the treatments of human neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease (Vitale et al., 2009; Phillips et al., 2014; Mitchell et al., 2018).

However, the procedures that rhesus macaques are exposed to in experiments of this nature and the conditions in which they are housed, raise valid concerns regarding their welfare. Neuroscience experiments can involve fluid restrictions, separation from any social group (where not singly housed), human interaction, and stressful transportation methods to the experiment area (Joint Working Group on Refinement, 2009); procedures that can lead to individuals experiencing at least mild stress (Pfefferle et al., 2018). Laboratory macaques involved in neuroscience procedures are sometimes fitted with head implants; head implants raise welfare concerns because of the stress of surgery to fit them, the potential for infections after, and the potentially painful maintenance of them (Johnston et al., 2016). Furthermore, rhesus macaques housed in neuroscience laboratories are typically singly or pair housed; putatively stressful for a species that naturally lives in large multi-male/ multi-female groups in the wild (Cawthon Lang, 2005).

UK parliamentary laws, European Directives, and treaties offer special protection to primates used in research due to their genetic similarity to humans, and because their complex sociality renders it difficult to meet their behavioural, social, and environmental needs in captivity (Chatfield & Morton, 2018). The most common funding bodies that fund research that may use primates within the UK and Europe are the MRC and Wellcome Trust, others include NERC, NC3Rs, BBSRC etc. Funders also have stringent guidelines and criteria to further protect the welfare of primates used in biomedical research. This is in line with The EU Directive 2010/63/EU, transposed into UK parliamentary law in 2012 by adding regulations to the Scientific Procedures Act 1986 that, *inter alia*, prohibits the use of primates in research where other models are available, and prohibits the use of wild caught primates in research. Furthermore, licence holders using specially protected species under Section 5C of the Animals (Scientific Procedures) Act 1986, like primates, must ensure that no alternative species is available (UK Home Office, 2022). However, this is often scrutinised by voices against non-human primates use as not being suitably critiqued or justified in most cases (RSPCA, 2011).

Despite the efforts of funders and the laws put in place there are still valid concerns about the welfare of primates used in research both from the scientific community and the general public (For example, see Bailey & Taylor, 2016). As such, researchers are continually trying to gain a better understanding of how I can further improve the welfare of primates in medical research. For example, the Joint working group on refinement 9th report (2009) and Buchanan-Smith *et al*. (2023) review where refinements can be made and potential barriers to implementation. Also, the most recent EU SCHEER report at the time of writing reports that primates are still needed in medical research and a timeline for when they will no longer be needed cannot be predicted. However, the report highlights that there are numerous potentials to apply the 3Rs to research using primates (SCHEER, 2017). Evidence from the literature corroborates this opinion as it is clear that researchers are continually looking to address the principles of the 3Rs (replacement, reduction, and refinement; Russell & Burch, 1959) and devise methods to assess the cumulative severity of animals in a research context as many refinements have been made and continue to be made.

1.2 The 3Rs: Replacement, Reduction, and Refinement

The 3Rs principles, first proposed by Russell and Burch (1959) are replacement, reduction, and refinement. Replacement can be completely replacing the animal model with a non-animal model or replacing an animal model for what is perceived to be a less sentient animal model. Replacement is difficult for primate models in biomedical research given their close phylogenetic proximity to humans and the need for their output to translate to human health. Reduction involves creating ways that reduce the number of a model species needed in experiments, typically by improving statistical modelling procedures and the experimental techniques. Refinement aims to decrease the severity of adverse procedures that the animal model is subjected to (reviewed by Vitale et al., 2009). Due to the difficulty of reducing and replacing macaques used in biomedical research, refinement is typically the most common principle addressed. Refinement can involve either refining husbandry, methodological, or handling methods. Some examples of where refinement has been implemented successfully include training to willingly go into a transport chair (rhesus macaques; Mason et al., 2019), handling rodents by tubes rather than tail holding (various strains of mice; Hurst & West, 2010), and the development of non-invasive head immobilisation techniques to replace the need for head implants (rhesus macaques; Slater et al., 2016). All funding bodies in the UK will only fund studies that use animals in research as long as they address questions relating to the principles of the 3Rs. And opinion polls suggest that the general UK public are more accepting of research that addresses the principles of the 3Rs and where no alternative model species is available (Ipsos MORI, 2018).

One proposed candidate for refinement in laboratory primates is the age at which primates are permanently separated from their mothers (herein, weaned) (Prescott et al., 2012). This suggestion is based on the knowledge gathered from empirical studies about the effects of maternal deprivation on macaque health, behaviour, immunology, and temperament.

1.3 Effects of early maternal separation

In the context of laboratory animal studies, early separation of the infant from its mother can be thought of as a model of early-life adversity. There is a vast array of literature documenting both the long-term negative effects of early-life adversity on the adult soma, and short-term physiological, behavioural, and cognitive effects of stress (Hertzman & Boyce, 2010). Studies using maternal deprivation stem from the well-known early work of Harlow and colleagues that studied the effects of maternal separation (brief separation, i.e., not weaning) on infant rhesus macaques in the context of attachment theory (Harlow et al., 1965). There are a number of reasons why researchers may be interested in using early separation as a model of early-life adversity in their work: some are interested in understanding the behavioural and physiological responses from a developmental psychobiological perspective (e.g., Capitanio et al., 2005); others for understanding physiological and neural mechanisms that moderate susceptibility to later negative health outcomes e.g. from alcoholism (e.g. Barr et al., 2004).

Negative effects of early separation from the mother have been documented in a number of mammalian species including humans (Gray, 1989), rats (*Rattus norvegicus;* Kikusui et al., 2008), pigs (*Sus scrofa domesticus;* Widowski et al., 2008), horses (*Equus caballus;* Waran et al., 2008), and lambs (*Ovis aries;* Napolitano et al., 2008). Evidence shows that this early separation can have negative effects on behaviour, where individuals separated early from their mother exhibit behaviour associated with psychological stress e.g., belly-nosing in pigs (Widowski et al., 2008), bar-biting in ICR-mice (*Mus musculus;* Würbel & Stauffacher, 1997), stereotypies in mink (*Mustela sp.;* Lathan & Mason, 2008), and wool-sucking in cats (Lathan & Mason, 2008). Immediate behavioural responses are also evident in animals removed from their mother that suggest the individual perceives the event to be a psychosocial stressor, for example, depressive phase in monkeys are associated with less activity and more crying vocalisations (Seay et al., 1963; Prescott et al., 2012); similar responses are also seen in domestic horses (*Equus caballus*: Waran et al., 2008).

In rhesus macaques in particular, early separation from the mother has been shown to affect temperament, resulting in infants that are more neophobic and anxious compared to naturally reared controls (Timmermans et al., 1994). Immunological consequences of early separation are evident, and studies have shown adverse rearing conditions can result in lower proportion of $CD8^+$ cells and natural killer cell activity (Lubach et al., 1995; Laudenslager et al., 1985). Furthermore, there is evidence that early separation can have negative health outcomes for rhesus macaques measured through *Campylobacter* incidence and diarrhoea (Goo & Fugate, 1984).

Given that rhesus macaques are a popular model organism for biomedical research and the evidence suggesting that early weaning can have negative effects on behaviour, immunology, and health, it is important that data are available to inform the best management practices of the infants in captivity that are to be used in such experiments. The idea that improving welfare is not only beneficial for the individual animal but also for the validity and reliability of the data resulting from their use in research is not novel. Poole (1997) highlighted this in their paper “Happy animals make good science”. Since then, it is widely accepted that maintaining animals in conditions that promote the best welfare possible is not only the ethically correct approach but also a prerequisite to improved data quality (Schapiro & Hau, 2023). Doing so will better comply with laws and guidelines to improve the welfare of macaques in science, potentially reduce the cumulative severity of experiments on the animals, and lead to scientific output that is more reliable.

1.4 Weaning laboratory rhesus macaques

In the UK there are four main Universities with neuroscience laboratories that the Medical Research Council’s Centre for Macaques (breeding colony; CFM) predominantly supplies to: Cambridge University, Oxford University, University

College London (UCL), and Newcastle University. The current NC3Rs guidelines, at the time of writing, for the weaning of captive bred macaques is to leave them in their natal group for as long as possible. Where this is not possible, weaning should occur no earlier than 10 to 14 months (Prescott et al., 2012). Although, sometimes early weaning is unavoidable from a colony management and animal welfare perspective such as if the mother dies or rejects her infant, or if the infant is receiving too much aggression. However, while it is intuitive that weaning at the earliest of the guidelines will be better for the individual’s welfare than even earlier weaning, for example, weaning at 10 months is better than weaning at 6 months, no data exist that systematically tests the benefits/costs of later weaning ages after 12 months i.e., is 10 to 14 months old adequate? Data that can address this question have the potential to affect policy guidelines and improve the welfare of captive bred macaques.

In the wild, rhesus macaques live in a female philopatric social groups where females remain in their natal group for their lives and form close social bonds with other females in their matrilines; males emigrate to new groups in early adolescence (typically 5 years old; Cawthon Lang, 2005). They are seasonal breeders and females only have one offspring per year. During the first year of life, the mother typically is very controlling of the infant’s environment. The mother will stop infants wandering off, restricting their experience of the social group, and provide nutritional sustenance. Over the first year (up to around 14 months) the mother gradually decreases this control, and the infant has increasing access to its environment and access to interact with peers and other members of the social group. The infant is fully nutritionally weaned at around 14 months of age and at this time the mother is likely pregnant and beginning to invest in her next offspring.

Therefore, the guidelines for weaning captive bred macaques and the natural weaning age of wild macaques are somewhat comparable. There is, however, one important exception: the wild conspecifics will then remain in their natal group for at least a few years (males) or for the remainder of their life (females). It is likely that the mother still provides emotional and agonistic support to their offspring subsequent to weaning that is important for their psychosocial development (Burkett & Young, 2012; Lindell et al., 2012; Dettmer et al., 2016; Minami et al., 2022); support that the captive-bred weaned individuals will not receive.

There are possible costs to a later weaning that have been previously highlighted in the literature. For example, Goo and Fugate (1984) found that later weaning resulted in decreased productivity. However, more recent data have failed to find this effect (Prescott et al., 2012; Rox et al., 2022). Furthermore, Goo and Fugate (1984) compared the effects of weaning at 6, 8, 10, and 12 months of age, therefore it is unclear how weaning after 12 months, in line with the current guidelines, affects individuals' welfare and productivity.

1.5 Aims and objectives

This thesis aims to test the hypothesis that a later weaning age in captivity will be beneficial for rhesus macaque welfare and health. No study to date has systematically tested this hypothesis after 12 months of age but extrapolations from studies on differential rearing and early weaning allow us to develop predictions relating to the potential benefits (1-3) and costs of later weaning (4-5):

1. Later weaning age will negatively correlate with an anxious and inhibited temperament measured by a novel object and human intruder test.

2. Later weaning age will better immune function: higher Neutrophil to Lymphocyte ratio.

3. Later weaning will correlate with improved health measures: decreased incidence of *Campylobacter*, decreased prevalence and severity of alopecia, and typical weight.

4. Older juveniles in breeding groups will receive more aggression and injuries that require veterinary attention than similarly aged monkeys in weaning groups.

5. Dams with juveniles that are weaned later will be slower to reproduce than dams with earlier weaned juveniles.

Following these predictions, the objectives of the thesis are to:

- Systematically review the literature on weaning and maternal separation and stress response measured from cortisol (Chapter 3).

- Test monkeys using a novel object and human-intruder test paradigm to quantify their temperament and analyse the association between weaning age and temperament (Chapter 4).

- Utilise individual health records to quantify alopecia severity and prevalence (Chapter 5), weights of matched samples of weaned and non-weaned monkeys (Chapter 6), *Campylobacter* infections in weaned and breeding groups (Chapter 6), injuries received in breeding and weaning groups (Chapter 7), and productivity of dams with infants of varying weaning ages (Chapter 7).

- Collect new data on aggression within breeding groups by recording groups around feeding times where most aggression will occur to test is the adult male disproportionately directs aggression towards juveniles in the group (Chapter 7).

- Collect more regular alopecia scores of similarly aged weaned and non-weaned monkeys to control for potential seasonal effects of alopecia (Chapter 5).

- Utilise annual health screen blood samples of Neutrophil and Lymphocyte counts to test immunological parameters of weaning age (Chapter 6).

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Chapter 2. Materials and Methods

2.0 Chapter summary

This chapter will cover the general background of the Medical Research Council's Centre for Macaques, where this study was undertaken. A description of the study species, their housing and husbandry procedures, and the observational nature of the study. Details are given to the datasets that will be presented throughout the thesis and how data and analysis scripts will be stored and available.

2.1 Study location

This study was conducted at the Medical Research Council's Centre for Macaques (CFM). The CFM is a breeding colony of rhesus macaques (*Macaca mulatta*) established in 2003 with support from the Medical Research Council (MRC), Wellcome Trust, Oxford and Cambridge Universities to breed and supply rhesus macaques to UK Research Council and Wellcome grant-holders based at UK universities for use in basic and applied research. The CFM supplies around 30 macaques each year, predominantly to UK Universities with neuroscience laboratories such as Newcastle University, Oxford University, Cambridge University, and University College London. There are 22 enclosures at the CFM distributed along 2 corridors. As of 5th July 2023 there are 182 animals (119 in breeding groups; 63 in weaning groups) with eight adult breeding males and 51 adult breeding females. Actual numbers of macaques in the colony at a given time varied over the course of data collection as monkeys are born, supplied, or removed from the colony each year.

2.2 Rhesus macaques

Rhesus macaques (*Macaca mulatta*) are a species of nonhuman primate belonging to the family *Cercopithecidae* that originate from Southeast Asia and occupy the largest distribution of any nonhuman primate and a diverse range of habitats across this distribution including artificial/terrestrial, forest, savannah, and shrubland (Southwick et al., 1996; Timmins et al., 2008). Rhesus macaques are abundant in their habitat and are classified as Least Concern by the IUCN red list for endangered species, likely due to their high ecological tolerance allowing them to thrive in a broad array of environments (Cords, 2012).

A sexually dimorphic species, adult male rhesus macaques weigh, on average 7.7 kg and are around 1.75 ft tall; adult females weigh, on average 5.34 kg and are around 1.54 ft tall (Fooden 2000; Cawthon Lang, 2005).

The diet of Rhesus macaques is heavily influenced by human activities, making it challenging to define their natural dietary habits. Studies have shown that up to 93% of their diet may consist of food obtained either directly from humans, such as handouts, or indirectly through agricultural products (Richard et al., 1989; Southwick & Siddiqi, 1994). In urban and agricultural areas, they often feed on various fruits and vegetables by raiding crops or scavenging through garbage (Goldstein & Richard, 1989; Richard et al., 1989). In India, particularly around temples, they are known to inhabit areas where they are fed by locals as part of religious practices. Common temple offerings include bread, bananas, peanuts, seeds, and a variety of fruits and vegetables, along with more unusual items like ice cream and fried bread (Wolfe, 2002). In habitats less influenced by humans, rhesus macaques focus on a diet consisting of natural foods like fruits, flowers, leaves, seeds, gums, buds, grass, clover, roots, bark, and an array of insects such as termites, grasshoppers, ants, and beetles. They are also known to consume bird eggs, shellfish, fish, and even mushrooms (Fooden, 2000). During dryer seasons, they have been observed eating dirt from termite mounds, likely to supplement their diet with minerals and nutrients (Lindburg, 1971).

Rhesus macaques live in large multi-male/multi-female groups, that have an average of 10 to 80 individuals, and are a female philopatric social groups where females remain in their natal group for their lives and form close social bonds with other females in their matrilines; males emigrate to new groups in early adolescence (typically 5 years old; Melnick et al., 1984; Cawthon Lang, 2005). They have strict linear dominance hierarchies that remain relatively stable over time and are largely based around the matriline: young females inherit rank from their mother and each subsequent female offspring ranks above her predecessors so that once the head of a matriline dies, her youngest daughter takes her rank (Seth, 2000). Male dominance rank is less stable over their lifetime compared to females, whilst they do inherit their mothers rank at birth, as they mature, they must compete for higher social status with other males in the group and start this process again once they emigrate (Lindburg, 1971).

Early maternal investment is biased toward male offspring, evidenced by a delay in inter-birth-interval following the rearing of male offspring (Bercovitch & Berard, 1993; Bercovitch et al., 2003). Mothers take primary parental care, although infants are routinely handled by close female relatives, known as 'auntying' (Lindburg, 1971). Maternal styles vary from protective to rejective/abusive (Maestripieri, 1998; Maestripieri, 2005) and involve behaviours, including protectiveness, rejection, grooming, cradling, nursing, and physical contact with the infant (McCormack et al., 2014). Protective mothers will restrain infants, preventing them from independent exploration, at more frequent rates than is typical; rejective mothers will reject the infants attempts at contact more than is typically seen (Maestripieri, 1998; Maestriperi, 1999b). Variation in maternal styles has been shown to result in individual differences in socioemotional processing (Mandalaywala et al., 2014). Typically, individuals start to explore their own environment more frequently away from the mother gradually from birth (Rawlins & Kessler, 1986) and will be much more independent by the birth of their younger sibling the subsequent birthing season, although offspring receive more maternal investment in the form of grooming and support during agonistic encounters later life and females receive disproportionately more than males (Fairbanks, 1996).

Communication in rhesus macaques involves both vocal and non-vocal methods, playing a critical role in their social interactions. Non-vocal communication, including facial expressions, body postures, and gestures, is particularly significant for interactions at close distances (Partan, 2002). A widespread facial expression in Rhesus macaques, as well as other macaque species, is the "silent bared teeth" face, typically displayed by lower-ranking or submissive individuals towards those of higher rank (Maestripieri, 1999a). Another expression, the "fear grimace," often paired with a scream, is observed in frightened animals and serves to appease or redirect aggression (Rowe, 1996). Dominant macaques may use a silent "open mouth stare" as a threat, characterized by a straight tail and a quadrupedal stance (Partan, 2002). The "present rump" gesture, where the tail is raised to expose the genitals, is another common signal in hierarchical contexts (Maestripieri, 1999a).

In terms of vocal communication, Rhesus macaques use a variety of sounds. "Coos" and "grunts" are often heard during group movements, affiliative interactions, and grooming sessions (Hauser, 1998). Sounds like "warbles," "harmonic arches," and "chirps" are typically associated with the discovery of high-quality or rare food items.

The “shrill bark,” a loud, high-pitched alarm call, is emitted in threatening situations (Lindburg, 1971). Aggressive encounters may involve vocalizations such as “screeches,” “screams,” “squeaks,” “pant-threats,” “growls,” and “barks” (Lindburg, 1971). Infants of Rhesus macaques have a distinct set of vocalizations, including “geckers,” which are harsh, staccato sounds made during weaning conflicts, often accompanied by convulsive body jerks reminiscent of a human child's temper tantrum (Lindburg, 1971; Partan, 2002).

Rhesus macaques are a common animal model used in biomedical and neuroscience research and are an appropriate species to study the effects of husbandry procedures on their welfare because of this.

2.3 Enclosures

Each enclosure at the CFM typically houses one social group. There are two types of groups at the CFM: breeding groups and weaning groups. The breeding groups contain 1 adult male and up to 12 adult females plus their offspring. The weaning groups are predominantly single sex groups of around 15 age mates that were weaned around similar times. In some male weaning groups, an older male is included within the group to be the alpha male and promote group stability. Individuals are usually weaned into their weaning group with one or more siblings from their breeding group once they weigh at least 1.5 kg and are displaying competence in being independent from their mother.

Each enclosure has a playpen with enrichment and sawdust. The playpen measures 8.04 m long x 3.35 m wide x 2.8 m height. Adjoining the playpen is a cage area that the group can access via small hatches. The cage area measures 8.0 m long x 1.5 m wide x 2.8 m. The hatches between the playpen and cage area can be closed by staff in order to separate or contain individuals into one area for husbandry procedures. Each playpen has a large window at the back allowing visual access between neighbouring groups (see figure 2.1). In addition, there are two cage areas within a cage room, allowing further visual access between two neighbouring groups. Enrichment is changed by the care staff regularly.

Figure 2.1 (A) Image of a play pen (B) Image of an adjoining cage room, monkeys have access to the play pen and cage room via hatches in the wall (C) Layout of the colony rooms. The numbered areas indicate the play pens and the grey shaded areas indicate the cage rooms

Each enclosure is kept at a temperature of 18˚C - 20˚C and lights are on between the hours of 0700hrs and 1900hrs. Monkeys are fed daily by the care staff, typically between 0800hrs and 1000hrs and their diet is decided by the Named Animal Care and Welfare Officers (NACWOs) of the centre. The diet is predominantly pellets that are designed to meet the nutritional requirements of nonhuman primates. Additionally, the pellets are supplemented with a mixture of lentils, oats, seeds, and nuts to promote natural foraging behaviour, and a variety of fruit and vegetables that change daily including carrots, cabbage, melon, bananas, and hard-boiled eggs. Water is available *ad libitum* and is accessed via a number of water dispensers located in both the playpen and cage room. The enclosures are cleaned bi-weekly where the sawdust bedding is stripped, playpen and cage room are cleaned, and new wood shavings bedding is provided.

There are 22 enclosures evenly distributed across two corridors. One corridor is at the front of the building and faces towards the Defence Science and Technology Laboratory (DSTL) site where the CFM is based. The monkeys in the first corridor will regularly see people walking past and up to the entrance of the building. Corridor 2 at the rear of the building faces the perimeter of the site; monkeys on corridor 2 are less likely to see humans walking past.

2.4 Life history and weaning at the CFM

At the CFM, the typical life history of captive macaques post-birth follows one of four distinct routes (figure 2.2). Some macaques are weaned and grouped with others of the same sex, where they will then either be supplied (figure 2.2: black line) or retained for breeding (figure 2.2: red line). Another pathway involves the direct transportation of macaques from the Central CFM from the natal group to the designated laboratory (figure 2.2: green line). Additionally, certain macaques are selected to remain at the CFM to become part of the breeding stock directly from the natal group (figure 2.2: blue line). This decision is influenced by the immediate needs of research facilities and the objectives of the breeding program. These varied trajectories reflect the operational requirements of the breeding program and the demands of the research facilities.

The CFM follows available guidelines and aims to leave individuals in their natal groups for as long as possible. At the CFM, weaning age is determined by a number of different factors including aggression within the group; number of individuals in the groups; the availability of rooms for weaning groups to move into; the age of the individual; and maternal rejection/abuse/death. Therefore, the majority of the variation of weaning ages at the CFM ranges from 12 months to 36 months. This study takes advantage of the variation to study the effects of weaning ages on macaque temperament, health, and welfare. The age at which the individuals’ were separated from their mother and placed into their weaning group was not manipulated as part of this study as conducting the large experiments necessary to establish the benefits of weaning later than current guidelines would be financially prohibitive and difficult to justify ethically. By taking an observational approach, no monkeys were used for this study which allowed a large sample size. Weaning practices at the CFM are largely independent of the characteristics for each individual monkey and are driven largely by enclosure availability. As such, the design of this study is pseudo experimental.

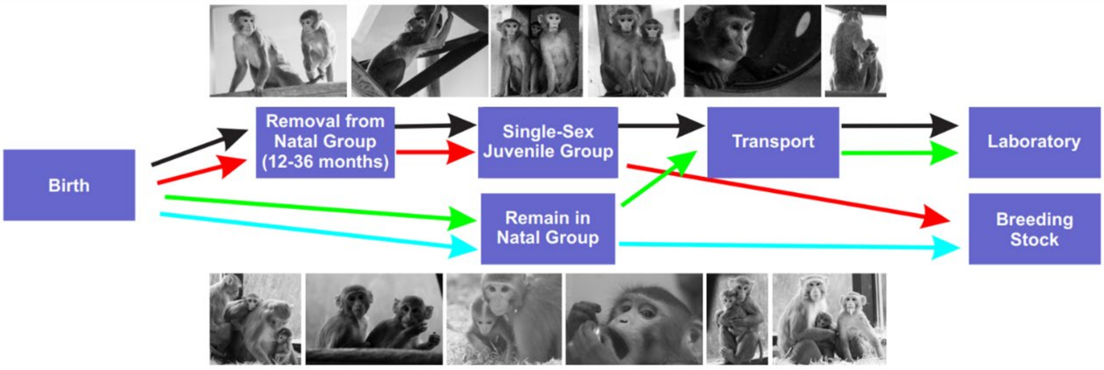

Figure 2.2 Typical life history trajectories from birth for macaques at the CFM. 1) weaning and subsequent transport to the laboratory (black line); weaning to then be retained for breeding (red line); remaining in the natal group prior to being transported to the laboratory (green line); or remaining in the natal group and being kept for breeding stock (blue line)

2.5 Annual Health Screens and individual health records

All macaques at the CFM undergo annual health screening performed by the attending veterinary surgeon (overseen by the Named Veterinary Surgeon; NVS) and care staff. Macaques are checked for various measures of physical health whilst sedated with an injection of Ketamine Hydrochloride. Measures taken include body weight, coat condition (Honess et al., 2005), body score, teeth condition, and for any apparent injuries. Additionally, blood samples are taken by the attending veterinary surgeon and samples are sent to the laboratory at MRC Harwell haematology and serum chemistry. Rectal swabs are also taken to be sent to the laboratory to test for the presence of bacterial and parasitic infections. In the past this was done with 3 swabs taken over 3 days but since 2022 is done with a single swab on the day of the health screen.

All macaques' health records from birth to death or supply are stored as paper copies and on the CFM database. Individual's health records include most measures taken from annual health screens and health measures taken outside of the health screen as well as other useful information such as training, habituation, temperament tests (Chapter 4), pedigree, involvement in any other sub threshold procedures, injuries, treatments, weaning, location changes, births, dates of removal from the colony and reasons, as well as supply location and date.

Annual health records are available for all animals that have been in the CFM since its establishment in 2003, although earlier records vary in consistency and reliability compared to records from around 2008 onwards. A large portion of the data presented in this thesis utilised these individual records (Chapters 5, 6, and 7) for recorded weights, alopecia scores (from 2008 onwards), injuries, and C*ampylobacter* incidence (see figure 2.3 for the timeline from which various datasets used in this thesis were derived). Age and weaning ages ranged differently depending on the dataset utilised and these are described in individual chapters.

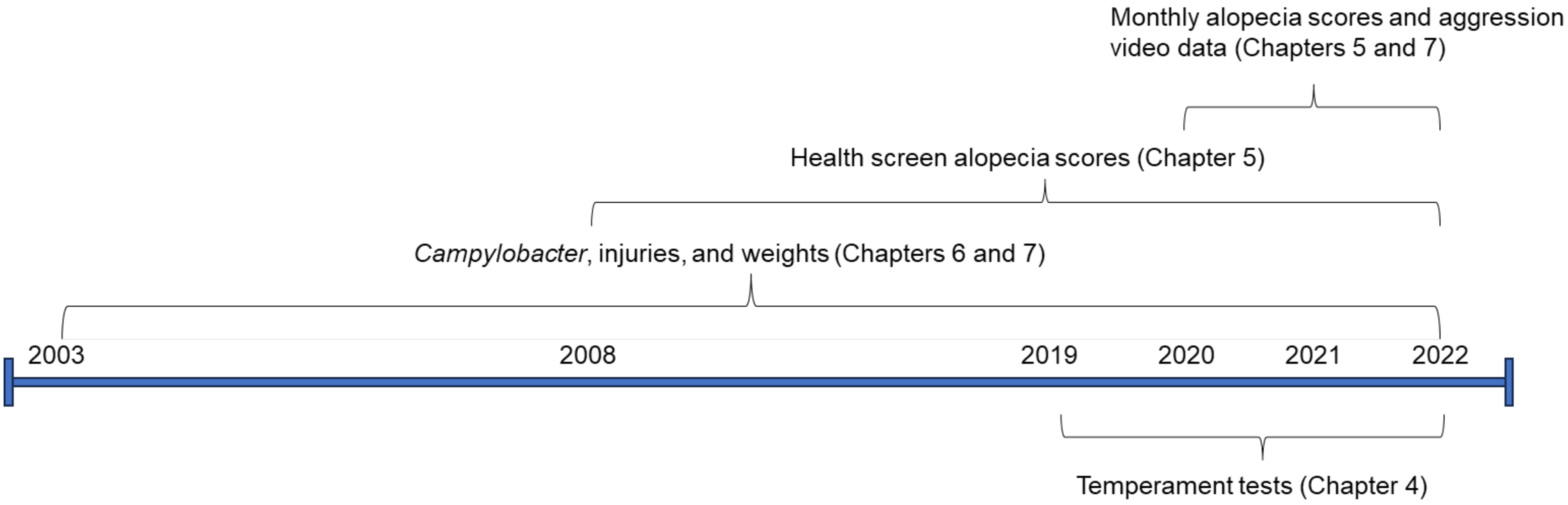

Figure 2.3 Timeline showing years each dataset presented in data chapters span.

2.6 Video data collection

Temperament testing data (Chapter 4) and aggressive behavioural data (Chapter 7) were collected using 2 HD camcorders (Sony HDR-CX625). All original videos are stored on the Newcastle University server and will be available upon reasonable request. Videos were processed using AISEESOFT (Version 9.2.38).

2.7 Statistical analysis

Analysis was conducted using RStudio (Version 4.1.1.) and MATLAB (Version R2019a). All datasheets and R scripts used to generate results presented in this thesis will be available on the Newcastle University data archive: https://data.ncl.ac.uk/

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Chapter 3. Effects of Differential Rearing of Laboratory Rhesus Macaques (*Macaca mulatta*) on Hypothalamic-Pituitary-Adrenal Axis Activity: A Systematic Review and Meta-Analysis

3.0 Chapter summary

Extensive research has explored the relationship between early-life adversity, cortisol, and the hypothalamic-pituitary-adrenal (HPA) axis in rhesus macaques. The literature presents a complex and often conflicting picture regarding the impact of early-life adversity on HPA axis activity. This inconsistency highlights the need for a comprehensive and systematic review of the available research to identify trends, variations, and areas where further investigation is required. This chapter presents a systematic review and meta-analysis analysis of 24 studies published between 1989 and 2023, encompassing various measures of cortisol from plasma, hair samples, and saliva. The majority of studies focused on cortisol levels within the first year of life, with a few examining adult responses. All studies included experimentally manipulated the early rearing environment. The results revealed a complex pattern of cortisol responses to adverse rearing conditions. In the included studies, stressors were applied later to compare the stress responses of adverse versus control reared macaques. While a similar number of studies reported higher and lower cortisol values in response to stress and basally, there were some instances of both higher and lower measurements within the same study. The most common stressor employed in the studies was separation, followed by pharmacological methods and assessments. Furthermore, the study meta-analysed cortisol responses between primates reared in adverse conditions (Peer-reared, PR) and typically reared monkeys (Mother-reared, MR). The analysis showed that PR monkeys tended to exhibit lower cortisol responses to stressors when samples were taken in the morning than afternoon, but this effect did not reach statistical significance. For basal measurements, PR monkeys had lower cortisol values in the morning, while no significant difference was observed in the afternoon. Based on the findings, several possible explanations were proposed, including variations in stress reactivity and coping strategies among individuals, developmental timing of adverse experiences, and the influence of environmental factors. In summary, this study contributes to our understanding of the relationship between adverse rearing conditions and cortisol regulation in primates. It highlights the complex nature of cortisol responses to adversity, and suggests possible explanations for the observed patterns.

3.1 Introduction

3.1.1 Background on cortisol and allostasis

Cortisol is a steroid hormone secreted by the adrenal glands in response to hypothalamic-pituitary-adrenal (HPA) axis activation and it is often used as a physiological marker of stress in nonhuman primates (Anestis, 2011). The question at the heart of this study revolves around the impact of early-life adversity, particularly maternal separation and weaning, on cortisol regulation and stress response in rhesus macaques. Stress can be defined as a disruption to homeostasis, and the HPA axis activation in response to stress functions to preserve homeostasis (Miller & O'Callaghan, 2002). Furthermore, if an organism is exposed to repeated stressors, the organism may respond by altering its typical behaviour and baseline levels of circulating hormones in response to the potential for the environment to contain further stressors – this ability to achieve homeostasis through change is termed allostasis (McEwan, 2000; Miller & O'Callaghan, 2002; Novak et al., 2013). It is well recognised that these systems that act to protect the body can also paradoxically damage the body; the price paid by the organism for utilising this function is termed allostatic load (McEwan, 2000).

Allostasis refers to the process by which an organism achieves stability through change in response to environmental challenges and stressors (Offidani et al., 2013). It involves the interaction of different physiological systems at varying degrees of activity to maintain overall homeostasis (Guidi et al., 2020). Allostasis goes beyond the traditional concept of homeostasis, which focuses on maintaining stability through constancy, by recognising the need for adaptive changes in response to dynamic conditions (Woods & Ramsay, 2007). Allostasis has been studied in various areas of research, including stress, sleep regulation, cognitive function, health outcomes, and behaviour regulation. In the context of stress, allostasis refers to the body's ability to adapt and respond to stressors by activating the hypothalamic-pituitary-adrenal (HPA) axis and other physiological systems involved in the stress response (McEwen, 1998). Chronic stress can lead to allostatic overload, where the body's adaptive mechanisms become dysregulated and fail to return to baseline, resulting in long-term physiological and psychological consequences (Guidi et al., 2020).

It is also well recognised that experiencing adversity early in life leaves individuals' particularly vulnerable for experiencing negative health outcomes later in development (Hertzman & Boyce, 2010). As such, a breadth of research has been devoted to understanding how HPA axis activity acts as a mechanism that mediates the link between early life adversity and psychopathologies later in life.

3.1.2 The Hypothalamic-Pituitary-Adrenal axis and stress response

When exposed to acute stress, the HPA axis activates to meet the demand of stress through synthesis/release of three hormones: corticotrophin-releasing hormone (CRH); adrenocorticotrophin hormone (ACTH); and a species-specific glucocorticoid, in the case of humans and nonhuman primates, cortisol (Miller & O'Callaghan, 2002). The HPA axis is a negative feedback system, the hypothalamus releases CRH, this triggers the anterior pituitary to release ACTH, the adrenal cortex then releases the glucocorticoids which inhibit further release of the other hormones (see figure 3.1). In humans the time taken for cortisol to reach peak levels in the blood is around 20-40 minutes (Pollard & Ice, 2006; see figure 3.2).

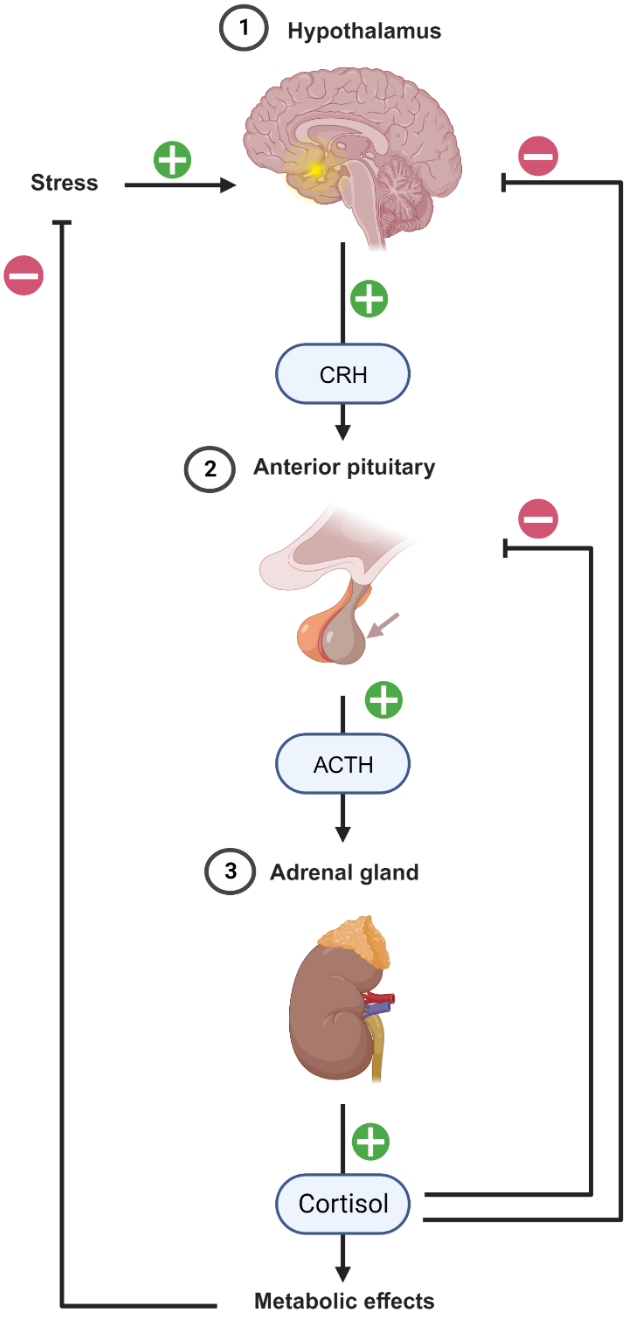


Figure 3.1. Schematic of the Hypothalamic-Pituitary-Adrenal Axis activity in response to stress. Adapted from Camila Maria Fontana, University of Padova. Created with BioRender.com.

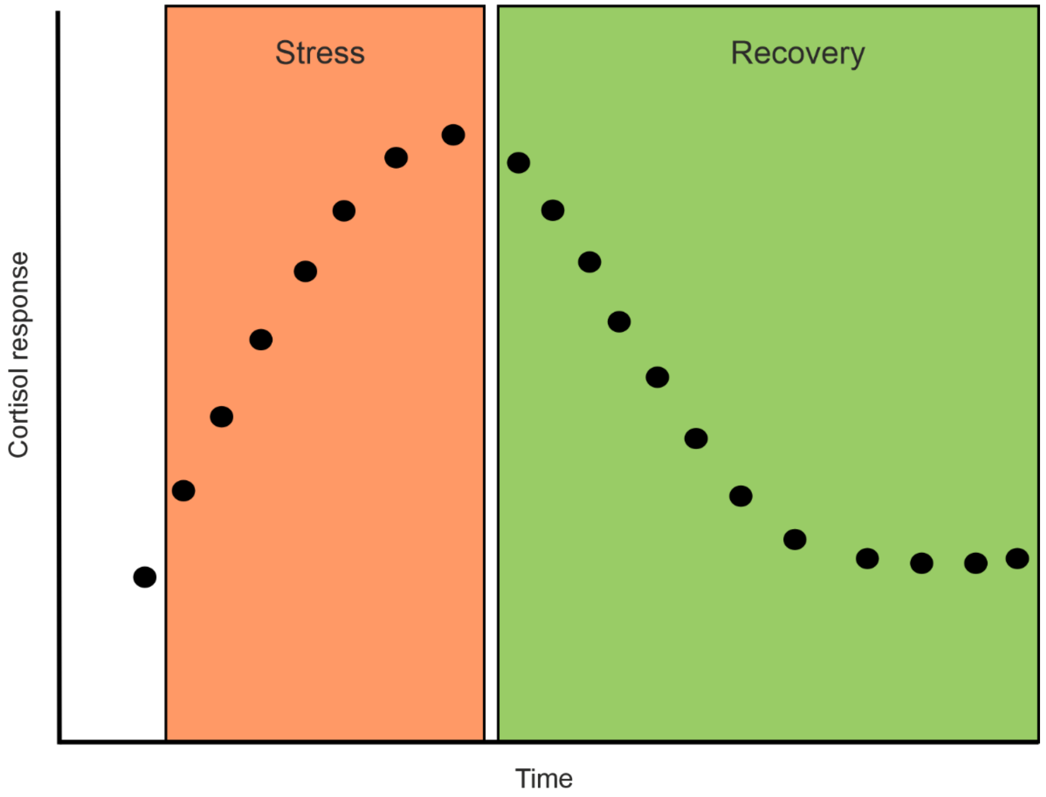


Figure 3.2. Time course of cortisol in response to a stressor. Adapted from McEwan (1998).

In addition to variation caused by stress, cortisol levels follow a circadian rhythm (see figure 3.3). The circadian rhythm of cortisol involves the natural fluctuation of cortisol levels throughout a 24-hour period. The awakening cortisol response is a distinct part of this rhythm, with cortisol concentrations increasing in the first 30 minutes after awakening and gradually dropping throughout the day (Clow et al., 2004).

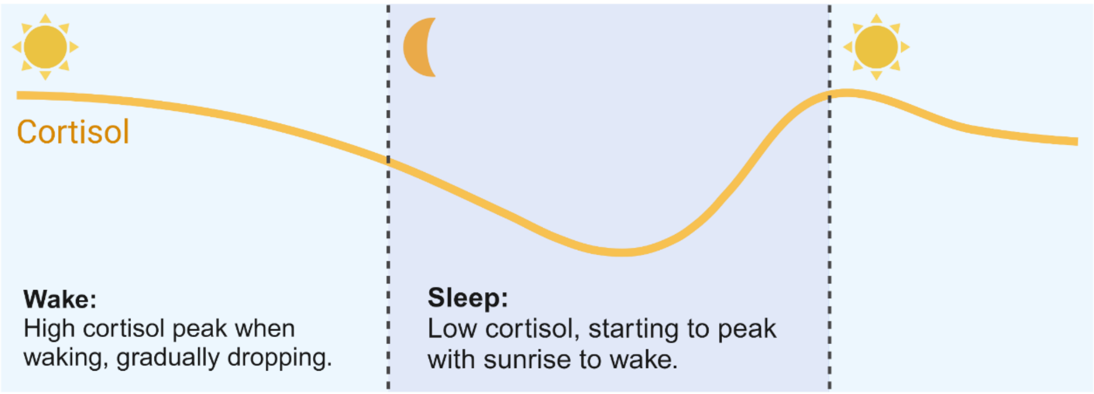


Figure 3.3. Schematic showing circadian rhythm of cortisol. Image created with BioRender.com

The HPA axis is influenced by various factors, including acute stress, trauma, and sex. Chronic stress can lead to HPA axis dysfunction and studies have shown that chronic stress induces both functional and structural adaptations within the HPA axis, resulting in long-term alterations in neuroendocrine reactivity to subsequent stressors (Ostrander et al., 2006). Furthermore, there are sex differences in the HPA axis response to stress, with females typically displaying heightened responses (Young & Korszun, 2009; Sze & Brunton, 2020). Dysregulation of the HPA axis has been implicated in various neuropsychiatric disorders, including depression. Studies have shown that individuals with depression often exhibit abnormalities in HPA axis function, such as atypical responses to the dexamethasone suppression test, higher baseline cortisol values, and an overactive response to psychological stressors (Lopez-Duran et al., 2009). Similarly, dysfunctions in the HPA axis have been reported in other mental disorders, such as bipolar disorder and schizophrenia (Murri et al., 2016).

Saliva, hair, blood, and urine samples can all be collected to measure cortisol levels. The choice of sample depends on the specific research or clinical context, as well as the desired timeframe of cortisol assessment. Saliva and hair samples offer non-invasive and convenient methods for measuring cortisol (saliva measuring acute; hair measuring chronic), while blood and urine samples provide more immediate measures of cortisol levels. These different sampling methods have also been used in research involving rhesus macaques.

3.1.3 Current state of knowledge with research involving rhesus macaques

Rhesus macaques (*Macaca mulatta*) are widely used as a model species to understand these effects as they share similarities with humans in cognition and neurophysiology (Kalin & Shelton, 1989; Frith & Frith, 2007). In humans, childhood adversity, such as maltreatment or socioeconomic disadvantage, has been found to contribute to higher allostatic load in adulthood (Widom et al., 2018; Gale et al., 2015). The effects of chronic stress and adverse experiences early in life can have long-lasting impacts on physiological functioning and increase the risk of health problems later in life (Gale et al., 2015; Finlay et al., 2023).

To model early life adversity in laboratory rhesus macaques, the early rearing environment is manipulated. The basic paradigm for the adverse rearing condition typically involves; early separation of mother and infant (usually within 72 hours of birth); receiving extensive human contact; being exposed to the rearing environment between 3-12 months; and being compared to infants exposed to typical social development i.e., reared with the mother (Novak & Sackett, 2006), although the protocols for adverse rearing conditions differ slightly between studies and laboratories. Once infants are exposed to early weaning are around 3 months of age, studies separate between slightly different adverse rearing conditions to be compared with typically reared monkeys: total isolation, partial isolation, surrogate reared, peer reared, and surrogate-peer reared (Novak & Sackett, 2006); however, some studies manipulate the adverse conditions further in an attempt to detect possible subtle differences between discreet scales of adversity e.g. by continuously re-pairing peer reared infants with novel age-mates and comparing to peer reared infants with no rotation (Rommeck et al., 2013).

The consensus on the validity of these rearing paradigms to model early life adversity stem from Harlow and colleagues early work demonstrating the negative behavioural outcomes of infant rhesus macaques that have experienced maternal deprivation (Harlow, Dodsworth, & Harlow, 1965). Studies that investigate the effects of early life adversity on HPA axis activity and allostatic load rely on cortisol samples 1) in response to an acute stressor, to understand the effects of early life adversity on stress responsivity, and 2) in the absence of a stressor, to understand how stressful the individuals perceive their adverse environment and how early life adversity may alter the basal levels of individuals typical cortisol levels. The acute stressors imposed on the infant macaques, to measure acute stress response, tend to be similar across experiments and almost always involve a separation from the rearing group (which will involve human handling). Not to be confused with rearing paradigm, these separation stressors involve separation from the natal cage (in the case of control reared monkeys) or removal from the home cage (in the case of adversely reared monkeys). Other stressors that are imposed on the subjects include pharmacological challenges and neonatal assessments.

3.1.4 Macaque rearing history and HPA axis activity

Rearing history has been found to have a significant impact on the stress response in rhesus macaques. Studies have shown that different rearing conditions, such as mother-reared, peer-reared, or surrogate-peer-reared (see table 3.1 for definitions), can lead to variations in emotional and physiological responses to stress (Raper et al., 2013). For example, surrogate-peer-reared macaques exhibited an age-dependent increase in defensive behaviours and freezing responses during stress-inducing conditions (Raper et al., 2013). Genetic factors also interact with rearing history to influence stress response in rhesus macaques. Polymorphisms in genes related to the serotonin transporter (5-HTTLPR) and the µ-opioid receptor (OPRM1C77G) have been associated with altered stress response and increased alcohol consumption in macaques with histories of early-life stress (Barr et al., 2003; Barr et al., 2007). These genetic variations can interact with environmental stressors, such as early-life adversity, to influence the incidence of depression, anxiety, and aggression (Barr et al., 2003; Barr et al., 2004; Kinnally et al., 2010). Additionally, early-life stress and rearing history can affect the development and function of the immune system in rhesus macaques. Early-life stress, such as nursery-rearing, has been shown to impact immune development and function, leading to differences in immune responses to stress or separation (Cirulli et al., 2009; Oxford et al., 2015). Furthermore, macaques reared in adverse social conditions, such as peer rearing or surrogate-peer rearing, exhibit enhanced expression of genes involved in inflammation, cytokine signalling, and T-lymphocyte activation, as well as suppression of genes involved in innate antimicrobial defences Cole et al. (2012).

Despite the extensive research conducted in this field, the existing literature presents a mosaic of findings, with some studies reporting increased basal cortisol levels in adversely reared macaques and others showing varying responses to stressors. This incongruity highlights a pressing problem: the need to synthesise and clarify the current state of knowledge regarding the effects of early-life adversity on HPA axis activity in rhesus macaques. For example some studies have reported adversely reared rhesus macaques to have higher basal levels of cortisol (Champoux et al., 1989; Dettmer et al., 2017) and higher levels in response to stress (Higley et al., 1991; Higley et al., 1992; Fahlke et al., 2000); others find the opposite of this effect (Shannon et al., 1998; Clarke et al., 1998; Barr et al., 2004b; Barr et al., 2004c), and some studies find no significant differences between mother reared and peer reared rhesus macaques (Barr et al 2004a). Therefore, it is difficult to determine if individuals who have faced adversity develop more stress responsive phenotypes or less stress responsive phenotypes. Furthermore, in one of the most extensive studies done, Capitanio et al. (2005) show evidence that HPA axis activity of adversely reared infants may have altered and possibly matured quicker in response to the environment.

To date, some groups of researchers have highlighted this issue and provided informative reviews of the literature (e.g., Novak et al., 2013; Otovic & Hutchinson, 2015). While these studies have undoubtedly expanded our knowledge, they have also left a significant gap. The disparities in findings and the absence of a cohesive synthesis of existing research make it challenging to draw definitive conclusions about the effects of early-life adversity on cortisol regulation in rhesus macaques. To navigate this complex landscape, I seek to address this critical gap by conducting a systematic review and meta-analysis of the literature. By synthesising and evaluating the available evidence, I aim to provide a more comprehensive understanding of the relationship between early-life adversity, cortisol, and HPA axis activity in this model species.

3.1.5 Aims

Initially, cortisol was planned to be used as a measure to test variation by weaning age in line with the aims of this thesis (Chapter 1). However, it was unclear what predictions one would draw given the conflicting findings published. As such, the aims of this chapter were to conduct a systematic review and meta-analysis to ascertain how differential rearing paradigms affect HPA axis activity in basally and in response to stress.

Through this approach, I intend to provide a comprehensive and cohesive synthesis of the current state of knowledge in this field. This study aims not only to address the existing gap but also to lay the groundwork for more targeted and informed research into the complex interplay between early experiences, cortisol regulation, and stress response in rhesus macaques.

3.2 Methods

All methods were described initially in detail in a protocol provided in supplementary material (Appendix 1).

3.2.1 Literature search and data collection

A PRISMA diagram for the study is available as supplementary material (Appendix 1). The literature search was conducted on 1st October 2019 using the PubMed and Scopus databases. A search for papers including the word “cortisol” or “hypothalamic-pituitary-adrenal axis” or “HPA axis”, and “rearing”, and “rhesus” returned 88 records. After removing duplicates, the total number of records were 36. A protocol for screening the returns of the literature search is available as supplementary material (Appendix 1). Briefly, papers were removed from the dataset for this study that: 1) were not complete original research papers available in the English language 2) used species other than rhesus macaques in their sample 3) did not manipulate the early rearing environment 4) did not report associations between rearing conditions and levels of cortisol concentration (plasma, hair, salivary, faecal etc.) either basal or in response to an acute stressor or both 5) did not report statistics needed to obtain an effect size for the association/ did not report sample sizes. 24 studies passed the screening procedure and were included in this systematic review (see Appendix 2 for search record).

To combine the standard errors of groups that had been split into more groups e.g. to display sex x rearing interactions, formulae provided in Baker and Nissim (1963) was used:

$$e_3^2 = \frac{1}{n(n-1)}\left[n_1(n_1-1)e_1^2 + n_2(n_2-1)e_2^2 + \frac{n_1n_2}{n}(m_1-m_2)^2\right]$$

Where n_1, n_2 are the sample size of the first and second group of a particular rearing condition, $n = n_1 + n_2$; m_1, m_2 are the means of two groups; and e_1, e_2 are the standard errors of the two groups.

All effects were converted to standardised mean differences (Cohen’s *d*) and their variance using formulae provided in Borenstein *et al*. (2009) and Pepper *et al.* (2018;

supplementary R script). Where means and their standard error/deviations were available these were converted directly to Cohen's *d*:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{Swithin}$$

Where $\bar{x}_1$ and $\bar{x}_2$ are the means of the two groups, in this case the means of the control and adverse rearing conditions respectively. *Swithin* was calculated using the formulae:

$$Swithin = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

Where *n1*, *n2*, and *S1, S2* are the sample sizes and standard deviations of the control and adverse rearing conditions, respectively.

Variance of *d* (*Vd*) was calculated using the formulae:

$$v_d = \frac{n_1 + n_2}{n_1 n_2} + \frac{d^2}{2(n_1 + n_2)}$$

Where F-Ratios were reported, these were first converted to the correlation coefficient (*r*) and then to Cohen's *d* using the formulae:

$$r = \sqrt{\frac{\mathrm{F}}{\mathrm{F} + \mathrm{d}f}}$$

Where F is the reported F-statistic and d*f* is the associated degrees of freedom.

Variance of *r* (*Vr*) was calculated using the formulae:

$$v_r = \frac{(1 - r^2)^2}{(n - 1)}$$

Converting *r* to Cohen's *d* was achieved using the formulae:

$$d = \frac{2r}{\sqrt{1 - r^2}}$$

The Variance of *d* (*Vd*) was calculated from *Vr* using the formulae:

$$v_d = \frac{4v_r}{(1 - r^2)^3}$$

The final dataset with all effect sizes contained 110 effects from 23 studies. However, sample was reduced to make comparisons easier to interpret (see below).

3.2.2 Data analysis

All analyses were conducted in RStudio (Version: 4.1.1) using the package metafor (Viechtbauer, 2010).

Rearing conditions in this review can be compared to experimental treatments (adverse versus control; see table 3.1), and the review aimed to understand the effects of treatment on the cortisol concentrations in response to acute stress/basal cortisol concentrations. See Table 3.1 for definition of rearing conditions. Briefly, rearing conditions where the individual was reared with continuous access to its mother were treated as control; all other rearing conditions were treated as adverse.

Table 3.1. Operational definitions of rearing conditions for this meta-analysis.

Rearing condition	Abbreviation	Definition
Control		
Mother-peer-reared	MPR	Individuals remain with their mothers as part of a social group.
Dyad mother-reared	MR	Individuals remain with their mothers as a dyad.
Adverse		
Peer-reared	PR	Individuals are reared with at least one other age mate but not part of a larger group. Peer-reared individuals are usually separated from their mothers within 72 hours of birth, hand-reared by a human in an incubator for around 37 days, and then placed with other identically reared age mates.
Surrogate-peer-reared	SPR	Individuals are reared by an inanimate surrogate that provides milk. In this condition, individuals tend to have limited access to peers for socialisation (usually around 2hrs per day).
Surrogate-reared	SR	Individuals are reared by an inanimate surrogate but do not have access to peer interactions.
Isolation	I	Individuals do not have access to socialise with peers and do not have an inanimate surrogate.
Other	O	In the case where the manipulations are slight variations of the above rearing conditions/do not fit any of the above definitions.

Table 3.2. Variables extracted from papers and their definitions.

Variable	Definition
Socialisation in the adverse rearing condition	Provided access to conspecifics during rearing condition (yes or no)
Age tested	The age at which cortisol samples were taken
Stressor category*	Pharmacological/separation
Sample time for basal cortisol measurement	Time in minutes between disturbance and collection of what is reported as a basal sample.
Laboratory	The name of the laboratory where the study was conducted.

*Not the rearing stressor. Separation stressor refers to separation from the home cage in these studies.

Variability between study protocols (see table 3.2) meant that actual analysis differed from the original protocol outlined in Appendix 1. Meta-analysis was performed on only the most comparable studies. As such, I conducted random-effect meta-analyses assuming heterogeneity of the true effect sizes between samples as rhesus macaques are a heterogeneous species and studies were conducted among a variety of groups and populations of macaques. Analyses were performed only where the adverse rearing condition was PR, cortisol was extracted from plasma, and macaques were infants or juveniles. Separate analyses were performed for time of day (morning or afternoon), and response test (stress or basal).

3.3 Results

24 studies published between 1989 – 2022 were included after screening. Of the 24 studies, 22 measured cortisol from plasma, three from hair samples, and one from saliva. The overlap arises from two studies: Davenport *et al*. (2003) measured cortisol from plasma and saliva; Feng *et al*. (2010) measured cortisol from hair and plasma. 15 studies measured basal cortisol and cortisol response to a stressor. Three studies measured only basal cortisol, and six only in response to stress. A similar number of studies reported the adverse rearing condition to have higher and lower cortisol values than the control (see table 3.3). Furthermore, there were a

similar number of effects that were reported to be statistically significant (10 where adverse rearing had higher cortisol values; 9 where they had lower). The majority of studies measured cortisol when the subjects were within the first year of life, one study measured cortisol between 1 – 3 years old (Davenport et al., 2003), and two studies measured adult responses (Parr et al., 2012; Zhang et al., 2016). Sample sizes ranged from 8 to 257 with large variation between studies (Mean±SD = 74.93±75.26). There were some studies that reported higher and lower measurements within the same study. Five studies reported the adverse rearing condition to have both higher and lower cortisol values in response to stress; two studies reported adverse rearing conditions to have both higher and lower basal cortisol values; one study reported both within the same study (see table 3.3).

The most common stressor employed was separation (16 studies) and separations ranged from 30 minutes to 7 days, next was pharmacological (8 studies) where monkeys were administered either dexamethasone, ACTH, or ethanol, and last an assessment (5 studies). Where time between stressor and sample collection was reported, measurements were taken between 5 – 30 minutes following a stressor. Most samples were taken in the morning or afternoon (morning: 0600hr – 1130hr; n = 11; afternoon: 1200hr – 1600hr; n = 11), with two studies taking samples in the evening (1800hr – 2000hr).

Table 3.3. Number of studies reporting adverse rearing conditions having higher or lower cortisol values in response to stress and basally than control rearing conditions.

	basal		stress	
	higher	lower	higher	lower
n	11	10	14	12

Table 3.4. Studies reporting the adverse rearing condition to have both higher and lower cortisol values than the control condition and possible reasons.

Response type	Study	Adverse rearing condition	Details	Protocol
Stress	Davenport *et al*. (2003)	SPR	Had lower values at 14 days and one month of age following a temperament assessment; had higher values at 3, 4, and 5 months of age following a separation.	Sampled both plasma and saliva, did not report time of day sample was taken nor the time between disturbance and sample collection. Adversely reared animals were separated from birth, provided an inanimate surrogate, and socialised with peers at 28 days of age. Stressors employed were 30 minute separation and assessment.
	Dettmer *et al*. (2012)	PR	Had higher values at 6, 12, and 18 months of age following a group change and lower values at 24 months	Sampled hair. Adversely reared animals were separated at birth socialised with peers at 37 days of age
		SPR	Had lower values at 6 months of age, and higher values at 12, 18,	Sampled hair. Adversely reared animals were separated at birth, provided an

Table 3.4. Studies reporting the adverse rearing condition to have both higher and lower cortisol values than the control condition and possible reasons.				
Response type	Study	Adverse rearing condition	Details	Protocol
			and 24 months of age following group change.	inanimate surrogate, and socialised with peers at 37 days of age
	Feng *et al.* (2010)	PR	Had lower values when sample was taken 15 minutes, and higher values after 30 minutes following handling stressor.	Sampled hair and plasma. All plasma samples were taken between 1330 – 1500 hours within 30 minutes of disturbance. Adversely reared animals were separated at birth and socialised with peers at 30 days of age. The stressor involved a 30 minute assessment.
	Shannon *et al.* (1998)	PR	Had lower values at 14 and 30 days old in response to a neonatal assessment, and lower values at 120 days in response to a separation but higher values at 90 and 150 days old in response to separation.	Sampled plasma between 1130 – 1430 hours and did not report the time between disturbance and sample collection. Adversely reared animals were separated from birth and socialised with peers at 37 days of age. Stressor involved a 30 minute separation.

Table 3.4. Studies reporting the adverse rearing condition to have both higher and lower cortisol values than the control condition and possible reasons.

Response type	Study	Adverse rearing condition	Details	Protocol
		SPR	Had lower values at all ages	Sampled plasma between 1130 – 1430 hours and did not report the time between disturbance and sample collection. Adversely reared animals were separated from birth, provided an inanimate surrogate, and socialised with peers at 37 days of age. Stressor involved a 30 minute separation.
	Rommeck *et al.* (2011)	PR	Had lower value in response to separation, and higher values for other stressors.	Sampled plasma between 0900 – 1600 hr and did not report the time between disturbance and sample collection. Adversely reared animals were separated from birth and socialised with peers at 30 days of age. Stressors involved ACTH administration, as well as separations for either 2 hours or 23 hours.

Table 3.4. Studies reporting the adverse rearing condition to have both higher and lower cortisol values than the control condition and possible reasons.

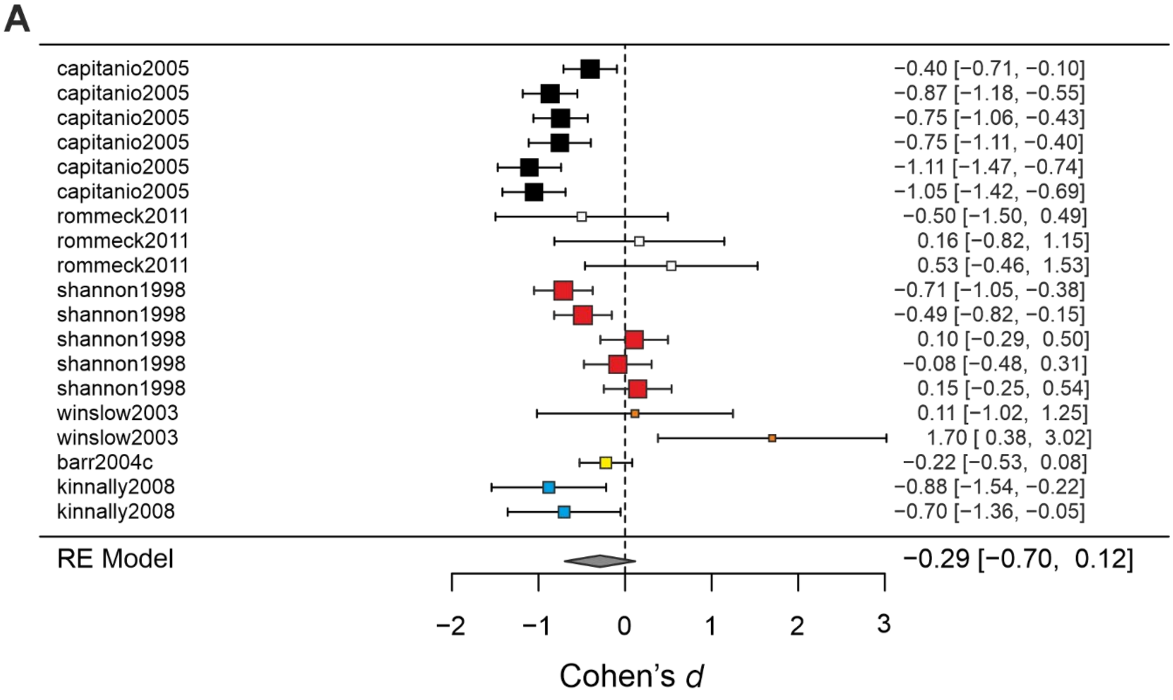
Response type	Study	Adverse rearing condition	Details	Protocol
		Other (continuous rotational pairing)	Had lower values in response to separation and higher values in response to human intruder and pharmacological stressor.	Same as PR except subjects were continuously paired with another infant, but partners were rotated once per week within a group of four infants such that infants were exposed to three different social partners throughout their stay in the nursery.
		Other (intermittent pairing)	Lower values for all stressors	Same as PR except subjects were paired for 8 hours a day, from 0700 to 1500 hours, and were then separated by an opaque divider for the rest of the time.
Basal	Clarke *et al*. (1993)	PR	Measured at 6 and then 8 months of age.	Sampled plasma between 1300 – 1500 hours and within 6 minutes of disturbance. Adversely reared animals were separated from birth and socialised with peers at 42 days of age.

Table 3.4. Studies reporting the adverse rearing condition to have both higher and lower cortisol values than the control condition and possible reasons.

Response type	Study	Adverse rearing condition	Details	Protocol
	Feng *et al.* (2010)	PR	Higher plasma cortisol at 24 months of age and lower hair cortisol at 24 and 41 months of age.	As above.

PR monkeys were estimated to have lower cortisol responses to stressors than MR when samples were taken in the morning and afternoon, although neither met the criteria for statistical significance (see table 3.5; figure 3.4).

For basal measurements, where samples were taken in the morning, PR monkeys were estimated, overall, to have cortisol values that were around 0.6 standard deviations lower than typically reared monkeys (Estimate±SE = -0.65±0.1, z = -6.55, p = <0.0001, CI(lower, upper) = -0.85, -0.46; see figure 3.5 and table 3.5). Whereas, for basal measurements taken in the afternoon, PR monkeys were estimated to have higher cortisol values, although this effect did not meet the criteria for statistical significance (see table 3.5).



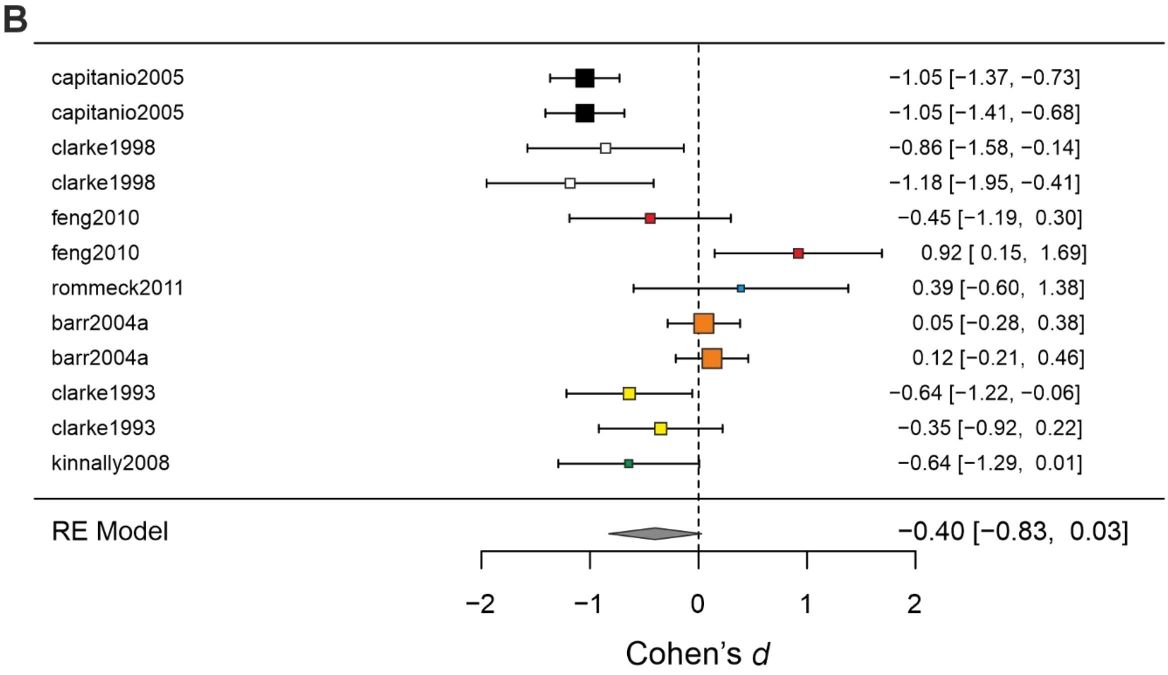


Figure 3.4. Forest plots of Cohen's *d* estimate and variance around the effect for different studies comparing infant and juvenile stress response of PR and CR A) where samples were taken in the morning. B) Where samples were taken in the afternoon. Effect size boxes weighted by sample size and coloured by study ID.

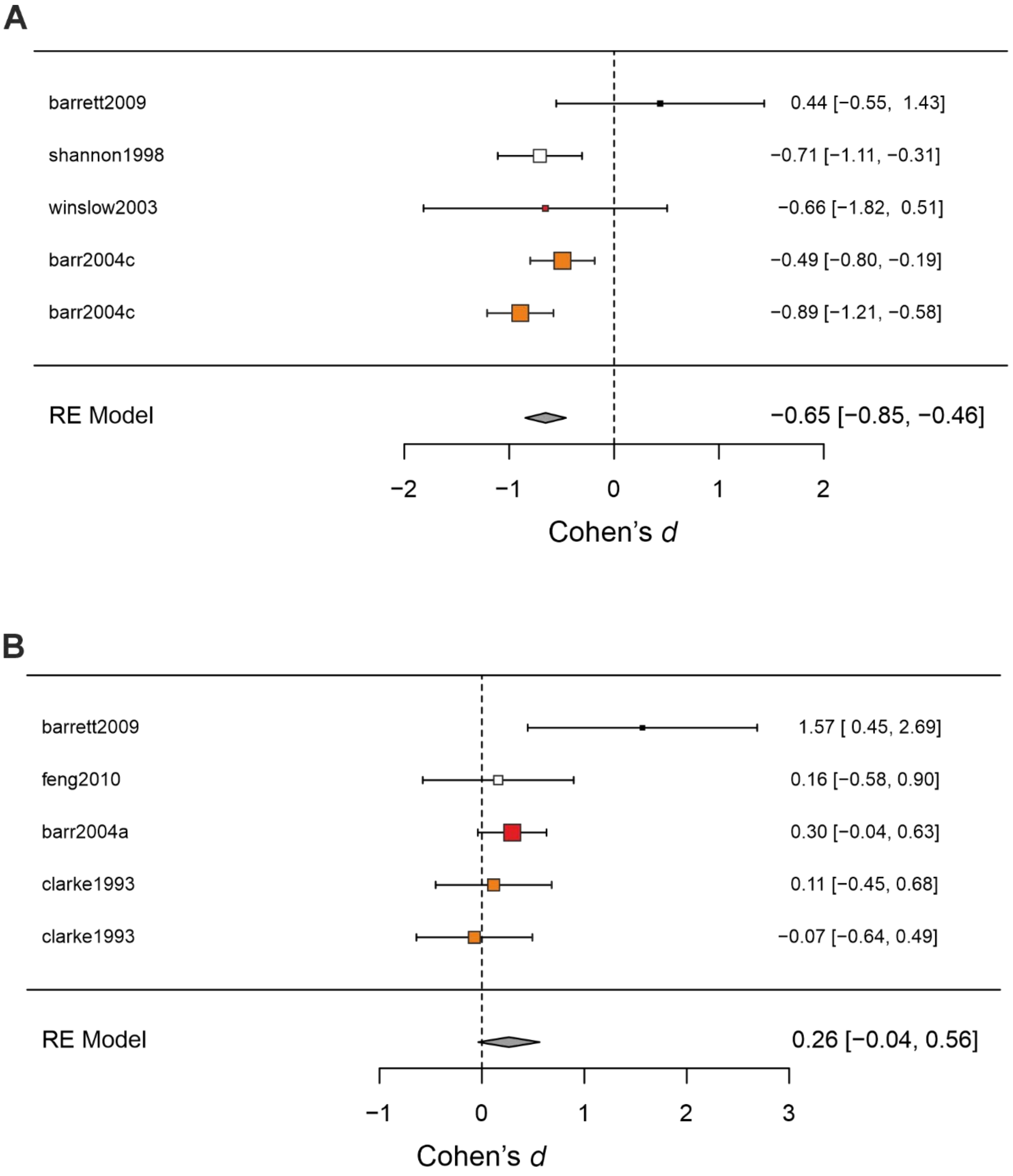


Figure 3.5. Forest plots of Cohen's *d* estimate and variance around the effect for different studies comparing infant and juvenile basal cortisol of PR and CR A) where samples were taken in the morning. B) Where samples were taken in the afternoon. Effect size boxes weighted by sample size and coloured by study ID.

Table 3.5 Coefficients of metafor models for different conditions

Model	Estimate	SE	z	p	CI(lower)	CI(upper)	*n*
Stress (morning)	-0.29	0.21	-1.4	0.16	-0.7	0.12	6
Stress (afternoon)	-0.4	0.22	-1.83	0.07	-0.83	0.03	7
Basal (morning)	-0.65	0.1	-6.55	<0.0001	-0.85	-0.46	4
Basal (afternoon)	0.26	0.15	1.72	0.09	-0.04	0.56	4

3.4 Discussion

3.4.1 Summary

The present systematic review and meta-analysis examined the relationship between adverse rearing conditions and HPA axis activity in rhesus macaques. The analysis included 24 studies published between 1989 and 2022, which collectively provided insights into the impact of adverse rearing on cortisol measurements from various sources, including plasma, hair, and saliva.

The results of the systematic review revealed mixed findings regarding the effects of adverse rearing conditions on basal HPA axis activity and HPA axis activity in response to acute stressors. Some studies reported higher basal cortisol levels in adversely reared rhesus macaques, while others found lower levels or no significant differences compared to control conditions (Cirulli et al., 2009). Similarly, the response to stressors varied across studies, with some reporting higher cortisol levels in adversely reared macaques and others finding lower levels or no significant differences.

Specifically, 10 studies reported higher cortisol values, while nine studies reported lower cortisol values in adverse-reared rhesus macaques. These divergent findings suggest that adverse rearing conditions can have complex effects on cortisol regulation, which may be influenced by factors such as the specific stressor employed, the age range of the primates, and the timing of cortisol sample collection.

3.4.2 Possible explanations for variability

Regarding the types of cortisol measurements, most studies assessed both basal cortisol levels and cortisol responses to stressors taken from blood samples. This approach provides a comprehensive understanding of the impact of adverse rearing on cortisol regulation. However, it is noteworthy that a subset of studies focused exclusively on either basal cortisol or cortisol response to stress, limiting the ability to draw definitive conclusions about the overall effects of adverse rearing. The variability in the results may be attributed to several factors, including the specific rearing conditions, age at testing, and the type of stressor employed. Furthermore, it was not possible to identify how the sample used may be more or less sensitive to detect chronic or acute stress between rearing conditions as nearly all studies used plasma samples. There are a number of reasons this may have been preferred (see table 3.6 for pros and cons of each sample type).

Table 3.6. Pros and cons for different sampling matrices.

Sample	**Pros**	**Cons**
Plasma	Immediate measure.	Invasive and can induce a stress response.
	Commonly employed.	Limited sampling frequency
Hair	Long-term measure for chronic assessment	Limited temporal resolution. Does not capture short-term fluctuations
	Non-invasive.	Potential external contamination.
	Cortisol is incorporated into hair as it grows, making hair cortisol levels stable and less susceptible to short-term fluctuations.	May not be suitable for all individuals. Alopecia is common in captive rhesus macaques (see Chapter 5).
Saliva	Non-invasive.	Saliva samples can be affected by oral health conditions.
	Easier than blood to collect at multiple time points.	Potential contamination.
	The rate of equilibrium between blood and saliva for cortisol is very fast.	Training required for primates to chew on a swab.

The protocols for adverse rearing conditions differed slightly between studies and laboratories, which may have introduced variability in the results. For example, Rommeck *et al*. (2011) continuously re-paired peer reared infants with novel age-mates, Capitanio *et al.* (2005) had different housing conditions for different PR groups, and Champoux *et al*. (1989) reared the adverse group in isolation. Furthermore, the age at which the adversely reared group were separated from their mothers differed between studied. In most studies, infants were separated from birth, however, Parr *et al*. (2012) separated the adversely reared group at 18 months of age, Fahlke *et al.* (2000) separated infants at six months of age, and Sanchez *et al*. (2005) separated infants between three and six months of age. Additionally, the included studies used different methodologies and measured cortisol levels in various biological samples, such as plasma, hair, saliva, and faeces. This variability in methods and sample types, meant I had to deviate from our original analysis protocol (Appendix 1), focusing instead on meta-analysing a small number of comparable studies, and may have contributed to the mixed findings observed.

Several possible explanations can be considered to account for the diverse findings observed in the relationship between adverse rearing conditions and cortisol levels. Adverse rearing conditions encompass a wide range of factors, such as social deprivation, maternal separation, weaning, and environmental stressors. The variations in the specific conditions experienced by primates across different studies may contribute to the divergent cortisol responses observed. Factors such as the intensity, duration, and timing of adverse experiences may influence the physiological stress response, leading to variations in cortisol levels.

Furthermore, the age at which adverse rearing occurs can significantly impact cortisol regulation. Most studies in this analysis focused on measuring cortisol within the first year of life where infants were separated shortly after birth, indicating a critical period for the development of stress reactivity. The early postnatal period is characterised by heightened vulnerability and sensitivity to environmental influences, potentially resulting in long-lasting effects on the stress response system. As only 2 studies measured HPA axis activity in adulthood (Parr et al., 2012; Zhang et al., 2016), there is very little evidence of long-term effects of adverse rearing on HPA axis dysregulation.

Individual differences likely play an important role in individual variations in response to adverse rearing conditions. Factors such as genetic predispositions, temperament, and coping mechanisms can influence the degree of cortisol dysregulation. Some individuals may be more resilient and demonstrate adaptive responses to adverse experiences, leading to normalised cortisol levels over time. Conversely, others may be more susceptible to the effects of adverse rearing, resulting in sustained alterations in cortisol regulation. There is some evidence that this may be transmitted intergenerationally (Kinally & Capitanio, 2015) and it is likely there are genetic differences between animals from different colonies/laboratories.

The timing of cortisol sample collection also emerged as a crucial factor as, depending on the time of day animals were sampled, PR were estimated to have both higher and lower basal cortisol. The majority of samples were taken in the morning (between 0600 – 1200 hours) or afternoon (any time after 1200 hours), with a few studies collecting samples in the evening. These different time points may have implications for interpreting cortisol levels, as the diurnal variation in cortisol secretion can influence the results. Future studies should consider standardising the timing of sample collection to enhance comparability across studies.

The most frequently employed stressor in the included studies was separation, ranging from brief separations to more prolonged periods. Other stressors, such as pharmacological interventions and assessments, were also utilised in a subset of studies. Different stressors, such as separation, pharmacological interventions, or assessments, may elicit distinct physiological and emotional responses, leading to different cortisol profiles. The duration, intensity, and controllability of stressors can also influence the magnitude and direction of cortisol changes. Therefore, the specific stressor employed in each study may contribute to the discrepancies observed in cortisol levels between adverse-reared and control groups. Unfortunately analysing the effects of different types of stressors was not possible due to the small sample size.

3.4.3 Meta-analysis

The meta-analysis conducted in this review focused on estimating the effects of adverse rearing conditions on cortisol levels. The results indicated that peer-reared (PR) monkeys tended to exhibit lower cortisol responses to stressors compared to mother-reared (MR) monkeys when samples were taken in the morning and afternoon. However, these differences did not reach statistical significance. For basal cortisol measurements taken in the morning, PR monkeys showed significantly lower cortisol values compared to typically reared monkeys. In contrast, for basal cortisol measurements taken in the afternoon, PR monkeys exhibited higher cortisol values, although this effect was not statistically significant. These findings suggest that adverse rearing conditions may impact diurnal cortisol rhythms and basal cortisol levels, albeit with variations depending on the time of day.

3.4.4 Limitations

It is important to acknowledge the limitations of the current review. The wide variation in sample sizes, age ranges, and methodologies across studies introduces heterogeneity and potential bias into the meta-analysis. Additionally, the complex interplay of genetic, environmental, and individual factors influencing cortisol regulation warrants further investigation. Some studies, for example Barr *et al*. (2004c), examined the interaction between rearing condition and rh5-HTTLPR polymorphism on HPA axis activity. For this analysis effects of different groups were pooled together. As such, I could not examine potentially important gene x environment interactions. Furthermore, this study did not analyse potentially important sex differences. This was because some studies did not report sample sizes for sex or sex was pooled together in figures from which data were extracted.

The study conducted a systematic literature review, but it is important to acknowledge the potential for publication bias. It would have been beneficial to address how publication bias might have influenced the findings and discuss any efforts taken to mitigate this bias, such as searching for unpublished studies or including studies from non-English sources.

Another limitation is there may be non-independence arising from having multiple effects from the same lab. While study ID was included as a random effect in this meta-analysis, providing some control for this issue, future studies should aim to quantify variations between sampling populations.

3.4.5 Future directions

Future studies should aim for greater standardisation in methodologies, including sample collection and analysis techniques, to improve comparability across studies. Consistency in stressor types, duration, and timing of sample collection would enhance the ability to synthesise findings and draw robust conclusions.

Given the ethical implications of adverse rearing practices, future studies should include comprehensive assessments of animal welfare throughout the study. Incorporating measures of behavioural, physiological, and psychological well-being would allow for a more comprehensive evaluation of the effects of adverse rearing conditions and help guide ethical considerations in primate research. This should be reported transparently in all studies (Pomerantz et al., 2022).

Furthermore, where colonies have ageing populations of macaques that have previously been exposed to differential rearing conditions, follow-up studies aiming to understand the potential long-term effects of differential rearing on HPA axis activity would be valuable, as this is currently lacking. Although, due to the body of evidence of the adverse effects of early weaning, guidelines should prioritise the prevention and mitigation of adverse rearing conditions to promote optimal cortisol regulation and overall health.

3.4.6 Conclusion

In conclusion, the complex interplay of adverse rearing conditions, developmental factors, individual differences, stressor types, timing of sample collection, and methodological variations collectively contribute to the diverse findings observed in the relationship between adverse rearing and cortisol levels.

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Chapter 4. The Association between Weaning Age and Neophobia in Captive Rhesus Macaques (*Macaca mulatta*)

4.0 Chapter summary

Evidence is clear that very early weaning or adverse rearing conditions have negative and long-lasting consequences on behaviour e.g., evidence shows that macaques reared by an inanimate surrogate were more avoidant or neophobic of novel objects compared to mother reared counterparts and this persisted up to two years of age. However, no data exist on the potential benefits of delaying weaning beyond current guidelines. I tested the prediction that a later weaning age would correlate with reduced neophobia. Macaques underwent novelty tests consisting of three two-minute phases (one novel food and two novel objects). The monkeys in this study were weaned macaques at the Centre for Macaques, a UK breeding centre that supplies monkeys to UK neuroscience. All monkeys had been permanently separated from their mothers into same-sex peer groups and were tested once between 2019 – 2021, close to their 3rd birthday. I measured latencies to approach/interact with the novel items; time spent hiding; escape attempts; and time spent in the freeze position. Principal component analysis revealed one component relating to neophobia which had positive loadings for latencies, time spent freezing and hiding. This principal component was the outcome variable in a linear model that included weaning age and sex as predictors. I found, contrary to our prediction, that a later weaning age correlated with increased neophobia. Does this mean that later weaned monkeys are indeed more neophobic? Perhaps there is a sensitive window where monkeys are more suggestible to removal and any later is detrimental. However, I cannot draw this conclusion from these data due to a problem with the study design. That is, later weaned monkeys would have spent less time in their new group prior to testing, as relocation can be a major stressor for captive macaques, this result could suggest short term effects of weaning on neophobia. More research is needed to disentangle these possible effects.

4.1 Introduction

4.1.1 What is temperament?

Stevenson-Hinde *et al*. (1980) wrote “Anyone who works with primates, or for that matter many other species, becomes conscious of individual differences”. These individual differences were once generally disregarded or considered noise around an adaptive mean, likely because of traditional optimality approach, which tends to consider variation as nonadaptive. (Stevenson-Hinde et al., 1980; Francis, 1990, Stevenson-Hinde & Hinde, 2011, Coleman, 2020). The history of animal personality research can be traced back to the early 20th century, with early studies focusing on the behaviour of primates (Kinnaman, 1902a, b; Crawford, 1938; Stevenson-Hinde & Zunz, 1978; Stevenson-Hinde et al., 1980a, b; Higley & Suomi, 1989; Schenider et al., 1991). Researchers became interested in animal personality because they observed that animals within a given population show consistent behaviour across situations and contexts. This led to questions about why such behavioural inflexibility evolved and what mechanisms might underlie the phenomenon (Whitham & Washburn, 2011). However, it wasn't until the 1990s that the concept of animal personality gained widespread attention. In the past, the noticeable differences between animals missed any acceptable framework by which to measure it. As such, the recent surge since the 1990s result of cumulative evidence collectively morph into testable framework and the reviews of this evidence that gained significant interest (Gosling & John, 1999; Gosling, 2000; Jones & Gosling, 2005; Gosling, 2008; Freeman & Gosling, 2010; Whitham & Washburn, 2011).

Since then, research has been conducted on a wide range of animal species, including fish, birds, and mammals, while expanding and flourishing in numerous disciplines, including behavioural ecology, developmental psychobiology, genetics, and comparative psychology. Now these individual differences are considered interesting and important to quantify in their own right as personality research has led to the development of a clear theoretical framework, supported by empirical evidence (Stevenson-Hinde et al., 1980; Gyuris et al., 2008; Gosling, 2008; Weiss, 2017; Mary Ley & Bennett, 2007; Highfill et al 2013; Chira, 2014; Blaszczyk, 2019; Weiss & Gartner, 2016, Coleman, 2017; Coleman, 2020).

These individual differences are referred to as, often interchangeably, temperament, personality, or behavioural syndromes (Capitanio, 2011; Coleman, 2020).

Temperament or personality can be thought of as a biological predisposition to the way an individual responds to a stimulus that is relatively consistent across time and context (Stamps & Groothuis, 2010; Coleman, 2020), and will herein be referred to as temperament. There is evidence that genetic variation can affect information processing in the brain individual differences in brain function, which, in turn, shapes behaviour and risk for psychopathology (Hariri & Weinberger, 2003; Bogdan et al., 2012; Hyde et al., 2013; Hyde, 2015). Furthermore, there is evidence that genetic polymorphisms can shape personality traits. For example, polymorphisms of monoamine neurotransmission, such as dopamine receptor D4 and SERT promoter region polymorphisms, have been found to be associated with personality traits such as novelty seeking, extraversion, neuroticism, and harm avoidance (Pogue-Geile et al., 1998; Riese et al., 2006; Calapoglu et al., 2010; Inoeu-Murayama et al., 2011). Although, gene x environment interactions cannot be discounted as evidence suggests that developmental plasticity can contribute to individual differences in a variety of traits. For example, Cordova-Palomera *et al*. (2014) found, in their study of human twins, that differences in birth weight can lead to differences in working memory and epigenetic signatures in IGF2 and related genes.

Temperament components in humans have been shown to be heritable with around 40% of variability due to genetics (Jang et al., 1996; Vukasovic et al., 2015). Similarly, evidence has shown temperament to be somewhat heritable in macaques (Williamson et al., 2003; Sullivan et al., 2011; Brent et al., 2014; Arnaud et al., 2017). Heritability estimates of temperament from free-ranging rhesus macaques range from 0.14-0.35 (Brent et al., 2014) and from 0.23-1.0 in captive macaques (Williamson et al., 2003; Rogers et al., 2008; Fawcett et al., 2014). The close relationship between mother and offspring is thought to create a mechanism for transmission of behavioural and psychological similarities from mother to offspring (Suomi, 1999; Sullivan et al., 2011).

In humans, there are several theoretical frameworks for human personality. One of the most widely used frameworks is the five-factor model, which proposes that human personality can be described in terms of five broad dimensions: openness, conscientiousness, extraversion, agreeableness, and neuroticism (five factor model; McCrae & Costa, 1987; Dingman, 1990; Tatalovic-Vorkapic et al., 2015; Simpson et al.,2019). Other theoretical frameworks include Happenstance Learning Theory to identify the constructs of human agency, hardiness, and proactive personality for their effectiveness in facilitating resilience during transitional and stressful circumstances (Greenleaf, 1970); the biopsychosocial model has been used to study the relationships between health outcomes and balancing work and family demands (Lee et al., 2002); as well as human ecology, systems theory, and social exchange theory (Ndofirepi et al., 2022). The five-factor model of personality is widely accepted within the field of psychology as an empirically verified and theoretically sound framework (Cooper et al., 2012).

There are several theoretical frameworks for animal personality research. Primate models of temperament seem to align with those from the human literature (King & Jose Figueredo, 1997). For example, The Hominoid Personality Questionnaire (HPQ: Weiss et al., 2011) has been used in studies of nonhuman animals. Studies in rhesus macaques that have assessed temperament using ratings from care takers that are familiar with the animals have found similarities to humans in the structure of temperament, with rhesus macaques reported to have six components: Confidence, friendliness, dominance, anxiety, openness, and activity (Weiss et al., 2011). Furthermore, there is evidence that monkeys undergo robust ontogenetic shifts in gaze following across early development, adulthood, and aging that are similar to those of humans (Rosati et al., 2016). Evidence suggests these traits emerge as young as 3 – 7 months of age (Sullivan et al., 2011; Simpson et al., 2019) and are correlated with subjective well-being (Simpson et al., 2019). The framework used to assess personality will typically be informed by the research question. Some theoretical frameworks focus on the ecology and evolution of animal personality (Reale et al., 2010; Gherardi et al., 2012; Blaszczyk, 2019) and Tinbergen's four questions model have been used as frameworks to study animal personality (Santana, 2023).

The shy-bold continuum is recognised as one theoretical framework of personality. A highly conserved axis of natural individual behavioural variation found in every study system it has been tested in to date including humans, primates, fish, birds, and marine predators (Coleman, 2020; Schapiro & Coleman, 2022), The shy-bold continuum refers to individual variation in their propensity to take risks and has been used to study consistent individual personality in a variety of contexts, including foraging behaviour, risk responsiveness, and fitness.

4.1.2 Common methods to assess temperament in captive primates

There are a number of ways to quantitatively assess the temperament of animals: typically, temperament is assessed either through standardised tests where behaviour is quantified in response to a stimulus, or through trait ratings where caretakers who have experience with the animal rate them on a Likert scale from a list of adjectives relating to different aspects of temperament (see Freeman & Gosling, 2011; Freeman et al., 2011; Coleman & Pierre, 2014 for reviews of the various methods). Behavioural tests are considered more objective as the technician/researcher can quantify overt behaviours in response to the stimulus which can make comparisons between individuals easier. However, subjective trait ratings by caretakers that have experience with the individuals have proved valid and reliable while being less time intensive than behavioural coding. Freeman, Gosling, and Schapiro (2011) review the pros and cons of the various methods in more detail. Ultimately, the method employed to assess temperament will depend on the resources and constraints of the facility.

Trait ratings of personality and well-being can be less intensive and require much less time than behavioural coding methods. For trait ratings to be feasible, the rater needs to have experience with the individuals they are rating and know them well – which is more than likely the case for technicians that work day in and out with the animals in their care.

These methods typically require the rater scoring the individual on a Likert scale from a list of adjectives relating to different aspects of temperament. A study by Robinson *et al*. (2021) employed 3 questionnaires:

- To assess welfare that included questions relating to the animals quality of life such as social relationships, social environment, hair loss, dominance status etc. (Robinson et al., 2016,2017; the 16-item animal welfare survey can be found at https://www.drlaurenrobinson.com/surveysdesigned/)

- To assess subjective well-being which included questions such as how happy is the animal, how happy would the rater be if they were that animal for a week etc. (King & Landau, 2003; Weiss et al., 2006; the 16-item animal welfare survey can be found at https://www.drlaurenrobinson.com/surveysdesigned/)

- To assess personality/temperament – the Hominoid personality Questionnaire where raters are asked to rate animals based on adjectives such as friendly, confident etc. (Weiss et al., 2009; Weiss et al., 2017; the 16-item animal welfare survey can be found at https://www.drlaurenrobinson.com/surveysdesigned/)

The key benefit of trait rating methods is that they can also be used to assess for positive indications of welfare.

Another way to assess temperament is through the use of standardised tests undertaken in the animals' home or in a novel environment, where their responses to a stimulus are recorded. Different tests can be performed depending on the aspect of temperament of interest. For example, in the human intruder test (Kalin & Shelton, 1989), the stimulus is a novel human. Human intruder tests take place in a controlled setting and involve an unfamiliar human entering. A human intruder test typically involves 3 phases:

1. Back to the monkey.
2. Profile phase: the intruder presents its profile to the monkey and does not look at it.
3. Eye contact phase: the intruder makes eye contact with the monkey for the duration of the phase.

The human intruder test typically measures reactiveness and fearfulness in response to a potentially threatening stimulus (the intruder). Measures taken usually include cooing, which is usually seen more in the alone phase (back to monkey) with no stimuli and is like a call for help. Freezing is predominant in the profile phase thought of as detection avoidance and direct aggression as an appropriate response to the eye contact phase (Fairbanks & Jorgensen, 2011). The magnitude of these measures is considered to reflect anxiety experienced by the individual. Human intruder tests have been shown to produce reliable trait-like differences that are predictive of future performances later in life (Kalin & Shelton, 1998; Kalin, 2001). Furthermore, these measures have been pharmacologically validated (Kalin et al., 1998; Kalin et al., 2005; Kalin et al., 2007).

It is important to note that the human intruder test can be viewed as particularly invasive, and the technician/researcher is encouraged to explore if other strategies are feasible, for example, Bethell *et al*. (2019) suggest a dot-probe task that measures response slowing and inhibition as a sensitive and less invasive alternative.

One of the most common tests that have been performed in nonhuman animals are novel object tests. When performing novel object tests, as the name suggests, novel objects are added either to the individuals home environment or the animal is taken to a testing area and is presented with different novel stimulus (Coleman & Pierre, 2014). Common measures taken during these tests include latencies to approach and interact with novel food and objects, exploration of the novel objects, alongside behavioural indicators of affective states. Studies have found latencies to approach and inspect to be positively correlated within individuals for different types of novel objects – indicating that indeed, response disposition is being measured. Furthermore, these measures are typically independent of escape attempts and locomotion indicating that the test reveals differences in different dimensions of temperament. With response to the objects relating to neophobia if avoided and other measures such as locomotion indicative of anxiety (Fairbanks & Jorgensen, 2011).

4.1.3 Novel object tests and neophobia

Novel object tests reveal an individual's disposition on the shy-bold continuum. Kagan *et al.* (1988) studied this variation in children and the authors labelled children who responded more negatively to novel situations as 'behaviourally inhibited'. Behavioural inhibition in children was characterised by longer latencies to approach an unfamiliar person or object along with behavioural displays of distress such as crying (Garcia Coll et al., 1984); many studies in animals has been modelled on this early work (Fairbanks & Jorgenson, 2011). The term behavioural inhibition has alternatively been labelled in the literature as reactivity, timidity, fearfulness, anxiety, introversion, shyness, or neophobia (Higley et al., 2011). Herein, I will use the term neophobia to describe an individual's tendency to approach or avoid novel objects, people, or situations.

Temperament in humans has been shown to correlate with later outcomes such as academic and career success, and health and well-being later in life (Poropat, 2009; Seibert & Kramer, 2001; Sutin et al., 2016; Strickhouser et al., 2017). Work in humans has shown that children that score high on neophobia are more predisposed to later negative health outcomes such as anxiety, depression, and other psychopathologies (Hirshfeld et al., 1992; Rosenbaum et al., 1993; Schwartz, Snidman, & Kagan, 1999, Coleman, 2020). Similar results have been found in rhesus macaques, for example, Gottlieb *et al*. (2018) found macaques that were rated as more nervous, gentle, and vigilant to be more at risk of having diarrhoea.

Neophobia is potentially detrimental to the welfare of laboratory rhesus macaques. This fear response can lead to increased stress and anxiety when these animals are exposed to novel situations, which is a common occurrence in research settings. Such heightened stress not only affects their physiological health, potentially altering immune function (Lubach et al., 1995), but also their psychological well-being, often manifesting in behavioural issues (Vandeleest et al., 2011). In the context of neuroscience research, adaptability is crucial as macaques are frequently required to adjust to new handlers, environments, and experimental protocols. A high level of neophobia complicates this adjustment process, potentially leading to longer acclimatisation periods and impacting the validity and reliability of research data due to the animals' stress responses (Schapiro & Hau, 2023). Moreover, the training and handling of neophobic macaques may become more challenging (Coleman et al., 2005; Fox et al., 2021), making routine care and experimental procedures more complex and time-consuming. From a research perspective, the presence of neophobic behaviours introduces variability and confounding factors that can skew research results, as these behaviours might interfere with the animals' natural responses in experimental settings. Ethically, ensuring the well-being of animals used in research is paramount, and exposing neophobic macaques to high-stress situations raises significant concerns about animal welfare. Therefore, understanding and mitigating neophobic tendencies in laboratory rhesus macaques is essential for their well-being, the safety and efficiency of their handling and training, and the integrity and ethical conduct of the research.

4.1.4 The importance of quantifying temperament for captive primate management and welfare

There are a number of non-mutually exclusive challenges that facilities housing rhesus macaques for research face where assessing temperament can be valuable (see table 4.1 for summary of findings where temperament can be important for colony management).

Table 4.1. Summary of literature findings assessing temperament that are useful for colony management.

Predictive value	Reference	Findings	Species
Injuries	Boyce *et al*. (1998)	Inhibited monkeys received more injuries during a relocation stressor where the group moved to a smaller enclosure.	*Macaca mulatta*
	Robinson *et al*. (2018)	Monkeys rated higher on confidence and anxiety were less likely to receive an injury.	*Macaca mulatta*
	Doelling *et al*. (2021)	Monkeys rated as friendly received fewer injuries in the breeding season; monkeys rated higher on openness received more injuries in the non-breeding season.	*Macaca fuscata*
Developing abnormal behaviour	Vandaleest *et al*. (2011)	Monkeys rated as nervous or gentle and reared in isolation with the mother were more likely to develop stereotypies than similarly rated monkeys reared in more typical conditions.	*Macaca mulatta*
	Gottlieb *et al*. (2013)	Animals rated as low on gentle, active in response to human intruder, and bold on novel object contact expressed more stereotypic behaviour.	*Macaca mulatta*
	Gottlieb *et al*. (2015)	Bolder monkeys expressed more stereotypic behaviour.	*Macaca mulatta*
Susceptibility to inadequate health status	Capitanio (1999)	Monkeys rated as highly social had a slower onset of Simian Immunodeficiency Virus compared to low social monkeys.	*Macaca mulatta*

Table 4.1. Summary of literature findings assessing temperament that are useful for colony management.

	Gottlieb *et al*. (2018)	Monkeys that were rated as nervous, gentle, vigilant, and not confident were more at risk of experiencing chronic diarrhoea.	*Macaca mulatta*
Maintaining stability in groups	McCowan *et al*. (2011)	Males that were rated as equable and had high social power were more successful at conflict intervention than males with high social power that were rated as bold or excitable.	*Macaca mulatta*
Response to training	Coleman *et al*. (2005)	Inhibited monkeys were more difficult to train on a simple target task than explorative monkeys.	*Macaca mulatta*
	Fox *et al*. (2021)	Infants that scored high on behavioural inhibition were less likely to take a food reward from a human later in life.	*Macaca mulatta*
Response to husbandry procedures	Waitt *et al*. (2002)	Monkeys that were rated as friendly had more positive interactions with animal caretakers and displayed less behavioural indicators of anxiety in response to cage cleaning.	*Macaca arctoides*
Formation and maintenance of bonds in group housing	Weinstein & Capitanio (2008)	Monkeys rated as high on adaptability and equability in infancy associated more with individuals that were rated similarly as yearlings.	*Macaca mulatta*
	Weinstein & Capitanio (2012)	Relationship stability in pair housed monkeys from 1-2 years old was predicted by being rated	*Macaca mulatta*

Table 4.1. Summary of literature findings assessing temperament that are useful for colony management.

		similarly high on equability in infancy.	
Compatibility for pair housing	McMillan *et al*. (2003)	Pair housed monkeys with similar temperaments were more compatible and engaged in more positive social behaviours.	*Macaca mulatta*
	Baker (2010)	Females that were similarly aggressive towards caretakers, and males that were similarly fearful towards caretakers, were more compatible when paired	*Macaca mulatta*
	Capitanio *et al*. (2015)	Female pairings were more successful based on similar temperaments when temperaments were assessed up to 10 years prior to pairing. Male pairing success rate was higher when they had low mean values of gentle and nervous.	*Macaca mulatta*
	Pomerantz & Capitanio (2021)	Pairs with similar emotionality scores displayed more affiliative behaviour and those with similarities in ratings of nervousness were less dominant/submissive to one another.	*Macaca mulatta*

Whilst temperament can be powerful for predicting outcomes relating to management, it is important to bear in mind that there are factors which can affect how individuals respond to stimuli in their environment i.e., temperament (Gottlieb et al., 2013). For example, prenatal exposure to stressful conditions can influence how emotionally responsive the infant will be (Herrington et al., 2016). Postnatal exposure to atypical rearing conditions can also influence the temperamental attributes of an individual (Timmermans et al., 1994; Gottlieb et al., 2013).

4.1.5 Developmental influences on temperament

Although the definition of temperament suggests that it is consistent and stable across time and contexts, there is a growing body of evidence that suggests that personality can change over development. Cross-sectional and longitudinal studies have shown that personality traits can change in adulthood, challenging the assumption that personality traits are stable over time (Staudinger & Kunzmann, 2005; Neyer, 2006; Roberts & Mroczek, 2008; Feichtinger & Honer, 2015; Kandler et al., 2015; Allemand & Fluckiger, 2017; Geukes et al., 2017; Lenhausen et al., 2020; Bleidorn et al., 2021).

Furthermore, there is a rich repertoire of literature documenting the effects of early life experience on individual differences later in life. Childhood adoption, childhood maltreatment, negative early-life experiences, childhood abuse, exposure to armed conflict in childhood, and childhood trauma have all been linked to altered trait and expression of personality in adulthood (Smyer, 1998; Brents et al., 2015; Tanwani & Sivalingam, 2015; Beilharz et al., 2019; Benjet et al., 2020; Miller, 2022). Early life experience has also been found to be associated with temperament later in life in rats (Gracceva et al., 2011; Rodel & Meyer, 2011); great tits (Naguib et al 2011), cavies (Siegeler et al., 2011), macaques (Timmermans et al., 1994), and rabbits (Hudson et al., 2011; Eccard & Rodel, 2011).

European starlings (*Sturnus vulgaris*) have proved a good model for studying these effects, producing good experimental evidence of the association. Studies investigating the effects of early life experience using starlings have employed a variety of experimental manipulations including cross-fostering to expose siblings to divergent early experience (Nettle et al., 2015), manipulating food availability (Swaddle & Witter, 1997), and administering differential quality diets to different groups (Geluso & Hayes, 1999). The documented consequences of these manipulations include individual differences in feeding behaviour (Andrews et al., 2015), stress responsiveness (Dickens et al., 2009), judgment bias (Gott et al., 2018), performance on cognitive tasks (Nettle et al., 2015), singing behaviour (Buchanan et al., 2003), reproductive behaviour (Archawaranon), and dominance (Bedford et al., 2017). There are also evident somatic effects of early life adversity as

Nettle et al. (2017) found that both nutritional input and begging effort in the nestling period affect cellular aging and adult inflammation in the starling.

4.1.6 The potential effect of weaning age on temperament in rhesus macaques

The effects of early life stress exposure on subsequent behaviour has been studied in three contexts in NHPs: maternal separation, maternal behaviour, and variable foraging demand (Parker & Maestripieri, 2011). Studies have shown that social experiences, such as maternal interactions, early life stress, and paternal experiences, can influence emotional behaviour, stress reactivity, and attachment behaviour in rhesus (Stevenson-Hinde & Simpson, 1988; Maestripieri, 2005; Maestripieri et al., 2006; Barr et al., 2008; McCormack et al., 2009; Kinnally & Capitanio, 2015; Paukner et al., 2017; Rayson et al., 2021). Maternal effects play a key role in shaping later infant behaviour. For example, macaques that were rejected by their mothers engaged in more solitary play (Maestripieri et al., 2006) and maternal anxiety and abuse have also been found to influence maternal behaviour and the intergenerational transmission of infant abuse in macaques (Maestripieri, 2005; Maestripieri, 2010). Paukner and colleagues have shown that social experiences, such as interactions with mothers and exposure to different faces, can shape early face preferences in rhesus macaques (Paukner et al., 2009; Paukner et al., 2017). Furthermore, the early rearing environment of the mother can predict their maternal competence (Goin & Gust, 1998). Paternal effects have been less well studied but there is evidence that paternal experiences can influence macaque biobehavioural makeup through non-social mechanisms (Kinnally & Capitanio, 2015).

Numerous evidence shows that adverse rearing practices can alter behaviour, neuroendocrine, and immune functioning and these effects can be long lasting (Capitanio 1986; Capitanio et al. 2005; Coe et al. 1989; Lubach et al. 1995; Sackett et al. 2006; Shannon et al. 1998; Capitanio et al., 2006). Furthermore, these adverse rearing practices have been linked to impulsive aggression, incompetent social behaviour, and increased behavioural and endocrine responsivity to stress in macaques (Barr et al., 2003; Kinnally et al., 2013; see Chapter 3 for a review of this). Early work from Harlow and colleagues demonstrated the detrimental effects of disruption to the mother-infant bond by separating infant rhesus macaques from their mother at birth rearing them in total social isolation: these monkeys were excessively fearful a year later (Harlow et al., 1965). The undeniable stress this places on both the infant and mother was widely recognised and as such used as a model of early life stress (Reinhardt, 2002). Thankfully, this practice is now considered ethically unacceptable, although slightly less extreme practices such as peer rearing, surrogate peer rearing, and early weaning have been found to produce similar results when compared to more typically reared counterparts (Reinhardt 2002; Prescott et al., 2012). Furthermore, monkeys reared in the natal group were more likely to survive than peer-reared counterparts (Goin & Gust, 1998).

Specific to rearing and neophobia, Timmermans *et al*. (1994) found that cynomolgus macaques that were reared in isolation with an artificial surrogate were more likely to develop neophobia than monkeys that were reared with their mothers in more typical conditions. The authors also found these neophobic tendencies to persist up to 2 years old (Timmermans et al., 1994).

There is plenty of evidence presented previously indicating that a severe disruption to natural development i.e., very early weaning can affect an individual much later in life. However, no evidence exists on the potential benefits of weaning monkeys later than is current practice under UK guidelines (see Chapter 1 for guidelines).

4.1.7 Aims, objectives, and predictions

The aims of the study presented in this chapter were to test the hypothesis that a later weaning age would be beneficial for the welfare of captive rhesus macaques that are destined to be used in neuroscience research, specifically that a later weaning age would result in monkeys that are less fearful during testing conditions and more confident in response to unfamiliar objects. To do this, temperament was quantified by testing monkeys in a controlled environment for their response and behaviour towards novel food and objects to assess how their responses would vary as a function of their weaning age. Monkeys were left alone and recorded during tests for later scoring of various behaviours to assess temperament. I predicted that later weaned monkeys would be less neophobic and less anxious, and this would be apparent by shorter latencies to approach novel food and objects and fewer displays of validated behavioural indicators of anxiety.

4.2 Methods

4.2.1 Ethical statement

All methods were approved by the Animal Welfare Ethics Review Board (AWERB) from the Centre for Macaques (Reference: CFM2019E001) and Newcastle University (Reference: 830).

4.2.2 Subjects

This study was undertaken at the Medical Research Council Centre for Macaques (CFM). Subjects were 136 rhesus macaques (*Macaca mulatta*; 59 females) from four birth cohorts underwent novel object tests as close to their 3rd birthday as possible (ages ranged from 2.97 – 3.51 years, mean±SD=3.07±0.1) between March 2019 – August 2022. All monkeys were reared by their mothers in social groups with one adult male and multiple adult females and their offspring. Weaned monkeys (n=122; females = 46) were separated from their mothers either due to death of the dam or for usual colony management (weaning ages range from 0.87 – 3.14 years, mean±SD=2.04±0.54). At the CFM, weaned monkeys are housed in groups of up to 16 animals, and their living areas are cleaned bi-weekly. Monkeys are fed daily a mixture of commercial food pellets designed for the dietary requirements of primates; forage mix containing different seeds, lentils, and oats; a variation of fruit and vegetables; and raisins and nuts during training and habituation sessions. All monkeys have access to water *ad libitum*. Lights are on between 0700hrs and 1900hrs.

4.2.3 Temperament tests

At the CFM, rhesus macaques have use of a playpen and adjoining cage area. In the cage room (CR), there are two cage areas opposite each other with approximately a 1.5 m gap in between. This allows two separate groups visual access to one another.

Prior to testing, the group opposite the group containing the individual to be tested were confined to their playpen with no visual access to the test. Similarly, individuals within the same group as the test subject were confined to their playpen and had no visual access to the test. This was done so that no monkey could see the novel food or objects before they were tested.

For testing, the individual was separated from the rest of the group in an adjoining cage room parallel to their playpen, measuring 8.0m long x 1.5m wide x 2.8m high.

The cage area in the CR has three sections (see Figure 4.1); for the tests the animal was contained to the middle section using panels to block access to the upper and lower section. The resulting section measuring 6.0m long x 1.5m wide x 1m high. A black sheet was placed on the front of the cage furthest away from door where observers entered and covered approximately 1/3 of the cage area. This was done to provide a visual barrier that the individual could choose to hide behind. The cage area was set up with one camera in front on a tripod and one camera at the side of the cage attached to a clear plastic divider panel.

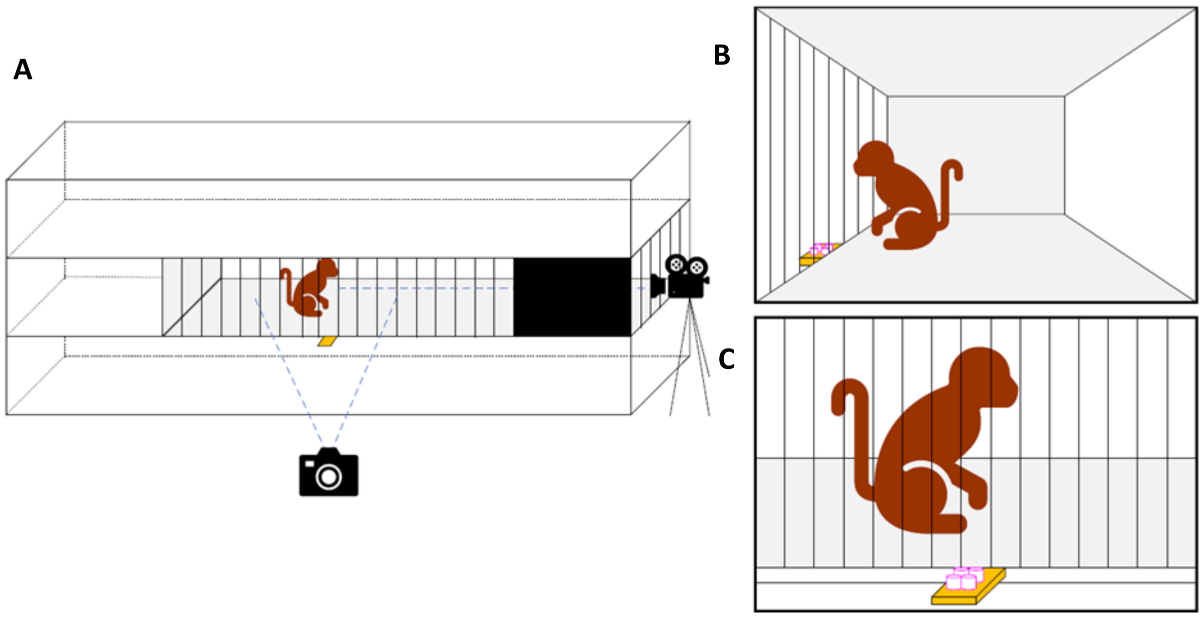

Figure 4.1. Cage setting. A) Cage room was set up with a wooden shelf outside of the cage where the different items used for the test were positioned. The tests were recorded using two cameras, one positioned on the side of the cage and the other on the front of the cage. B) Camera view from the side of the cage. C) Camera view from the front of the cage.

There were two sets of stimuli for the novel food and novel object tests that remained consistent throughout and were randomly assigned per test (see Figure 4.2). Two stimulus sets were used to mitigate for potential disruptions to the test: pilot studies conducted revealed that a disruption such as monkeys from the group gaining access to the testing area would result in data loss. By having another novel stimulus set, a monkey could be re-tested if there was a problem with the first test. In the middle of the cage area was a piece of wood that acted as a shelf for the food items and novel objects (see Figure 1). The temperament test consisted of 5 phases. All tests took place between 0900hr and 1400hr allowing the monkeys time to have been fed and eaten so that hunger was not a motivation to engage with novel items (particularly the novel food).

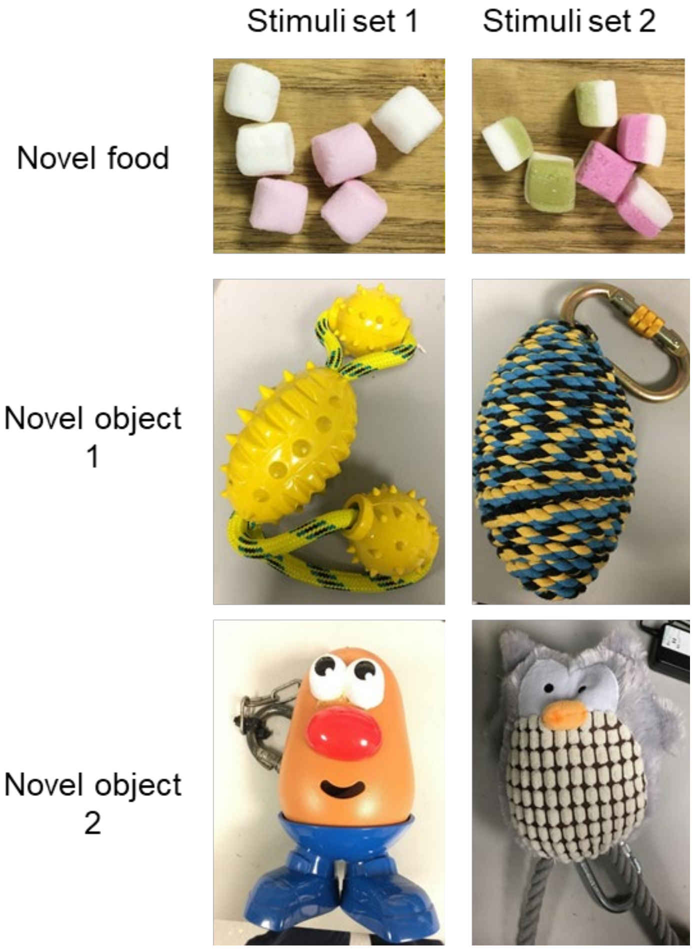

Figure 4.2. Images of the novel food and objects for each stimulus set.

4.2.3.1 Familiar food phase (FF)

The start of the testing was the FF phase. A handful of raisins were used as the familiar food throughout all tests as the animals at the CFM receive these during routine training and habituation sessions. An observer entered the CR, placed the raisins onto the wooden shelf, and left immediately. The individual was then left alone with the familiar food for 5 minutes. This phase was included to allow the monkey time to acclimatise to the testing area as being separated from the group is a stressor for captive macaques.

4.2.3.2 Novel food phase (NF)

Immediately following the FF phase, the observer entered the CR, removed any familiar food that was left from the previous phase, placed the novel food onto the wooden shelf, and left immediately. The individual was then left alone with the novel food for 2 minutes. The novel food was either marshmallows or dolly mixtures, with the specific type depending on the stimulus set (see figure 4.2). Each test consistently used six pieces of novel food, with colours evenly distributed. When using the dolly mixture set, three purple and three green pieces were presented. In tests with marshmallows, three pieces were white and three pink, maintaining colour balance across all tests.

4.2.3.3 Novel object phases (NO-1 and NO-2)

Immediately following the NF phase, the observer entered the CR, removed any NF that remained, rested the first novel object onto the wooden shelf attaching it to the cage using a carabiner, and left immediately. The individual was then left alone with the NO-1 for two minutes. Following this, the observer entered the CR, removed the NO-1, and followed the same steps as in the NO-1 phase for the NO-2 phase. The individual was then left alone with the NO-2 for 2 minutes. Subsequently, the observer entered the CR, removed the NO-2, and left. The individual was then left alone for 2 minutes before being let back into the group.

4.2.4 Outcome measures

Videos were coded by DM who was blind to the identity and weaning age of individuals for 33 tests. For 103 tests DM could not be completely blind to individuals or their weaning ages due to working within the CFM. Instead, the first 10 seconds of video was removed (the identity of the individual is announced when the cameras are turned on) and videos were given a randomised identity tag before being processed and coded by DM. Behaviours were coded as from an ethogram (see table 4.2) made up of behaviours commonly used for assessing temperament in rhesus macaques (e.g., see Coleman et al., 2005; Bliss-Moreau & Moadab, 2016; Arnaud et al., 2017; Coleman et al., 2017) and were either states (durations) or events (frequencies/latencies) (Altmann, 1974). Certain behaviours were coded throughout all phases of temperament testing, while others were coded exclusively during familiar and novel food testing (see table 4.2 for detailed ethogram). Video coding was carried out in BORIS (Friard & Gamba, 2016; version 7.9.8).

Table 4.2. Operational definitions and outcome measures for behaviours coded from temperament test videos.

Behaviour	Operational definition	Outcome variable
Withdrawal	Individual makes a quick jerky motion to a different location, can occur within the same location e.g. behind the visual barrier.	Frequency
Displacement	Individual displays: Yawns (wide opening of the mouth displaying teeth) Body shakes (rapid shake of the body) Self-scratches (quick repetitive scratching of its own skin/fur)	Frequency
Aggressive	Individual displays: Open mouth (staring with mouth and eyes open) Head bob (head moves up and down/ side to side with direct eye contact) Ears back (individuals ears retreat flat against its head with eyebrows raised) Threat vocalisation (bark) Lunge (individual makes quick forward movement in slightly crouched position with direct eye contact)	Frequency
Cage agitation	Individual slaps, bites, or shakes cage	Frequency
Lipsmack	Rapid lip movement, lips pursed, lower jaw moves up and down, usually with audible smacking sound	Frequency
Vocal coo	Medium pitch, clear call with rounding of the lips	Frequency
Fear grimace	Wide grin with teeth showing	Frequency
Behind visual barrier	Individual is sat, moving, or climbing in the area of the cage behind the visual barrier. Freeze duration to be recorded separately	Duration
Freeze	Individual is in a tense posture with ventrum pressed down or towards floor for more than 2 seconds.	Duration

Table 4.2. Operational definitions and outcome measures for behaviours coded from temperament test videos.

Escape attempts	Individual tries to squeeze one or more limb through the wooden slats; each attempt is separated by 2 seconds	Frequency
Approach	The first time the individual initiates movement toward the direction of the novel object/food whilst looking in that direction	Latency
Eat	The first time the individual places food in its mouth and starts to chew	Latency
Touch	individual touches the novel object/food with tongue or fingers	Latency and frequency
Inspect	Subject is looking at the object without touching it whilst being close enough to touch e.g. within 10cm	Latency and frequency

Grey shaded cells indicate behaviours that were included for analysis (explained in Section 4.5.2.1)

4.2.5 Statistical analysis

4.2.5.1 Data treatment

For analysis, only a subset of the behaviours defined in table 4.2 were considered (shaded in table 4.2). This is because macaques rarely performed most of the behaviours in the ethogram. 109 out of 122 monkeys touched or inspected the novel food or objects following approach: it was found that if a macaque was to touch or inspect the novel food or objects, this was almost always immediately following approach (touch: $r = 0.95$; inspect: $r = 0.98$). Of the monkeys that did touch/inspect there was little variation in the number of times they did so (touches: range = 0-7, mean±SD = 0.64±0.9; inspections: range = 0-3, mean±SD = 0.25±0.46). This lack of variation led to the exclusion of these measures. Similarly, for the novel food phase out of the 89 monkeys that approached the novel food, 85 proceeded to inspect or touch it, of which, 80 proceeded to eat it; eating the novel food happened almost instantly after approaching ($r = 0.99$). Additionally, only the latency to approach the novel food and objects were included, while inspections and touches were disregarded.

4.2.5.2 Intraclass correlation coefficients to assess reliability

30% of all videos were randomly chosen (41/136) and were coded by another coder to assess inter-observer reliability. The coder was provided training on each behaviour in the ethogram and shown videos displaying each behaviour. The additional coder was blind to the monkeys' identity and their weaning age. Reliability was assessed by computing intraclass correlation coefficients *ICC*(2,1), which assesses absolute agreement between coders was assessed using a two-way random-effects model assuming the measurement from a single coder will be the basis of the measurement (Shrout & Fleiss; 1979; McGraw & Wong, 1996; Koo & Li, 2016). *ICC*s were computed using the ICC function in the '*psych*' package in R (Revelle, 2020). Reliability was assessed only for the variables that were included in the analysis.

4.2.5.3 Principal component analysis

Principal component analysis was used to reduce the dimensionality of the dataset. To do so, the variables 'behind visual barrier', 'escape attempts' and 'freeze' were summed across the three novel phases to gain an overall representation of fearfulness, whereas the latency variable for each phase were included separately in case monkeys were responding differently to the different food and objects. All variables to be included in the PCA were scaled to have a mean of 0 and a standard deviation of 1. The number of components to retain was decided by conducting parallel analysis using the 'paran' package in R (Dinno & Dinno, 2010; Horn, 1965).

Parallel analysis revealed one component to retain, as such no rotation was necessary for the PCA, which was conducted using the 'principal' function of the psych package (Revelle, 2020). Differentially weighted component scores were then extracted to be used in further analysis.

4.2.5.4 Model fitting

Multiple regression was used with the 'lm' function in R to analyse the combined influence of weaning age, sex, and their interaction, while controlling for the stimulus set used and cohort on the principal component score. Various diagnostics of model validity and stability were then checked (Cook's distance, DFBetas, DFFits, leverage and Variance Inflation Factors; distribution of residuals, residuals plotted against fitted values), and none of these indicated obvious influential cases, nor obvious deviations from the assumptions of normality and homogeneity of residuals (Quinn &

Keough, 2002; Field, 2005). To test the significance of the predictors as a whole, the fit of the full model was compared with the fit of a null model comprising only the intercept (Forstmeier & Schielzeth, 2011).

4.3 Results

Overall, all measures were reliable with *ICC*(2,1) estimates ranging from 0.78 to 0.82 (see Table 4.3.), as such, these measures were retained for the analysis.

Table 4.3. Inter-rater reliability for variables used in analysis

Variable	*ICC*(2,1)	CI (lower, upper)
Latency to approach	0.9	0.87, 0.93
Behind visual barrier	0.94	0.89, 0.97
Freeze	0.78	0.62, 0.87
Escape attempts	0.82	0.53, 0.92

105 monkeys approached the familiar food (female = 42), 89 monkeys approached the NF (female = 36), 80 monkeys approached NO1 (females = 42), and 78 monkeys approached NO2 (female = 35). See table 4.4 for sample sized based on approach behaviour.

Table 4.4. Number of monkeys that approached novel food and/or objects

Approach	Females	Males	Total
Only NF	5	12	19
Only NO1	6	5	11
Only NO2	1	4	5
Only NF and NO1	5	6	11
Only NF and NO2	3	12	15
Only NO1 and NO2	8	4	12
All novel phases	23	23	46
None	8	11	19

Two components that had eigenvalues greater than 1 and parallel analysis for component retention revealed one component was greater than what would be expected from random data (210 iterations) at the 95th percentile. This single component had salient loadings (>0.4) for latency to approach in all of the phases and freeze behaviour (Table 4.5) and explained 30% of the total variance between individual variables. This component was named 'neophobia score' as it was associated with longer latencies to approach in all phases and more time spent in the freeze position across all phases, indicating fearfulness and reluctance to approach the novel food and objects.

Table 4.5. Loadings of the variables into the principal component

Variable	Loading	h^2
Latency (FF)	0.61	0.37
Latency (NF)	0.68	0.46
Latency (NO1)	0.61	0.38
Latency (NO2)	0.65	0.43
Escape attempts	-0.25	0.07
Behind visual barrier	0.37	0.14
Freeze	0.52	0.27

With the general linear model of neophobia score, the predictors were found to influence the neophobia score (full-null model comparison: $F_{7,114} = 2.27$, $p = 0.03$; see Table 4.6 for model output). There was a significant interaction of weaning age and sex on neophobia score: for every unit increase in weaning age, neophobia score

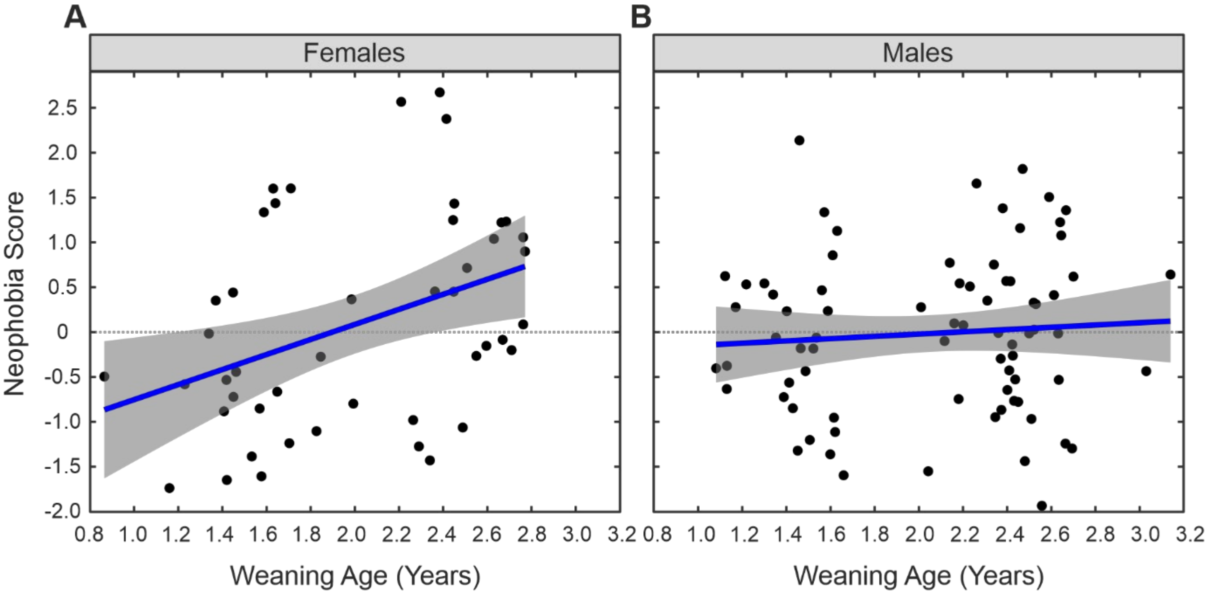

Figure 4.3 Interaction between weaning age and neophobia score. A: Females; B: Males. Dots represent each individual animal. Neophobia is scaled to have a mean of zero and a standard deviation of one. Dotted line across zero represents that average neophobia score. Blue line indicates regression model and grey shading around the line represents 95% confidence intervals.

was estimated to increase by around 80% of a standard deviation (Esimate±SE = 0.8±0.27, t = 2.95, p = 0.004), furthermore the slope was steeper for females (β = 1.35) than for males (β = 0.63; Esimate±SE = -0.72±0.34, t = -2.10, p = 0.04; Figure 4.3).

Interestingly, it was found, compared to the 2019 cohort, that the 2022 cohort had neophobia scores that were around half a standard deviation larger (Esimate±SE = 0.53±0.26, t = 2.03, p = 0.04). While monkeys from the 2020 and 2021 cohort were estimated to have larger neophobia scores, these effects did not reach statistical significance (table 4.6). Post-hoc comparisons of the same model with the factor relevelled for each cohort to be the reference category revealed no significant differences between any of the other cohorts (Figure 4.4). The stimulus set used was not found to influence the neophobia score.

Table 4.6. Model output results

Variable	Estimate	Std. Error	T value	P	CI	R^2
Intercept	-1.81	0.58	-3.14	0.0022*	-2.95, -0.67	
Weaning age	0.80	0.27	2.95	0.0039*	0.26, 13.4	
Sex†	1.35	0.71	1.90	0.0601	-0.06, 2.76	
Stimulus set	-0.03	0.19	-0.18	0.8605	-0.41, 0.34	0.0003
Cohort 2020‡	0.40	0.25	1.61	0.1093	-0.09, 0.9	0.04
Cohort 2021‡	0.24	0.28	0.87	0.3881	-0.31, 0.8	
Cohort 2022‡	0.53	0.26	2.03	0.0447*	0.01, 1.06	
Weaning age*Sex	-0.72	0.34	-2.10	0.0381*	-1.39, -0.04	0.04

*Indicates significance at $\alpha<0.05$

†Female as reference

‡2019 as the reference category

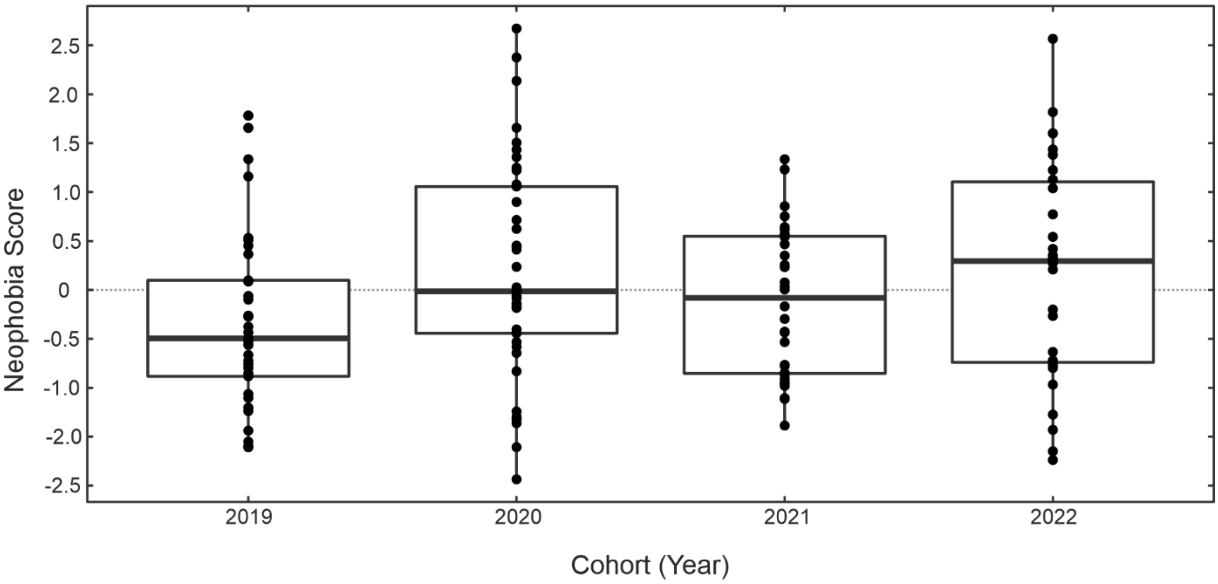

Figure 4.4. Boxplots displaying neophobia score by cohort. Dots represent individual data points. Boxes represent the lower and upper quartiles. Solid horizontal lines represent the median. Solid vertical lines represent the range of the data. Dots that are disconnected from the solid vertical line represent outliers that are 1.5 times the inter-quartile-range larger or smaller than the upper or lower quartiles, respectively. Dashed horizontal line represents mean neophobia score at zero.

4.3.1 Post-hoc analysis with time since wean, father ID, and number of days treatment was required

Due to a confound arising from the study design (weaning age was correlated with time spent in the new group prior to being tested; r = -0.98). Post-hoc analysis was undertaken to assess only individuals that had been weaned at least one year prior to being tested, this left 54 animals for analysis (female = 23). While this excluded the latest weaned monkeys, there was still a range from 0.87 – 2.34 years (mean±SD = 1.5±0.26) with the average weaning age still being later than current guidelines. While the correlation between time spent in the new group and weaning age was still evident and strong in this sample (r = -0.92), it makes it more possible that any effects were not attributed to the acute effect of separation, assuming individuals will have had enough time to acclimatise to their weaning group. In this model, the interaction term between weaning age and sex, and their main effects, were no longer evident and thus dropped to just include the individual effects. There was no effect of weaning age or sex on neophobia score in this sample, although monkeys from cohort 2022 had significantly higher neophobia scores than those in 2019 (Estimate±SE = 0.93±0.37, t = 2.6, p = 0.01), and, as in the full model, this was the only significant cohort difference found.

To try and control for potential genetic factors and the influence of social status, an additional general linear mixed effect model was fitted to the data with the same structure as the full model but with the addition of maternal rank (factor: high, mid, or low) as a fixed effect and Father ID included as a random effect. The sample size for this model was 92 monkeys (female = 36) from 9 fathers. The same weaning age and sex interaction and cohort effect was found, as for the full model. There was no significant effect of maternal rank. There was a standard deviation of 0.13 at the level of Father ID with a residual standard deviation of 0.98, suggesting there was more variation in the neophobia scores of monkeys with the same father than there was between.

To assess potential effects of prior experience to being removed from the group in the testing area, the number of days a monkey received an injection was quantified and modelled as a predictor variable with neophobia score as the response. At the CFM, when a monkey requires an injection, they must be separated from the group into the cage area where the temperament tests took place. As such, monkeys that have received more treatments will have more experience being separated in the testing area which could affect their response in either direction i.e. they may be more habituated and quicker to approach, or more fearful of separation due to the putatively negative valence of receiving treatment, and be more fearful during testing. However, there was no evidence of an effect of treatment days on neophobia score (general linear mode: p = 0.2).

4.4 Discussion

This study is the first to assess the potential effects of weaning much later than is currently recommended under guidelines e.g. NC3Rs on temperament in rhesus macaques.

The main finding of this study is contrary to the prediction: a later weaning age was associated with a larger neophobia score for females at 3 years old and weaning age appeared to explain little variance in neophobia for males at 3 years old.

4.4.1 Weaning age on neophobia

There are a number of ways this result could be interpreted. Later weaned females are more neophobic and this could be because they have had time to form strong and stable bonds within their matriline and subsequent disruption to these bonds can negatively impact their affective state and subsequent cognition. However, this result should be interpreted with caution due to a confound arising from the study design: later weaned monkeys have had less time to acclimatise to their new groups prior to being tested. Age at testing was restricted to 3 years to control for potential confounds of age but this introduced the confound of time in the new group. If an animal was weaned, for example, at 2 years of age and tested at 3 years of age, this animal had one year between being weaned and tested to settle in its new group whereas an animal weaned at 1 year of age would have had 2 years to settle. The removal of an animal from its natal group into a new group is a major stressor in the lives of captive macaques and Dettemer *et al.* (2018) found behavioural indicators of anxiety did not return to near baseline values until 12-18months post relocation in their sample. As such, this result may indicate the acute effect of weaning on the individuals state affect which in turn affects their response to the novel testing situation and being separated from the group, and perhaps females are more sensitive to this effect than males are.

Further research is needed to test this prediction by testing animals of similar ages post relocation stress comparing both within-individual variation in response to relocation with between-subjects variation of those who have not been relocated. Indeed, when the conducting the post-hoc analysis of monkeys that had been weaned at least one year prior to being tested, the effect of weaning age was no longer found, providing some support to this theory, although caution should be taken with interpretation as the sample size was significantly reduced (n = 54) and the effect found in the full model was small ($R^2 = 0.04$), therefore it is possible that this analysis with the reduced sample did not have enough statistical power to detect the effect if true. Another notable limitation of this study is the lack of temperament data on the animals prior to weaning. This data could have provided a valuable baseline for the animal, allowing for a more nuanced understanding of the impact of weaning age on neophobia over time. The absence of such pre-weaning temperament data limits our ability to discern whether the observed neophobia is a direct result of the weaning process or merely a continuation of pre-existing temperamental tendencies. By incorporating multiple assessments over stages of development, future studies would gain a better understanding of the stability and change in the personality trait of neophobia across time and context (a crucial aspect of personality research).

It is also important to consider that neophobia is not a binary trait but exists on a continuum. The extent to which different levels of neophobia, such as a score of 1.4 versus 1.2, or the time taken to inspect an object (30 seconds vs. 20 seconds), meaningfully impact the animals is an aspect that requires further exploration. There may also be a threshold effect, where extreme cases of neophobia, characterised by complete avoidance of inspection and the display of numerous anxiety behaviours, are of particular concern. In future studies, categorising animals as “highly neophobic” and “not neophobic” based on these considerations, and comparing this with weaning age, could provide additional insights. This approach might reveal more nuanced relationships between weaning age and neophobia.

4.4.2 Housing and husbandry

Earlier work has found adverse rearing conditions to produce neophobic monkeys (Timmermans et al., 1994) and other work has found adverse rearing conditions to produce monkeys that with different temperamental profiles than those that were socially reared (Gottlieb et al., 2013). In the current study, while weaning ages varied, all monkeys were reared identically as what would be described in the literature as socially reared/mother-peer-reared/typically reared. All of the monkeys in the current study were reared by their mother in social groups until at least 0.8 years of age (for the earliest weaned monkey). Even in the case of early weaning in this sample (where the mother died) the infant was still reared by its mother for much longer than in atypical rearing conditions described in the literature that produce different temperament proclivities. As such, it is possible that removal very early in life produces these effects seen on cognitive development and socio-emotional processing that is evident later in life, whereas removal at 0.8 years (roughly 9.6 months; just below European guidelines) is enough time to allow the infant to learn species typical behaviour in response to stimulus from its mother. That is not to advocate for the earliest weaning age in the current guidelines being good enough, as other factors should still be taken into consideration; but rather a testament to typical rearing conditions producing a normal variation in response to novelty.

4.4.3 Heritability

Individual differences are studied now in their own right and as is evident from this sample there is variation in neophobia across all weaning ages suggesting other uncontrolled factors are having stronger effects on this variation than the weaning age of monkeys reared in what can be considered as more typical conditions. Temperament has been shown to be somewhat heritable in humans (Jang et al., 1996; Vukasovic et al., 2015) and macaques (Williamson et al., 2003; Sullivan et al., 2011; Brent et al., 2014; Arnaud et al., 2017). One limit to this current study is it was not possible to collect data from mothers or fathers to assess similarity between the temperament of parent and offspring. To try and control for this, an additional model was fitted with Father ID specified as a random effect for a subset of the data. Father ID was chosen as there are fewer fathers to the offspring than mothers, with the CFM operating as a one-male, multi-female breeding colony. As such, there would not have been enough observations at each level of Mother ID to include as a random effect. This model revealed more variation in the neophobia scores of infants from the same father than between. This is, of course, not evidence that temperament is not inherited as this could not properly be assessed. However, it could be interesting to further investigate the heritability of temperament from fathers as previous evidence has shown similarities between maternal and offspring temperament, this result suggests that the temperament of individuals from the same father are not hugely similar to one another (assumably the father too) and inheritance mechanisms may work though mitochondrial transmission (Kinnally & Capitanio, 2015).

4.4.4. Cohort differences

It was found that the monkeys from the 2022 cohort to have, on average, a higher neophobia score than those tested in 2019. One interesting thought for this finding is in relation to the COVID-19 pandemic. The monkeys that were tested in 2022 would have been born in 2019 (birth dates range from March 21st to July 30th) and as such would have been between the age of 8 months – 1 year at the start of UK national lockdown in March 2020. During this initial lockdown, to prevent transmission of the virus within and between monkeys and staff, staff were divided into two teams that worked on alternate days. Therefore, monkeys were exposed to fewer staff on a given day, the staff they were exposed to suddenly had increased PPE and were potentially unrecognisable, and routine habituation and training was significantly reduced to prioritise cleaning and feeding with a reduced staff load on each day. As such, at a critical stage in their development, the monkeys tested in 2022 suffered from a lack of habituation in the years previous. Evidence does suggest captive (and habituated) animals are more bold and engage in more novelty seeking behaviour than wild (and unhabituated) conspecifics (Forss et al., 2021), and studies have found increased habituation to human presence to increase novelty seeking behaviour in wild vervet monkeys (Forss et al., 2021). Although this interpretation should be interpreted with caution as habituation was not directly quantified in this study, rather cohort could be considered a proxy. Furthermore, the monkeys tested in the 2020 and 2021 cohort were alive during the COVID-19 pandemic and as such received the same levels of habituation for that period without displaying significantly more neophobia than the 2019 cohort. Potentially this is due to them having received the standard amount of habituation at critical developmental windows in the first 3 years of life (2020 cohort were around 3 years old during the initial lockdown and 2021 cohort monkeys were around 2 years old).

4.4.5 Conclusion

In conclusion the data presented in this chapter do not provide support for the hypothesis that a later weaning age will increase tolerance to laboratory procedures that require interactions with novel humans or objects, although they provide some support to the idea that monkeys in the laboratory could benefit from a good habituation programme which could result in reduced neophobia. Future research would benefit from quantifying individual levels of habituation and testing individuals before and after a stressor such as relocation to elucidate the effect of weaning and time since weaning.

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Chapter 5. The Association Between Weaning Age and Alopecia in Captive Rhesus Macaques (*Macaca mulatta*)

5.0 Chapter summary

Alopecia, or hair loss, is a potential welfare concern in captive rhesus macaques with multiple factors known to influence the aetiology. To investigate the potential impact of weaning on alopecia prevalence and severity, while controlling for age, sex, and season, both acute (within six months of weaning) and chronic (beyond six months of weaning) assessments were conducted using a retrospective dataset and newly collected data. This study is the first to explicitly test the association between weaning age and alopecia in captive macaques, shedding light on the importance of the immediate post-weaning period as a critical window for alopecia development. I found that being weaned was a significant predictor for alopecia severity and prevalence at the second health screen, with weaned females exhibiting around 40% more severe alopecia and being approximately 2.3 times more likely to have alopecia than females that remained in the natal group. The acute effect of weaning on alopecia was supported by the monthly dataset, revealing approximately 17% more severe alopecia and 20 times higher alopecia prevalence in weaned monkeys compared to non-weaned ones. Furthermore, the retrospective dataset indicated that for every unit increase in weaning age, monkeys were around 48% less likely to display any alopecia in the short-term. However, I did not observe a significant effect of weaning age on alopecia severity or prevalence in the chronic dataset. This discrepancy suggests that the impact of weaning age on alopecia may be time-limited or influenced by other factors such as aging or social dynamics. Our study also revealed clear seasonal patterns, with higher rates of alopecia occurrence and severity during the Spring and Summer months. Moreover, males displayed less severe alopecia and were less likely to develop alopecia than females. While our findings contribute valuable insights into the associations between weaning, sex, and season on alopecia in captive macaques, limitations include the retrospective design and potential biases. I recommend that facilities aiming to reduce alopecia prevalence in macaques delay weaning while closely monitoring newly weaned monkeys. Future research should explore specific interventions and mechanisms underlying alopecia development to promote primate welfare effectively.

5.1 Introduction

5.1.1 What is alopecia

Alopecia is a medical term used to describe partial or complete hair loss from a part of the body where it exists naturally and has been observed in many mammalian species. Alopecia is often presented as a multi-faceted welfare issue that is prevalent in many primate facilities (Novak & Meyer, 2009; Kroeker et al., 2017; Lutz et al., 2019). Alopecia occurs as a result of disruption to any stage of the hair cycle. The hair cycle consists of an anagen (growth), telogen (resting), and catagen (shedding) phase. The catagen phase is a transitional phase where hair growth stops, and the hair follicle shrinks. Finally, during the telogen phase, the hair follicle is at rest, and the hair is shed (Mak & Chan, 2003; Tamura et al., 2018; Lin et al., 2022; Natarelli et al., 2023). In humans, there are various types of alopecia, including alopecia areata, androgenetic alopecia, citacricial alopecia, and non-citacricial alopecia (Beigi, 2018). Alopecia areata, a common form of alopecia, is characterised by chronic, recurrent, and non-scarring hair loss. It includes subtypes such as alopecia areata in patches, alopecia totalis, and alopecia universalis, which vary in severity and the areas affected by hair loss. Additionally, androgenetic alopecia, another non-scarring type, is the most common hair loss disorder worldwide and is characterised by the gradual miniaturisation of hair follicles (Beigi, 2018). Different patterns of alopecia are associated with disruptions during different phases of the hair cycle. For example, one of the most common forms of hair loss is telogen effluvium. As the name suggests, telogen effluvium is associated with disruptions during the telogen phase of the hair cycle (Headington, 1993).

5.1.2 The role of stress on alopecia

Evidence from humans suggest there is a link between stress and resulting hair loss (Botchkarev, 2003; Salhab et al., 2022). Several studies have found that psychosomatic factors, such as poor social support, life events, and anxious attachment are associated with the initiation and exacerbation of alopecia areata (Mehran et al., 2012; Rajabi et al., 2018). For example, Brajac *et al*. (2003) found that significantly more patients presenting with alopecia areata had experienced stressful life events in the 6 months prior to the study, suggesting a possible acute effect of stress in the aetiology of alopecia.

One mechanism of this link can be through cortisol induced dysregulation of the hair follicle (Thom, 2016). Furthermore, experimental evidence from mice has shown that stress alters the hair cycle, leading to premature termination of the normal duration of active hair growth (anagen), prolonging the telogen stage, and delaying subsequent anagen and catagen stages (Arck et al., 2003; Aoki et al., 2003; Katayama et al., 2007; Liu et al., 2013). These effects are mediated by neuropeptide substance P and reactive oxygen species (Liu et al., 2013; Wang et al., 2014).

Another mechanism could be behavioural via self-directed hair plucking (trichotillomania; Chamberlain et al., 2010), a behaviour also noted in macaques (Lutz et al., 2013). In humans, Trichotillomania is associated with traumatic and negative events in childhood (Ozten et al., 2015), anxiety and obsessive-compulsive disorders (Grzesiak et al., 2017), boredom (Wang & Zhang, 2022), and nutritional deficiencies (Zhao et al., 2021). The role of hair plucking in the aetiology of alopecia in macaques can be difficult to determine: in group housed macaques, the behaviour could be self-directed or performed by another individual in group housed primates (Lutz et al., 2013). Furthermore, where hair plucking has been determined, the rates are considerably lower than the rates of alopecia within the colony, suggesting a small association (Kramer et al., 2011; Lutz et al., 2013); similar results have been found in cats (*Felis catus*: Waisglass et al., 2006; Luchins et al., 2011). Where hair plucking is performed by another individual, it tends to be directed down the hierarchy (Heagerty et al., 2017). Similarly, lower ranking vervet monkeys (*Cercopithecus aethiops*) show more pronounced hair loss than higher ranking conspecifics, suggesting alopecia could be linked to social stress (Isbell, 1995).

There is currently no experimental evidence of an association between stress and hair loss in primates as methodologies similar to studies in mice would be difficult to justify ethically. Since it is difficult to directly assess the link between stress and alopecia in primates, studies often rely on assessing the correlation between cortisol measures and documented alopecia. These findings suggest that there is likely some correlation between affective state and hair loss in macaques. Novak *et al*. (2014) found that severe alopecia was associated with high levels of hair cortisol concentrations and Novak *et al*. (2017) found that decreasing levels in hair cortisol over time was associated with subsequent hair regrowth in female macaques. These findings are currently the best evidence for an association between stress and hair loss in captive primates. However, the direction of this relationship is unclear and as

Novak *et al.* (2017) discusses: a confounding variable may cause hair loss which causes stress /may cause stress which causes hair loss.

5.1.3 The role of alopecia on stress

There is evidence also pointing to the role of alopecia in the aetiology of stress related disorders in humans, suggesting a possible bi-directional relationship between alopecia and stress. For example, the trauma of chemotherapy-induced alopecia has also been well-documented in the psychological literature (Trusson & Pilnick, 2017). Studies evaluating the sexes separately have found that alopecia is associated with feelings of low self-esteem or insecurity in both sexes (Davis & Callender, 2018, Abbas et al., 2021), with females potentially more at risk because of their greater risk for developing stress related disorders (Chaplin et al., 2008). Furthermore, systematic reviews of psychological interventions for quality of life, mental health, and hair growth in alopecia areata and scarring alopecia found that alopecia is associated with significant psychological burden (Cash, 1999; Engel et al., 2023). In macaques, this link is not well studied, although welfare consequences of hair loss are conceivable, for example, issues with thermoregulation and disruption to natural grooming behaviour (Lynch et al., 2011; Chen et al., 2021).

5.1.4 Other documented correlates of alopecia and the difficulties inferring welfare from alopecia in non-human primates

Scientific effort has been put into understanding the causes of alopecia in rhesus macaques given that they are one of the most commonly used model species for neuroscience, biomedical science, and immunological science and many are housed in laboratories and breeding facilities across the world. Alopecia has been suggested as a possible biomarker for stress (Novak et al., 2017) and therefore a proxy for welfare. However, it remains unclear just how reliable and valid alopecia is as a marker of welfare.

The existence of a link between alopecia and poor welfare is unclear since there are multiple variables associated with the aetiology of alopecia, some of which are more convincingly associated with stress such as more relocation events (Lutz et al., 2016), being singly housed (Kroeker et al., 2014; Lutz et al., 2016), and HPA axis activity (Novak et al., 2014); others of which could be either a consequence or cause of poor welfare; and others which could be natural. To elaborate, alopecia in rhesus macaques has been shown to correlate with:

- Sex: females tend to have more alopecia (Beisner & Isbell, 2009; Kroeker et al., 2014; Lutz & Sharp, 2015).
- Increasing age (Steinmetz et al., 2006; Kroeker et al., 2014; Beisner & Isbell, 2009).
- Bold temperament (Coleman et al.,2017).
- Pregnancy (Davis & Suomi, 2006; Dettmer et al., 2017).
- Season: more alopecia in the Spring months (Steinmetz et al., 2006; Kroeker et al., 2014), and end of the mating season in free ranging macaques (Vessey & Morrison, 1950).
- Hair plucking, both self-directed, and by other individuals (Reinhardt et al., 1986; Kramer et al., 2011; Luchins et al., 2011; Lutz et al., 2013; Heagerty et al., 2018).
- Prior facility (Kroeker et al., 2017).
- Consequence of infection (Lair et al., 2009).
- Diet: Pig tail macaques given a high fat diet for 3 months had chronic inflammation of the GI tract, weight loss, and alopecia (Mustafa et al., 2019).

Take season for example, this could be natural variation in response to gonadal hormones during breeding vs non-breeding season (Vessey & Morrison, 1970), or something more complex. One study in mice found that the pituitary hormone prolactin regulates hair follicle cycles (Craven, 2001). Melatonin has also been shown to be synthesised in the hair follicle and to express functional melatonin receptors, which are expressed in a hair cycle-dependent manner (Trueb, 2010). Both melatonin and prolactin are influenced by natural light suggesting annual changes in photoperiod could be a mechanism by which hair is shed. Furthermore, the seasonal changes in immune function may be driven by annual changes in photoperiod and melatonin secretion (Freeman et al., 2007), and therefore determining if an individual presenting with alopecia is a welfare concern or not can be difficult.

As the evidence suggests at least the possibility of an association between alopecia and stress and therefore welfare, it is important to gain more of an understanding of possible causes that do present as a potential welfare issue. Doing so will better inform intervention and treatment strategies such as social housing where the cause is the stress of single housing (Novak et al., 2017).

5.1.5 The role of early life experience in the aetiology of alopecia. Could weaning age be associated with alopecia?

The role of early life experience in the aetiology of alopecia is not well understood. However, it is certainly conceivable that early life experience should play at least an indirect role. As previously discussed in this chapter, stress, ageing, and temperament have all been found to correlate with alopecia (Steinmetz et al., 2006; Kroeker et al., 2014; Beisner & Isbell, 2009; Novak et al., 2017; Novak et al., 2014). All of these variables have been shown to be influenced by early life experience: Numerous evidence shows early life experience can be responsible for individual differences in behaviour in a number of species (reviewed in Chapter 4), stress reactivity and immune function (reviewed in Chapter 3); and cellular ageing (Nettle et al., 2017).

A major stressor in the lives of captive macaques is the separation of young macaques from their mother, as such alternative rearing paradigms have been used as a model of early life stress (Reinhardt, 2002). Numerous evidence shows that adverse rearing practices can alter behaviour, neuroendocrine, and immune functioning and these effects can be long lasting and covary with alopecia (Capitanio 1986; Capitanio et al. 2005; Coe et al. 1989; Lubach et al. 1995; Sackett et al. 2006; Shannon et al. 1998; Capitanio et al., 2006).

In the most relevant study relating to the question of weaning and alopecia to date, Conti *et al*. (2012) utilised a dataset of 231 rhesus macaques where different rearing paradigms were in place to model early life adversity and create animal models of depression and found that the peer reared females were more likely to experience hair loss than other rearing conditions. The authors note that this group also displayed more aggression, making it difficult to determine the effect of early life stress on hair loss where it could be a result of aggressive hair plucking. Despite the evidence that early and adverse rearing conditions can have long lasting consequences on monkeys' behaviour and health, no study has directly assessed the impact of separation on alopecia or the potential effects of age of separation on alopecia.

5.1.6 Aims and predictions of this chapter

The aim of the present study was to test the hypothesis that weaning of individuals (removing them from their mother and natal group), a putative psychological stressor, would result in hair loss and that the age that an individual is weaned would moderate the length and magnitude of this effect. Current guidelines in the UK state that individuals should not be weaned before 10-14months of age and that they should be left for as long as possible.

Predictions are as follows:

- Acute effect: Weaning will be associated with increased prevalence and severity of hair loss and, for the weaned animals, a later weaning age will negatively correlate with prevalence and severity of hair loss.
- Chronic effect: Weaning age will be negatively correlated with prevalence and severity of hair loss later in life.

To test these predictions, historical health screen records where alopecia was taken as a measure were utilised (see Chapter 2). To test the acute prediction, in addition to the historical records, a new dataset was collected from similarly aged weaned and non-weaned individuals from November 2020 – July 2022 to better control for season as health screen observations historically have taken place in the Spring and Summer months.

5.2 Methods

5.2.1 Retrospective dataset

This study was conducted at the Medical Research Council's Centre for Macaques (CFM). At the CFM, the animal technicians, and the named veterinarian screen macaques annually. During annual health screens, individuals are sedated with ketamine and examined by the veterinarian for physical signs of health: coat condition, body condition, weight, rectal temperatures, body measurements etc. Additionally, rectal swabs are taken on the day of the health screen and for two days following the initial check to screen for the presence of bacterial infections such as, Salmonella, Shigella, and Campylobacter. All recorded measures are stored in the individuals' paper health record and uploaded to the colony database Oracle based database provided by ENOS.

As pregnancy is well known to be associated with alopecia in rhesus macaques (Novak & Meyer, 2009; Lutz et al., 2019), observations from the database were not collected for any females that were parous or pregnant during a given health screen. Pregnancy status was estimated based on infant birth dates e.g., if an individual was health screened in July on a given year and gave birth in September of the same year, data would not be collected from her as rhesus macaque gestation is around 166 days.

All monkeys were indoor housed in social groups. I collected 1274 health screen observations from 470 monkeys (females = 247) monkeys, screened between the years 2009-2022.

The CFM has 22 enclosures that are all very similar in size. Enclosures consist of a larger play pen area that contains enrichment equipment, sawdust, and bedding material (8.04 m length x 3.35 m width x 2.8 m height) and an adjacent cage room area that the individuals can access via hatches in the play pen (6.12 m length x 1.5 m width x 2.8 m height). All enclosures exceed the EU Directive guidelines for non-human primates.

5.2.2 Alopecia scoring in the retrospective dataset (2009-2019)

Alopecia scoring was based on the scoring system described by Honess *et al*. (2005). This scoring system consists of 5 scores (definitions in Table 5.1).

Table 5.1. Alopecia scores and definitions from Honess *et al*. (2005)

Score	Definition	Example
1	Very good coat condition; complete back cover	
2	A few small patches of alopecia (2–5cm2)	
3	Large patches of alopecia (≥ 5cm2), or numerous small ones totalling 25–50% of the surface of the back	
4	Generalised alopecia (not patchy), involving more than 50% of the back	
5	Back completely bald (i.e. more skin visible than hair; Image from Honess et al., 2005)	

There were some inconsistencies between the scoring system employed by the veterinarians and the one of Honess *et al*. (2005) up until the 2020 health screen year. Prior to 2020, coat condition was scored between 0 and 5 (adding an extra score) and the scores were flipped: 0 being bald and 5 being a full coat. Furthermore, there were some half measures in the dataset. To be more in line with the current literature, I flipped the scores around so a score of 5 would indicate severe hair loss and rounded up half measures. The main difference between the scoring system employed by veterinarians at the CFM and that of Honess *et al*. (2005) is the additional score of 0. To bring this more in line with the Honess *et al*. (2005) method, the two worst hair scores were pooled into one to create scores of 0-4, 1 was then added to these scores to create scores of 1-5

5.2.3 Alopecia scoring from 2020 onwards

From 2020, alopecia scoring was consistent with that descried by Honess *et al*. (2005). Additionally, photographs were taken during the Health Screen for later assessment of inter- and intra-observer reliability of alopecia assessments.

Monthly alopecia scores were also taken from September 2019 – July 2022 in the playpen, again employing the Honess *et al.* (2005) method. To assess the consistency between scores derived from play-pen assessments and those taken by the NVS during the Health Screen, Individuals that had a Health Screen were scored in their play pen on the same week.

Furthermore, I employed the alopecia assessment described in Bellanca *et al*. (2014), known as the rule-of-nines, on photographs taken at the health screen to assess agreement between the two scoring systems. This scoring system involves assessing various regions of the monkeys’ body and assessing for presence or absence of hair loss greater than half an inch in that region. The body is split into 12 sections, each representing 9% total body coverage, except for the tail which represents 1%.

As there were differences in the way alopecia was measured at health screens prior to 2020, before including the scores in the same dataset, distributions were checked using Kologorov-Smirnov tests. Distributions of alopecia scores were compared between a dataset containing only observations prior to 2019 and a dataset with distributions including the most recent observations from 2019 to 2022. The test indicated the distributions were significantly different from one another ($D = 0.0.2$, $p <$

0.0001). However, because it is not clear if these differences were a result of the scoring system employed or another unmeasured factor, the data were analysed together.

5.2.4 Analysis of acute effect of weaning (retrospective)

To examine the acute effect of weaning, I sampled the full dataset for individuals that had two health screen observations either side of weaning taking place, with the second observation being at least 6 months after weaning. This left us with paired observations for 163 monkeys (70 females). To control for the possibility of changes in alopecia score between health screens being explained by aging between health screens, I took another sample of the full dataset for monkeys that had two back-to-back observations where they remained in their natal group with their mother. This produced observations for 86 monkeys (74 females). For this analysis, males were excluded as there were very few (n = 12) that had back-to-back observations in the natal group for comparison with those that were weaned.

After reducing the dataset to females that had health screen observations that were no more than 2 years apart, the remaining sample size was 111 females. 60 females could only be in the wean group i.e. did not have back-to-back observations in the natal group; 41 females could only be in the remain group; 10 females could be in either. To balance groups as much as possible, the 10 females that had back-to-back observations both in the natal group and either side of weaning were placed in the remain group, and their observations either side of weaning were excluded from the dataset.

There is evidence that season is associated with alopecia in indoor housed rhesus macaques (Steinmetz et al., 2005). To control for season, a season variable comprising of three factors was created based on daylight length: ‘short’ for months November to February; ‘long’ for months March to July; and ‘mid’ for other months. As there was only one female whose observation was made in the short season, this observation was excluded prior to analysis.

The final dataset for this analysis consisted of 110 (weaned = 60) females aged between 2.3 – 4.9 years (mean±SD = 3.2±0.6). Weaning ages for the 60 weaned females ranged from 1.5 – 4.2 years (mean±SD = 2.3±0.4).

To assess if being weaned (TRUE or FALSE) was associated with alopecia severity, a generalised linear model was fit with a Poisson error structure. Alopecia score at the second health screen was the outcome variable and predictor variables were if the monkey was weaned at the health screen (true or false), age at second health screen, season, and alopecia score at the first health screen. The dispersion parameter for this model was 0.53, indicating suitability of the Poisson error structure. Collinearity between the predictor variables was tested by calculating variance inflation factors (VIF: Zuur et al., 2009). Collinearity was not an issue (maximum VIF = 1.19). To assess if being weaned was associated with alopecia prevalence, alopecia was treated as binomial where any score above 1 was labelled 1 and all scores of 1 were labelled 0. a generalised linear model was fit to with a binomial error structure using the same predictors as the Poisson model.

To assess if weaning age was associated with alopecia severity at the second health screen, the full dataset with all monkeys with back-to-back observations either side of weaning was used (n = 162; females = 70). Ages ranged from 2 – 4.9 years (mean±SD = 3.1±0.5) and weaning ages ranged from 1.5 – 4.2 years (mean±SD = 2.4±0.5).

A generalised linear model was fit with Poisson error structure. Alopecia score at the second health screen was the outcome variable and predictor variables were weaning age, sex, age at health screen, season, and alopecia score in the first health screen. Overdispersion was not an issue for this model (dispersion parameter = 0.53) indicating Poisson error structure was suitable. Calculating the VIF revealed collinearity between weaning age (VIF = 4.4) and age (VIF = 4.7) to be an issue.

To control for this, the data was then reduced to monkeys aged between 2.5 – 3.5 years (mean±SD = 2.97±0.23) and weaning ages ranged from 1.6 – 2.8 years (mean±SD = 2.3±0.27). This age range was decided based on visual inspection of a histogram of ages that shown the majority of the sample was within this range. The sample size for this model was 128 monkeys (females = 52). The model was then fit to this dataset excluding age as a covariate. Again, overdispersion was not an issue (dispersion parameter = 0.53). Collinearity was not an issue in this model (maximum VIF = 1.03). To assess the effect of weaning age on alopecia prevalence at the second health screen, alopecia was treated as binomial and a generalised linear model was fit with a binomial error structure using the same predictors as the Poisson model.

5.2.5 Analysis of acute effect of weaning (monthly measures)

To elucidate potential seasonal effects, monthly alopecia scores were taken from 79 monkeys that were either weaned or remained in the natal group (see table 5.2 for sample sizes of groups by sex) between November 2020 – July 2022.

Table 5.2. Sample size for monthly dataset

n	Weaned		Not weaned	
	F	M	F	M
	17	20	22	20

To assess the potential effects of weaning and weaning age on alopecia, alopecia was treated as either binomial (to assess associations with prevalence) or as the raw score (1-5: to assess associations with severity) and these were the outcome variables for the models. Four models were fit to the data: two models assessing the difference in alopecia (1) severity (poisson error structure) and (2) prevalence (binomial error structure) between weaned and not weaned monkeys; two models assessing the difference in severity and prevalence by weaning age for the weaned monkeys (n = 37). All models included sex and season. Season was treated as a factor with 4 levels (Winter: 1st December – 28th February; Spring: 1st March – 31st May; Summer: 1st June – 30th August; Autumn: 1st September – 30th November). To account for repeated measures, the random intercept of animal ID was included in the models. Overdispersion was not an issue with this model (maximum dispersion parameter = 0.22), nor was collinearity (maximum VIF = 1.01).

5.2.6 Analysis of chronic effect

To examine the potential long-term effect of weaning on hair loss, a sample was taken from the full dataset to include only weaned monkeys that were screened close to their 3rd birthday (to eliminate age as a confound; mean±SD = 2.98±0.24) and had been weaned at least 6 months prior to the health screen. Observations made in the season with the shortest days were similarly excluded from this dataset due to having few observations (n = 6). The dataset for this analysis contained observations from 247 monkeys (females = 98). Weaning ages ranged from 0 – 2.76 years (mean±SD = 1.75±0.54).

To assess the association between weaning age and alopecia severity close to an individual's 3rd birthday, a generalised linear model was fit with a Poisson error structure. Alopecia score was the outcome variable and predictor variables were weaning age, sex, and season. Overdispersion was not an issue with this model (dispersion parameter = 0.55), nor was collinearity (maximum VIF = 1.05). As with the acute models, a binomial model as fit to assess alopecia prevalence with the same predictor variables as the poisson model.

5.3 Results

5.3.1 Acute analysis results (retrospective)

Being weaned was the only significant predictor for alopecia severity and prevalence at the second health screen after controlling for age, season, and alopecia at the first health screen (see table 5.3).

Table 5.3 Coefficients of retrospective acute model (females)

Variable	Estimate	SE	z	p
(Intercept)	0.404479	0.468164	0.864	0.3876
Weaned at Health Screen†	0.337250	0.151298	2.229	0.0258
Age	0.036294	0.127943	0.284	0.7767
Season‡	- 0.211905	0.164134	- 1.291	0.1967
Alopecia Score at First Health Screen	0.009397	0.105163	0.089	0.9288

† False as the reference factor.

‡ Long as the reference factor.

Weaned females displayed alopecia scores that were around 40% more severe and were around 2.3 times more likely to have alopecia at the second health screen than the group that remained in the natal group (prevalence: Estimate±SE = 0.84±0.42, z = 1.99, p = 0.047; severity: Estimate±SE = 0.34±0.15, z = 2.23, p = 0.026; Figures 5.4A and 5.4B, respectively).

The second model with the males included assessing the association between weaning age and alopecia score at the second health screen after weaning revealed no evidence of an effect of weaning age on alopecia severity but a significant effect of weaning age on alopecia prevalence, with later weaned monkeys being around 48% less likely to display any alopecia with every unit increase in weaning age (Estimate±SE = -0.73±0.33, z = -2.2, p = 0.03; see figure 5.1).

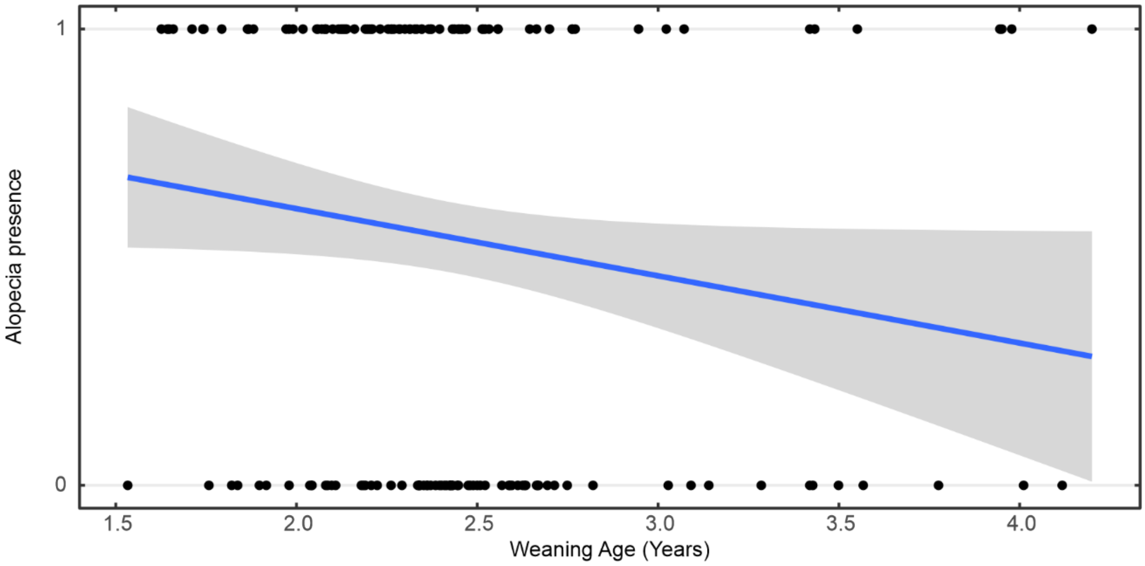

Figure 5.1 Alopecia prevalence by weaning age. Dots represent monkeys that either had (1) or did not have (0) alopecia. Blue line and grey shading represent model regression estimate and 95% confidence intervals.

Males were also found to display alopecia that was around 76% less severe than females (Estimate±SE = -0.27±0.12, z = -2.34, p = 0.02, figure 5.4C), and were around 45% less likely to display any alopecia (Estimate±SE = -0.8±0.34, z = -2.33, p = 0.02; see table 5.4).

Table 5.4 Coefficients for retrospective acute severity and prevalence models.

	Severity model				Prevalence model			
Variable	Estimate	SE	z	p	Estimate	SE	z	p
Intercept	1.1	0.3	3.6	0.0003	2.8	0.9	3.	0.003
Weaning age	-0.07	0.11	-0.6	0.55	-0.72	0.33	-2.2	0.03
Sex†	-0.27	0.12	-2.3	0.02	-0.8	0.34	-2.3	0.02
Season‡	-0.23	0.13	-1.7	0.08	-0.7	0.36	-1.9	0.06
Alopecia in the first HS	-0.05	0.08	-0.6	0.54	-0.13	0.23	-0.6	0.55

†Females as reference factor.

‡Long as reference factor.

5.3.2 Acute analysis results (monthly)

Models fit to the monthly data revealed a significant effect of weaning and season on both prevalence and severity. Weaned monkeys had around 17% more severe alopecia (Estimate±SE = 0.16±0.06, z = 2.5, p = 0.014, figure 5.3A) and were around 20 times more likely to have alopecia (Estimate±SE = 3.0±0.84, z = 3.53, p = 0.0004, figure 5.3C). Furthermore, there were clear seasonal patterns present with monkeys experiencing alopecia in the Spring and Summer months, and the alopecia being more severe (severity: p = 0.042; prevalence: p = <0.0001; figure 5.3B and 5.3D, respectively). For the weaned group, no evidence that weaning age was associated with prevalence or severity was found (see table 5.5 for model coefficients).

Table 5.5. Coefficients of severity and prevalence model for monthly dataset

	Severity				Prevalence			
	Est	SE	z	p	Est	SE	z	P
Intercept	-0.012	0.08	-0.2	0.82	-7.83	1.09	-7.16	<0.0001
Weaned†	0.16	0.064	2.46	0.014	2.95	0.84	3.52	0.0004
Sex‡	-0.05	0.064	-0.8	0.44	-0.17	0.75	-0.2	0.8
Season (Spring)§	0.19	0.09	2.08	0.037	3.3	0.6	5.5	<0.0001
Season (Summer) §	0.22	0.09	2.44	0.015	3.7	0.6	6.09	<0.0001
Season (Winter) §	0.05	0.1	0.52	0.6	1.5	0.63	2.37	0.02
Weaning age*	0.03	0.07	0.46	0.65	0.36	0.54	0.67	0.5

†False as the reference factor.

‡Female as the reference factor.

§Winter as reference factor.

*Fit to dataset with just weaned animals.

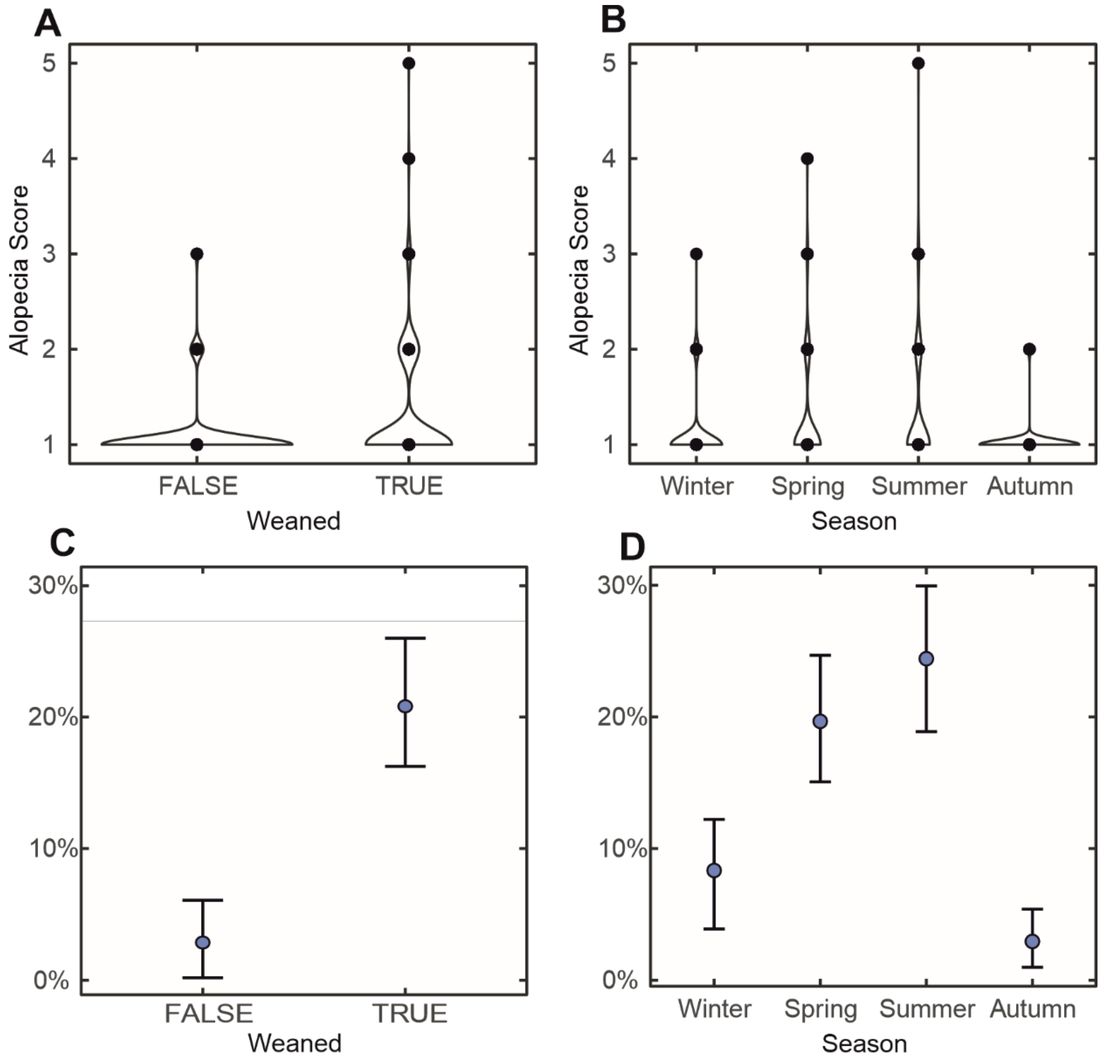

Figure 5.2 A. Violin plot displaying the difference in the distributions of alopecia severity score between the weaned group and the group that were in their natal groups. B. Violin plot displaying the differences in the distribution of alopecia severity scores by season. C. differences in the percentage of individuals that presented with alopecia between the weaned group and the group that remained in their natal groups. Error bars represent bootstrapped 95% confidence intervals. D. Differences in the percentages of individuals that presented with alopecia by season. Error bars represent bootstrapped 95% confidence intervals.

5.3.3 Chronic analysis results

The model assessing the potential chronic effects of weaning age on alopecia at the health screen close to the monkeys 3rd birthday revealed significant effects of sex and season: similarly to the acute dataset, males displayed alopecia that was around 20% less severe than females (Estimate±SE = -0.23±0.93, z = -2.43, p = 0.015, figure 5.4D), and less alopecia altogether, although this effect was near significant (Estimate±SE = -0.51±0.28, z = -1.85, p = 0.065). Furthermore, monkeys that were screened in the ‘mid’ season were less likely to have alopecia with 80% fewer monkeys displaying any alopecia, and had around 38% less severe alopecia those screened in the ‘long’ season (severity: Estimate±SE = -0.48±0.12, z = -3.94, p < 0.0001; prevalence: Estimate±SE = -1.41±0.33, z = 4.29, p < 0.0001, figures 5.1E and 5.4F, respectively). No effect of weaning age was found in the chronic dataset.

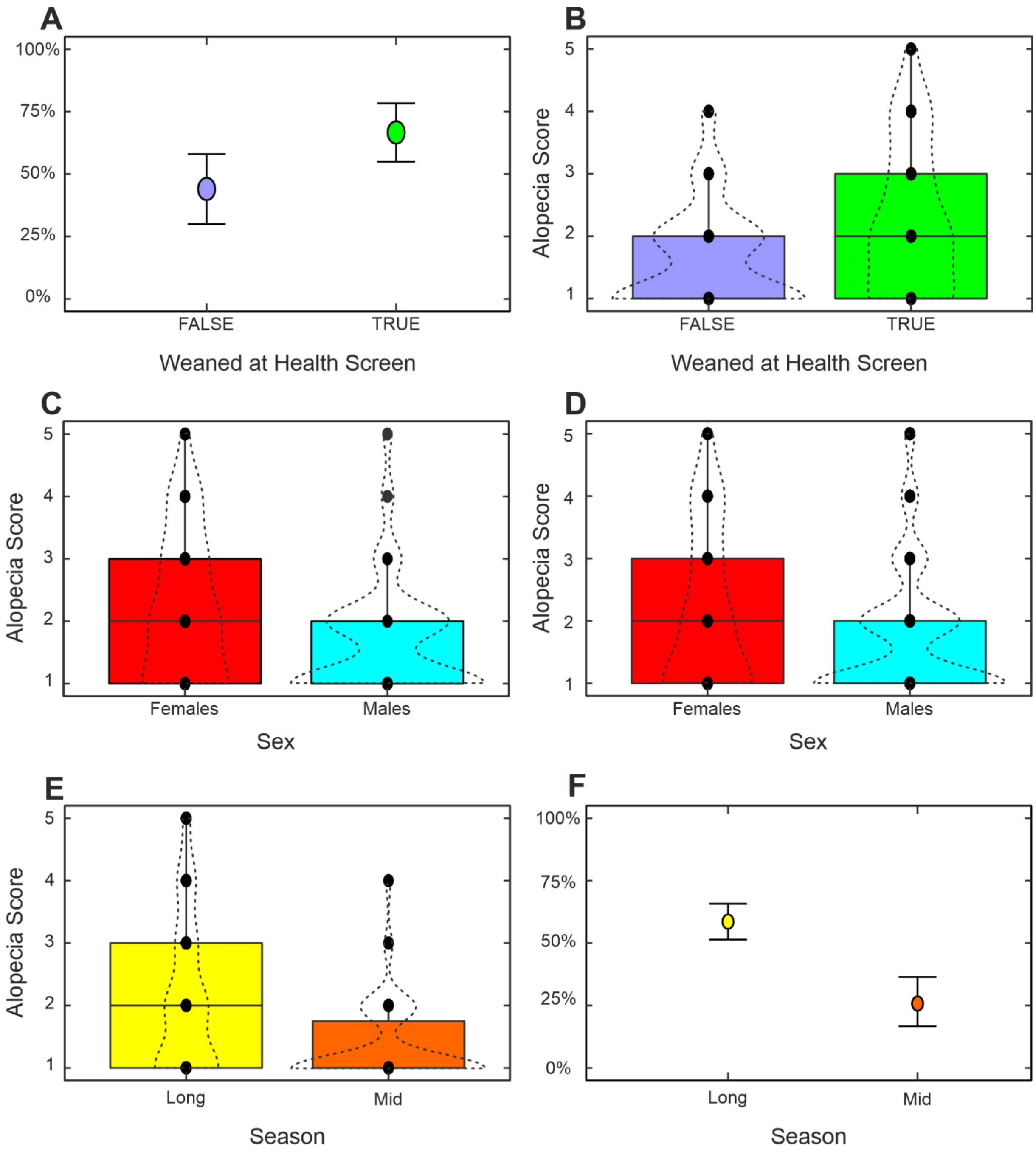

Figure 5.3. A. Difference in the percentage of individuals that presented with alopecia between those that were weaned at the health screen vs those that remained in the natal group. Error bars show bootstrapped 95% confidence intervals. B. Boxplot of differences in alopecia severity at second health screen between those that were weaned vs those that remained in the natal group. Dotted lines represent overlaying violin plot. C. Boxplot of differences in alopecia severity between males and females at the second health screen. Dotted lines represent overlaying violin plot. D. Boxplot of differences in alopecia severity between males and females at observation close to their 3rd birthday. Dotted lines represent overlaying violin plot. E. Boxplot of differences in alopecia between seasons at observation close to 3rd birthday. Dotted lines represent overlaying violin plot. F. Difference in the percentage of individuals that presented with alopecia between seasons at observation close to their 3rd birthday. Dotted lines represent overlaying violin plot.

5.4 Discussion

5.4.1 Summary

The aim of this study was to investigate the potential effect of weaning on the prevalence and severity of alopecia in captive rhesus macaques destined for use in neuroscience research, whilst controlling for age, sex, and season. The analysis encompassed both acute and chronic assessments of alopecia, providing valuable insights into the factors influencing these. In the retrospective acute analysis, weaning emerged as a significant predictor for alopecia severity and prevalence, particularly in females. Weaned females showed significantly higher alopecia scores compared to their non-weaned counterparts. Additionally, males had less severe alopecia than females. The monthly analysis revealed that weaning and season significantly influenced alopecia prevalence and severity. Weaned monkeys experienced more severe alopecia, with a noticeable increase in spring and summer. No association was found between weaning age and alopecia in the weaned group. The chronic analysis focused on the potential long-term effects of weaning age on alopecia and found significant effects of sex and season on alopecia severity and prevalence, with males showing less severe alopecia than females and a lower overall prevalence. Screening season also influenced alopecia, with fewer cases in the 'mid' season compared to the 'long' season. However, no significant effect of weaning age was observed in this dataset.

This study is the first to explicitly test the associations between weaning age and alopecia in captive macaques and highlights the importance of considering the immediate post-weaning period as a critical window for alopecia development, potentially mitigated by weaning age. Understanding the acute effects can inform the development of targeted intervention strategies aimed at reducing stress and promoting the welfare of weaned macaques.

5.4.2 Acute vs chronic effect of weaning age on alopecia

In the retrospective analysis, I found that being weaned was a significant predictor for both the severity and prevalence of alopecia at the second health screen. Weaned females were around 2.3 times more likely to have alopecia and displayed alopecia scores that were approximately 40% more severe than the females that remained in the natal group. This effect was independent of season, age, and previous alopecia. I were unable to test if this effect was present in males using the retrospective dataset as there were too few males who had observations either side of weaning taking place. This is because of breeding colony dynamics: historically, weaned males would be supplied shortly after weaning whereas more females tend to be retained for breeding. Whilst the effect in females were independent of season, health screens have historically taken place in the winter and summer months. As such, monthly observations were conducted to elucidate potential seasonal patterns, albeit with a reduced sample size. In the monthly dataset including both males and females, and controlling for season, this effect was still present: weaned monkeys had approximately 17% more severe alopecia and were approximately 20 times more likely to have alopecia compared to non-weaned monkeys, suggesting greater certainty in the acute effect of weaning on alopecia development. I did not find evidence of an effect of weaning age on alopecia severity. However, there was a significant effect of weaning age on alopecia prevalence in the retrospective dataset, with later-weaned monkeys being approximately 48% less likely to display any alopecia in the short-term. This effect was not replicated in the monthly dataset. One possible reason for this could be due to the sample size being too small to detect the effect in the monthly dataset (n = 37 weaned monkeys) compared to retrospective dataset (n = 162 monkeys).

I did not observe a significant effect of weaning age on alopecia severity or prevalence in the chronic dataset. This discrepancy between the acute and chronic analyses suggests that the impact of weaning age on alopecia may be time-limited or that other factors, such as aging or changes in social dynamics, could have influenced the chronic alopecia outcomes. It is possible that the impact of weaning age on alopecia is more pronounced during the immediate post-weaning period. This could reflect the stress and adjustment associated with the separation from the mother and the transition to a new social group (Dettmer et al., 2018). This acute effect may gradually diminish over time as the monkeys adapt to their new environment and social dynamics. Macaques may exhibit compensatory mechanisms or coping strategies over time to mitigate the effects of early-life stressors like weaning. As the monkeys mature and gain more experience in the social group, they may develop adaptive behaviours or physiological responses that help buffer the long-term consequences of weaning, resulting in a reduced chronic effect. Furthermore, the chronic analysis in the study was conducted at a health screen close to the monkeys' third birthday, indicating a substantial time gap between weaning and the chronic assessment. During this period, the macaques undergo significant physical and behavioural changes associated with growth and maturation, which may introduce additional factors influencing alopecia development. It is possible that these factors, such as aging processes or other environmental influences, overshadow the direct impact of weaning age on chronic alopecia.

This is, to our knowledge, the only study to date to directly test the impact of this early life event on subsequent alopecia development in rhesus macaques. In humans, stressful life events have been attributed to the acute development of alopecia (Brajac et al., 2003). A similar study by Conti *et al.* (2012) found that peer reared females were more likely to experience hair loss than other rearing conditions. Although it is not clear whether this result was acute or chronic as the authors do not mention the time between rearing and alopecia measurements. The authors note that this group also displayed more aggression, making it difficult to determine the effect of early life stress on hair loss where it could be a result of aggressive hair plucking.

Similarly, I cannot directly speak to the mechanisms by which the monkeys in the present dataset lost hair following weaning. One potential mechanism could be physiological: cortisol induced dysregulation of the hair follicle (Thom, 2016). Equally, it could be a result of hair plucking, both self-directed, and by other individuals as monkeys settle into their new group (Reinhardt et al., 1986; Kramer et al., 2011; Luchins et al., 2011; Lutz et al., 2013; Heagerty et al., 2018).

Future studies would do well to try and identify these mechanisms in order to inform the best management practices for welfare. Nevertheless, whatever the mechanisms, this study provides evidence to support the acute effect of weaning on alopecia development and some evidence that weaning age can mitigate this effect. As such, I recommend that facilities aiming to reduce alopecia prevalence in macaques delay weaning as long as possible whilst monitoring newly weaned monkeys closely. The statistical model presented in this chapter estimates suggest that for every year weaning is delayed, the likelihood of the monkey developing alopecia can be expected to reduce by around 50% in the short term.

Additionally, this study notably lacks an examination of potential non-linear effects. This oversight is significant, as there could be a complex, non-linear relationship where either exceptionally early or late weaning might disproportionately influence the incidence and severity of alopecia. The linear model used might mask such nuances, potentially overlooking critical thresholds or turning points in weaning age that could have a more pronounced impact on alopecia outcomes. This gap suggests the need for further research employing models capable of detecting non-linear patterns, to more accurately determine the optimal weaning age that minimises alopecia risk. Such models would be valuable in identifying any critical periods where the stress of weaning could either be particularly detrimental or, conversely, have less impact on the health and welfare of the animals.

5.4.3 Seasonal patterns

In terms of seasonal patterns, I observed clear associations between the occurrence and severity of alopecia and specific seasons. Monkeys exhibited higher rates of alopecia in the Spring and Summer months, with the condition being more severe during these periods. This finding is consistent with the Steinmetz *et al*. (2006) which both reported seasonal variations in alopecia occurrence and severity among monkeys. This observation suggests a potential environmental influence on the development and severity of alopecia. At the CFM factors such as temperature, humidity, and diet are controlled as much as possible. One possible mechanism for this could be hormonal changes with exposure to natural light across the varying photoperiod. Both melatonin and prolactin have been shown to be important in the hair cycle (Craven, 2001; Trueb, 2010) and vary as a function of natural light, although future research is needed to test this hypothesis. Furthermore, not all monkeys develop alopecia during these seasons suggesting factors such as individual genetics could be important in identifying which animals are more at risk during these seasons. Nevertheless, as alopecia is often used as a welfare indicator and facilities concerned about alopecia development should take seasonal variations into consideration when assessing individual welfare. It is possible that only a subset of monkeys displaying alopecia during these months could be experiencing poor welfare while the rest are undergoing natural variation.

5.4.4 Sex differences

In both the acute and chronic retrospective dataset, I observed that males were less likely to develop alopecia and displayed less severe alopecia than females. This is supported by most studies investigating sex differences at multiple ages (Beisner & Isbell, 2009; Kroeker et al., 2014; Lutz & Sharp, 2015). In the present study, I excluded parous and pregnant females and can therefore conclude that this difference is not driven by pregnancy related alopecia (Davis & Suomi, 2006; Dettmer et al., 2017). Differences may be attributed to hormonal factors associated (Vessey & Morrison, 1950) or could indicate that females are more at risk of experiencing poor welfare as female humans have shown to be more at risk of developing stress related disorders (Chaplin et al., 2008). Again, facilities aiming to reduce alopecia might want to focus on intervention strategies targeting females.

5.4.5 Limitations to consider and future directions

5.4.5.1 Generalisability of findings

This study was conducted at one breeding facility located in the UK. Past research has shown that facilities can differ in their rates of alopecia (Novak et al., 2014) and that prior facility can affect alopecia (Kroeker et al., 2017). As such, it is unclear how generalisable these results are to the wider population of captive macaques. Future research including multiple facilities or collaborating with other research institutions could help gather a more diverse and representative sample of macaques.

5.4.5.2 Retrospective dataset

The study utilised a retrospective design, which may introduce biases and limitations. Retrospective data collection relies on existing records and may be subject to incomplete or inconsistent information, potentially impacting the accuracy and reliability of the findings. By collecting data new data focusing on following macaques from birth through weaning and beyond, future studies could provide more accurate and reliable information on the temporal relationship between weaning age and alopecia. This study aimed to do just that but was limited to a small sample size and limited time frame.

Additionally, the small sample size per group precluded the inclusion of group as a random factor in the model. Given that social hair pulling could contribute to alopecia in the animals, the absence of group analysis is a notable limitation. Future research could consider exploring this aspect. Furthermore, a significant limitation of this study is the lack of behavioural data to determine whether animals were pulling out their own hair or if alopecia was a result of social hair pulling. This omission has certainly limited our understanding of the potential causes of alopecia. The inclusion of such behavioural data in future studies will undoubtedly yield deeper insights into the underlying causes of alopecia in macaques.

5.4.5.3 Lack of Intervention or treatment analysis

This study primarily focused on identifying associations between weaning age and alopecia without investigating specific interventions or treatments. Future studies could explore the effectiveness of interventions targeting alopecia associated with weaning such as enrichment strategies (Novak et al., 2017), medical treatments (Uno & Kurata, 1993), or dietary supplements (Hamel et al., 2017) as this might lead to some understanding of the mechanisms by which monkeys are losing hair and how it relates to welfare.

5.4.6 Conclusions

In conclusion, our study provides valuable insights into the associations between weaning, sex, and season, on the prevalence and severity of alopecia in a captive rhesus macaque colony. It is evident that weaning plays a significant role in the development and progression of acute alopecia in this sample and that early weaning may increase the risk of alopecia, both in terms of prevalence and severity. This information is crucial for primate caregivers and researchers, as it highlights the potential welfare implications of early weaning practices. Efforts should be made to evaluate and adjust weaning protocols to minimise the negative impact on primate welfare, particularly in relation to alopecia development.

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Chapter 6. Does Weaning Age Predict Health Outcomes in Captive Purpose-Bred Rhesus Macaques (*Macaca mulatta*)? A Retrospective Study

6.0 Chapter summary

There is robust evidence that very early weaning has long-lasting negative consequences on monkeys' behaviour (increased neophobia and stereotypies) and general health (reduced weight, increased susceptibility to gastrointestinal infections and disorders, increased prevalence of alopecia, and higher probability of being wounded). Whilst the link between early weaning and subsequent health and behaviour consequences is well established, no study has systematically tested the hypothesis that weaning later than the current UK guidelines (of no earlier than 10 to 14 months) will have further welfare benefits. To test this, I took advantage of historical records at the Medical Research Council's Centre for Macaques. During annual health screens, animal technicians and the Named Veterinary Surgeon take measures of weight, Campylobacter presence from rectal swabs, and blood samples for haematology analysis. To test acute effects of weaning, I sampled records for monkeys that had two consecutive observations: one group either side of weaning; one group that remained in the natal group (n = 160). To test chronic effects, I sampled the data for observations of individuals near their 3rd birthday (excluding those that were weaned within 6 months of the observation; n = 212). I found an acute effect of weaning on Campylobacter incidence: weaned individuals were more likely be infected with Campylobacter. Furthermore, a later weaning age appeared to mitigate these effects: later weaning age was associated decreased probability of Campylobacter infection. I found that weaning age did not explain variation in weights, but weaned monkeys were slightly lighter (80g) overall than monkeys that remained in the natal group. I found no evidence that weaning age was associated with immunology as measured by neutrophil to lymphocyte ratios. Taken together, these results corroborate earlier research highlighting the benefits of a later weaning age, particularly for reducing infection rates within colonies.

6.1 Introduction

6.1.1 Campylobacter

Campylobacter is a genus of gram-negative bacteria that is commonly found in the gastrointestinal tract of many animals, including rhesus macaques (*Macaca mulatta*). In humans, it is one of the leading causes of bacterial gastroenteritis, which is characterised by symptoms such as diarrhoea, abdominal pain, and fever (Kienesberger et al., 2012). Campylobacteriosis is one of the most common bacterial causes of foodborne illness in the world. *Campylobacter* bacteria are typically found in the intestines of animals, particularly poultry, and can be spread to humans through the consumption of contaminated food or water, or through contact with infected animals or their faeces. The bacteria can also be spread from person to person.

Campylobacter infection is a significant health concern for both humans and animals, with an estimated 1.3 million cases reported in humans annually in the United States alone. Rhesus macaques have been used as a model organism for biomedical research due to their genetic and physiological similarity to humans. This includes the study of infectious diseases such as *Campylobacter*. However, the susceptibility of rhesus macaques to *Campylobacter* infection and the potential effects of early-life stress on their susceptibility to this disease are still not well understood.

Campylobacter is also prevalent in many facilities housing non-human primates for research and clinical symptoms include weight loss, dehydration, and in severe cases, death (Baze & Bernacky, 2002; Prongay et al., 2013; Nunez et al., 2015; Laing et al., 2018). Due to the potential for this bacterium to cause significant morbidity and mortality in research animals, it is essential to understand the factors that may predispose macaques to *Campylobacter* infection.

Campylobacter infections in rhesus macaques are typically treated with antibiotics, but as with any infection, prevention is key. To prevent the spread of *Campylobacter* in animal research facilities, it is important to maintain good hygiene practices, such as frequent hand washing, cleaning, and disinfecting animal enclosures, and monitoring animals for signs of illness. Vaccination against *Campylobacter* may also be an option for some animal populations in the future, as studies testing vaccine efficacy, using macaques as a model organism, are showing promising results (Islam et al., 2006; Quintel et al., 2020). To the best of my knowledge at the time of writing, no vaccine is commercially available.

Several factors have been identified as risk factors for *Campylobacter* infection and subsequent clinical signs and mortality in macaques. These include age, sex, housing conditions, and immune status (Prongay et al., 2013). Younger macaques are more susceptible to fatal outcomes of infection than adults (Prongay et al., 2013). Males have been found to be more frequently infected with *Campylobacter* than females, although this may be due to behavioural differences between the sexes (Andrade et al., 2007). Furthermore, *Campylobacter* prevalence has been shown to vary with season, with cases of *Campylobacter*iosis peaking during summer and autumn months (Nylan et al., 2002; Kovats et al., 2004; Jorgensen et al., 2011; Djennad et al., 2019; Dou et al., 2021). This variation is likely due to seasonal fluctuations in immune system status (Mann et al., 2000).

Infections in macaques are typically acute. Not all macaques show clinical signs of *Campylobacter* infection, and some individuals may carry the bacteria without displaying any symptoms (Kalashnikova et al., 2002; Islam et al., 2005; Andrade et al., 2007). However, even asymptomatic carriers can shed the bacteria in their faeces and potentially transmit the infection to other animals or humans for around 43 days following infection, after which infection is usually eliminated (Fitzgeorge et al., 1981). Macaques with previous exposure can mount a stronger immune response and may exhibit more mild or asymptomatic infections but can still shed the bacteria and potentially transmit the infection to other animals or humans, albeit for a reduced time (Fitzgeorge et al., 1981). Therefore, it is important to gain a deeper understanding of the risk factors associated with the prevalence of the disease.

6.1.2 Campylobacter and the gut microbiome

The presence of *Campylobacter* in the gut can disrupt the balance of the microbial community and alter the diversity and abundance of different bacterial species. Studies have shown that *Campylobacter* infection can reduce the diversity of the gut microbiome, with a decrease in the relative abundance of commensal bacteria such as Bacteroidetes and an increase in potentially pathogenic bacteria such as Proteobacteria (Rendina et al., 2019). Additionally, the severity of infection may be correlated with changes in the gut microbiome composition. The mechanisms by which *Campylobacter* alters the gut microbiome are not fully understood, but it is thought to involve the production of virulence factors and the induction of an inflammatory response, which can create a less favourable environment for some commensal bacteria. Furthermore, if antibiotics are used to treat infection this will impact the gut microbiome further, although most infections resolve without the need for such interventions (Moore et al., 2005; Alfredson & Korolik, 2007).

Disruption of the gut microbiome by *Campylobacter* infection can potentially affect the welfare of the host. The gut microbiome plays an important role in various physiological functions, including nutrient absorption, immune system development and function, and gut-brain communication. Dysbiosis or disruption of the gut microbiome can lead to various negative health outcomes, such as increased susceptibility to infection, inflammation, and metabolic disorders (Dettemer et al., 2019; Skonieczna-Zydecka et al., 2018; Dupjan & Stamp Dawkins, 2022).

6.1.3 Body weight

In captive rhesus macaques, body weight typically follows a growth curve similar to that seen in the wild population. Infants experience rapid weight gain during their first year of life, while juvenile and subadult macaques experience slower, steady growth until they reach adulthood (Turcotte et al., 2021). Once they reach adulthood, body weight may stabilise, although changes in weight can still occur due to age-related changes in metabolism or changes in diet, activity level, or health status. In general, adult rhesus macaques have a weight range of 4 to 15 kg, depending on age, sex, and other factors.

It is important to note that individual variation exists, and single cross-sectional measurements of body weight may not be an accurate reflection of an individual macaque's health or welfare status. Therefore, it is necessary to establish individual baseline measurements and to use multiple welfare measures in conjunction with longitudinal body weight monitoring to fully assess the health and welfare of captive rhesus macaques.

Many factors can affect weight such as genetics (Ha et al., 2002; Kanthaswamy et al., 2014), dam age and weight (Bercovitch et al., 1998; Wilson et al., 1978), milk quality, maternal behavior, and environmental stress (Haertel et al., 2018). For example, Schapiro and Kessel (1993) found that juvenile macaques provided with environmental enrichment weighed significantly more than those in comparatively barren enclosures. Furthermore, the enriched macaques were more similar in weight to free ranging conspecifics. This study highlights how measures that reduce stress and increase the opportunity for natural behaviours can affect typical growth patterns of young macaques, and similar results have been found in baboons (*Papio Anubis*: Garcia et al, 2009). Chronic stress can lead to a variety of physiological changes that may affect weight. For example, stress can increase the release of the hormone cortisol, which can increase appetite and cause the body to store fat, particularly around the abdominal region (Sapolsky, 2004). As being obese or underweight can be indicative of poor welfare (Bauer et al., 2011; Zijlmans et al., 2021), measures such as infection status, habitat quality, physiological, and behavioural are important in conjunction with weight to gain a more comprehensive understanding of welfare (Prescott et al., 2023).

6.1.4 Neutrophil to Lymphocyte Ratio (NLR)

The neutrophil-to-lymphocyte ratio (NLR) is considered a marker of systemic inflammation. Neutrophils are typically the first type of white blood cell to arrive at the site of an infection, and they play an important role in fighting off invading pathogens. Lymphocytes, on the other hand, are a type of white blood cell that are involved in the immune response and can help to identify and destroy foreign substances, such as bacteria and viruses. An elevated NLR has been associated with a number of different health conditions, including inflammation, infection, and cancer (Neal Webb et al., 2020; Capitanio et al., 2023).

In the context of biomedical research, the NLR may be used as a measure of immune function and overall health in animal models, including macaques. A higher NLR may indicate a greater likelihood of infection or other health problems, while a lower NLR may suggest that the animal is in good health and has a well-functioning immune system.

When an individual experiences stress, the body releases stress hormones, such as cortisol and adrenaline, which can alter immune function and impact the balance of white blood cells in the body. Specifically, stress can increase the number of circulating neutrophils and decrease the number of circulating lymphocytes, leading to an increase in NLR (Capitanio et al., 2023).

6.1.5 Weaning and health measures

Maternal separation involves removing infant primates from their mothers for a period of time and has been shown to have various effects on the behaviour and physiology of rhesus macaques. For example, very early separation has been shown to correlate with altered regulation of HPA axis activity in response to stress (Higley et al., 1992; Capitanio et al., 2005; Kinnally & Capitanio, 2015), reduced expression of genes involved in antiviral and antimicrobial response (Cole et al., 2012), lowered immune competence (Coe et al., 1989; Gust et al., 1992; Lubach et al., 1995; Clarke et al., 1996; Lewis et al., 2000; Kinnally & Capitanio, 2015), negative health outcomes (Conti et al., 2012) and heightened reactivity to stress and novelty (Suomi, 1991; Timmermans et al., 1994; Kinnally et al., 2010; Gottlieb & Capitanio, 2013). Hence it is why historically maternal deprivation has been used as a model of early life adversity (McKinney, 1974; Sackett et al., 2002). However, there is limited research on the specific effects of maternal separation on *Campylobacter* susceptibility in rhesus macaques. Maternal separation and weaning are common stressors experienced by infant primates, including rhesus macaques, that can have long-lasting effects on their health and welfare. There is growing evidence that early-life stress, including early weaning, can alter immune function and increase susceptibility to infectious diseases, such as *Campylobacter* infection.

Evidence suggests that weaning could affect *Campylobacter* susceptibility by various mechanisms: Munoz-Zanzi *et al*. (1999) found that monkeys that were lighter at weaning were 3 times more likely to experience chronic diarrhoea. Furthermore, *Campylobacter* has been shown to be associated with diarrhoea (Gottlieb et al., 2018) and diarrhoea has been found to be associated with decreased linear weight by up to 34% in infant rhesus macaques (Haertel et al., 2018). Furthermore, there may be a bi-directional link between *Campylobacter* and immunity through gut microbiome and gut-brain-axis disruptions thus affecting psychological well-being (Dupjan & Stamp-Dawkins, 2022). For example, Dettemer *et al.* (2019) found that typically reared macaques had more Bacteroides than adversely reared, as well as marked differences in other genera of bacteria. Dettermer *et al.* (2019) suggest these findings can serve to explain the differences in immune function between differentially reared macaques reported by Lubach and colleagues (1995). An example highlighting the potentially bi-directional nature of the relationship between *Campylobacter* and immunity comes from Clemmons *et al*. (2014). The authors found that in immunocompromised individuals (one that was on an Simian Immunodeficiency Virus study and another that was young), *Campylobacter* infection could spread extra intestinally and become fatal.

A study by Sackett *et al*. (2002) found that surrogate-peer-reared (SPR) macaques did not differ in survival, growth, clinical treatments, injuries, or mortality compared to mother-reared (MR). However, the MR paradigms employed still rely on removing macaques from their mothers earlier than would be acceptable under modern UK guidelines. Therefore, a comparison with more typically reared animals may produce different results. Although other studies have suggested that environmental factors can be associated with growth (Schapiro & Kessell, 1993; Suzuki et al., 2000).

The potential link between early weaning and neutrophil to lymphocyte ratio (NLR) in rhesus macaques is unknown. Neal Webb *et al* (2020) studied chimpanzees and found, counter-intuitively, that more typically reared chimpanzees had higher NLR than atypically reared. In a study in rhesus macaques, Capitanio *et al.* (2023) found that negative early-life experiences were associated with low NLR and low NLR was also associated with later negative outcomes. Again, this finding is counter to patterns found from human subjects and more research is needed to understand the effects of early-life experience on this immunological parameter.

6.1.6 Impact on data quality of model organisms

The welfare of macaques is an important consideration in biomedical research. In addition to ethical concerns, poor welfare can also affect the validity and reliability of scientific data obtained from these animals (Schapiro & Hau, 2023).

Infections can cause a range of physiological and behavioural changes in animals, including alterations in immune function, metabolism, and stress responses. These changes can potentially affect the outcomes of experiments and the reliability of the data collected. For example, a *Campylobacter* infection in a rhesus macaque could cause inflammation in the gut, which may impact the absorption and metabolism of drugs or nutrients being studied. To ensure the validity and reliability of experimental results, researchers must take steps to minimise the impact of any infections or other health conditions that may affect the animals being studied. This may involve careful monitoring of animal health and behaviour, appropriate treatment of infections, and potentially the exclusion of data collected during periods of illness or recovery (Schapiro & Hau, 2023).

6.1.7 Aims of the chapter

This study aims to assess the association between various health measures routinely taken at the Centre for Macaques (CFM) and weaning age to test the predictions that a later weaning age would be associated with:

- Decreased *Campylobacter* infection.
- Decreased weight after controlling for the polynomial age curve and sex.
- Decreased NLR.

6.2 Methods

All animals at the CFM undergo annual health screens (see Chapter 2 for detailed information). Briefly, rectal swabs were performed on each monkey on the day of the health screen and two days following for bacterial assays performed by subcontracting companies for the CFM. Weights were taken from each sedated monkey at health screen and at other various points in an individual’s life. For this analysis I focused only on weights taken while the animal was under sedation as these are more reliable measures. Weights recorded during animal sedation are considered more reliable due to the animal's stillness, which ensures consistent and accurate scale readings, in contrast to weights taken when the animal is awake and potentially moving, which could result in fluctuating and inaccurate measurements. Furthermore, ~2ml of blood was taken from each monkey at an annual health screen by the veterinarian and blood samples were sent to MRC Harwell for haemotology. Samples were analysed using a Advia 2120 analyser.

6.2.1 Campylobacter acute analysis

The total dataset contained 3489 *Campylobacter* tests from 800 animals (Females = 437) from 2004 – 2021.

To analyse the acute effect of weaning, the full dataset was sampled for animals that had (1) consecutive observations either side of weaning and (2) consecutive observations where they remained in the natal group (similar methodology employed for retrospective analysis of alopecia: Chapter 5). Out of the 800 monkeys, 167 monkeys only had observations either side of weaning, 57 monkeys only had observations where they remained in the natal group, and 103 monkeys could be placed into either group i.e. had consecutive observations either side of weaning and in the natal group. To keep groups as balanced as possible, the observations where the monkeys remained in the natal group were kept. This dataset contained 160 monkeys (females = 64) in the ‘remain’ group and 167 monkeys (females = 66) in the ‘wean’ group

To assess the effect of weaning on *Campylobacter* infection (binomial: 1 for positive and 0 for negative), a generalised linear model with binomial error structure was fit to the data. Whether the animal was weaned at the health screen (TRUE or FALSE) was the factor of interest and I controlled for sex, *Campylobacter* infection (binomial) at the first health screen, and *Campylobacter* infection in the group (binomial).

To then assess the effect of weaning age on *Campylobacter* infection in the second health screen for the monkeys that were weaned at the second health screen, a generalised linear model with binomial error structure was fit with weaning age, sex, and *Campylobacter* infection at the first health screen as predictor variables.

There is unavoidable pseudoreplication in the current design as some monkeys will share the same enclosure at a given health screen and will therefore be more likely to contract *Campylobacter*. However, it was not feasible to include group ID as a random effect as there were too few groups with enough animals. To get the group ID to an appropriate level for modelling restricts the data to just 38 monkeys. As such, *Campylobacter* presence within the group (binomial) was included to control for this.

6.2.3 Campylobacter chronic analysis

To assess the potential chronic effect of weaning age on subsequent *Campylobacter* infection risk, the full dataset was sampled for weaned animals that had health screen observations close to their 3rd birthday as possible and were weaned at least 6 months prior to the health screen. The sample size for this analysis was 212 monkeys (females = 90) aged between 2.7 – 3.3 years (Mean±SD = 2.97 ±0.17)

In this dataset, 37 monkeys were weaned but remained in the natal group (mother removed from colony or died), 31 of which were female. As such, males and females were analysed separately. Males that were weaned but remained in the natal group were excluded from analysis due to small sample size (n = 6).

A generalised linear model with binomial error structure was then fit to each dataset (female or male) with *Campylobacter* presence (binomial: 1 for positive test; 0 for negative test). Weaning age, group size, and group *Campylobacter* presence were included as predictor variables. The model fit to the female dataset included the same predictors as the fit to the male dataset, with the addition of group type (factor with two levels: breeding or weaning).

6.2.3 Weight analysis

The total weight dataset contained 11924 weights from 830 monkeys (females = 469). To analyse the effect of weaning age on weight whilst controlling for weight trajectories across age groups and repeated measures, a linear mixed effect model was fit to the data. Males and females were analysed separately because of the sexual dimorphism and difference in growth rates and trends (Turcotte et al., 2021).

Data were restricted to monkeys that were equal to or less than 3 years old. The final dataset contained 2553 weights from 350 males, and 2478 weights from 314 females.

A polynomial term of age was included and visually inspected, the best fit for males was with 4 terms, and 3 terms with females to reflect the additional growth spurt that males undergo from adolescence to adulthood. Weaning age was included as a covariate in the models, and individual ID was included as a random effect to control for repeated measures.

6.2.4 NLR analysis

NLR was calculated by dividing the absolute counts of neutrophils by absolute counts of lymphocytes. NLR was then log transformed to follow a normal distribution. This variable was then included as the outcome of a generalised linear mixed effect model with weaning age, sex, weight, injury (binomial), and time since previous injury as predictors. Sample size for this analysis was 757 observations from 309 monkeys (females = 175) screened between 2017 – 2019.

6.3 Results

6.3.1 Acute dataset

After controlling for *Campylobacter* in the first health screen and *Campylobacter* presence within the group (both not significant), I found significant effects of weaning and sex (see table 6.1). Weaned animals were around twice as likely to test positive for *Campylobacter* at the second health screen than the group that remained in the natal group (Estimate±SE = 0.72±0.3, z = 2.5, p = 0.014; see figure 6.1A). Males were more at risk overall in this sample, being over 2.5 times more likely to present with *Campylobacter* infection (Estimate±SE = 1.02±0.3, z = 3.3, p = 0.001; see figure 6.1B). For the animals that were weaned at the second health screen, I found that a later weaning age negatively correlated with incidence of *Campylobacter*, that is for every unit increase in weaning age, a monkey was estimated to be just over half as likely to present with *Campylobacter* infection (Estimate±SE = -0.82±0.26, z = -3.2, p = 0.0014; see figure 6.1C).

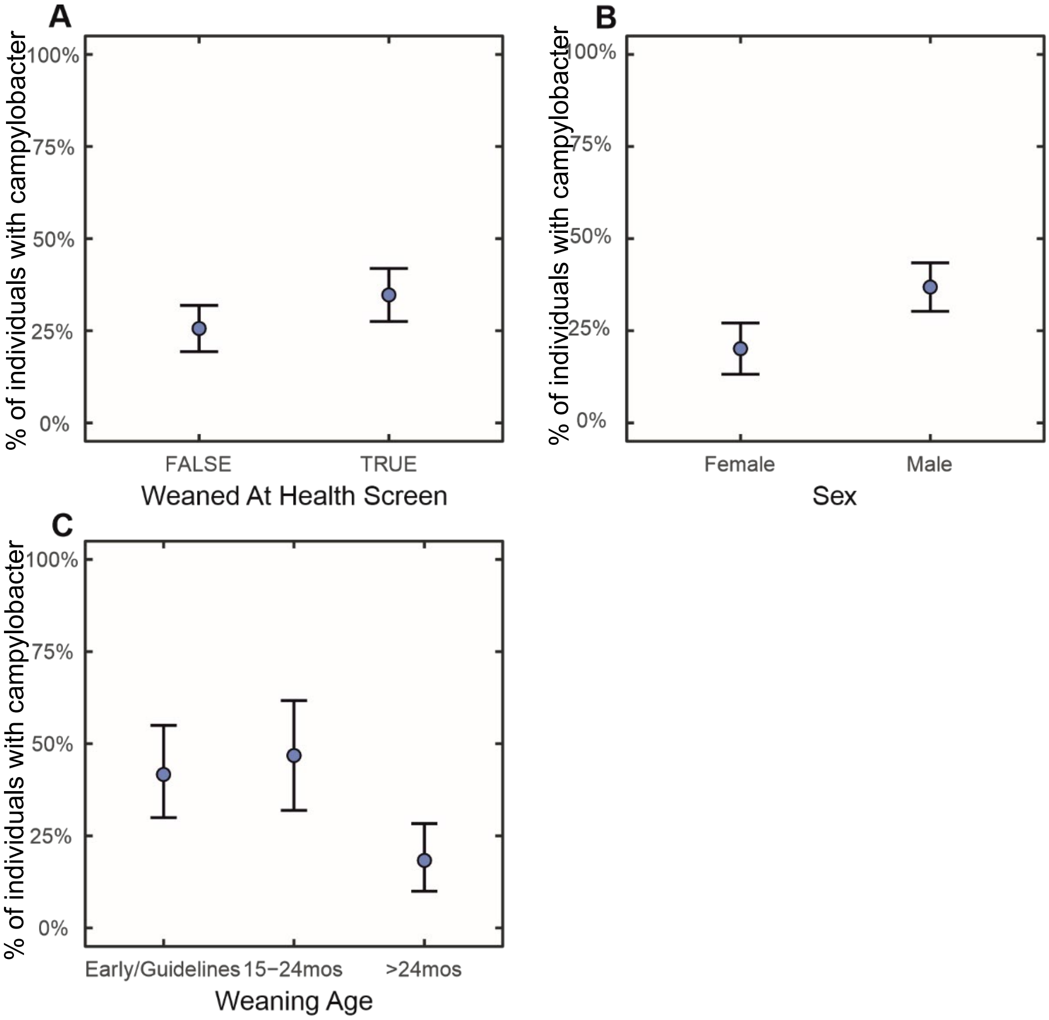

Figure 6.1 Comparison between the proportion of individuals that tested positive for campylobacter between A) those that were weaned or remained in the natal group at the second health screen. B) Males and females. C) Weaning age, here depicted as categorical for visualisation purposes. Dots represent to proportion of the group that tested positive for campylobacter. Error bars represent bootstrapped 95% confidence intervals.

Table 6.1 Model coefficients for acute *Campylobacter* model

Variable	Est	SE	z	p
Intercept	-20.73	974.05	-0.02	0.98
Weaned	0.72	0.29	2.45	0.014
Sex*	1.016	0.31	3.34	0.0009
Group *Campylobacter*	19.44	974.05	0.02	0.98
Campylobacter	0.53	0.34	1.55	0.12
Weaning age	-0.68	0.26	-3.12	0.0014

*Females as the reference category

6.3.2 Chronic dataset

Analysis of weaning age and *Campylobacter* presence for animals that were weaned and where observations were made close to their 3rd birthday, no significant effects were revealed in the male dataset model (see table 6.2 for model results). In the female dataset model, no evidence that weaning age explained *Campylobacter* infection was found. However, it was found that weaned females that had been placed into weaning groups (compared to those that remained in the natal group) were close to 6 times more likely to test positive for *Campylobacter* (Estimate±SE = 1.78±0.8, z = 2.3, p = 0.023; see table 6.2 and figure 6.2).

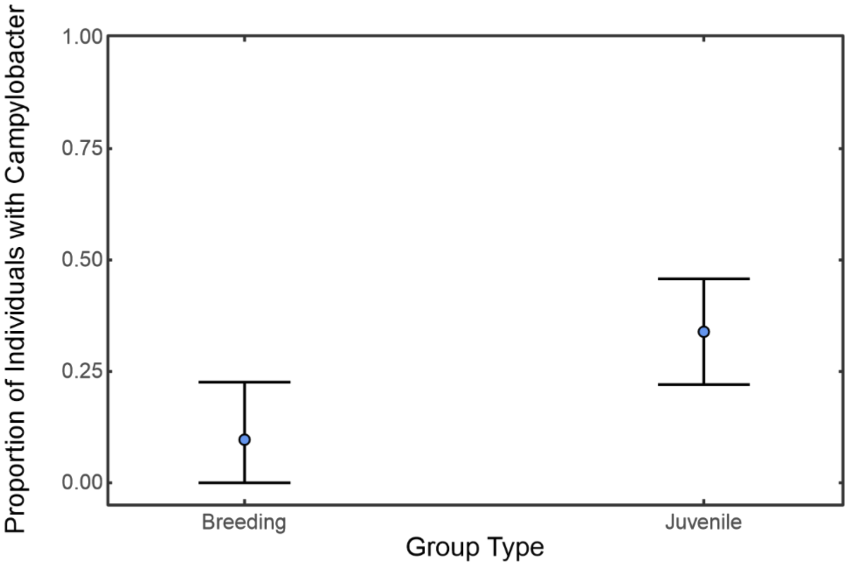

Figure 6.2 Comparison between the proportion of individuals that tested positive for campylobacter between females that remained in the natal group (Breeding) and those that were weaned into same sex weaning groups (Juvenile). Dots represent to proportion of the group that tested positive for campylobacter. Error bars represent bootstrapped 95% confidence intervals.

Furthermore, it was estimated that for every unit increase in group size, the odds of *Campylobacter* infection decreased by around 17%, although this effect did not meet the criterion for statistical significance (Estimate±SE = -0.19±0.1, z = -1.9, p = 0.065).

Table 6.2 Model coefficients for female and male chronic *Campylobacter* models

	Est	SE	z	p
Females				
Intercept	-19.95	1904.72	-0.01	0.9
Weaning age	0.63	0.53	1.18	0.24
Group type	1.78	0.79	2.27	0.02
Group size	-0.19	0.09	-1.85	0.065
Group *Campylobacter*	19.33	1904.72	0.01	0.9
Males				
Intercept	-19.63	1466.48	-0.013	0.9
Weaning age	0.48	0.42	1.16	0.25
Group size	-0.07	0.09	-0.74	0.46
Group *Campylobacter*	19.52	1466.48	0.013	0.9

6.3.3 Weights

After controlling for weight gain across age, I found that weaned females and males weighed around 80g less than counterparts that remained in the natal group (females: Estimate±SE = -0.084±0.03, t = -3.2, p = 0.016; males: Estimate±SE = -0.087±0.023, t = -3.8, p = 0.0001; see table 6.3).

Table 6.3 Model coefficients for female and male weaning weight models.

	Est	SE	t	p
Females				
Intercept	3.15	0.026	119.38	<0.0001
poly(Age, 3)1	82.88	0.61	136.61	<0.0001
poly(Age, 3)2	-6.1	0.45	-13.64	<0.0001
poly(Age, 3)3	0.27	0.43	0.63	0.53
Weaned*	-0.08	0.027	-3.16	0.0016
Males				
Intercept	3.26	0.025	130.55	<0.0001
poly(Age, 3)1	112.26	0.62	180.91	<0.0001
poly(Age, 3)2	6.67	0.44	15.02	<0.0001
poly(Age, 3)3	7.37	0.40	18.24	<0.0001
poly(Age, 4)4	-4.9	0.39	-12.76	<0.0001
Weaned*	-0.087	0.023	-3.81	0.00014

*FALSE as the reference category

After controlling for weight gain across age with polynomial terms, weaning age was not found to affect weight for males or females (see table 6.4).

Table 6.4 Model coefficients for weaned female and male weight models

	Est	SE	t	p
Females				
Intercept	3.94	0.11	37.54	<0.0001
Poly(Age)1	52.44	0.74	70.62	<0.0001
Poly(Age)2	-6.38	0.69	-9.13	<0.0001
Poly(Age)3	1.27	0.49	2.55	0.01
Weaning age	-0.03	0.06	-0.57	0.57
Males				
Intercept	4.37	0.09	50.87	<0.0001
Poly(Age)1	79.19	0.77	103.56	<0.0001
Poly(Age)2	7.38	0.78	9.5	<0.0001
Poly(Age)3	4.29	0.62	6.89	<0.0001
Poly(Age)4	-5.15	0.48	-10.68	<0.0001
Weaning age	-0.06	0.05	-1.1	0.27

6.4.4 NLR

I found no association between weaning age and NLR (see table 6.5) after controlling for weight, if the monkey had presented with an injury at the health screen, and time since their last injury. I did find that males were estimated to have NLR that were around 1.19 lower than females. Heavier monkeys were estimated to have larger NLR, for every kg increase in weight NLR was estimated to increase by 0.12. Furthermore, injured monkeys had NLR that were around 1.67 larger than monkeys without an injury (see table 6.5).

Table 6.5 Model coefficients for NLR model				
	Est	SE	t	p
Intercept	2.49	0.55	4.51	
Weaning age	-0.13	0.19	-0.69	0.49
Sex*	-1.19	0.29	-4.02	<0.0001
Weight (Kg)	0.12	0.05	2.47	0.014
Time since injury (days)	-0.0004	0.0004	-1.09	0.28
Injury	1.67	0.44	3.85	0.00014
*Females as the reference category				

6.4 Discussion

6.4.1 Summary

The purpose of the study was to investigate the relationship between weaning age and various health outcomes, specifically focusing on *Campylobacter* infection, weight differences, and neutrophil-to-lymphocyte ratio (NLR) in captive purpose-bred rhesus macaques. The analysis encompassed both acute and chronic assessments of *Campylobacter* infection, providing valuable insights into the factors influencing these. This study is the first to explicitly test the associations between weaning age and *Campylobacter*, weight, and immunological measures in captive macaques and highlights the importance of considering the immediate post-weaning period as a critical window for *Campylobacter* susceptibility, potentially mitigated by weaning age. Understanding the acute effects can inform the development of targeted intervention strategies aimed at reducing stress and promoting the welfare of weaned macaques.

6.4.2 Acute vs chronic effect of weaning age on Campylobacter

In the acute analysis, I found that weaned animals were around two times more likely to test positive for *Campylobacter* at the second health screen compared to those that remained in the natal group and males were more likely than females to be infected. Furthermore, this effect was found to be independent of the presence of *Campylobacter* in the first health screen and *Campylobacter* presence within the group, suggesting that weaning itself increases the susceptibility to *Campylobacter* infection. For the weaned animals, a later weaning age was negatively correlated with *Campylobacter* infection. For every year increase in weaning age, likelihood of being infected with *Campylobacter* was halved, suggesting a later weaning age could mitigate the effect of weaning on *Campylobacter* infection.

I did not find evidence for any chronic effect of weaning age on *Campylobacter* infection for males or females. However, I found that weaned females placed into weaning groups were approximately six times more likely to test positive for *Campylobacter* compared to those remaining in the natal group. This suggests that the group change is more important in determining *Campylobacter* infection than weaning. Unfortunately, as very few weaned males had remained in the natal group, I could not test if this effect was replicated in both sexes.

There are several potential mechanisms or hypotheses that could explain the observed associations between weaning and *Campylobacter* infection in the study. While the specific mechanisms cannot be definitively determined without further investigation, the following hypotheses provide plausible explanations.

The finding that weaned animals in the acute dataset were more likely to test positive for *Campylobacter* suggests that the weaning process itself may contribute to an increased risk of infection, potentially through decreased immunity. This finding aligns with previous studies that have demonstrated an association between removal from the mother and natal group and an elevated susceptibility to infectious diseases (Lubach et al., 1995). Further support for this comes from the finding that weaned animals that remained in the natal group were less likely to experience *Campylobacter*. Furthermore, the finding that a later weaning age was associated with decreased risk in this group could suggest that separation at a time when the immune system is more immature, leaves individuals more susceptible (Lubach et al., 1995). As weaning is a critical period during which animals transition from maternal dependency to independent feeding. This transition may coincide with an immaturity or developmental stage of the immune system, making younger weaned individuals more susceptible to *Campylobacter* infection.

Other experimental research has found that macaques with previous exposure can mount a stronger immune response and may exhibit more mild or asymptomatic infections (Fitzgeorge et al., 1981). This suggests that later weaned animals may have been less likely to be infected because of a previous infection in the natal group. However, I did not find evidence that previous infection was associated with increased or decreased susceptibility in this sample, suggesting that later weaning can mitigate the acute effect of weaning on infection susceptibility. Could that then result in later weaned monkeys becoming more prone to infection later in life? In this study, the lack of a chronic association with weaning age and infection provides support against this hypothesis as later weaned animals were no more likely to be infected later in life than earlier weaned animals. Furthermore, earlier weaned monkeys were more likely to experience acute but not chronic infection, suggesting following initial infection they developed immunity.

Unfortunately, with the data available, I are unable to determine which of the infected animals presented with any clinical signs or symptoms that pertain to compromised welfare such as diarrhoea (Baze & Bernacky, 2002; Prongay et al., 2013; Nunez et al., 2015; Laing et al., 2018), as this was not recorded during health screens. Although it is worth noting that whilst *Campylobacter* can be associated with mortality in rhesus macaques (Prongay et al., 2013), there was no such incidence in this colony. As such, it is difficult to quantify exactly if later weaned animals experienced improved welfare and future research is needed to investigate this. However, if monkeys are more susceptible to this infection, it is possible they are more susceptible to others. Furthermore, where animals are purpose bred to be used in research, infections can potentially affect the outcomes of experiments and the reliability of the data collected (Schapiro & Hau, 2023). Campylobacter infection in could cause inflammation in the gut, which may impact the absorption and metabolism of drugs or nutrients being studied, potentially requiring more animals to be used in the experiment or animals being used where they are not suitable. To this point, methods that can reduce infections will have benefits in terms of the animal's welfare and lead to a reduction of animals needed for studies. The present findings indicate that a later weaning age is promising for achieving this aim. I recommend that colonies looking to reduce infection rates consider leaving macaques in their natal groups for longer than is currently recommended, although further research is needed to identify how weaning age may be associated with other potentially important infections.

The low incidence of illness observed at the CFM is a positive indicator of animal health and sanitary conditions. However, this finding also suggests that a single-time-point measure may not be sensitive enough to detect subtle variations in infection rates or to fully assess the health impacts of *Campylobacter* in the colony. it is advisable for future research to consider longitudinal studies that track cumulative illnesses over time. Such an approach would allow for a more nuanced understanding of infection dynamics, including the frequency, severity, and duration of *Campylobacter*-related illnesses. Moreover, longitudinal data could help in identifying potential risk factors and effective control measures, thus contributing to better health management in animal colonies.

6.4.3 Weaning and weight

The weight dataset investigated the influence of weaning age on weight differences and compared the weights of weaned individuals to those remaining in the natal group, controlling for weight gain across age. The analysis found that both weaned females and males weighed around 80g less than their counterparts. Furthermore, weaning age did not have a significant effect on weight for males or females. Whilst the effect of weaning met the criteria for statistical significance, the observed effect is arguably negligible. Take an example of 3-year-olds in the dataset. The average weight for 2.7 - 3-year-old macaques in the current dataset is 4.67 Kg (SD = 0.66, n = 205). At the CFM, in line with Home Office standards, a reduction in weight that is greater than 5% of the body weight is the first point at which monitoring is advised to take place, following this, reductions of 10% of body weight is cause for intervention, and at a 20% reduction, euthanasia discussions will take place with named persons. In the case of the 3-year-olds in this example, the difference in weights is around 2% of the average body weight for that age range and is unlikely to alone be an indicator of poor welfare or cause for concern in these monkeys. One possible explanation for this slight difference could be that the monkeys in weaning groups are with more peers and may therefore be more active as they have more access to play.

The only other study to date that has investigated weight differences between monkeys weaned at different ages is one by Goo and Fugate (1984). These authors found that monkeys that had remained with their mother for the first year of life were over 200g heavier at 1 year of age than monkeys that were weaned at 6 months of age. One possible reason why this study did not find similar evidence of weight disparities is because the average weaning age in our sample was 1.3 years old and only 27 monkeys were weaned earlier than 1 year old. Suggesting that allowing the infant to remain with its mother for at least the first year of life can nullify the observed effect of weaning on weight disparities.

6.4.4 Weaning age and NLR

The findings regarding the association between weaning age and NLR (neutrophil-to-lymphocyte ratio) suggest that there was no significant relationship between these variables after controlling for weight, presence of injury, time since last injury, and sex. This implies that weaning age alone does not appear to be a strong predictor of NLR in the studied population. The NLR ratio is often used as an indicator of systemic inflammation and immune response (Neal webb et al., 2020). A higher NLR is typically associated with increased inflammation and can be indicative of various health condition, making the finding that monkeys with an injury had a higher NLR intuitive.

It is worth noting that the absence of a significant association between weaning age and NLR does not necessarily imply that weaning age has no impact on immune function or inflammatory response. Other unmeasured variables or interactions with other factors not considered in this study may play a role in determining NLR levels. For example, previous research has found differences in immunological parameters when measuring lymphocyte proliferation (Laundenslager et al., 1990). Additionally, effects of early life experience may be context dependant, for example, Capitanio *et al.* (2023) suggest these effects become apparent when measures are taken under stressful conditions.

It is also possible that I were not able to identify any effects due to the limited sample size for this analysis. As these measures were only available from 2017 – 2019, I were unable analyse potential acute effects of weaning on NLR and perform comparisons between weaned and non-weaned animals like with the other datasets. It is possible that any effects of weaning and weaning age are acute like with *Campylobacter* infections, but the testing of this hypothesis will have to be left to future studies.

6.4.5 Conclusions

Overall, the study's findings have important implications for understanding *Campylobacter* transmission dynamics, risk factors, and the potential impacts on health outcomes. They provide valuable insights for developing targeted prevention strategies and interventions to mitigate the risk of *Campylobacter*iosis and its associated consequences. Additionally, the study expands our understanding of how early-life events, such as weaning, can have long-term effects on disease susceptibility and physiological outcomes.

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Chapter 7. Assessing the Perceived Costs of Delaying Weaning in a Captive Breeding Colony of Rhesus Macaques (*Macaca mulatta*)

7.0 Chapter summary

This chapter investigates the perceived costs of delaying weaning in captive rhesus macaques, aiming to shed light on the implications of this practice on aggression, injuries, and productivity within the groups. The study analysed aggression video data and injury records from both weaning and breeding groups, as well as productivity data to understand the factors influencing inter-birth intervals. Results from the aggression video data revealed that breeding males directed significantly less mild and intense aggression compared to displacements, possibly due to their higher social rank. However, a non-significant trend indicated slightly more aggression towards juveniles, suggesting potential competition for resources or rank establishment. Aggression levels also showed a marginal increase on high-value food days. In terms of injuries, older juveniles in both breeding and weaning groups faced higher risks, with steeper slopes of injuries in weaning groups. Factors such as the age of the oldest male and the introduction of new males influenced injury rates in breeding groups. Contrary to assumptions, delaying weaning did not significantly impact inter-birth intervals. However, maternal age was found to be a significant factor, with older mothers displaying shorter intervals between births. The study challenges the assumption that earlier weaning improves maternal productivity and emphasises the importance of considering multiple factors that may impact social dynamics and well-being within captive macaque populations. It highlights the need for colonies to carefully evaluate the age of the male in the group, the introduction of new males, and the age range within weaning groups before making decisions about weaning practices.

7.1 Introduction

7.1.1 Aggression and injuries in primate societies

In primate societies, aggression is a common and natural part of social interactions, serving as a means of establishing dominance, resolving conflicts, and maintaining social order and can be influenced by various factors (Hosey et al., 2016). Dominance plays a significant role in primate social systems (Weiss et al., 2007) and dominance-like personality dimensions have been observed in nonhuman primates, such as chimpanzees and bonobos (Weiss et al., 2007; Garai et al., 2016). Another factor is reproductive competition, which can lead to aggression between females in promiscuous primate societies (Baniel et al., 2018). Furthermore, access to resources like food or territories can elevate forms of intragroup and intergroup aggression in both humans and non-human primates (Kasumovic et al., 2017).

Aggression can sometimes result in fight-related injuries, but the frequency and severity of these injuries can vary depending on various factors such as species, social dynamics, and environmental conditions. Injuries resulting from aggression can range from minor wounds to more severe injuries, and in some cases, mortality (Behera et al., 2020). In species with despotic social styles and strict dominance hierarchies, fight-related injuries may be more common due to the intense competition for resources and reproductive opportunities. On the other hand, in species with more tolerant social styles and relaxed dominance hierarchies, aggression may be less frequent, and injuries may be less common.

In species with strong dominance hierarchies and intense competition, such as some macaque species, fight-related injuries are common (Muller, 2017). In these species, males may engage in aggressive encounters to establish dominance and secure mating opportunities, resulting in more injuries (Muller, 2017). For example, male mandrills (*Mandrillus sphinx*) have been observed to target sexually receptive females with aggression, inflicting costs and increasing their long-term mating success (Smit et al., 2022).

Primates have also evolved various behavioural strategies to manage and mitigate aggression within their societies. Reconciliation is one such strategy observed in many primate species, where individuals engage in friendly contact following a conflict to repair social relationships and reduce tension (Zhao et al., 2016). Grooming and other forms of conciliatory behaviour have been shown to increase in primate groups prior to potentially stressful events, such as feeding or crowding, as a means of tension reduction (Judge et al., 2006). These behaviours help to maintain social cohesion and minimise the risk of escalated aggression and injuries. Social stability in primate societies can be maintained through subordination signalling networks, which communicate formal and settled dominance relationships (Beisner et al., 2016; McCowan et al., 2022).

7.1.2 Aggression and injury in macaque societies

Macaque societies can exhibit different types of social organisation, ranging from egalitarian to despotic (Thierry, 1985; Aureli et al., 1997; Thierry, 2004; Palagi et al., 2015), which can affect the frequency and intensity of aggression (Hemelrijk et al., 2005; Woods et al., 2019). Phylogeny plays a role in shaping the social behaviour and organisation of macaques and some core aspects of social behaviour and organisation in macaques are highly conserved, suggesting a high degree of phylogenetic inertia (Thierry et al., 2000). Comparative studies have revealed correlated variations between species in multiple social traits, such as dominance relationships, aggression patterns, reconciliation, socialisation, and access to resources (Thierry, 2013; Thierry, 2021). These linkages between behavioural traits act as constraints that limit evolutionary responses to external pressures (Thierry, 2013).

Social tolerance is another important factor influencing the social organisation of macaques. Macaque species can be classified on a scale of social styles, ranging from the least tolerant to the most tolerant (Thierry, 2004; Thierry, 2021). Differences in social tolerance influence various behaviours, including aggression and affiliation patterns, dominance relationships, and play (Palagi et al., 2015). Macaque species with more tolerant social styles, such as the Sulawesi macaque (*Macaca nigra*), exhibit relaxed dominance, higher levels of conciliatory behaviour, and a more egalitarian social structure (Wendland et al., 2005; Palagi et al., 2015). In contrast, species with less tolerant social styles display, like rhesus macaques (*Macaca mulatta*) and Japanese macaques (*Macaca fuscata*), have steeper dominance hierarchies, higher levels of aggression, and stronger nepotism (Wendland et al., 2005; Palagi et al., 2015). Rhesus macaques fall on the more despotic social style and as such, their social status within groups is determined by a strict linear dominance hierarchy (Michopoulos et al., 2012). They exhibit a degree of variation in aggression-related behaviour that is uniquely high, making managing them in captivity a challenge (Bernstein & Gordan, 1974).

7.1.3 Managing aggression and injuries in captive macaques

Frequent and severe aggression can negatively impact the welfare of rhesus macaques, and management actions such as veterinary care and temporary or permanent removal of individuals may be necessary (Beisner & Isbell, 2011). Furthermore, aggression can also disrupt social dynamics and hierarchies within macaque groups, leading to instability and potential welfare issues. The high levels of injury associated with the formation of unfamiliar macaque groups highlight the need for understanding and managing aggression in captive settings (Woodell et al., 2022).

Macaques have evolved their own conflict management systems. Effective conflict managers use low-intensity aggression or threats when intervening in conflicts, often intervene impartially, and sometimes terminate conflicts through mere approaches (Flack et al., 2005). Furthermore, policing behaviours by third parties, involving impartial intervention upon others' conflicts, can reduce the intensity and duration of aggression (Beisner et al., 2011). Reconciliation, which involves friendly interactions between former opponents after a conflict, can help reduce tension and restore social bonds. Post-conflict third-party consolation involves affiliative interactions by bystanders with the victim of aggression, providing social support (Kutsukake & Castles, 2004). However, Composition of breeding groups in captivity is driven by the size, purpose and philosophy of the colony and can have several significant differences to the groups in the wild, meaning effective management strategies are required to minimise aggression and injuries. Research into this has uncovered a number of factors associated with injuries in group housed captive macaques:

- Group stability: groups with fragmented matrilines exhibited more fighting with kin and higher rates of injuries (Beisner et al., 2011). Furthermore, disruptions to previously stable social structures can increase aggression (Balasubramaniam et al., 2021).

- Natal male alliances: The presence of multiple natal males in rhesus macaque groups may act in opposition to group stability and potentially contribute to aggression (Beisner et al., 2010).

- Seasonality: Wounding in captive macaques may be influenced by seasonality. For example, wounding has been reported to increase during the birth season rather than the breeding season in zoo housed Japanese macaques (Cronin et al., 2020).

- Stress and chronic stressors: Chronic stressors, such as prolonged single housing, recurrent invasive procedures, and other potentially stressful conditions, can contribute to the development and maintenance of self-injurious behaviour and aggression in macaques (Bourgeois et al., 2007).

- Environmental conditions: Factors such as housing type, group density, and environmental conditions during pregnancy can influence aggression, submission, competition, and injuries sustained by pregnant female macaques (Silk, 1987).

Furthermore, crowding has been associated with an increase in severe injuries in rhesus macaques (Baker et al., 2000). The social structure and composition of macaque groups can also influence aggression and welfare. Changes in social group composition and social density can affect conflict management mechanisms and increase aggression in captive macaques (Cowl et al., 2021). The stability of hierarchical relationships within macaque groups is important for their welfare, and disruptions to social structure can lead to increased stress and aggression (Balasubramaniam et al., 2021).

- Introducing new males to a breeding group: injury rates are much higher the first few months following the integration of a new male into a breeding group compared to established groups (Massey et al., 2022).

- Personality: individuals with low confidence or high anxiety have been found to receive more injuries (Norman et al., 2021). Sociability (extraversion) has been positively associated with affiliative interactions, while confidence has been associated with aggressive behaviours (Simpson et al., 2019). These personality dimensions have also been linked to lifetime injury incidence and well-being in rhesus macaques (Simpson et al., 2019).

A lesser studied aspect of the management of macaque colonies is looking at the factors that affect productivity (the probability of a female getting pregnant and/or successfully raising the infant). Factors that affect injury rates in macaques can also affect productivity. For example, Massey *et al*. (2022) found a boost in productivity if a new adult male in his prime is added to the group but there was also a trade-off with this resulting in increased injuries.

7.1.4 Factors affecting reproductive success in primates

Many of the factors associated with aggression and injury rates are also associated with reproductive success:

- Social bonds: Social bonds provide support, protection, and access to resources, which can increase the survival and reproductive success of individuals (Silk et al., 2009; Schülke et al., 2010; Silk et al., 2013).

- Dominance rank: In many primate societies, high-ranking individuals, both males and females, tend to have higher reproductive success (Gesquiere et al., 2011). Dominance rank can provide better access to mates, resources, and protection, leading to increased reproductive opportunities (Sadalla et al., 1987; Massen et al., 2011).

- Mating strategies: Females may choose high-quality mates based on various factors, including dominance rank, physical characteristics, and social alliances (Rusch, 2014). Male-male competition for access to females can also influence reproductive success (Baniel et al., 2018; Zhang et al., 2021).

- Social networks: The size and quality of social networks can influence reproductive success in primates (Silk et al., 2009). Having a larger social network and strong social connections can provide advantages in terms of resource sharing, protection, and access to mates (Noonan et al., 2014; Kalbitzer et al., 2017; de Vevey et al., 2022).

- Resource availability: Access to resources, such as food and territory, can affect reproductive success in primates. Females with better access to high-quality food resources may have higher fertility rates and better offspring survival (Riedel et al., 2010). Resource availability can also influence male-male competition and dominance hierarchies (Nakamura et al., 2013).

- Parental care: Investment in parental care, particularly by males, can impact reproductive success in primates and males that provide care to offspring may enhance their own reproductive success by increasing offspring survival and attracting mates (Muller, 2017).

7.1.5 Strategies to maximise productivity and reduce injuries in captive macaques

There are methods that are well established for reducing aggression and injuries in captive macaques which often involve providing the animals with more favourable conditions that maximise welfare. For example, providing environmental enrichment, such as increased access to natural substrates, grass, or produce, can reduce aggression and promote positive behaviours in macaques (Beisner & Isbell, 2011; Wooddell et al., 2019). Ensuring stable hierarchical relationships and minimising disruptions to social structure can contribute to a more harmonious social environment. Implementing preventive measures, such as socialisation of isolate-reared individuals with normal infants, can help reduce the development of abnormal behaviours (Bellanca & Crockett, 2002). Ensuring sufficient food resources and avoiding food shortages can help reduce aggression associated with competition for resources (Boccia et al., 1988; Little & Sommer, 2002; Alami et al., 2012; Vaglio et al., 2021).

Similarly, maximising productivity involves making group level adjustments that can minimise stress and maximise the welfare of the animals (Cleveland et al., 2003). For example, providing stable social groups (Rox et al., 2022), ensuring adequate nutrition (Bercovitch, 1997; Anderson, 2022), and minimising the risk of disease transmission (Kanaujia et al., 2004; Kanthaswamy et al., 2013) can all aid the goal of maximising productivity. Furthermore, where females are bred to be productive, it is important to balance the timing of breeding debut with the females' physical condition. Young mothers that initiate reproduction shortly after adolescence may have fewer bodily reserves available for lactation, which can affect milk synthesis and trade-offs between reproduction and their own growth (Hinde, 2009).

One strategy that is claimed to maximise productivity and reduce injuries is weaning juveniles from their mother and natal group early (Prescott et al., 2012), despite their being limited evidence to support this claim. The main piece of evidence supporting this justification for early weaning comes from a well powered study by Goo and

Fugate (1984), where they randomly assigned 750 infant rhesus macaques to be removed at 6, 8, 10, or 12 months and found that dams of macaques removed at 6 months had better reproductive rates. Similarly, the study by Goo and Fugate found that remaining with the dam increased the likelihood of receiving and injury. Some studies have corroborated this finding in other species. For example, Kotera *et al*. (1975) found that birth rate increased from around 50% to 75.36% by weaning at 3 months old (Japanese macaques) and Maninger *et al*. (2000) found that a later weaning age correlated with postpartum amenorrhea and longer inter-birth intervals in pig-tailed macaques (*Macaca nemestrima*).

However, a study in baboons (*Papio hamadryas*) did not find a similar effect (Wallis & Valentine, 2001). Similarly, Prescott *et al*. (2012) found no evidence to support the hypothesis that an earlier weaning age enhances productivity in rhesus macaques or cynomolgus macaques (*Macaca fascicularis*) at two separate facilities. Furthermore, a more recent study by Rox *et al*. (2022) found that multigenerational group housing and group-rearing enhanced reproductive success in rhesus macaques, as indicated by higher birth rates, shorter IBIs, and improved offspring survival rates. The latter study was a retrospective analysis using data collected at the Biomedical Primate Research Centre in Rijswijk between 1996 and 2019 and provides strong support against the notion that earlier weaning maximises productivity.

One possible explanation for the contrasting findings between Goo and Fugate (1984) and Rox *et al*. (2022) in rhesus macaques is that group structure is more important in influencing productivity than weaning age alone. The key differential factor between these studies lies in the housing and social structures of the macaques. While Goo and Fugate (1984) suggest a correlation between early weaning and increased maternal reproduction in a harem-based housing structure, Rox *et al*. (2022) indicates that in a more natural, multigenerational group setting, the early weaning of infants is not a requisite for enhanced maternal reproduction. This finding is significant as it suggests that group structure, particularly the presence of matrilines and a more natural social environment, plays a crucial role in determining and improving maternal productivity. Furthermore, the multigenerational group structure reflects a more natural social organisation, which will likely have positive welfare implications. The similarity in reproductive success between multigenerational and peer groups, despite differences in weaning age, underlines the importance of considering natural social structures in captive breeding programs.

This approach not only aligns with the natural inclinations of the species (female philopatry) but also potentially enhances the overall welfare and social well-being of the animals.

Aside from the Goo and Fugate (1984) study, there is little evidence that later weaned macaques will receive more injuries. Although, there is clear logic behind the rationale for this prediction as older juveniles in the group will increasingly be seen as competition for resources by older animals. However, disruptive early experiences such as early weaning has been found to lead to several negative outcomes including increased aggressive behaviours (Barr et al., 2003). As such further investigation into these perceived costs of later weaning is imperative.

7.1.6 Aims

Two separate datasets were utilised in this study: newly collected behavioural data (aggression) and colony records (injury and productivity) as no aggressive behavioural data was collected in the colony prior to this study. The aims of this chapter were to assess the assumptions that leaving young animals in their natal group for longer increases the likelihood for injury and aggression. If this assumption is correct, I hypothesised that the rates of aggression in breeding groups would be higher to juveniles than adult females, and that injuries to older juveniles in breeding groups would be higher than to similarly aged peers in weaning groups. Rates of aggression in the breeding groups was assessed by collecting data on aggressive instances from the breeding male to the other members of the group, hypothesising that the male would disproportionately direct behavioural aggression towards juveniles. Injuries were assessed from colony records, I extracted the number of injuries each individual received per month between the ages of 1 and 3 years from colony records between 2008 - 2022. Rates were compared between group types (breeding or weaning). Furthermore, if the justification of weaning individuals early is to improve welfare, it is important to ensure that earlier weaned animals are not receiving more injuries than later weaned conspecifics. To assess this, I analysed just weaned animals, hypothesising there would be no difference in injury rates as a function of weaning age. To test the assumption that weaning individuals early would maximise reproduction, colony records were utilised to assess the association between weaning age of an individual and the time (days) to the birth of their mother's subsequent offspring, whilst controlling for maternal rank and age effects.

7.2 Methods

7.2.1 Video data collection

Subjects for this analysis were 6 breeding males (all breeding males that were present in the colony at the time of data collection). Video cameras were set up on the selected breeding groups between 07:00 and 12:00hrs using a (Sony HDR-CX625) on Tuesdays and Thursdays between October 2020 – December 2021. At the CFM, the diet is predominantly pellets that are designed to meet the nutritional requirements of nonhuman primates. Additionally, the pellets are supplemented with a mixture of lentils, oats, seeds, and nuts to promote natural foraging behaviour, and a variety of fruit and vegetables that change daily including carrots, cabbage, melon, bananas, hard-boiled eggs etc. Tuesdays and Thursdays were selected as they represent low value vs high value feed days as they contained tomatoes and cucumbers vs bananas and eggs, respectively.

A total of 57 videos were collected. On three occasions, videos were recorded on a different day (two on Wednesday, one on Friday) to maximise data collection where videos could not be recorded on a Tuesday or Thursday because of husbandry practices. For some groups, there are missing days for either Tuesday or Thursday because of husbandry procedures in the group (see table 7.1).

Table 7.1. Sample size of videos per breeding group by day

Breeding male ID	Number of videos per day				Grand Total
	Tuesday	Wednesday	Thursday	Friday	
Plum	4		4		8
Sol	3		3		6
Star	2	1	2		5
Thorn	7		6	1	14
Vincent	6	1	4		11
Will.I.Am	7		6		13
Total	29	2	25	1	57

Videos were edited and processed using Aiseesoft Video Editor to cut 30 minutes around the feed time (15mins before and after the care staff placed food into the playpen).

7.2.2 Behavioural coding of video data

Videos were coded using BORIS (Friard & Gambia, 2016). The adult male in the breeding group was the focal subject for observations and continuous recording for all instances of discrete aggressive events directed to another member of the group were recorded (see table 7.2 ethogram) along with an identifier of the recipient of the aggression (adult female or juvenile).

Table 7.2 Ethogram of behaviours for aggression video data coding

Behaviour	Operational definition	Category
Displacement	Nonaggressive approach by one individual followed by the other individual moving away	Displacement
Brief chase	Chase < 6m	Mild
Contact	Mild push or slap	Mild
Low level pursuit	Threatening while approaching	Mild
Lunge	Actor lunges in the direction of the recipient	Mild
Threat	Open mouth/head bob/ears back/threat grunt	Mild
Bite	Actor places mouth on recipient	Intense
Grapple/wrestle	Actor grabs hold of recipient and does not let go within 2 seconds	Intense
Long chase	Chase > 6 m	Intense

7.2.3 Analysis of video data

All analysis was conducted in RStudio (version 4.1.1). To test the prediction that the adult male would direct more aggression towards older juveniles than other age groups, a general linear mixed effect model with Poisson error structure was fit to a subset of the full dataset excluding observations that were not on a Tuesday or Thursday, if there were no difference in aggression by day then the full dataset would be analysed. The count of aggressive acts was calculated per category (see table 6.1) for a given observation day and was the outcome variable in the model. Predictor variables were the interaction between victim age class (factor with two levels: adult female or juvenile) and aggression category (factor with 3 levels: displacement, mild, or intense), day of the week (factor with two levels: Tuesday or Thursday) and the number of juveniles and number of females in the group (continuous) were also controlled for in this model. Additionally, the model included a random intercept of breeding male ID.

7.2.4 Treatment of health record data for injury

Treatment of the health record data for injuries and productivity have previously been described in Massey *et al*. (2022). Health and breeding information were recorded on individual health records for each monkey and husbandry information (including feeding and cleaning) was recorded on day books for each group. These data were transferred to an electronic oracle-based database (ENOS) by technical and scientific staff. The database information was checked for errors by a senior member of staff (CW). Prior to analysis the data was exported from the database to an excel spreadsheet and subsequently analysed in Matlab (www.mathworks.com; version 2020a) and R (version 4.2).

Injuries were included in the dataset if they required some form of veterinary attention (such as antibiotic and/or analgesic injection or suturing). All injuries listed in the database were sorted into those most likely to be caused by fighting (slice wounds, puncture wounds, lacerations, amputations, de-gloving injuries), and those where the cause was unclear and were more likely to be accidental (bruising, swelling, limping with no obvious wounds, broken bones) were excluded.

To reduce the data set to a meaningful size for modelling I used a time resolution of one month, calculating the number of injuries. For all the continuous predictor variables such as age I calculated their average value across the month. Predictor variables were either individual level (applied to the individual animal such as age) or group level (such as the number of animals in the group).

As the study covered a period of 14 years, the composition of the groups changed over time. Each group was assigned a unique group ID. If a group had a substantial change, a new ID was assigned and if the group was divided in two, each of the smaller groups were assigned a new ID.

7.2.5 Analysis of injury data

The final dataset contained the count injuries per individual per month for 616 monkeys (females = 299) aged between 1 and 3 years (mean +- SD = 1.98+-0.57). The data were used to assess two predictions, the first prediction being that older juveniles in breeding groups would receive more injuries than younger juveniles (group comparison study). The second being that earlier weaned monkeys in the weaning groups would receive more injuries (weaning group study). To assess these two predictions, two negative binomial models were fit to the data as the injury variable was overdispersed.

Sample size for model 1 was 616 monkeys (see table 7.3 for sample size by sex, and group type) from 88 unique group IDs.

Table 7.3. Breakdown on sample size by group type and sex

n	Group			
	Breeding		Weaning	
	Females	Males	Females	Males
	271	272	157	282

NB. Sample size appear larger because some monkeys have observations in both group types. This was controlled for in modelling by including the random intercept of ID

Both models (group comparison study and weaning group study) were fit with a negative binomial distribution. The outcome variable for both models was the count of injuries per individual per month. For the group comparison study, a model selection approach was used to determine the best combination from all possible combinations of predictors (group type, age and sex) by comparing AIC using the 'MuMIn' package in R (Barton, 2020). The model with the smallest AIC value was selected for further analysis.

For the weaning group study, as I was interested in the effect of weaning age on injuries this was included as a covariate along with age, age range, group size, and sex. While interaction terms between these variables may be important, they were not included in the model as I was only interested in testing the effect of weaning age.

For both models, all the numerical predictor variables were centred on zero by taking the values and subtracting the mean. Collinearity between the predictor variables were tested by calculating the variance inflation factors for each predictor variable. All predictor variables included in the model had a variance inflation factor of less than 2. For both models individual ID and unique group ID were modelled as random intercepts.

7.2.6 Analysis of productivity data

To test the prediction that dams of earlier weaned offspring would reproduce faster than those with offspring that were weaned later, records from 402 infant births from 108 mothers were analysed. Inter-birth-interval (IBI) was extracted by calculating the time between the birth of an infant and their younger sibling to assess if weaning age of infant correlated IBI of younger sibling. A general linear mixed effect model was fit to the data with IBI as the outcome variable and weaning age as the predictor variable. I also controlled for maternal rank (factor with the levels: low, mid, high), age of the mother (continuous), sex of the infant (factor with two levels: male or female), and the random intercept of mother ID.

To assess model assumption of homoscedascity was met, fitted values were plotted against the residuals of the models. This revealed an increase in residual variance along the fitted values suggesting this assumption was not met. IBI was then transformed using Tukey's Ladder of Power (Tukey, 1977). This transformation was performed using the function 'transformTukey' of the R-package rcompanion and provides a λ value that maximised the Shapiro-Wilk W statistic or minimises the Anderson-Darling A statistic (Mangiafico, 2016; Mangiafico, 2019). Fitting the model with the Tukey transformed outcome variable improved model stability but did not change the conclusions drawn from model output. As such, the model with raw IBI data will be reported to facilitate interpretation.

7.3 Results

7.3.1 Aggression video data results

In the model from video data, there was a significant main effect of aggression type: breeding males directed around 75% and 98% less mild and intense aggression, respectively, compared to displacements (mild: Estimate±SE = -1.42±0.25, $z = -5.7$, $p < 0.0001$; intense: Estimate±SE = -3.73±0.71, $z = -5.23$, $p < 0.0001$; see figure 7.1).

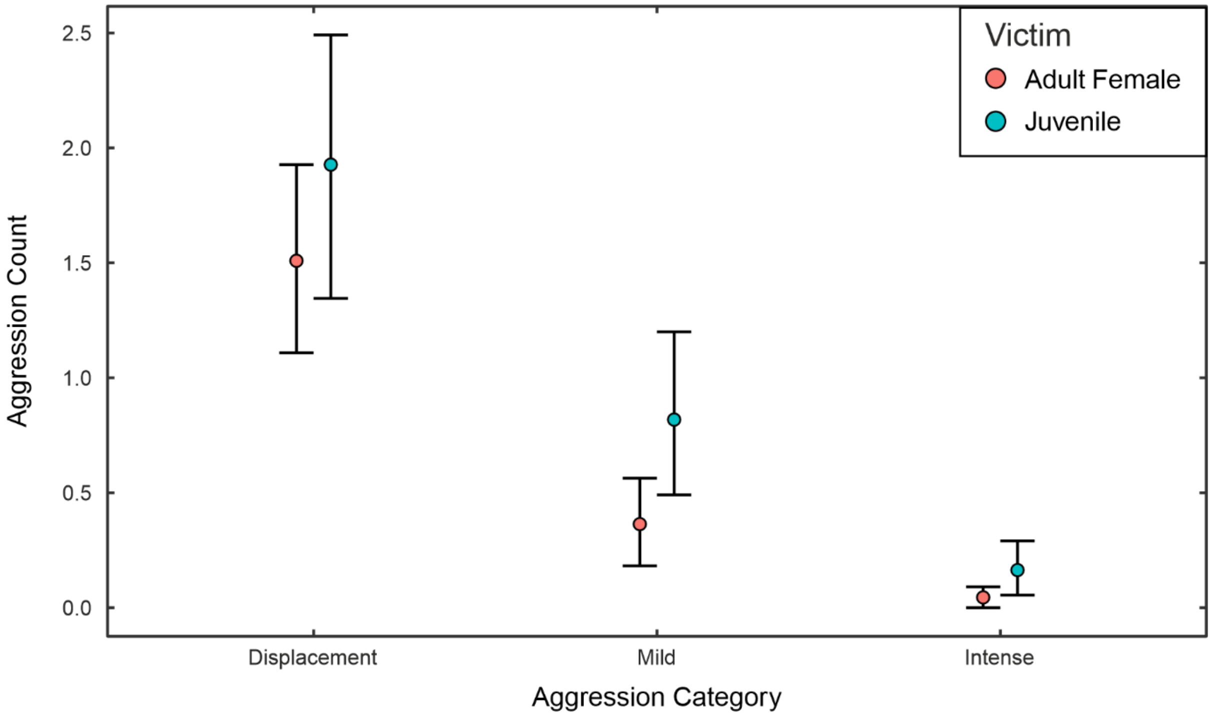

Figure 7.1. Differences in aggressive behaviours that breeding males displayed by category and victim. Dots represent the mean. Error bars represent bootstrapped 95% confidence intervals.

The model estimated that males directed around 27% more aggression towards juveniles than adult females, although this effect did not meet the criteria for statistical significance (Estimate±SE = 0.25±0.15, z = 1.7, p = 0.09). For the interaction between these two variables, the difference in intense aggression towards juveniles and females was no different than displacements and there was a trend for males to direct more mild aggression towards juveniles than females (Estimate±SE = 0.57±0.31, z = 1.9, p = 0.06; see figure 7.1). Day of the week was estimated to effect aggression counts: there were around 12% fewer instances of aggression on Tuesdays compared to Thursdays, although this effect was marginally non-significant (Estimate±SE = -0.24±0.12, z = -1.9, p = 0.054). No evidence was found that the number of females or juveniles in the group explained any of the variation in instances of aggression (see table 7.4).

Table 7.4. Coefficients of aggression video data model with counts of aggressive behaviours as the response.

Variable	Estimate	SE	z	p

Intercept	0.93	0.77	1.21	0.23
Victim*	0.25	0.15	1.68	0.09
Aggression (Mild)†	-1.42	0.25	-5.73	<0.0001
Aggression (Intense)	-3.73	0.71	-5.23	<0.0001
Day	-0.24	0.12	-1.93	0.054
Females	-0.026	0.068	-0.38	0.7
Juveniles	-0.035	0.059	-0.59	0.6
Victim*Aggression(Mild)	0.57	0.31	1.86	0.06
Victim*Aggression(Intense)	1.26	0.79	1.59	0.1
*Adult female as the reference factor †Displacements as the reference level				

7.3.2 Injury data results

7.3.2.1 Group comparison study

Model selection revealed the best model to be one that included the interaction of group type and age (see table 7.5).

Table 7.5. Model selection coefficients with number of injuries an animal had received in a given month as the response.

Model	df	logLik	AICc	Delta	Weight
Group type*Age	7	-1609.94	3233.9	0	0.49
Group Type*Age + Sex	8	-1609.9	3235.8	1.93	0.19
Group Type+Age	6	-1612.34	3236.7	2.80	0.12
GroupType*Age + Sex + Age Rank	9	-1609.377	3236.8	2.89	0.12
Group Type*Age + Age*Sex + Group Type*Sex + Age Rank	11	-1607.98	3238.0	4.1	0.063
Group Type*Age*Sex + Age Rank	12	-1607.7	3239.4	5.54	0.03
Age	5	-1621.41	3252.8	18.95	0
Group Type	5	-1633.75	3277.5	43.63	0

In the first model I found significant main effects of age and group type, as well as a significant interaction between them. For every unit increase in age, a monkey was likely to receive around 68% more injuries (Estimate±SE = 0.52±0.14, z = 3.8, p = 0.0001. Monkeys in weaning groups were likely to receive around 33% fewer injuries (Estimate±SE = -0.39±0.12, z = -3.21, p = 0.01). Furthermore, this slope of age was steeper for weaning groups (β = 0.52) than for breeding groups (β = 0.29; see figure 7.2).

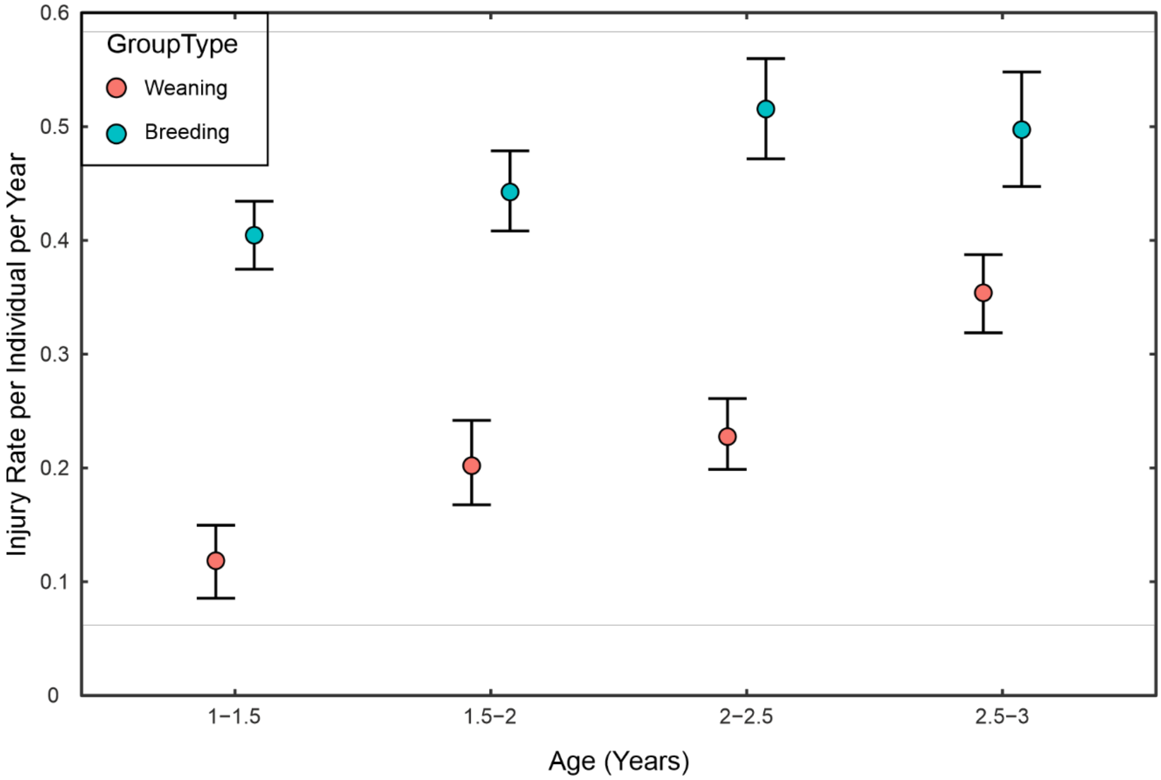

Figure 7.2 Effect of age and group on injury rate and their interaction. Dots represent the mean injury rate per individual per year, error bars represent bootstrapped 95% confidence intervals. Age is presented here as categorical to aid visualisation although it was modelled as continuous.

7.3.2.2 Weaning group study

Within the weaned animals, there was no significant association between weaning age, sex, and group size on injury. I found significant effects of age and age range (see table 7.6 for model results). The effect of age, of course, is unsurprising given results from model 1 (see figure 7.2). Interestingly, per unit increase in age range within the weaning group, individuals were estimated to be likely to receive around 16% more injuries (see figure 7.3).

Table 7.6. Coefficients of weaning group injury data model with number of injuries an animal had received in a given month as the response.

Variable	Estimate	SE	z	P
Intercept	-3.9	0.28	-13.9	< 0.0001
Age	0.43	0.14	2.9	0.003
Weaning age	0.01	0.15	0.09	0.9
Sex	0.02	0.32	0.06	0.9
Age range	0.14	0.05	3.1	0.002
Group size	-0.03	0.03	-1.2	0.2

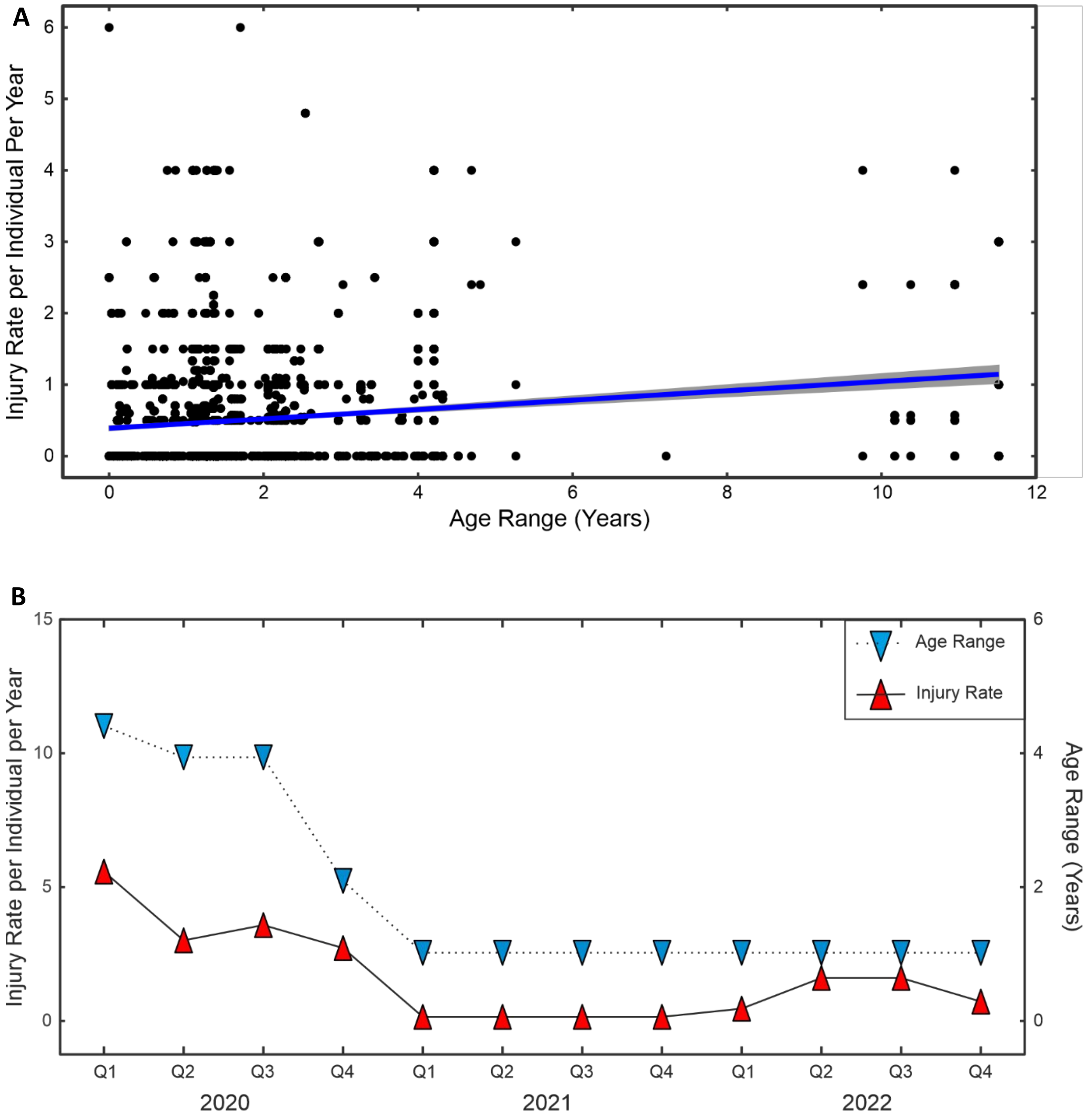

Figure 7.3 A. Relationship between the age range withing the weaning group and the injury rate per individual per year. Dots represent total injuries per individual each year. Blue line represents regression line and grey shaded area around the line represents 95% confidence intervals. B. Case study depicting mean injury rate per individual per year (red triangle; y axis) and the corresponding age range within the group (blue triangle; secondary y axis), over 3 years. Injury rates were extremely high (around 5 per individual per year) when the weaning group was formed (2020 – Q1). Between 2020 – Q3 and 2020 – Q4, the oldest male was removed from the group leaving the group with more similarly aged peers. Following this, the observed data indicate a progressive decline in injury rates, converging towards a level consistent with colony norms.

7.3.3 Post-hoc analysis of factors associated with injury in young animals in breeding groups

To assess other variables that might be important for reducing injuries of young animals in a large model was fit to the data with sex, age, and their interaction; new male (factor with 3 levels: established, new male introduction phase, and new male), sex, and their interaction; maximum age (age of the breeding male); and the number of adults in the group. This was then inspected, and interaction terms dropped if non-significant. This resulted in the final model not including any interaction terms.

In this model, unsurprisingly, age was found to have a significant effect (see figure 7.1). Maximum age (age of the breeding male) was estimated to effect injuries, such that for every unit increase in maximum age individuals were likely to receive around 9% fewer injuries (Estimate±SE = -0.09±0.02, z = -3.8, p = 0.0001; see figure 7.4A). Furthermore, having a new male in the group that is past the introduction phase (first 3 months following introduction) resulted in the injuries increasing by around 50% compared to established groups (Estimate±SE = 0.63±0.26, z = 2.56, p = 0.01), this appears to be a gradual increase as injuries in the introduction phase were not significantly different from established group (see figure 7.4B). Furthermore, There were no effects of sex or number of adults present in the group (see table 7.7 for model coefficients).

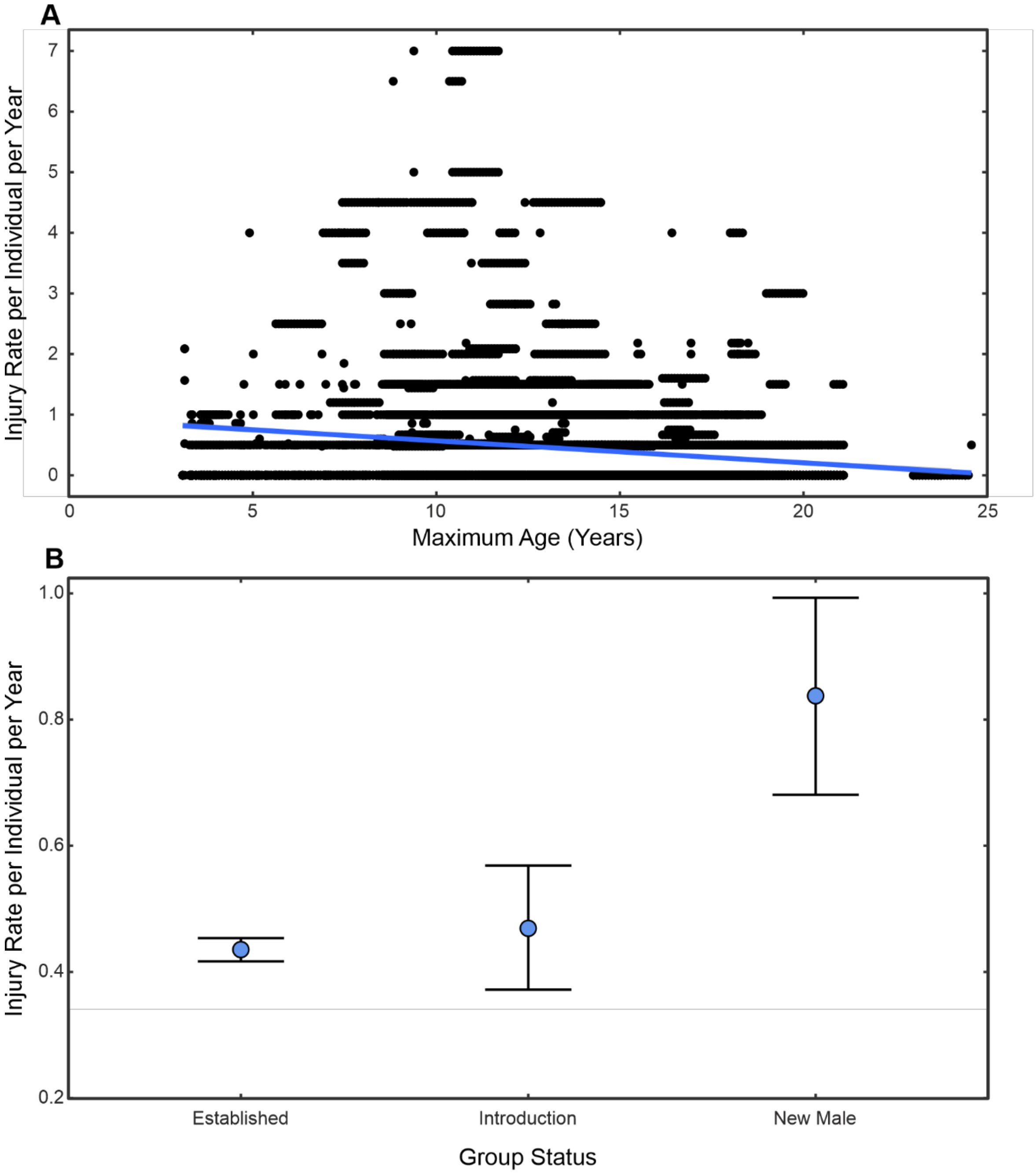

Figure 7.4 A. Relationship the maximum age (age of the breeding male) in the breeding group and the injury rate per individual per year. Dots represent total injuries per individual each year. Blue line represents regression line and grey shaded area around the line represents 95% confidence intervals. B. Association between injury rate per individual per year and the status of the breeding group (see text for definitions). Dots represent the mean. Error bars represent bootstrapped 95% confidence intervals.

Table 7.7. Coefficients for model fit to breeding group injury data with number of injuries an animal had received in a given month as the response.

Variable	Estimate	SE	z	p
Intercept	-3.85	0.22	-17.95	<0.0001
Age	0.76	0.13	6.02	<0.0001
Sex*	0.13	0.18	0.72	0.47
Group status (Introduction)†	-0.23	0.14	-1.61	0.11
Group status (New male)†	0.41	0.18	2.32	0.02
Maximum age	-0.09	0.024	-3.84	0.0001
Number of adults	-0.026	0.039	-0.66	0.51

*Females as the reference level

†Established as the referencelevel

7.3.4 Productivity analysis results

Contrary to assumptions, weaning age did not effect IBI, the estimated correlation was in the negative direction although this did not reach the criterion for significance (Estimate±SE = -44.1±34.6, z = -1.3, p = 0.2; see figure 7.5A). I found that for every unit increase in maternal age (year), a mother was estimated to give birth to their subsequent offspring around 1 month sooner (Estimate±SE = -28.63±9.5, z = 3.02, p = 0.003; see figure 7.5B). No significant effects were found for sex of the infant or maternal rank (see table 7.8 for model coefficients).

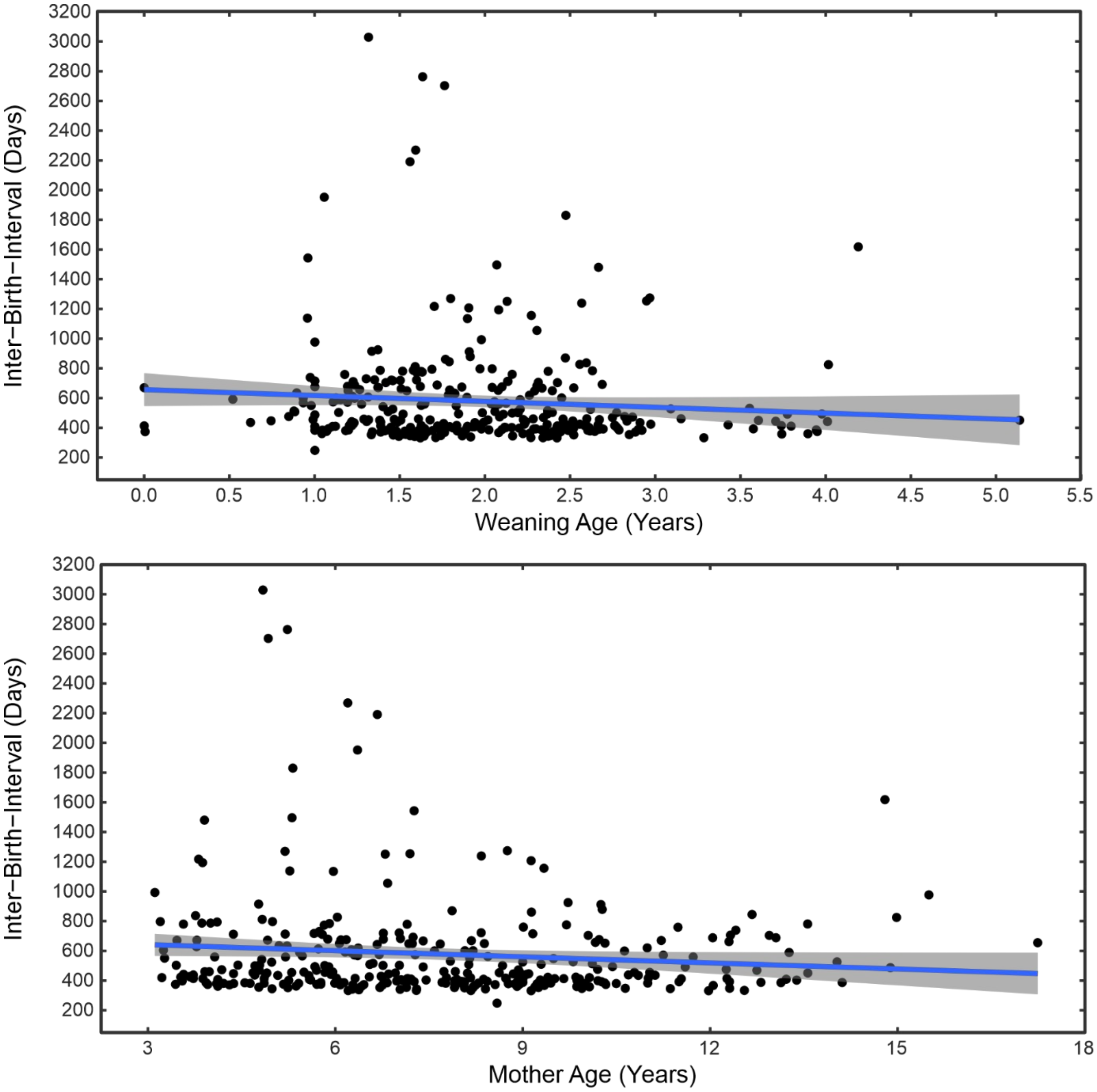

Figure 7.5 Relationship between inter-birth-interval to next maternal sibling and A. weaning age; B. Mother age. Dots represent individual data points. Blue lines represent regression line and grey shaded area around the line represents 95% confidence intervals.

Table 7.8. Coefficients for model fit to productivity data with IBI as the response variable.

Variable	Est	SE	df	t	p
Intercept	539.99	47.18	70.31	11.46	<0.0001
Weaning age	-44.09	34.62	214.35	-1.27	0.2
Mother age	-28.63	9.47	211.8	-3.02	0.003
Maternal rank (LOW)*	-36.99	53.55	35.68	-0.69	0.5
Maternal rank (MID)*	85.37	56.49	42.98	1.51	0.14
Sex†	-40.64	45.24	214.73	-0.89	0.37

*High as the reference level

†Female as the reference level

7.4 Discussion

7.4.1 Summary

The findings of this study provide insights into the perceived costs of delaying weaning in captive rhesus macaques. The analysis of aggression frequency revealed that the breeding males directed significantly fewer instances of mild and intense aggression compared to displacements, suggesting the breeding males may use displacements as a more common strategy to establish dominance and maintain social order within the group. The trend indicating slightly more aggression towards juveniles compared to adult females may be attributed to the breeding males perceiving juveniles as potential competitors for resources and mates. There was increased risk for older juveniles and juveniles in breeding groups to receive more injuries, although overall rates were low. Furthermore, factors other than the age of offspring separation appear to be more important in female productivity.

7.4.2 Video data aggression

In the aggression behaviour video dataset, I found that males displayed very few instances of mild and intense aggression compared to displacements. This finding is unsurprising given that rhesus macaques have a strict linear dominance hierarchy (Michopoulos et al., 2012). As the only adult male within the group, the breeding male will be the alpha. As such, the breeding male does not need to display as much aggressive behaviours as lower ranking individuals will signal subordination and move out of his way, reducing the need for physical aggression.

There was a non-significant trend for the breeding male to exhibit more mild aggression towards juveniles than adult females (see table 6.1). One possible reason is the influence of social rank and dominance. Aggression is used by rhesus macaques to establish and reinforce social rank, and higher-ranking individuals may exhibit aggression towards lower-ranking individuals, including older juveniles to maintain social order and prevent disruptions within the group (Amici et al., 2019; Johnston et al., 2020). Alternatively, the male may view the growing juveniles as competition for resources. There was a marginally non-significant trend for breeding males to display more aggression on Thursdays compared to Tuesdays. During the period when video data were collected, Thursdays were considered a high value food day. On these days the diet consisted of the usual forage mix and chow, with the addition of boiled eggs and bananas. Whereas on Tuesdays, in addition to the forage mix and chow, the diet consisted of tomatoes and cucumbers. Experienced care staff highlighted these days as low vs high reward food days. Therefore, it is possible that the aggression that the male exhibited was related to food competition.

However, it is important to note that there may be an issue of pseudo-replication in the design of this study. As identification could not be reliably recorded when coding video data, age categories were instead used. If the breeding male directed more aggression to a particular juvenile in the group, then a more appropriate model would include recipient ID as a random effect. As such, the results from this dataset should be treated with caution. There was one breeding male who might have been expected to display aggression disproportionately to certain juveniles in the group as this breeding male had one juvenile male in his group that he did not sire. Post-hoc analysis with this male removed found, similar to the full model, that rates of mild and intense aggression were significantly lower than displacements. However, the interactive effect between victim and aggression type was no longer apparent. This could suggest that this one male did indeed drive this marginally non-significant result in the full dataset. However, without this male included, sample size was reduced, and it is possible there was insufficient statistical power to detect the effect.

Furthermore, aggression has the potential to result in injuries to the older juveniles (Kanthaswamy et al., 2011; Amici et al., 2019). However, in the post-hoc analysis of injuries during the periods when aggression video data was obtained revealed, there was no correlation between the two variables. Although it is possible that adult females in the group were targeting the older juveniles more, I could not assess this with the current dataset.

As such, this study provides little evidence to support the assumption that older juveniles in breeding groups are any more likely to receive overt signals of aggression than other members of the breeding group from the breeding male.

7.4.3 Age and group effects on injuries

In the retrospective analysis of injury data, I found clear age and group type effects, as well as their interaction. Injuries were estimated to increase with age in weaning and breeding groups, juveniles in breeding groups were estimated to receive more injuries than those in weaning groups, and the slope of injuries over age was steeper for juveniles in weaning groups than breeding groups.

The age-related behaviour and development of rhesus macaques may contribute to the higher injury rates in older juveniles. As juveniles reach sexual maturity, they become larger, more aggressive, and engage in more physically aggressive behaviour and this increase in aggression may result in older juveniles receiving more injuries compared to younger juveniles (Amici et al., 2019). Additionally, the hormonal and physiological changes associated with sexual maturation may contribute to increased aggression and risk-taking behaviour in older juveniles (Amici et al., 2019). It is possible that the slope was steeper for weaning group juveniles because they will start to try and establish dominance amongst peers, whereas in breeding groups, the presence of adults limits the rank that juveniles can expect to achieve. Furthermore, dominant males can act as third-party interveners to maintain group stability (Flack et al., 2005). It is possible that slope is steeper in weaning groups as there are no dominant males to intervene as juveniles try to establish their rank within the group.

When looking further into the factors associated with injuries in these different groups, I identified other potentially important factors. In breeding groups, the maximum age in the group (age of the oldest male) and if the group had recently had a new breeding male added to the group both effected the number of injuries a juvenile could expect to receive. These results are similar to a previously published dataset that analysed the factors associated with injury rates of breeding females at the same colony (Massey et al., 2022). One explanation for the result of age is that older males are more experienced and are better able to manage group stability (Beisner et al., 2013). However, it is likely that there is a selection bias in the colony where aged males are ones that have proven to be less aggressive and therefore have longevity within the colony as breeding males (Massey et al., 2022). Furthermore, juveniles were found to be a greater risk of receiving injury in the 3 months after a new male was introduced into the group. This finding is unsurprising given that new males that join a group in primate societies tend to direct more aggression towards younger members of the group and sometimes engage in infanticide (Bartlett et al., 1993).

In the weaning groups, I found no evidence that weaning age was associated with number of injuries received. Other studies have found that very early rearing results in monkeys that are much more aggressive and prone to injuries (Conti et al., 2012). However, these rearing paradigms remove individuals from their mothers much earlier than is recommended by UK guidelines. Even in the case of the earliest weaning age at the CFM, this would be considered more typical rearing compared to other studies. As such, it is possible that even the earlier weaned monkeys in this sample are behaviourally adjusted enough to appropriately status signal to minimise injury risk.

Interestingly, the age range within the weaning groups was found to be important in determining injuries. At the CFM weaning groups are formed with similarly aged peers that are usually within a year or two of age. However, there were some weaning groups that were formed with the addition of an older individual, expecting that this individual would act as a third-party intervener and support group stability. However, it became apparent to technicians that this was not the case, as is highlighted by the finding presented in this chapter that a greater age range resulted in more injuries each individual could expect to receive. The reasons behind this are unclear. In other colonies where macaques are pair housed, pairing an adult with a juvenile tends to be successful in maximising the welfare for both individuals (Ori Pomerantz, personal communication). However, in this case, monkeys are group housed and the older individual is still a young adult. It is possible that the group is too large for the inexperienced older animal to manage. Massey *et al*. (2022) provide some support for this where they found, in the same colony, older males were more effective at managing larger groups and younger males where better with smaller groups of 2 – 5 individuals. In the case study presented in Figure 6.3B, the older juvenile was placed in a group of 12 other juveniles. However, this observation is anecdotal and warrants further formal scientific investigation.

The interactive effect of age and group type does provide some support to the assumption that delaying weaning will put older juveniles in breeding groups at greater risk of injury. However, the data presented in this chapter suggest this assumption has been overstated. Whilst the injury rates were greater for older juveniles in breeding groups, they were also greater for older juveniles in weaning groups. Suggesting that juveniles risk increases with age regardless of weaning age. Furthermore, the actual rates for older juveniles in both groups were relatively low. In breeding groups, juveniles aged 2 – 3 years received on average around 0.5 injuries per year; in weaning groups, individuals of the same age received on average between 0.2 and 0.4 injuries per year. Additionally, this study has identified that other factors within breeding groups might be more important in determining injuries than the age they are removed.

7.4.4 Productivity

Contrary to our hypothesis, the analysis of inter-birth intervals did not find a significant effect of weaning age on the time to the birth of the mother's subsequent offspring. This suggests that delaying weaning may not have a direct impact on reproductive patterns, as previously assumed. There was an estimated negative correlation between weaning age and IBI, although this did meet the criteria for statistical significance. A recent study found that multigenerational group housing and group-rearing enhanced reproductive success in rhesus macaques, as indicated by higher birth rates, shorter IBIs, and improved offspring survival rates (Rox et al., 2022). This is similar to the group composition at the CFM where the group contains multiple related females and their offspring. It is likely that the structure is more important in maximising productivity than the age the infant is separated. Although, a necessity to maintaining this structure is to leave offspring in the natal group for as long as possible.

One factor that was statistically significant in explaining variation in IBI was maternal age. Older mothers tended to have shorter inter-birth intervals highlighting the potential influence of maternal age and reproductive senescence on reproductive success. It is possible that older mothers prioritise reproduction and invest more resources in current offspring, leading to shorter inter-birth intervals, known as the terminal investment hypothesis (Hoffman et al., 2010; Hoffman & Maestripier, 2011).

Furthermore, the social dynamics and dominance rank within the group may influence inter-birth intervals in older female rhesus macaques. Dominance rank has been shown to be independent of male age, and high-ranking males tend to have the highest reproductive success (Widdig et al., 2004), and females may prefer to mate with high-ranking males, which can result in shorter inter-birth intervals (Bercovitch, 1997). These factors can contribute to shorter inter-birth intervals in older females. However, in the current dataset I did not find any effects of rank on productivity. The most likely explanation for this is the reliability of historically recorded rank data (see section 7.4.5).

The approach used for this dataset did not account for the possibility of non-linear effects, which could have significant implications in understanding the dynamics of weaning age and reproductive patterns. Specifically, the study did not explore scenarios where extremely early or unusually late weaning might have a different impact on IBI compared to moderate weaning ages. Such non-linear dynamics, if present, could offer a more nuanced understanding of the optimal weaning period that maximises maternal productivity. Investigating this aspect could refine our understanding of the optimal weaning ages for maximising productivity in purpose-bred macaques.

In summary, I did not find any evidence to corroborate the assumption that earlier weaning maximises maternal productivity.

7.4.5 Limitations and future directions

It is important to note that this study has some limitations. As previously mentioned, there is the potential for pseudo-replication with the video recorded behavioural dataset. Furthermore, for the behavioural data collected from breeding groups, the sample size was relatively small with only six breeding males. As the CFM is quite a small colony compared to facilities outside of the UK, there are few breeding groups established at one time. Aggressive encounters can be rare to observe, and sometimes macaques perform them when there is no observer present. To try and maximise the number of aggressive encounters recorded, videos were recorded around feed times. A better approach would be to record more hours of each group so to not miss instances of aggression, however, this would have been too time consuming for the scope of this project. A further crucial limitation of this study was that the videos could not be scored blindly. This was because identification of individual animals was needed, and this level of recognition was only possible for those familiar with the group and the study at CFM. Moreover, due to time constraints, the videos were not scored twice, precluding reliability analysis. Despite these constraints, measures were taken to minimise observational bias. A clear and consistent ethogram, aligned with published studies on macaque aggressive behaviour, was employed to standardise the recording and interpretation of behaviours. For future studies, the CFM now has CCTV monitoring each group and future studies would benefit from utilising this alongside potentially automated methods for coding behavioural data from videos including females within the analysis, as females may be the aggressors.

The retrospective dataset was constrained to only include that information that was recorded reliably and consistently in the health records. Some factors, such as dominance rank, could not be included in the main study as they were not recorded reliably during this period. Other factors, such as the identification of the animals causing the injuries and the time of day the injuries occurred, would only be possible to obtain through constant monitoring like has been set up.

This study focused on a specific breeding colony of rhesus macaques. Therefore, caution should be exercised when generalising the findings to other primate species or other captive populations with different group structures. At the CFM, breeding groups consist of one adult male, multiple females, and their offspring. Other captive populations can have key differences in group structure. For example, the group size can be much smaller (single-male groups with a small number of females) or much larger (such as the large multi-male–multi-female groups at the Californian National Primate Center in the USA). The CFM keeps macaques in matrilineal groups to mimic the situation in the wild (Rox et al., 2021), whilst others do not retain female offspring in their natal groups. Furthermore, the age at which male offspring are removed from their natal groups can be much earlier (Prescott et al., 2012) or later than in the case of macaques at the CFM.

7.4.6 Conclusions

In conclusion, this study provides valuable insights into the perceived costs of delaying weaning in captive rhesus macaques. The findings suggest that delayed weaning may have implications for aggression and injuries within the group, with older juveniles being more vulnerable to aggression. However, the increased risk is small with factors other than weaning potentially playing an important role. Colonies with a similar structure to the CFM should consider leaving animals in the group for as long as possible, especially if the breeding male is old and has proven to manage groups without frequent aggressive incidents. If a new male is to be introduced into a breeding group, then weaning may benefit the juveniles, however the introduction should be delayed as long as possible to allow the juveniles in the group more time with the mother prior to weaning. When juveniles are to be weaned, the smallest age range possible in newly set up weaning group should aim to be achieved. Colonies with a different philosophy and structure should also consider these recommendations while examining other factors that could reduce injuries and maximise productivity. The results presented in this chapter suggest, at the very least, that decisions to wean macaques earlier should not lie on assumptions.

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Chapter 8. General Discussion

8.0 Summary of aims and objectives

The aims of this thesis were to test the hypothesis that a later weaning age in captivity will be beneficial for rhesus macaque welfare and health. Testing the following predictions (also see figure 8.1):

- Later weaning age will negatively correlate with an anxious and inhibited temperament measured by a novel object and human intruder test.
- Later weaning age will better immune function: higher Neutrophil to Lymphocyte ratio.
- Later weaning will correlate with improved health measures: decreased incidence of *Campylobacter*, decreased prevalence and severity of alopecia, and typical weight.
- Older juveniles in breeding groups will receive more aggression and injuries that require veterinary attention than similarly aged monkeys in weaning groups.
- Dams with juveniles that are weaned later will be slower to reproduce than dams with earlier weaned juveniles.

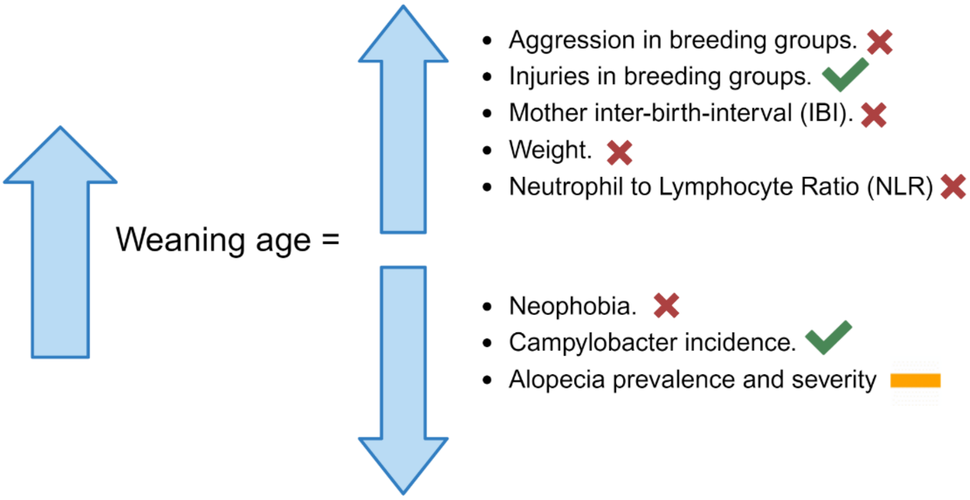

Figure 8.1. Diagram of the predictions tested in this thesis and evidence to support them.

8.1 Summary of key findings from data chapters

8.1.1 Chapter 3

In Chapter 3, a systematic review and meta-analysis was conducted on the relationship between adverse rearing conditions and HPA axis activity in rhesus macaques. The review included 24 studies published between 1989 and 2022, examining the impact of adverse rearing on cortisol measurements from various samples: plasma (21 studies), hair (3 studies), and saliva (1 study).

The findings of the systematic review revealed mixed results regarding the effects of adverse rearing conditions on basal HPA axis activity and the response to acute stressors. Some studies reported higher basal cortisol levels in adversely reared macaques, while others found lower levels or no significant differences compared to control conditions. Similarly, the response to acute stressors also varied across studies, with some reporting higher cortisol levels in adversely reared macaques and others finding lower levels or no significant differences (see Table 8.1 for number of reported effects in either direction per study).

Table 8.1. Number of effects per studies reporting adverse rearing to have higher or lower cortisol compared to control reared.

Study	Higher	Lower
Barr *et al.* (2004a)	3	-
Barr *et al.* (2004b)	-	4
Barr *et al.* (2004c)	-	3
Barr *et al.* (2009)	1	-
Barrett *et al.* (2009)	3	-
Capitanio *et al.* (2005)	-	12
Champoux *et al.* (1989)	3	-
Chen *et al*. (2010)	2	2
Clarke *et al.* (1993)	4	6
Clarke *et al*. (1998)	-	2
Davenport *et al*. (2003)	3	3
Dettmer *et al.* (2012)	6	2
Fahlke *et al.* (2000)	1	1
Feng *et al.* (2010)	2	3
Higley *et al.* (1991)	2	-
Higley *et al.* (1992)	3	-
Kinnally *et al.* (2008)	-	3
Parr *et al.* (2012)	1	1
Rommeck *et al.* (2011)	5	11
Sanchez *et al.* (2005)		3
Shannon *et al.* (1998)	2	8
Winslow *et al.* (2003)	2	1

Table 8.1. Number of effects per studies reporting adverse rearing to have higher or lower cortisol compared to control reared.		
Zhang *et al.* (2016)	2	

The divergent findings suggest complex effects of adverse rearing conditions on cortisol regulation, influenced by factors such as the specific stressor employed, age range of the primates, and timing of cortisol sample collection. However, the data presented in Chapter 3 can only speak to the point of timing; future studies are needed to address the other speculative points. Most studies assessed both basal cortisol levels and cortisol responses to stressors. However, some studies focused exclusively on either basal cortisol or cortisol response, limiting definitive conclusions about the overall effects of adverse rearing. Additionally, the protocols for adverse rearing conditions differed slightly between studies and laboratories, which may have introduced variability. Furthermore, different methodologies and sample types for cortisol measurement (plasma, hair, saliva) were used, contributing to the mixed findings observed.

8.1.2 Chapter 4

Chapter 4 investigated the potential effects of weaning age on temperament in rhesus macaques, hypothesising that later weaned monkeys would be less neophobic and that neophobia score would correlate negatively with weaning age. The main finding of the study was contrary to the prediction, with later-weaned females exhibiting a higher neophobia score at 3 years old. However, the interpretation of this result should be approached cautiously due to a confound in the study design related to acclimatisation time in new groups: the later a monkey was weaned, the shorter amount of time they had to acclimatise to the new group prior to being tested.

The higher neophobia observed in later-weaned females could be attributed to the disruption of strong and stable bonds formed within their matriline. However, it is also possible that the time between weaning and testing may have impacted their affective state, subsequent cognition, and response to the novel testing situation (Dettmer et al., 2018). This finding also suggests that females may be more sensitive to the acute effects of weaning on their affective state compared to males.

Additionally, a post-hoc analysis of monkeys weaned at least one year prior to testing showed no significant effect of weaning age, supporting the theory that time since weaning and acclimatisation to new groups may influence neophobia.

All monkeys in the study were reared under typical social conditions, suggesting that removal very early in life may have more pronounced effects on cognitive development and socio-emotional processing; whereas the earliest removal at around 0.8 years allowed sufficient time for infants to learn species-typical behaviour from their mothers. However, it emphasises the need to consider the paradigm by which macaques were weaned as opposed to just weaning age.

Data were not collected from parents to assess the similarity between parent and offspring temperaments. When the effect of Father ID was explored, the results indicated more variation in neophobia scores among infants from the same father than between them. This suggests that temperament may not be highly similar within offspring of the same father, but further investigation is needed to understand the heritability of temperament from fathers. I could not similarly assess the effect of Mother ID in this study as the number of mothers in the dataset was insufficient to model as a random effect (see Chapter 4).

Chapter 4 also explores the potential influence of the COVID-19 pandemic on the study results. Monkeys tested in 2022, who were born in 2019, experienced reduced habituation due to changes in staff schedules and routines during the pandemic. This lack of habituation at a critical stage of development may have contributed to their higher neophobia scores. However, caution is advised in interpreting this finding as habituation was not directly quantified in the study.

In conclusion, the data from the study do not support the hypothesis that later weaning age increases tolerance to laboratory procedures involving interactions with novel humans or objects. However, the differences between cohorts implies that implementing a good habituation program could potentially reduce neophobia (towards humans and ergo procedures) in laboratory monkeys. Future research should quantify individual levels of habituation and conduct pre- and post-stressor testing, such as relocation, to further examine the effects of weaning and time since weaning.

8.1.3 Chapter 5

Chapter 5 investigated the potential effects of weaning age on the prevalence and severity of alopecia in captive rhesus macaques. The study aimed to control for age, sex, and season while examining both acute and chronic assessments of alopecia.

The data presented in this study are the first to directly test the associations between weaning age and alopecia in captive macaques. The study relied on three datasets:

- The monthly dataset containing alopecia scores from 79 monkeys (Females = 39);
- the acute dataset which sampled colony records at health screens and compared monkeys with health screens either side of weaning to those that remained in the natal group, this dataset contained 110 females;
- and the chronic dataset which contained 247 weaned monkeys (Females = 98) close to their 3rd birthday.

The results highlight the importance of considering the immediate post-weaning period as a critical window for alopecia development since monkeys were more likely to display alopecia shortly after weaning. Understanding the acute effects of weaning can inform targeted intervention strategies to reduce stress and promote the welfare of weaned macaques.

The retrospective analysis found that weaning was a significant predictor for both the severity and prevalence of alopecia at the second health screen when monkeys were around 4 years old. Weaned females were more likely to have alopecia, and their alopecia scores were more severe compared to females that remained in the natal group. This effect was independent of season, age, and previous alopecia. However, due to limited data on males, it was not possible to test the effect of weaning on alopecia in males compared to a group of males that remained in the natal group.

The monthly alopecia score data corroborated the findings from the retrospective (colony records) dataset. Weaned monkeys had a higher likelihood of displaying alopecia and exhibited more severe alopecia compared to non-weaned monkeys, even after controlling for season. However, the analysis did not find evidence of an effect of weaning age on alopecia severity in either dataset. In the retrospective dataset, later-weaned monkeys were less likely to display any alopecia in the short-term, but this effect was not replicated in the monthly dataset, potentially due to a smaller sample size.

However, I did not find a similar effect in the chronic dataset. This discrepancy between the acute and chronic analyses suggests that the impact of weaning age on alopecia may be time limited. Other factors, such as aging or changes in social dynamics, may influence chronic alopecia outcomes (Steinmetz et al., 2006; Kroeker et al., 2014; Beisner & Isbell, 2009). The acute effect of weaning on alopecia may diminish over time as monkeys adapt to their new environment and develop coping strategies. Furthermore, the chronic analysis in the study was conducted at a health screen close to the monkeys' third birthday, indicating a substantial time gap between weaning and the chronic assessment, during which various factors may have influenced alopecia development.

While this study is the first to directly test the impact of early-life events on subsequent alopecia development in rhesus macaques, the mechanisms by which monkeys lost hair are unclear. Potential mechanisms could involve cortisol-induced dysregulation of the hair follicle or hair plucking by the monkey itself or other individuals. Facilities aiming to reduce alopecia may consider intervention strategies targeting recently weaned animals, particularly females.

8.1.4 Chapter 6

Chapter 6 investigated the relationship between weaning age and various health outcomes, specifically *Campylobacter* infection, weight differences, and neutrophil-to-lymphocyte ratio (NLR) in captive rhesus macaques. The study aimed to understand the acute and chronic effects of weaning on these health measures and provided insights into the potential mechanisms underlying the observed associations. The *Campylobacter* datasets in this study were treated identically to those presented in Chapter 5, i.e. the acute dataset contained paired observations from weaned monkeys that had observations either side of weaning to compare with those that remained in the natal group. Sample size for the *Campylobacter* acute dataset was 327 monkeys (Females = 130). The chronic dataset contained all weaned monkeys that had health screens close to their 3rd birthday (*n* = 212; Females = 90). The weight dataset contained weights from 664 monkeys (Females = 314). Finally the NLR dataset contained data from 309 monkeys (Females = 175).

The acute analysis revealed that weaned animals were more likely to test positive for *Campylobacter* compared to those that remained in the natal group. Males were also more likely than females to be infected. This effect was independent of *Campylobacter* presence in the first health screen and within the group. A later weaning age was associated with a decreased likelihood of *Campylobacter* infection, suggesting that delaying weaning could mitigate the susceptibility to *Campylobacter*.

However, no evidence was found of a chronic effect of weaning age on *Campylobacter* infection. Weaned females placed into weaning groups were more likely to test positive for *Campylobacter* than those remaining in the natal group. The group change appeared to be more important in determining *Campylobacter* infection than weaning. Further investigation is needed to determine if this effect is replicated in both sexes.

Several hypotheses are presented to explain the observed associations between weaning and *Campylobacter* infection. The weaning process itself may contribute to an increased risk of infection, potentially through decreased immunity because of the stress response associated with weaning (Coe et al., 1989; Gust et al., 1992; Lubach et al., 1995; Clarke et al., 1996; Lewis et al., 2000; Kinnally & Capitanio, 2015). The finding that a later weaning age was associated with decreased risk suggests that separation at a time when the immune system is more immature leaves individuals more susceptible. The study also considers the possibility that previous exposure to *Campylobacter* may result in a stronger immune response and milder or asymptomatic infections in later weaned animals.

In terms of weight differences, the study found that weaned females and males weighed slightly less than their counterparts that remained in the natal group. However, the observed weight difference was relatively small and unlikely to be a significant indicator of poor welfare or cause for concern. The most likely explanation for this finding is that increased activity due to being in weaning groups with similarly aged peers may contribute to this slight difference in weight, although this is purely speculative and no data are available to test this.

Regarding the neutrophil-to-lymphocyte ratio (NLR), the study did not find a significant relationship between weaning age and NLR after controlling for other factors. This suggests that weaning age alone may not be a strong predictor of NLR in the studied population. Other unmeasured variables or interactions with other factors may influence NLR levels.

8.1.5 Chapter 7

Chapter 7 examined the perceived costs of delaying weaning in captive rhesus macaques. The analysis focused on aggression frequency, injuries, inter-birth intervals (IBIs), and factors influencing them (weaning age, group size and type, sex, and age range). The study provided insights into the social dynamics and potential risks associated with delayed weaning in these macaques. For this study, two separate datasets were utilised: newly collected behavioural data (aggression) and colony records (injury and productivity). The newly collected behavioural data contained numbers of aggressive encounters from 6 breeding males. The injury dataset had observations from 616 monkeys (Females = 428). Lastly the productivity dataset contained 402 infant births from 108 mothers.

For the newly collected behavioural data, aggression was categorised by severity: displacements (least severe); mild; and intense. The analysis of aggression frequency revealed that breeding males directed fewer instances of mild and intense aggression compared to displacements, indicating the use of displacements as a dominant strategy for establishing and maintaining social order within the group (Hosey et al., 2016). There was a trend suggesting slightly more aggression towards juveniles compared to adult females, possibly due to breeding males perceiving juveniles as potential competitors for resources and mates (Goo & Fugate, 1984). The findings also suggested that aggression displayed by the breeding male may be influenced by food competition on high-value food days. There were around 12% fewer instances of aggression on Tuesdays compared to Thursdays. On Thursdays, monkeys were fed a diet that contained eggs, whereas on Tuesdays they were fed a diet contained tomatoes. Monkeys are known to prefer eggs at the CFM (NACWO, personal communication).

Regarding injuries, older juveniles and juveniles in breeding groups were found to have an increased risk of receiving injuries, although overall injury rates were low. The age-related behaviour and development of rhesus macaques, including increased aggression and risk-taking behaviour as juveniles reach sexual maturity, may contribute to higher injury rates in older juveniles (Amici et al., 2019). Another potential reason that has been highlighted in the literature are factors such as the presence of dominant males and group stability can also influence injury rates (Beisner et al., 2010; Beisner et al., 2011; Balasubramaniam et al., 2021). Additionally, the study highlighted the importance of group composition and the potential challenges of introducing older individuals into weaning groups. The finding that juveniles in breeding groups received more injuries compared to other groups, while statistically significant, should be interpreted in the context of the actual injury rates and the overall welfare considerations. While the injury rates were higher for juveniles in breeding groups (around 0.5 injuries per individual per year, with weaning groups having around 33% fewer injuries), it is important to note that the absolute number of injuries was relatively low. This suggests that the risk of injury, although slightly elevated, may not necessarily outweigh the potential benefits of delayed weaning.

Another assumed cost of delaying weaning that I tested in this study is that mothers with older infants in the group will be slower to reproduce. Contrary to the assumed costs, the analysis did not find a significant effect of weaning age on inter-birth-intervals. The results suggested that other factors, such as group structure and social dynamics, may play a more significant role in determining reproductive patterns and maternal productivity. Maternal age was identified as a significant factor, with older mothers having shorter inter-birth intervals, potentially due to the terminal investment hypothesis (Hoffman et al., 2010; Hoffman & Maestripieri, 2011).

8.2 Possible explanations for the effects of weaning age on alopecia and Campylobacter

There are several possible explanations for the similar findings of an acute effect of weaning on alopecia and *Campylobacter* presented in Chapters 5 and 6. Delaying the weaning process allows macaques to have a more mature immune system, which can enhance their ability to tackle infections and reduce the likelihood of developing conditions such as alopecia or *Campylobacter* infection. Early weaning, on the other hand, may coincide with an immaturity or developmental stage of the immune system, making younger weaned individuals more susceptible to these health issues.

The delayed weaning age may allow macaques to receive a longer period of maternal care and milk consumption. Maternal milk provides essential nutrients and antibodies that can confer passive immunity to the infant, protecting them from infections. By prolonging the weaning age, macaques may receive a higher level of maternal antibodies, which can help in combating *Campylobacter* infection and maintaining overall health, potentially reducing the occurrence of alopecia.

Delaying weaning age may result in extended interactions with the mother and natal group, providing greater social support and reducing stress for the infant macaques. Lower stress levels can contribute to better overall health, including reduced susceptibility to infections and conditions such as alopecia (Conti et al., 2012).

Weaning is a crucial period for establishing a healthy gut microbiome, which plays a vital role in immune function and overall health. Delaying weaning age may allow macaques to have prolonged exposure to the maternal microbiome and a more gradual transition to solid food. This extended exposure and gradual transition could positively influence gut microbial diversity and composition, promoting a healthier gut environment. A healthy gut microbiome can contribute to better immune function and potentially reduce the risk of *Campylobacter* infection (Dettmer et al., 2019; Dupjan & Stamp Dawkins, 2022).

It's important to note that these explanations are speculative and the exact mechanisms underlying the observed associations between later weaning age and reduced alopecia and *Campylobacter* infection would require further investigation and research.

8.3 Costs and benefits of the pseudo-experimental design of the studies

As outlined in Chapter 2, this project did not manipulate the weaning age of any of the subjects and was a pseudo-experimental design. The rational for describing this thesis as relying on a pseudo-experimental design is that the weaning practices at the CFM are largely independent of the characteristics for each individual monkey and are driven largely by enclosure availability. The use of pseudo-experimental designs, where weaning age is not directly manipulated but rather based on natural variation in colony management decisions, comes with several limitations:

- Confounding factors: this study lacked the control and randomisation typically found in experimental designs. As a result, there may be confounding factors that influence the relationship between weaning age and the outcomes of interest. These confounding factors can introduce bias and make it difficult to establish a causal relationship between weaning age and the observed effects.

- Potential reverse causality: it is possible that the observed association between weaning age and outcomes is not due to the effects of weaning age itself, but rather reflects underlying differences in other factors related to colony management decisions. For example, colonies may choose to delay weaning for certain individuals based on their health status or social dynamics, which can confound the observed effects. However, weaning at the CFM is predominantly determined by age of the animal and space availability.
- Limited generalisability: this study relied on the existing variation in weaning age within the CFM. The findings may be influenced by factors unique to that colony, such as colony management practices, group dynamics, or environmental conditions. This limits the generalisability of the findings to other captive populations or species with different management approaches.

Although, despite these limitations there are clear benefits from employing the pseudo-experimental design in this context:

- Less ethically challenging: as this study did not introduce additional interventions or procedures that may disrupt the animals' routines or welfare, this non-invasive approach minimises the potential impact on the macaques and is ethically favourable.
- Increased sample size and more cost effective: related to the point above, since conducting the large experiments necessary to establish the benefits of later weaning would be financially prohibitive and difficult to justify ethically. By taking an observational approach, no monkeys were used and significantly more data was able to be collected.
- Longitudinal assessment: the observational approach allowed for the utilisation of existing records. This longitudinal perspective provides valuable insights into the long-term consequences of weaning age decisions.
- Real-world application: the data reflect the practical realities of colony management decisions and practices. The findings can inform and guide decision-making in captive macaque populations, helping to optimise colony management strategies, animal welfare, and health outcomes.

While the pseudo-experimental design can provide valuable insights into the relationship between weaning age and outcomes, it is important to interpret the findings cautiously and consider the limitations associated with this design. Complementary experimental studies that directly manipulate weaning age while controlling for other variables can help strengthen the evidence and provide a clearer understanding of the causal effects of weaning age on the observed outcomes.

8.4 Future Directions

Findings from Chapter 3 suggests future studies should aim for greater standardisation in methodologies, including sample collection and analysis techniques, stressor types, and timing of sample collection. The results from this chapter highlight the importance of comprehensive assessments of animal welfare and the inclusion of measures of behavioural, physiological, and psychological well-being in primate research.

Future studies are recommended to investigate the mechanisms underlying hair loss following weaning to inform management practices for welfare. The study suggests that delaying weaning as long as possible while closely monitoring newly weaned monkeys could help reduce alopecia prevalence. Seasonal patterns in alopecia occurrence and severity were observed, indicating potential environmental influences that should be considered when assessing individual welfare.

Conducting longitudinal studies that follow monkeys from birth to adulthood would provide a more comprehensive understanding of the long-term effects of various factors on behaviour, health, and welfare outcomes. This would allow researchers to capture developmental trajectories and detect changes over time and predict performance and welfare once animals arrive in the laboratory.

The group structure, such as the size, composition, and social dynamics, can significantly influence the behaviour, health, and welfare of captive macaques. Different colonies may have variations in group size, male-female ratios, dominance hierarchies, and social interactions, which can affect the dynamics and outcomes studied in the research. Therefore, it is necessary to exercise caution when directly applying the findings to other colonies with different group structures.

Additionally, the findings may be influenced by other factors such as housing conditions, management practices, and genetic background, which can vary across different colonies. These factors can interact with group structure and influence the outcomes of interest. Therefore, it is important to consider the specific context and conditions under which the studies were conducted when assessing the generalisability of the findings.

To establish broader generalisability, it would be beneficial to conduct similar studies in diverse colonies with different group structures, housing conditions, and management practices. Comparative studies involving multiple colonies would provide a more comprehensive understanding of the relationship between weaning age and various health and behavioural outcomes across different contexts.

Furthermore, future studies could explore the use of automated methods, such as computer vision or sensor technologies, to monitor behaviour, social interactions, and health outcomes in a non-invasive and continuous manner. This would allow for larger-scale data collection and analysis, providing a more detailed understanding of the monkeys' lives.

Integrating more biological measures, such as hormone levels, immune function, or genetic factors, into studies examining the effects of weaning age on behaviour, health, and welfare would be beneficial. This would provide a more mechanistic understanding of the underlying processes and potential biomarkers related to welfare outcomes.

Promoting the standardisation of data collection protocols and variables across different research facilities to facilitate better comparability and meta-analyses of findings. This would enhance the cumulative knowledge in the field and improve the validity of the results. The methodology presented in Chapter 4 has already been applied to laboratories at Oxford University where the CFM supplies macaques. Furthermore, there is current discussions with the laboratories at Newcastle University to apply the temperament testing methodologies to the monkeys they receive there. In the future, this will allow a comprehensive assessment of how early life experiences shape temperament and coping strategies in the laboratory environment.

Overall, future research should aim to address the limitations of the current studies, expand the scope of investigations, and focus on practical applications to enhance the well-being of captive rhesus macaques and other captive primate species.

8.5 Other potential measures of interest for future studies

Cognitive tests and assessments can be conducted to evaluate the cognitive abilities, learning, memory, and problem-solving skills of macaques weaned at different ages. Assessing their cognitive functioning can offer insights into the potential cognitive impacts of weaning age on macaques' welfare once they begin their life in the laboratory.

Evaluating the impact of weaning age on macaques' utilisation and response to environmental enrichment can provide insights into their welfare. Assessments can include the use of novel objects, social enrichment, cognitive puzzles, and opportunities for physical exercise. Monitoring engagement, preference, and stress reduction associated with different enrichment strategies can help determine the effectiveness of enrichment for macaques weaned at different ages.

Assessing additional physiological measures such as heart rate variability, cortisol levels, immune markers, and other stress-related indicators can provide a more comprehensive understanding of the physiological impact of weaning age on macaques' welfare. These measures can help identify potential stressors, health implications, and overall physiological well-being.

Evaluating positive welfare indicators, such as positive affective states, contentment, and the presence of positive behaviours, can provide a more holistic understanding of macaques' welfare. Measures such as playfulness, affiliative behaviours, vocalisations, and other positive indicators can be assessed to determine the impact of weaning age on the promotion of positive welfare experiences.

It is important to consider a multidimensional approach to welfare assessment, combining various measures from different domains (behavioural, physiological, cognitive, social, and affective) to gain a comprehensive understanding of the impact of weaning age on macaques' welfare. Incorporating such measures can help capture the complexity of welfare and provide more nuanced insights into the effects of weaning age on macaques' overall well-being.

8.6 Implications for macaques used in research

Delaying the weaning age may contribute to improved welfare for macaques in research laboratories. The studies suggest that early weaning can have negative consequences, such as increased alopecia and susceptibility to *Campylobacter* infection. By allowing macaques to remain with their mothers for a longer duration, there is a potential to mitigate these negative effects and promote better overall welfare. Furthermore, as infections can potentially affect experimental outcomes and compromise the reliability of data collected, by reducing infection rates through a later weaning age, research studies can be conducted with a lower risk of confounding variables related to infections (Schapiro & Hau, 2023).

Delaying weaning and improving the welfare of macaques in research laboratories can potentially reduce the need for additional animals in studies. If infections and other health issues are minimised through a later weaning age, fewer animals may be required to achieve statistically significant results. This reduction in animal usage aligns with the principles of the 3Rs (Replacement, Reduction, Refinement) and promotes ethical and responsible research practices, and the refinement of delaying weaning is a relatively simple change to make (Prescott et al., 2012).

8.7 Conclusions and recommendations

One of the main findings presented in this thesis is the relationship between weaning age, sex, and season on *Campylobacter* infection, alopecia prevalence and severity in captive rhesus macaques. The result underscores the impact of early weaning on acute alopecia and *Campylobacter* infection. Delaying weaning may mitigate these risks, benefiting animal welfare and research data quality.

Additionally, the perceived costs of delaying weaning, notably increased aggression and injuries among older juveniles, were investigated. While these concerns are valid, the actual risks are relatively low, and factors such as group composition, male age, and weaning group age range age play a more significant role in determining aggression and injuries. Considering the long-term welfare implications, the benefits of delayed weaning in social development and overall health may outweigh the slight risk of injuries.

Contrary to assumptions, earlier weaning does not necessarily maximise maternal productivity; other factors like maternal age and social dynamics within the group have a more substantial impact on reproductive patterns.

The fact that the data did not consistently prove the benefits of later weaning within the colony's existing practices (see figure 8.1), suggests that the current guidelines strike a balance between macaque developmental needs and practical colony management. Nonetheless, evidence hints at potential advantages in extending weaning age, such as reduced alopecia and lower susceptibility to *Campylobacter* infection, with perceived costs likely overstated. Hence, colonies should consider the option of extending weaning age for better macaque welfare and health outcomes.

In addressing the welfare of rhesus macaques housed in colonies, it is recommended to focus on several key areas. Firstly, consider extending the weaning age, as this may reduce susceptibility to issues such as *Campylobacter* infection and alopecia. This should be tailored based on individual and group health and behaviour observations. Alongside this, implement routine health monitoring, specifically focused on the post-weaning period to promptly detect and manage health issues.

Furthermore, facilitating extended interactions with mothers and the natal group can significantly support the social development of juveniles, reducing stress and potentially improving overall health and temperament. Developing and applying stress reduction strategies, particularly around the weaning process, is also vital. This could encompass environmental enrichment and minimising disruptions.

Careful consideration of group composition when introducing weaned juveniles into new groups is crucial. The aim should be to create socially stable groups that minimise aggression and competition, especially for older juveniles.

Instituting habituation programs can acclimate macaques to human interaction and novel stimuli, reducing neophobia and enhancing adaptability to various environments. Monitoring and investigating the causes of injuries, especially in older juveniles and breeding groups, will aid in developing strategies to mitigate these risks while maintaining social and environmental enrichment.

Finally, collaborating with other colonies and research institutions to share best practices and stay updated on the latest welfare research is beneficial. Regular review and adaptation of these practices are crucial as more research and insights become available, ensuring a balance between the practical aspects of colony management and the goal of optimising the welfare of rhesus macaques.

8.8 Chapter references

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