Assessing the welfare of laboratory-housed marmosets (*Callithrix jacchus*): Effects of breeding and infant rearing background

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“Be kind whenever possible. It is always possible.”

“Our prime purpose in life is to help others. And if you can’t help them, at least don’t hurt them.”

Dalai Lama

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Abstract

The common marmoset is the most frequently used New World primate in laboratory research and testing. In the UK, their use is strictly controlled by the Animals (Scientific Procedures) Act, which is underpinned by the principles of humane science: Replacement, Reduction and Refinement. Despite their use, there are a number of problems associated with the breeding of marmosets, including low dam longevity and increasing litter sizes. Large litters have led to high infant mortality and the need for human intervention to improve infant survival, which involves removal from the family for substantial periods of time. Previous research in a range of primate species shows that early life family separation is associated with numerous adverse behavioural and physiological effects. This project therefore sought to systematically investigate the effects of breeding and infant rearing practices, integrating a number of measures to assess the welfare of laboratory- housed marmosets.

Potential predictors of dam longevity and litter size were first identified in three captive UK colonies, over four decades. Dam longevity was found to be approximately 6 years, with heavier dams living longer, but overall there was no consistent improvement in longevity over the decades. As longevity varied widely between colonies and over time, environment may be one of the most important factors. Approximately half of all births at each colony were litters larger than two, and these larger litters had greater infant mortality. Only dam weight at conception was useful in predicting litter size, with heavier dams producing larger litters.

The consequences of large litters and early separation from the family for supplementary feeding were then investigated. Although twins had lower body weight than 2stays (two infants remaining with the family after death of the other littermate/s) and supplementary fed triplets, they also had the fewest health problems. There was also some evidence that animals from larger litters were more at risk of suffering from extreme low weight. Some minor differences were found in behavioural development between litter sizes.

Singleton infants received more rejective rearing, while 2stays received more protective rearing, perhaps following the loss of an infant. While twin infants gained independence earlier than singletons or 2stays, they did not appear to cope better with stress in adulthood, displaying more significant increases in stress-related behaviour following the routine stressor of capture and weighing, compared to 2stays and supplementary fed triplets. While overall cortisol unexpectedly decreased from baseline to post capture, there were only significant fluctuations in 2stay marmosets. Instead, there were some increases in positive behaviour in supplementary fed triplets following the stressor, suggesting enhanced coping ability. However, in another group of supplementary fed triplets, there were subtle increases in depressive-like symptoms, measured using cognitive bias and preference tests, suggesting a reduced expectation of and interest in rewards. There were however no differences between family-reared and supplementary fed marmosets in time to learn a visual discrimination task, or in responses to temperament tests.

Therefore, while it was hypothesised that early family separation would have adverse developmental consequences, there were actually very little differences between marmosets of different litter sizes and rearing backgrounds, across the range of measures. Results suggest that the current supplementary feeding programme, along with a regular human socialisation programme, minimises any potential negative effects. However, we should always be finding ways to improve the lives of animals in our care. Possible Refinements include reducing dam weight to increase twin births and improve infant survival, and training to allow supplementary feeding on the carrier’s back, to prevent infant separation and reduce disruption to the family. These Refinements could reduce fear and allow monkeys to become more resilient to the laboratory environment.

CHAPTER 1: Primates in Research, Animal Welfare and Rearing Practices

Abstract

It is of ethical and scientific importance to protect the welfare of primates bred and housed in the laboratory. Strict regulations govern their use in the UK, which are underpinned by the principles of humane science, the 3Rs. These require researchers to Replace animals if possible, or else Reduce the numbers needed and maximise welfare through Refinement. While all mammals can suffer and so need careful consideration, primates may be special candidates as their complex cognitive, social and psychological needs can be more challenging to meet in laboratories than other commonly used species. Their use must be clearly justified, achieved by weighing the harms to the animals against the potential benefits. It is critical that welfare is maximised. Improved welfare can also have significant scientific merit, allowing the most reliable and valid results to be obtained from the animals.

Animal welfare is based on three approaches: biological functioning, leading a natural life and subjective state. The viewpoints shape the methods used for assessment. However, animal welfare is a multidimensional concept, best assessed with a holistic approach that integrates a variety of measures. In the laboratory, animal welfare is based on Refinement. Although the common marmoset is the most frequently used New World primate in research and testing, there are several problems associated with their breeding that would benefit from such Refinements. These problems include low dam longevity and increasing litter sizes, leading to high infant mortality and the need for human intervention to improve survival of larger litters. Young marmosets are routinely separated from their family, despite there being much evidence that this can have adverse effects on their development. Two types of primate model have been used to investigate this: Parental Separation Models and Maternal Behaviour Models. While such work has offered profound insights, early life stress exposure may not always be so deleterious. The present thesis therefore examines the effect that litter size and

early parental separation has on a broad range of welfare measures, including development and longevity, affective state and the stress response.

1.1 The use primates in research

1.1.1 Legislative requirements

Many animals are used every day in biological, medical and psychological research, including non-human primates. In the UK, this is subject to provisions of the Animals (Scientific Procedures) Act 1986 (A(SP)A) and its Codes of Practice (Home Office, 1986, 1989; 1995). The new European Directive (2010/63/EU) on the protection of animals used for scientific purposes (European Union, 2010) has recently been enforced to harmonise the laws and regulations of Member States. The legislation contains mandatory minimum standards for controls, transparency, accommodation and care, which should result in better provision for animals in laboratories internationally and is viewed as essential in eliminating trade barriers (Miller, 1998). Research institutions can maintain their existing provisions if they are stronger than the Directive's requirements, although this is not mandatory as it could be seen as 'gold plating' (RSPCA, 2011). Within the UK, the NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research) produce guidelines on primate care, accommodation and use that are higher than UK legislation.

Procedures under the A(SP)A are then controlled through a licensing system, including designated establishment, project license (including estimated severity) and personal licensees. As well as the Establishment Licence Holder and Named Veterinarian, a Named Animal Care and Welfare Officer is appointed to ensure the day to day care and welfare of the animals (Hubrecht, 2014).

Legislative requirements are put in place to ensure procedures are justified and to minimise any harm to animals during breeding, transport and use in research (Hubrecht, 2014). Much of the current British and European legislation adopts a utilitarian approach to dealing with the dilemma of using animals in research (Home Office, 1986). From this viewpoint, ethical reviews involving a cost-benefit judgement, must be carried out, in which the suffering of animals is weighed against the potential benefits of the research (Home Office, 1986). Retrospective reports on the actual level of pain, suffering, distress and lasting harm are required (EU, 2010). Reassurance that things are being done properly is also important for the public perception of animal use, with support more likely to be maintained if researchers are open about their work and promote the welfare of their animals (Rose, 2011).

Special justification is required for the use of dogs, cats, Equidae and primates under the Act. Permission to use primates is only granted if the research aims to avoid, prevent, diagnose or treat debilitating or life-threatening clinical conditions in humans. The Secretary of State must be satisfied that there is no alternative test or animal (Home Office, 1986), and no great apes have been used since its introduction. *In vivo* testing is however still a requirement for regulatory approval (EU, 2010), with a second, non-rodent species required prior to clinical trials in humans (APC, 2002). Typically, if a dog is ruled out, primates are then considered (APC, 2002). Although their use in research is due to their phylogenetic similarity to humans, this may also make them a special case (Boyd Group, 2002). While there is little evidence of a *Scala Naturae*, which suggests that different species have different capacities to suffer (Hubrecht, 2014), a larger brain has evolved in primates to deal with their complex physical and social world, and providing for such complexities given the laboratory constraints can be challenging. Primates can therefore suffer from boredom or fear, and inappropriate rearing and environments can have adverse effects on welfare (Buchanan-Smith, 2010a). Although it is possible that different species have different needs and so are more affected by certain procedures (Hubrecht, 2014), there are problems with comparing the suffering of primates to other commonly used species, as subjective experiences are difficult to understand (Boyd Group, 2002).

1.1.2 3Rs: Ethical importance

Much legislation is underpinned by the principles of the 3Rs: Replacement, Reduction and Refinement, first proposed by Russell and Burch in 1959. These are now widely accepted as the fundamental ethical framework of humane science, and should be applied from project design to execution (Home Office, 1986). Replacement refers to finding alternatives to using protected animals (living vertebrates and cephalopods), which can be achieved by using insentient material, such as *in vitro* methods or *in vivo* studies on cells or tissue cultures. Use of computer models, human volunteers and invertebrates (eg. *Drosophilia*) could also be possible. If it is not possible to find Replacements to animals, Reduction should be considered, in which the fewest number of animals are used to obtain the same goals or the same number of animals are used to obtain more information. Reduction is predominantly achieved through good planning and experimental design, such as use of improved statistical analysis, data sharing and modern techniques, like imaging, to allow longitudinal studies of the same animals (Hubrecht, 2014). The third R, Refinement, is defined as minimising pain, suffering and lasting harm, while enhancing the wellbeing of animals that must still be used. This applies to the life to death experience of the animals, and so includes housing, husbandry and breeding, as well as specific research procedures (Buchanan-Smith et al, 2005).

The main ethical dilemma is whether humans are morally justified in causing pain to animals during research aimed at alleviating human suffering (Rollin, 2007). As well as the potentially painful procedure, they are often kept in smaller, less complex and more predictable environments (Bowell, 2010), which often restricts behaviour (Olsson et al, 2003). Humans, and the control we have over their environment, may be the largest source of fear, which can be reinforced by handling or performing routine husbandry. Various practices are thought to be at least moderately distressing, particularly if prolonged, including noise (Cross et al, 2004), isolation or removal of a companion (Norcross and Newman, 1999) and crowded conditions

(Baer, 1998). Separation from the family early in life, such as for hand-rearing, could also lead to adverse developmental consequences (Dettling et al, 2002; 2007; Buchanan-Smith, 2010b).

There are therefore many opportunities to Refine housing, husbandry and procedures (JWGR, 2009; Prescott, 2010). It is important to provide an environment that meets the animal's needs, as well as to reduce the stress of handling and other husbandry requirements. As these requirements affect all animals throughout their lives, Refinements in this area have the potential to improve the wellbeing of all animals used in research (Hubrecht, 2014). Enrichment, to allow species-specific behaviour, complexity and control, may include modifications to the enclosure structure, housing in compatible social groups or changes to feeding methods. For marmosets, wooden items and artificial gum trees provide opportunities for gnawing, while perches, branches and ropes give options of where to move and rest (Hubrecht, 2014). Complex physical and social environments are important in the development of behaviourally and physiologically normal animals, that are able to cope with stressors later in life (Hubrecht, 2014). Positive reinforcement training (PRT) is also increasingly being used to allow animals to co-operate with husbandry or experimental procedures, removing the need for restraint (Prescott et al, 2005) and allowing them to gain positive associations with such aspects of the laboratory environment (Tasker, 2012). PRT is common in primate management. For example, marmosets have been trained to hold a target, which can be used to direct them to sit on a weigh scale in the home cage (McKinley et al, 2003). Providing the ability to predict aversive events has also been shown to reduce the stress response (Weiss, 1970), while an unpredictable schedule of positive appetitive events is recommended (Rennie and Buchanan-Smith, 2006b; Bassett and Buchanan-Smith, 2007). Refinements must however be assessed scientifically to prove that they are indeed of benefit (Bayne, 2005), and not instead causing any stress or injury. High quality care and competent use of laboratory-housed primates are probably the most important factors influencing welfare.

1.1.3 3Rs: Scientific importance

It is increasingly being recognised that the welfare of animals used in laboratory research can influence the results derived from them. Good laboratory research is based on normal, healthy animals, with no confounding factors (unless disease or stress is the subject of investigation). However, it is known that poor psychological wellbeing can alter many different biological parameters, including neurological, endocrine and physiological stress systems. Poor welfare is therefore a confounding variable, just as disease alone is (Poole, 1997). Breeding, rearing and housing conditions can all affect behaviour and physiology, which increases variability and non-repeatability of data derived from the animals, and could obscure treatment effects (Howard, 2002).

Research using primates should be high quality and solve important problems, producing unambiguous results by keeping unwanted variation to a minimum (eg. Poole, 1997). Healthy, unstressed animals at the starting point of study are likely to produce better models and provide more consistent, meaningful results, than animals with compromised behaviour and physiology due to poor welfare (Poole, 1997; Weed and Raber, 2005). Improving welfare could therefore also improve science. Fewer animals would then be required to produce statistically significant results. Indeed, recent research has increased our understanding of how welfare and quality of science are related. Tasker (2012) found that enhanced socialisation led to lower baseline heart rates in cynoglgus macaques (*M. fascicularis*), which improved the sensitivity of cardiovascular measures derived from them. Further, Hall (2014) found that dogs (laboratory-bred beagles) in a negative affective state had increased anxious behaviours and higher blood pressure at baseline, as well as greater responses following challenges, and so data obtained from these animals are different to those in a positive affective state.

1.2 Animal Welfare

The field of animal welfare science is based on the belief that humans have a moral and ethical obligation to ensure the welfare of the animals whose lives they influence, and that we should continue to work to improve their quality of life (Sandøe et al, 1997; Appleby, 1999). The concept of animal welfare is broad and multi-dimensional (Dawkins, 2004), lying on a continuum of poor to good (Broom, 1999). Its study requires a scientific approach (Hubrecht, 2014), and now encompasses many discpliplines, including physiology, ethology, neuroscience and veterinary medicine, which can all be used synergistically to address animal welfare issues (Fraser, 2008). However, there is no universally accepted definition. Duncan and Fraser (1997) have presented a practical approach, where opinions are categorised into those supporting one of three approaches: biological functioning, natural living and feelings-based. The welfare of animals used in laboratory research is predominantly based on the concept of Refinement. Species-specific welfare indicators in marmosets are discussed in Chapter 5.

1.2.1 Biological functioning

The biological functioning approach is concerned with factors such as injury, disease, reproduction and growth, and so it is relatively easy to measure welfare objectively with this viewpoint (Duncan and Fraser, 1997). Some scientists may therefore see this as the only way to assess welfare. Reductions in growth, reproduction and survival clearly show a problem with the body's normal functioning. Improving basic functioning, by providing better physical and social environments, must indicate improvements in welfare (Fraser, 2008).

According to this approach, welfare is regarded as an animal's attempts to cope in its environment (Broom, 1986). Homeostasis keeps physiological variables at their set point, maintaining an internal equilibrium and allowing the animal to survive challenges (Frandson and Spurgeon, 1992). Low welfare occurs when an animal cannot cope with chronic or intense environmental challenges, leading to disruption of health and functioning, and even death.

Meanwhile, when an animal successfully copes with environmental challenges, there is good health, functioning and longevity, resulting in high welfare (Fraser and Broom, 1990; Duncan and Fraser, 1997).

Allostasis has since been proposed, to also take into account environmental challenges that may lead to under stimulation (Korte et al, 2007). Allostasis involves changing the physiological variable to meet anticipated demands (Sterling and Eyer, 1988), with natural selection shaping responses to meet the most likely of these (Korte et al, 2007). However, if mechanisms are activated outside of the allostatic range, the animal may continue to respond when the challenge no longer exists or may mount an inadequate response (Korte, 2001). Over-stimulation may therefore lead to pathologies (McEwen and Lasley, 2002), while under-stimulation could result in depression, allergies and autoimmune disorders (Sternberg, 1997). If captivity does not meet the animal's adaptations, changes in reactivity and resilience can also occur.

As 'stress' refers to the negative physiological and psychological state experienced when an individual is threatened (Moberg, 2000), demonstrating this in an animal would be clear evidence for compromised welfare, with impairment in coping reflected in several physiological and biochemical measures (Fraser, 2008). When stressed, the body undergoes a set of characteristic changes in the nervous and endocrine systems. The sympathetic nervous system (SymANS) releases norepinephrine, causing increases in heart rate and blood flow to muscles, which has been termed the 'flight or fight' response (Sapolsky, 1992). This system works in conjunction with the hypothalamic pituitary adrenal (HPA) axis, which responds to virtually any type of challenge. During activation of the HPA axis, the hypothalamus releases CRH (corticotrophin releasing hormone), causing the pituitary gland to release ACTH (adrenocorticotrophic hormone) into the blood, which in turn causes the adrenal gland to increase the output of glucocorticoids (Sapolsky, 1992). Cortisol is the main hormone in many mammals, with numerous studies using it as an indicator of welfare. Cortisol increases the amount of glucose, making more energy available for immediate use. It also reduces processes of reproduction and growth, as well as lowers immune activity. The stress system therefore has complex interactions with many other systems.

While activation of the SymANS and HPA axis is an adaptive response, preparing the body for increased demands, very strong or prolonged periods of activation could lead to a substantial array of diseases, failure to reproduce or abnormal behaviour (Fraser, 2008). This can therefore have implications for the health and wellbeing of animals throughout their life. The intensity of the response is thought to reflect the degree of averseness, with large changes in cortisol or catecholamines indicating unusually high activation of the stress response, and so greater psychological and physiological stress (Fraser, 2008). Changes in other physiological parameters, including heart and respiration rate, have been shown to be associated with stressful occurrences. Reproductive and growth hormones, as well as immune parameters, may also be useful indicators. Non-invasive methods of measuring levels of stress are therefore essential tools for animal welfare researchers.

However, physiological measures require careful interpretation. The stress response systems are not only activated by unpleasant situations, but also from natural or presumably pleasurable activities (eg. exercise, mating) (Rushen and de Passile, 1992). In these cases, elevated levels do not necessarily denote a welfare issue (Fraser, 2008). It is also difficult to separate normal adaptive fluctuations from changes that signify a significant welfare problem (Fraser, 2008). It has been suggested that a sustained increase of 40% or more of free corticosteroids in blood plasma could provide evidence for reduced welfare (Barnett and Hemsworth, 1990). However, this threshold has been questioned, as several studies have found decreases in growth and survival, without corresponding changes to corticosteroid concentrations (Rushen, 1991). Animals could also be disease free, well-fed and breeding, although may not have high welfare (Mendl, 1991). Therefore, while often these are straightforward indicators of animal welfare, caution is needed.

1.2.2 Natural living

The natural-living approach suggests that animals have a genetically predisposed nature, referred to as ‘*teleos*’ (Rollin, 1993). An animal should be kept in an environment where it can express the full repertoire of natural species-specific behaviours they have evolved to perform (Kiley-Worthington, 1989). These can be anatomical, physiological, behavioural, affective and cognitive. Further, animals have a fundamental requirement to carry out certain ‘behavioural needs’ (Pool, 1992). These are necessities, as the animal is internally driven and strongly motivated to perform them. If they cannot, their welfare is jeopardised (Duncan, 1998). Even domestic animals have been found to show virtually all the behavioural repertoire of their wild ancestors. Despite them serving little function in the captive environment, their performance is likely to be very important to their welfare (Duncan, 1998).

In some cases, the motivating behaviour is impossible for captive animals to perform, which could lead to frustration (Fraser, 2008). However, having food provided and no need to escape predators could also lead to boredom (Hediger, 1955). These animals may then over react to unusual or surprising events (Wemelsfelder, 2005). Unnatural housing could also cause behavioural abnormalities, such as aggression or self-biting (Fraser, 1959). For example, solitary housing has been found to lead to self-injury in macaques (*Macaca* sp.). The most effective treatment is being housed with a companion (Reinhardt and Rossell, 2001).

‘Stereotyped behaviour’, defined as ‘repetitive, unvarying and apparently functionless’ (Mason, 1991, p103), has been used most extensively in the assessment of animal welfare. These behaviours are often performed for long periods, in the same place or at the same time, and animals have difficulty stopping. Many are normal ‘source behaviours’, the most common involving the mouth or general locomotion, which are ‘redirected’ to an inappropriate target, generally where the normal target is missing (Mason, 1991). However, it is difficult to conclude what these behaviours mean for the animals welfare.

Research has found stereotypies to be associated with dysfunction in the brain (Garner and Mason, 2002) and poor reproductive abilities (Diez-Leon et al, 2013: *Neovison vison*). They could also indicate unpleasant states, or that either the past or present environment has not allowed the animal to react in a normal way (Fraser, 2008). While Mason and Latham (2004) concluded that most situations that elicit stereotypies do reduce welfare, stereotyping individuals often have higher welfare than non-stereotyping individuals within a sub-optimal environment. They therefore suggested that stereotypies may be a form of ‘do it yourself enrichment’, providing a substitute for natural behaviour, or repetition could have calming ’mantra effects’. Such behaviour could also become habit, or be due to a general perseveration. In these cases, stereotypies may be neutral or even beneficial for welfare, and so should not be used as the sole indicator (Mason and Latham, 2004).

Behavioural observations are however a useful, simple and non-invasive method of assessing welfare by this viewpoint. Increases in the amount or intensity of certain activities could signify an underlying problem. For example, non-human primates have been seen to perform self-directed activities, such as grooming and scratching, in challenging situations, which can be alleviated with anxiolytic drugs (Troisi, 2002). Certain behaviours have also been characterised as agonistic or submissive, or are associated with alarm and fear. Meanwhile, other behaviours, including play, and relaxed postures and facial expressions, are likely to be indicative of a positive mental state and so good welfare. Wild counterparts can serve as useful benchmarks (Duncan and Fraser, 1997). Their study is useful in understanding the species’ normal range and frequency of behaviour, and their requirements (Roder and Timmermans, 2002). It could therefore also provide theoretical foundations for Refinements (eg. enrichment: Buchanan-Smith, 2010a).

An animal would be considered to have good welfare if it was displaying a normal range and frequency of behavioural patterns, with no abnormal behaviour (Poole, 1997). Normal behaviour would suggest that the captive environment shares similar characteristics to natural habitats, indicating it has met behavioural needs (Poole, 1992). Giving the animal choice over which conditions and behaviours to perform could be the best way of accommodating the concern for naturalness (Fraser, 2008). For example, allowing animals to forage and hunt in the manner in which they are adapted (Fraser, 2008). Normal interactions with the environment, both physical and social, are especially important for the development of young animals.

However, this approach is not free of criticism. Firstly, while enriched cages are encouraged, it can be difficult to actually make conditions natural in the laboratory environment. A common concern is that Refinements may bias results and increase variation, decreasing reliability and replicability of data (Buchanan-Smith, 2010a). However, more environmentally enriched conditions should lead to more ‘normal’ animals, with barren housing more likely to lead to abnormal behaviour (Garner, 2005). Secondly, it is not clear what occurrence, frequency, duration or quantity of deviation in behaviour from wild counterparts would indicate changes in welfare (Novak and Drewsen, 1989). Thirdly, natural environments do not always provide the best quality of life. More natural environments may include adverse conditions, such as lack of food, harsh weather and predators. Animals may therefore still face serious welfare problems (Fraser, 2008). Rather than replicating the animals’ natural environment, their natural behaviour can be accommodated (Fraser, 2008). Some natural behaviour may however also be detrimental, such as shivering and fleeing (although this may give the animal a sense of control). In these cases, not requiring an animal to exercise the adaptation may not pose a problem. Therefore, performance of natural behaviour may not always be a practical indicator of welfare (Dawkins, 1998).

1.2.3 Feelings-based

For many people, concern about the subjective state of animals (their emotions) is the most important element of animal welfare (Fraser, 2008). Legislation in many countries is designed to protect animals under human care from negative subjective states, such as ‘pain’, ‘distress’ and ‘suffering’, while positive affective states, such as happiness (Poole, 1997), are inherent for good welfare. Table 1.1 provides definitions for such positive and negative subjective states. While some see it as inappropriate to use science to try to understand affective states in animals, as it cannot be observed and is too anthropomorphic, others value the view, suggesting it promotes concern for animal wellbeing and conscientious, empathetic care.

Although there has been debate over whether animals feel emotion at all, or instead react to stimuli automatically with no conscious subjective experience, it is unlikely that the ability to feel emotion evolved solely in humans. The extent to which an animal can suffer may depend on their ability to feel emotion, and so level of consciousness, which could be related to cognitive ability (Rogers, 2010). However, Bekoff (2002) proposed that suffering may be equal in individuals that experience “this is painful” and ones that consciously “feels pain”. Therefore, greater cognitive ability does not necessarily mean they can suffer more, and may even help them to cope better (Broom, 2010). There may however be increased possibilities of pleasure (Broom, 2010) and pain, such as empathising with others and dreading future events, in more cognitively complex individuals (Mendl and Paul, 2004).

While the conscious experience of emotion in animals cannot be assessed directly, the use of several indirect measures could serve as indicators of affective state, including changes in behaviour and physiology. It is assumed that much behaviour is motivated by pleasant or unpleasant states (Fraser and Duncan, 1998), with negative states warning animals of threats and positive states rewarding particular behaviours (Barnard and Hurst, 1996). Affective states can therefore have a strong influence on what animals prefer and are motivated to do. The possibility of asking animals what they want has therefore been raised (Fraser and Matthews, 1997). Preference tests can be used to obtain the animal’s own view, in which they are offered a choice between two environments. Objective measures are recorded, with more time in one environment or shorter latencies to approach, indicative of a preference (Bateson, 2004).

Subjective value judgements are therefore unnecessary (Hughes and Black, 1973). However, such preference does not necessarily indicate strength of motivation (Duncan and Fraser, 1997).

Table 1.1: Definitions of negative and positive subjective states (adapted from Tasker, 2012)

Subjective state	**Definition**	**Reference**
Affect	Involves positive and negative feelings.	Yeates and Main, 2008
NEGATIVE		
Pain	An aversive sensory and emotional experience, caused by the awareness of tissue damage. The individual changes its behaviour and physiology to avoid or reduce damage, and promote recovery.	Molony and Kent, 1997
Suffering	A negative emotional state, due to adverse physical and psychological events that overload an individual's coping mechanisms.	Morton and Hau, 2002
Stress	The physiological and psychological changes experienced when there is a threat to an individual's homeostasis.	Moberg, 2000
Distress	An aversive state that occurs when exposure to stressors over-taxes an individual's coping ability, and so they fail to return to their physiological or psychological homeostasis.	Moberg, 2000
Fear	An emotional state caused by the perception of well-defined threats, leading to defensive reactions.	Janczak, 2010a
Anxiety	A fearful emotional state shown in healthy animals, where the source of the threat is unclear.	Janczak, 2010b
Depression	A negative low arousal state, associated with experiences of loss or lack of reward.	Mendl et al, 2010a
Boredom	A low arousal state, due to a chronic lack of opportunity to interact with the environment.	Wemelsfelder, 2005
Frustration	A negative emotional experience, caused when an animal is motivated to express a behaviour, but is prevented from doing so.	Keeling and Jenson, 2009
POSITIVE		
Liking	The positive feelings experienced following a rewarding event.	Yeates and Main, 2008
Wanting	The psychological state caused by motivation to gain a reward.	Yeates and Main, 2008
Pleasure	A positive state, due to rewarding physical or emotional experiences.	Balcombe, 2011
Happy	The animal displays a wide range of normal behaviour, and no abnormal behaviour. They are able to relax and are confident, rather than fearful, toward non-threatening stimuli.	Poole, 1997

Strong preferences are likely to influence welfare more than weak preferences, and so measures of how badly an animal wants a particular environment or to perform a certain behaviour have been developed (Dawkins, 1990). The simplest method is to give the animal an instrumental task, such as pushing a lever or weight, or running down a runway, to gain access to or avoid a particular option. Dawkins (1990) further proposed that elasticity of demand could be used to demonstrate how important different commodities are to animals. Some commodities, such as food, are inelastic 'necessities', while others are more elastic 'luxuries'. If the animal has to work, it is expected that they would put more time and effort into obtaining important rewards. They may only engage in other activities, such as play, when the cost is low. Therefore, the easiest way to understand the importance of a resource is to look at the highest price they are willing to pay (Kirkden, 2003). However, animals may favour what they are most familiar with, or they may not choose what is beneficial for them, and so this method does not take into account long-term consequences (Duncan, 1978). Preferences may also vary depending on context (Bateson, 2004), so it is only limited to short-term motivations.

More novel approaches to describe affective states have since been developed, such as 'Free choice profiling', which is a qualitative approach that involves rating the overall 'expressive qualities' or body language of an animal, such as confident and excitable, using the observer's own descriptors (Wemelsfelder et al, 2001). The method has been validated in a number of species, and has shown good agreement with quantitative measures of behaviour (Rousing and Wemelsfelder, 2006) and physiology (Wemelsfelder, 2007).

Cognitive bias has also recently emerged as a promising tool. Cognition refers to information processing, such as attention, memory, learning and decision making. Cognitive appraisals of situations can be influenced by emotional state (Mendl et al, 2009). Background mood states are most apparent when there is no current strong emotion-inducing event, which may otherwise mask them (Mendl et al, 2010a). For example, it is known that depressed humans tend to view ambiguous stimuli more pessimistically then non-depressed individuals.

Harding and co-workers (2004) investigated this in rats, training them to press a lever for a food reward when one tone sounded, and avoid pressing to prevent unpleasant noise when another tone sounded. Intermediate tones were then presented. Rats exposed to unpredictable adverse conditions were more likely to interpret the ambiguous tones as negative, pressing the lever less often and less quickly than those that were spared the events, suggesting a more depressive-like state. Such biases have since been demonstrated using a variety of species and methods. Cognitive bias testing could therefore be used to detect enhanced expectations of positive or negative events, and so the valence of underlying emotion. This is further discussed in Chapter 6.

Although subjective feelings are adaptive and often promote biological functioning, an animal could be happy, although have a terminal disease. In this case, it would not be seen as having good welfare. Alternatively, an animal could have good health, but not necessarily be happy. While there has been much respected work in this area, more research is still needed to sufficiently understand the subjective feelings of animals (Duncan and Fraser, 1997).

1.2.4 Integrated approaches and use of science to assess animal welfare

Animal welfare is a highly complex concept (Fraser, 2008) and given the range of methods used, it is important to understand how the different types of information fit together (Fraser, 2008). Despite certain advantages and disadvantages of each approach, one would expect broad agreement between the measures (Fraser, 2008). For example, there is the assumption that if pain and distress are eliminated in laboratory animals, they are able to function more normally, leading to more valid results. Table 1.2 shows the range of animal welfare measures, depending on the viewpoint taken.

Duncan and Fraser (1997) proposed that we should consider aspects of all ideas in combination, rather than regarding them as separate points of view, in order to aid interpretation and provide validation of results (Dawkins, 1980; Dawkins, 1983; Fraser &

Broom, 1990; Broom, 1991; 1996; Dawkins, 1998). For example, asking whether an animal's health is compromised or if they show signs of wanting to escape a situation can help to interpret measures of 'stress hormones' (Dawkins, 2004). However, in some situations, increasing welfare by one criteria may actually reduce welfare according to another criteria, in which case a decision must be made (Dawkins, 2012).

Table 1.2: Measures of animal welfare depending on viewpoint

Biological functioning	**Natural living**	**Feelings based**
Reproductive success	Behavioural repertoire	Preference tests
Body weight and condition	Time budgets	Motivation tests
Injury	Stereotyped behaviour	Free choice profiling
Disease	Self-injurious behaviour	Cognitive bias tests
Longevity	Agonistic/ submissive behaviour	Facial expressions
Heart rate	Anxiety related behaviour	
Blood pressure	Social interactions	
Respiration rate	Posture	
Catecholamines	Play	
CRH and ACTH	Locomotion	
Corticosteroids	Vocalisations	
Growth/ reproductive hormones		
Immune parameters		

* Highlighted areas indicate those used in the present thesis

It is critical that animal welfare arguments are sound, solidly backed by scientific evidence, to withstand any criticism (Dawkins, 2012). It is therefore essential to make clear what is being measured and how these data can be used to make inferences about welfare (Mason and Mendl, 1993). Dawkins (2004) argues that questions about consciousness are currently beyond science to explain, although this does not prevent concern about the ethical treatment of animals. Rigouous studies can be carried out without reference to consciousness, to provide practical proposals to improve welfare. This idea led to the suggestion that animal welfare assessment should address two key questions: Are they healthy and do they have what they want? These capture both the biological functioning and natural living approaches, and give a basis for evidence-based welfare.

Good physical health is the starting point, which includes an absence of indicators of future ill health. Detecting preclinical signs of poor health, such as weakened immune function (Dantzer, 2001) or disturbed behaviour (Wemelsfelder, 2007), is beneficial, as pre-emptive action can then be taken. For example, robust research has been carried out in detecting reliable early signs of pain, with a practical behaviour based scoring system being developed and validated in laboratory rats following surgery (Flecknell and Roughan, 2004). It is also important to attend to the wider behavioural adaptations of animals, which allow them to cope in their environment. Ethology has greatly increased the tools available to assess the welfare of animals (Hubrecht, 2014), including the production of ethograms and our understanding of adaptation through the study of behaviour in the wild. Tinbergen (1951) provided a distinction between proximate mechanisms of 'wants' and ultimate functions of 'needs', which is crucial in defining and measuring animal welfare, as an animals 'needs' can be met, although they may still 'want' to perform the behaviour associated with it. Controlled studies, such as choice tests, when designed correctly to give the most useful answers, can provide information on what animals want and don't want (Dawkins, 2012). Experiments can then also be used to demonstrate that changes do actually improve health or give animals what they want (Hubrecht, 2014).

A comprehensive programme of scientific measures, which incorporates basic health and functioning, pain and distress, as well as behaviours in accordance with an animal's motivation, should therefore be better than one than investigates only one or two of these (Fraser, 2008). Measures taken may however depend on what is being assessed. For responses to an experiment, a quick assessment of pain or disease in the animal may be most relevant, rather than long-term measures of homones. However, in order to establish how best to keep an animal, long-term measures, including more behavioural indicators, may be most appropriate (Hubrecht, 2014).

Several welfare measures, from each approach, are integrated in the current thesis. These are highlighted in Table 1.2. Reproductive success, body weight and condition, as well as injury, disease and longevity are measured throughout life. Behavioural repertoire, including play, locomotion and vocalisations, are assessed in infancy and adulthood. Measures of corticosteroids, as well as preference and cognitive bias tests, are also conducted in adulthood.

1.3 Use of the common marmoset in scientific research

The common marmoset is widely used as a non-human primate model in biomedical research (Hart et al, 2012), as their evolutionary similarity to humans allows better translation of data to therapeutics in patients (Sachs, 2003). Due to their similar immune system, they are used in modelling autoimmune disorders, such as rheumatoid arthritis and multiple scherosis. They also have comparable brain morphology, and so are used as models of neurodegeneration, such as in Parkinson’s disease. Similar cognitive tasks can be given to those used in the diagnosis of human neuropsychiatric disorders (Spinelli et al, 2004), including home cage CANTAB testing (Crofts et al, 1999). As they are small primates, allowing relative ease of handling, with an absence of many zoonoses, marmosets are less expensive to keep than larger macaques (Tardif et al, 2011).

The Home Office publishes annual reports on the number of animals used in scientific procedures in the UK. There was a 7% increase in procedures using non-human primates in 2013, compared to 2012, although the majority of these were Old World monkeys (2,928 procedures performed on 1,922 animals). Despite an increase in 2009 and 2010, changing patterns of research have lead to an overall decline in the number of marmosets used over the past 20 years (Home Office, 2014). In 2013, 308 procedures were performed on 280 New World monkeys (marmosets and tamarins). The majority of these studies was for the purpose of fundamental biological research (223 animals used) and applied studies in human medicine or dentistry (57 animals used).

Like most primates, marmosets are highly intelligent and have complex social lives, which means that their needs can be more challenging to meet than other commonly used laboratory species. They may therefore be more at risk of compromised welfare (Buchanan-Smith, 2010a). For this reason, the welfare of common marmosets must be carefully considered, both during the design of experimental procedures and in routine husbandry of the colony, with their use yielding valid and reliable results.

1.4 Breeding common marmosets

1.4.1 Rearing practices

It is not permitted to use any wild-caught primates under the A(SP)A. Most marmosets are therefore bred for purpose in the UK, either onsite or by commercial breeders. Due to the common marmoset's high fecundity, with multiple ovulations per cycle and a 5 month inter-birth interval (Smucny et al, 2004), they can be bred and maintained in captive colonies in sufficient numbers to meet research requirements (Poole and Evans, 1982). Variations in reproductive output are examined in Chapter 3. However, there are problems associated with their breeding, including low dam longevity, increasing litter sizes and high infant mortality.

The common marmoset is characterised by twin births (Stevenson and Rylands, 1988) and the care and co-operation of all members of the family in raising the young (Ingram, 1977). Infant development to independence is described in Chapter 4. However, triplets are becoming increasingly common in captive colonies. As marmoset families generally cannot care for more than two infants at a time, larger litters are routinely hand-reared to improve their survival.

A survey of management practices at the 4 main UK marmoset colonies revealed that various methods of rearing have been developed. Table 1.3 provides full descriptions of each. Complete hand-rearing was carried out at one facility, if infants were abused or rejected, although this was rare. This facility routinely performed partial hand-rearing, in which one infant was removed from the family group for certain periods of the day. Another colony practiced rotational hand-rearing, involving one infant being removed per day on a rotational basis. A third colony carried out supplementary feeding, in which all three of the infants were removed. During this time, infants were kept in incubators, with a woollen pad to cling on to, and hand-fed by care staff at regular intervals. Infants at each facility were occasionally fostered, if an appropriate breeding female was available. The fourth colony routinely euthanized the smallest triplet, to prevent any suffering and avoid the need for hand-rearing, as well as provide the remaining two infants with a better chance of survival.

However, such human intervention means that the young monkeys are separated from their family for substantial periods of time, which is biologically unnatural (Dettling, 2002). They also receive considerable handling by carestaff. Early life experience can have a significant long-term effect on development (Parker and Maestripieri, 2011), with many studies looking at the effect of infant-parent relationships and removal from the family on social, emotional and cognitive capacities in non-human primates.

Table 1.3: Description of commonly used rearing practices for infants from triplet litters in the laboratory (personal correspondence)

Rearing	**Practice**	**Duration**
Complete hand-rearing	One infant is removed from the family group and reared in an incubator, either alone or with peers.	Permanent family absence, although the animal is later reintroduced to marmosets.
Partial hand-rearing	One infant (usually the smallest or largest triplet) is temporarily removed from the family group, for hand feeding.	8 hours during the day (usually from 8:00 to 16:00), for the first 6-8 weeks of life. After this time, the family remains undisturbed.
Rotational hand-rearing	One of the three infants is temporarily removed from the family group per day, on a rotational basis, for hand feeding.	8 hours during the day (from 8:00 to 16:00), every 3 days, for the first 6-8 weeks of life. After this time, the family remains undisturbed.
Supplementary feeding	All three of the infants are temporarily removed from the family group together, for hand feeding.	2 hours twice a day (8:00-10:00 and 16:00-18:00), for the first 6-8 weeks of life. After this time, the family remains undisturbed.
Fostering	The infant is introduced to another family, if a suitable dam is available (ie. has given birth less than a week before and no longer has her own young or has only one infant of a similar age).	The infant remains with the new family group.

1.4.2 Parental separation

Early separation studies aimed to investigate mechanisms underlying mother-infant attachment (Harlow, 1959). Since then, two types of primate model have been developed, to evaluate the effect of variation in the early life environment. Parental Separation Models involve complete or short, repeated infant separations from the family. They include isolate-rearing, peer-rearing, parental separations and stress inoculation. Although ‘quantity’ of early social experience is important, ‘quality’ can also play a part. Maternal Behaviour Models therefore involve variation in caregiver behaviour, either by manipulating caregivers’ ability to provide care (eg. altering foraging demands or administering drugs), or by looking at naturally occurring differences in maternal style and maternal abuse. They are more likely to be experienced by infants if free-living, than more experimental separations (Parker and Maestripieri, 2011). Table 1.4 describes the main features of each type of model.

Research has generally found that the stress of loosing a parent in early life is deleterious, enhancing fear and anxiety (Capitanio, 1986), increasing anhedonia (Paul et al, 2000) and impairing cognition (Pryce et al, 2004), as well as altering baseline activity and stress responsivity of the HPA axis (Capitanio et al, 2005). In human populations, childhood traumatic experiences can also impair coping ability and increase the risk of mood and anxiety disorders (Parker and Maestripieri, 2011). Parental separation paradigms have been used to provide a primate model for human major depression (Pryce et al, 2004). Results of such parental separation studies are described in detail in Chapters 5 and 6. However, variations of the parental separation model are commonly practised as basic husbandry in colonies of marmosets bred for use in research and testing. Rearing background could therefore effect welfare, as well as the results derived from those animals.

Table 1.4: Primate models of early life stress (adapted from Parker and Maestripieri, 2011)

Animal model	**Procedure**	**Stage and duration**	**Species**	**References**
Parental Separation Models				
Isolate-rearing	The infant is reared in a cage alone, on a surrogate or by a human carer.	Total maternal/ family absence from birth. May be later introduced into a group.	Rhesus macaques *(Macaca mulatta)*	Novak and Harlow, 1975; Kraemer, 1992; Paul et al, 2000.
Peer-rearing	The infant is reared in a cage, in a small group of age-matched peers.	Total maternal/ family absence from birth. May be later introduced into a group.	Macaques (*Macaca sp.)*	Capitanio, 1986; Capitanio et al, 2005; Higley et al, 1992a; Parr et al, 2002.
Repeated parental separations	The infant is removed from the family for brief periods. Alternatively, the mother can be removed, or both can be removed from conspecifics.	Variable: 3-14 days from birth for macaques. 30-120 min from birth for New World monkeys.	Macaques (*Macaca sp.*) Common marmosets (*Callithrix jacchus*)	Spencer-Booth and Hinde, 1971; Caine et al, 1983. Dettling et al, 2002; Pryce et al, 2004; Dettling et al, 2007.
Stress inoculation	The infant is removed from the family for brief periods.	Weekly 1 hour separations, from the age of 10 weeks to 17 weeks.	Squirrel monkeys (*Saimiri sciureus*)	Parker et al, 2004.
Maternal Behaviour Models				
Maternal style	The mother either physically rejects or protects the infant, to varying degrees.	Throughout infancy, from birth. Rejection episodes last a few seconds.	Macaques (*Macaca sp.)* Vervet monkeys (*Cercopithecus aethiops)*	Bardi and Huffman, 2006; Maestripieri et al, 2006a,b. Fairbanks and McGuire, 1993.
Maternal abuse	The mother hits, bites, drags or throws the infant.	Throughout infancy, from birth. Abuse sessions last a few seconds.	Macaques (*Macaca sp.)*	Maestripieri and Carroll, 1998.
Foraging demands	The mother is exposed to unpredictable foraging demands, involving varied access to food.	Foraging conditions switch from high to low demand every 2 weeks, at 3-6 months old.	Bonnet macaques (*Macaca radiata*) Squirrel monkeys (*Saimiri sciureus*)	Andrews and Rosenblum, 1994; Rosenblum, 1994. Parker et al, 2006.
Experimentally reduced care	Family members are administered a dose of tranquilliser (eg. Fluphenazine).	8 days at 4 weeks old (fathers) and 14 weeks old (older siblings).	Common marmosets (*Callithrix jacchus*)	Locke-Haydon, 1984; Chalmers and Locke-Haydon, 1986.

1.4.3 Early life stress

Exposure to differing interactions with caregivers in early life can have important consequences for development, affecting reactivity (Chapter 5) and personality (Chapter 6) later in life (Bowlby, 1969). The resilience model of stress development assumes a J-shape relationship between early stress intensity and later stress vulnerability (see Figure 1.1). Overcoming moderate levels of stress may increase perception of control, leading to adaptive responses to challenges and stress resilience (Parker and Maestripieri, 2011). Parker et al (2004) has described this as 'inoculating'. However, too little or too much stress could prevent the young animal from developing the ability to cope with challenges, especially if outside the normal range experienced by the species (Parker and Maestripieri, 2011).

Features of early life stress, including type, duration and frequency, developmental timing, ecological validity, as well as the degree of social, sensory and motor deprivation and magnitude of stimulation provided by human caretakers, can all play a part in producing diverse developmental outcomes (Parker and Maestripieri, 2011). It is therefore possible that, as marmosets are adapted to being transferred between carriers from a young age (Ingram, 1977), separation from the family with warmth and food may not provide insights into deprivation. Remaining with litter mates, so infants are not isolated, as well as predictable timing of separation and positive experiences with humans may all minimise potential stress.

A particular stressful experience may also not lead to the same outcome in different individuals (Cicchetti, 1993). Gender differences in coping may be present. For example, Parker et al (2006) exposed squirrel monkey (*S. sciureus*) infants to high foraging-demands (HFD) or low foraging demands (LFD), finding that males had significantly lower levels of cortisol at baseline and after stress exposure than females, in adulthood. Further, only HFD males demonstrated diminshed HPA activation than LFD males following stress. Males may therefore have reduced reactions when later exposed to stressful situations (Parker et al, 2006). However, there is contrasting evidence from studies of other primate species. Male rhesus macaques (*M. mulatta*) had higher ACTH values than females, after being moved to a new cage (Clarke, 1993). Stress-induced neural damage has also been discovered in male vervet monkeys (*C. aethiops*), but not in females (Uno et al, 1989).

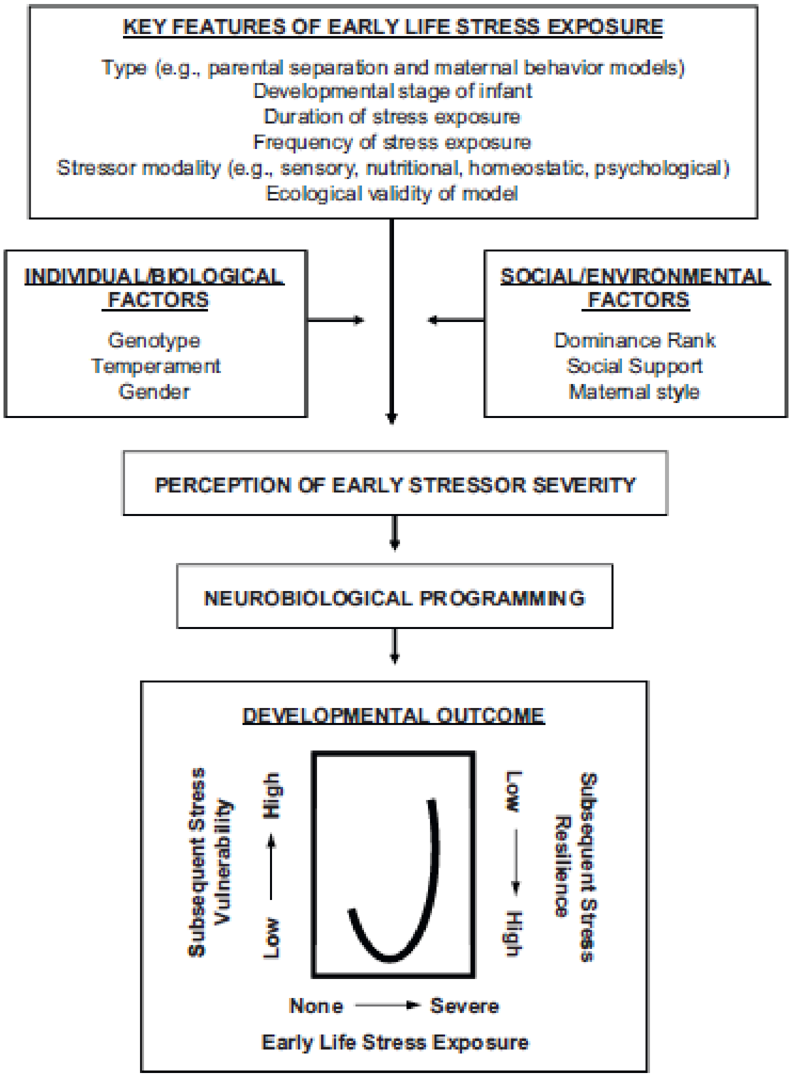


Figure 1.1: Diagram illustrating the features and perception of stress, risk and protective factors and developmental outcomes (Parker and Maestripieri, 2011)

Stress related disorders, such as depression and anxiety, as well as resilience to stress, are unlikely to be due to a single adverse event. Outcomes instead depend on the balance of risk and protective factors, which include temperament and genes, as well as social support and maternal style (Parker and Maestripieri, 2011). Figure 1.1 shows these interactions. Increasing resilience would be of great importance in reducing stress and enhancing the welfare of captive primates.

1.5 Overall objective of the thesis

Many studies have found that separation from the family in early life can affect behaviour, physiology and cognition later in life. However, family separation is commonly practiced when breeding common marmosets for use as models in laboratory research and testing. It is not only of ethical importance, but also scientific importance, to understand the effect of this rearing practice on welfare. The overarching aim of this thesis is therefore to assess the welfare of marmoset infants born and raised under different conditions, including supplementary feeding of larger litters.

The first aim was to identify factors associated with dam longevity and larger litters (Chapter 3). Second, the behavioural and physical development of infants reared under different conditions was examined (Chapter 4). Third, the long-term effects of such practices were investigated in adult marmosets, in their response to stressors (Chapter 5), as well as their temperament and affective state (Chapter 6). An integrated approach was used to measure welfare. Behavioural observations were combined with body weight, as well as physiological measures and cognitive tests. Based on previous research, it was hypothesised that larger litters would be associated with greater infant mortality, and that early family separation would have adverse developmental consequences, leading to lower body weight and increased insecurity as infants, as well as an increased stress response and depressive-like symptoms as adults. Given that twins are the naturally adapted litter size, it was predicted that marmoset-reared twins would have the lowest mortality and highest welfare, displaying greater independence and security, lower stress responsivity and little depressive-like symptoms. Evidence-based recommendations, to promote twin births and Refine practice for dealing with large litters, is then discussed, to enhance the welfare of common marmosets housed in breeding facilities.

CHAPTER 2: General Methods

2.1 The common marmoset

The common marmoset (*Callithrix jacchus*) is the study species of the present thesis. It is therefore important to be familiar with the ecology of wild groups, in order to better understand the behaviour of captive animals. Common marmosets are small (approximately 320g (Araujo et al, 2000)) arboreal primates. They are an incredibly adaptable species, able to survive in a wide range of conditions (De la Fuente et al, 2014). Common marmosets are distributed across northeast Brazil, colonising a number of habitats, from humid, tropical forests to dry semiarid scrublands (Rylands and de Faria, 1993). They have a variable home range, from 0.5-6.5 ha (Hubrecht, 1985), which is small compared to other callitrchids, possibly due to their exploitation of tree exudates, a stable food resource (Hubrecht, 1985). These exudates are a major part of their diet, although they also consume fruit and insects (Stevenson, 1978).

Wild populations have a relatively stable social structure, of extended family groups of 3-15 individuals (Hubrecht, 1984; Pontes and Da Cruz, 1995). Sizes do however vary, due to births, immigrations, emigrations and disappearances (Ferrari and Digby, 1996). Although monogamous groups have been observed (Albuquerque et al, 2001), polygamy has also been documented in the wild (Arruda et al, 2005; Sousa et al, 2005). Common marmosets reach sexual maturity at 18-24 months (Hearn, 1982). They usually produce twins (Sousa et al, 1999) every 5-6 months, mainly at the start of the dry season and beginning of the wet season (Hubrecht, 1984). All group members co-operate in raising the young, which is a prominent aspect of callitrichid social orgaisation (Yamamoto, 1993). Table 2.1 displays comparative life history data from wild marmoset groups.

Table 2.1: Life history data from studies of wild common marmoset populations

	Data	**Reference**
Group size	3-15	Hubrecht (1984)
Age at first reproduction	4.5-5 years	Tardif et al (2008)
Inter-birth interval	5-6 months	Hubrecht (1984)
Average litter size	2	Sousa et al (1999)
Average weight	Male: 317.9 Female: 322.0	Araujo et al (2000)
Average longevity	11.7 years	Ross (1991)

It is important to consider the life history and activity budget of wild individuals, to give benchmarks for the assessment of welfare in captivity (Veasey et al, 1996). However, there has been considerable variation in the amount of time spent in certain behaviours between field sites. Table 2.2 displays activity budgets found in wild groups. Natural environments have varied physical and social aspects, and marmosets will adjust their behavioural patterns to cope with different environments. For example, reductions in activity, such as foraging and locomotion, accompanied by increases in resting, have been found at a semiarid site during high temperatures (De la Fuente et al, 2014). Marmosets now also live close to human settlements, in habitats different from those they originally evolved in, where previously unseen behaviours may develop (eg. feeding from rubbish bins) (Veasey et al, 1996).

Comparisons between the behaviour of captive animals and their wild counterparts can therefore be problematic. Wild activity budgets may be inaccurate, as marmosets can be difficult to observe in the field, and as they are affected by geographic and temporal variations, it may be difficult to generalise across the species (Veasey et al, 1996). As well as this, due to space and other restrictions, group composition and activity budgets of captive populations are often very different from wild populations (Badihi, 2006). Although performance of the full natural behavioural repertoire, for similar proportions of time as in the wild, may be desirable, an animal that is not doing this may not necessarily be suffering. The performance of natural behaviour in captivity may also not be the best way to achieve functional results (Duncan and Fraser, 1997), and so new behaviours may indicate an adaptation (Veasey et al, 1996). The significance of certain behaviours in particular circumstances could however provide more accurate judgements of animal welfare (Duncan and Fraser, 1997). For example, research to show which behaviours are associated with positive and negative situations can give observations a solid base to interpret what is seen (Dawkins, 2012). Therefore, while it is important to understand the breadth of information gathered in the wild, this is not necessarily comparable to captivity. In the present study, differences in activities, reproductive output and rearing background were compared in the same environment, to assess welfare.

Table 2.2: Activity budgets from studies of wild common marmosets (adapted from Badihi, 2006).

	Stevenson and Rylands (1988)	**Alonso & Langguth (1989)**	**Ferrari and Digby (1996)**
Locomotion	35% (including foraging)	11%	
Foraging		24%	
Feeding	10%	27%	
Inactive	53%	18%	
Social activities	10%	15% (grooming)	37% (including inactive)
Interactions with other groups		5%	

2.2 Study animals

Study animals were common marmosets (*Callithrix jacchus*) housed at Dstl, Porton Down, UK. All animals were purpose-bred in captivity, and none had been involved in experiments prior to the study. All animals over 12 months of age wore a numbered tag around their neck, to aid identification.

Three studies were conducted, and reported in Chapters 4-6. In the first study (Chapter 4), 35 infants born into the marmoset colony were studied, in each of three conditions (twins, 2stays and singletons). Records from 34 adult animals (twins, 2stays and supplementary fed triplets) were also accessed, to look at weight. Studies reported in Chapters 5 and 6 involved stock animals (ranging in age from 1 to 3 years) raised under one of three conditions (twins, 2stays and supplementary fed triplets). In some cases, animals were used in both Chapters 5 and 6. As this was for different studies, it was not considered to adversely impact on the results. However, re-use was not possible for all stock animals. As the facility breeds for the purpose of use in internal programmes, animals were often issued for experiments that arose during the study period. A total number of 69 common marmosets were used over the studies (Table 2.2). Further details of animals used in each study are provided in the appropriate chapter.

While an even male: female ratio was aimed for in each study, in order to avoid gender bias, the majority of triplets available in stock rooms were male. An unbalanced sex ratio is due to the chimerism occurring in this species, which involves an exchange of cells between animals in utero (Sweeny et al, 2012). When male and female embryos are paired, chimeras tend to differentiate into males (Nagashima et al, 2004: chimeric pigs).

2.3 Rearing conditions

Conditions were based upon practices currently carried out at the breeding facility, and so no manipulations were used. Four rearing conditions were studied over the present thesis:

1. Marmoset reared infants from twin litters, as control infants.

2. Marmoset reared singletons, either born as the only infant or when a sibling dies less than one week old.
3. Marmoset reared infants from triplet litters, where two infants remain with the family (when one sibling dies less than one week old). These will be known as '2stays'.
4. Supplementary fed triplets (full schedule detailed below).

Foster rearing was also used at the facility, if a suitable dam had given birth less than a week before and no longer had her own young, or had only one infant of a similar age. The dam's scent was rubbed on the foster infant, before being introduced. They were then closely monitored, to ensure their health and integration into the new family. However, fostering was very rare, and so a large enough sample size could not be generated.

Table 2.2: Number of marmosets in each condition in all studies

	Twins	**2stays**	**Triplets**	**Singletons**	**Total**
Chapter 4					
Infant	14 (7M, 7F)	16 (10M, 6F)	None	5 (5F)	35
Adult	10 (5M, 5F)	13 (5M, 8F)	11 (8M, 3F)	None	34[a]
Chapter 5	6 (3M, 3F)	8 (3M, 5F)	7 (6M, 1F)	None	21
Chapter 6	8 (4M, 4F)	9 (4M, 5F)	8 (5M, 3F)	None	25[b]

[a] For all animals studied, growth was also recorded.
[b] 11 animals were used in both Chapter 5 and Chapter 6.

2.3.1 Supplementary feeding of triplets

On postnatal day 1, the family member carrying the infant was encouraged towards the front of the homecage with a piece of marshmallow, so that restraint was not necessary, and the infant removed from their back. All three infants were taken out of the family group together, weighed and a small amount of back or tail hair shaved for identification. They were then wrapped in towelling together and placed in an incubator in the play cage room. The litter of infants were removed from the family daily, following a fixed schedule, for 2 x 2 hours (8:00-10:00am, 16:00-18:00pm). The infants were hand-fed at the beginning and end of each 2 hour period, during which each infant was handled individually for approximately 5 minutes, until they had finished eating. At the end of the incubator session, the infants were returned to the opening in the home cage, where their parents immediately retrieved them. They received 4 feeds per day until they were 20 days old. This was reduced to 3 feeds, with 2 feeds in the 2 hour morning session in the incubator and one afternoon feed, after which they were immediately returned to the family, until they were 25 days old. After this age, there was no incubator time, with feeds reduced to 2/ day between 26 and 30 days old, and to 1/ day between 31 and 41 days old. Plates 3.1 and 3.2 show the infants receiving supplementary feeding and the set up within the incubator. Supplementary feeding was however stopped in October 2012, due to a management change, after which the lightest infant was euthanised by a sodium pentobarbital overdose.

Plate 2.1: Supplementary feeding of infants

Plate 2.2: A litter of infants in the incubator

2.3.2 Husbandry of all infants

Infants from all rearing backgrounds were captured and removed from their family group at day 10 for weighing, and subsequently every month when every marmoset in the room was weighed. The carrying family member was caught, and briefly restrained while the infants were removed. The adult was then weighed, and the infants placed back on the carrier one at a time, while in the weigh box, to record their weight. This took approximately 2 minutes, with the infants removed from the carrier for under a minute. For the remainder of their time they were left with the family. All animals received a human socialisation programme, which involved the technicians offering food to the whole family (either through the bars or by taking in a bowl of forage) and sitting in the home cage with them for 5 minutes. The marmosets were also trained to accept milkshake from a syringe. Banana Nesquik was given to each family through the bars, for 1 minute or until each individual had consumed 2ml. Both husbandry practices were carried out once a week.

2.4 Housing

2.4.1 Breeding rooms

There were 3 rooms of breeding animals. Each room contained 20 individual cages, 10 in each row, which could be opened into double cages for larger groups, by means of sliding plastic partitions. Each row contained between 4 and 6 groups of marmosets. The gap between the two rows of cages was approximately 1.6m, which allowed clear visual contact, while being wide enough to limit territorial aggression between families on opposite sides.

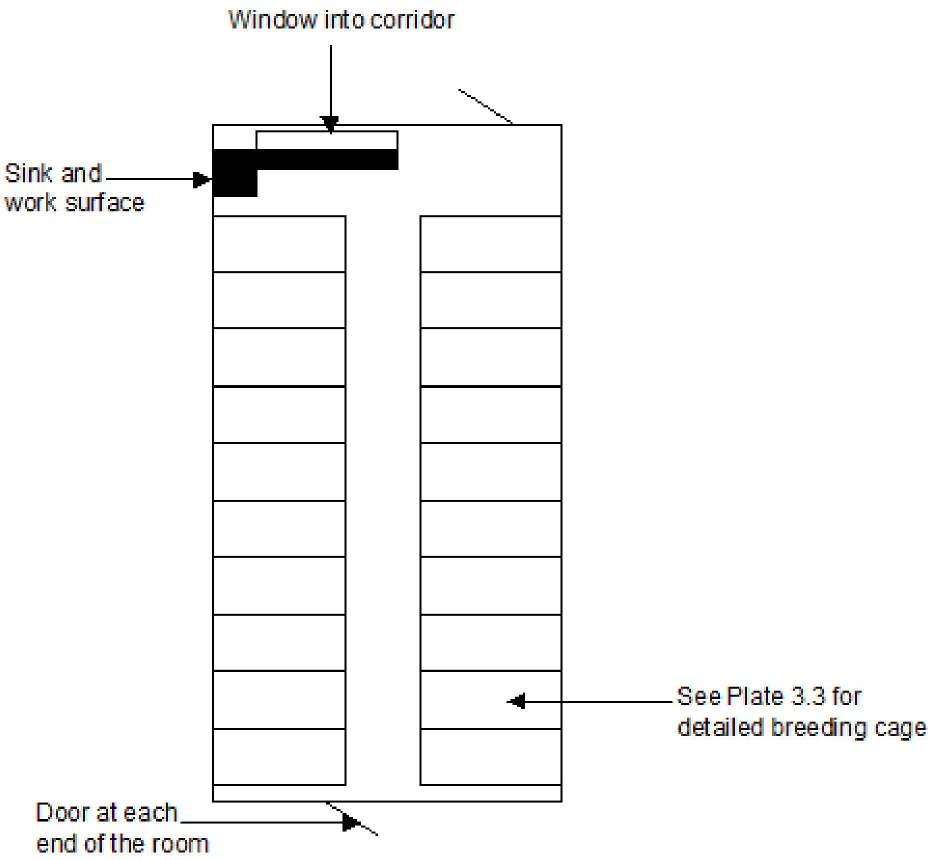


Figure 2.1: Schematic diagram of a marmoset breeding room (not to scale)

Family housed marmosets (2-10 individuals), of monogamous pairs and their offspring, were housed in cages measuring 1.52m wide x 1.22m deep x 2.15m high. Cage floors were covered in a deep layer of sawdust, and had a walk in door. Two food trays were attached to the front of the cage, with openings to take food in and out. The cages were also furnished with a nestbox, several branches and logs of various orientations, ropes, platforms and perches. Branches are the ideal substrate for the expression of several behaviours, including chewing, climbing and scent-marking, and so are important elements of cage furnishings for marmosets. The cages also contained suspended toys, including ladders, buckets or helmets, boxes, tea towels, rubber matting, wellies, hanging baskets, tubes and food devices. These toys increase environmental complexity and choice (Badihi, 2006), encouraging behaviours such as climbing, foraging, exploration and play. Calm locomotor activity is desirable in captive primates, and may provide an indicator of welfare (Buchanan-Smith et al, 2004). Internal cage furnishings can also be used to rest within, or provide visual barriers should the animal require privacy. Each family had access to a veranda on top of the cage, to allow a wider visual range. Figure 2.1 depicts the layout of a breeding room, and Plate 2.3 shows a breeding cage.

2.4.2 Stock rooms

There were 3 rooms of vasectomised male mixed-sex stock pairs at the start of the study. Each room contained between 10 and 18 individual metal wire cages, half along each opposite wall. Pairs of marmosets were housed in cages measuring 100cm wide x 60cm deep x 180cm high. Floors trays, which could slide in and out for cleaning, were covered in wood chippings. A metal shelf was attached to the front of the cage, with openings to take food in and out. The cages were also furnished with a nestbox, wooden platforms, perches, ropes and suspended toys. A wire veranda (67cm wide x 24cm deep x 18cm high) was attached to the front central section of the cage and on top of the cage, allowing animals to be in visual contact with neighbouring pairs. A tray filled with chippings and food could be attached under the veranda, to promote foraging. Plate 3.4 shows a stock cage.

All the animal rooms were all connected with corridors at the bottom and top of each. A window at the far end of each room allowed animals to see into the corridor, allowing the marmosets to see care-staff going about their duties. Temperature and humidity were thermostatically controlled at 23-24ºC and 55 +/- 10% respectively. Lighting was provided on a 12 hour light/dark cycle, coming on at 6:45 and off at 18:45, with a dawn and dusk phase.

Plate 2.3: Breeding room cage

Plate 2.4: Stock room cage, with one marmoset in the top veranda and one in the front veranda

2.5 Husbandry

All marmosets had *ad libitum* access to water from one bottle on the front of each cage. Food was delivered twice a day. All animals were fed primate pellets between 8:00 and 9:00, on metal trays placed onto the food shelves. The primate pellets were presented after softening in water each day (40/pair and 80/family), and were also mashed for the infants in breeding rooms. Pellets mashed with gum was given twice a week to all animals. A variety of fruit (one piece per animal) was then provided between 13:30 and 14:30, alternating between two of either banana, apple, grapes, melon and pear. Malt loaf, egg, rusk, mealworms, dates, peanuts and bread were also provided on alternating days. Milkshake was given twice a week. Vitamin D supplement was added to the milkshake on Sundays. Gum arabic, presented in suspended cardboard cups, was provided twice a week. Mealworms and forage mix, consisting of dried fruit, seeds and rice krispies, were also scattered on top of the breeding cages twice a week. Forage mix was provided in stock room forage trays on the cage floor and under the veranda, to provide a constant supply.

A maintenance regime was carried out in which floors and corridors were swept and washed between 8:00 and 12:30 each day. These were also disinfected once a week. Visual checks for health and welfare were carried out at this time. Fresh water was provided in the morning, and the previous day's food was removed. Bottles were changed and wet shavings in breeding rooms were picked up each week. Weighing took place every Tuesday (breeding room) and Wednesday (stock room), between 9:00 and 13:00, with each room weighed every month on a rota basis. Full cage cleaning occurred every 8 weeks in breeding rooms, in which the walls and floors were scrubbed and hosed, and all furniture cleaned and replaced. Fresh sawdust was then provided. A scented perch was always transferred to the clean cage. Scent marking is important in marmoset communication, playing a role in sexual, territorial and social behaviour (Epple, 1970). On the alternate month, only perches were removed and cleaned. Stock pairs were moved to a clean cage each month, and the previous one removed for washing. Bottom trays were cleaned weekly, and clean buckets and perches provided fortnightly.

Enrichment was introduced every Friday, where paper parcels, cardboard boxes, mealworm feeders (breeders) or bottles (stock) were provided with forage mixed into sawdust. Access to one of two play cages was given to a family for 3 days, via connection of ducting from the home cage across the corridor. These were large cages, with access to different toys and feeding devices, as well as a one-way mirror with an outside view. One pair of stock animals was allowed access to a 'bug box' (a large wooden box containing sawdust and locusts) for two days at a time, on a rotational basis. As described above, every animal was syringe trained once a week, to aid with the administration of medication. Human socialisation was also carried out, to maintain positive staff-animal relationships. Any treatments were given in the morning and afternoon. Clinics were scheduled for twice a week, with any routine surgeries (eg. vasectomies, dentals) carried out once a week.

2.6 Behavioural observations

2.6.1 Observation protocol

Coded behavioural data were collected directly onto a Psion Workabout (hand-held computer), using Observer V8.0 event recording software (Noldus Information Technology). All observations were made in full view, in which the author stood approximately 1m from the front of the cage. As circadian rhythms in behaviour have been found in captive (eg. Erkert, 1997) and wild (eg. Stevenson and Rylands, 1988) marmosets, timing of observations were matched between conditions to minimise the potential confound of time of day.

All animals were habituated to the presence of an observer, for one hour a day over two weeks, prior to behavioural observations. The observer entered the room quietly, approached slowly, speaking softly, before sitting and standing in various places for the remainder of the habituation period (based on Bowell, 2010).

A pilot study was carried out, to investigate the success of the habituation period:
Formal habituation to an observer was conducted, for one hour a day over 10 days (3 hours for each room). Ten family groups were observed before habituation, after 5, 6, 8 and 10 days, and again after 20 days of working in the rooms. Focal sampling of one animal per group (N= 5 males; 5 females) was conducted for 2 minutes, between 9:30 and 12:30. Five behaviours were recorded as indicators of disturbance, using all occurrence of short duration behaviours (tsik call, raised tail present and scratch) and 10 second instantaneous sampling for longer duration behaviours (tail pilo-erection and watching observer). Repeated measures ANOVAs revealed no significant difference over time in any of the behaviours. There was a general decrease in tsik calls, scratching, tail pilo-erection and raised tail present, although these all occurred at very low frequencies throughout the observation period. Watching remained high after 10 days.

There was however a general decrease, which was approaching significance by day 20 (p=0.079). Figure 2.2 shows the mean frequency of the behaviours at each time point.

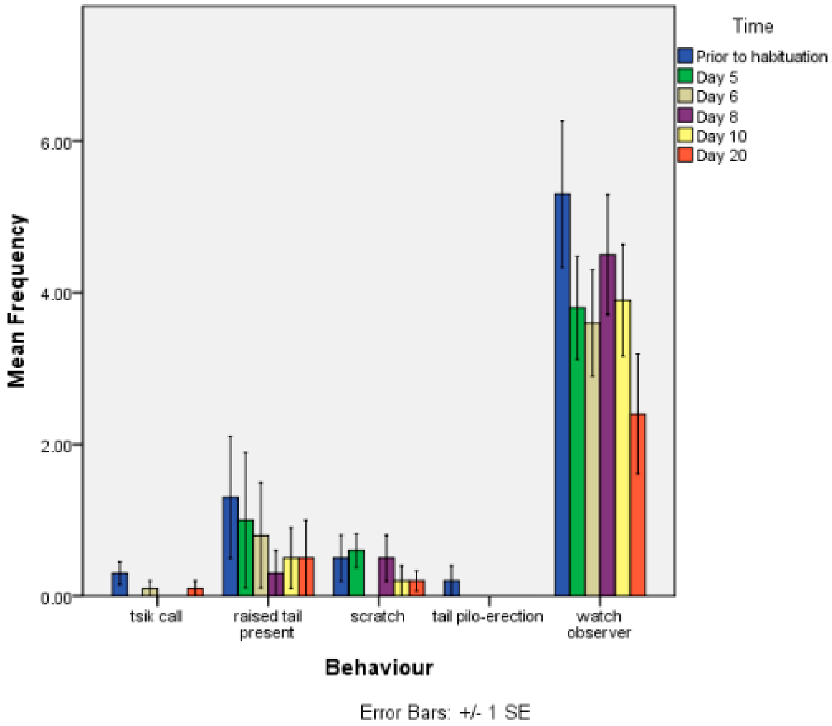


Figure 2.2: Mean (+/- 1SE) frequency per 2 minutes of tsik calls, raised tail present and scratch, and mean number of point samples (max 12) for tail pilo-erection and watch observer over the 6 time points (N=10).

2.6.2 Sampling methods and recorded behaviours

Focal animal sampling was used in Chapters 4 (of both infants) and 5 (of both animals in the pair). Behaviours were recorded using 30 second instantaneous sampling (for longer duration behaviours) and all-occurrence sampling (for short duration/rare behaviours), expressed as estimated percentage of sample time when in view and frequency per sample time respectively. Two observations, lasting 15 minutes each, were conducted in Chapter 4, one in the morning and one in the afternoon. Two 5 minute observations were conducted in Chapter 5, both between 9:00 and 10:30.

A wide range of behaviours were recorded. Infant and parental behaviours were recorded in Chapter 4, based upon those already published for the marmoset (Stevenson and

Poole, 1976, Locke-Haydon and Chalmers, 1983, Dettling et al, 2002, Ventura and Buchanan-Smith, 2003). Adult behaviours were recorded in Chapter 5, based on previous work investigating marmoset responses to stressors (Bassett et al, 2003; Badihi, 2006; Dettling et al, 2007). Full behavioural categories are provided in the relevant chapters.

2.7 Additional sources of data

In each study, further data were collected, in addition to the behavioural observations. In Chapter 4, weight and body condition scores were also recorded, in order to study both behavioural and physical development. In Chapter 5, salivary cortisol was collected, as well as behavioural data, in response to a stressor. In Chapter 6, responses in temperament tests, cognitive bias tests and preference tests were measured to look at affective state. Correlations between the measures of welfare used were investigated in both Chapter 5 and 6. Each additional source of data is described in detail in the relevant chapters.

2.8 Statistical analysis

Data were summarised and analysed using SPSS statistical software. In all statistical analysis, Bonferroni adjustments were not made, despite multiple analyses being carried out, to reduce the risk of Type II errors (false negatives). As only small sample sizes were available, the tests have low statistical power, and so it may be difficult to find significant results, particularly if the effect size is small. Therefore, the significance level remained at 0.05. This allowed maximum information to be extracted from the data, and independent assessment of the validity of results (Caldwell et al, 2005). Where data were not normally distributed, non-parametric tests were considered the most appropriate method of analyses, due to their greater power in detecting large, important differences between the groups when assumptions of normality have been violated (Field, 2009). Only significant results are presented.

CHAPTER 3: Long-term data on reproductive output and longevity in captive female common marmosets (*Callithrix jacchus*)

Abstract

Significant variation in reproductive output has been found among female common marmosets, compared to other anthropoid primates. The study explores this reproductive variation, focusing on potential predictors of dam longevity and litter size, as well as changes over time. Back-record analysis was conducted, yielding litter information and reproductive summaries of 360 dams housed at three UK marmoset colonies over 4 decades (1970s-2000s). Results revealed differences among the colonies, as well as within colonies over decades, suggesting environment may play an important role. Cox proportional hazards regression analyses revealed significant effects of mean litter size and yearly production on dam longevity. Decade, mean inter-birth interval and mean dam weight were found to be significant factors explaining dam longevity when looking at colonies individually. The most commonly recorded reason for death involved management decisions to euthanize due to 'poor condition'. Linear regression models found that no reproductive variable was useful in explaining mean litter size, except dam weight at conception, data which was only consistently recorded at one colony. While triplets were common at all three colonies, these larger litters were consistently associated with higher infant mortality, despite human intervention to improve survival. This study increases understanding of marmoset reproduction, and possible improvements to practical aspects of colony management to enhance survival and welfare are discussed.

3.1 Introduction

3.1.1 Reproduction in the common marmoset

Callitrichidae produce more offspring per delivery, with more variation in litter size, than any other anthropoid primate (Smucny et al, 2004). There are routinely multiple ovulations per cycle. Twins are the norm, although triplet litters are common. Inter-birth intervals (IBIs) are also often short (approximately 5 months), with females able to conceive again shortly after birth (Smucny et al, 2004). This means they can produce two litters a year (Tardif et al, 2008). However, their high fertility is accompanied by high rates of pregnancy losses and infant mortality (Jaquish et al, 1991). There can therefore be significant variation in reproductive output per year, as well as over a females lifetime (Smucny et al, 2004).

An overview, combining data from published literature and a large American multi-colony database (5 institutions; 479 known-age dams), reported that breeding females had an average longevity of 5-7 years and a maximum of 16.5 years (Tardif et al, 2011). Animals had a reproductive life span in captivity of around 2 years (Smucny et al, 2004). An average of 4.0 litters were produced in a female's lifetime, with a yearly weaned production of around 2.3 infants (Tardif et al, 2003). However, Nishijima et al (2012) found an unexpectedly longer average female life span of approximately 9 years at an established Japanese breeding colony (73 males and 80 females, born 1982-2006), although a similar maximum age of 16.7 years. Males lived for an average of 12.4 years, significantly longer than females, with a maximum of 21.7 years. This clear sex-difference in survival was attributed to the reproductive costs of gestation and parturition in the females, as noted by others (eg. Tardif et al, 2008). In a report of another colony, maintained at the University of Cambridge (Ridley et al, 2006), 80% of breeders (males and females) were alive at 10 years of age. These animals were allowed to live out their optimum captive lifespan, only being euthanized for welfare reasons.

Due to difficulties acquiring data, there is little known about longevity in wild common marmosets. Results from a wild population (9 free ranging groups of 209 animals), followed for 10 years at a field site in Northeastern Brazil, suggest that early life mortality is relatively high compared to other age groups (66.7% infant survival). Females began reproducing around 4.5-5 years, as time is needed to find a breeding slot, and continued until they were 8-9 years old. Tenure therefore averaged 3.5 years, which is similar to in captivity (Tardif et al, 2008).

Females can breed until relatively close to their maximum life span, with a rather abrupt reproductive decline, associated with follicular depletion, or inability to maintain behavioural dominance (Tardif et al, 2008). Whilst longevity and infant survival may be expected to be higher in captivity than in the wild, as captive marmosets are protected from predators and dominance competition, as well as have ample food provided, this may not be true for some common marmoset breeding colonies.

3.1.2 Factors affecting litter size and dam longevity in captivity

Few studies have looked at variables that influence the number of infants born per reproductive attempt in callitrichids (Bales et al, 2001). Jaquish et al (1996) investigated environmental and genetic determinants of phenotypic variation in average born litter size in three species of captive callitrichids (951 saddle back tamarin (*S. fuscicollis)*, 524 cotton top tamarin (*S. oedipus,*) 195 common marmoset (*C. jacchus)* at Oak Ridge Associated Universities, 1962-1992). There was low heritability of litter size, with only husbandry changes significant in the common marmoset. Increased cage volume and complexity, combined with increased protein content in the diet, were associated with a greater number of triplets. A good quantity of usable space has been found to maximise well-being and breeding success in cotton-top tamarins (Savage, 1995). Maternal body weight is also known to be important in marmosets, influencing ovulation number, losses during gestation and live-born litter size (Tardif et al, 1997). Bales et al (2001) found that higher pre-pregnancy body mass was associated with a greater number of live births (wild golden tamarins of known age, for 162.5 female-seasons). Evidence therefore suggests that females can adjust reproductive output in response to energetic factors (Jaquish et al, 1996).

The most important factor in infant survival is litter size (Tardif et al, 2003). Several studies following the production of a single captive breeding colony over a number of years report that litter sizes have increased since establishment (Box and Hubrecht, 1987: 543 infants over 12 years; Poole and Evans, 1982: 204 infants over 6 years). However, larger litters generally result in higher infant mortality (Jaquish et al, 1991). The likelihood of all triplet infants surviving is greatly increased if one or all infants are partially or completely hand-reared (Hearn and Burden, 1979). However, the welfare consequences and effect on subsequent scientific output of these rearing practices have been questioned (Buchanan-Smith, 2010b).

It is also important to examine factors affecting dam longevity in captive colonies. Longevity in the current study is defined as the animals’ life span in the colony, which often involves decisions to euthanise due to health or breeding management. Smucny et al (2004) pooled data across five American marmoset colonies, gathering information from 1,649 litters and reproductive summaries from 400 dams. Cox proportional hazards regression analysis revealed dam longevity to be significantly affected by number of litters, age at first parturition and site (Smucny et al, 2004). Other studies have also found a positive relationship between longevity and age at first parturition (Jaquish et al, 1991), with dams first reproducing later in life (4 years and over) tending to live longer than those first reproducing at younger ages (less than 2.5 years and 2.5-3.99 years). Although it may expected that larger litters would be associated with high energetic cost (Tardif et al, 1993) and reductions in life span, there is no evidence that this is the case (Jaquish et al, 1991; Smucny et al, 2004). Changes in longevity over time have however been found at an American captive colony (Southwest National Primate Research Centre). Average life span extended from 4.82 years in 1994-1999 during colony establishment, to 7.07 years in 2000-2005, when the colony was stable. Mortality however increased with associated changes to the colony, including new animals and housing conditions (Tardif et al, 2011). With greater experience of colony management and husbandry practices, as well as increases in basic biological knowledge, one might expect improved welfare and less infant mortality from colony establishment to present day.

3.1.3 Aim

The present study examined reproductive information from three large well-established UK captive *Callithrix jacchus* colonies, each using different infant rearing practices, over a period of four decades. Patterns of change between establishments and over time in litter size, infant mortality and dam longevity were determined, to increase understanding of reproductive variation, particularly factors affecting dam longevity and born litter size. It was hypothesised that litter size would increase over time, and be associated with increased dam weight, although larger litters would have higher infant mortality. It was also predicted that dam longevity would increase over the years, with corresponding improvements to husbandry, and be associated with age at first parturition. Such data have the potential to aid in the management of captive common marmoset colonies (Smucny et al, 2004), many of which are housed for breeding purposes to provide non-human primate models for biomedical research (Hart et al, 2012).

3.2 Method

3.2.1 Population Description

Reproductive information was obtained from records of marmoset dams used for breeding or in reproductive studies at three UK colonies. One colony was a commercial breeder, the other two bred marmosets primarily for use on site. The first dams in the records, which began breeding early in each decade, were selected. Data were collected from 120 dams at each site. At Colony A (CA), 30 dams in each of four decades (1970s, 1980s, 1990s and 2000s) were selected. As there were no data available from the 1970s at Colony B (CB) and Colony C (CC), data from 40 dams in each of three decades (1980s, 1990s and 2000s) were collected from these sites. This yielded information from 360 dams. Fifteen wild-caught and fifteen in-house bred animals were sampled in the 1970s at CA (no difference was found between the two in number of litters (t=0.00 (28), P=1.00) and litter size born in captivity (t=1.14 (134), P=0.256)). All other animals were bred in-house. This produced data from 2712 litters (CA 527; CB 1237; CC 967 litters). Loss of archived data at CB meant that born litter size was lost from all files in the 1980s, although weaned litter size could still be extracted. The data therefore consisted of dam information for 5588 born infants (CA 1287; CB 2004; CC 2297 infants). Lack of records during the early 1980s at CC also meant that survived litter size could not be extracted.

Two sets of back-record data were examined for each colony. The breeding file contained litter information for each dam, and the stock file contained individual dam life histories (including dates of birth and death, and manipulations for experimental or management purposes). These data sets were cross-referenced to provide a full account of each female's life in the colony. Dams euthanised at the end of an experiment were not included, although many sampled at CA were manipulated for non-terminal studies (e.g. given implants, injected with hormones and bled periodically).

3.2.2 Litter Information

Litter information consisted of data from each particular dam, regarding dates of birth for each litter, litter size, sex ratio and inter-birth intervals. Survival of each infant at birth (CA, CB and CC) and to weaning age (6 months; CB and CC) was recorded. Data for the first litter following intentionally aborted pregnancies or contraception administration were excluded when calculating mean IBI. Contraception was generally only used once or twice towards the end of a female's breeding life, usually if there was a health problem. If contraception was stopped, females did occasionally become pregnant again.

3.2.3 Reproductive Summaries

Reproductive history was also summarised for each female. Reproductive output variables included mean litter size born, mean litter size survived, number of litters produced and mean IBI. Longevity, age at first parturition, reproductive life span (calculated as the years between a dam's first and last birth), lifetime production, lifetime survived production, production per reproductive year and survived production per reproductive year (calculated by dividing lifetime production or survived production by (reproductive life span + 0.67)). The figure 0.67 years represents the average in utero investment in the first litter (5 months), plus the lactation investment in the last litter (time until weaning (3 months)) (Smucny et al, 2004). Table 3.1 shows the number of dams sampled for each variable at each colony.

3.2.4 Infant rearing practices

At CA, one infant from each triplet litter was either fostered or hand-reared in the 1970s. In later years, no intervention was carried out when triplets were born. At CB, infants from triplet litters were partially hand-reared (one was removed for 8 hrs/day from the family and given supplementary food), in an attempt to improve survival. Triplets were also fostered if an appropriate dam was available, or completely hand-reared if the family rejected or abused their young. At CC, triplets were supplementary fed, in which all infants were removed from the family for 2 hours twice a day for hand feeding. Very light infants (<27g) were routinely euthanised at day 1.

Table 3.1: Number of dams included for each variable in each colony

Variable	Colony A	Colony B	Colony C
Dam longevity	105 (Ex 15 wild caught in 70s)	120	115 (Ex 4 ex breeders still alive in 2000s and 1 purchased in 80s)
IBI	93 **(**Ex 27 primiparous)	115 (Ex 5 primiparous)	108 (Ex 12 primiparous)
Age at 1st parturition	105 (Ex 15 wild caught- may have had previous litters)	120	119 (Ex 1 purchased in 80s)
Lifetime production	105 (Ex 15 wild caught)	80 (Ex 40 in 80s- no record of born litters)	119 (Ex 1 purchased in 80s)
Survived production	105 (Ex 15 wild caught)	120	80 (Ex 40 in 80s- no record of losses)
Production/yr	120	80 (Ex 40 in 80s)	120
Survived production/yr	120	120	80 (Ex 40 in 80s)
Reproductive life span	80 (Ex 40 wild caught and primiparous)	115 (Ex primiparous)	107 (Ex primiparous and 1 purchased in 80s)
Litter size	120	80 (Ex 40 in 80s)	120
Survived litter size	120	120	80 (Ex 40 in 80s with missing data)
Number of litters	105 (Ex 15 wild caught)	120	119 (Ex 1 purchased in 80s)
Maternal body weight at conception	0	0	118 (Ex 2 in 80s with missing data)
Number in dam litter	0	0	118 (Ex 2 in 80s with missing data)

* Ex= excluding

3.2.5 Maternal body weight and number in dam litter

As all animals are weighed every month at CC, this information was available on individual records. Weights at likely conception dates or early in pregnancy, approximately 5 months prior to the birth date, before significant gain from the fetuses (Tardif and Jaquish, 1997; Bales et al, 2001), were recorded and used in analysis. Table 3.2 displays the mean weights of females at each litter size. The number of infants in the dam's litter at her birth was also recorded at CC, and so this was included to look at any potential genetic influence in mean litter size. Neither weights nor dam's own litter size was recorded consistently at CA or CB.

Table 3.2: Mean dam weight at conception of each litter size

Litter size	**Dam weight (g)**
Singleton (N=47)	366.06g ± 49.39
Twin (N=489)	373.80g ± 41.57
Triplet (N=376)	396.49g ± 45.74
Quadruplet (N=10)	391.20g ± 40.16

3.2.6 Statistical analysis

Descriptive statistics were carried out to summarise the reproductive output of the 120 dams at each colony. The percentages of each born litter size and their associated losses, as well as changes in litter size and dam longevity over time were also examined.

Descriptive statistics were also conducted to summarise cause of death over all three colonies (N=356). These were divided into 'euthanised', 'died naturally', or 'not stated' (some within this category gave a cause of death, but did not specify whether the animal was euthanised or died naturally). This was further divided into 'health' or 'breeding management' reasons for death, as well as if this was ‘not stated’ (in some cases it was recorded that the animal was euthanised or died naturally, but the reason was unknown).

Mean litter size

Multi-linear regression procedures using the Enter method were performed on 258 dams for whom there were complete data on all independent variables (IVs), to describe the amount of variation in the dependent variable (DV) mean litter size. Preliminary Spearman’s Rank correlations were first used to look for potential multicollinearity between variables. Number of litters was not included in the analyses, due to the strong correlation with dam longevity ($r=0.89$, $P< 0.001$), although no other variable was highly correlated ($r >0.60$) with another. R^2 change values for each additional variable entered in the regression model were used to describe the variance explained by each IV separately. The criterion for entry into the model was $P<0.05$. Although DVs were not normally distributed, models can still be used to make valid conclusions from this sample (Field, 2009). Colony and decade were regression control variables. Independent variables of longevity, mean IBI, age at first parturition and yearly production (following Smucny et al, 2004) were entered into the model.

It became clear from comparisons that the colonies showed different patterns. There were also different issues that arose, including data from wild-caught animals in the 70s at CA, missing data in the 80s at CB and CC, and no weights or dam litter size recorded at CA and CB. Each colony was therefore analysed separately, to prevent important information being lost. A one way ANOVA was also conducted to look at differences in weight between litter sizes at CC, with follow up Tukey HSD tests to compare the groups.

Survival analysis

Cox proportional hazards regression was performed to investigate which reproductive output variables could affect dam longevity. This is appropriate as it can be used to evaluate the effect of two or more continuous or categorical variables on whole-life survivorship. It also handles censored cases, so animals without a completed lifespan can be included (Jaquish et al, 1991).

Survival analysis was conducted for 262 dams of known birthdates, using the Enter method, with covariates of mean litter size, mean IBI, age at first parturition and yearly production. Site and decade were included as control variables. Each colony was also analysed separately, with decade as a control variable. Additional covariates of number of dam litter and dam weight at likely conception were included for CC. For dams with known date of death, longevity was the time of death. For dams still alive in the colonies (N=4), longevity was the age at censor date. This was defined as the date of the last update in the colony records.

3.3 Results

3.3.1 Variation in reproductive output

Reproductive output variables for the dams of the three colonies (combined decades) are summarised in Table 3.2. The values represent grand mean and medians calculated from the mean values of all dams. For CA data, no measured parameter was normally distributed (P>0.05) and so median values are most appropriate. For CB data, ‘yearly production’ and ‘yearly survived production’ were normally distributed, and for CC, ‘dam longevity’ and ‘weight at conception’ were normally distributed, and so mean values are most appropriate for these.

Table 3.3: Variation in dam reproductive variables (Colonies A, B and C, combined decades)

Variable	**Mean and SD**			**Median, min-max**		
	A	B	C	A	B	C
Dam longevity (yrs)	5.31± 2.06	7.39± 2.60	6.04± 2.47	4.98 (1.31- 11.34)	6.99 (2.80- 16.20)	5.76 (1.88- 13.59)
Inter-birth interval (days)	229.17± 81.71	190.87 ± 39.22	192.05± 81.85	206.00 (151.00- 669.00)	180.00 (151.00- 337.00)	170.20 (149.67- 754.00)
Age at first parturition (yrs)	2.68± 0.82	2.32± 0.68	2.30± 0.63	2.49 (1.19-5.17)	2.19 (1.14-6.69)	2.13 (1.33-5.62)
Lifetime production (no of infants born)	10.77± 9.16	25.05± 17.10	18.88± 13.72	9.00 (1.00-42.00)	21.00 (1.00-59.00)	16.00 (1.00-59.00)
Survived production (no of infants)	9.74± 8.61	19.05± 12.68	13.45± 11.54	8.00 (0.00-42.00)	16.00 (1.00-53.00)	10.50 (0.00-46.00)
Production/year (infants born/ yr of RL)	3.84± 1.19	4.67± 1.31	4.32± 1.05	3.62 (1.49-7.71)	4.60 (1.49-7.74)	4.42 (1.49-6.48)
Survived production/year (infants/yr of RL)	3.34± 1.29	3.39± 0.88	2.99± 1.47	3.24 (0.00-6.58)	3.39 (0.76-5.49)	3.43 (0.00-5.44)
Reproductive lifespan (yrs)	2.61± 1.91	4.75± 2.26	3.77± 2.39	2.15 (0.42-9.06)	4.61 (0.63-13.36)	3.58 (0.41-11.68)
Litter size (no of infants born)	2.37± 0.53	2.55± 0.55	2.32± 0.43	2.33 (1.00-4.00)	3.00 (1.00-4.00)	2.33 (1.00-3.50)
Survived litter size (no of infants)	2.06± 0.65	1.87± 0.37	1.56± 0.71	2.00 (0.00-3.23)	2.00 (1.00-3.00)	1.75 (0.00-3.00)
Number of litters (litters/dam)	4.37± 3.37	10.31± 6.15	7.93± 5.49	3.00 (1.00-14.00)	9.00 (1.00-30.00)	7.00 (1.00-23.00)
Weight at conception (g)			373.39± 43.44			369.13 (283.00-503.00)
Number in dam litter			2.42± 0.53			2.00 (1.00-4.00)

*Reproductive life span (RL) is summarised for multiparous females only. Survival age and age at first birth were calculated for dams born into the colony, and so exclude wild caught animals. Inter-birth intervals were calculated excluding abortions and after a change of mate.

*For CA, medians are most appropriate for all values. For CB 'yearly production' and 'yearly survived production', and CC 'dam longevity' and 'dam weight at conception' mean values are most appropriate.

3.3.2 Changes in mean litter size and dam longevity

Figures 3.1 and 3.2 display median dam longevity and median of the mean litter size, for each colony over the decades. These graphs reveal different patterns of change over the decades between the sites.

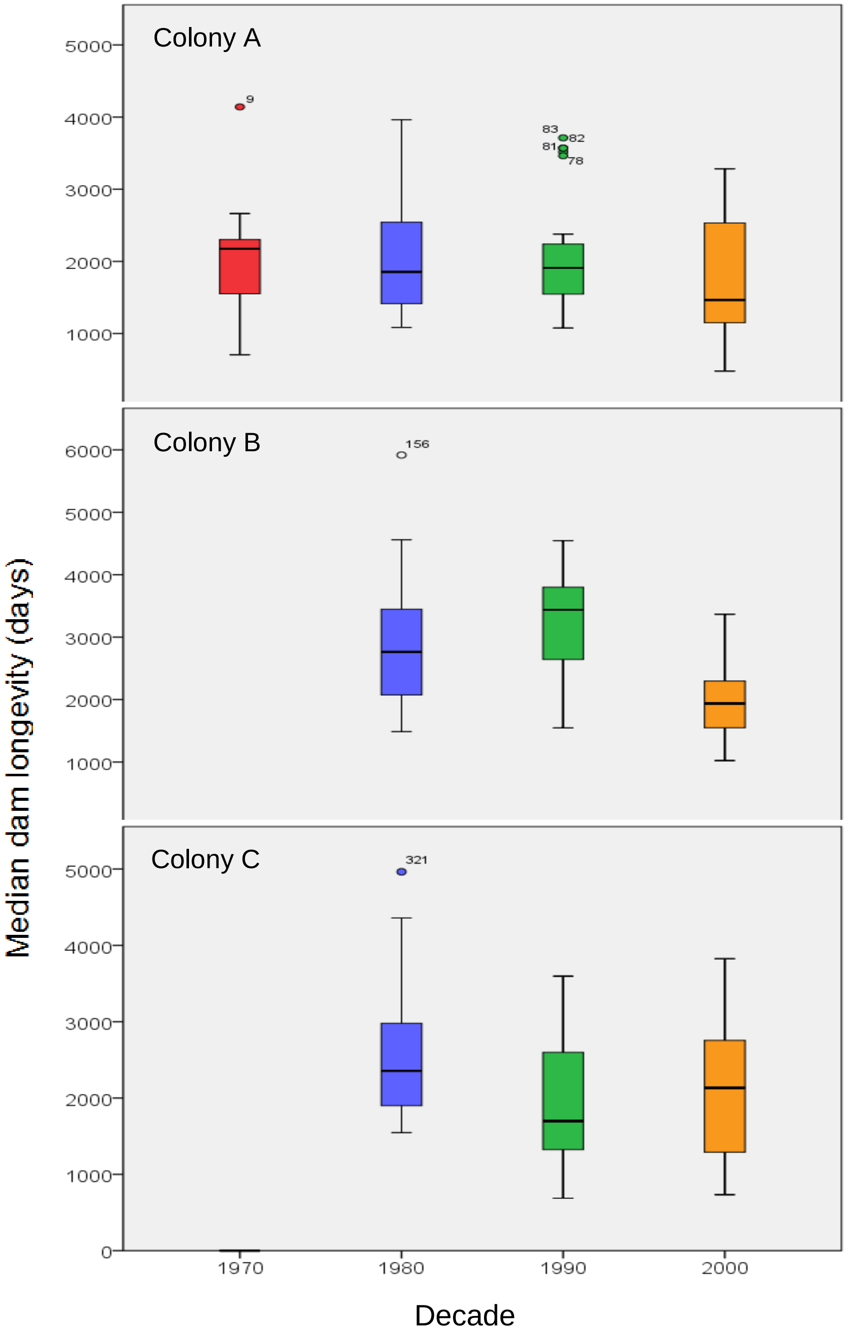


Figure 3.1: Median dam longevity (N=105 CA; 120 CB; 115 CC) for each colony over four decades. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: circles.

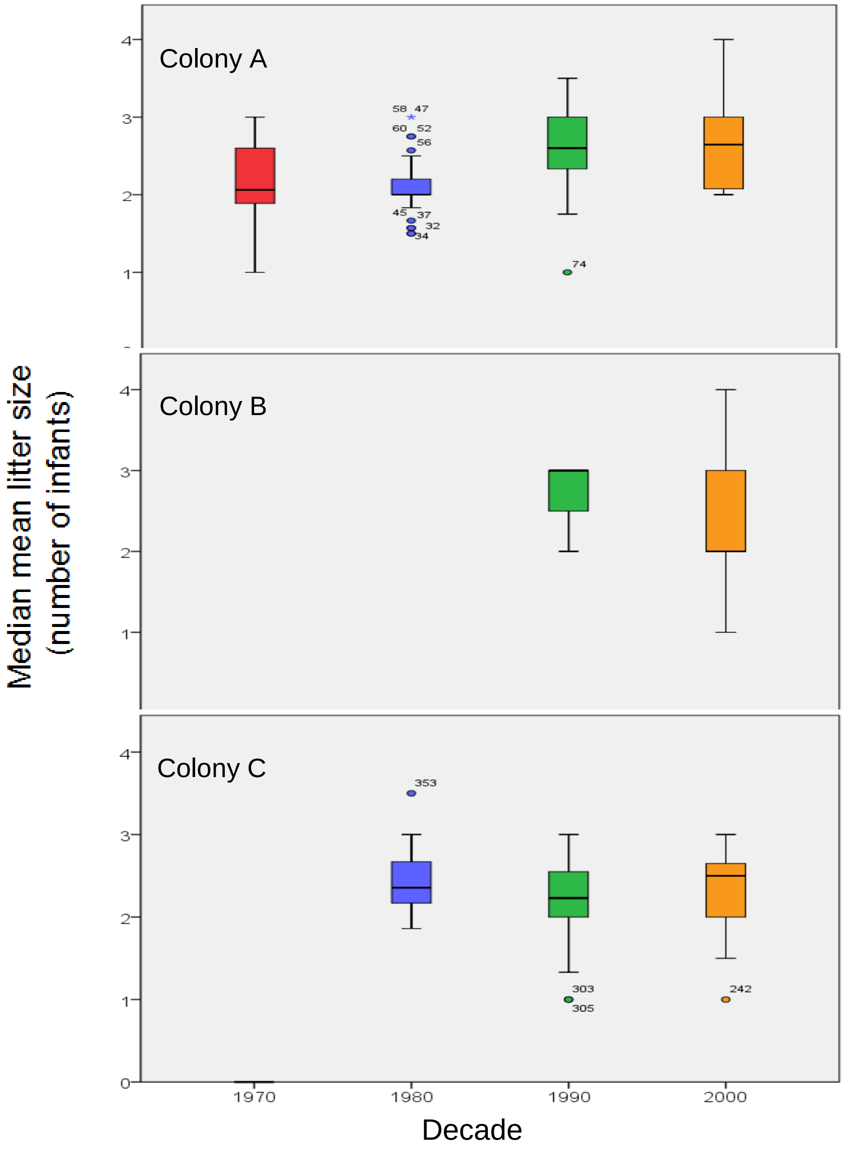


Figure 3.2: Median of mean litter size (N=120 CA; 80 CB; 120 CC) for each colony over four decades. Mean litter size calculated as sum of number of infants in each litter, divided by total number of litters, for each dam. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: circles.

3.3.3 Litter sizes and associated losses

Figure 3.3 displays the percentage of births at Colonies A, B and C. Compared to twins, triplet births were equally as common at CA, more common at CB and a little less common at CC, when data from all four decades were combined. Table 3.4 shows the total percentage of mortality (number of infants) associated with each litter size at each colony at birth, within 6 months and in total. In the majority of cases, these were by natural causes or euthanasia due to poor growth. Infant mortality was highest in quadruplet and quintuplet litters.

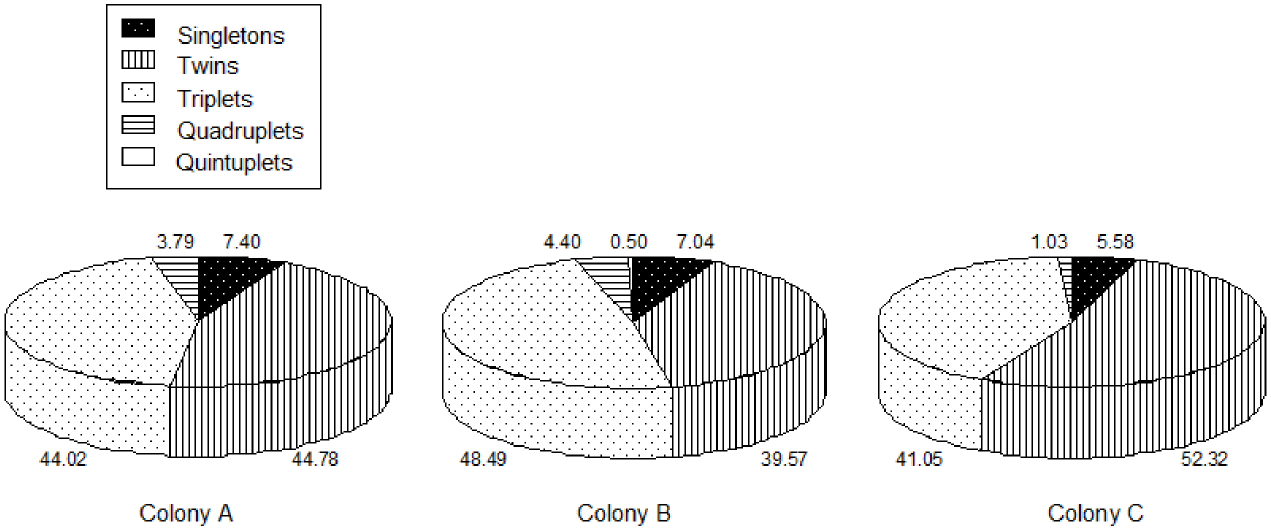


Figure 3.3: Percentage of litter sizes at birth at Colonies A (N=527), B (N=796) and C (N=967)

Table 3.4: Percentage of each litter size, together with their associated mortality (all three colonies)

	Singletons			**Twins**			**Triplets**			**Quadruplets**			**Quintuplets**		
Colony	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Number of litters born	38	56	54	235	315	506	228	386	397	20	35	10	0	4	0
Number of Infants born	38	56	54	470	630	1012	684	1158	1188	80	140	40	0	20	0
Number of infant losses at birth	3	3	5	38	17	45	82	54	104	6	20	8	0	0	0
% losses at birth	7.89	5.36	9.25	8.09	2.70	4.45	11.99	4.66	8.75	7.50	14.29	20.00	0.00	0.00	0.00
Number of infant losses at 6 months	N/A	9	3		84	44		302	205		43	6		13	0
% losses at 6 mnths	N/A	16.07	5.55		13.33	4.34		26.08	17.26		30.71	15.00		65.00	0.00
Total number of infant Losses	N/A	12	8		101	89		356	309		63	14		13	0
% total losses	N/A	21.43	14.81		16.03	8.79		30.74	26.01		45.00	35.00		65.00	0.00

*NA= no data on infant mortality after the day of birth

3.3.4 Dam cause of death

Descriptive statistical analysis was carried out on 356 dams from all three colonies. Table 3.5 shows the number of animals that were euthanised or died naturally, as well as when this was not stated, and the associated percentages of each cause of death (health, breeding management or unknown). Where this information was recorded, the most common reason for death involved management decisions to euthanasise on welfare grounds, due to the animal's poor condition.

Table 3.5: Percentages of each cause of death when animals were either euthanised, died naturally or when this was not recorded (N=356)

	Euthanised (N=274)	**Natural death (N=22)**	**Not stated (N=60)**
% Health	**65.69**	**27.27**	**48.33**
Gastrointestinal	1.45	4.55	1.67
Injury	1.82	0	0
Neurological	2.19	0	1.67
Poor condition	44.90	13.64	33.33
Reproductive	7.30	9.09	10
Respiratory	3.28	0	1.67
Surgical complications	1.09	0	0
Tumour	3.28	0	0
Optic	0.36	0	0
% Breeding management	**1.82**	**0**	**0**
Removed from breeding	1.09	0	0
Not breeding	0.36	0	0
Infanticide	0.36	0	0
% Unknown	**32.48**	**72.72**	**51.67**

3.3.5 Mean litter size

A linear regression model of mean litter size was estimated (R^2 = 0.45), explaining 44.8% of the variance in mean litter size for the combined colonies. Two hundred and fifty eight cases were included in the analysis. Control variables for decade, and colony were included in the model. Significant differences in mean litter size were found between colonies (explaining 45%), with CC having significantly lower mean litter size than CA and CB. CA and CB were not significantly different. A significant difference was also found between decades (explaining 42%). Mean litter size in the 90s was significantly higher than in the 80s. No other comparisons were significant. Net of the control variables, yearly production had the highest explanatory value (44.7%, positive effect) followed by longevity (9.8%, positive effect), with both being significant. Table 3.6 summarises the results of the Multiple Linear Regression analysis.

A linear regression model of mean litter size was estimated for each colony. For CA, 80 cases were included, and 45.9% of the variance was explained. Control variables for decade were included in the model (explaining 23.6%). Mean litter size in the 70s and 80s were significantly lower than in both the 90s and 2000s. Net of the control variables only yearly production was significant (22.3%, positive effect).

For CB, 75 cases were included, and 47.8% of variance was explained for mean litter size. As all cases in the 1980s were incomplete, only those in the 1990s and 2000s were included. Mean litter size was significantly higher in the 90s than the 2000s. Net of the control variables (explaining 13.1%), only yearly production was significant (34.6%, positive effect).

For CC, 102 cases were included, and 55.7% of the variance in mean litter size was explained. No decade was significantly different to another. Net of the control variables yearly production had the highest explanatory value (51.3% positive effect), followed by mean dam weight (21.7%, positive effect), with both significant. An ANOVA found a significant difference in dam weight at likely conception between born litter sizes, with post hoc Tukey HSD tests showing dam weight to be higher in triplet births than twin and singleton births. No difference was however found in quad births. Table 3.7 shows the significant results of this analysis. While dam's own litter size was included in analysis, this was not found to contribute significantly to the model.

Table 3.6: Summary of regression results for mean litter size born (combined, N=258 and separate colony analysis)

Model variables	**R^2**	**Adjusted R^2**	**R^2 change**	**Significance of Added variable**
COMBINED COLONIES (N=258 complete cases)				
Whole model r2=.448, adjusted .432				
Site	0.05	0.04	0.05	P<0.01
Site AvC				P<0.05
Site BvC				P=0.001
Decade	0.04	0.03	0.04	P<0.05
Decade 90v80				P<0.01
Yearly production	0.45	0.43	0.37	P<0.001
Dam longevity	0.10	0.08	0.02	P<0.01
COLONY A (N=80 complete cases)				
Whole model r2=.459, adjusted .43				
Decade	0.24	0.21	0.23	P<0.001
Decade 70v100				P=0.01
Decade 80v100				P<0.001
Decade 90v70				P<0.05
Decade 90v80				P<0.001
Yearly production	0.46	0.43	0.22	P<0.001
COLONY B (N=75 complete cases)				
Whole model r2=.478, adjusted .463				
Decade	0.13	0.12	0.13	P=0.001
Decade 90v100				P=0.001
Yearly production	0.48	0.46	0.35	P<0.001
COLONY C (N=102 complete cases)				
Whole model r2 =.557, adjusted .539				
Mean dam weight	0.26	0.23	0.22	P<0.001
Yearly production	0.55	0.54	0.51	P<0.001

Table 3.7: Significant differences in dam weight at conception between litter sizes

Test	df	F	P
Weight at conception	3, 918	21.61	<0.001
Between litter sizes			
Triplets>Twins			<0.001
Triplets>Singletons			<0.001

3.3.6 Survival analysis

A whole-life survivorship analysis revealed that colony, mean litter size and yearly production were significant (P<0.05) factors affecting dam longevity. CA had significantly lower survival than CC and CB, although CB and CC were not significantly different. Decades 80 and 90 were significantly higher than in the 2000s, although no other comparison was significant. Increases in mean litter size and yearly production were both significantly associated with higher dam longevity.

Analysis of individual colonies revealed that only mean IBI had a significant relationship (positive) with dam longevity at CA. Dams with longer mean IBI demonstrated higher longevity than those with shorter mean IBI. There were no significant differences in longevity between the decades at CA. Only decade was significant at CB. Females breeding in the 90s lived for longer than those breeding in the 2000s. At CC, mean litter size (positive), yearly production (positive), mean IBI (negative) and mean weight (positive) were all significant factors affecting dam longevity. Females with higher mean litter size, higher yearly production, shorter mean IBI and higher weight showed greater longevity. No significant differences in longevity were found between decades at CC. While dam's own litter size was included in analysis, this was not found to contribute significantly to the model. Table 3.8 summarises the results of the Cox Proportional Hazards Regression from combined and separate colony analysis.

Table 3.8: Summary of cox proportional hazard regression results for dam longevity (combined, N=262 and separate colony analysis)

Covariate	Estimate	SE	Wald statistic	df	P	Relative risk	Lower 95% CI for Relative risk	Upper 95% CI for Relative risk
COMBINED COLONIES (N=262)								
Whole model (X^2=43.92)								
Site			18.29	2	<0.001			
BvA	-0.70	0.17	17.85	1	<0.001	0.50	0.36	0.69
CvA	-0.43	0.15	7.90	1	=0.005	1.53	1.14	2.06
Decade			11.94	3	<0.01			
80v100	-0.36	0.17	4.18	1	<0.05	0.70	0.50	0.99
90v100	-0.51	0.51	11.42	1	=0.001	0.60	0.45	0.81
Mean litter size	-0.44	0.15	8.43	1	<0.005	0.64	0.48	0.87
Yearly production	-0.23	-0.06	13.81	1	<0.001	0.79	0.70	0.90
COLONY A (N=80)								
Whole model (X^2=5.15)								
Mean IBI	-0.004	0.002	4.56	1	<0.05	1.00	0.99	1.000
COLONY B (N=75)								
Whole model (X^2=38.22)								
Decade								
90V100	-1.82	0.30	36.07	1	<0.001	0.16	0.09	0.29
COLONY C (N=106)								
Whole model (X^2=30.17)								
Mean litter size	-0.69	0.33	4.25	1	<0.05	0.50	0.26	0.97
Mean IBI	0.003	0.001	6.15	1	<0.05	1.00	1.00	1.01
Yearly production	-0.58	0.13	21.84	1	<0.001	0.56	0.44	0.71
Mean weight	-0.01	0.003	12.17	1	<0.001	0.99	0.98	1.00

3.4 Discussion

3.4.1 Reproductive output and dam longevity

The present study summarised the reproductive output of captive marmosets housed at three UK colonies over a period of 4 decades. Overall, many values are similar to those previously described (Smucny et al, 2004; Tardif et al, 2003; Box and Hubrecht, 1987), although several are greater in the UK colonies. These higher UK values appear to be due to the lifetime production and number of litters at CB in particular, where there was also the highest reproductive life span and shortest IBIs. While some females had a reproductive life span of only one or two litters, others had consistently high production over many years. There was therefore considerable variation between female common marmosets. Table 3.9 provides comparative data from previous research.

Over all three colonies, average longevity was approximately 6 years in the UK, which is similar to other establishments from the 1980s (Box and Hubrecht, 1987) to the 2000s (Smucny et al, 2004). It appears that while the majority of animals were euthanised, rather than died naturally, this was due to health and welfare reasons, most commonly ‘poor condition’. More detailed records would however be beneficial, including a more specific cause of death. Management decisions can also be made regarding which animals are most suitable to keep in breeding, depending on their reproductive success and the number of animals needed for research, and so longevity could be related to production (Essl, 1998). However, only a very small portion of those with adequate records, were euthanised due to breeding management. Dam health and longevity is therefore a concern. While one may expect increased longevity in captivity compared to the wild, as predators and food shortage are not constraints, this does not appear to be the case at some colonies.

Table 3.9: A summary of results from previous studies of captive colonies, including combined results from all three sites in the present study

Variable	Current study (N=302^{1};304^{2};305^{3};316^{4};320^{5};340^{6}; 344^{7} dams)	Smucny et al (2004) (N=272^{1};287^{2};400^{3} dams)	Tardif et al (2003) (N=479 dams)	Box & Hubrecht (1987) (N=543 infants)
Dam longevity (years)				
Mean	6.29^{6} +/- 2.55	5.74^{3}+/-2.46	5.99 +/-2.31	6.00
Median	5.94^{6}			
IBI (days)				
Mean	202.54^{4} +/- 71.27	216.70^{1}+/-98.53		
Median	181.21^{4}		162.00	158.00
Age at 1st parturition (years)				
Mean	2.42^{7} +/- 0.73	2.91^{3}+/-1.16		
Median	2.25^{6}			
Lifetime production (number of infants born)				
Mean	17.70^{2} +/- 14.48	8.03^{3}+/-7.15	7.75	
Median	14.00^{2}		6.00	
Survived production (number of infants)				
Mean	14.38^{3} +/- 11.80 [a]	4.37^{3}+/-4.36 [b]		
Median	11.00^{3} [a]			
Production/yr (infants born/yr of RL)				
Mean	4.23^{5} +/- 1.21	3.66^{3}+/-1.57	2.30	
Median	4.23^{5}			
Survived production/yr (infants/yr of RL)				
Mean	3.27^{5} +/- 1.21	1.87^{3}+/-1.29 [b]		
Median	3.37^{5}			4.00 [b]
Reproductive life span (years)				
Mean	3.84^{1} +/- 2.51	2.08^{2}+/-1.55		
Median	3.33^{1}			
Litter size (number of infants born)				
Mean	2.40^{5} +/- 0.50	2.22^{3}+/-0.56		
Median	2.33^{5}			
Mode	2.00		2.00	3.00
Survived litter size (number of infants)				
Mean	1.86^{5} +/- 0.61	1.87^{3}+/-0.68 [b]		
Median	2.00^{5} [a]			
Number of litters (litters/dam)				
Mean	7.67^{7} +/- 5.72	3.54^{3}+/-2.84	3.45	
Median	6.00^{7}		4.00	

a. Survived the day of birth and up to 6 months
b. Survived up to 1 month after birth
+/- SD

3.4.2 Factors affecting dam longevity

A whole-life survivorship analysis, combining data from all three UK colonies, found that site, decade, yearly production and mean litter size were all significant predictors of dam longevity. Dam longevity and lifetime productivity at CA, where experimental manipulations were often carried out for reproductive studies, was the lowest of the three colonies, and very similar to those obtained by Smucny et al (2004). Average longevity was 5.31 years, which was relatively similar in each decade. However, many animals were placed on terminal experiments in the 2000s, which did limit the available sample in this decade. Dam longevity and lifetime productivity at CB, a commercial facility in which breeding pairs were rarely disturbed, was the highest. Average longevity was 9.58 years in the 1990s, which is similar to the University of Cambridge (Ridley et al, 2006). However, longevity significantly decreased in the 2000s, after a change in diet and moves between buildings. Differences in housing and husbandry could therefore be important factors in dam longevity. Results from CC, an establishment that bred for purpose, fell between those obtained at the other two sites. Longevity remained at around 6 years over the decades, which is similar to data published by Tardif et al (2003). Data therefore suggests that longevity in captivity does not appear to have improved significantly, despite increased understanding of the species' biological and psychological needs, and concurrent improvements in their care. While there were insufficient details to investigate which specific environmental factors are most important, it appears that appropriate housing and particularly a diet that meets nutritional needs is necessary, as is a stable, closed colony with minimal stress (Tardif et al, 2011).

Although the costs of high reproduction might be expected to reduce condition and longevity (Tardif et al, 2008), there was no evidence that this was the case. In fact, dams with larger mean litter sizes, producing more infants per year, tended to have higher longevity. Previous research (Jaquish et al, 1991; Smucny et al, 2004) has found no relationship between litter size and dam longevity. Although larger litters did not appear to be detrimental to physical health, there is evidence that they may be stressful for parents. Tardif et al. (2002) found that dams spent less time carrying and nursing triplet infants, compared to twin infants. There was also a higher frequency of triplet-infant initiated interactions, associated with increased harassment by mothers, than for twins. These findings suggest that dams could only tolerate a limited amount of time with their young, and that larger litters seem to disrupt maternal behaviour (Tardif et al, 2002).

As differences between colonies were found, they were also analysed separately. Only mean IBI was significant in explaining dam longevity at CA, with dams experiencing longer inter-birth intervals surviving longer. Mean IBI was also significant at CC, although a negative association was found at this colony, which may be due to poor health causing failure to conceive or early abortion, prolonging IBI (Poole and Evans, 1982). Instead, heavier dams survived for longer at CC, where weight was recorded. This finding may be because lactation is relatively costly for marmosets, with small mothers experiencing substantial mass loss and high risk of mortality following twin litters (Tardif et al, 2002). While it is possible that the constant high energetic demand of pregnancy and lactation could reduce longevity, and so increasing time between births may give females time to recover body condition, this effect does not span all three colonies and so no robust conclusions can be made. However, it could be interesting area for future research to explore.

Although results from previous studies suggest that delaying the onset of breeding in captivity may increase longevity (Jaquish et al, 1991), with early age at first reproduction having detrimental health consequences, no association was found between age at first parturition and dam longevity in the present study. However, age at first parturition was generally around 2.0 years, with very few after this time (see Table 3.3 and Table 3.9). It is common for captive female marmosets to first begin breeding at 18 months (Tardif et al, 2003), while first parturition in wild females is at a much later age (Tardif et al, 2008). Perhaps if more females had begun breeding after 4 years, a similar result to Smucny et al (2004) would be found. This may be another useful area of future research, and a possible consideration in the management of breeding marmosets. While it is important to consider age-related pathologies, marmosets could be managed to survive for longer before degeneration occurs (Tardif et al, 2011).

3.4.3 Litter size and infant mortality

Although twins are the norm in the wild, triplet litters are common in captivity. Litters larger than two accounted for approximately half of the births examined in each colony. However, larger litters did have considerably greater perinatal mortality than twins, ranging from 30% of infants from triplet litters to 65% from quintuplets. High infant mortality has been reported previously in captive colonies (Jaquish et al 1991), primarily due to the large proportions of triplets born.

As marmoset families are rarely able to rear more than two infants at a time (Poole and Evans, 1982), these young are unlikely to survive without some form of human intervention. While CA did not intervene when triplet litters were born in later decades, CB and CC both consistently carried out supplementary feeding of triplet infants. Despite hand rearing in attempt to improve survival, large litters still resulted in higher mortality than twins. While it was rare for all three triplets in a litter to die, there was often one infant loss within the first few weeks. These rearing practices also involve infant removal from the family for extended periods of time, which has been associated with adverse developmental outcomes (Dettling et al, 2002; Pryce et al, 2004). Although triplet losses at birth were higher at CC than CB, due to routine euthanisia of very light infants, losses at 6 months were lower. This suggests that their practice of rotational hand-rearing may have been more successful, as litter mates remained together and were separated from the family for shorter periods of time. Due to our ethical obligation to ensure good welfare, as well as the importance of raising animals that are ‘fit for purpose’, potential factors affecting mean litter size were also studied.

3.4.4 Factors affecting born litter size

A linear regression model, combining data from all three UK colonies, found that 44.8% of variance in mean litter size born was explained by site, decade, yearly production and dam longevity. CC had the lowest mean litter size of the three colonies. Differences over time were also found at CA, where births changed from predominantly twins in the 70s and 80s to predominantly triplets in the 90s and 2000s. The tendency for litter size to increase with the age of the colony has been well-documented (Box and Hubrecht, 1987; Poole and Evans, 1982). However, litter size fell significantly in CB, although remained similar at CC.

Inspection of colonies separately showed that only yearly production was significant at CA and CB. However, these findings are somewhat obvious or unavoidable, and so are not useful predictors. They are therefore of little interest, as they will not contribute to Refinements. Mean dam weight at likely conception was a significant predictor of mean litter size at CC, with heavier dams producing larger litter sizes. Dam weight was also significantly higher prior to triplet births compared to twin or singleton births. Tardif and Jaquish (1997) also showed that higher weight was associated with higher number of ovulations. Much variation occurred within females, with 90.9% of subjects weighing more when ovulating 3-4 than when ovulating 2. However, mothers that lose mass during pregnancy, due to nutritional restriction, can reabsorb fetal material, leading to litter size reduction in utero (Tardif and Jaquish, 1997). Litter size could therefore change from date of conception, which may explain why this factor did not explain more of the variation.

The dam’s own litter size was not significant in predicting litter size, a finding reported by previous authors (Tardif and Jaquish, 1997; Jaquish et al, 1991), and so genetic variance does not appear to play a major role. Tardif and Jaquish (1997) found that much variation in number of ovulations was seen within, rather than between, females. Low repeatability of final litter size per dam has also been discovered (Jaquish et al, 1991). It is therefore unlikely that selecting breeding females who were born to twin litters themselves would be a successful way of promoting twin births in captive colonies. Litter size instead appears to be flexible (Jaquish et al, 1996), determined by environmental variables affecting energy availability, such as diet or physical activity. Captive marmosets can weigh as much as 600g (Poole and Evans, 1982), and are significantly heavier than their wild counterparts, weighing around 320g (Araujo et al, 2000), which may account for captive females producing larger litters than those in the wild. Maintaining dams at lower healthy weights may help to reduce larger litters, and the associated higher infant mortality. Structural enrichment to increase activity, as well as a reduced calorie diet at likely dates of conception, could therefore be investigated as a possible Refinement. This must however be applied carefully, as heavier dams also seem to have greater longevity.

3.4.5 Conclusion

The present study provides information on reproduction and life history in female marmosets housed at UK breeding colonies, in comparison to similar international establishments. Areas of concern include high rates of infant deaths and dam health. Potential predictors of mean litter size and dam longevity were therefore examined, and possible ways of aiding with practical aspects of managing these animals discussed. Maintaining a colony of experienced breeders, with longer healthy life spans and an increased incidence of twin births could have far-reaching implications to improve the quality of life for marmosets in breeding facilities.

CHAPTER 4: The impact of rearing background on the welfare of common marmosets *(Callithrix jacchus):* Effects on behavioural and physical development

Abstract

Early life environment can have a substantial impact on an individual's physical and behavioural development. The current study investigated the effect of litter size and rearing background on infant care and behaviour, as well as growth, health and survival. Although twins had lower body condition scores as adults than 2stays and triplets, records suggest that individuals from any litter size could suffer from extreme low weight, but particularly when born to larger litters. Twins gained independence earlier, although were more reliant on caregivers at this young age. Singleton infants received more rejective parenting than 2stays, and were more active when off their carriers than previous research has described. Meanwhile, 2stays received more protective family rearing than twins, although developed social play earlier. While early independence and rearing with a same age sibling may promote security and ability to cope, there were few major overall differences in activity between twins and the other litter conditions. Therefore, while there was some small effects of litter condition, this may not have substantially affected development and welfare.

4.1 Introduction

4.1.1 Infant care and development in the common marmoset

Callitrichids are characterised by several unique features. These include twin births (Stevenson and Rylands, 1988) and the intense care and co-operation of all members of the family in caring for the young (Ingram, 1977). Twinning in the Callitrichidae has been explained as an evolutionary adaptation allowing small primates to give birth to large-brained offspring (Leutenegger, 1973). Communal infant rearing may have resulted from this reproductive biology and the high energetic demands of infant care (Tardif et al, 1993), enabling them to successfully rear the twins.

Many detailed observations of captive common marmosets have found that the behavioural and physical development of marmosets show a series of distinct changes from dependence to maturation, which are most evident in the infant phase (reviewed in Yamamoto, 1993). Throughout infancy, common marmosets also develop characteristically different relationships with each group member (Locke-Haydon and Chalmers, 1983), as their relative importance changes with age. In the review below, statements refer to common marmosets, unless stated otherwise.

Newborns are carried almost continuously for the first three weeks of life, with babies being handed back to the mother for suckling. Caregivers are extremely tolerent of infants at this time, frequently accepting their attempts to climb on (Yamamoto, 1993), and are highly responsive to infant vocal cues (Sanchez et al, 2014). Various family members take turns in carrying the infants, although some studies have reported the father to be the main caregiver in the first week (Locke-Haydon and Chalmers, 1983). However, there is much evidence of high variability among families in patterns of care (Arruda et al, 1986; Ingram, 1977; Yamamoto, 1993).

In later infancy, caregivers begin to encourage the infants to get off, picking them up less frequently and increasing the amount of rejections. The father may become the main caregiver at this time, with studies finding that there were more successful attempts to climb on to him than the mother (Yamamoto, 1993). After week 4, many independent behaviours begin to appear (Yamamoto, 1993). Infants are more mobile and leave their carriers increasingly more to explore and play socially, which is thought to allow young animals to develop complex patterns of adult behaviour (Box, 1975b). Weaning occurs after week 8, although solid food is often first tasted prior to this though sharing or stealing. Agonistic behaviours may appear, as well as scent-marking (Yamamoto, 1993), which is known to be an important aspect of communication in the marmoset (Epple, 1993).

By the end of the infant phase, there is marked decrease in dependence on caregivers (Yamamoto, 1993). This has also been found in field studies, in which young marmosets were seen to move long distances themselves by the end of the infancy (Stevenson, 1988). However, the carrying period may be extended in the wild (Yamamoto et al, 1996; Stevenson and Rylands, 1988). By 8 weeks, wild infants are still carried up to 50% of the time, while captive infants spend approximately 90% off their carriers at this age (Ingram, 1977). Differences in carrying may be due to the larger daily ranges and risk of predation in the wild (Yamamoto, 1996). Infants did however gain independence at similar rates. There were few differences in the first appearance of key behaviours between captive and wild common marmosets, except for social play (see Table 1.1). Captivity may also influence care patterns. Ingram (1978) found that infants of wild born parents in captivity were nursed more often and spent more time at a distance, while those with captive born parents spent more time seeking proximity and were rejected more often.

At this time of reduced parental care, interactions with the twin increase significantly. Support is also found in older siblings (Yamamoto, 1993). The number of helpers in a family group has been associated with differences in infant care. Maternal carrying has been found to decrease when more caregivers were present (Ingram, 1977; Cleveland and Snowdon, 1984: *S. oedipus*). However, in some cases maternal care can increase in large groups (Santos et al, 1997: *Callithrix* and *Leontopithecus*). In others, it was the father's care that decreased considerably in the presence of more helpers (McGrew, 1988: *S. oedipus*). This difference may be due to the broader definition of care used in McGrew's study (Yamamoto, 1993). Both captive and field studies have found that the age of siblings may lead to differential interactions. Juveniles rarely help with infant carrying (Captive: Box, 1975b; Wild: Yamamoto et al, 1996), with adult helpers having a more active role in infant care.

Table 1.1: First appearance of developmental markers in captive (reviewed in Yamamoto, 1993) and wild (Stevenson, 1988; Alonso, 1984) infant common marmosets

Behaviour	**Mean and range in captivity (days)**	**First day in wild (days)**
Off carriers	15.4 (11-20)	13 (Alonso, 1984) 25 (Stevenson, 1988)
Foraging	28.6 (25-34)	32 (Stevenson, 1988: fruit) 38 (Alonso, 1984: animal prey)
Solitary play/ exploration	19.6 (11-25)	25 (Alonso, 1984)
Social play	31.6 (25-49)	61 (Stevenson, 1988)
Scent mark	62.8 (33-73)	61 (Stevenson, 1988)

4.1.2 Factors affecting infant care and development

Litter size

As infant marmosets are adapted to be born as one of a twin pair (Leutenegger, 1973; Table 2.1), a principle aspect of their environment is the close presence of an exact age peer. They develop a close proximity during many types of activity, such as playing, exploring, resting and foraging (Box, 1975b). Having a twin allows the infants to develop socially together, perhaps being important in promoting security and independence (Yamamoto, 1993). Cleveland and Snowdon (1984) compared the early development of twin and singleton cotton-top tamarins (*S. oedipus*) over the first 20 weeks of life. They found that twins spent more time in social play than singletons, independent of the number of siblings available to play with. Twins also spent more time in solitary play and away from their parents, usually with each other, while singletons had no other companion (Yamamoto, 1993). As singleton infants spend less time playing and exploring, their learning and development may be restricted (Box, 1991). For example, Menzel et al (1963: *Pan troglodytes*) found that restricted experience lead to increased dependency on others and over reactivity to novel objects. On the other hand, exposure to an enriched environment elicited a greater repertoire of behaviours and increased developmental rate (Ventura and Buchanan-Smith, 2003).

However, caregivers may reject and transfer twins more than singletons, as greater effort is needed to carry them (Price, 1991: *S. oedipus).* While smaller infants from triplet litters, in which only two remain due to loss of the third, could be easier to carry for longer periods of time, encouragement to get off and stay off the carrier is an important part of infant development (Yamamoto, 1993). There is also some evidence that litters larger than two could be stressful for parents and disrupt maternal behaviour. Tardif et al (2002) observed that mothers spent less time carrying and nursing triplet infants, and were harassed by the infants more often, compared to twin infants, suggesting that mothers could only tolerate a limited amount of time with their young (Tardif et al, 2002).

Evidence from studies of captive marmosets (Jaquish et al, 1991) has also demonstrated that twins have significantly higher survival than singletons and triplets during the first month of life. As both very small and very large infants (under 26g and over 35g) were less likely to survive than infants of mid-range birth weight, risk does not seem to be due to lower birth weights in larger litters (Jaquish et al, 1997). Parents are simply unable to care for more than two young, because of the mother's limited milk supply and competition between the infants' for nursing time. Meanwhile, large singletons may also be vulnerable, due to problems in delivery or mothers being unable to meet their energetic demands (Jaquish et al, 1997). Although after the first month of life, when weaning from mothers milk typically occurs (Yamamoto, 1993), litter size no longer affected survival, twins still tended to have significantly higher whole life survivorship than triplets (Jaquish et al, 1991). Therefore, litter size could have considerable implications for infant development and the survival of marmoset monkeys (Jaquish et al 1997).

4.1.3 Early life stress

Family rearing style

There is increasing evidence that early life events, including caregiver behaviour, can have a substantial impact on the development of an individual. Although general patterns of caregiving are consistent among groups of marmosets, there can be significant inter-group differences (Ingram, 1977), including the amount of care group members are prepared to give and the amount of care that infants seek. Family members could provide little care, and so infants may continue to seek interactions and be highly rejected. Locke-Haydon and Chalmers (1983) found that infants with rejecting fathers tended to have rejecting mothers and siblings, and so there may be no compensation for behaviour between family members. However, in other families, caregivers may offer plentiful attention, and so one individual may provide the majority of care an infant requires and will compensate for any lower amounts given by other group members.

Primate models of maternal behaviour have indeed shown marked individual differences in care, along two dimensions of Protectiveness and Rejection (Maestripieri, 1998). Protectiveness is measured by the degree to which the mother physically restrains the infant’s exploration, initiates contact and provides nurturing behaviour, such as grooming. Rejection involves the degree to which the mother limits the duration of carrying, suckling and contact with the infant. Aspects of such maternal behaviour include maternal attraction, the interest they have for their infant, and maternal anxiety, the perception of danger for their offspring. Maternal anxiety can be triggered by forced separation (Maestripieri, 2011), enhancing maternal motivation and the expression of protective behaviours. Studies have found that infants reared by highly protective mothers had delayed independence, and were more fearful and inhibited (Fairbanks and McGuire, 1993: *C. aethiops*). Meanwhile, infants of more rejecting mothers acquired independence earlier (Bardi and Huffman, 2006: *M. mulatta* and *M. fuscata*), but tended to be more anxious and impulsive (review in Parker and Maestripieri, 2011).

Exposure to different rates of rejection is comparable to exposure to a stressor of different intensity levels, with too little or too much leading to stress vulnerability. Evidence from primate models supports the resilience model of stress development, which assumes a J shaped curve, with moderate levels of stress leading to adaptive responses (Parker and Maestripieri, 2011). While rejection is likely to be physically and psychologically stressful for young primates, protectiveness in itself is not. Protective parenting could however provide different opportunities to explore and learn how to overcome challenges (Parker and Maestripieri, 2011).

Parental separation

As well as rearing style, separation from the family can have a substantial effect on development. It has been known for many years that litter size has been increasing in captive breeding colonies (Stevenson and Rylands, 1988). This creates a problem as infants from litters larger than two usually do not survive without human intervention. The likelihood of survival is greatly increased if one infant is partially or completely hand-reared (Hearn and Burden, 1979), and so early separation from the family is commonly practised in primate colonies (Poole and Evans, 1982). However, such husbandry practices are often advocated without a sound scientific understanding of their welfare implications (Buchanan-Smith, 2010b).

In marmosets, infants are in full, continuous body contact with their carriers throughout the first several weeks of life (Yamamoto, 1993), and so interruption of this contact for even brief periods is unnatural and likely to be extremely stressful. Early separation may therefore undermine an individual's ability to cope with subsequent stressors (Parker and Maestripieri, 2011). Since Harlow's (1959) early experiments of maternal deprivation, more recent studies have looked at the effect of experimentally induced parental separation. Dettling et al (2002) demonstrated that early deprivation in common marmosets resulted in significantly lower body weight than control infants, after the first month of life. Reductions in weight may be due to stress-induced catabolic processes, reduced nursing time or decreased milk quality or quantity from stressed mothers. It is therefore possible that long term regulation of energy balance can be programmed during early infancy (Reilly et al, 2005). Early deprivation also caused an increase in distress vocalisations and a higher proportion of time in the suckling position, perhaps motivated by increased comfort seeking. However, the infants received a similar amount of parental care to controls (Dettling et al, 2002), suggesting that separation did not affect parental protective behaviours. Early parental deprivation may also effect the activity and development of the sympathetic nervous system (SymANS) (Pryce et al, 2004) and hypothalamic pituitary adrenal (HPA) axis (Dettling et al, 2007), which could have implications for health. Indeed, Capitanio (2011: *M. mulatta*) found remarkably long term changes in physiological and immune responses, which could alter the rapidity and severity of disease progression.

Rearing background could therefore have an impact on welfare, and on scientific output. It is possible that infant separation from the family in the current or even in previous litters, as well as loss of an infant, could increase the level of caregiver protective behaviours. Separation from the natal group could also influence the long-term development of marmosets, as family care is important for normal neurobiology, physiology and behaviour (Pryce et al, 2004). It is likely that twins have the highest welfare, tending to be family-reared with no disruptions and having the security of a same-age sibling.

4.1.4 Aim

This study followed marmosets throughout life, to investigate the effect of litter size and rearing background on physical and behavioural development. Infants of different litter sizes were observed, to investigate any differences in caregiver behaviour, as well as first emergence and amount of key behaviours. Records of these animals were accessed to look at effect of litter size on growth, health and survival. Records of adult animals were also examined, to investigate the long-term effect of rearing background, including supplementary feeding, on growth, health and survival. It was hypothesised that twin marmosets would have the lowest mortality and fewest health problems, as well as earlier emergence of key behaviours and greater percentages of time spent in independent behaviour, compared to other litter conditions.

4.2 Method

4.2.1 Study animals

Infants from 16 breeding pairs were studied. Only healthy, multiparous females, in family sizes of 4-9 were included. No significant difference was found in family size between each condition (One way ANOVA: F(2,17)=1.168, P=0.335). Thirty-five infants of three rearing conditions were involved in behavioural development observations (conditions outlined in 2.2), and followed to investigate growth, health and survival. These included seven marmoset reared twins (7 males, 7 females), eight marmoset reared 2stays (10 males, 6 females) and five marmoset reared singletons, from birth or when a sibling dies under a week old (5 females). While an even male: female ratio was aimed for, only singleton females were born into the colony during the study period. Although a sample of supplementary fed triplets had also been planned, this practice was stopped at the colony during the study and so a large enough sample could not be generated. After this time, the lightest of the triplets or quadruplets was routinely euthanised, to provide the remaining 2 infants with the best possibility of survival. All observations began after 1 week, to ensure the infants were assigned to the appropriate condition. Table 4.1 describes the infant and family information for each litter.

Table 4.1: Litter and family information for each infant studied

Rearing	**Cage**	**Gender**	**DoB**	**Litter size at birth**	**Family size (excluding infants)**	**Comments**
Singletons (N=5)	12M	F	13/9/12	1	4	
	14M	F	12/12/12	1	6	
	393M	F	2/4/13	3	4	2 euthanised day 1
	61F	F	8/4/13	3	5	1 stillborn, 1 euthanised day 7
	414N	F	4/5/13	1	5	
Twins (N=7)	14M	M, F	14/7/12	2	8	
	55K	M, F	4/8/12	2	7	
	421N	M, F	20/11/12	2	9	
	47P	M, F	22/12/12	2	4	
	423N	M, M	19/2/13	2	3	
	108L	F, F	15/3/13	2	6	
	160L	M, F	17/3/12	2	4	
2stay (N=8)	414N	M, M	30/6/12	3	6	One stillborn
	92K	M, M	30/7/12	3	4	One euthanised day 1
	18L	M, F	28/8/12	3	7	One euthanised day 6
	86K	M, M	2/9/12	4	6	One euthanised day 1, one day 5
	101K	M, F	1/10/12	3	7	One euthanised day 5
	45G	M, F	4/10/12	3	8	One euthanised day 4
	108L	F, F	13/10/12	3	6	One euthanised day 1
	414N	M, F	2/12/12	3	5	One euthanised day 1

* Infants were euthanised as they were not thriving (low/ losing weight)

Records of a further 34 adult animals were used to investigate long-term growth, body condition, health and survival. This included 10 marmoset reared twins, 13 marmoset reared 2stays and 11 supplementary fed triplets (full schedule in 2.2.1) involved in Chapters 5 and 6. Information for each adult studied is provided in the appropriate chapter (Tables 5.1 and 6.1). This gave a total of 69 animals.

Housing and husbandry

For details of housing and husbandry, see 2.4.1 and 2.5.

4.2.2 Physical development

Body Weight and Condition

All infants were weighed at day 10, with only infants from larger litters consistently weighed at birth. Marmosets were then weighed monthly. Cross sectional weights for age were plotted for each infant, from birth (if available) to 6 months of age, to investigate any differences in physical development between the litter sizes. All had weight up to 6 months, after which the sample size for singletons dropped below 5. There was also one 2stay male loss at 6 months. Cross sectional weights for age were plotted for the additional adult animals, from 12 to 24 months. Due to husbandry practices, these older animals were only consistently weighed monthly after 12 months. Health records of all animals studied were accessed, to look at any illness and mortality.

Body condition was routinely scored at each monthly weighing, from approximately 12 months of age (and so infants included in behavioural development were not old enough to be scored). Staff used a scale based on Wolfensohn and Honess (2005: macaques), which involves palpating the lumbar area, to determine the amount of fat and muscle present. Scores range from 1 (emaciated) to 5 (obese), with a score of 3 being ‘normal’. Appendix A presents this scoring system. A pilot test was conducted, in which 10 dams were scored by 3 care-staff. Pearsons correlations revealed significant positive correlations between all 3 raters, with a moderate to strong level of association (ranging from 0.667, P=0.035 to 1.00, P<0.001), indicating high inter-rater reliability of the scoring system. However, its validity in the marmoset remains to be established with direct measures of fat, such as ultrasound measurements.

4.2.3 Behavioural development

Behavioural observations

Infant behaviour was recorded in the home cage from 2 to 8 weeks of life. Two sets of 15 minute observations were scheduled a day, one in the morning and one in the afternoon, for three days a week, from week 2 to week 6. One behavioural observation was then conducted per week at weeks 7 and 8. These were evenly distributed between AM and PM (8-12 and 12-4), avoiding cleaning and feeding times to minimise disturbance (Dettling et al, 2002), and matched between conditions. The infants were the focal subjects. Each were marked for identification at 10 days, using fur trimming.

Behaviours were recorded using the protocol outlined in 2.6. Behavioural elements of particular interest were: parent-infant (carry, anogenital lick, retrieve, rub off, rejection, agonistic behaviour); infant-parent (attempt to get on, terminate carrying, proximity, suckling position); infant-infant (social play, proximity) and infant alone (distress vocalisation, scratch, eat, explore, solitary play) (based on Dettling et al, 2002). The first appearance of key behaviours was recorded, e.g. explore, social play, solitary play, eat/forage, to identify which conditions promote early development and independence.

Table 4.2: Behavioural categories used

Behaviour	*Definition*
Caregiver-infant interactions	
Carry [a]	The infant clings to the back of a group member, with its weight supported. The member of the group (mother, father or sibling) was recorded.
Anogenital lick [b]	The caregiver licks the infant's anal region. This is an affiliative behaviour, which also has a cleaning function.
Retrieve[b]	A caregiver picks up an infant not already on the back of another individual, initiating a bout of carrying.
Caregiver transfer	One caregiver passes the infant to another. The caregiver often rolls or vocalises, and another member of the group takes the infant from them (Box, 1977).
Rub off [b]	The parent terminates a bout of carrying, by forcing the infant off, eg. rubbing them against part of the cage (Locke-Haydon and Chalmers, 1983).
Rejection[b]	A caregiver prevents the infant from climbing onto them.
Agonistic [b]	Aggressive behaviour, such as the caregiver lunging at, grasping, snap-biting or cuffing the infant, as well as arch-bristle and 'erh erh' vocalisations. These are usually accompanied by the infant squealing and withdrawing (Stevenson and Poole, 1976). The member of the group (mother, father or sibling) was recorded.
Allogroom [a]	A family member cleans the fur or skin of the infant with its hands or mouth.
Infant-caregiver interactions	
Attempt to get on [b]	The infant approaches and tries to climb on, initiating a bout of time on the caregiver. This may or may not be successful.
Terminate carrying[b]	The infant leaves the carrier's back spontaneously, ending a bout of time on the caregiver.
Proximity to caregiver [a]	The infant is stationary, sitting, crouching or lying next to a caregiver, with some form of physical contact. The member of the group (mother, father or sibling) was recorded.
Suckling position [a]	The infant is carried ventrally (on the mother), with its head in the nipple region.
Active transfer [b]	The infant moves from one carrier to another by itself (Ventura and Buchanan-Smith, 2003).
Infant-infant interactions	
Social play [a]	High activity social interactions involving close, non-aggressive physical contact with the twin, such as wrestling, chasing, grasping, pouncing, back-hugging, batting, biting and mutual investigation. This is often accompanied by the open mouth play face (Stevenson and Poole, 1976). The member of the group (mother, father or sibling) was recorded.
Proximity to same-age sibling [a]	The infant is stationary, sitting, crouching or lying next to the twin, with some form of physical contact.
Infant	
Distress vocalisation [a]	A squeal or 'nga' call made by the infant. ('Tantrum'- Epple, 1978).
Tail pilo-erection [a]	The hair on the tail stands on end. This is associated with some degree of disturbance to the animal.

Table 4.2 continued: Behavioural categories used

Behaviour	*Definition*
Scratch [b]	The animal rapidly moves its hand or foot, drawing its claws across the fur or skin.
Eat/forage [a]	The animal is engaged in any activity directly related to aquiring or ingesting food.
Gouge[a]	The animal makes an indentation in a piece of wood with its teeth.
Inactive [a]	The animal remains stationary whilst alone, without engaging in any other behaviour.
Locomotion [a]	The animal travels between locations by walking, running, climbing or jumping.
Explore [a]	The animal investigates objects in the environment by handling, sniffing, gently biting, licking or attending to them whilst walking around them.
Solitary play [a]	High activity behaviour performed alone, such as hanging and swinging on a rope, chasing tail or gnawing stationary objects, accompanied by rapid movement around the cage (Stevenson and Poole, 1976).
Out of sight [a]	The animal cannot be seen by the observer.
Other [a]	Any other behaviour not noted above.

a. Instantaneous sampling
b. All-occurrence sampling

4.2.4 Statistical analysis

Weight and body condition data

All data were tested for normality using Kolmogorov-Smirnov tests. Cross sectional weights for age were plotted for infants and adults. As data were normally distributed, 2x2 ANOVAs (rearing x gender) were conducted to identify any differences in weight at specific time points. Although those born singletons were consistently heavier than those raised as singletons, there was no significant difference in weight at any time point ($P>0.05$). Therefore, to increase the sample size for singletons, these were analysed together. Mean body condition was calculated, from monthly scores between 12 and 24 months old. As data were not normally distributed, a Kruskall Wallis test was conducted to look at differences in average adult body condition score, between rearing backgrounds. Mann Whitney tests were conducted to find where any differences lay. The association between body weight and condition at each month (12-24 months) was also investigated, using Spearman's rank correlations.

Health records

Descriptive statistics were carried out to summarise the number of health problems recorded for the marmosets studied. Infants and adults were examined separately. Health was divided into 'weight loss/ diarrhoea', 'physical' and 'behavioural'. Those that died either naturally or were euthanised during the course of the project were included, as well as those with no problems recorded. The percentage of each category in each litter condition was calculated.

Behavioural development data

As the 2 infants were recorded simultaneously for practical purposes, there is the potential for pseudoreplication and autocorrelation in observations (Lazic, 2010). Spearman's rank correlations were therefore conducted between litter mates of known ID, using the mean of each behaviour over the observation period (excluding any families with missing data), to see if infants of the same litter were independent. Fifteen of the 23 correlations were significant. Due to dependent data points, as well as not all infants being reliably identifiable, means of the litter mates were used in statistical treatment. Therefore, cage was the unit of measurement.

As no transformations were successful in making data normally distributed (due to the large number of zeros in the data set), as well as the small sample size, two-tailed Kruskal Wallis tests and follow-up Mann Whitney tests were used for all statistical comparisons of behaviour. Total frequencies and estimated percentage of sample time spent in activies were analysed, as well as at weeks 2-5 and weeks 5-8. Each specific week was also analysed. Data from families missing observations (N=5) were excluded from totals and appropriate weekly groupings (where this could artificially reduce overall frequencies and percentages of time), but included in weekly analyses, to prevent data from being lost. As singletons were all female and means were taken for other litter conditions, activities could not be tested between sexes. Means are presented when medians are zero.

For analysis of first emergence of key behaviours, values for both infants were included in analyses. Four groups with missing IDs were excluded (as it was not possible to determine if both infants displayed the behaviour). A ceiling value of 8 weeks was used if the infant did not display the behaviour within the observation period. Data from families were also excluded when there was a missing week of data around a similar time (approximately 1 SD) as when the behaviour should be emerging.

As weight and number of helpers in a family have been shown to affect infant care, Spearman's rank correlations were conducted between these factors and caregiving behaviours. Mean frequencies and estimated percentage of sample time spent in caregiver behaviours were calculated in each week, to approximately match the weigh date. Robust inter-group differences have also been reported. Therefore, differences in total caregiving behaviours (analysis excluded data from families with missing observations) between families with the same litter size were also investigated, using Kruskal Wallis tests.

4.3 Results

4.3.1 Weight

Infants: birth-6 months

Birth weight was only recorded for 4 twins and 0 singletons, and so due to the small sample size, was not analysed further. No significant difference was found between singleton, twin and 2stay infant weight at any time point, except at 1 month (Table 4.3). There was a significant effect of litter size, with twins (mean 69.94 +/- 9.59 (SD)) heavier than singletons (mean 60.84+/-12.93, P=0.046) and 2stays (mean 62.21+/-6.82, P=0.010). There was no main effect of gender. However, there was a significant rearing*gender interaction at this age. Female twins (mean 74.30+/-7.55) were heavier than male twins (mean 65.59+/-9.90), however male 2stays (mean 64.78+/-6.11) were heavier than female 2stays (mean 57.92+/-6.09).

Adults: 12 months-24 months

There was no significant difference in weight between adult twins, 2stays or supplementary fed triplets at any age. Figure 4.1 reveals that triplets were lighter than both twins and 2stays at 12 months. However, by 24 months, twins were lighter than 2stays and triplets.

4.3.2 Body condition

A significant difference in body condition was found between rearing backgrounds (Table 4.3). Adult 2stays and supplementary fed triplets both had significantly higher scores than twins. There was however no difference between 2stays and supplementary fed triplets. There was also no difference between males and females. Figure 4.2 displays the median body condition score (calculated from individual means) for each condition. There was a highly significant, but weak correlation between body weight and body condition score (Table 4.3).

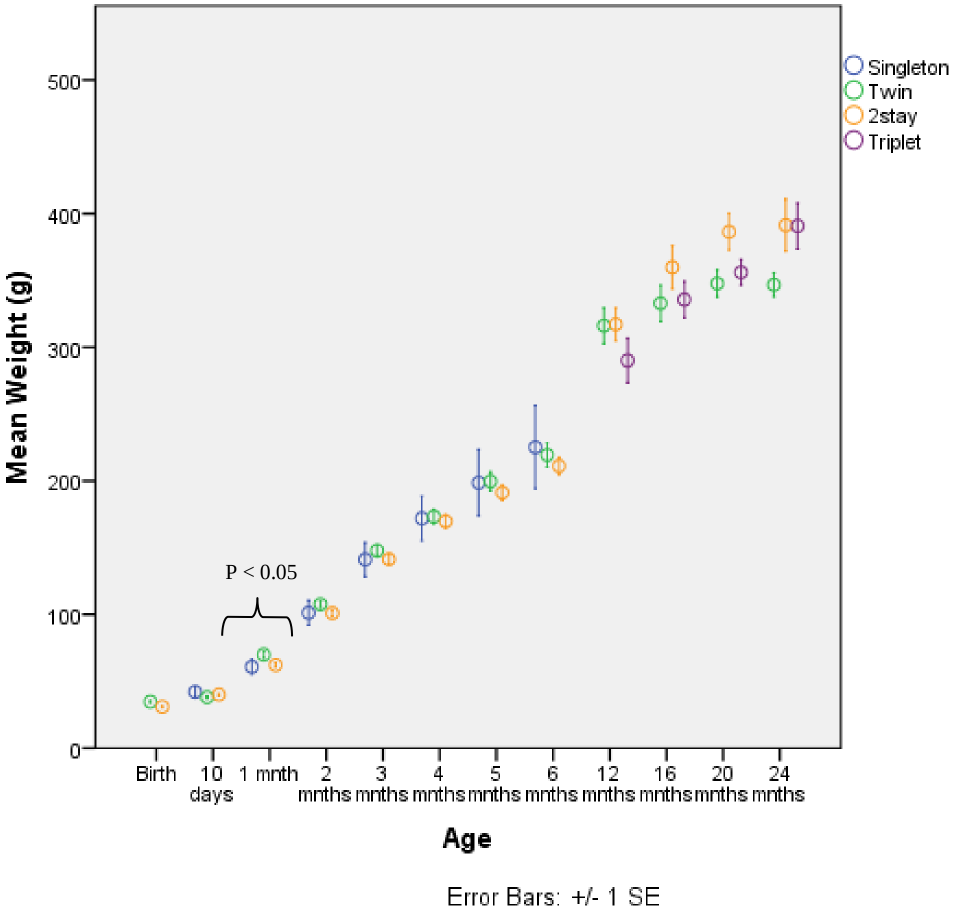


Fig 4.1: Mean (+/- 1 SE) longitudinal cross-sectional mean weight for age in each condition from birth to 6 months (infants) and 12-24 months (adults). Twins weighed significantly ($P<0.05$) more than singletons and 2stays at one month.

Table 4.3: Results of significant tests comparing weights of infants born to different litter sizes and body condition scores of adults raised under different conditions

Test	Analysis	df	Test statistic	P
Weight at 1 month	Rearing	2, 30	F= 4.60	0.018
	Rearing*Gender	1, 30	F= 6.22	0.018
Body condition	Rearing	2	X^2= 9.26	0.010
	Twins < 2stays		U= 25.50	0.005
	Twins < Triplets		U=32.00	0.040
Correlation between weight and body condition			r=0.34	0.001

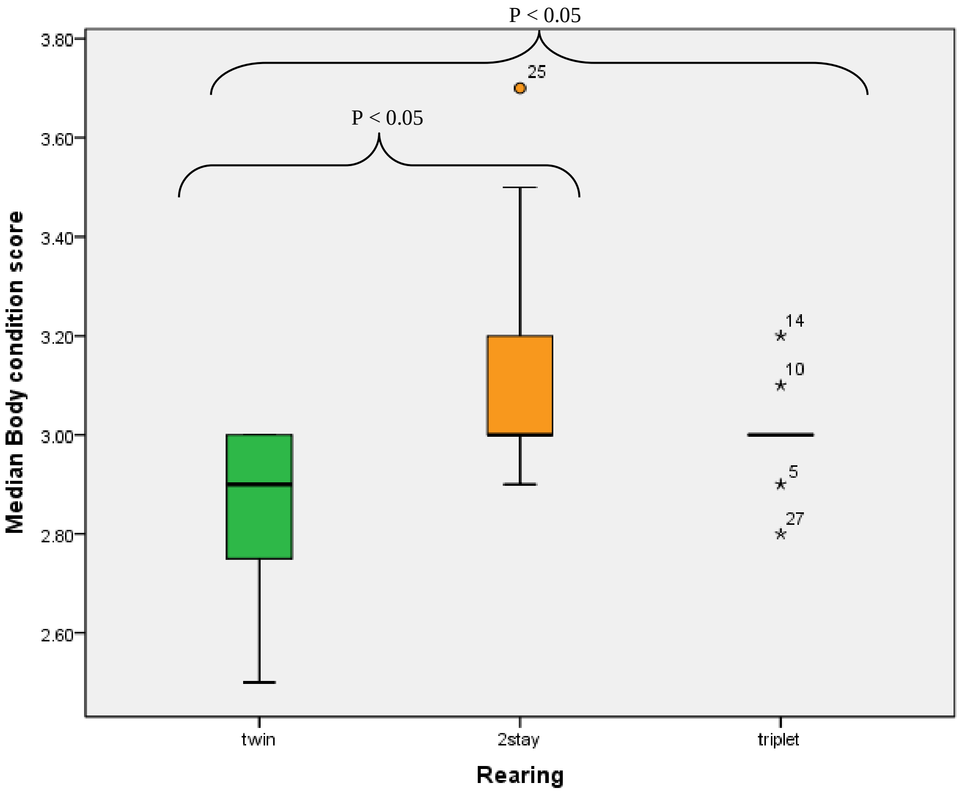


Fig 4.2: Medians for each rearing background of mean body condition score (from 12 months to 24 months) (twins N=11, 2stays N=13, and triplets N=11) Twins had a significantly ($P<0.05$) lower body condition score than 2stays and triplets. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

4.3.3 Health records

Table 4.4 shows the number and percentage of infant and adult marmosets in each rearing condition that experienced weight loss/ diarrhoea, physical or behavioural problems, as well as those that died or had no problems recorded. Six individuals were included in more than one category.

Table 4.4: Health problems recorded in infants and adults studied in each rearing condition

AGE	**Adult**	**Adult**	**Adult**	**Infant**	**Infant**	**Infant**
REARING	**Twin (N=11)**	**2stay (N=13)**	**Triplet (N=11)**	**Singleton (N=5)**	**Twin (N=14)**	**2stay (N=16)**
% Dead	0	7.69	0	20	0	12.50
Total number	**0**	**1**	**0**	**1**	**0**	**2**
Euthanised	0	1	0	1	0	1
Died	0	0	0	0	0	1
% Low weight and diarrhoea	27.27	23.08	36.36	40	7.14	31.25
Total number	**3**	**3**	**4**	**2**	**1**	**5**
Diarrhoea	0	0	2	1	0	0
Low weight	1	2	0	0	0	3
Low weight and diarrhoea	2	1	2	1	1	2
% Physical	72.73	53.85	45.46	0	7.14	31.25
Total number	**8**	**7**	**5**	**0**	**1**	**5**
Reproductive	0	2	0	0	0	0
Dental	1	1	2	0	0	2
Injury	7	4	3	0	1	3
% Behavioural	18.18	0	0	0	0	0
Total number	**2**	**0**	**0**	**0**	**0**	**0**
Repaired due to fighting with partner	2	0	0	0	0	0
% No problems recorded	9.09	38.46	36.36	60	71.43	31.25
Total number	**1**	**5**	**4**	**3**	**10**	**5**

* All animals were euthanised due to low weight and persistent diarrhoea. The singleton euthanised was born to a triplet litter. The 2stay infant died of suspected choking on food.

4.3.4 Behavioural development

Observations over the total 8 week period

Caregiver behaviour

Carrying and suckling were performed for a similar estimated percentage of time over the total observation period between litter sizes. Infants in each litter size were also rubbed off at similar frequencies. There was a trend for total retrievals to be different between litter sizes, with

2stays retrieved significantly more often than singletons. Total frequency of rejections was also approaching significance, with singletons rejected more than 2stays. There was a significant effect of litter size on total number of caregiver transfers. Singletons and 2stays were both transferred more than twins. There was a significant difference between litter sizes in total anogenital licking. Singletons and 2stays both received more than twins. The total estimated percentage of time caregivers spent grooming infants was similar between litter sizes. Agonistic behaviour from caregivers, directed at infants, was very rare, and no difference was found between litter sizes. Table 4.5 shows the results for significant caregiver behaviours. Figures 4.3 and 4.4 show all significant behaviours over the total observation period.

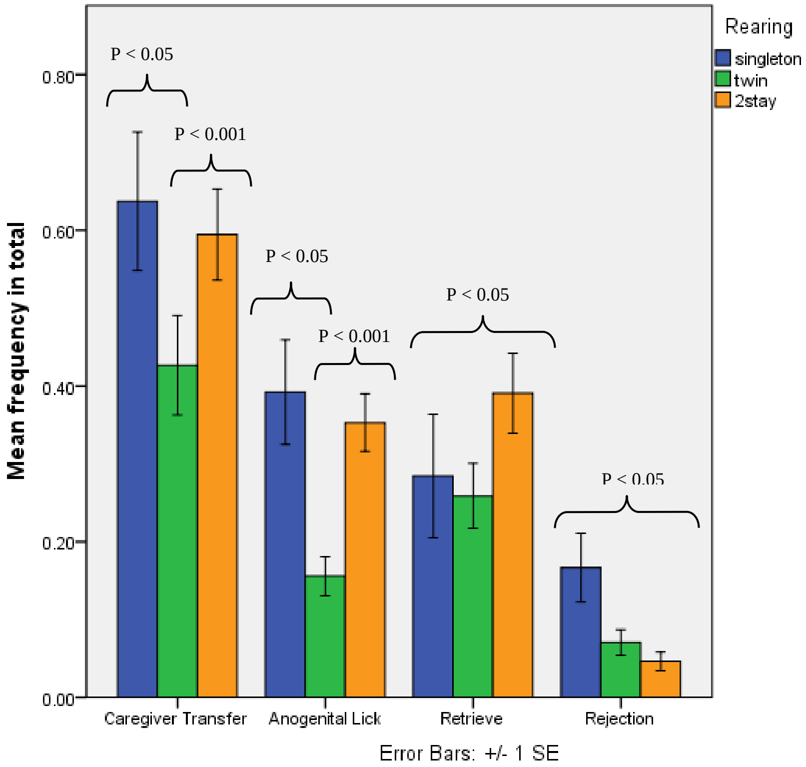


Fig 4.3: Mean (+/- 1 SE) frequency of occurrence (per 15 minutes) of significant behaviours over the total 8 weeks, across litter conditions (Means are presented as medians are zero.) Twins had significantly ($P<0.05$) less caregiver transfers than singletons and 2stays; twins had less anogentital licks than singletons and 2stays; 2stays had more retrievals than singletons; singletons had more rejections than 2stays.

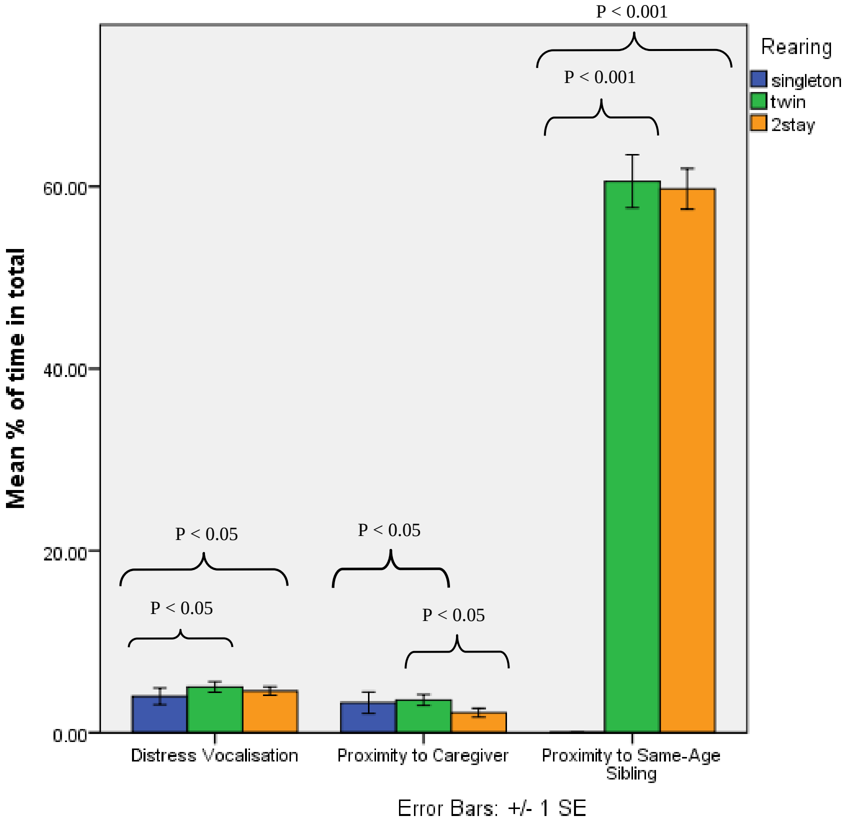


Fig 4.4: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in significant behaviours when in view, over the total 8 weeks, for each litter condition. (Means are presented as medians are zero.) Singletons had significantly ($P<0.05$) less distress vocalisations than twins and 2stays; twins had more proximity to their caregiver than singletons and 2stays; singetons had less proximity to a same age sibling than twins and 2stays.

Infant care-seeking

Overall, infants attempted to get on carriers as frequently in each litter size. Infants terminated carrying bouts very rarely, which was again similar between litter sizes. A significant difference in total proximity to caregiver was found, with twins spending longer close to their carers than singletons and 2stays. Infants of different litter sizes also significantly differed in total distress vocalisations emitted. Twins and 2stays both vocalised more than singletons. Table 4.6 shows the results of significant infant care seeking behaviours.

Key infant behaviours

Infants in each litter size spent a comparable estimated percentage of time inactive, as well as in locomotion and exploration, over the total observation points. In all litter sizes, infants also foraged, gouged and scratched for similar frequecnies and estimated percentages of time. Total proximity to a same-age sibling was however highly significant, as both twins and 2stays were in proximity to their same-age sibling, while singletons were alone. No significant difference was found between litter sizes in total estimated percentage of time spent in social or solitary play. However, Figure 4.5 reveals that twins and 2stays played socially more often than singletons, which is easily explained by singletons not having a same-age companion. Figure 4.6 shows that singletons instead played alone more than twins and 2stays. Table 4.7 displays the results of significant key infant behaviours.

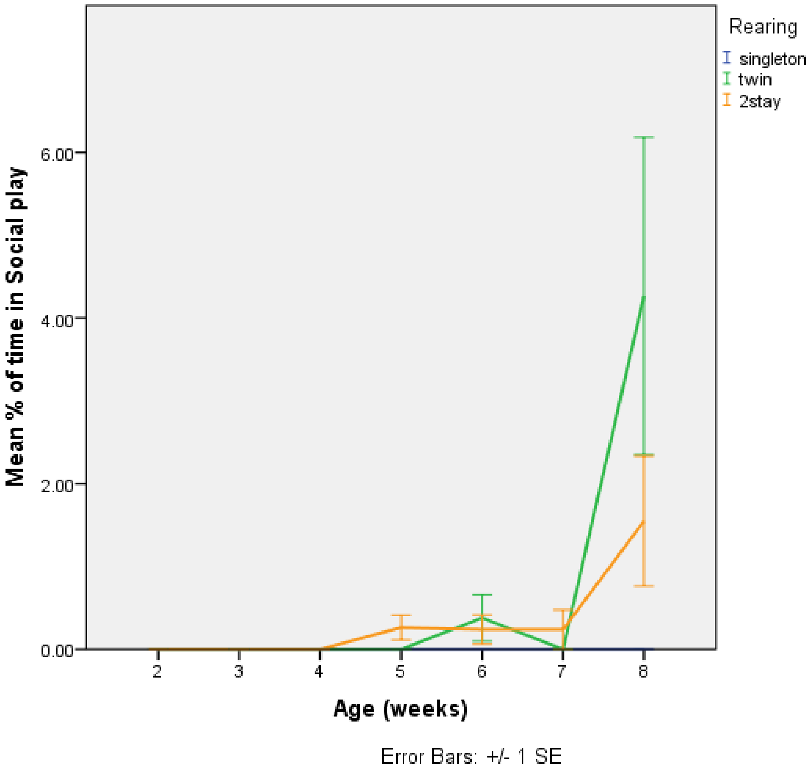


Fig 4.5: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in social play when in view, at each time point, for each litter condition. (Means are presented as medians are zero.)

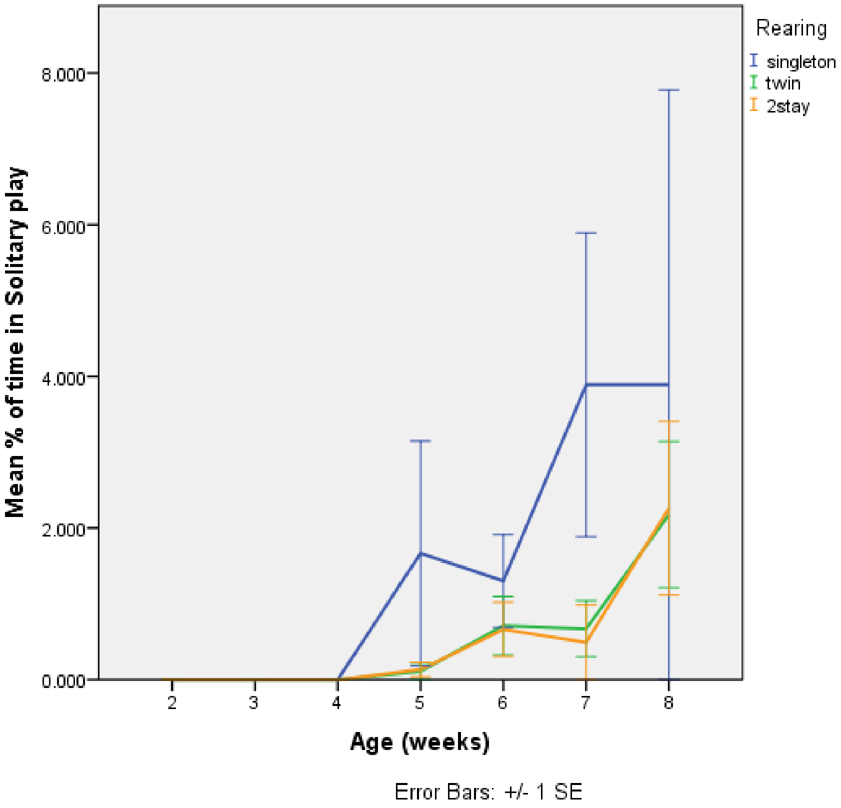


Fig 4.6: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in solitary play when in view, at each time point, for each litter condition. (Means are presented as medians are zero.)

Table 4.5: Significant caregiver behaviours in infant common marmosets (*Callithrix jacchus)* in different litter sizes (Kruskal Wallis H tests and follow-up Mann Whitney U tests)

Caregiver	**Total**			**Weeks**			**Weeks**			**Weekly**		
Behaviour				**2-4**			**5-8**					
	df	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**
Carry				2	H=7.53	0.023	2	H=5.30	0.071	WK 2 2	H=6.31	0.043
Singleton >2stay					U=2754.00	0.012					U=444.00	0.012
Singleton > twin								U=1499.00	0.037		U=360.00	0.037
										WK 4 2	H=6.70	0.035
Singleton > 2stay											U=456.00	0.035
Twin > 2stay											U=653.00	0.031
										WK 7 2	H=8.38	0.015
Twin > singleton											U=34.00	0.033
2stay > singleton											U=28.00	0.006
Suckling position										WK 2 2	H=6.85	0.033
2stay > twin											U=621.00	0.016
Retrieve	2	H=5.39	0.068	2	H=6.23	0.044						
2stay > singleton		U=10690.00	0.022		U=2998.50	0.027						
Twin > singleton					U=2071.00	0.012						
Rejection	2	H=5.48	0.065	2	H=8.03	0.018						
Singleton > 2stay		U=11209.00	0.023		U=3276.00	0.030						
Caregiver transfer	2	H=7.54	0.023				2	H=6.38	0.041	WK 3 2	H=6.04	0.049
Singleton > twin		U=7584.50	0.041								U=335.50	0.017
2stay > twin		U=17536.50	0.009					U=3750.50	0.011			
Anogenital lick	2	H=15.82	<0.0001	2	H=12.09	0.002	2	H=6.19	0.045			
Singleton > twin		U=7453.00	0.011		U=1871.50	0.005						
2stay > twin		U=16344.00	<0.0001		U=4388.50	0.001		U=3774.00	0.015			

Weeks 2-4

Caregiver behaviour

Estimated percentage of time spent carrying in weeks 2-4 was significantly different between litter sizes, with singletons carried more than 2stays. Twins and 2stays were both retrieved more often than singletons. Singletons were instead rejected more frequently than 2stays. A difference was also found between litter sizes in anogenital licking, with parents of singletons and 2stays performing more than parents of twins. Figures 4.7 and 4.8 show all significant behaviours at weeks 2-4. Table 4.5 shows the results for significant caregiver behaviours.

Infant care-seeking and key infant behaviours

During weeks 2-4, active transfers were more frequent in 2stays than twins. Twins and 2stays emitted more distress vocalisations than singletons. Twins however spent longer next to their sibling than 2stays. No other differences were found in infant behaviours at this time point. Table 4.6 shows the results of significant infant care seeking behaviours, and Table 4.7 displays significant key infant behaviours.

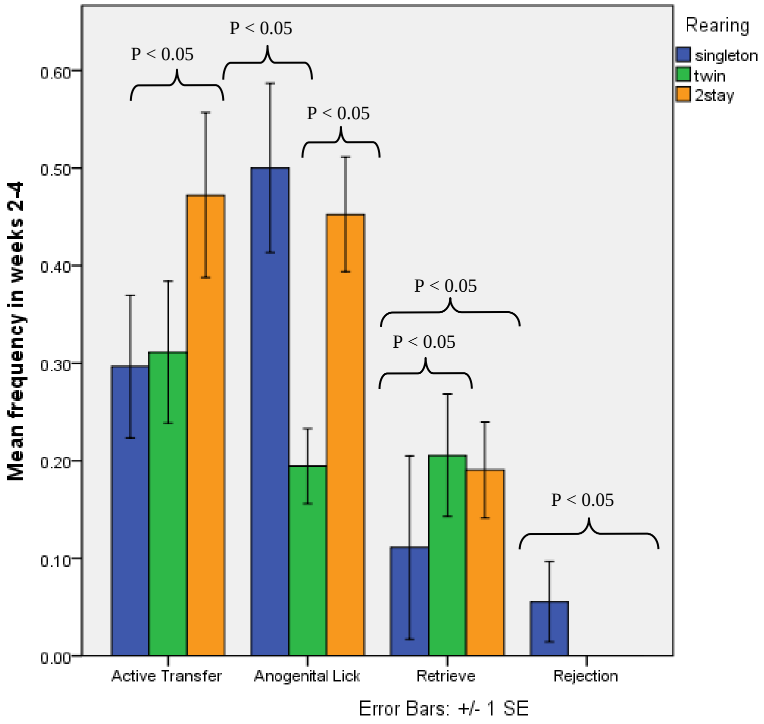


Fig 4.7: Mean (+/- 1 SE) frequency of occurrence (per 15 minutes) of significant behaviours in weeks 2-4, across litter conditions (Means are presented as medians are zero.) 2stays had significantly (P<0.05) more active transfers than twins; twins had less anogenital licks than singletons and 2stays; singletons had less retrievals than twins and 2stays; singletons had more rejections than 2stays.

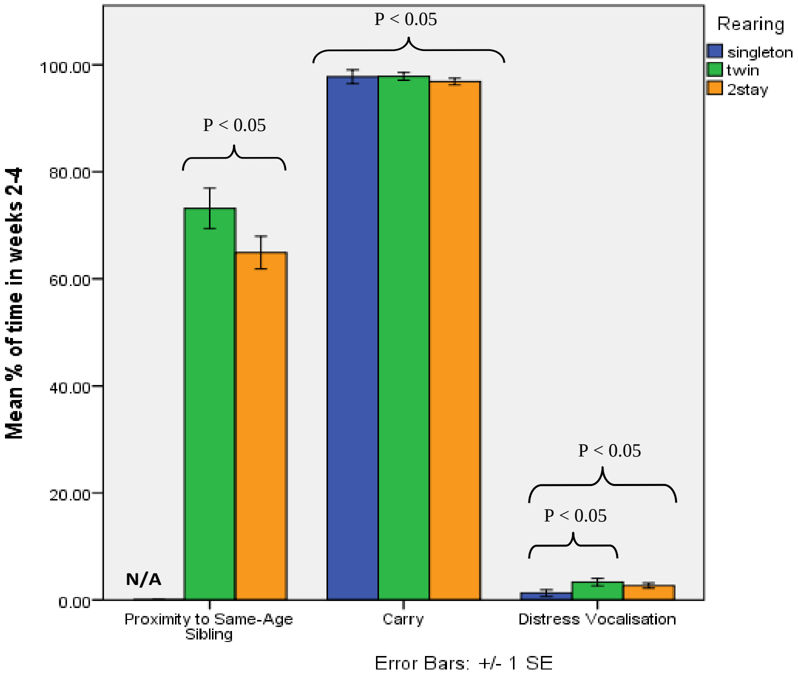


Fig 4.8: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in significant behaviours when in view, in weeks 2-4, for each litter condition. (Means are presented as medians are zero.) Twins had significantly (P<0.05) more proximity to their same-age sibling than 2stays; singletons were carried more than 2stays; singletons had less distress vocalisations than twins and 2stays.

Table 4.6: Significant care seeking behaviours in infant common marmosets (*Callithrix jacchus)* in different litter sizes (Kruskal Wallis H tests and follow-up Mann Whitney U tests)

Infant Care-seeking	**Total**			**Weeks 2-4**			**Weeks 5-8**			**Weekly**		
	df	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**
Active transfer				2	H=6.73	0.035				WK 2 2	H=6.96	0.031
2stay > twin					U=4748.00	0.017					U=689.00	0.050
2stay> singleton											U=427.50	0.029
										WK3 2	H=6.83	0.033
2stay > twin											U=712.50	0.008
Proximity to caregiver	2	H=8.17	0.017				2	H=13.09	0.001	WK 6 2	H=8.02	0.018
Twin > singleton		U=7596.00	0.034					U=1458.00	0.018			
Twin > 2stay		U=17784.00	0.009					U=3210.00	<0.0001		U=538.50	0.002
Distress vocalisation	2	H=6.71	0.035	2	H=6.30	0.043						
Twin > singleton		U=7279.00	0.013		U=1988.00	0.018						
2stay> singleton		U=10452.50	0.024		U=2824.00	0.020						

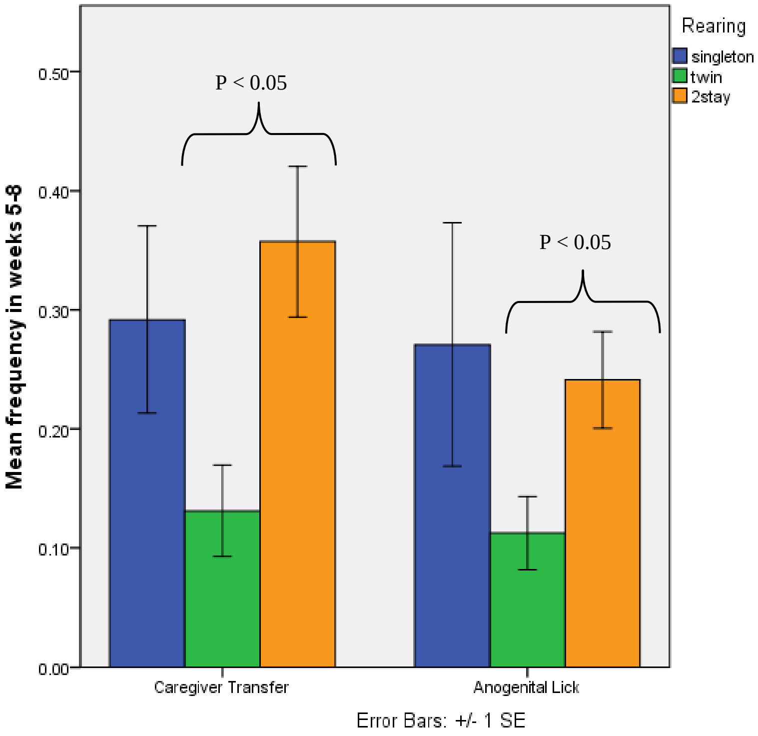
Weeks 5-8

Caregiver behaviour

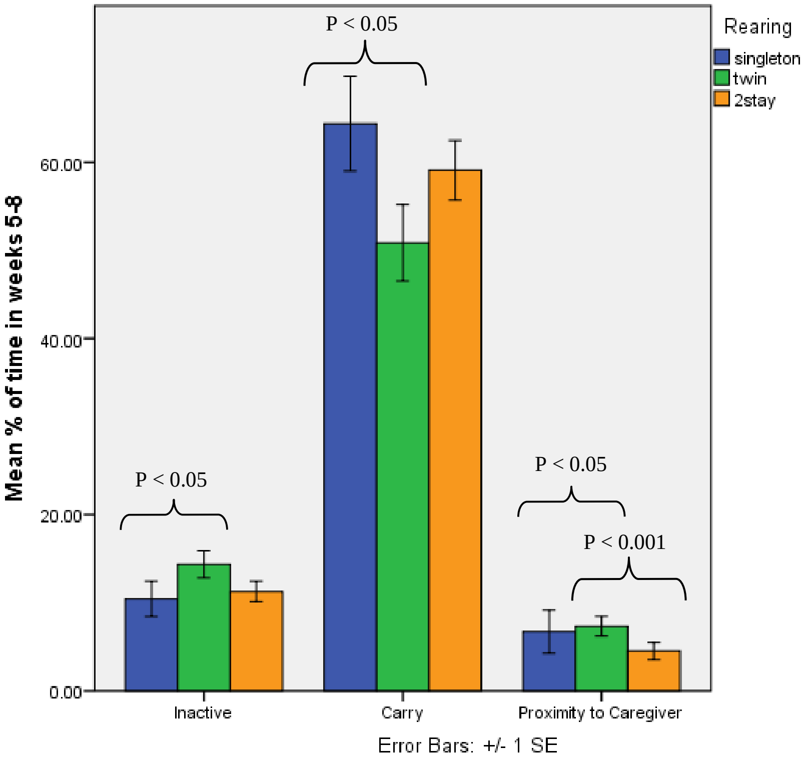
There was a trend for estimated percentage of time spent carrying in weeks 5-8 to be different between litter sizes, with twins carried less than singletons. 2stays were transferred between caregivers significantly more frequently than twins. 2stays also received more anogenital licks from parents than twins. Figures 4.9 and 4.10 show all significant behaviours at weeks 5-8. Table 4.5 shows the results of significant caregiver behaviours.

Infant care-seeking and key infant behaviours

There was a significant difference between litter sizes in proximity to caregivers at weeks 5-8, with twins spending longer in proximity to carers than singletons and 2stays. There was also a trend for twins to spend more time inactive than singletons. Table 4.6 shows the results of significant infant care-seeking behaviours, and Table 4.7 displays significant key infant behaviours.



Figures 4.9: Mean (+/- 1 SE) frequency of occurrence (per 15 minutes) of significant behaviours in weeks 5-8, across litter conditions. (Means are presented as medians are zero.) Twins had significantly (P<0.05) less caregiver transfers than 2stays; twins had less anogenital licks than 2stays.



Figures 4.10: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in significant behaviours when in view, in weeks 5-8, for each litter condition. (Means are presented as medians are zero.) Singletons were significantly (P<0.05) less inactive than twins; singletons were carried more than twins; twins were in proximity to their caregiver more than singletons and 2stays.

Table 4.7: Significant key behaviours and first emergence behaviours in infant common marmosets (*Callithrix jacchus)* in different litter sizes (Kruskal Wallis H tests and follow-up Mann Whitney U tests)

Infant Behaviour	**Total**			**Weeks 2-4**			**Weeks 5-8**			**Weekly**		
	df	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**
Inactive							2	H=5.29	0.071			
Twin > singleton								U=1499.00	0.036			
Proximity to same-age sibling	2	H=203.52	<0.0001									
Twin > singleton		U=867.00	<0.0001									
2stay > singleton		U=1224.00	<0.0001									
Twin > 2stay					U=4338.50	0.003						
Locomotion										WK7 2	H=10.74	0.005
Singleton > 2stay											U=34.00	0.034
Singleton > twin											U=18.50	0.001
										Wk 8 2	H=6.06	0.048
Twin > 2stay											U=58.00	0.025
Explore										Wk 7 2	H=5.22	0.074
Singleton > 2stay											U=41.50	0.036
Solitary play										Wk 7 2	H=11.45	0.003
Singleton > twin											U=36.00	0.030
Singleton > 2stay											U=31.50	0.002
Forage										Wk 7 2	H=7.33	0.026
Singleton > 2stay											U=33.00	0.006
First emergence												
Social play	2	H=9.08	0.011									
2stay < singleton		U=10.00	0.021									
2stay < twin		U=21.00	0.022									

Weekly

Caregiver behaviour

Analysis of carrying by individual weeks revealed a difference between litter sizes at week 2, when singletons were carried for longer than twins and 2stays. There were also differences at week 4, when 2stays were carried less than singletons and twins, and week 7, when singletons were carried less than twins and 2stays. 2stays spent longer in the suckling position than twins at week 2. Singletons were transferred between parents significantly more than twins at week 3. Table 4.5 shows the results of significant caregiver behaviours.

Infant care-seeking and key infant behaviours

2stays performed more active transfers than singletons and twins at week 2. Active transfers were also more frequent in 2stays than twins at week 3. At week 6, twins spent more time in proximity to caregivers than 2stays. Singletons spent more time in locomotion than twins and 2stays at week 7. However, by week 8, twins were spending longer in locomotion than 2stays. At week 7, there was a trend for singletons to explore more than 2stays. At this time, singletons also foraged for longer than 2stays, as well as spent more time in solitary play than both twins and 2stays. Figure 4.11 displays the significant behaviours at week 7, while Table 4.8 shows the means of all other significant weekly behaviours. Table 4.6 shows the results of significant infant care-seeking behaviours, and Table 4.7 displays significant key infant behaviours over all the analysed time points.

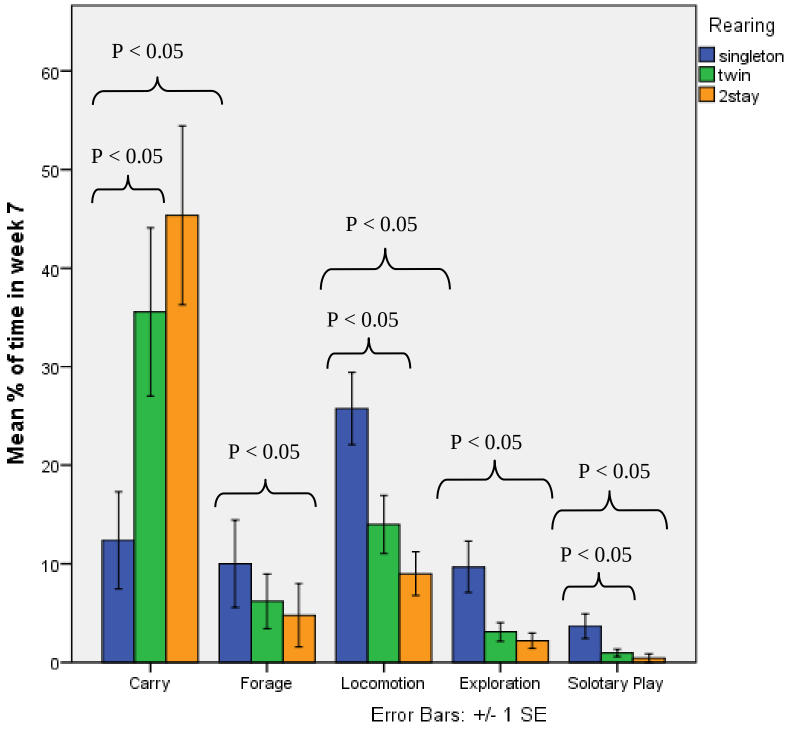


Figure 4.11: Mean (+/- 1 SE) estimated percentage of the 15 minute observation period spent in significant behaviours when in view, at week 7, for each litter condition. (Means are presented as medians are 0.) Singletons were carried significantly ($P<0.05$) less than twins and 2stays; singletons foraged more than 2stays; singletons spent more time in locomotion than twins and 2stays; singletons explored more than 2stays; singletons spent more time in solitary play than twins and 2stays.

Table 4.8: Other significant weekly behaviours (Means +/- SD). (Statistical data in Tables 4.5, 4.6 and 4.7)

Behaviour	**Singletons**	**Twins**	**2stays**
Caregiver behaviour			
Carry			
Week 2	100.00	99.40+/-1.67	99.06+/-2.40
Week 4	94.37+/-13.78	94.96+/-10.70	92.72+/-10.31
Suckling position			
Week 2	14.51+/-28.85	12.38+/-28.18	22.14+/-28.79
Caregiver transfers			
Week 3	1.17+/-0.92	0.69+/-1.06	0.76+/-0.91
Infant care-seeking			
Active transfer			
Week 2	0.13+/-0.34	0.21+/-0.51	0.36+/00.55
Week 3	0.42+/-0.58	0.30+/-0.60	0.58+/-0.70
Proximity to caregiver			
Week 6	7.88+/-13.56	7.56+/-10.36	4.20+/-10.03
Infant behaviour			
Locomotion			
Week 8	11.91+/-12.11	21.26+/-12.41	12.71+/-10.29

4.3.5 First emergence of key behaviours

There was no difference between litter sizes in first recorded attempt to get on carriers, with medians all around 4 weeks, or to terminate carrying (median: singletons 4.5, twins 4.0 and 2stays 6.0 weeks). All infants were first seen engaging in locomotion and exploration at around week 4. Infants from each litter condition started to forage between weeks 5 and 6, and to gouge between weeks 7 and 8. First day to solitary play was not significantly different between litter sizes, although medians reveal that singletons engaged in solitary play earlier (week 5) than twins and 2stays (weeks 7.5 and 8.0 respectively). Only first day to social play was significant, with singletons and twins both later than 2stays. Table 4.7 shows the results of key infant behaviours that were significantly different between litter sizes. Figure 4.5 displays the mean estimated percentage of the 15 min observation period each litter condition spent in social play when in view. 2stays first played socially at week 5, followed by twins at week 6. Singletons had not played socially by week 8.

4.3.6 Additional factors influencing caregiving: Family ID, family size and infant weight

Significant differences in caregiver behaviours were found between families with infants of the same litter size. Table 4.10 displays caregiver behaviours that differed significantly between families within each litter size.

Table 4.10: Differences found in caregiving between families of the same litter size.

Litter size	Behaviour	df	X^2	P
Singletons	Retrieve	2	11.43	0.003
	Carry	2	9.77	0.008
	Caregiver transfer	2	6.55	0.038
Twins	Rejection	4	14.91	0.005
	Carry	4	10.66	0.031
2stays	Anogenital lick	6	27.75	<0.001

Significant correlations and trends were found between family size and caregiver behaviours at each time point, as well as between weight and caregiver behaviour, when each litter size was combined and within each litter size. Table 4.9 displays these correlations. Although rejections of singletons in week 4 were not significantly correlated with weight (r=0.707, P=0.182), 4 infants (weighing 50-60g) were not rejected at all, while one heavy infant (85g) was rejected more frequently.

Table 4.9: Spearman's rank correlations between caregiver behaviours and infant weight or family size

Age	Behaviour	Weight		Family size	
		r	P	r	P
Overall					
Week 2	Anogenital lick	0.588	0.021		
Week 4	Carry	-0.444	0.057 (NS)		
Week 8	Retrieve			-0.467	0.038
Singletons					
Week 2	Suckling position			-0.949	0.051 (NS)
Week 4	Carry	-0.975	0.005		
Week 8	Rejection	0.872	0.054 (NS)		
Twins					
Week 2	Caregiver transfer			0.783	0.066 (NS)
Week 8	Retrieve			-0.746	0.054 (NS)
2stays					
Week 2	Carry	0.750	0.052 (NS)	0.673	0.067 (NS)
Week 4	Suckling position			-0.840	0.018
Week 8	Carry	-0.671	0.069 (NS)		

4.4 Discussion

4.4.1 Physical development

Body weight, condition and health

The body weight and condition of marmosets born and raised under different conditions were investigated, to look at any potential influences on physical development. Although previous research has found differences in birth weight between litter sizes (Jaquish et al, 1991), there were insufficient data to compare this in the present study, as only litters larger than two were consistently weighed at birth. There was however no difference between the litter sizes at the first weighing (day 10), or at any time point, except 1 month. At this age, twins were heavier than singletons and 2stays. While reduced body weight has been found in primate models of early parental separation (Dettling et al, 2002), data from adults suggest that there was no evidence for long-term reductions in body weight in marmosets that had experienced separation from the family for supplementary feeding.

Although there was no difference in adult weight from 12 to 24 months, average body condition during this time was significantly lower in twins than in supplementary fed triplets and 2stays, with twins also weighing approximately 45g less at 24 months (approximately 11.5% reduction). However, they may be lean and so have an average score a little below the 'normal' body condition of 3. As wild marmosets tend to weigh less than captive animals (Araujo et al, 2000), they are also likely to be rated as underweight (score of 2), although be healthy. As well as this, while significant, there was only a weak positive correlation between body condition and actual body weight, with a score of 3 having a large weight range. This may be due to differences in frame size, and so body condition is likely to provide a useful adjunct to weight alone (Tasker, 2012).

As well as birth weight, several other factors can affect growth and adult body weight (Tardif and Bales, 2004). As large litters are often born to larger mothers (Tardif and Jaquish, 1997), it is possible that genetic mechanisms, increased lactation (Tardif and Bales, 2004) or shared environment, such as food preferences (Reilly et al, 2005), lead to 2stays being heavier than twins. Early post-natal diet and gene-nutrient interactions can also influence weight gain and fat disposition (Martinez et al, 2012). Supplementary feeding of triplets in early life could therefore have an impact on later physical development. Environmental cues from the mother, such as stress from separation, and over or under nutrition, can also affect the neonate (Langley-Evans and McMullen, 2010), with programmed outcomes depending on the stimuli.

There may therefore be a relationship between rearing background and health in adulthood (Tardif and Bales, 2004).

While the early life stress of separation from the family has been found to interfere with physiological needs of young marmosets (Parker and Maestripieri, 2011), examination of each animal’s records suggests that animals in any litter size could experience low weight and diarrhoea. Although this was highest in adult triplets and infant singletons, sample sizes were not equal in each condition. Injuries were also common in adults, particularly in twins, due to fighting when paired. Infant singletons had no injuries, possibly as they had no twin to establish dominance with in the home group. The majority of twin infants had no problems recorded. While Jaquish et al (1997) did not look directly at health, they did find that survivorship was not effected by litter size after one month of age. Instead, larger infants of any given litter size had higher survival at 6 months than their lighter counterparts (Jaquish et al, 1997).

All animals in the current study were followed past 6 months of age, when mortality is low. Only four deaths were recorded, in infant singletons, and in both infant and adult 2stays. Three were euthanised due to low weight and persistent diarrhoea, while the fourth died unexpectedly, possibly due to choking. All of those euthanised were born to triplet litters. There is therefore some evidence that infants from triplet litters, where at least one infant was lost at birth, had lower survivorship than other litter conditions. As well as physical development, litter size could have an impact on behavioural development.

4.4.2 Behavioural development

Caregiver behaviour

The type of family interactions alter as young monkeys achieve independence and grow increasingly interested in their siblings and the environment around them (Ingram, 1977). These species-typical interactions provide specific forms of stimulation, and are essential in promoting growth and development (Dettling et al, 2007). Infants in three distinct litter conditions were studied from week 2 to week 8. Although litter size appeared to have few major overall effects on behaviour, some consistent differences did emerge.

All infants were carried almost continuously for the first 4 weeks of life. Singletons were carried more than 2stays in weeks 2-4 and more than twins in weeks 5-8. Ingram (1977) found that all singletons were carried for longer than twins by their father. However, while female twins and singletons spent a similar amount of time off their carriers, the male singleton spent more time being carried and suckled than male twins. 2stay infants were carried in the suckling position for longer than twins at week 2. They were also retrieved more and rejected less than singletons over all the observations. While some previous work has found that twins are more likely to be rejected and transferred than singletons, as greater effort is required to carry them (Caperos, et al, 2012), Ingram also found that the single male was rejected more than twins (Ingram, 1977). As frequency of rubbing off and attempts to get on were similar in each litter condition, singletons seemed to spend more time on the carrier, although once off, attempts to get back on were rejected more. Locke-Haydon and Chalmers (1983) also found that caregivers are less likely to accept infants, rather than rub them off more, with number of rejections failing to negatively correlate with time spent being carried. Only singletons were rejected in weeks 2-4. However, this was due to one infant in particular, who was heavier at an early age, which reflects issues with the small sample size. There was also a gender bias when interpreting data. As only female singletons were available to study, results may not be generalisable to males.

Parents of twins in the current study transferred the infants, as well as anogenitally licked them, less in total than in the other 2 conditions. Both behaviours also occurred less frequently in twins than 2stays in weeks 5-8. Anogenital licking, which has been linked to the marmoset’s extensive scent marking repertoire (Stevenson and Poole, 1976), in early infancy has been found to be associated with reduced fear responses and increased exploration later in life (Kaplan and Rogers, 1999). It is possible that the young have certain behavioural or physiological attributes that trigger anogenital licking and also increased exploration (Kaplan and Rogers, 1999).

These results suggest that 2stay infants could receive more tolerant family rearing, being suckled more early in life and retrieved more often, perhaps following loss of an infant. Singletons however may receive more rejective rearing, as parents appear more intolerant towards their dependent young, with infants seeking more care than some of their caregivers were prepared to give (Locke-Haydon and Chalmers, 1983). However, certain caregivers appeared to compensate for the lack of attention from other group members, as singletons were carried more in each weekly grouping. Locke-Hydon and Chalmers (1984) suggest that while some families may not compensate for the lack of caregiving by certain group members, particularly highly caring individuals may offer more care than other family members. There can be considerable variation in the mother's capacity or willingness carry their young (Santos et al, 1997), which may be due to weight of the infant or differences in maternal style. Ingram (1977) observed that the mother rejected infants' attempts to be carried more frequently than the father, suggesting that she may be more responsible for promoting infant independence. Instead, the father appeared more tolerant and receptive to the infants' attempts to be carried (Yamamoto, 1993). As 2stays and singletons received more anogenital licks and more transfers between parents than twins, both may receive more protective rearing from certain caregivers, as they seemed more willing to carry the young monkeys, taking them off others, rather than leaving them alone.

Over protective parenting, in which infants are kept in close proximity, or highly rejecting parenting, involving limited nurturing behaviour, can influence the development of young animals (Maestripieri, 2011). Infants reared by highly rejecting mothers can become anxious later in life. Studies have found that they generally develop independence at an earlier age than infants with less rejecting mothers (Bardi and Huffman, 2006: *M. mulatta* and *M.fuscata*), and engaged in more solitary play (Maestripieri et al, 2006: *M. Mulatta*). Conversely, infants reared by more protective mothers had delayed acquisition of independence and tended to be more fearful. Attachment theory (Bowlby, 1969) therefore predicts that differences in exposure to caregiving behaviour and responsiveness early in life influences reactivity to the environment (Parker and Maestripieri, 2011). These can become stable and persistent responses throughout life, and are further emphasised in stressful situations (Box, 1991). While exposure to too much or too little of these caregiver behaviours could lead to insecurity and stress vulnerability, infants exposed to moderate levels may become more resilient to later life stress (Parker and Maestripieri, 2011).

Infant care-seeking and key behaviours

Although time being carried appears to be controlled by caregivers, increases in play and other key behaviours tend to be initiated by infants. Independence is therefore a product of both caregiver and infant behaviour (Locke-Haydon, 1984). Some significant differences in this were found between litter sizes. Possibly as singletons were carried for longer estimated percentages of time, they emitted less distress vocalisations than twins and 2stays. Twins were carried less than singletons in weeks 5-8, when independence is being encouraged, instead spending a longer amount of time inactive. The data therefore suggest that twins may gain independence earlier than the other litter conditions, or at least singletons, spending more time off their carriers and learning to cope alone.

Twin infants continued to seek proximity to their caregivers for a longer estimated percentage of time than both other litter conditions at this early age. Increases in caregiver contact are often associated with decreases in carrying in marmoset families (Ingram, 1977). Results are contrary to previous reports that singletons spend more time in contact with group members than twins (Ingram, 1977; Cleveland and Snowdon, 1984: *S. oedipus*). Twins were also in proximity to their same-age sibling for a longer amounts of time than 2stays during weeks 2-4. Locke-Haydon and Chalmers (1983) observed that while twins do not tend to interact with each other at first, they do become more important during early independence (Yamamoto, 1993). As singletons were the only infant, they unavoidably spent no time in proximity to a same-age sibling.

Although there were similar overall estimated percentages of time spent in key behaviours, differences were found at specific weeks, particularly at week 7. At this time, singletons were carried less than the other two conditions, instead exploring and foraging more than 2stays, as well as engaging in more solitary play than both other litter sizes. The increased foraging may be a sign of maturity, regardless of caregiver attitudes, and perhaps due to higher levels of activity (Ventura and Buchanan-Smith, 2003). The result supports Box's (1975a) suggestion that singletons would spend more time in solitary play, due to lack of a twin. However, it is contrary to previous observations of cotton-top tamarins by Cleveland and Snowdon (1984), who found that singletons engaged in less of all types of play than twins. While no significant difference was found in social play between litter conditions, graphs suggest that twins and 2stays engaged in more of this behaviour than singletons, particularly at week 8. As Cleveland and Snowdon (1984) only found a significant increase in twins, compared to singletons, after week 15, observations were perhaps not carried out over a sufficiently long period of time for a significant difference to be seen. During the last 2 weeks of the observation period, when infants tend to spend much less time on the carriers back, differences in locomotion were seen. Infants from 2stay litters spent less time in locomotion than singleton infants at week 7, and less time than twin infants at week 8. However, as missing data were a problem, differences between individual weeks and weekly groupings may be due to exclusion of some families at certain time points.

While previous work has suggested that singletons are more insecure than twins, results from the current study reveal that singletons were actually quite independent when off their carrier. This could be associated with the higher levels of rejections, leading to more independent infants. However, this was only at week 7. No overall or continuing effect at week 8 was seen. Instead, 2stays appear to be less active, particularly than singletons, towards the end of the observation period, which may be related to a more protective rearing style, providing different opportunities to explore and develop coping strategies (Parker et al, 2011). As exploration and locomotion is reduced, development may be narrowed in terms of what they learn about their environment (Box, 1991). Restricted experience of manipulation of objects in infancy has been found to lead to generalised caution or over reactivity to novel objects (Menzel et al, 1963: *Pan troglodytes*). Any reduced general activity, as well as rearing style and attachment type (Bowlby, 1969), could therefore influence temperament and stress resilience.

4.4.3 Infant independence

The first occurrence of certain key behaviours was used as markers of infant development (Yamamoto, 1993; Ventura and Buchanan-Smith, 2003). Many of these emerged a similar time in the different litter conditions. Getting on and off carriers, as well as locomotion and exploration were seen at around 4 weeks (28 days), while foraging emerged at 5 weeks (35 days). Gouging, solitary and social play appeared a little later, at around 7 weeks (49 days). These average ages are similar, if a little later than, those found in other captive marmoset infants, particularly for social and solitary play. The differences in first emergence may be because other authors observed the infants for a longer period of time (eg. Yamamtoto, 1993: 3 hours/week). Yamamtoto (1993) found that on average infants first left the caregiver on day 15.4 and first tasted food on day 28.6. Solitary play first appeared at 19.6 days, while social play first occurred at 31.6 days. Similarly, wild infants first left their carrier on day 13 (Alonso, 1984). They were foraging by 32 days, although playing socially at 61 days (Stevenson, 1988; Alonso, 1984), which is closer to results in the present study. However, the range can be substantial (see Table 1.1), and first behaviours can be difficult to observe in the field.

Although 2stays appeared to engage in less locomotion than the other litter conditions, they were first seen playing socially earlier than both singletons and twins. There did appear to be some variation between 5 and 8 weeks for twins and 2stays, while the only first emergence was at 8 weeks for singletons. Box (1975b) recorded consistent social play between twins developing at 5-7 weeks old. However, this was only one family, with successive litters. Play increases as the infants get older, especially when in settled family groups with large, appropriately designed cages (Box, 1975a). Therefore, while twins and 2stays often first play together, singletons had no other companion (Yamamoto, 1993). Singletons instead only played with other family members when they were older, or engaged in solitary play.

Being raised with a same-age sibling allows young marmosets to develop socially together and may help to promote security. It has been suggested that play may have an adaptive function, improving spatial cognition and motor skills (Bertenthal and Campos, 1987), as well as enhancing emotional skills (Pellis and Pellis, 2009), which could both promote coping abilities in captivity (Ventura and Buchanan-Smith, 2003). The ability to respond flexibly to environmental change is necessary for primates, particularly given their complex social relationships and interactions (Box, 1991). Playing and interacting with the twin may therefore be particularly important in learning and developing independence (Box, 1991; Yamamoto, 1993; Cleveland and Snowdon, 1984: *S. oedipus*).

The increases in time spent in social or solitary play towards the end of the observation period in all three conditions could also be considered a sign of good welfare (Lee, pers. corr). Play behaviour is thought to be extremely desirable in captive primates, tending to be displayed when stress is minimal (Lee, 1983). For example, substantial drops in play behaviour have been found in rhesus macaques during food shortage (Loy, 1970), and will disappear completely in injured animals (Fagen, 1981). Social play is also thought to be associated with opioid-mediated pleasant emotional experiences (Fraser and Duncan, 1998), and could become a prominent behaviour due to its rewarding properties, although this has yet to be established for solitary play (Held and Spinka, 2011). However, increases in play can occur following challenging conditions (Loranca et al, 1999), or could lead to stress if used to establish dominance (Mendl et al, 2010). Individual differences, such as age (Held and Spinka, 2011) and personality (Biben and Champoux, 1999), can also influence frequency of the behaviour. Despite these potential difficulties with interpreting the welfare implications of play, animals feeling well will often play more, and gain psychological benefits from it. Play may therefore not only result from good welfare, but could also cause it (Held and Spinka, 2011).

4.4.4 Additional factors influencing care and development

Although litter size appears to have some effect on caregiver behaviour, several other factors could have an influence, including family rearing style, infant weight and family size. Correlations indeed revealed that increased weight was associated with increased anogenital licking (over all litter sizes) and decreased carrying (in singletons). There was also a trend for increased weight to be associated with increased rejections. Results support previous work, which has found caregivers reduce travelling speed when carrying heavier infants, suggesting that greater effort is required (Caperos et al, 2012). Artificially increasing the weight carried also lead to earlier maternal rejection of infants and subsequent paternal carrying in Goeldi's monkeys (Anzenberger et al, 2007).

Much previous research has found that parents may alter how much care they provide to their offspring depending on the potential contributions from helpers (McGrew, 1988: *S. oedipus*). Greater family sizes in the current study was correlated with decreased retrievals and decreased suckling position. There were also trends for family size to be associated with increased infant transfers between caregivers and increased carrying. Ingram (1977) and Cleveland and Snowdon (1984*: S. oedipus*) found that the mother decreases the amount of care she gives to her young when other caregivers are available, which could explain the decrease in suckling position with larger family sizes. The mother may limit her role to the provision of food for infants, leaving other family members to provide transportation and comfort behaviours. Competition to carry infants is also greater within large groups, with group members pulling an infant from another's back (Cleveland and Snowdon, 1984: *S. oedipus*), which may explain the increased caregiver transfers in larger groups. Jaquish et al (1997) further showed that the presence of helpers had a positive relationship with survival. Evidence therefore suggests that responses to infants, and perhaps their subsequent survival, is linked to the number of family members able to help with their care.

Although there are general similarities in overall patterns of caregiver behaviour, there can be marked differences between family groups, as well as within family groups over time (Box, 1977), which can have a considerable effect on the behaviour of infants (Dettling et al, 2007). Differences in some caregiver behaviour was found between families of the same litter size, including carrying, retrievals, rejections, transfers between caregivers and anogenital licking. Locke-Haydon and Chlamers (1983) have also found substantial differences in the amount of care group members are willing to give, and amount of anogenital licking has been found to vary considerably between individuals (Kaplan and Rogers, 1999). Other factors, including maternal age, prior social experience and adequacy of lactation, may also play a role in the rearing of young (Tardif et al, 1984). Therefore, there may be a complex inter-relationship between elements, including litter size (Ingram, 1977).

4.4.5 Conclusion

The present study provides information on differences in caregiving and key infant behaviours, as well as growth, health and survial, of marmosets born and reared under different backgrounds. Possible differences in family rearing style and level of independence were revealed. Differences in adult body condition score and probability of suffering from low weight were also found. Overall, results suggest that, while there were no major differences, litter size could have some small impact on welfare. Early life stress, including high levels of parental rejection or protection, as well as separation from the family, could also influence the stress response and temperament of adult animals.

CHAPTER 5: The impact of rearing background on the welfare of common marmosets *(Callithrix jacchus):* Effects on behavioural and physiological responses to routine stressors

Abstract

There is much evidence that early life experience, including separation from the family, can influence both vulnerability and resilience to stress. The current study investigated the effect of rearing background (twins, 2stays and supplementary fed triplets) on the behavioural and HPA axis response to the routine stressor of capture and weighing in adult common marmosets. Overall, and unexpectedly, salivary cortisol decreased from baseline to post capture, although individual variation was high. The decrease in cortisol was accompanied by significant increases in stress-related behaviour. While there were no significant differences in cortisol level between rearing conditions, 2stays demonstrated significantly greater deviations from baseline than the other conditions. Although there were no significant differences between rearing conditions, twins displayed significantly more behavioural disturbance than the other conditions following the stressor. Instead, there were increases in some positive behaviour post capture in supplementary fed triplets. This provides some evidence to support the model of stress resilience, suggesting that moderate early life stress (as in the supplementary fed triplets) could contribute to an enhanced coping ability to later stressful life events. While family separation is not recommended, early positive interactions with humans could reduce fear and improve the welfare of marmosets used in laboratory research and testing.

5.1 Introduction

5.1.1 Stress in the common marmoset

Primates face a number of potentially stressful experiences when kept in laboratories, resulting from the captive environment and routine husbandry procedures, as well as experimental manipulations (Bassett et al, 2003). Several indicators of stress have been identified in the common marmoset, including changes in the display of certain behaviours. Bassett et al (2003) observed a reduction in inactivity following capture and removal from the homecage for weighing. Increases in self-scratching, thought to be a displacement activity in primates, were also seen. These behavioural changes persisted for at least four hours post stressor, before returning to baseline levels. Administration of the anxiolytic drug diazapam decreased the frequency of self-scratching and scent-marking, suggesting these behaviours were associated with stress and, as allogrooming increased, was not due to the muscle relaxation properties of the drug (Cilia and Piper, 1997). While these are all natural behaviours for the common marmoset, increases in their expression are likely to be indicative of an underlying welfare problem.

Increased cortisol levels have also been well documented in primates following stressors such as isolation (Cross et al, 2004: *C. jacchus*), restraint (Reinhardt et al, 1995: *M. mulatta*) and maternal separation (reviewed in Hennessy, 1997). Removal from the home cage (Line et al, 1987: *M. mulatta*) and human handling (Hennessy et al, 1982: *S. sciureus*) have also both been shown to be physiologically stressful. Cortisol is the main hormone involved in the hypothalamic pituitary adrenal (HPA) axis, with levels often used as an index of stress (Kirschbaum and Hellhammer, 2000). It can be sampled from several mediums, including blood, saliva, urine, faeces and hair. Saliva sampling is non-invasive, as animals can be trained to voluntarily chew on collection devices (Norvak et al, 2012), and can provide a reflection of acute changes in hormone level (Higham et al, 2010). It is thought to reflect the unbound ‘free’ cortisol, which is the biologically active fraction of the hormone. The enzyme-linked immunosorbent assay (ELISA) can be used to quantify this response. However, the validity of the assay must first be tested for reliability and any species-specific problems (Reimers and Lamb, 1991).

5.1.2 Parental separation and stress

In the laboratory, infant marmosets are often routinely hand-reared, to improve survival of larger litters, which involves either one or all infants being removed for supplementary feeding (see Chapter 1). However, this necessitates separation from the family for substantial periods of time. As marmoset infants are adapted to be immersed in a rich social environment from birth, family life is extremely important for their development (Dettling et al, 2007). There is remarkable brain plasticity early in life, allowing maximal opportunity for experience to program the brain in long lasting ways (Knudsen, 2004). Much research has demonstrated that the stress of early parental loss can increase anxiety and fear, as well as alter baseline activity and stress responsivity of the HPA axis (reviewed in Parker and Maestripieri, 2011).

Several studies have used the parental separation paradigm, to look at later responses to separation and novelty. In some cases, the mother is removed from the group, while in others the infant is removed and isolated. Rhesus macaques (*M. mulatta*) exposed to short mother-infant separations (mother removed for 6 or 13 days at 21-32 weeks old) showed little behavioural differences from mother-reared animals at 12 and 30 months old, in interactions with their mothers or tendency to approach novel objects in the home cage. However, they showed significantly greater behavioural disturbance and less exploration of objects in a novel environment (Spencer-Booth and Hinde, 1971). Separated pigtail macaques (*M. nemestrina*, mother removed for 10 days at 4-8 months old) were rated as less sociable than controls (Caine et al, 1983). They also showed more disturbance behaviour and a longer latency to retrieve food in a novel environment (Capitanio et al, 1986). Suomi et al (1983: *M. mulatta*) found that separated infants (isolated at 4 days between 3 and 9 months old) only showed differences from non-separated individuals in the presence of their mother. While they spent more time in contact with her following reunion as infants, they were less interested in interacting when exposed to her following permanent separation. Reite (1987) suggested that these effects may be due to altered maternal behaviour following early separation, which may have affected attachment.

Peer-reared primates have also been studied. Researchers found high levels of fear and hyperemotional behaviour in these individuals, compared to mother-reared animals (Capitanio, 1986: *M. nemestrina*). There also appears to be some dysregulation of the HPA axis, with lowered plasma cortisol levels compared to mother-reared infants (Champoux et al, 1989: *M. mulatta*). Reduced responsiveness has also been reported in peer-reared monkeys, following social separation, a dexamethosone suppression test and ACTH (adrenocorticotrophic hormone) challenge (Capitanio et al, 2005: *M. mulatta*). The investigators suggested that the reduced responsiveness may be due to an altered set point of the HPA axis. However, other studies have failed to find differences in cortisol response (Winslow et al, 2003: *M. mulatta*) or have found heightened reactions when separated (Higley et al, 1992a: *M. mulatta*).

To provide a primate model for affective disorders, common marmoset infants have been exposed to unpredictable daily parental separations for the first month of life. Early deprived (ED) infants spent more time in contact with their parents (Parker et al, 2006) and less time in social play (Dettling et al, 2002), as well as exhibited reduced mobility and contact calls when isolated in a novel cage as juveniles (Dettling et al, 2007). Repeatedly separated infants were therefore more anxious and behaviourally inhibited, suggesting a lack of social stability. Early deprivation was also associated with altered physiological parameters, including diminished basal cortisol levels (Dettling et al, 2002). Elevated norepinephrine levels and systolic blood pressure have been found across the first year of life in these ED marmosets (Dettling et al, 2007; Pryce et al, 2004). Separation therefore altered both the psychological and physiological needs of the monkeys. Such evidence all emphasise the detrimental consequences of early parental separation, increasing subsequent fear and vulnerability to stressors (Parker and Maestripieri, 2011). Very few studies have however followed animals beyond one year of age, and so more longitudinal studies in marmosets would yield important information.

5.1.3 Parental separation and coping

The ability to cope with challenges is of great importance for the wellbeing of an individual, especially in a laboratory environment. While separation from the family early in life can lead to deficits in behavioural development, as well as alterations in physiology (Parker and Maestripieri, 2011), there is accumulating evidence that exposure to early life stress could promote resilience to stress in adulthood. Features of early life stress, including type, duration, frequency, ecological validity, sensory modality and developmental stage, can all play a role in the diverse range of developmental outcomes. Variation in the early social environment may therefore be a source of stress, which could have significant long lasting developmental effects. These may be negative, as previously described, or positive (Parker and Maestripieri, 2011).

Parker et al (2004) used the parental separation model in squirrel monkeys, first raising them in undisturbed natal groups, before exposing them to a 10 week stress inoculation protocol at approximately 17 weeks old. The protocol consisted of weekly one-hour separations from the natal group. The infants were surrounded by monkeys housed in adjacent cages, and were in auditory contact with their family group. These repeated short separations caused distress calls, agitated locomotion and acute increases in cortisol level. However, in response to subsequent stressors, these animals were better able to regulate negative emotional arousal, displaying less maternal clinging, as well as more exploration and food consumption, in a novel environment, and demonstrated diminished HPA activation. Results therefore suggested they were less anxious than non-inoculated monkeys (Parker et al, 2004). In another study, removal of the mother at weaning resulted in fewer distress calls, more time in proximity to peers and smaller increases in cortisol in stress inoculated monkeys (Lyons et al, 1999). This suggests they were more successful at response inhibition (Parker et al, 2004) and better able to control impulsive reactions. No significant long-term changes in maternal behaviour were observed, suggesting resilience was not maternally mediated.

These separations from the family were employed in late infancy, when wild infants develop independence and often have to cope with being separated from their mother while she forages. At this developmental stage, the stressor does not seem to overwhelm the young animal's ability to cope. As the protocol was administered weekly, there was also sufficient opportunities for recovery. Young animals may therefore develop the capacity for enhanced emotional regulation, and so this process is more likely to produce stress resilience (Parker and Maestripieri, 2011). However, it is unknown whether these effects persist across the lifespan (Parker and Maestripieri, 2011).

Evidence from a variety of primate models of human development suggest that early life experience, including disruption of the parent-offspring relationship, can influence both vulnerability and resilience to stress-related psychopathology (Parker and Maestripieri, 2011). As monkeys exposed to separations exhibited reduced stress responses to a later novel environment, compared to monkeys that remained undisturbed (Parker et al, 2006), resilience appeared to be due to the prior stressful experience. Stress inoculated monkeys may therefore be better equipped to deal with challenges. While exposure to excessive early life stress, or no stress at all, may undermine coping ability and leave individuals vulnerable, overcoming mild or moderate stress may enhance competence and protect individuals against adverse effects (Parker and Maestripieri, 2011). The resilience model of stress development therefore assumes a J shaped curve (Parker and Maestripieri, 2011: see Chapter 1). As marmoset infants are in almost constant body contact with a family member for the first several weeks of life, separations very early on are 'non-biological' events (Dettling et al, 2002). It is therefore possible that marmosets separated from the family in early life for supplementary feeding would be more vulnerable to stress.

5.1.4 Aim

Capturing for weighing is a regular management routine, which can be stressful for marmosets (Bassett et al, 2003; Bowell, 2010). This study aims to investigate the behavioural and cortisol responses of this stressor in adult animals. Validation of the use of salivary cortisol samples from marmosets was first conducted, and correlations between the welfare measures were investigated. Analysis was then conducted to assess the welfare and coping ability of individuals born and reared under three different backgrounds: family-reared twins and 2stays, and supplementary fed triplets. Adults exposed to family separations for supplementary feeding in early life were therefore compared to those that had remained undisturbed. Based on previous research, it was hypothesised that supplementary fed marmosets would display altered HPA axis function and heightened behavioural agitation to capture and weighing, compared to the other litter conditions.

5.2 Method

5.2.1 Study animals

Twenty-one marmosets were studied, aged between 1 year 7 months and 2 years 7 months at the start of data collection (excluding pilot study animals). They were all housed in vasectomised male-female pairs, as stock animals (from approximately 20 months old, following a period of same-sex housing after weaning from the natal group at 18 months). In six pairs, only one member was sampled, often as their partner did not fit one of the conditions, while on all other occasions both animals in the pair were studied individually. Although it was not ideal to record both simultaneously, as one member of the pair can influence the other, leading to pseudoreplication (Lazic, 2010), this was necessary in order to increase the sample size. Animals in three rearing backgrounds were included (Table 5.1). This comprised seven supplementary fed triplets (6 male, 1 female), eight 2 stays (3 male, 5 female) and 6 twins (3 male, 3 female). For details of rearing conditions see Section 2.2.

Table 5.1: Information for each animal studied

Rearing background	Gender	Litter size at birth	Comments
Supplementary fed triplets (N= 7)			
14R	M	3	
109R	M	3	
123R	M	3	
108R	M	3	
110R	M	3	
9R	M	3	*Only 1 post weigh
124R	F	3	*Only 1 post weigh
2stays (N=8)			
128R	F	3	One infant euthanised day 3
9T	F	3	One euthanised at day 7
82R	M	3	One stillborn infant
149R	F	3	One euthanised day 4
38T	F	3	One euthanised day 1
35T	M	3	One stillborn
6T	F	3	One euthanised day 2
18R	M	3	One found dead day 2 *Only 1 post weigh
Twins (N=6)			
76R	M	2	
139R	M	2	
28T	F	2	
29T	F	2	
106R	M	2	
12T	F	2	

* In 3 cases, only one cortisol sample and behavioural observation (directly after weighing) was collected.

Housing and husbandry

For details of housing and husbandry, see Sections 2.4.2 and 2.5.

Weighing procedure

Weighing is a necessary routine event, carried out each month, that provides a good opportunity to assess how individuals cope with a mild stressor, without inflicting any stress for the sole purpose of the study. One mixed-pair stock room was weighed and moved to a clean cage each week, on a rota basis. A standardised procedure was employed, based on current practice.

Weighing took place between 9:00 and 10:00. To look at order effects, approximately half of the animals in each condition were among the first to be weighed in the room, while the others were among the last to be weighed. The present home cage was first divided, to enclose the animal in the top right section. The marmoset was then caught by grasping the base of the tail and then holding the animal around the chest. After a brief health check, the marmoset was placed into a small, plastic box and weighed on the scales. They had no visual or olfactory contact with their pair member, although were within auditory contact. The box was opened in the new clean cage and the animal allowed to leave at will. This meant that the first marmoset was hand-caught, health checked and weighed, followed by a short isolation period in the new cage, while the second had a short isolation period in the old cage, before being caught, health checked and weighed. The time spent out of the home cage was approximately 2 minutes, with the whole process lasting approximately 5 minutes. The old cage was then removed for washing.

5.2.2 Behavioural response

Behavioural observations

Baseline and post-weighing data were recorded for each animal. Baseline behavioural data were collected over three days a week before weighing, to match the post-weigh time points, and averages calculated. Behaviour was then observed immediately after weighing and 30 minutes after weighing. Behavioural recording followed the protocol in 2.6. Behaviours of interest included activity (locomotion, inactivity, exploration), arousal (tail pilo-erection, vocalisation), social (proximity, initiate and terminate contact) and stress-related (self-scratch and scent mark).

Table 5.2: Stress-related behavioural categories

Behaviour	*Definition*	*Prediction if stressed* *(+ = increase; - = decrease)*
Locomotion and inactivity		
Inactive alert [a]	The animal remains stationary, alert and aware of the surroundings, without engaging in any other activity.	+
Inactive rest [a]	The animal is stationary, usually with the tail curled around the body or through the legs, its eyes open or closed.	-
Agitated locomotion [a]	The animal moves between locations rapidly. Its gait is not relaxed.	+
Calm locomotion [a]	The animal travels between locations by walking, running, climbing or jumping, its gait relaxed.	-
Individual behaviour/arousal		
Explore [a]	The animal investigates objects in the environment by handling, sniffing, gently biting, licking or attending to them whilst walking around them.	-
Autogroom [a]	The animal cleans its own fur or skin with hand or mouth.	+
Scratch [b]	The animal rapidly moves its hand or foot, drawing its claws across the fur or skin.	+
Scent mark [b]	The animal sits and rubs its anogenital area on a branch or other area of the enclosure (anal scent mark), or rubs its sternal area along a substrate (sternal scent mark).	+
Solitary play [a]	High activity behaviour performed alone, such as swinging on a rope, chasing tail or gnawing stationary objects, accompanied by rapid movement around the cage (Stevenson and Poole, 1976).	-
Agitated vocalisation [b]	The animal emits alarm vocalisations audible to the observer, such as ‘tsiks’ and ‘seeps’.	+
Calm vocalisation [b]	The animal emits calm vocalisations audible to the observer, such as ‘trills’ and ‘chirps’.	-
Tail pilo-erection [a]	The hair on the tail stands on end. This is associated with some degree of disturbance to the animal.	+
Eat/forage [a]	The animal is engaged in any activity directly related to aquiring or ingesting food.	-
Social behaviour		
Social play [a]	High activity social interactions involving close, non-aggressive physical contact with other individuals, such as wrestling, chasing, grasping, pouncing, back-hugging, batting, biting and mutual investigation. This is often accompanied by the open mouth play face (Stevenson and Poole, 1976).	-
Allogroom [a]	The animal cleans the fur or skin of another individual with its hands or mouth.	-/+
Proximity [a]	The animal is stationary, sitting, crouching or lying next to another individual, with some form of physical contact.	+

Table 5.2 continued: Stress related behavioural categories

Behaviour	*Definition*	*Prediction if stressed* *(+ = increase; - = decrease)*
Initiate contact [b]	The animal moves toward the pair member, to within 2cm.	+
Terminate contact [b]	The animal moves away from the pair member, terminating contact.	-
Watch observer [a]	The animal remains stationary, attending to the observer, either at the back of the cage or on the wire front.	+
Out of sight [a]	The animal cannot be seen by the observer.	
Other [a]	Any other behaviour not noted above.	

a. Instantaneous sampling
b. All-occurrence sampling

5.2.3 Cortisol response

Saliva collection

Two saliva samples were collected from study animals, at 0-5 minutes and 25-30 minutes after capture and weighing (post 0 mins and post 30 mins), prior to the behavioural observations. Saliva was sampled between 9:00-10:00 on three days of the previous week (within half an hour of each other), to ensure compatibility and avoid variation due to circadian rhythm (Cross and Rogers, 2004). Mean values could then be calculated, along with the matching behavioural observations (Bowell, 2010).

Salimetrics Oral Swabs were used to collect the saliva. These are made of a polymer, have verified recoveries of salivary cortisol, and do not cause a change in sample Ph (Salimetrics). The swab was first rubbed into a banana, to coat it with the fruit. One end was then presented through the wire wall of the home cage, with the other held by the experimenter, and the marmoset allowed to lick and chew the end, depositing saliva onto the swab (Cross et al, 2004). After approximately 5 minutes, the swab was removed and the marmoset given a small piece of banana.

Previous studies have shown this is an effective, non-invasive method for saliva collection in the marmoset, able to consistently obtain samples to monitor acute changes in free cortisol levels (Pearson et al, 2008). Banana has been found to be the preferred flavour, reliably encouraging chewing, and variations in banana concentration are likely to have minimal effects on the assayed cortisol concentration.

The swab was then taken for processing (after checking for traces of blood, which would effect the cortisol assay). The swab was first cut to a small size, to fit into the storage tube, and sealed. Samples were marked with subject ID, stage and date. The tubes, with their contents, were frozen at -20 ^{o}C for less than week. The samples were then placed into a centrifuge and spun for 15 minutes at 1500 RPM. They were then stored at –80 ^{o}C, until being assayed within 6 months. Storage time should not exceed 9 months (Aardal and Holm, 1995).

Cortisol assay

In collaboration with Dr. T. Smith at the University of Chester and staff at Dstl, I analysed samples using Salimetrics Salivary Cortisol Enzyme Immunoassay Research Kits. Saliva samples were first diluted with assay diluent by 1:5000, following results from the pilot study (see Section 5.2.3), to ensure they were within the range of the assay kit standards. The plate was first prepared with NSB wells. Saliva samples, standards and controls (25μl) were pipetted into the appropriate wells. Assay diluent (25μl) was also pipetted into zero and NSB wells. A 1:1600 dilution of conjugate (200μl) was added to each well, and the plate mixed for 5 minutes at 500rpm, before being incubated for 55 minutes at room temperature. The plate was then washed 4 times with wash buffer. TMB solution (200μl) was pipetted into each well, the plate mixed (5 minutes at 500rpm) and incubated for an additional 25 minutes. Stop solution (50μl) was added to each well, the plate mixed (3 minutes, 500rpm), and the assay read. Unbound cortisol levels were determined using a standard curve. Each sample was in duplicate, and a mean calculated.

5.2.4 Methodology Validation

Assay 1

A pilot study was first conducted to validate the use of the Salimetrics assay in common marmosets, by showing aspects of analytical validation. Twenty samples were tested from 4 animals (2 male, 2 female). Three baseline and 2 post capture (at 0-5 min and 5-10 min) were taken from each animal. Serial dilutions of the pooled samples were made, which showed parallelism with the synthetic standards provided in the kit, demonstrating high specificity. Increasing amounts of synthetic cortisol (0-3μg/dL) were then added to known quantities of sample (0.088μg/dL), finding an average recovery of 102.82%. Comparison of coefficients of variation (CV) of quality controls (N=3) showed high inter-assay precision (High: 7.28%; Low: 4.54%). High intra-assay precision (N=22) of 2.39% was also found. Sensitivity, the minimal concentration of cortisol measurable in the working range of the assay, is 0.007μg/dL (Salimetrics).

Assay 2

A further assay was conducted to look at biologically relevant changes in cortisol, between baseline and post capture, in order to give increased confidence that the assays are providing valid data. It was expected that cortisol concentration after a potentially stressful husbandry event would be significantly higher than at baseline. Baseline cortisol values (average of the 3 days) had a mean of 5023.68 +/- 1650.58 (SD) nmol/L (uncorrected for banana). While some were quite consistent in the values they produced (eg. Animal 1: 5453.62+/-283.16), others were more variable (eg. Animal 2: 5821.49+/-1140.24 nmol/L). Repeated measures ANOVAs revealed no significant difference (P>0.05) between average baseline cortisol level and at either 0-5 min (mean 4117.81+/-2109.67nmol/L) or 5-10 minute post capture (mean 3945.37+/-1529.86 nmol/L), although there was a tendency for cortisol to be lower post capture than at baseline. Times were standardised as much as possible, and so this unexpected result could be due to the provision of food, or higher levels of activity at baseline (Hingam et al, 2010). There may also have been a variable time lag between the stressor and maximal cortisol levels. In humans this is 10-30 minutes after stress cessation (Kirschbaum and Hellhammer, 2000). Variation within individuals at baseline was however deemed small enough to provide reliable data. Therefore, with results also informing timings of saliva collection, larger sample sizes were collected.

Assay 3

Having identified ten times the amount of cortisol in samples compared to previously published data, a further study was conducted to compare the use of Salimetrics Oral Swabs (SOS) with cotton buds, both with and without banana. Three pairs (N=6) of marmosets were presented with each swab, firstly without banana (cotton bud, then SOS). They were then allowed to chew each swab with banana (again cotton bud then SOS), to avoid contamination of the first samples. Cotton bud samples were pooled and serial dilutions were made, to compare with previous SOS results.

The assays identified a 1:1000 dilution was necessary for cotton bud sample readings to fall within the linear range of the standard curve, while a 1:5000 dilution was necessary for SOS samples. This confirms that SOS had the potential to recover 5 times as much cortisol as cotton buds. Cotton bud samples without banana had significantly higher cortisol concentrations than those with banana, as expected. A highly significant positive Spearman's rank correlation was also found between cotton buds with and without banana. The relationship fit the following equation: without banana= with banana/0.55. This is similar to that found by Cross et al (2004). A significant difference was found between SOS samples. However, those with banana had significantly higher cortisol levels than those without banana. Samples were also not significantly correlated. These results perhaps suggest that previous exposure to the banana on the cotton bud may have increased cortisol levels for the subsequent SOS sample. As a correction factor for SOS was difficult to identify, all data presented were uncorrected for banana.

Table 5.3: Mean concentrations of cortisol for each collection device, with and without banana.

Collection device	**Banana**	**Mean conc (nmol/L)**	**Mann-Whitney U**	**P**	**r**	**P**
Salimetrics Oral Swab	With	5191.73	1.00	0.011	0.70	0.188 (NS)
	Without	1593.20				
Cotton bud	With	1822.03	0.00	0.001	0.98	<0.001
	Without	3341.81				

5.2.5 Statistical Analysis

Means were calculated from the three baseline cortisol and behavioural values for each individual, to obtain one baseline value for use in the analysis, in attempt to reduce any large variations. Overall, cortisol data were not normally distributed (using Kolmogorov-Smirnov), and so Friedman tests were conducted to look at overall differences in cortisol over the time points (baseline, post 0 mins and post 30 mins). Follow-up Wilcoxon tests were conduced to find where the difference lay. Mann Whitney tests were used to look at any gender differences. As data were approximately normally distributed within the rearing conditions, parametric tests could be conducted to look at differences between baseline and post capture cortisol values in each of the three conditions. Due to some missing data points (where samples were not collected or analysed successfully), each time point was examined using paired samples t tests, to prevent any data from being lost in repeated measures ANOVAs, which only include subjects with all data points. Independent samples t tests were also conducted to look at any gender differences within the conditions. A one way ANOVA was conducted to look at any differences between the rearing conditions at each time point. Any effect of order weighed in the room on cortisol at post 0 mins and post 30 mins was analysed using Independent samples t tests.

No transformation was successful in making behavioural data normally distributed, and so non-parametric tests were conducted. Friedman's tests were used to look at the overall effect of observation point on each behaviour, with follow-up Wilcoxon tests. Mann Whitney tests were also used to look at any gender differences at each time point. Friedman tests were used to look at within subject time point differences in each rearing condition. Significant results were followed up with Wilcoxon post hoc tests. Mann Whitney tests were used to look for differences between males and females in each rearing condition. Kruskal Wallis tests were used to compare each time point between the rearing conditions. Significant results were followed up with Mann Whitney post hoc tests. Order effect was analysed using Mann Whitney tests. Spearman's rank correlations were also conducted, to examine any associations between cortisol and each behaviour.

5.3 Results

5.3.1 Cortisol Data

Overall response to stressor

Of the 81 samples attempted, 4 were either not successfully collected or analysed, meaning a total of 77 samples were successfully analysed. This equates to 95.06% of samples. Combining data from all animals (using all 3 baseline measurements), the mean cortisol baseline level was 7710.56 +/- 6735.65 (SD) nmol/L. Variation across cortisol measurements was high, ranging from 614.10 nmol/L to 28917.10 nmol/L. There was a significant difference between time points. Cortisol significantly decreased from baseline to post-capture 0 mins and from baseline to post-capture 30 mins. Although there was no room order effect at post 0 mins, results showed that those animals weighed last had significantly higher cortisol than those weighed first at post 30 mins.

Twins: comparison between baseline and post capture data

Although cortisol was lower at post 0 mins and post 30 mins than at baseline, this was not significant. Figure 5.1 shows the individual variation in cortisol between each twin marmoset studied, at each time point. Table 5.4 shows statistical details.

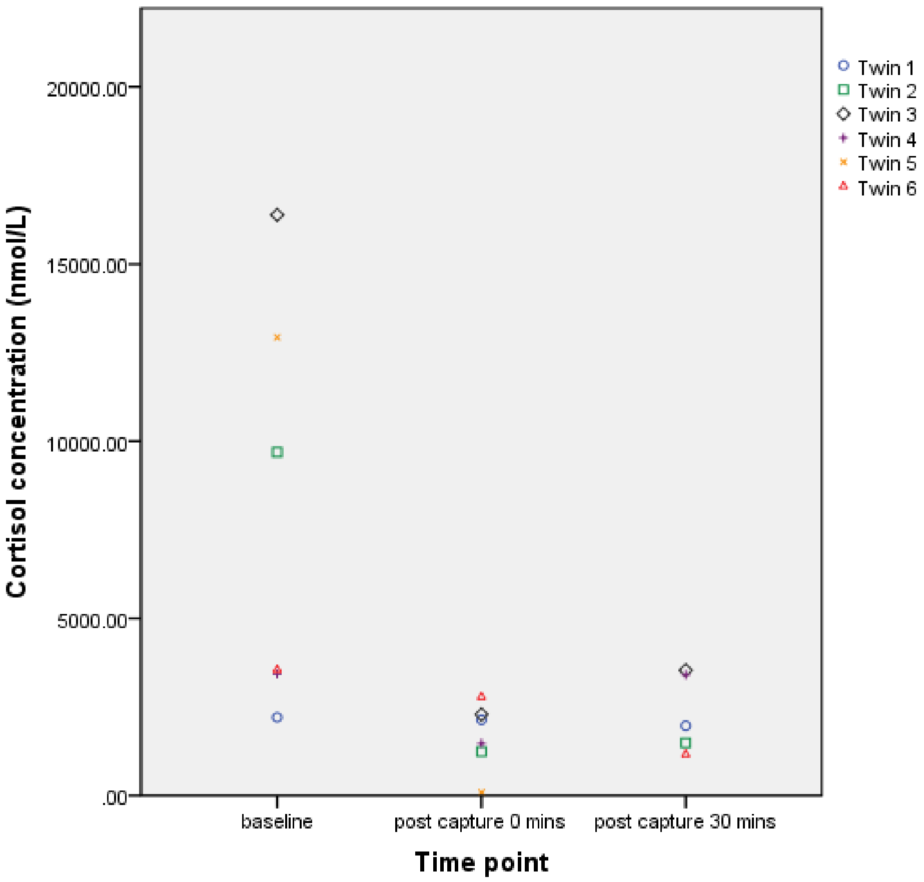


Fig 5.1: Individual variation in salivary cortisol concentrations (nmol/L) for twins across time points

2stays: comparison between baseline and post capture data

2stay cortisol level was significantly higher at baseline than at post 0 mins and at post 30 mins. There was no significant difference between cortisol levels at post 0 mins and post 30 mins. Figure 5.2 shows the individual variation in cortisol between each 2stay marmoset studied, as well as the significant changes found, at each time point. Table 5.4 shows the statistical results.

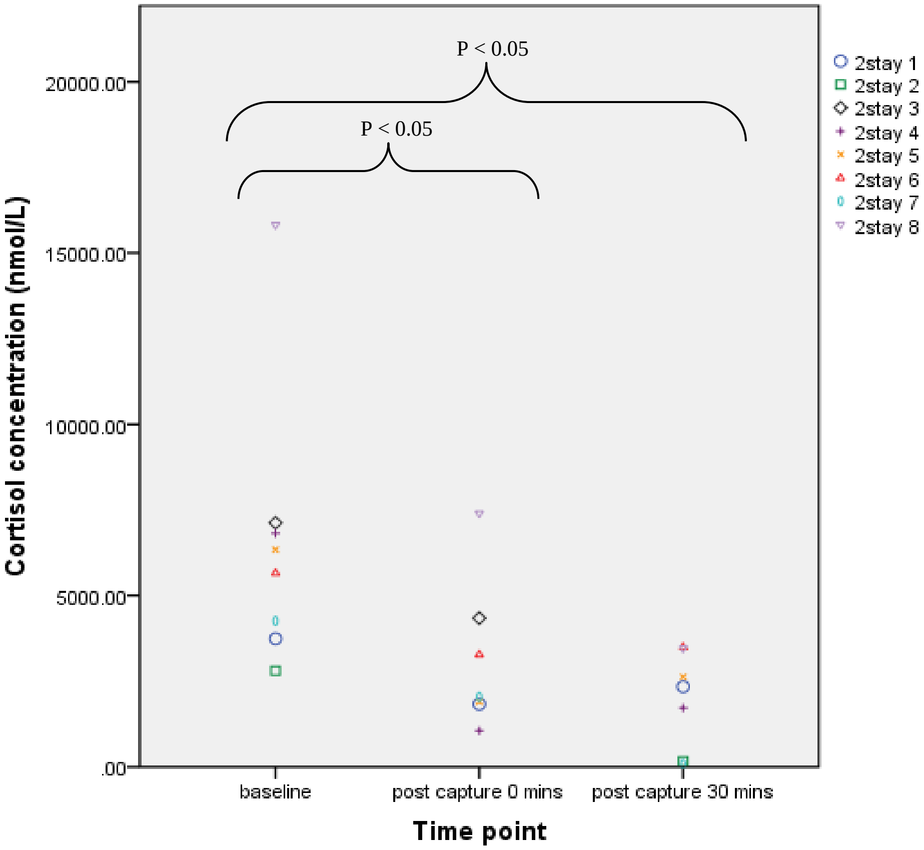


Fig 5.2: Individual variation in salivary cortisol concentrations (nmol/L) for 2stays across time points. Cortisol at basline was significantly ($P<0.05$) higher than at post capture 0 mins and post capture 30 mins.

Supplementary fed triplets: comparison between baseline and post capture data

Although there was a decrease in cortisol level from baseline to post 0 mins and post 30 mins, this was not significant in supplementary fed triplets. There was an increase in cortisol from post 0 mins to post 30 mins, although this also was not significant. Figure 5.3 shows the individual variation in cortisol between each supplementary fed triplet marmoset studied, at each time point. Table 5.4 shows statistical details.

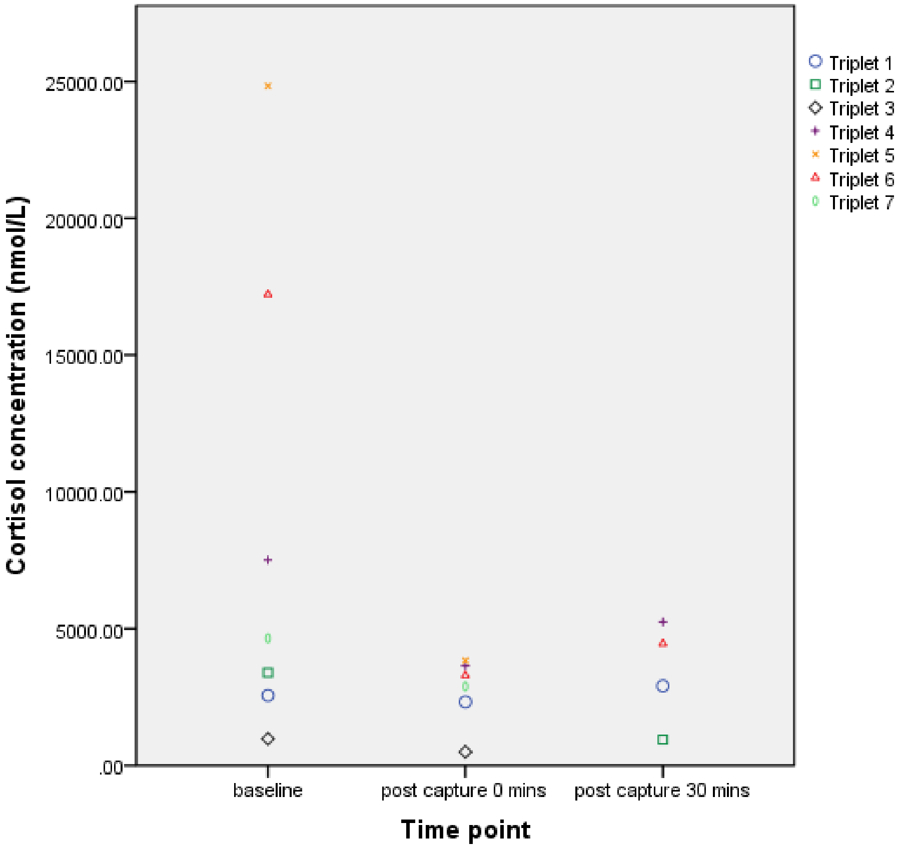


Fig 5.3: Individual variation in salivary cortisol concentrations (nmol/L) for supplementary fed triplets across time points

Gender differences

Overall, females had higher baseline cortisol values than males (mean 9473.34 +/- 7833.69 (SD) nmol/L versus 6388.47+/- 5530.48 nmol/L). However, this result was not significant. There was no difference in twin cortisol between genders at baseline. However, male twins (mean 2404.47 +/- 338.91 nmol/L) had significantly higher cortisol at post 0 mins than female twins (mean 936.48 +/- 741.33 nmol/L). There was no difference in gender at post 30 mins. There was no significant difference in cortisol level between 2stay males and females at any of the time points. As only one female supplementary fed triplet was included, no gender analyses were conducted for this condition.

Table 5.4: Significant changes in cortisol concentration (overall and within each rearing condition)

Test	Analysis	df	Test statistic	P
Overall cortisol	Time	2	X^2= 19.86	<0.001
	Base to Post 0 mins		Z= -3.82	<0.001
	Base to Post 30 mins		Z= -3.36	<0.001
	Room order		t=-2.86	0.013
Twin cortisol	Post 0 min, Gender	4	t= 3.12	0.036
2stay cortisol	Base to Post 0 mins	6	t= 4.40	0.005
	Base to Post 30 mins	6	t= 3.24	0.018

Differences in cortisol between rearing conditions

There was no significant difference in baseline cortisol between the rearing conditions. Cortisol levels at post 0 mins and post 30 mins were also not significantly different. There was however huge variation at baseline, particularly for supplementary fed triplets. Figure 5.4 displays the mean cortisol values at each time point for each rearing condition.

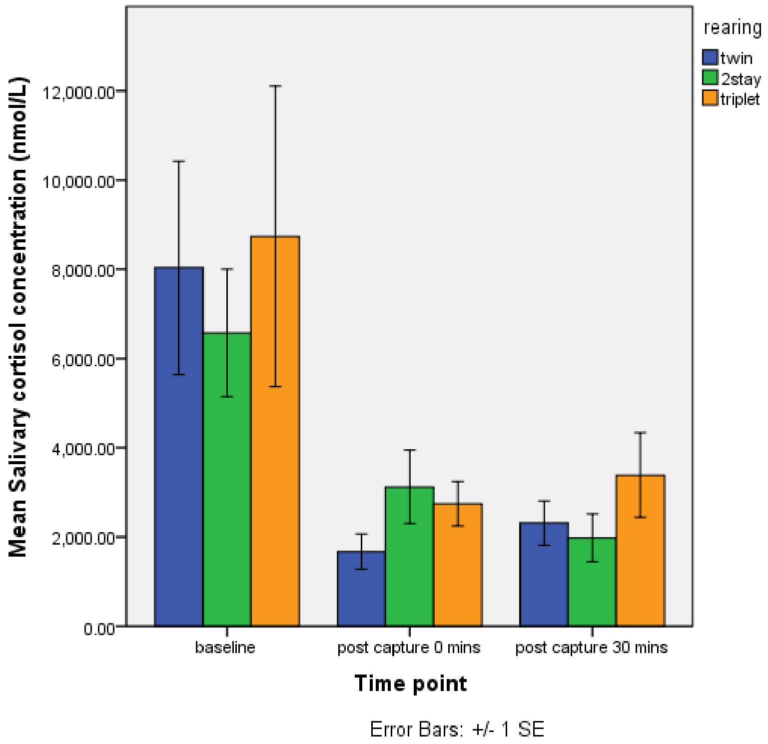


Fig 5.4: Mean (+/- 1 SE) salivary cortisol concentration (nmol/L) in each rearing condition at baseline, capture 0 mins and capture 30 mins time points

5.3.2 Behavioural Data

Overall response to stressor

There was significantly more scratching at baseline than at post 0 mins. Scent marking was higher at post 0 mins and post 30 mins than at baseline. There was no significant difference in agitiated vocalisations over time, although there were almost 4 times as many at post 0 mins (mean 1.944 +/- 2.53 (SD)) than at baseline (0.50 +/- 0.734). Inactive alert was higher at baseline and post 30 mins than at post 0 mins. There was a highly significant difference in agitated locomotion over the observation points, with significantly more at post 0 mins and post 30 mins than at baseline. A significant difference was also found in calm locomotion over time points, with more at baseline than post 0 mins and post 30 mins. Proximity was higher at baseline than at post 0 mins and post 30 mins. Watching was also significantly higher at baseline than post 0 mins and post 30 mins. Foraging was higher at post 0 mins than at baseline. Table 5.5 shows the significant changes in behaviour over the three observation points.

Some behaviours were also significantly different between order of weighing at post 30 mins. Inactive alert was higher in those weighed first than last, while explore was higher in those weighed last than those weighed first. At post 0 mins, there was a trend for animals weighed last in the room to have a greater frequency of terminate contact than those weighed first.

Table 5.5: Significant changes in overall behaviour

Behaviour	df	Test statistic	P
Scratching	2	X^2= 10.26	0.006
Base>Post 0 mins		Z=-2.65	0.008
Scent marking	2	X^2=17.82	<0.001
Post 0 mins> Base		Z=-3.34	0.001
Post 30 mins>Base		Z=-3.62	<0.001
Inactive alert	2	X^2= 8.21	0.016
Base>Post 0 mins		Z=-2.98	0.003
Post 30 mins>Post 0 mins		Z=-2.06	0.039
Agitated locomotion	2	X^2=17.22	<0.001
Post 0 mins>Base		Z=-3.31	0.001
Post 30 mins>Base		Z=-3.31	0.001
Calm locomotion	2	X^2= 9.91	0.007
Base>Post 0 mins		Z=-1.83	0.067 (NS)
Base>Post 30 mins		Z=-2.67	0.008
Proximity	2	X^2=11.32	0.003
Base>Post 0 mins		Z=-2.02	0.043
Base>Post 30 mins		Z=-2.78	0.005
Watching	2	X^2=18.00	<0.001
Base>Post 0 mins		Z=-2.96	0.003
Base>Post 30 mins		Z=-2.69	0.007
Foraging	2	X^2=5.71	0.058 (NS)
Post 0 mins>Base		Z=-2.85	0.004
Order effects			
Post 30 mins Inactive alert		U=17.00	0.035
Post 30 mins Explore		U=20.00	0.014
Post 0 min terminate contact		U=29.50	0.079 (NS)

Twins: comparisons between baseline and post capture data

Scratching at baseline was significantly higher than at post 0 mins in twins. Scent marking at baseline was significantly lower than at post 0 mins and at post 30 mins. There was a significant difference over the observations points for inactive alert, with this behaviour significantly lower at post 0 mins than at baseline and at post 30 mins. Agitated locomotion was significantly higher at post 0 mins than at baseline. There was also a trend for this behaviour to be higher at post 30 mins than at baseline. As well as this, calm locomotion was significantly higher at baseline than post 0 mins and post 30 mins. There was a trend for proximity to partner to be different across the time points. Proximity was significantly higher at baseline than post 0 mins. Figures 5.5 and 5.6 display the median frequencies and estimated percentage of sample time spent in each significant behaviour when in view, for twins over the time points. Table 5.6 shows the significant results of statistical analysis.

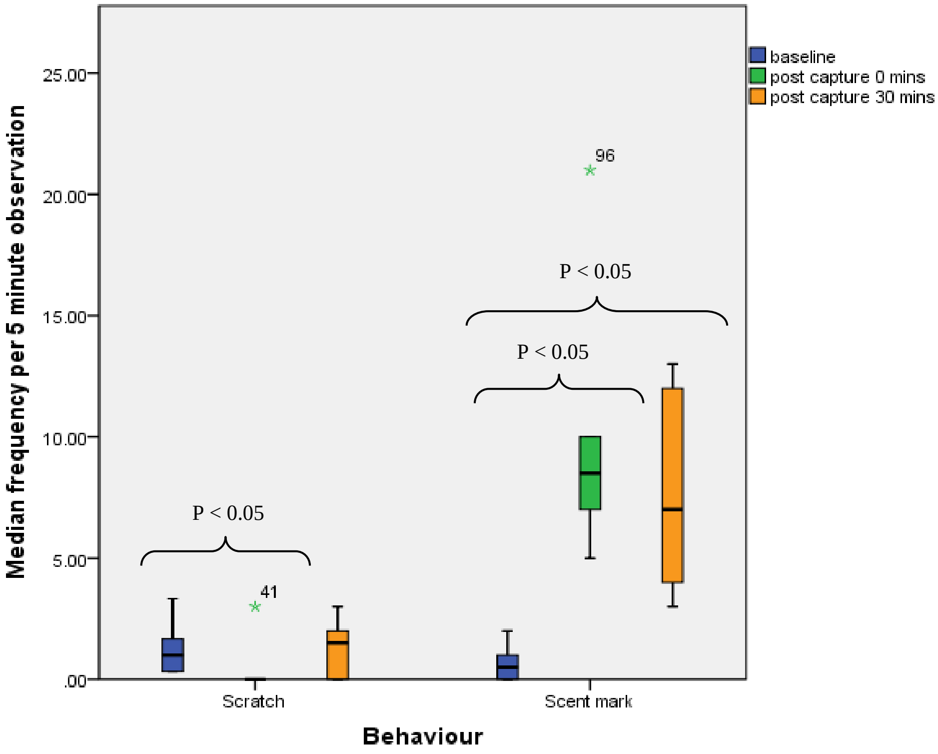


Fig 5.5: Median frequency of occurrence (per 5 minutes) of significant behaviours over each time point for twin marmosets. Scratching was significantly ($P < 0.05$) higher at baseline than post capture 0 mins; scent marking was significantly lower at baseline than at post capture 0 mins and post capture 30 mins. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

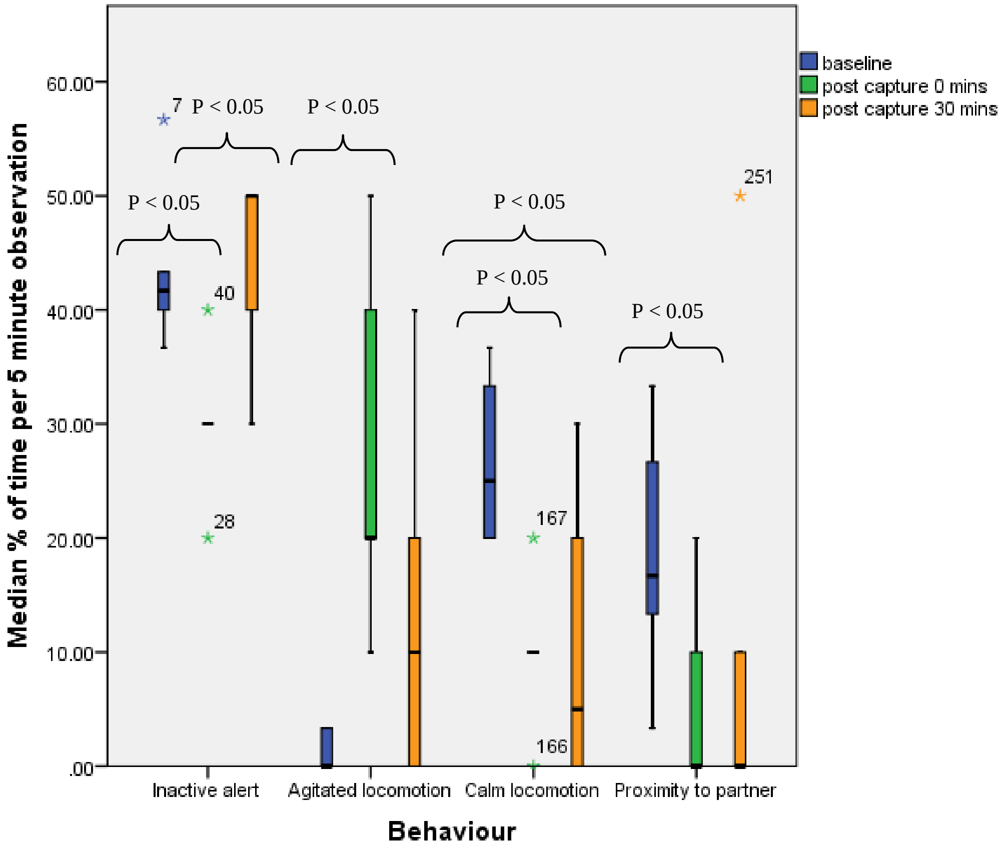


Fig 5.6: Median estimated percentage of the 5 minute observation period spent in significant behaviours when in view, over each time point for twin marmosets. Inactive alert was significantly ($P<0.05$) lower at post capture 0 mins than at baseline and post capture 30 mins; agitiated locomotion was lower at baseline than at post capture 0 mins; calm locomotion was higher at baseline than post capture 0 mins and post capture 30 mins; proximity to partner was higher at baseline than post capture 0 mins. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

2stays: comparisons between baseline and post capture data

There was a trend for agitated locomotion to be different in 2stays over the time points. Agitated locomotion was higher at post 30 mins than at baseline. A significant difference in foraging was found over the observations. Foraging was significantly higher at post 0 mins than baseline and post 30 mins. Figure 5.7 displays the median estimated percentage of sample time spent in each significant behaviour when in view, for 2stays over the time points. Table 5.6 shows the significant results of statistical analysis.

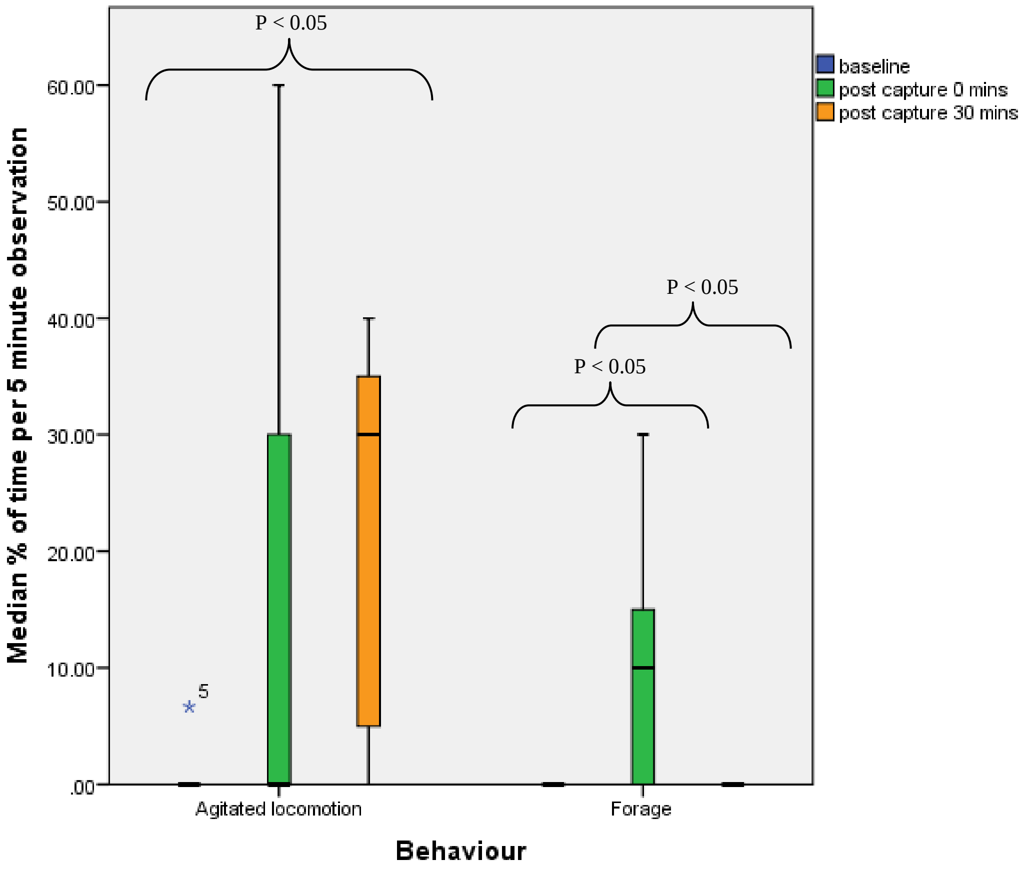


Fig 5.7: Median estimated percentage of the 5 minute observation period spent in significant behaviours whenin view, over each time point for 2stay marmosets. Agitated locomotion was significantly ($P<0.05$) lower at baseline than at post capture 30 mins; foraging was higher at post capture 0 mins than baseline and post capture 30 mins. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

Supplementary fed triplets: comparisons between baseline and post capture data

There was an increase in scent marking from baseline to post 0 mins and post 30 mins in supplementary fed triplets, although this was not significant. A significant difference was found in initiation of contact over the time points, which was significantly lower at baseline than at post 0 mins and post 30 mins. Agitated locomtion was significantly lower at baseline than at post 0 mins and post 30 mins. Watch was significantly higher at baseline than at post 0 mins and post 30 mins. There was also a trend for exploration to be different over the time points, with this behaviour significantly higher at post 0 mins than at baseline. Figures 5.8 and 5.9 display the median frequencies and estimated percentages of sample time spent in each significant behaviour when in view, for supplementary fed triplets over the time points. Table 5.6 shows the significant results of statistical analysis.

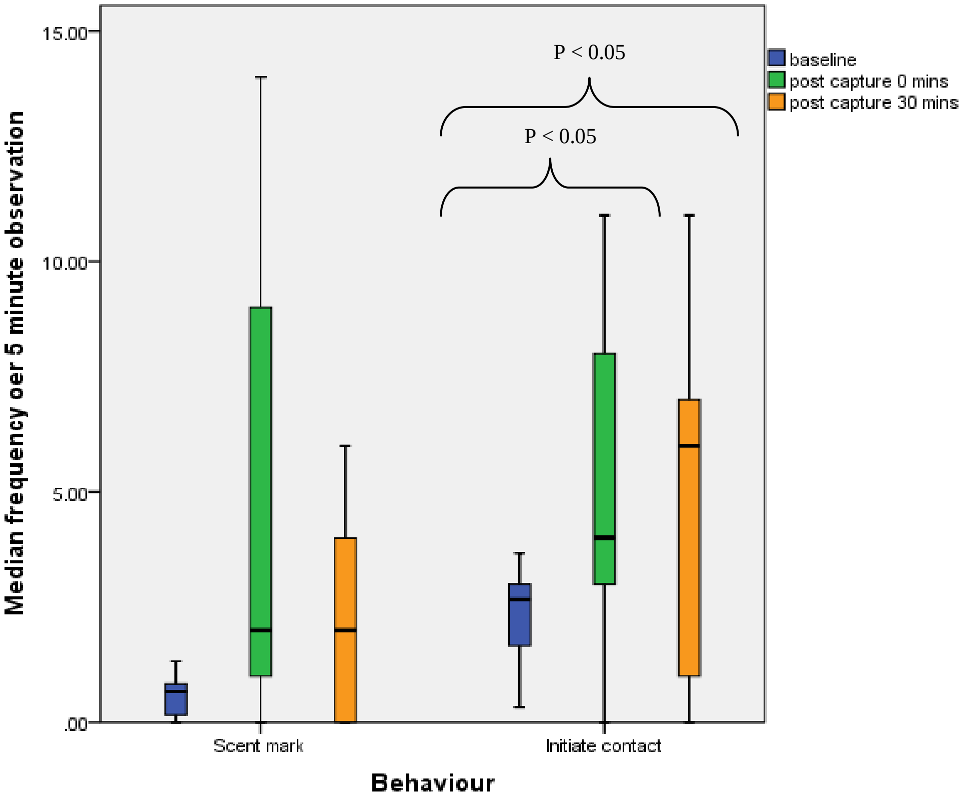


Fig 5.8: Median frequency of occurrence of significant behaviours over each time point for supplementary fed triplet marmosets. Initiate contact was significantly ($P<0.05$) lower at baseline than at post capture 0 mins and post capture 30 mins. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers.

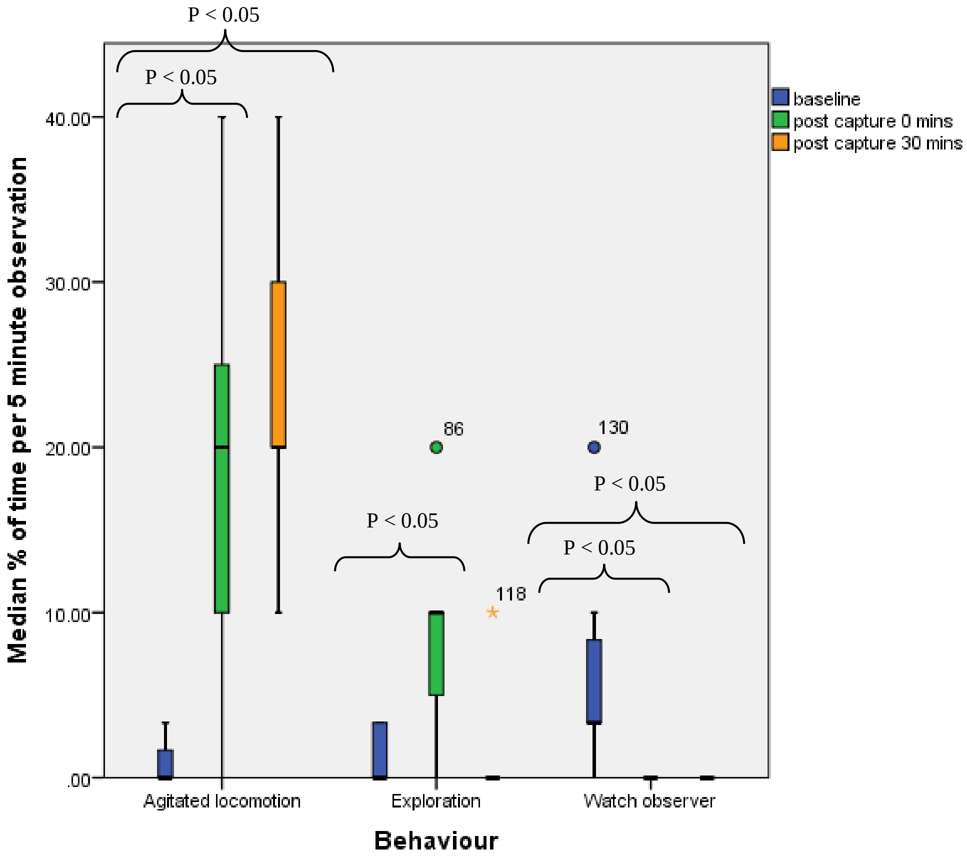


Fig 5.9: Median estimated percentage of the 5 minute observation period of significant behaviours when in view, over each time point for supplementary fed triplet marmosets. Agitated locomotion was significantly (P<0.05) lower at baseline than at post capture 0 mins and post capture 30 mins; exploration was higher at post capture 0 minutes than at baseline; watch observer was higher at baseline than at post capture 0 min and post capture 30 mins. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

Table 5.6: Significant changes in behaviour within each rearing condition

	Twin			**2stay**			**Supplementary fed triplet**		
Behaviour	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**	**df**	**Test statistic**	**P**
Scratching	2	X^2= 6.70	0.035						
Base>Post 0 mins		Z= 2.23	0.026						
Scent marking	2	X^2= 9.33	0.009				2	X^2= 6.53	0.038
Base<Post 0 mins		Z= -2.20	0.028					Z= -1.89	0.058 (NS)
Base<Post 30 mins		Z= -2.20	0.028					Z= -1.83	0.068 (NS)
Inactive alert	2	X^2= 6.82	0.033						
Base>Post 0 mins		Z= -2.03	0.042						
Base>Post 30 mins		Z= -2.04	0.041						
Agitated locomotion	2	X^2= 8.67	0.013	2	X^2= 5.16	0.076	2	X^2= 6.63	0.036
Base<Post 0 mins		Z= -2.21	0.027					Z= -2.03	0.042
Base<Post 30 mins		Z= -1.84	0.066		Z= -2.04	0.041		Z= -2.02	0.043
Calm locomotion	2	X^2= 6.52	0.038						
Base>Post 0 mins		Z= -2.20	0.028						
Base>Post 30 mins		Z= -2.00	0.046						
Proximity to partner	2	X^2= 5.16	0.076						
Base>Post 0 mins		Z= -2.00	0.046						
Foraging				2	X^2= 10.00	0.007			
Base<Post 0 mins					Z= -2.06	0.039			
Post 30 mins<Post 0 mins					Z= -2.06	0.039			
Initiation of contact							2	X^2= 8.32	0.016
Base<Post 0 mins								Z= -2.04	0.041
Base<Post 30 mins								Z= -2.02	0.043
Exploration							2	X^2= 5.29	0.071
Base<Post 0 mins								Z= -2.04	0.041
Watch observer							2	X^2= 10.00	0.007
Base>Post 0 mins								Z= -2.23	0.026
Base>Post 30 mins								Z= -2.03	0.040

Gender differences

Overall, males initiated contact significantly more than females at post 0 mins. Females instead foraged more than males at post 0 mins. There were only differences between gender in twin marmosets, and not in the other rearing conditions. At post 0 mins, twin females foraged for a longer estimated percentage of sample time (median: 20.00) than twin males (0.00). At post 30 mins, twin males initiated contact (median 7.00) more frequently than twin females (4.00). At the same time point, twin females terminated contact more (median: 4.00) than twin males (1.00). Table 5.7 displays the significant gender differences in behaviour.

Table 5.7: Significant gender differences in behaviour overall and within rearing conditons

Rearing	**Time point**	**Behaviour**	**Mann Whitney U**	**P**
Overall	Post 0 mins	Initiate contact	23.00	0.025
	Post 0 mins	Forage	19.00	0.008
Rearing				
Twins	Post 0 mins	Forage	0.00	0.034
Twins	Post 30 mins	Initiate contact	0.00	0.050
Twins	Post 30 mins	Terminate contact	0.00	0.046

Differences in behaviour between rearing conditions

There were no significant differences in any behaviour recorded between twins, 2stays and supplementary fed triplets at baseline, post 0 mins or post 30 mins.

5.4.3 Relationship between behaviour and cortisol

When all marmosets were analysed together, scent mark, agitated vocalisation, agitated locomotion and forage were significantly negatively correlated with cortisol level. Calm vocalisation and proximity were significantly positively correlated with cortisol level.

Rearing conditions were then looked at separately. For twins, cortisol concentration was significantly negatively correlated with scent mark, initiate contact, agitated locomotion and forage. Cortisol concentration was positively correlated with inactive alert, calm locomotion and explore. For 2stays, only proximity was significantly positively correlated with cortisol level. Again, for triplets, only proximity was significantly positively associated with cortisol level. Table 5.8 shows the significant correlations between cortisol concentration and each behaviour overall, as well as within each rearing condition.

Table 5.8: Significant correlations between cortisol concentration and behaviour

Behaviour	**Overall**		**Twins**		**2stays**		**Supplementary fed triplets**	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Scent mark	-0.28	0.006	-0.49	0.006				
Agitated Vocalisation	-0.21	0.038						
Agitated Locomotion	-0.33	0.001	-0.55	0.002				
Forage	-0.24	0.017	-0.37	0.047				
Calm vocalisation	0.23	0.021						
Proximity	0.28	0.005			0.38	0.017	0.37	0.040
Initiate contact			-0.46	0.011				
Inactive alert			0.42	0.024				
Calm locomotion			0.49	0.007				
Explore			0.42	0.024				

5.4 Discussion

5.4.1 Salivary cortisol

Measuring salivary cortisol proved to be successful, confirming that this is a promising non-invasive method of measuring acute changes in cortisol. The Salimetrics assay met typical validation criteria. It was found to have high specificity, accuracy and precision, as well as high sensitivity. Mean baseline cortisol in the present study was 7710.56 +/-6735.65 (SD) nmol/L. Females had approximately one-third higher baseline levels than males, which is similar to previous reports in common marmosets (Johnson et al, 1996: blood cortisol). However, there was considerable individual variation at this time, and removal of high outliers would have resulted in very small sample sizes. It is possible that there was an interfering substance effecting the validity of the assay, leading to increased values, although the high specificity suggests that cortisol in the samples and standards did react in a similar manner with the antibody (Reimers and Lamb, 1991), with no interference from other molecules. It may however be necessary to conduct an ACTH challenge, to confirm the validity of the assay (Hubrecht, pers. corr). Administration of ACTH is followed by significant elevations of glucocorticoid metabolites (Romero and Wingfield, 2001), and so detection of these increases would confirm the assay's ability to find biologically meaningful changes. This method has been used to validate assays in several species, such as mourning doves (*Zenaida macroura*: Washburn et al, 2003), spotted hyenas (*Crocuta crocuta*: Goymann et al, 1999) and white tailed deer (*Odocoileus virginianus*: Millspaugh et al, 2002).

A considerably higher amount of cortisol was also recovered using the present method, compared to previously published data on common marmosets. For example, Cross et al (2004) found mean concentration at undisturbed baseline periods to be 561 nmol/L, which rose to almost 4500 nmol/L in disturbed periods in certain individuals (mean 1198 +/- 179 (SD) nmol/L). There has however been substantial variation between studies using the same medium, with Bowell (2010) also finding salivary cortisol to be much greater than this previous work (1222.0 +/- 122.0 (SD) nmol/L). Differences between studies may be due to time of sample collection, as Cross et al (2004) collected their samples later in the day (16:00-17:00), when cortisol has decreased from morning levels. Salimetrics Oral Swabs collect more cortisol than cotton buds (Salimetrics, 2012), and Salimetrics assay (0.19 nmol/L) is more sensitive than those previously used (eg. Orion Diagnostica: 0.8 nmol/L), which could have increased the cortisol recovered. As very high values were only seen at baseline in the current study, there was some consistency across the samples. Direct comparisons between studies may therefore not be useful, although relative differences can be found within studies.

5.4.2 Overall response to the stressor

After the marmosets had been hand-captured, weighed and placed in a new cage, cortisol levels significantly decreased. Changes in cortisol concentration were therefore detected, albeit in the opposite direction to predicted. Despite the decrease in cortisol concentration, there were increases in stress-related behaviours post capture, which suggests the marmosets did find the experience stressful. It is also unlikely that they had habituated to the capture and weighing process. Scent marking and agitated locomotion were both higher following capture than at baseline, which was associated with reductions in calm locomotion. While exploration of a new environment is associated with an increased frequency of scent marking in mammals in general (Eisenberg and Kleiman, 1972), Bassett et al (2003) found increases in scent marking following removal from the homecage and human handling, suggesting it is an anxiety-related behaviour in marmosets. Badihi (2006) reports that scent marking was lowest in outdoor condtions, when marmosets only had olfactory and auditory contact with other groups, suggesting that close proximity to neighbouring animals may also increase levels of scent marking (Stevenson and Pool, 1976).

Proximity and watching were higher at baseline than during post-capture observations. Bowell (2010) also found that contact sitting and watchful behaviour were not affected by a stressor, suggesting these behaviours do not seem to be an indicator of stress in marmosets. Scratching and inactive alert were also significantly higher at baseline than directly after capture. Inactive alert appeared to return to baseline levels by 30 minutes after the stressor. Bassett et al (2003) also found that exposure to a stressor led to decreases in inactivity in marmosets. However, foraging increased directly after being placed in a new cage. Some gender differences were also seen. Males initiated contact more than females, following a stressor. Meanwhile, females tended to forage for longer after being placed into a new cage, which may be due to males deferring to the females (Box, 1997).

While some studies have found significant elevations in salivary cortisol, following social isolation and a period of noise and human activity in the animal house (Cross et al, 2004), others have found reductions in cortisol post stressor. Bowell (2010) found that cortisol level had decreased signficantly from baseline levels by 30 minutes after capture. Cross and Rogers (2006) also found a consistent decrease in cortisol levels in all marmosets after presentation of a snake-model stimulus. This response was unexpected, given the increase in stress related behaviours, including tsik calls, agitated movement and mobbing responses. In a further study, cortisol levels doubled in magnitude when marmosets were isolated from peers in an unfamiliar room. However, playback of mobbing calls from a familiar conspecific when isolated lead to decreases in cortisol (Cross and Rogers, 2006). The researchers suggested that social communication may be stress reducing, at least physiologically, by reinforcing intra-specific bonds.

Stress reduction could be due to social buffering, the ability of a companion to ease the stress of challenging situations (Gilbert and Baker, 2010), resulting in a reduced cortisol peak and faster recovery (Novak et al, 2012), compared to when facing the situation alone. Much physiological evidence has been found for this (eg. Winslow et al, 2003: *M. mulatta*). Although marmosets in the present study were in auditory contact with conspecifics, this still does not explain the behavioural changes observed. However, the order of weighing in a room had an affect. Cortisol was higher 30 minutes after capture, when marmosets were weighed last in the room than when they were weighed first, perhaps as they had been anticipating capture for longer. At this time, those weighed first spent a longer estimated percentage of time inactive alert, watching others being weighed, while those weighed last engaged in more exploration, as all weighing was then complete. Previous research has also found a positive relationship between order of blood sampling in a room and plasma cortisol concentrations (Flow and Jaques, 1997: *M. fascicularis*). Watching other monkeys undergo routine husbandry or procedures can therefore be stressful.

5.4.3 Comparisons between cortisol and behaviour

Attempts to map cortisol data onto behavioural data found contradictory results. Scent mark, agitated locomotion, agitated vocalisation, initiate contact and forage were all negatively correlated with cortisol concentration. Calm vocalisation, proximity, inactive alert, calm locomotion and explore were positively correlated with cortisol concentration. However, none of these correlations was particularly high. While there may be some use for measuring cortisol (Cross et al, 2004), behaviour does appear to provide a better representation of the marmoset's response to a routine stressor (Bowell, 2010). As well as this, it cannot be assumed that cortisol level is a direct index of stress, as lower values are not necessarily good, while higher values are not always bad (Novak et al, 2013). Hubrecht and Mason (1993) concluded that the short-term cortisol response may be an inappropriate measure of stress, failing to tell the whole story, and so integrated measures are more appropriate (Dawkins, 1998).

There may also have been methodological issues with timing when collecting cortisol. Saliva samples could either have been taken too early or too late to catch the peak. It is possible that by the time the marmosets were back in the home cage, the danger has passed and the parasympthetic nervous system has dampened the stress response. Other factors (food, blood contamination) could also have elevated baseline levels. As habituation to the swabs was carried out, it is unlikely the higher cortisol levels were due to stress during saliva collection. Alternatively, while a passive response is associated with increased activation of the parasympathetic system, resulting in greater fluctuations of cortisol, more active responses, such as those seen following capture, involves increased activation of the sympathetic system, which release adrenaline (Cross and Rogers, 2006). A possible area of future research could therefore be to look at Alpha Amylase, a proxy for adrenaline found in saliva (Higham et al, 2010). Indeed, norepinephrine was found to be more sensitive to early deprivation stress in marmosets than cortisol (Dettling et al, 2002).

5.4.4 Effect of rearing condition

Variation in early interactions with caregivers can have an important role in development, with the quality of early experience enhancing stress vulnerability or resilience (Parker and Maestripieri, 2011). Rather than attachment style, response to parental separation may be better explained by the individual's responsiveness to stress (Insel, 1992). However, the present study found minimal differences between conditions in their response to a stressor. Cortisol levels were similar in each rearing condition, with all decreasing from baseline to post capture. Although there was no main effect of rearing at any time point, some differences in cortisol were seen when looking at each rearing condition separately. There was no significant difference in cortisol between the time points in twins or supplementary fed triplets. However, there was a significant difference within 2stay marmosets between the time points, with cortisol significantly higher at baseline than at post 0 mins and post 30 mins. Although this may demonstrate greater changes in cortisol, there was less variation between individuals in this rearing condition, which may account for the significant result. Due to the small sample size and missing data, as well as high variation (at least at baseline), results should be taken cautiously.

The previously reported diminished basal cortisol levels in early separated common marmosets (Dettling et al, 2002) were therefore not seen in the current study. However, studies investigating the effect of rearing background on HPA axis activity have been inconsistent. For example, bonnet macaque (*M. radiata*) infants exposed to variable foraging demand (VFD) when they were 4-17 weeks old, were rated as less sociable and less dominant than low foraging demand (LFD) animals at 2.5-3.5 years (Andrews and Rosenblum, 1994). They also displayed over activity of the HPA axis and blunted noradrenergic responsivity (Rosenblum et al, 1994). Some peer-reared rhesus macaques also show higher basal cortisol levels than mother-reared animals (Higley et al, 1992b), while others show lower basal cortisol levels (Shannon et al, 1998) or similar cortisol levels (Clarke, 1993).

Despite little change in cortisol, marmosets in all conditions exhibited striking increases agitated locomotion following capture and weighing. Scent marking also increased in both twins and supplementary fed triplets following the cage change. Some interesting differences also emerged between the rearing conditions. Behaviour changed more significantly from baseline to post capture for twins, compared to the other conditions. There was significantly less calm locomotion at both post capture points and less self-scratching at post capture 0 mins, than at baseline. Inactive alert was lower at post 0 mins than at baseline and after 30 minutes. Proximity was also lower at 0 mins than at baseline. It therefore appears that there is more unsettled movement post capture. Female twins foraged for longer than males directly after capture. While twin males initiated contact more than females, the reverse was true for terminate contact. There were few significant behavioural changes in 2stay marmosets, except for an increase in foraging at post 0 mins, compared to baseline and post 30 mins.

Supplementary fed triplets engaged in more initiations of contact at both post capture observations points, than at baseline. Watch observer was lower post capture than at baseline, which may be because they are more unsettled or interested in the events going on around them during weighing. Exploration was higher at post 0 mins than at baseline. Therefore, while there were some negative behavioural changes in 2stays and supplementary fed triplets, there were also some increases in positive behaviours. There were however no significant differences found between the rearing conditions in any behaviour studied.

Results are therefore contrary to previous work, which has reported early deprived marmosets to be more anxious and behaviourally inhibited (Dettling et al, 2007). Peer-reared individuals have also been found to show more distress in novel environments (Higley et al, 1992a: *M. mulatta*) and greater fear in acoustic startle response tests (Parr et al, 2002: *M. mulatta*). Instead, results are more consistent with Parker et al’s (2004) stress innoculation study in squirrel monkeys *(S. sciureus),* which demonstrated that brief separations from the family group lead to less negative arousal, and instead more exploration and food consumption in a novel environment. Supplementary fed animals in the current study may therefore be better able to deal with challenges in the laboratory. As there appeared to be more behavioural disturbance in the twin marmosets studied, they may be more vulnerable to stress.

5.4.5 Effect of separation from the family

Rearing primates in isolation can have devastating effects on development and behaviour (Parker and Maestripieri, 2011). However, marmosets in the current study were taken out with their siblings for brief periods, to be supplementary fed, and integrated back into the family as soon as possible, which could have minimised any adverse affects. Effects of family separation tend to be greater when they deviate significantly from the norm, particularly when the individual is separated very early on, and the deprivation is longer lasting and more complete. Risk and protective factors, such as social support, temperament and genes, may also contribute to the outcome. As common marmosets are co-operative rearers (eg. Ingram, 1977), infants are naturally adapted to be passed between helpers, which may help to explain the differences found between this species and the very maternally bonded macaques used in many primate models of parental separation.

Supplementary fed marmosets also have more extensive interactions with human carers than family-reared animals. Prior experience of increased positive human interactions has been shown to reduce stress in the common marmoset. Bassett et al (2003) found that anxiety related behaviour significantly increased following a stressor (capture and weighing) in untrained animals, but not in trained animals. The study provides evidence that increased human contact can reduce fear responses and so reduce the negative reaction to being handled and removed from the homecage. Early exposure to positive human interactions may therefore improve ability to cope with routine laboratory procedures. Although removal from the family is not recommended, if it is possible to keep infants in the group, training to allow co-operation with laboratory procedures may be beneficial in reducing stress for all animals. Training allows a degree of predictability and control, which has been found to have a positive impact on welfare (Bassett and Buchanan-Smith, 2007). Simple positive interactions with humans could also reduce the physiological stress of capture just as much as training (Bowell, 2010).

5.4.6 Conclusion

The present study investigated whether rearing background had a long-term effect on response to a routine stressor in common marmosets. Salivary cortisol was successfully analysed using an assay previously unused in marmosets. Behaviourally, capture elicited some stress in all animals, although this was not reflected physiologically, highlighting the problems of welfare assessment using single dimensions. Some differences were found between rearing conditions in cortisol and behavioural deviations, following the mild stressor. Although it was hypothesised that separation from the family early in life would lead to stress vulnerability, it appears that moderate life stress, as well as increased positive interactions with humans, may have contributed to some stress resilience in supplementary fed triplets. Routine socialisation with humans is therefore recommended, to improve the welfare of laboratory-housed marmosets.

CHAPTER 6: The impact of rearing background on the welfare of common marmosets *(Callithrix jacchus):* Effects on fear and affective state

Abstract

Early life environment, including family separation, can have a major influence on affective state. The current study investigated the effect of rearing background in temperament tests, as well as responses in cognitive bias and preference tests, in adult common marmosets. No significant differences were found in latency to approach and obtain food from a human or a novel object between rearing conditions, suggesting no effect on these bold/shy temperament measures. There were no differences in cognitive bias task acquisition time, or proportion of responses to each ambiguous stimulus. However, only supplementary fed marmosets made fewer responses to the middle probe, compared to the probe nearest the rewarded stimuli, suggesting greater expectation of the negative outcome. Similarly, while no difference was found between rearing conditions in consumption of milkshake at different concentrations, only supplementary fed triplets demonstrated no preference for the lowest milkshake concentration over water, suggesting mild anhedonia. Correlations were also found between each of the tests. These results support other research, suggesting that early separation from the family lead to subtle increases in depressive-like symptoms. However, as these were only minor differences, the practice of supplementary feeding to reduce mortality in large litters of marmosets did not have a major impact on welfare, and so any effect on peformance in reward based scientific tasks is likely to be minimal.

6.1 Introduction

6.1.1 Emotion and cognitive bias in animals

The subjective experience of affective state is at the heart of animal welfare science (Fraser, 2008). Neuroscience evidence suggests that there are multiple levels of control in brain emotion-affective processing. It has been argued that raw affect arises from basic discrete emotional systems, at least in mammals, such as fear, rage, panic and play, which are rooted in particular brain sites (Panksepp, 2011). These ancestral psychological abilities to feel the sensation of pleasure and pain may have been the source of the consciously aware cognitive mind, with species differences in the expression of affective states (Panksepp, 2011). As well as the discrete emotion approach, researchers have further presented a dimensional view of emotions. This takes into account valence (positive versus negative), rather than arousal alone (Mendl et al, 2010a). A wide range of emotions can be integrated, represented as locations in two-dimensional space, and predictions for how these states arise can be made and measured. For example, relaxed and excited are both positively valenced states, but the latter involves a higher degree of arousal (Mendl et al, 2010a).

Mendl et al (2010a) brought the two approaches together, to provide a conceptual framework for studying animal emotion. They suggested that both systems may be present, and interact with each other to serve different functions. Regardless of whether animals consciously experience emotion, it is likely that there are evolutionary advantages of systems that represent the experience of reward and punishment. These systems are at the core of all emotional states (Nesse and Ellsworth, 2009). Mood states could therefore provide information on the presence of rewards and threats in the animal's environment, and how it copes with these (Prinz, 2004). However, as animals are unable to communicate, we have relied on indirect measures, such as levels of stress hormones and behaviour patterns, to assess their emotions (Matheson et al, 2008).

Cognitive bias has recently emerged as a promising tool for the assessment of emotion in animals (Mendl and Paul, 2004). This model is based on the theory that emotional feelings are important in guiding cognitive processes (Williams et al, 1997). Cognitive bias is described as the propensity of an individual to exhibit behaviour indicating anticipation of either a relatively positive ('optimistic') or negative ('pessimistic') outcome, in response to affectively ambiguous stimuli. Biases can therefore provide insight into the valence of the individual's affective state (pleasant/ unpleasant), and possibly even affective states of the same valence (anxiety/ depression) (Paul et al, 2005). The theory is supported by work from Harding et al (2004), which demonstrated that rats showed 'pessimistic' responses, as well as other depressive symptoms, when subjected to adverse conditions.

Negative cognitive biases are reliable indicators of self-reported stress in humans, and are implicated in affective disorders (Wilson et al, 2006). Anxious or depressed individuals tend to interpret ambiguous stimuli more pessimistically (Eysenck et al, 1987). People high in anxiety are more likely to expect a negative outcome from an ambiguous event than those low in anxiety (Eysenck et al, 1991). Similarly, humans experiencing depression are more likely have a reduced expectation of a positive outcome than non-depressed individuals (Miranda and Mennin, 2007).

There is also accumulating research demonstrating cognitive biases in animals, including dogs (Mendl et al, 2010b), rats (Burman et al, 2008), starlings (Matheson et al, 2008), sheep (Doyle et al, 2010), chicks (Salmeto et al, 2011), honeybees (Bateson et al, 2011) and rhesus macaques (Bethell et al, 2012). These studies often involved initial training on a 'Go/No-Go' task, in which animals learnt to respond to a rewarded stimulus, and to cease responding to an unrewarded or punished stimulus. During testing, they were presented with intermediate stimuli. More responses to intermediate probes would indicate a positive bias (optimism), while few responses would indicate a negative bias (pessimism). Active choice tasks have also been employed (eg. Parker, 2008: *Rattus norvegicus*; Matheson et al, 2008: *Sturnus vulgaris*), in which animals were required to make one of two responses, depending on whether they perceived the cue to be closer to the positive (more/ immediate food) or negative (less/ delayed food) stimuli. While this eliminates the question of reduced general activity, as a response is required for both cues, the use of food as positive and negative reinforcers could make the perceived affective difference small and so biases may not be evident (Mendl et al, 2009).

Generally, results of cognitive bias testing demonstrate that animals develop a more negative outlook following a stressful event, although develop a more positive outlook following positive events, such as enrichment. Changes in the environment therefore influence information processing and response to ambiguous stimuli (Bethell et al, 2012). A change in response to the probe nearest the rewarded stimuli (P+), but not to the probe nearest the unrewarded stimuli (P-), could implicate mechanisms sensitive to reward, while changes in response to P-, but not to P+, could implicate those sensitive to punishment (Bethell et al, 2012).

6.1.2 Effect of separation from the family

Temperament and cognition

Young marmosets are adapted to receive intense care from all members of the family during infancy, which is known to be important for their development (Dettling et al, 2007). However, as described earlier, hand rearing is a common practice in colonies of laboratory housed common marmosets, when families have litters larger than two. Several practices are employed, which involve either one or all infants being removed from the family for supplementary feeding, to improve their survival. Many aspects of the early life experience are therefore altered, including the physical and social environment (Feenders and Bateson, 2013). Early separation from the family could induce changes in their cognition and behaviour, increasing anxiety and fear in adult animals. In human populations, adverse experiences in childhood can also increase the risk of developing mood disorders (Parker and Maestripieri, 2011).

Previous work has used parental separation paradigms, to look at response to novelty later in life. Maternally deprived macaques were significantly more neophobic, showing greater behavioural disturbance, less exploration (Spencer-Booth and Hinde, 1971: *M. mulatta*) and a longer latency to retrieve food in a novel environment (Caine et al, 1983: *M. nemestrina*). However, there were marked individual differences in some studies (Spencer-Booth and Hinde, 1971). Peer-reared primates have also been found to display high levels of fear. Hyperemotional behaviour (Capitanio, 1986: *M. nemestrina*), as well as reduced responsiveness (Capitanio et al, 2005: *M. mulatta*) has been found, which could indicate differences in anxiety and depression.

Pryce et al (2005) studied the short and long-term effects of daily social isolation from biological parents as infants in common marmosets. Variable times of onset and duration were used, to introduce unpredictability. They trained adolescent (8-12 months old) marmosets on a two-way visual discrimination simple/ reversal learning task. Results showed that, while early deprived (ED) marmosets performed similarly to controls on the simple discrimination task, they made significantly more errors following visual reversal. They therefore took more sessions to learn the new task (Pryce et al, 2004), suggesting an impaired ability to acquire information about changes in the emotional significance of environmental stimuli. As ED marmosets spent more time responding to only one stimulus position following reversal, subjects may have had increased vulnerability to perceived loss of control with respect to rewarding events (Pryce et al, 2004). Early deprived animals may therefore be unable to respond flexibly to environmental change.

However, Feenders and Bateson (2013: *Sturnus vulgaris*) found some evidence that hand-rearing led to less neophobic and less impulsive animals, with no difference in learning speed, accuracy or perseveration, compared to mother-reared animals. Therefore, emotionally driven decision making was altered in a way generally associated with reduced developmental stress (Feenders and Bateson, 2013). In rats, early handling also led to animals that were less anxious and fearful, when exposed to later challenges (Pryce et al, 2003). However, differences between studies may be due to the species investigated, with macaques often being very maternally bonded.

Temperament traits may also be linked to cognitive bias. For example, reduced fearfulness (induced using diazapam) has been associated with a more positive judgement of ambiguous stimuli in lambs (Destrez et al, 2012). Neuroticism, involving anxiety, depression and impulsivity, is associated with a fear of uncertainty and shyness with unknown individuals, as well as the inhibition of behaviour following punished or unrewarded events. On the other hand, extraversion, including traits such as gregariousness, assertiveness and positive emotions, is associated with appetitive approach to novel and rewarded events (Ebstein, 2006). Responses in novel object and human interaction tests may therefore be useful in determining such temperament traits, related to the higher-order temperament dimension of boldness/shyness (Svartberg, 2002). They are objective measures that are easy to administer and interpret (Bowell, 2010), and have been used to determine temperament in several species of primate (eg. Bowell, 2010: *C. jacchus*; Coleman et al, 2005: *M. mulatta*). Subjective personality questionnaires have also been developed for a number of non-human primate species (eg. Stevenson-Hinde and Zunz: *M. mulatta*, 1978; King and Figueredo, 1997: *Pan troglodytes*; Weiss, King and Perkins, 2006: *Pongo* spp.), although these have yet to be validated for the marmoset. Cognitive bias has rarely been co-investigated with temperament, and so the relationship between them is of interest. As cognitve bias tests rely on food rewards, anhedonia may also be associated with responses.

Anhedonia

Anhedonia, a loss in interest in acts that are normally rewarding, has also been measured in animals exposed to early separations from the family. It is a core symptom of major depression (Paul et al, 2000), as well as a chronic depressed mood (sadness, irritability), which may be due to deficits in motivation or pleasure, and may have an influence on reward decision-making (Treadway and Zald, 2010). Anhedonia can be readily measured by changes in hedonistic responsiveness. For example, Wilner et al (1992) describes studies of chronic exposure to a variety of mild stressors, which lead to decreased preference for sweet liquids in rats, which can be reversed with chronic administration of antidepressant drugs.

Maternal deprivation in squirrel monkeys resulted in decreased consumption of novel fruit juices (Hennessy, 1986a). While this was interpreted as neophobia, it may also reflect anhedonia (Paul et al, 2000). Paul et al (2000) also found that maternal deprivation led to anhedonia-like states in adult rhesus macaques. They looked at changes in concentration on consumption, to identify preference thresholds, giving animals two-bottle choice tests with access to multiple concentrations of sweet and bitter solutions (sucrose and quinine, vs. tap water). Looking at the effect of stimuli with different hedonistic properties is of interest, due to the tendency of depressed individuals to focus on the aversive properties of events (Paul et al, 2000). All animals drank more sucrose than water, although there was a reduced preference in those maternally deprived, compared to controls. However, these animals also continued to consume more aversively bitter water at much higher concentrations than controls, which suggests a general weakened response to stimuli (affective flattening), and so it was suggested that anhedonia may be secondary to a sensory deficit (Paul et al, 2000).

Operant schedules of conditioned reinforcement can be used to separate the consumption of reward (liking) from motivation to obtain that reward (wanting) (Pryce et al, 2005). Infant marmosets that were exposed to repeated parental separations early in life were found to perform significantly fewer progressive ratio operant responses (touches of a stimulus to obtain a reward: Pryce et al, 2004), which is indicative of a diminished response to rewarding stimuli. Maternal care has also been manipulated, by varying the foraging demands on the mother (predictable high or low food supplies, or unpredictable food supplies). Bonnet macaques with mothers exposed to unpredictable foraging demands showed reduced sociability as adults (Rosenblum and Andrews, 1994), possibly reflecting an anhedonic state (Pryce et al, 2005).

Early deprivation in marmosets has therefore been found to induce depression-like symptoms (Pryce et al, 2005), as well as cause impairments in several domains of cognitive function and response to the environment (Ruedi-Bettschen et al, 2005), suggesting that it is a marked early life stressor (Pryce et al, 2005). It is therefore possible that early separated marmosets display more fear and depressive-like symptoms than undisturbed marmosets. However, it has been found that hand-rearing reduces level of fear in some species (Feenders and Bateson, 2013; Pryce et al, 2003). Therefore, it is important for welfare, as well as scientific findings, to understand the psychological consequences of such procedures (Bethell et al, 2012). Simple temperament tests, as well as cognitive bias and choice tests, may provide useful methods to assess affective state in marmosets.

6.1.3 Aim

The aim of this study was to establish the impact of rearing background on temperament, cognitive performance and affective state in adult common marmosets. Supplementary fed animals, exposed to early life family separations, were compared to undisturbed family-reared animals. Each subject was first given a human interaction and novel object test. A ‘Go/ No Go’ cognitive bias task was then developed for use with captive marmosets. Time to learn the task, as well as response to ambiguous probes, was measured. Cognitive bias testing was followed by a two bottle choice test, to measure anhedonia. Correlations between each of the welfare measures were also conducted. Based on previous research, it was hypothesised that supplementary fed marmosets would display greater latencies to approach novel objects and humans, as well as display a more negative cognitive bias and a reduced interest in an appetitive liquid.

6.2 Method

6.2.1 Study animals

Twenty five adult common marmosets, housed in vasectomised male mixed-sex pairs, were studied. Due to circumstances at the laboratory, these were a different group of animals to those studied in Chapter 5. The marmosets used were aged between 1 year 4 months and 3 years 1 month at the start of data collection. One animal per pair was sampled. Animals were studied from three rearing backgrounds (Table 6.1), comprising eight supplementary fed triplets (5 male, 3 female), nine 2stays (4 males, 5 females) and eight twins (4 males, 4 females). For details of rearing conditions see Section 2.2. One twin female failed to learn the cognitive bias visual discrimination task within the time period of 8 weeks, and so was not tested, although results were included from the novel object/human interaction and preference tests.

Housing and husbandry

For full details of housing and husbandry, see Sections 2.4.2 and 2.5.

Table 6.1: Information for each animal studied

Rearing	Gender	Litter size	Comments
Supplementary fed triplets (N= 8)			
35P	F	3	
82P	M	3	
89P	M	3	
34P	F	3	
14R	M	3	
124R	F	3	
123R	M	3	
108R	M	3	
2stays (N= 9)			
16R	F	3	One infant found dead day 2
29P	M	3	One infant euthanised day 6
10P	M	4	One infant euthanised day 2, one euthanised day 5
119P	F	3	One infant euthanised at 1 week
18R	M	3	One infant found dead day 2
128R	F	3	One infant euthanised day 3
38T	F	3	One infant euthanised day 1
82R	M	3	One infant stillborn
46T	F	3	One infant euthanised day 1
Twins (N= 8)			
11P	F	2	
31P	M	2	
72P	M	2	
48P	F	2	No cognitive bias test data.
76R	M	2	
140R	F	2	
29T	F	2	
139R	M	2	

6.2.2 Temperament tests

Response to novel object

The novel object test was conducted first, to prevent the marmosets from being influenced by an experimenter who had previously given them food (Bowell, 2010). Two plastic film canisters (one for each animal, to prevent one individual dominating the food source) were filled with pieces of chopped banana, as this is a favoured food (Caldwell et al, 2009, and pers. obs.), increasing motivation to obtain the reward. It also has a strong aroma, ensuring the marmosets were able to detect the presence of food. Tests were carried out between 9:00-11:00, after the animals had their morning feed. The pots were placed face down on a shelf, the door shut and a stopwatch started. The observer stood in front of an adjacent cage and avoided looking directly at the test cage, which can be threatening for marmosets. Latency from closing the door to when each subject first touched the canister and when they first obtained the banana was recorded. A time limit of 2 minutes (120 secs) was imposed (based on Bowell, 2010).

Response to human interaction

The novel object test was followed by the human interaction test. The marmoset's home cage was approached slowly, at an angle of approximately 45°. Standing approximately 30cm away from of the front veranda, without facing directly into the cage or looking at the marmosets, two pieces of dried papaya or pineapple (a favoured food as indicated by preference tests with non-study marmosets in the colony) was offered, one for each animal. Latency to take the reward from the hand was recorded for both members of the pair, up to 2 minutes (based on Bowell, 2010). These latencies provide information on the order in which each pair takes the food, as one member may be facilitated by the other. If the study animal's partner dominated the food source, or appeared to prevent the test subject from approaching, they were distracted by providing another piece of food lower in the cage, while the reward was offered to the study animal in the original position. The non-test animal was never rewarded in the test animal's location. Testing was carried out between 9:00 and 11:00.

6.2.3 Cognitive bias

Apparatus

An adult marmoset was enclosed in the veranda on the front of the home cage, to allow individual testing whilst maintaining contact with their partner. A visual discrimination task was then employed, based on the Wisconsin General Test Apparatus, an established method of testing cognitive abilities in non-human primates. Tubes of differing height were presented outside the veranda, on a tray attached to the front of the cage. Reference tubes (S+ and S-) were 2cm and 15cm in height. Three unreinforced ambiguous heights were evenly distributed at intermediate points between the two reference heights: one located midway (PI) between the reference points, while the other two (P+ and P-) were halfway between the central probe and each reference height (11.5cm, 8.5cm, 5,5cm) (based on Bethell et al, 2012). Plate 6.1 shows the cognitive bias stimuli. Stimuli were presented on the tray, on which the food (small pieces of rusk) was placed. Plate 6.2 shows the set-up of the apparatus in the animal's home cage.

Food was hidden under each stimulus, to prevent olfactory cues. When the reinforced height (S+) was presented, the reward was revealed, while at the unreinforced height (S-), the reward was unavailable (baited but inaccessible). No food deprivation was employed. Half of the animals were allocated the largest tube (15cm) as the reinforced stimulus, while the other half were allocated the smallest tube (2cm), to counterbalance the rewarded and unrewarded conditions (Bethell et al, 2012).

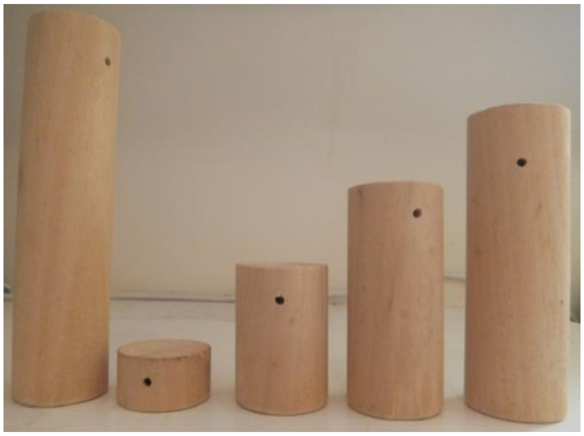


Plate 6.1: Cognitive bias stimuli

Plate 6.2: Cognitive bias apparatus set-up in the home cage

Training

Training sessions were carried out once a day at similar times (between 9:00 and 12:00), avoiding cleaning and feeding times to minimise disturbance. All sessions lasted for 5 minutes maximum. Sessions were also terminated if the animal earned the maximum amount of rewards (20 pieces). Order of training was counterbalanced between the conditions (twin, 2stay, supplementary fed triplet), over each group of animals being studied.

‘Go/No go’ task training sessions were conducted, in which single stimuli were presented (Burman et al, 2008). Correct ‘Go’ responses to S+ were rewarded with an accessible treat (100% fixed ratio schedule). Correct ‘No go’ responses to S- were unrewarded (inaccessible treat, with a 2 second inter-trial interval), while incorrect ‘Go’ responses were followed by a 5 second time-out punishment (Pryce et al, 2004). At the end of each daily session, the monkey was rewarded with a favoured piece of dried fruit. The number of trials taken to achieve criteron was recorded, to look at any differences between conditions in training performance (Mendl et al, 2009). There were three stages of training, to shape the behaviour gradually, allowing the animals to learn more easily. The training schedule was as follows:

Training session A (Rewarded)

The marmosets were first allowed 2 days to familiarise themselves with the apparatus. They were then presented with the rewarded height and encouraged to touch the tube to obtain the reward (following Pryce et al, 2004). A 5 second time limit was imposed for responses, with a maximum of 20 trials or 2 minute session, whichever occurred first. A new trial began when the animal either received the reward or 5 seconds had passed with no response. They were considered trained when the animal was calmly moving around the enclosed space, reliably touching the tube and taking the reward for 80% of presentations, over 3 consecutive days. Session B then began.

Training session B (Fixed rewarded and unrewarded)

The unrewarded height was introduced. In trials 1-22, the rewarded height was presented for two consecutive trials, the unrewarded height for the next two trials and this process repeated (Burman et al, 2008). The first and last trials were always rewarded, to maintain interest in the task. A 2 second response time was imposed. This presentation time was selected, as it allowed enough time for the animals to respond on 'Go' trials and ensure attention was maintained during 'No go' trials. This session continued until the animal responded correctly on 80% S+ trials and 80% S- trials, over 3 consecutive days, before Session C commences.

Plate 6.3: Marmoset reaching for the S+ stimulus

Training session C (Random rewarded and unrewarded)

A pseudorandom schedule was then used, with the 20 training entries divided between rewarded and unrewarded heights. No more than two rewarded or unrewarded heights occurred consecutively, and equal numbers of both were presented (Burman et al, 2008). The first and last trials were always rewarded. A 2 second response time was imposed, with a new trial starting if there was no response within this period. Training was considered completed when the animal was responding correctly on 80% S+ trials and 80% S- trials (Bethell et al, 2012), over 3 consecutive repetitions. Plate 6.3 shows the marmoset sitting within the front veranda and reaching for his S+ stimuli (the small tube).

Cognitive bias testing

Twenty trials were carried out during each test session. Three unreinforced ambiguous height trials (P+, PI, P-) were interspersed, on trials 6, 12 and 18. The overall sequence alternated between rewarded and unrewarded heights, starting and finishing with a rewarded trial. There was the same number of ambiguous trials following a rewarded height as an unrewarded height. The presentation order was counterbalanced over 3 test days, with heights depending on the learned S+ and S-, as follows:

Test day 1: P-, PI, P+
Test day 2: PI, P+, P-
Test day 3: P+, P-, PI

The number of 'Go/No go' responses to ambiguous heights were recorded. A pseudorandom training day was presented between the test days, to re-establish the learnt discrimination task and ensure the animals were performing to criterion (based on Bethell et al, 2012). If there was more than 10 seconds of persistent escape attempts at any time, the animal was allowed to leave before the test was completed, although this occurred rarely. Only cognitive bias sessions where correct responses were made on at least 80% of trained stimuli were included (noted during the session).

6.2.4 Reward Motivation

Two bottle preference tests

After completion of cognitive bias testing, monkeys were not tested for 1 week (following Pryce et al, 2004). Reward motivation was then assessed, using a two bottle preference test. A pilot study was first conducted, to assess the marmosets' preference for milkshake, compared to tap water. The animals are routinely given milkshake for syringe training (prepared with 200ml of water and 3 scoops of Nesquik banana milkshake powder) and on Sundays, so are familiar with the liquid. Three breeding pairs (3 males, 3 females) were presented with 2 bottles for 10 minutes, one of milkshake and the other of tap water, over four days. Total consumption for each pair was then measured. A paired samples t test revealed significantly more milkshake was consumed than water (t=6.42, df=11, P<0.001), although preference did tend to decrease over the test days. Figure 6.1 displays the mean amount of milkshake and water consumed over the pilot test days.

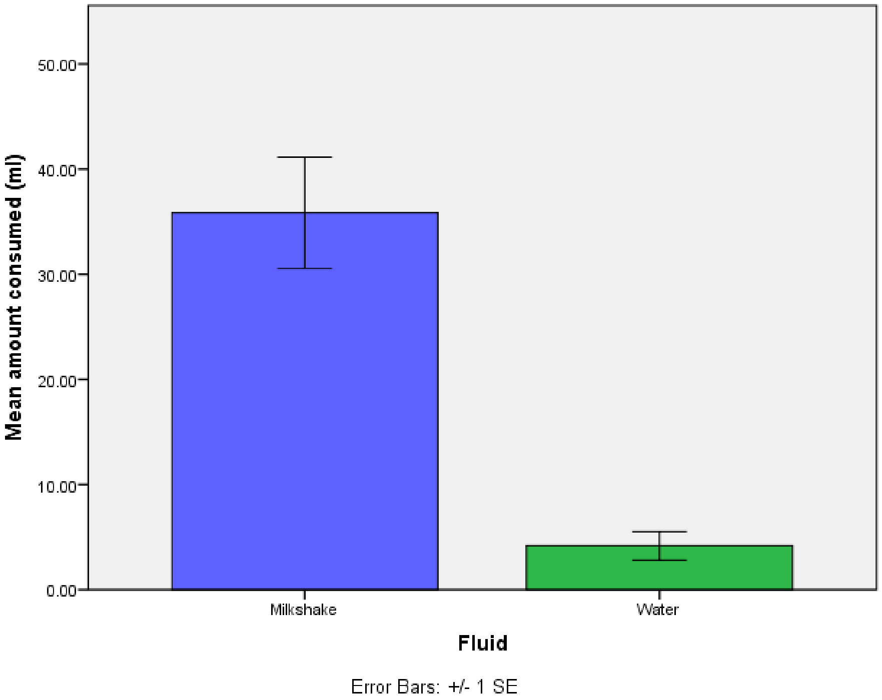


Fig 6.1: Mean (+/- 1 SE) amount of milkshake and water consumed per 10 minute test (max available 100ml) in the pilot study preference test

Testing was conducted in the home environment, to avoid potential confounds with separation, and neither food nor fluid management was employed. The marmoset was enclosed in the front veranda, to have exclusive access to the bottles whilst maintaining contact with their partner (Pryce et al, 2004). Animals were exposed to the bottles on two occasions prior to testing, with the veranda open.

Once a day, at a similar time (9:00-12:00), the animals were allowed access to the testing box for 2 minutes, to drink from a pair of identical 60ml drinking bottles. These were simultaneously presented in the middle of the veranda (Laska et al, 1997). One bottle contained tap water, and the other contained one of four concentrations of Nesquik banana milkshake (60ml water with 1 scoop of powder; ¾, ½ and ¼ scoops). There were therefore 4 trials, over 4 separate days. Bottle positions were alternated daily to control for position preference, and concentration pairs were counterbalanced between animals to avoid order effects. Consumption of water and milkshake was measured at the end of access. Pearson's correlation revealed that there was no significant association between body weight and average or total fluid consumed over the test days. Therefore, data were analysed in ml consumed, rather than ml/g. If there was more than 10 seconds of persistent escape attempts at any time, the animal was allowed to leave immediately, before the test time was completed.

Plate 6.4 shows the preference test set-up within the home cage.

6.2.5 Statistical analysis

Data were first checked for underlying assumptions of normality, using the Kolmogorov-Smirnov test. As not all data were normally distributed, Kruskal Wallis tests were conducted to look at differences between rearing conditions in latency to retrieve and obtain food in the human interaction and novel object tests. Mann Whitney tests were also conducted to look at differences between gender overall and within rearing conditions.

As data were normally distributed, a 2 way ANOVA (between rearing condition x between gender) was used to look at differences in cognitive bias task acquisition time. Exploratory analyses were conducted to assess possible effects of testing day on response to each probe. As non-parametric, binary responses were used, Cochran's Q tests (an extension of Kruskal Wallis test, for dichotomous data) were conducted. No significant difference of testing day was found on proportion of responses made to each probe, in any rearing condition, and so data were collapsed across the testing sessions. The proportion of 'Go' responses were calculated over the three test days (sum of responses/ number of days). No transformation was successful in making cognitive bias data normally distributed, and so Friedman tests were conducted to look at overall and within rearing condition differences in response to each probe, with follow up Wilcoxon tests. Kruskal Wallis tests were used to examine differences between rearing conditions in response to each probe. Mann Whitney tests were used to look at gender differences within rearing conditions.

Despite transformations, data remained non-normal, and so Friedman tests were conducted to look at overall effect of milkshake concentration on the amount of milkshake and water consumed. Milkshake consumption within the rearing conditions was normally distributed. Therefore, mixed factor 4x2 ANOVAs (within concentration x between gender) were conducted, to look at differences in amount consumed between milkshake concentrations. As no post hoc tests were possible, due to the repeated measures design, the ANOVA was followed by paired samples t tests to find which milkshake concentrations differed. Independent samples t tests were used to look at gender differences in consumption at each concentration. Despite transformations, water consumption data within the rearing conditions remained non-normal. Friedman tests were therefore used to look at differences in water consumption at each milkshake concentration, with follow-up Wilcoxon tests. Kruskall Wallis tests were conducted to look at effect of rearing condition on water consumption. Mann Whitney tests were used to look at gender differences. Wilcoxon tests were used to look at differences between milkshake and water consumption at each concentration overall and within each rearing condition. A two way 3x2 ANOVA (between rearing condition x between gender) was also conducted to investigate differences in consumption at each milkshake concentration.

Finally, Spearman's rank correlations were conducted, to look at any associations between all of the tests. Fifteen correlations were run in total. To correct for multiple comparisons, those with r values of 0.60 or above were considered most relevant. These are presented graphically.

6.3 Results

6.3.1 Human interaction test

In the human interaction test, 100% of the animals took food from the hand well within the 2-minute time limit. There was no significant difference between rearing conditions in latency to take food from a human. There was no overall or within rearing condition effect of gender.

6.3.2 Novel object test

Results showed that 100% of animals approached the canisters, with 96% obtaining the food (only 1 female 2stay didn't obtain the banana within the 2 minute time limit). There was no significant difference between the rearing conditions in latency to approach or obtain food from the novel object. There was no effect of gender overall or within any of the rearing conditions.

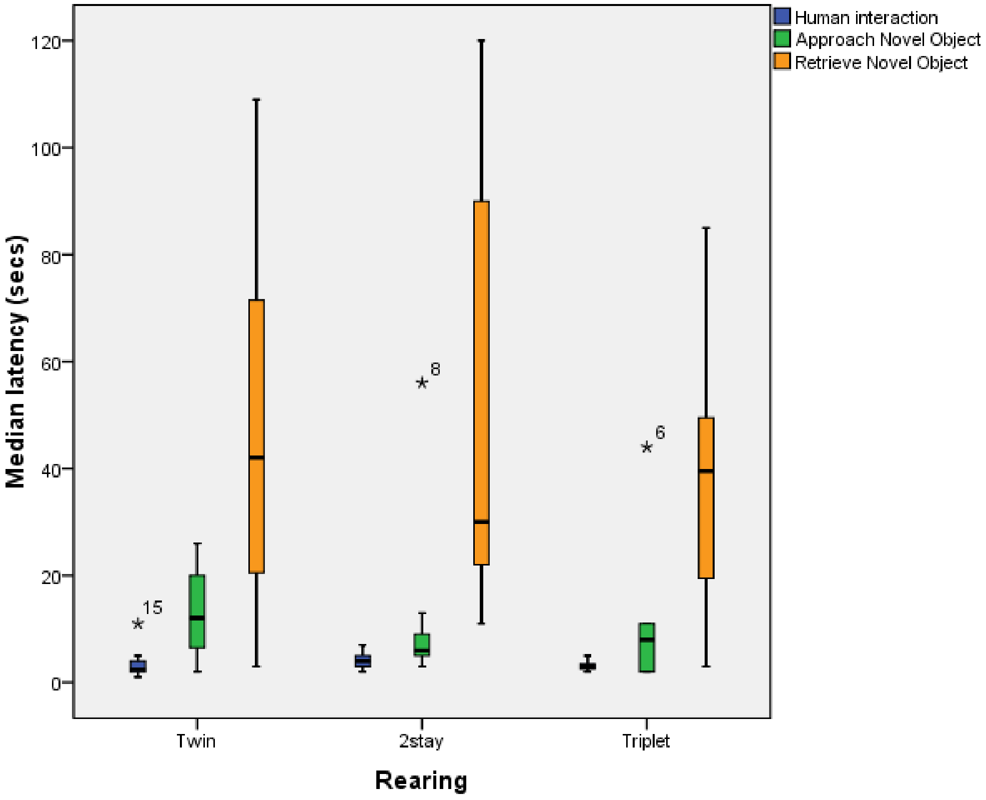


Fig 6.2: Median latencies (seconds) to obtain food from a human, and to approach and retrieve food from the novel object, in each rearing condition. Median: solid line; Interquartile range: boxes; Minium and Maximum value: whiskers; Outliers: stars.

6.3.3 Cognitive bias

Effect of rearing on task acquisition time

Training took a mean of 20.36 +/- 8.93 (SD) sessions to learn the task. Many learnt in less than 20 sessions (4 weeks), although some took the full 40 sessions (8 weeks). A ceiling value of 40 was used for the one individual that didn't learn. There was no significant effect of rearing condition or gender in time taken to complete the visual discrimination training.

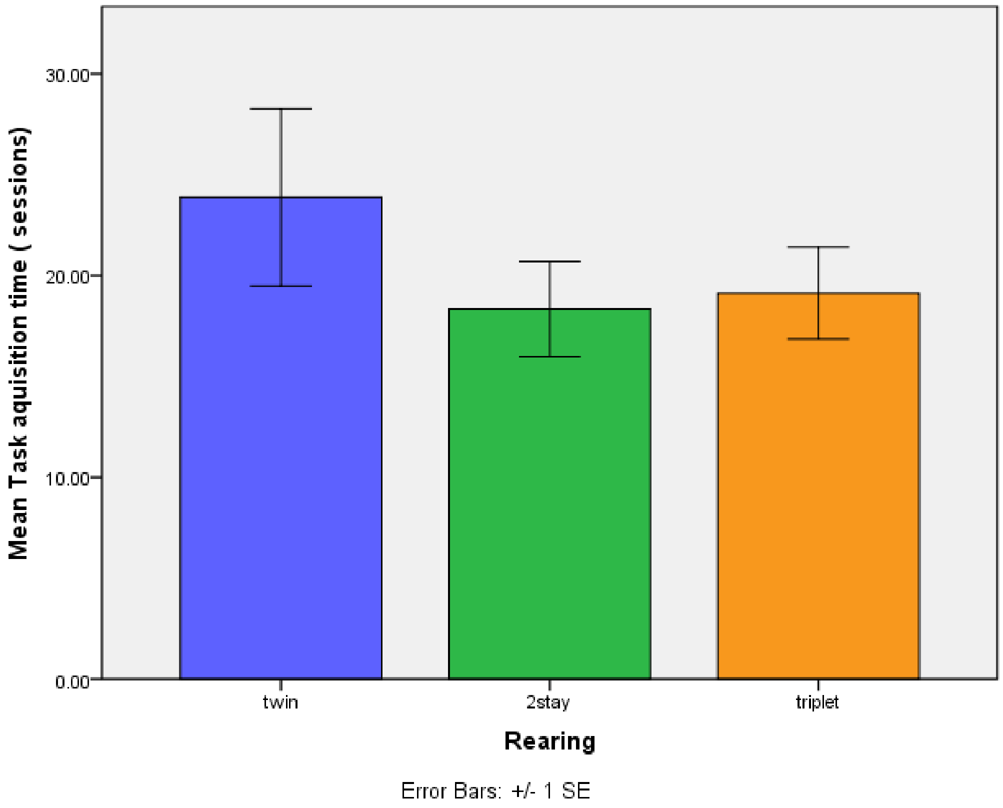


Fig 6.3: Mean (+/- 1 SE) number of sessions taken to complete training for the cognitive bias task in each rearing condition

Response to probes overall and within rearing conditions

Figures 6.4a-c display the mean responses to P+, PI and P- on the three test days for twins, 2stays and supplementary fed triplets. There was an overall difference in response to each probe. P+ received significantly more ‘Go’ responses than PI and P-. PI received significantly more ‘Go’ responses than P-. Table 6.2 shows the significant results of statistical analysis.

Each rearing condition was then examined separately. For twins and 2stays, there was a significant difference in the proportion of ‘Go’ responses to each stimulus. Although no significant difference was found between P+ and PI or P- and PI, there were significantly more responses to P+ than P-. There was also a significant difference in proportion of ‘Go’ responses to each stimulus for supplementary fed triplets. While no significant difference was found in response to P- and PI, there were significantly more responses to P+ than PI, well as P-.

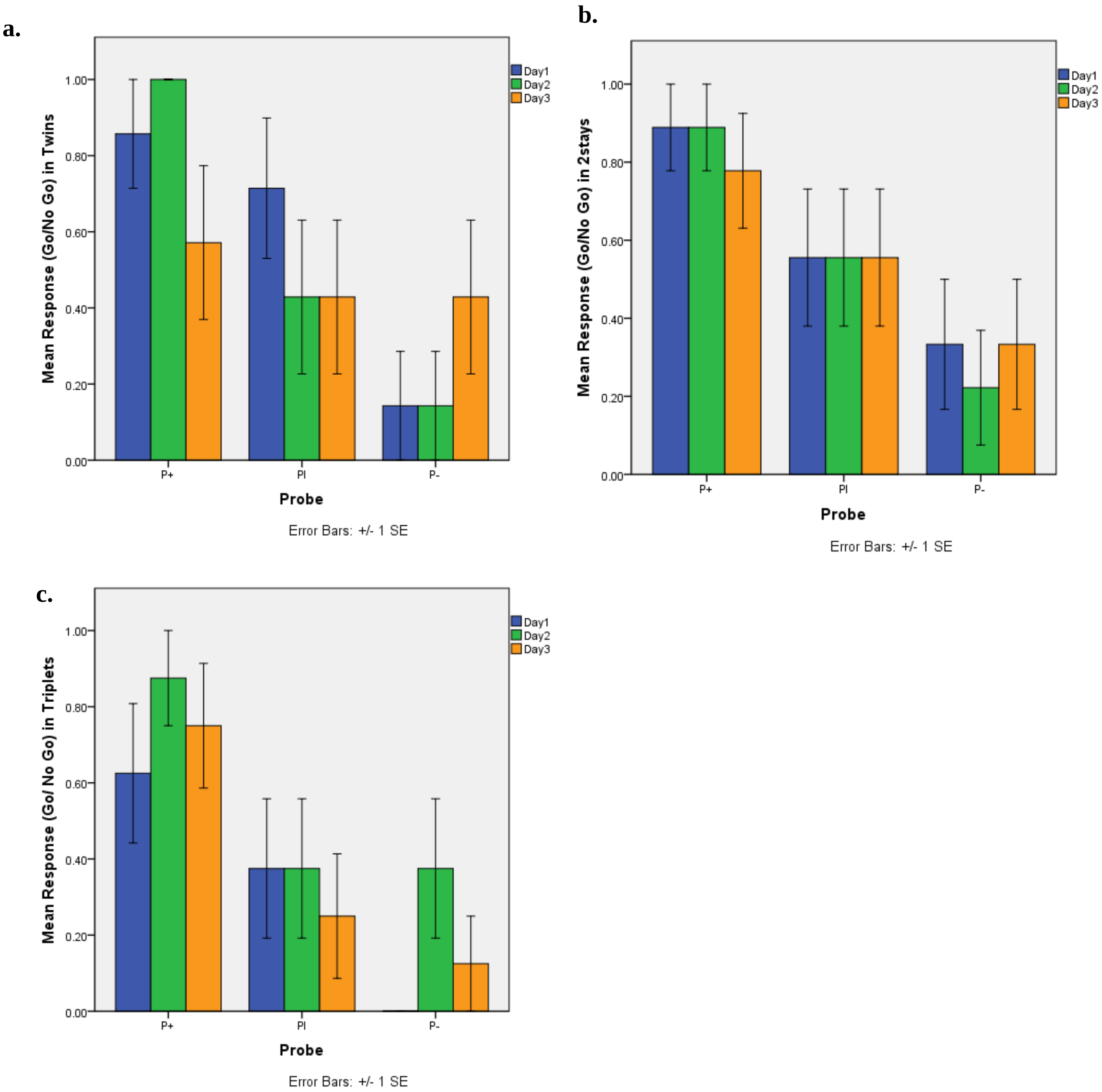


Fig 6.4 a, b and c: Mean (+/- 1 SE) proportion of responses (0= 'No go', 1='Go') to each probe on each of three test days for twin (a), 2stay (b) and supplementary fed triplet marmosets (c). 'No go' indicates no touch to the probe; 'Go' indicates a touch to the probe. (Means are presented as medians are zero.)

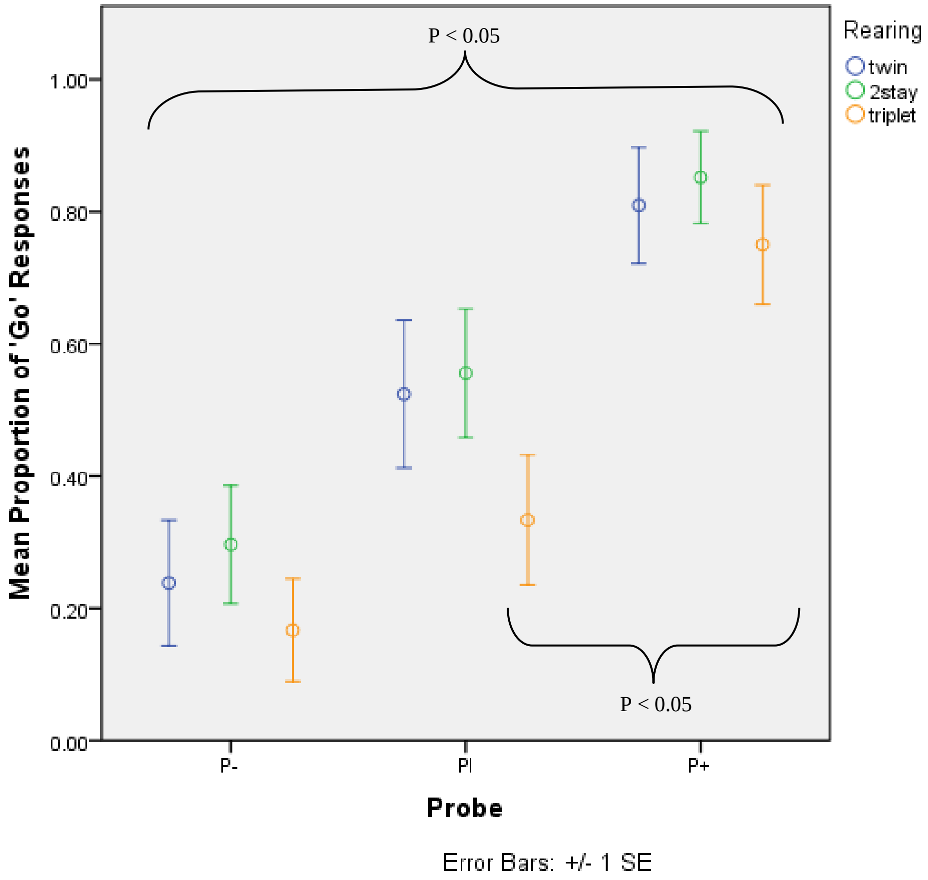


Fig 6.5: Mean (+/- 1 SE) proportion of responses on probe trials (P+, PI, P-) for twins, 2stays and supplementary fed triplets. (Means are presented as medians are zero.) All conditions had significantly ($P<0.05$) less responses to P- than P+; supplementary fed triplets also had significantly less responses to PI than P+.

Effect of rearing condition on response to probes

No significant difference was found between the rearing conditions in ‘Go’ responses to P+, PI or P-. Variation in responses to PI and P- was large, particularly in twins. No differences were found between males and females in any rearing condition for any probe. Figure 6.5 displays the mean proportion of responses to each probe in each rearing condition.

Table 6.2: Significant results for cognitive bias tests (overall and within rearing conditions)

Test	**Analysis**	**df**	**Test statistic**	**P**
Overall	Response to probe	2	X^2= 31.57	<0.001
	P+ > PI		Z=-3.20	0.001
	P+ > P-		Z=-4.80	<0.001
	PI > P-		Z=2.69	0.007
Twins	Response to probe	2	X^2=11.00	0.004
	P+ > P-		Z=-2.39	0.017
2stays	Response to probe	2	X^2=9.85	0.007
	P+ > P-		Z=-2.54	0.011
Triplets	Response to probe	2	X^2=11.12	0.004
	P+ > P-		Z=-2.54	0.011
	P+ > PI		Z=-2.03	0.042

6.3.4 Preference tests

Fluid consumption overall

When all data were combined, there was a significant difference between the milkshake concentrations in amount of milkshake consumed. Each higher concentration was consumed significantly more than the lower concentrations. There was also a significant difference in amount of water consumed, depending on which concentration of milkshake it was paired with. Water was consumed significantly more when paired with 0.50 than 0.25 and 0.75, as well as more at 0.25 and 0.50 than at 1.00. Significantly more milkshake was consumed than water at each concentration. Table 6.3 displays the significant results of statistical analysis in the preference test overall.

Table 6.3: Results of significant preferences overall, for different concentrations of milkshake in comparison to water, for each concentration of milkshake, and for water in each milkshake concentration

Analysis	**df**	**Test statistic**	**P**
Milkshake v Water			
0.25 milkshake > water		Z=-3.36	0.001
0.50 milkshake > water		Z=-3.93	<0.001
0.75 milkshake > water		Z=-4.22	<0.001
1.00 milkshake > water		Z=-4.38	<0.001
Milkshake consumed/ Concentration	3	X^2= 50.12	<0.001
0.25 < 0.50		Z=-3.60	<0.001
0.25 < 0.75		Z=-3.88	<0.001
0.25 < 1.00		Z=-4.38	<0.001
0.50 < 0.75		Z=-2.72	0.007
0.50 < 1.00		Z=-4.38	<0.001
0.75 < 1.00		Z=-3.19	0.001
Water consumed/ Milkshake concentration	3	X^2=22.00	<0.001
0.25 < 0.50		Z=-1.97	0.049
0.25 > 1.00		Z=-2.96	0.003
0.50 > 0.75		Z=-2.75	0.006
0.50 > 1.00		Z=-3.60	<0.001

Twins

There was a significant difference in amount of milkshake consumed between the concentrations for twin marmosets. No concentration*gender interaction was found. More milkshake was consumed at 0.75 and 1.00 than at 0.25, as well as more at 0.75 and 1.00 than 0.50. Females drank significantly more of 1.00 milkshake concentration than males. There was no significant difference in water consumption at each milkshake concentration. There was no significant gender difference in water consumption. Significantly more milkshake was consumed at each concentration, than water. Figure 6.6 shows the amount of water and milkshake consumed at each concentration for twin marmosets. Table 6.4 displays the significant results for the preference test in twins.

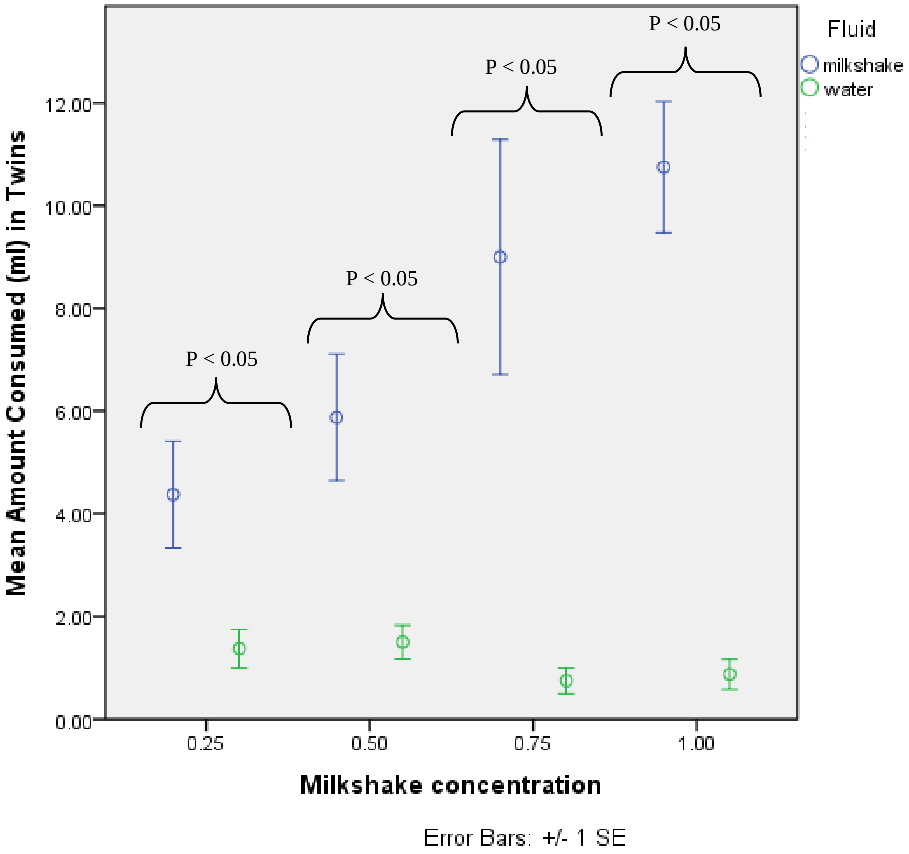


Fig 6.6: Mean (+/- 1 SE) amount of milkshake and water consumed (ml) at each milkshake concentration for twin marmosets. (Means are presented as medians are zero.) Milkshake consumption was significantly ($P<0.05$) higher than water consumption at 0.25, 0.50, 0.75 and 1.00 concentrations.

Table 6.4: Results of significant preferences in twins, for different concentrations of milkshake in comparison to water and for each concentration of milkshake.

Analysis	**df**	**Test statistic**	**P**
Milkshake v Water			
0.25 milkshake > water		Z=-2.38	0.018
0.50 milkshake > water		Z=-2.38	0.018
0.75 milkshake > water		Z=-2.53	0.012
1.00 milkshake > water		Z=-2.31	0.021
Milkshake consumed/	3	F=13.23	<0.001
Concentration			
0.25 < 0.75	7	t=-3.19	0.015
0.25 < 1.00	7	t=-6.50	<0.001
0.50 < 0.75	7	t=-2.70	0.031
0.50 < 1.00	7	t=-6.36	<0.001
Females < Males at 1.00	6	t=-2.69	0.036

2stays

There was a significant difference in amount of milkshake consumed between the concentrations for 2stays. No concentration*gender interaction was found. Significantly more milkshake was consumed at 0.50, 0.75 and 1.00 than at 0.25. Significantly more milkshake was also consumed at 1.00 than at 0.50 and 0.75. There was no significant difference between males and females in milkshake consumption. There was a significant difference in water consumption at each milkshake concentration. Tests found significantly more water was consumed at 0.50 than at 0.25, 0.75 and 1.00, as well as more at 0.25 than 1.00. There were no significant gender differences in water consumption. Significantly more milkshake was consumed at each concentration than water. Figure 6.7 shows the amount of water and milkshake consumed at each concentration for 2stay marmosets. Table 6.5 displays the significant results for the preference test in 2stays.

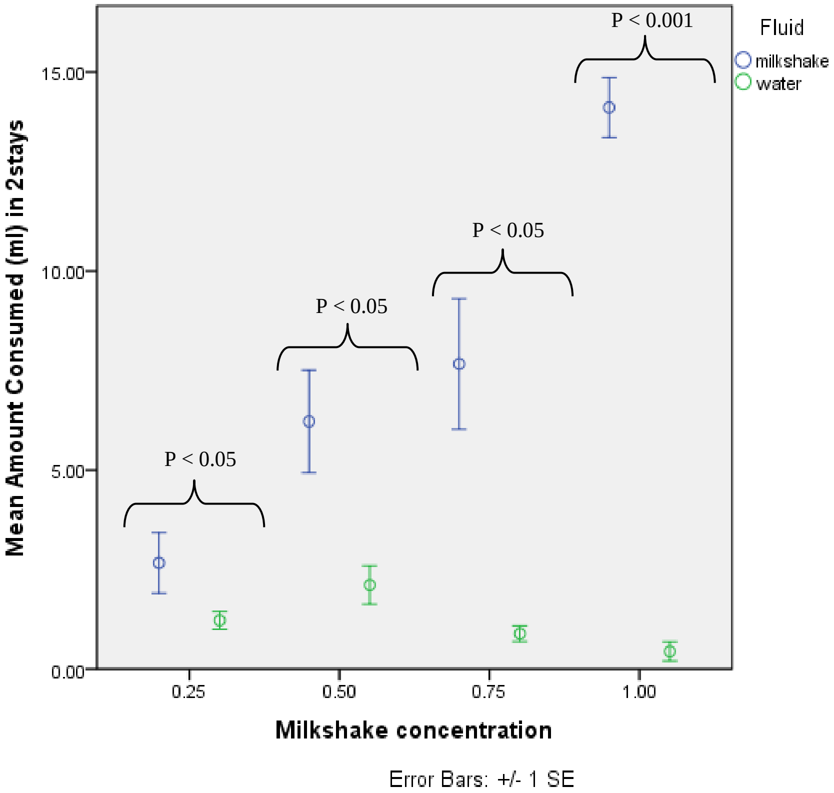


Fig 6.7: Mean (+/- 1 SE) amount of milkshake and water consumed (ml) at each milkshake concentration for 2stay marmosets. (Means are presented as medians are zero.) Milkshake consumption was significantly ($P<0.05$) higher than water consumption at 0.25, 0.50, 0.75 and 1.00 concentrations.

Table 6.5: Results of significant preferences in 2stays, for different concentrations of milkshake in comparison to water, for each concentration of milkshake, and for water in each milkshake concentration

Analysis	**df**	**Test statistic**	**P**
Milkshake v Water			
0.25 milkshake > water		Z=-2.03	0.042
0.50 milkshake > water		Z=-2.53	0.012
0.75 milkshake > water		Z=-2.55	0.011
1.00 milkshake > water		Z=-2.67	0.008
Milkshake consumed/ Concentration	3	F=21.59	<0.001
0.25 < 0.50	8	t=-2.71	0.027
0.25 < 0.75	8	t=-2.71	0.027
0.25 < 1.00	8	t=-10.71	<0.001
0.50 < 1.00	8	t=-6.42	<0.001
0.75 < 1.00	8	t=-4.87	0.001
Water consumed/ Milkshake concentration	3	X^2=11.88	0.008
0.25 < 0.50		Z=-2.12	0.034
0.25 > 1.00		Z=-2.07	0.038
0.50 > 0.75		Z=-2.00	0.046
0.50 > 1.00		Z=-2.39	0.017

Supplementary fed triplets

There was a significant difference in amount of milkshake consumed between the concentrations in supplementary fed triplets. No concentration*gender interaction was found. Significantly more milkshake was consumed at 0.50, 0.75, and 1.00 than at 0.25. Significantly more milkshake was also consumed at 1.00 than at 0.50. There was no significant difference between males and females in milkshake consumption. There were significant differences in water consumption at each milkshake concentration. More water was consumed at 0.25 and 0.50 than at 1.00. There was no significant gender difference in water consumption. Milkshake was consumed significantly more than water at 1.00, 0.75 and 0.50. Figure 6.8 shows the amount of water and milkshake consumed at each concentration for supplementary fed triplet marmosets. Table 6.6 displays the significant results for the preference test in supplementary fed triplets.

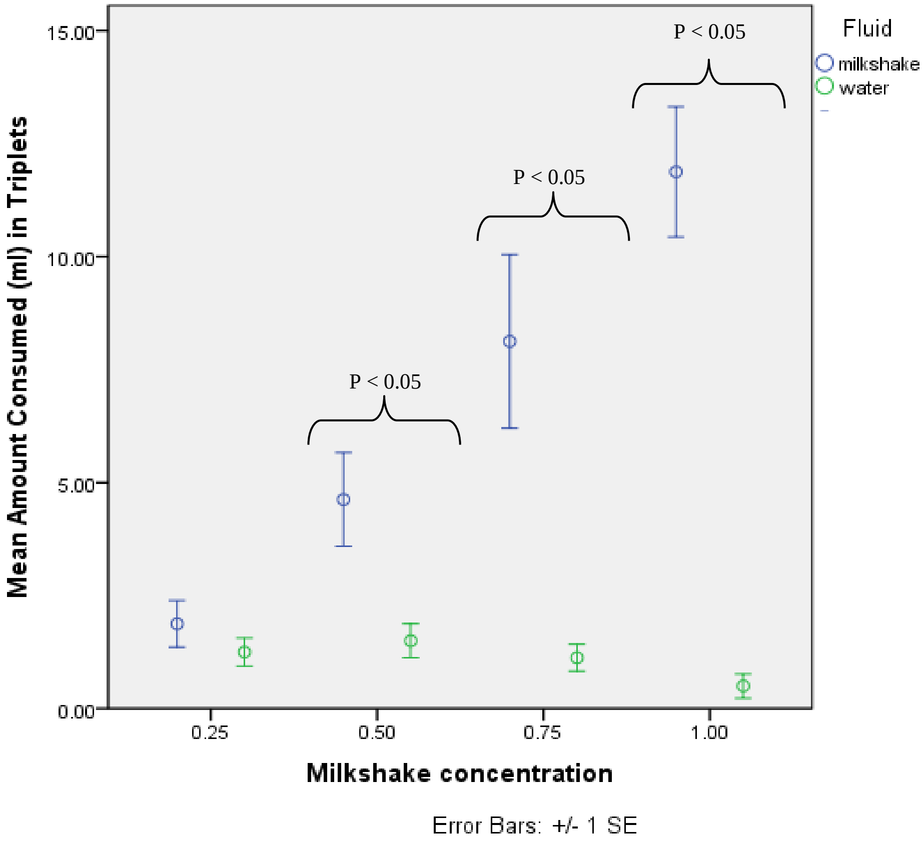


Fig 6.8: Mean (+/- 1 SE) amount of milkshake and water consumed (ml) at each milkshake concentration for supplementary fed triplet marmosets. (Means are presented as medians are zero.) Milkshake consumption was significantly ($P<0.05$) higher than water consumption at 0.50, 0.75 and 1.00 concentrations.

Table 6.6: Results of significant preferences in supplementary fed triplets, for different concentrations of milkshake in comparison to water, for each concentration of milkshake, and for water in each milkshake concentration

Analysis	**df**	**Test statistic**	**P**
Milkshake v Water			
0.50 milkshake > water		Z=-1.97	0.049
0.75 milkshake > water		Z=-2.53	0.012
1.00 milkshake > water		Z=-2.52	0.012
Milkshake consumed/ Concentration	3	F=11.73	<0.001
0.25 < 0.50	7	t=-3.27	0.014
0.25 < 0.75	7	t=-3.53	0.010
0.25 < 1.00	7	t=-6.11	<0.001
0.50 < 1.00	7	t=-5.09	0.001
Water consumed/ Milkshake concentration	3	X^2=10.32	0.016
0.25 > 1.00		Z=-2.45	0.014
0.50 > 1.00		Z=-2.27	0.023

Effect of rearing condition on fluid consumption

There was no significant effect of rearing, gender or rearing*gender interaction in amount of milkshake consumed at the 0.25, 0.50 or 0.75 concentrations. There was no main effect of rearing or gender at the 1.00 concentration. There was however a significant rearing*gender interaction at this concentration (F(2)=3.619, P=0.047), with twin males drinking less than twin females and supplementary fed triplet females drinking less than supplementary fed triplet males. Figure 6.9 shows the amount of milkshake consumed at each concentration for marmosets in each rearing condition.

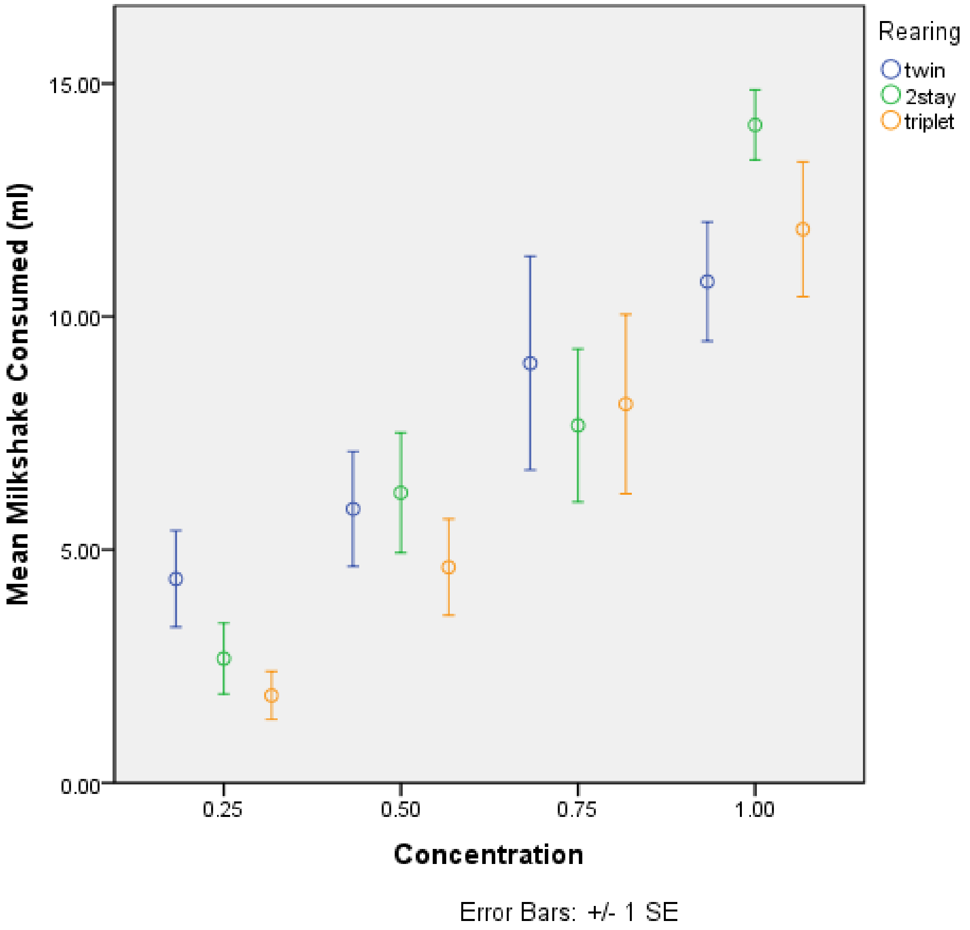


Fig 6.9: Mean (+/- 1 SE) amount consumed (ml) at each milkshake concentration (0.25, 0.50, 0.75, 1.00) for twins, 2stays and supplementary fed triplets.

6.3.5 Correlations between tests

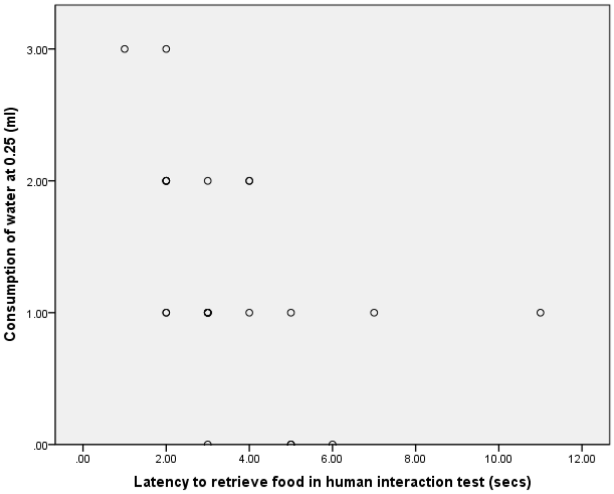
Latencies to approach and obtain food in the novel object test were significantly positively correlated. There was a significant positive correlation between latency to take food in the human interaction test and both latency to approach and latency to obtain food from the novel object. Latency in the human interaction test was positively correlated with cognitive bias (CB) task acquisition time and consumption of 0.25 milkshake, but negatively correlated with consumption of water at the 0.25 milkshake concentration (water at 0.25). Latency to obtain food from the novel object was negatively correlated with proportion of ‘Go’ responses to P+. CB task acquisition time was negatively correlated with amount of 0.25 and 0.50 milkshake consumed, as well as the proportion of ‘Go’ responses to PI.

Water 0.25 was positively correlated with water 0.50, 0.25 milkshake, 0.50 milkshake and 0.75 milkshake, as well as the proportion of ‘Go’ responses to P+. Water 0.50 was positively correlated with the proportion of ‘Go’ responses to P+ and the proportion of ‘Go’ responses to PI. Water 0.75 was positively correlated with water 1.00, while water 1.00 was negatively correlated with 1.00 milkshake. Consumption of 0.25 milkshake was positively correlated with consumption of 0.50 and 0.75 milkshakes. Consumption of 0.50 milkshake was positively correlated with consumption of 0.75 and 1.00 milkshakes, as well as the proportion of ‘Go’ responses to P+. Consumption of 0.75 milkshake was positively correlated with consumption of 1.00 milkshake and the proportion of ‘Go’ responses to P+. Consumption of 1.00 milkshake was also positively correlated with proportion of ‘Go’ responses to P+.

Proportion of ‘Go’ responses to P+ was positively correlated with proportion of ‘Go’ responses to PI, while proportion of ‘Go’ responses to PI was positively correlated with proportion of ’Go’ responses to P-. Graphs are presented for the highest correlations (r>0.60). Table 6.6 displays the significant correlations between each of the tests (correlations higher than r= 0.60 are highlighted as the most relevant results, to correct for the multiple comparisons made).

Table 6.6: Significant correlations between each of the tests conducted

Test	**HI**	**NO approach**	**NO obtain**	**CB training**	**0.25 shake**	**0.50 shake**	**0.75 shake**	**1.00 shake**	**Water at 0.25**	**Water at 0.50**	**Water at 0.75**	**Water at 1.00**	**P+ Go**	**PI Go**	**P- Go**
HI		r=0.46 P=0.02	r=0.40 P=0.05	r=0.42 P=0.04	r=0.45 P=0.03				R=-0.60 P=0.002						
NO approach	r=0.46 P=0.02		r=0.80 P<0.001												
NO obtain	r=0.40 P=0.05	r=0.80 P<0.001											r=-0.41 P=0.050		
CB task acquisition	r=0.42 P=0.04				r=-0.47 P=0.02	r=-0.73 P<0.001								r=-0.42 P=0.04	
0.25 milkshake	r=0.45 P=0.03			r=-0.47 P=0.02		r=0.59 P=0.002	r=0.46 P=0.02		R=0.59 P=0.002						
0.50 milkshake				r=-0.73 P<0.001	r=0.59 P=0.002		r=0.65 P<0.001	r=0.56 P=0.004	R=0.47 P=0.02				r=0.50 P=0.01		
0.75 milkshake					r=0.46 P0.02	r=0.65 P<0.001		r=0.54 P=0.005	R=0.56 P=0.004				r=0.67 P<0.001		
1.00 milkshake						r=0.56 P=0.004	r=0.54 P=0.005					r=-0.54 P=0.005	r=0.49 P=0.02		
Water at 0.25	r=-0.60 P=0.002				r=0.59 P0.002	r=0.47 P=0.02	r=0.56 P=0.004			r=0.52 P=0.007			r=0.46 P=0.02		
Water at 0.50									R=0.52 P=0.007				r=0.66 P<0.001	r=0.38 P=0.07	
Water at 0.75												r=0.43 P=0.03			
Water at 1.00								r=-0.54 P=0.005			r=0.43 P=0.03				
P+ Go			r=-0.41 P=0.05			r=0.50 P=0.01	r=0.67 P<0.001	r=0.49 P=0.02	R=0.46 P=0.02	r=0.66 P<0.001				r=0.50 P=0.01	
PI Go				r=-0.42 P=0.04						r=0.38 P=0.07			r=0.50 P=0.01		r=0.45 P=0.03
P- Go														r=0.45 P=0.03	



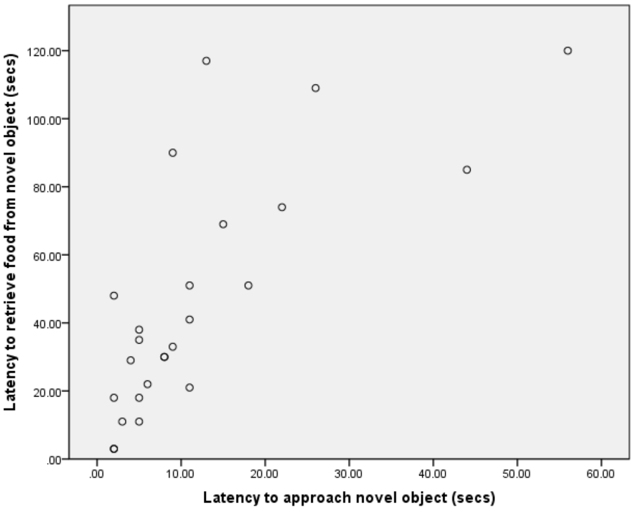
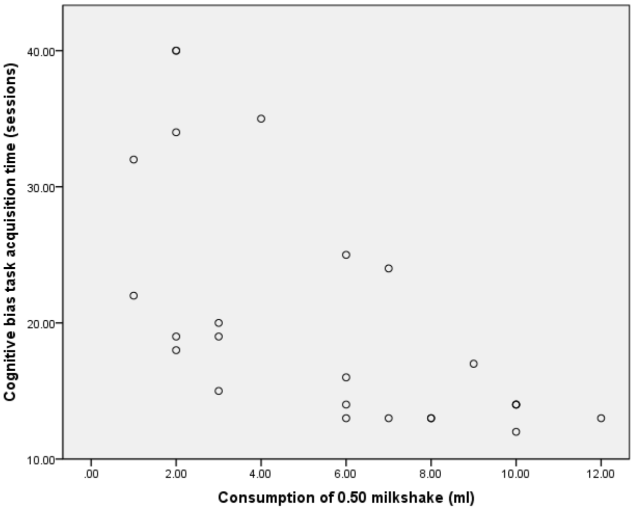


Fig 6.10: Negative correlation between consumption of water at 0.50 (mls) and latency to retrieve food from a human (secs)

Fig 6.11: Positive correlation between latency to approach and retrieve food in the novel object test (secs)



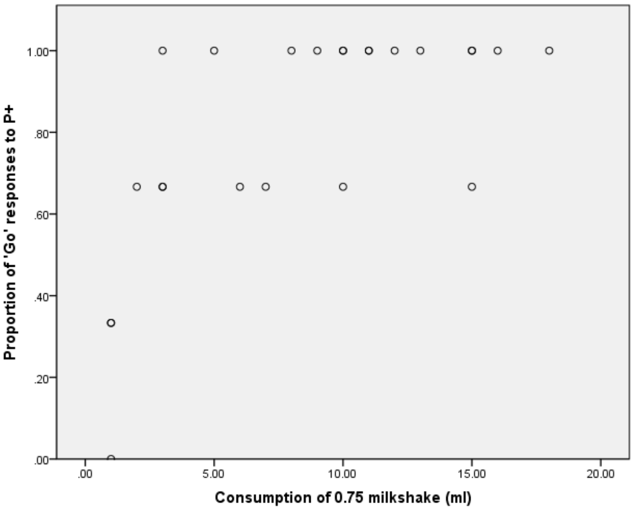
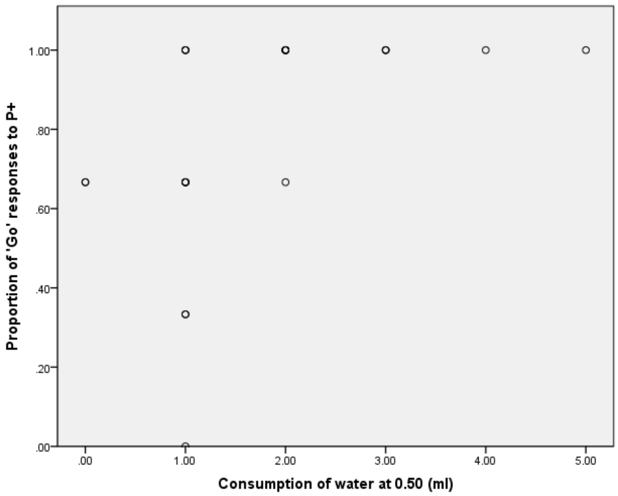


Fig 6.12: Negative correlation between cognitive bias task acquisition time (sessions) and consumption of 0.50 milkshake (mls)

Fig 6.13: Positive correlation between proportion of ‘Go’ responses to P+ and consumption of 0.75 mikshake (mls)



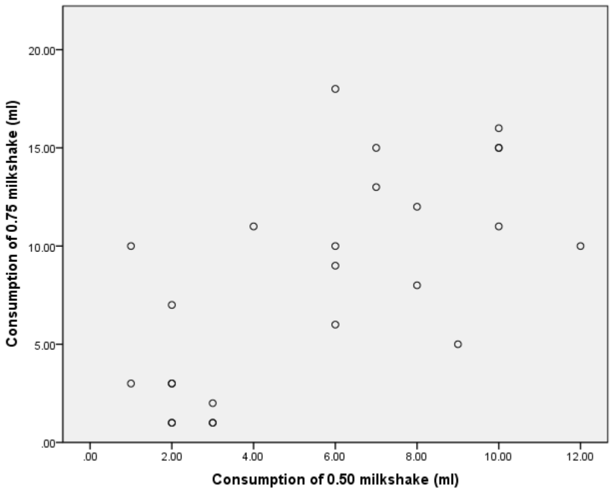


Fig 6.14: Positive correlation between proportion of ‘Go’ responses to P+ and consumption of water at 0.50 (mls)
Fig 6.15: Positive correlation between consumption of 0.50 and 0.75 milkshake concentrations (mls)

6.4 Discussion

6.4.1 Temperament tests

While some previous work has found increased neophobia in primates separated from the family early in life (Spencer-Booth and Hinde, 1971: *M. mulatta*; Caine et al, 1983: *M. nemestrina*), other work has found hand-rearing leads to less neophobic and impulsive animals (Feenders and Bateson, 2013: *Sturnus vulgaris*). There was no evidence that supplementary fed triplets in the current study were more fearful than family-reared marmosets. There was in fact no difference between animals raised under different conditions in time taken to retrieve food from an unknown human, or in latency to approach and obtain food from a novel object. All animals quickly accepted food from the hand, within 3-4 seconds, which is an encouraging finding, as it suggests they are not particularly fearful of humans. All animals studied in this colony approached the novel object, with 96% accessing the food. There was however large individual variation in time to obtain food in the novel object test, with some animals retrieving the reward very quickly and others failing to do so. These findings are in contrast to Bowell (2010), who found that only 80% of common marmosets were willing to touch the novel object, with only 47% successfully obtaining the food. Just under two thirds were willing to take food from the hand of an unknown person. There may therefore have been differences in husbandry between the facilities, with the present colony receiving more regular positive interactions with humans.

6.4.2 Cognitive bias

Few studies have looked at the effect of separation from the family on behavioural responses in depression-related tests, with many focusing on the hypothalamic pituitary adrenal (HPA) axis and monamine effects (Pryce et al, 2005). After learning a ‘Go/No Go’ task, the response to intermediate probes was used to quantify cognitive bias and so investigate underlying affective state. All but one of the marmosets was successfully trained in the visual discrimination task. Task acquisition took an average of 20 sessions, with no significant difference between rearing conditions. While other studies have suggested a link between early life stress and impaired learning in primate species, there was no evidence for this in the current study. Results are instead similar to work by Feenders and Bateson (2013), who found no difference in cognitive ability between hand-reared and family-reared starlings. Pryce et al (2004) also found no difference in learning a simple discrimination task, although impairments in early deprived common marmosets were evident following reversal.

While there was no significant difference in response to probes over the days, the data suggest there may have been some learning that responding to the ambiguous probes does not lead to a reward or punishment. Optimistic animals may therefore begin responding, as they do not receive a punishment, while pessimistic animals may cease responding, as they do not receive a reward. Overall, P+ received significantly more ‘Go’ responses than PI and P-, and PI received significantly more ‘Go’ responses than P-. Progressively fewer responses as the tube size neared S- was to be expected. There were no significant differences in response to each probe between the rearing conditions. However, there were some differences when each condition was analysed separately. For twins and 2stays, there were significantly more ‘Go’ responses to P+ than P-. However, in supplementary fed triplets, there were significantly more responses to P+ than both PI and P-. The data presented therefore suggest that there may be a minor difference in affective state between family-reared and supplementary fed marmosets, which can influence judgement of ambiguous stimuli. As there were fewer touches of the PI, relative to P+, in supplementary fed triplets, there may be a reduced expectation of the positive event in these individuals.

Although a reduced response to P+ during testing is expected in depression, while a reduced response to P- is expected in anxiety, individuals are presumed to be in more negative affective state if they perform less ‘Go’ responses to at least one of the ambiguous probes (Bethell et al, 2012). Animals that were separated from their family during infancy could therefore have reduced the probability of receiving the worst outcome (a time out) by refraining from touching the most ambiguous probe, akin to Harding et al’s (2004) experiment, where rats in unpredictable conditions withheld from pressing the lever. These individuals may therefore be more sensitive to the risk of a bad outcome, which is adaptive if responding would be detrimental to fitness (Matheson et al, 2008). Although only a minor difference, results are similar to Pryce et al’s (2004) study, which found that early deprived marmosets are more sensitive to loss of control with respect to rewarding events.

As there was large variation between individuals, with some responding to all probes and others failing to respond to any, cognitive biases did emerge. However, these differences were not exclusive to certain rearing conditions. As all animals continued to respond to the trained stimuli, differences in response to ambiguous cues were not due to reduced general activity or attention. However, it does not mean that these processes are complex, or involve conscious thought processes and subjective experience of emotion (Bateson et al, 2011). Alternative explanations may include differences in arousal, motivation and risk taking. Response may also depend on the salience of the positive or negative events used in training

(Bethell et al, 2012). As well as cognitive tests, the use of neuroscience approaches may be able to provide greater understanding of emotion in animals, offering insight into the mechanisms involved and tools to assess subjective state (Panksepp, 2011).

6.4.3 Reward motivation

Reduced consumption of appetitive food or drink have been found in choice tests and progressive ratio tests, as a marker of reward systems. Overall, marmosets in the current study preferred milkshake to water, when presented with a 2 bottle choice test. Each of the lower milkshake concentrations were consumed less than higher concentrations, as expected. More water also tended to be consumed when paired with lower milkshake concentrations, suggesting the marmosets were searching for the appetitive drink.

Although there were no differences between rearing conditions in milkshake consumption at each concentration, there were again some small differences when each condition was examined separately. In twins, more milkshake was consumed at the 2 highest concentrations than the two lowest concentrations. In 2stays, more milkshake was consumed at the full concentration than all other concentrations, and less milkshake was consumed at the lowest concentration than all other concentrations. More water was consumed when paired with milkshakes of the two lower concentrations than the higher concentrations. In supplementary fed triplets, less milkshake was also consumed at the lowest concentration than all other concentrations and more at the full concentration than the two lowest concentrations. More water was consumed when paired with the two lowest concentrations of milkshake than at the full concentration.

Subtle differences in preference were also found. A significantly greater amount of milkshake was consumed than water at each concentration in twins and 2stays. However, in supplementary fed triplets, this was only the case at 0.50, 0.75 and 1.00. There was no preference for the milkshake over water at the 0.25 concentration, which may suggest that these marmosets are less interested in reward, and so mildly anhedonic, at lower milkshake concentrations. Results therefore provide some evidence to support preference test studies that have found anhedonic-like states in maternally deprived rhesus macaques (Paul et al, 2000: *M. mulatta*). Pryce et al (2004) also found reduced motivation to obtain reward in early deprived common marmosets. However, differences between rearing conditions were very small. Other work, particularly in rats, has found no differences in appetitive fluid consumption between maternally separated individuals, compared to non-handled or early handled individuals (Crnic et al, 1981).

6.4.4 Correlations between tests

Correlations were conducted between all the tests, finding several links between temperament, cognitive bias and anhedonia. Overall, those that approached the novel object quicker also obtained the food quicker, which is not surprising. Those that were quicker to accept food from a human were also generally faster to approach and obtain food from the novel object, which suggests that both tests can identify traits related to boldness/shyness (Bowell, 2010). As animals took longer to access food in the novel object test, this may be more unfamiliar or challenging than interacting with a person, and so could also measure a different aspect of temperament, perhaps ability to problem solve. Those that were quicker to take food from a human were also quicker to learn the cognitive bias visual discrimination task. Previous research has also found that those reluctant to touch the novel object or obtain food from the hand were significantly slower to learn a training task than those that successfully retrieved the food (Bowell, 2010). Accepting food from the hand is vital to the progression of training. A marmoset who is calm and willing to approach a human is generally bolder, wasting less time in training and testing sessions, and so tends to make more progress than a more apprehensive marmoset. Similarly, birds that were less neophobic started pecking the stimulus quicker, which meant they often learnt the task quicker (Feenders and Bateson, 2013).

Marmosets that obtained the food from the novel object faster also responded more frequently to the P+. Bold animals are known to be less risk sensitive (Dammhahn and Almeling, 2012). Those that learnt the cognitive bias training task faster also drank more of the lowest two milkshake concentrations, which could be related to increased interest in reward, as well as responded more to the PI, which could reflect higher risk taking or lower sensitivity to reward/ punishment. Marmosets that consumed more water when paired with 0.25 milkshake concentration also consumed more of the 0.25, 0.50 and 0.75 milkshake concentrations. They also responded more often to the P+. Those that consumed more water when paired with the half milkshake concentration also responded more frequently to the P+ and PI. Greater consumption of the lowest milkshake concentration, as well as highest milkshake concentration, was associated with greater consumption of all other milkshake concentrations, as well as increased response to the P+. Those that touched more of the P+ also touched more of the PI, while those that touched more of the PI also touched more of the P-. Figure 6.16 displays the main correlations between the tests.

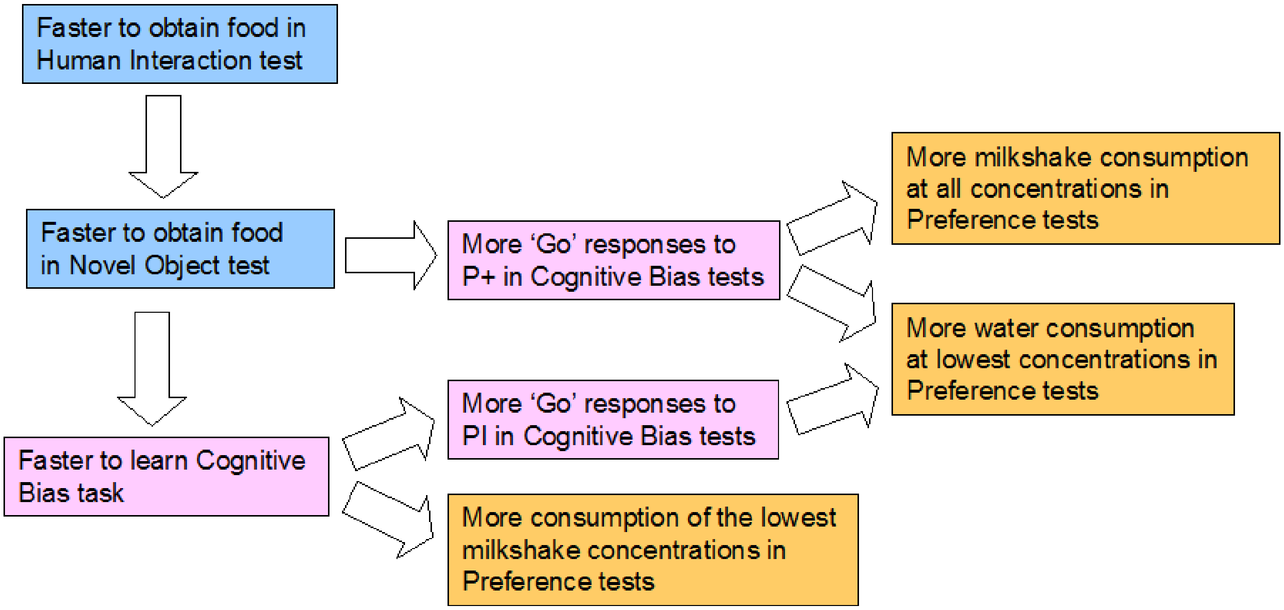


Fig 6.16: Positive correlations between temperament, cognitive bias and preference tests

The speed at which individuals learnt the cognitive bias visual discrimination task, and to a certain extent their response to the ambiguous stimuli in cognitive bias tests, could therefore be somewhat predicted by their responses in temperament and preference tests. Individuals that were less neophobic and more interested in the appetitive drink often displayed more optimistic cognitive biases. The novel object test could therefore perhaps be used as a proxy for cognitive bias, if there is insufficient time for large numbers of training trials, and so could be a practical and useful indicator of affective state in common marmosets. Personality, known as as individual differences in behaviour that are stable across time and contexts (Gosling and Vazire, 2002), has been associated with cognitive bias in birds. More neurotic parrots (*Amazona amazonica*) were found to have greater attention bias for environmental stimuli, being unable to disengage their attention from an unfamiliar observer (Cussen and Mench, 2014).

Further, studies of personality genetics have found associations between dopamine (involved with reward) and extraversion (Van Tol et al, 1992), as well as between serotonin (involved in negative emotions) and neuroticism (Lesch et al, 1996). The presence of the 5-HTTLPR short allele significantly reduces the rate of serotonin transporter expression (Lesch et al, 1996), and is associated with anxiety related personality traits and vulnerability to affective disorders in humans. Functional neuroimaging studies have strengthened this connection (Hariri et al, 2002). Similarly, variation in the dopamine receptor gene (DRD4) has been associated with personality type, with lowered transcriptional activity associated with reduced novelty seeking (Rogers et al, 2004). Balanced polymorphisms across the DRD4 gene may ‘fine tune’ dopaminergic function and maintain differences in reward-directed behaviour and aspects of cognition (Ebstein, 2006). There may therefore be underlying characteristics that predispose certain individuals to anxiety or depression, with personality being useful in predicting such behavioural outcomes.

6.4.5 Effect of separation from the family

Research has found that the stress of losing a parent early in life can produce abnormal neurobiology, increase anhedonia and impair cognition (see Parker and Maestripieri, 2011). Studies of maternal deprivation, the continuous absence of the mother and hand-rearing by humans, have found severe long-term effects (Pryce et al, 2005). However, supplementary fed triplets in the current study displayed no impairments in cognition and minor differences in affective state, compared to family-reared twins and 2stay marmosets. There was some evidence that supplementary fed triplets showed reduced expectation of reward and reduced interest in reward, both depressive-like symptoms. Although they did have prolonged periods away from the family, for 2 hours twice a day for the first 8 weeks of life, they were in continued contact with littermates. The separations were also as brief as possible, with infants reintegrated back into the family completely by 8 weeks old. This procedure could explain the lack of major differences, as seen in previous studies. Increased duration or severity of deprivation would likely have lead to greater differences between supplementary fed and family-reared marmosets. While Parker and Maestripieri (2011) have suggested that moderate stress in early life could increase resilience, severe stress could undermine an individual's ability to cope with challenges. The outcomes of early life stress are therefore broad, and depend on many factors, including personality and genes.

Supplementary fed marmosets also experience considerable human contact and increased exposure to novel situations in early life. Providing it is not overwhelming for the young animals, this could decrease fear as adults (Parker and Maestripieri, 2011). The lack of any major differences between rearing background in the current study could be due to the ongoing human socialisation and training programmes that all the animals receive throughout their life at the colony. Studies have found that positive interactions with humans can reduce negative indicators of welfare and increase positive indicators (Manciocco et al, 2009). Bassett et al (2003) further found evidence that training could benefit the welfare of marmosets. These are therefore important, practical husbandry Refinements. All staff can participate in the simple task of hand-feeding their animals, maintaining a positive human-animal relationship, which can reduce fear and substantially improve the welfare of laboratory housed primates (Bowell, 2010).

6.4.6 Conclusion

The present study investigated whether rearing background had a long-term effect on temperament, cognition and anhedonia in common marmosets. No major differences were found, although the minor differences provide some evidence to suggest that removal from the family in early life can alter long-term affective state, reducing expectation of and interest in rewards. However, the current study is useful in demonstrating that supplementary feeding may not have been a major source of stress, having little effect on affective state, at least following the husbandry practice at the colony studied. However, young marmosets should be kept within the family if possible, and receive regular socialisation with humans throughout their life.

CHAPTER 7: General Discussion and Recommendations

Abstract

Russell and Burch’s (1959) work on the principles of humane science, the three Rs, Replacement, Reduction and Refinement, have been of great importance, and now underpin much legislation on the use of animals in experiments (Chapter 1). Until we have found alternatives to animals, we must ensure that we minimise their suffering and enhance their welfare wherever possible. These Refinements should be applied to the life to death experience of the animal. Despite their wide use as a non-human model in biomedical research, the breeding of marmosets in the laboratory face a number of problems, which have not previously been systematically examined. Investigating their welfare is not only of ethical importance, particularly given the complex emotional, social and physical needs of primates, but also of scientific importance in reducing unwanted variability and obtaining the best possible results.

There were two main aims of the thesis:

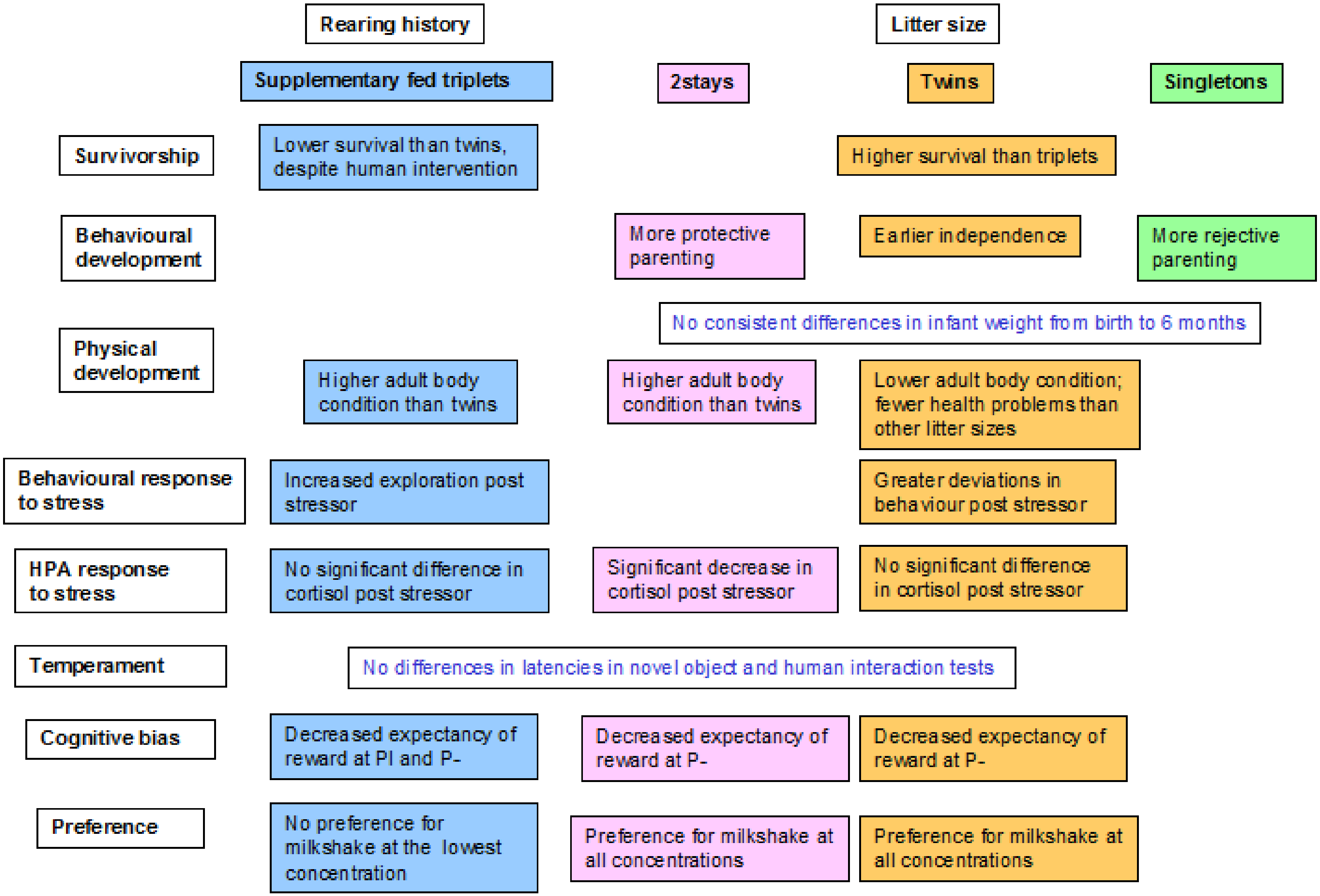
1. Determine factors associated with dam longevity and litter size
2. Compare the welfare of marmosets born and reared under different conditions

This thesis did identify potential predictors of dam longevity and litter size, as well as successfully assessed the consequences of large litter sizes and early separation from the family for supplementary feeding, piggy-backing on routine husbandry practices at a large research organisation. Results from the variety of measures used are discussed, and recommendations for future Refinements and research areas are explored.

7.1 Factors affecting litter size and dam longevity

Although marmosets are the most widely used New World primate in laboratory research and testing, there are problems associated with their breeding and rearing. Firstly, the health of breeding females is a concern, with records showing that ‘poor condition’ was the most common cause of death (Chapter 3). Dam longevity in the UK was found to be approximately 6 years, which is similar to previously described international establishments (Box and Hubrecht, 1987; Smucny et al, 2004; Tardif et al, 2003). However, no consistent improvements were found over time, which was surprising given the increases in biological knowledge and improvements to husbandry practices. Dam longevity instead varied widely between the colonies, as well as over the decades. Environment may therefore be one of the most important factors, with minimal levels of stress and particularly a diet that meets nutritional needs likely to increase longevity.

Litter size has also been increasing in captive colonies, with around half of all births at each of the colonies examined being litters larger than two (Chapter 3). However, this poses a significant issue, as marmoset families are rarely able to rear more than two infants at a time (Poole and Evans, 1982). Dam weight at likely conception was the only useful predictor of mean litter size, with heavier dams producing larger litters, a finding previously reported by others (Tardif and Jaquish, 1997). However, heavier dams also survived for longer (Chapter 3). Although large litters could be expected to drain maternal resources, there was no evidence this is the case. In fact, larger litters and more litters per year were associated with increased dam longevity. Supporting triplet, rather than twin, fetal growth therefore does not seem to be particularly costly. Similarly, another study of captive marmosets also found that there is no increase in energy intake in pregnant females carrying either twin or triplet litters, although there may be lower energy expenditure when carrying triplets, due to reduced movement (Nievergelt and Martin, 1999).



	Rearing history		Litter size	
	Supplementary fed triplets	2stays	Twins	Singletons
Survivorship	Lower survival than twins, despite human intervention		Higher survival than triplets	
Behavioural development		More protective parenting	Earlier independence	More rejective parenting
Physical development		No consistent differences in infant weight from birth to 6 months		
	Higher adult body condition than twins	Higher adult body condition than twins	Lower adult body condition; fewer health problems than other litter sizes	
Behavioural response to stress	Increased exploration post stressor		Greater deviations in behaviour post stressor	
HPA response to stress	No significant difference in cortisol post stressor	Significant decrease in cortisol post stressor	No significant difference in cortisol post stressor	
Temperament	No differences in latencies in novel object and human interaction tests			
Cognitive bias	Decreased expectancy of reward at PI and P-	Decreased expectancy of reward at P-	Decreased expectancy of reward at P-	
Preference	No preference for milkshake at the lowest concentration	Preference for milkshake at all concentrations	Preference for milkshake at all concentrations	

Figure 7.1: Diagram of similarities and differences between rearing and litter conditions across the studies

7.2 Comparing the welfare of marmosets

7.2.1 Effect of litter size

As animal welfare is a multidimensional concept, involving assessment of biological functioning, natural living and subjective state (Chapter 1), multiple measures were taken to produce an overall assessment of the welfare of marmosets born and raised under different backgrounds. Behaviour was combined with body weight and condition, as well as cortisol level. Responses in cognitive bias, preference and temperament tests were also recorded. Ideally, convergent validity would have been established between the measures of behavioural development, stress response and affective state, but due to circumstances at the laboratory, were each recorded in different groups of animals. Figure 7.1 displays the similarities and differences found between the litter sizes and rearing backgrounds over the studies.

Examination of back records revealed that litters larger than two were associated with greater infant mortality than twins, despite human intervention. Triplets may have increased competition for resources in utero, resulting in limited growth in late gestation due to uterine crowding, and so are often born smaller (Chambers and Hearn, 1985) and at risk of greater perinatal mortality (Chapter 3). In the wild, triplets are extremely rare, although have been found to survive. Dixson et al (1992) used DNA fingerprinting to confirm that three same-aged individuals were siblings. However, due to their higher mortality, many colonies euthanise the least thriving triplet for welfare reasons (Windle et al, 1999).

Despite an impact on survival, no real differences in weight were found in surviving marmosets of different litter sizes (Chapter 4). This result is contrary to previous work, finding triplets (Jaquish et al, 1991) had reduced body weight. However, there is evidence that triplet birth weight has increased over the years, while twin birth weight has remained similar, reducing the differences between the litter sizes. Higher maternal weights may play a part (Tardif et al, 2013). Although animals in any litter size could experience low weight, those from triplet litters where at least one infant was lost at birth did have lowest survivorship.

While twins had lower body condition as adults than 2stays and supplementary fed triplets, they also had the fewest health problems. As noted earlier, lower body condition may be nearer the wild state, and indeed be healthy. Tardif et al (2009) found that while only large twins at birth became overweight, it was small triplets that became obese, suggesting differences in prenatal factors that drive growth and final body mass (Tardif et al, 2013). Obesity may influence organ function and physiology (Lane et al, 1999; Power et al, 2013), and so have an impact on scientific tests.

Results from observations of singletons, twins and 2stays in the home cage showed very few major differences between the litter sizes in infant behavioural development (Chapter 4). Many key behaviours emerged at a similar time and were performed for similar estimated percentages of time. Some differences in family care did however emerge. Singletons received more rejective rearing, although this may have been associated with increased weight in these infants. High rejection levels in singletons has been found previously (Ingram, 1977), although is contrary to other studies finding that twins are more likely to be rejected (Caperos et al, 2012). 2stay infants instead received more protective rearing, being nursed for longer and retrieved more often, perhaps following the loss of an infant. These marmosets also had decreased activity during the last 2 weeks of the observation period, which could restrict learning (Box, 1991). It seems that twins gained independence earlier than singletons and 2stays, being carried less in weeks 5-8, instead spending more time in proximity to their caregivers. As singletons had no same-age companion, they spent more time in solitary play, although first engaged in social play a little later than twins and 2stays. Marmosets are a gregarious species, with a need for social companionship, and so the presence of a same-age sibling may enhance security and enable them to better cope with stressful events (Rennie and Buchanan-Smith, 2006a). Caregiver behaviour and independence could also affect stress reactivity (Parker and Maestripieri, 2011).

When comparing responses to a routine stressor in adulthood, between twin, 2stay and supplementary fed marmosets, it was the twins that displayed the most increases in unsettled, stress-related behaviour following capture and weighing (Chapter 5). This result was unexpected. While early independence and a same-age sibling are likely to promote security, adult twins did not seem to be coping better. Although overall cortisol levels decreased from baseline to post capture, only 2stay marmosets had a significant fluctuation in cortisol concentration, although this may be due to the larger sample size and lower variation in this group. While overprotective parenting has been associated with increased fear (eg. Fairbanks and McGuire, 1987: *Chlorocebus aethiops*), there was little evidence for this in the adult 2stays studied. However, it is difficult to make links between infant and adult behaviour in the thesis due to different animals being involved in each study.

Contradictory results were also found between the physiological and behavioural data over all the marmosets studied, with cortisol level decreasing and anxious behaviours increasing following capture and weighing (Chapter 5). Previous work has however found other physiological measures (heart rate, blood pressure and haematological analytes) to be correlated with behavioural responses in macaques (Tasker, 2012: *M. fascicularis*). Cardiovascular variables were also found to be correlated with behaviour, as well as responses in cognitive bias tests, in dogs (Hall, 2014). Therefore, these measures may be more useful than cortisol. Agitated locomotion and scent marking were found to be associated with the stressful event, both increasing significantly following capture and weighing, which has been found in previous studies of the common marmoset (Bassett et al, 2003). These behaviours may therefore be most sensitive to changes in welfare, and could provide information on affective state.

7.2.2 Effect of rearing background

Human intervention is often carried out to improve survival of infants from larger litters, including part and even complete hand-rearing. However, this involves separation from the family for substantial periods of time, which is known to be important in development. While it was planned to look at the care and behavioural development of supplementary fed triplets at the colony, this practice was briefly stopped during the study and so the sample size was too small to be analysed. However, adult marmosets were investigated to look at the long-term effects of separation from the family.

Cortisol levels were no different to twins and 2stays at baseline or post capture, which is contrary to previous reports finding diminished basal cortisol levels in early separated marmosets (Dettling et al, 2002). However, hypothalamic pituitary adrenal (HPA) evidence has been inconsistent (Higley et al, 1992b; Clarke, 1993; Shannon et al, 1998). Supplementary fed triplets also engaged in significantly more exploration following capture and weighing, suggesting lower levels of stress. Again, results are contrary to Dettling et al (2007), who found early separated marmosets to be more anxious and behaviourally inhibited, and instead are more consistent with Parker et al's (2004) 'stress inoculation' studies (Chapter 5).

Despite apparent increased coping in one group of supplementary fed animals, another group displayed subtle increases in depressive-like symptoms. While both twins and 2stays only had a reduced expectation of reward at the probe nearest the unrewarded stimuli (P-), compared to the probe nearest the rewarded stimuli (P+), supplementary fed marmosets responded less to both the middle probe (PI) and P- in cognitive bias tests. They also showed no preference for milkshake over water at the lowest concentration, while twins and 2stays both consistently preferred milkshake to water in preference tests (Chapter 6). Early separated marmosets may therefore have a reduced expectation of positive events, as well as a reduced interest in the reward, compared to the other conditions. Results therefore provide some evidence to support previous research, such as Pryce et al (2004), who found that early deprived marmosets are more sensitive to loss of control, as well as had a reduced motivation to obtain reward. Altered affective state could therefore affect responses in scientific tests, especially those that require animals to respond to stimuli to receive a reward (Hall, 2014).

However, there were no significant differences in average response to each probe or consumption of each milkshake concentration between the rearing backgrounds. Family-reared and supplementary fed marmosets also had similar latencies to approach and obtain food in the human interaction and novel object tests, suggesting no difference in these bold/shy aspects temperament. Similarly, no difference was found in the cognitive bias task acquisition time. There was therefore no increased caution or impaired cognitive ability in marmosets that had been separated from the family early in life, which has been found in previous studies of hand-reared animals (Feenders and Bateson, 2013: *Sturnus vulgaris*).

While there was little evidence for an effect of human intervention on long-term affective state, consistent responses were found throughout the tests. Marmosets that obtained food quicker in the temperament tests were faster to learn the cognitive bias task, which was associated with more ‘Go’ responses to the ambiguous probes (P+ and PI) in the cognitive bias test, as well as more consumption of the milkshake in the preference test. Such simple temperament tests of exploration/inhibition could therefore be used as predictors of training success and to some extent, responses in the cognitive bias and preference tests, and so could provide a quick indicator of traits that may predispose vulnerability to anxiety or depression. Motivation for food could however play a part, as well as other aspects of temperament, including motivation to work (Inglis et al, 1997) and level of distractibility. Sociability with humans may have the largest effect, which is likely to be related to previous experience, including rearing background (Bowell, 2010). The lack of difference in these tests between rearing backgrounds is possibly due to the regular human socialisation programme at the colony, with all animals very quickly taking food from the hand.

7.3 Rearing background and resilience

Primate models of maternal behaviour have been used to investigate the marked differences in caregiver behaviour between family groups. Manipulations of the post-natal social environment have also been made in monkeys, using parental separation models (Chapter 1). While such models have found increased stress responses, cognitive impairments and anhedonia in early separated primates, these procedures were likely to be more stressful for the young animals than the supplementary feeding investigated in this thesis.

One major difference is that the infants were not isolated during their time away from family. The marmosets were also fed at predictable times, building positive experiences with humans from an early age, and are naturally adapted to being passed between carriers (eg. Ingram, 1977), which could mean they are less stressed during separation from the mother than other primate species. These explanations may shed light on the minor differences found between family-reared and supplementary fed animals in the current study, compared to much previous work. Other rearing practices, including partial and rotational hand-rearing, and particularly complete human hand-rearing (Chapter 1), which do involve isolating the young marmosets for the whole day, could however have led to more severe developmental consequences.

While too much stress, such as complete deprivation from the family, or overly rejective or protective care, could undermine an individual's ability to cope and lead to mood disorders, successfully overcoming a moderate amount stress could in fact enhance future competence (Chapter 1). As well as this, rather than each 'dose' of early life stress increasing subsequent vulnerability, results demonstrate the range of developmental outcomes from early life stress. One group of supplementary fed animals had enhanced coping following a routine husbandry event, while another had a more negative affective state compared to the other rearing conditions.

Many factors contribute to the outcome of early life stress, including family rearing style, social support, personality and genes. Some previous studies have also found that gender may have an effect. As the level of HPA responsiveness has been found to be altered in male monkeys, but not females (Parker et al, 2006), rearing background seems to effect stress responsivity more significantly for males. However, Pryce et al (2004) found no gender differences in any of the physiological and cognitive effects of early deprivation. Overall, males had lower baseline cortisol levels than females in the current study (Chapter 5), although this was not significant. However, male twins had higher cortisol concentrations than female twins directly after weighing. Male twins also initiated contact more frequently and foraged for less time following capture and weighing, suggesting they may have been more effected by the stressor than female twins. Male twins also drank less of the highest milkshake concentration than female twins (Chapter 6), and so there may have been some gender difference in anhedonia in this rearing condition. In this case, too little early life stress may have undermined ability to cope.

It is possible that neuromodulatory systems, such as dopamine, serotonin and norepinephrine, are involved in reward and punishment systems (Burgdorf and Panksepp, 2006). The impact of early stress may therefore be dependent on genotype, specifically a gene central to the regulation of serotonin (Champoux et al, 2002), with those carrying the short serotonin transporter (SERT) allele being more affected by rearing background. Low serotonin in peer-reared animals (Higley, 2003) and those with highly rejecting mothers (Maestripieri et al, 2006a, b; Maestripirei et al, 1992) has been associated with high reactivity and impulsivity.

The degree of perceived control or predictability, following cognitive perception and appraisal of the situation (Veisser and Boissy, 2007), is also important, and can affect the magnitude of the stress response (Fraser, 2008). While those that can neither predict nor control the event are likely to have greater behavioural and physiological responses (heart rate, cortisol, flight, avoidance), those that can exert a degree of each should show a less severe response (Wiepkema and Koolhaas, 1993). Learning to deal with novel situations and human contact from an early age could increase perceived control, and so resilience in adulthood, which would enhance the welfare of the animals.

7.4 Recommendations for Refinements

7.4.1 Promoting dam health and twin births by reducing dam weight

As large litters resulted in higher infant mortality (Chapters 3 and 4), there is evidence that twin births should be promoted. Captive marmosets are now significantly heavier than their wild counterparts, as a consequence of diet and physical activity (Araujo et al, 2000), which has lead to an increase in these triplet births (Kirkwood, 1983). Higher weight at conception in large litters was indeed found in Chapter 3 of the thesis. Maintaining females at lower healthy weights, more similar to their wild counterparts, could therefore be an important management practice, helping to increase the incidence of twin births.

Most small-bodied primates have high metabolic rates, which require high energy and nutrient quality (Garber, 1987). However, fruit fed in captivity is very different nutritionally to that found in the wild (Oftedal and Allen, 1996), being high in sugar and low in fibre, protein, minerals and vitamins (Schwitzer et al, 2009). Dried fruit mixes also contain a lot of sugar. Captive diets are therefore very high in energy, without the associated foraging costs (Schwitzer et al, 2009), and so captive animals may be at risk of becoming overweight (EAZA Guidelines, 2010). Birth weight, as well as exposure to high fat diets, has been found to enhance the development of early life obesity, with large mothers also supporting higher pre-weaning growth rates (Tardif and Bales, 2004). Further, earlier intake of solid food and more efficient intake of food was related to obesity as juveniles (Ross et al, 2013).

As well as larger litters, obesity can lead to several health problems, including skeletal abnormalities and cardiovascular disease, which will affect longevity and welfare (Lane et al, 1999). In a study of the metabolic consequences of early onset obesity, overweight marmosets were found to have lower insulin sensitivity and more difficulty maintaining glucose homeostasis, showing both higher fasting blood glucose and higher insulin levels (Power et al, 2013), which could develop into diabetes. Diets low in fibre could also cause gastrointestinal problems (Edwards and Ullray, 1999), while high amounts of sugar could lead to poor dental health (Johnson-Delaney, 2008).

For overweight animals, the overall quantity of diet can be reduced, starting with a 5% decrease (Crissey et al, 1999). More crickets can be offered than mealworms, as they contain less fat and energy, and sugary treats can be reduced (eg. marshmallows) and replaced with rusk (effectively used in Chapter 6 as reinforcement). Plowman (2013) reviewed quite a substantial diet change, implemented for medium-sized primates at Paighnton Zoo, UK, in which all fruit, bread, eggs and seeds were gradually removed and changed to commercial pellets, fresh vegetables and small amounts of dog biscuits and brown rice. The new diet had higher levels of protein and fibre, and lower levels of readily digestible carbohydrate. Following the dietary change, primates had improved dentition (less gum disease, gingivitis, tartar build up and tooth removal), as well as sustainable weight loss in obese individuals and stable healthy weights in others, without limiting food intake. There was also a considerable cost saving (Plowman, 2013).

Dietary changes should however be considered carefully. Marmosets do not respond well to abrupt changes, being susceptible to gastrointestinal problems (Ludlage and Mansfield, 2003), and so changes should be gradual over several weeks (Tardif et al, 2006). The dominant breeding female may also consume more of the preferred food (JWGR, 2009) and so could have the most imbalanced diet. Multiple food bowls should therefore be provided. Lower value foods, such as those used by Plowman (2003), could also reduce monopolisation. Careful monitoring of body weight and condition, as well as the calorie content of produce provided and the amount consumed, should be carried out, to ensure animals do not lose too much weight too quickly (EAZA Guidelines, 2010).

Enrichment can also be used to reduce weight, and is an important Refinement. It can be physical, such as increasing enclosure size, design and complexity, including furnishings for climbing and leaping, outside runs or exercise rooms and novel, hanging objects to encourage exploration and play. It can also be food related, such as deep litter, whole fruits on sticks, insect dispensers and artificial gum trees to encourage foraging and exercise (Buchanan-Smith, 2010a). Feeding on gum may be a behavioural necessity and important for the gut. However, fruit must be decreased as more gum is offered (EAZA Guidelines, 2010). As well as reducing weight, enrichment can increase the performance of normal behaviours and increase ability to cope with challenges. It also adds choice and control to the environment (Buchanan-Smith, 2010a).

While such diet changes or increased enrichment could be used to maintain dams at lower weights, litter size in marmosets depends on maternal energy balance (Rutherford and Tardif, 2008). Larger females or those that are gaining weight (in a positive energy balance) during the follicular phase are more likely to have higher ovulation numbers (Tardif and Jaquish, 1997). Therefore, it may only be necessary to decrease energy intake at likely conception, putting females into a temporary negative energy balance. As inter-birth interval was generally around 5 months in the current study, females were often becoming pregnant very soon after the birth of their previous litter, and so this is likely to be the most effective time to implement a reduced calorie diet.

However, care is needed in decreasing female weight to reduce large litters, as heavier dams tend to live longer (Chapter 3). As females can lose weight after birth of a litter, due to the energetic demands of reproduction (Tardif et al, 1993), a low calorie diet may be particularly costly during lactation times. Studies have found that energy intake did not rise significantly during pregnancy, although increased by up to 100% during the fifth and sixth weeks of lactation, accompanied by a gradual loss of weight, despite decreases in activity (Nievergelt and Martin, 1999: *C. jacchus*; Kirkwood and Underwood, 1984: *S. oedipus*). As infant care is shared among group members, males could also lose significant amounts of weight, following the birth of infants (Achenbach and Snowdon, 2002: *S. oedipus*). Food intake is reduced in carriers (Price, 1992: *S. oedipus*). There are also increased costs of travelling with infants in common marmosets (Tardif, 1997) and performing other caregiving behaviours, such as vigilance. However, a study of common marmosets has found no change in energy intake or weight loss in fathers (Nievergelt and Martin, 1999).

Further research is therefore necessary to investigate the effect of using diet and physical activity to reduce dam weight and mortality from larger litters. It is important to establish with evidence whether a more natural diet and weight is connected to welfare. Increased health and performance of positive behaviours in dams may be indicators of good welfare. However, any improvements in mental state or that this is what they ‘want’ would also have to be established, and decisions may have to be made whether increased health is more beneficial than removing favoured food items (eg. Dawkins, 2012). Caution may also be required, as overall welfare for twins was not considerably greater than that of larger litter sizes.

There is evidence that delaying the onset of breeding can enhance dam longevity (Jaquish et al, 1991; Smucny et al, 2004), although this was not found in the thesis (Chapter 3). While captive female marmosets commonly begin breeding at 18 months (Tardif et al, 2003), first parturition in wild females is at a much later age and they can often breed until they are 8 or 9 years old (Tardif et al, 2008). Similarly, Smucny et al (2004) found that dams first reproducing at or above 4 years old had greater survival than those first reproducing before this age. Increasing the age of first reproduction could therefore be an important consideration in the management of breeding marmosets, which may also warrant further investigation.

7.4.2 Human socialisation programmes

The overall lack of major differences between the rearing backgrounds in the current study is likely due to the regular training and human socialisation programmes that all animals receive throughout their life at the colony. As the laboratory environment can be stressful, fear is likely to be a major factor in the lives of marmosets used in research and testing. Humans may be the largest source of fear (Bowell, 2010). Marmosets are often thought of as having a nervous disposition (Poole et al, 1989), and as a prey species, they are naturally vigilant (Koenig, 1998). However, they are an adaptable species, and even in the wild can live in close proximity to humans and overcome their fear when associated with positive interactions (personal observation).

Primates in laboratories have been found to benefit greatly from socialisation with humans, starting early in life (Laule, 2010). Regular positive interactions are associated with a reduction in anxiety related behaviours (Bassett et al, 2003), and fear responses to novel humans and situations later in life (JWGR, 2009). A recent study also found enhanced socialisation lead to lower baseline heart rates, improving sensitivity of cardiovascular measures derived from these animals (Tasker, 2012).

The current facility regularly spent time hand-feeding the whole family, and this is encouraged for all colonies. Socialisation takes little time and training, making routine implementation cheap and easy to fit around daily husbandry routines. However, it has the potential to improve the welfare of large numbers of captive primates (Rennie and Buchanan-Smith, 2006a). Staff can also take other opportunities to have positive interactions, while they are performing their duties (Tasker, 2012). Careful socialisation with all care-staff is necessary, as studies have found that not all monkeys generalise from a familiar to unfamiliar person (Bowell, 2010). Reduction in stress would benefit the animals and staff, as well as the scientific output. It is also an important prerequisite to training.

As marmosets did not appear to have habituated to being captured by young adulthood, displaying behavioural signs of stress and physiological signs of negative anticipation (Chapter 5), alternative methods of weighing could be considered. One such method is positive reinforcement training to shape the behaviour to enter a box, to be placed on a weigh scale. After being allowed to habituate to the box and move in and out freely, marmosets can be encouraged to remain in the box. The door can then be closed briefly, and for progressively longer periods of time, while they receive a reward (Tardif et al, 2006). Allowing the monkeys to enter a box voluntarily, rather than be captured, could be a quick and effective way of reducing stress, not only in those being weighed, but also in those watching others undergoing the routine husbandry event (eg. Flow and Jaques, 1997: *M. fasicularis*).

7.4.3 Training to allow supplementary feeding on carriers

While there were few differences found between the rearing conditions, separation from the family is not recommended, if it is at all possible to keep the infants with the natal group (JWGR, 2009). The effect of infants being removed on the family members was not investigated in the current study. Although some work has found no differences in parental care when infants were removed in early life, compared to undisturbed family groups (Dettling et al, 2002), forced separation could trigger anxiety in caregivers and increase the performance of protective behaviours (Maestripieri, 2011).

As well as stress to the family, maternal stress during pregnancy could adversely effect development of the next litter in utero (Buitelaar et al, 2003). Increased risk may be due to glucocorticoid hyper-exposure (Rice et al, 2010), with large amounts of maternal cortisol transferring to the fetus. Compromised physical growth (Schneider, 1992a: *M. mulatta*) and impaired motor dexterity (Pryce et al, 2011: *Callithrix jacchus*) has been found in prenatally stressed primates. They also display more disturbance behaviour in a novel environment (Schneider, 1992b: *M. mulatta*) and less exploration (Pryce et al, 2011: *M. mulatta*). Increased anxiety in approach-avoidance conflict situations is a consistent finding across studies. Effects were more pronounced when mothers were stressed early in gestation, which is when supplementary feeding is carried out, compared to in mid-late gestation and controls (Schneider et al, 1999: *M. mulatta*). Feeding motivation has however been found to increase in offspring of stressed mothers, possibly reflecting effects on the reward network (Pryce et al, 2011). There is also evidence of over-active or altered HPA axis function. In rhesus macaque (*M. mulatta*) infants, prenatal stress has been found to increase both baseline (Pryce et al, 2011) and post stress (Uno et al, 1994) cortisol levels, compared to infants of unstressed mothers. However, these findings have not been replicated in macaques (Scheider et al, 1998: *M. mulatta*), nor in common marmosets (Pryce et al, 2011: *C. jacchus*). Results are therefore generally similar to those seen in postnatally stressed primates.

Psychosocial stressors can effect activity of the HPA axis in marmosets (Smith and French, 1997: *Callithrix kuhli*). Significant cortisol elevations have been found in both mothers and infants following brief involuntary separations (Mendoza et al, 1980: *Saimiri sciureus*), as well as in adult pairs in response to the disturbance involved in establishing separation (Hennessy, 1986b: *Saimiri sciureus*). Therefore, daily removal of infants may lead to stress in families, both from the human disturbance and separation anxiety, which could increase levels of glucocorticoids in the mother and fetus. If this is the case, alternatives to the supplementary feeding routine may be necessary.

Training carriers to allow staff to provide supplementary feeding to the infants whilst they remain on their backs could therefore be a possible Refinement (Buchanan-Smith, 2010b), mitigating stress to the family and infants. Another better practice may be to cross-foster infants to well-experienced surrogate parents on contraception (Morris, 2010). As parents have been found to respond equally to calls from their own and unfamiliar dependent infants (Sanchez et al, 2014), fostering is likely to be a successful method, if an appropriate family is available.

Positive reinforcement training is increasingly being used to enhance care and wellbeing (Prescott et al, 2005), with primates being trained to co-operate with many procedures. Training allows animals a degree of control and predictability over their lives, which can lower the psychological impact of stressors, and act as enrichment to reduce boredom (Bowell, 2010). It would also promote positive animal-staff relationships, as well as aid in practical purposes (Bowell, 2010).

Carriers could be encouraged to take food from a syringe, so they remain in the same position, while the infants are fed. Such simple syringe training is routinely carried out at the facility. Appropriate training programmes could be developed and implemented (Prescott et al, 2005). Sessions can be short, incorporated into daily routines, and time investment is often recouped once trained (McKinley et al, 2003). Future research could therefore investigate the success of such training, and the impact on the infant and family could be compared to the original supplementary feeding regime. Table 7.1 lists the recommendations and evidence behind these suggestions.

Table 7.1: Recommendations for Refinements

Recommendation	**Reference**
Reduce dam weight	Thesis: Chapter 3 Literature: Kirkwood, 1983; Tardif et al, 1997
Delay onset of breeding	Literature: Jaquish et al, 1991; Smucny et al, 2004
Human socialisation programme	Thesis: Chapters 5 and 6 Literature: Basset et al, 2003; JWGR, 2009
Training for weighing	Thesis: Chapter 5 Literature: Tardif et al, 2006
Training for supplementary feeding on the carrier's back	Literature: Maestripieri, 2011; Morris, 2010; Prescott et al, 2005; Savastano et al, 2003

7.5 Relevance to other captive contexts

Much of the findings in the thesis are relevant to other captive settings. Marmosets are the most commonly kept 'pet' primate, although due to lack of regulation, their numbers are unknown (RSPCA, 2015). Many private breeders sell animals alone from a young age, when they would be dependent on family care, in unsuitable housing (RSPCA and Wild Futures, 2012; Born Free Foundation, 2014). Poor diets have lead to Metabolic Bone Disease (Power et al, 1995) or over/under weight animals (RSPCA and Wild Futures, 2012). Hand-rearing is also often carried out to ‘tame’ primates, and continued isolation of such a social animal is a considerable welfare issue (Novak et al, 2006). Zoos now try to avoid hand-rearing, and where it is necessary, resocialiastion with conspecifics as soon as possible is the primary objective (Porton, 1997). However, comparison of hand-reared and parent-reared individuals in zoos is rare (Porton and Nielbruegge, 2006).

An appropriate diet and environment is therefore very important for all marmosets kept in captivity (Lane et al, 1999; Buchanan-Smith, 2010a). It is not recommended in any context to separate young marmosets from their family, although training can be used to aid management. For example, primates in zoos have been syringe trained to facilitate palpation (Savastano et al, 2003), which may also be applied to other practices. Further research could be conducted into reproduction, longevity and hand-rearing in zoos and as ‘pets’, as the results found in this thesis may be specific to the laboratory. As welfare involves the personal experience of individual animals (Fraser, 2008), concerns raised in the thesis are not only for large scientific establishments, but may also apply to all those breeding marmosets.

7.6 Final conclusion

Husbandry practices are often advocated without a sound scientific understanding of their welfare consequences (Buchanan-Smith, 2010b). This thesis therefore examined the effect of breeding and rearing practices in the common marmoset. It included the welfare assessment of dams, as well as infants and adults of different litter sizes and rearing backgrounds. Although it was hypothesised, based on numerous primate models, that early family separations would lead to adverse developmental consequences, there was surprisingly little difference between marmosets of different litter sizes and rearing backgrounds, across the range of measures taken. Overall, supplementary feeding at this facility appeared to have little effect on development and welfare, and so may not be a major source of stress resulting in impairments. While effects are greater when deprivation is more long lasting and complete, infants at this facility were taken out for short periods, with their siblings, and integrated back into the family as soon as possible. Therefore, the current supplementary feeding procedure, along with a regular human socialisation programme, appears to minimise the potential adverse effects of early separation, and so this practice should be used if hand-rearing is necessary. The lack of major differences could also mean that unwanted variability is kept to a minimum, which would help to Reduce the number of animals used.

While this is a surprisingly encouraging finding, we should always be looking at ways to improve the lives of animals in our care. Possible Refinements to reduce dam weight and mortality from large litters, as well as to allow supplementary feeding on the carrier's back to prevent stress to the family, could be interesting areas of future research. Minimally aversive routines and environmental consistency, as well as closer human-animal interaction and positive reinforcement training for new situations, are encouraged for effective management of captive animals. These could reduce fear and allow the monkeys to become more resilient to the laboratory environment.

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Appendix A

Body condition scoring system (based on Wolfensohn and Honess, 2005)

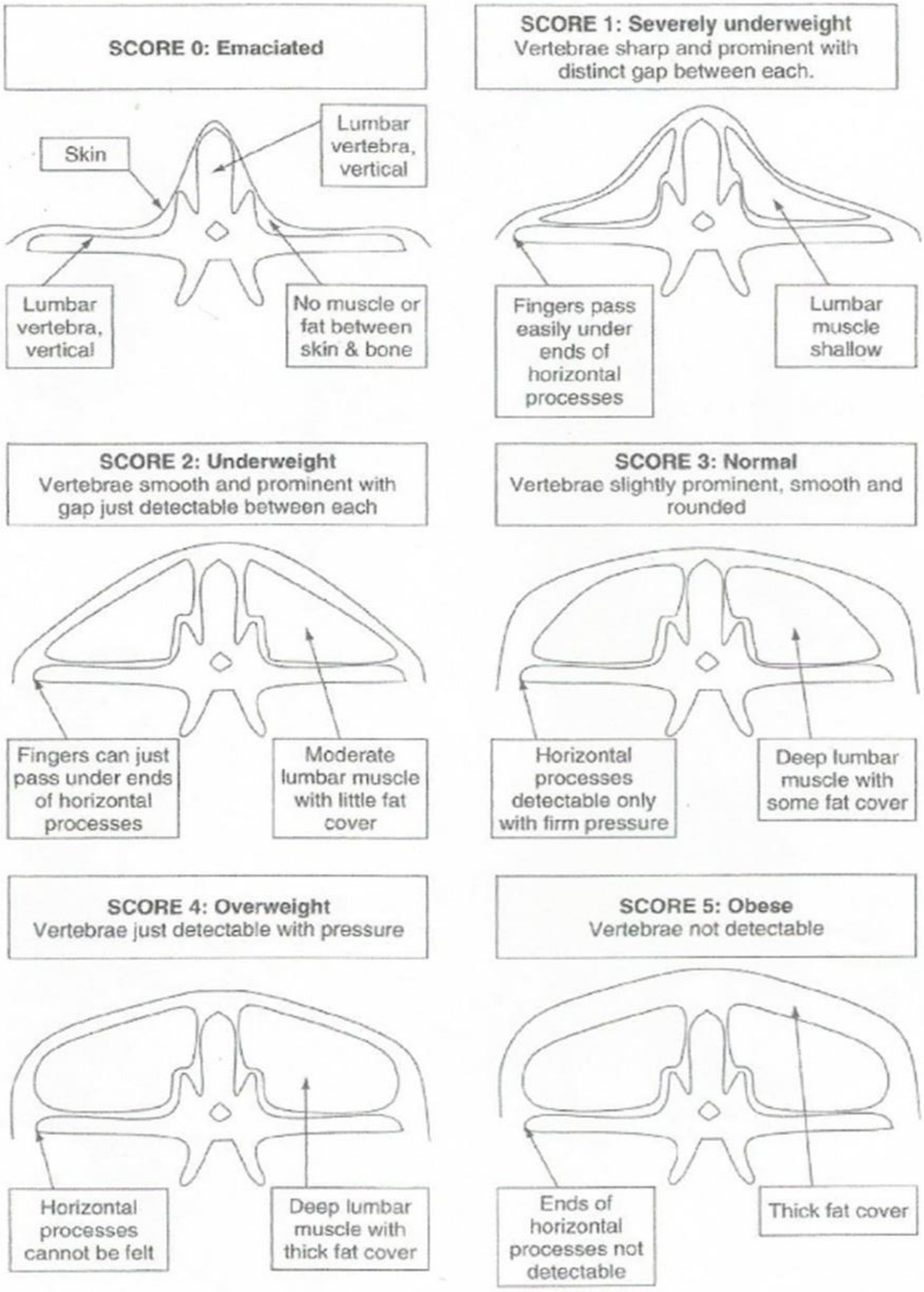

Figure 5.5 Condition scoring: vertebrae.

Appendix B

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(based on Chapter 3)

American Journal of Primatology

RESEARCH ARTICLE

Long-Term Data on Reproductive Output and Longevity in Captive Female Common Marmosets (*Callithrix jacchus*)

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The common marmoset (*Callithrix jacchus*) is widely used in biomedical research, with many housed for breeding purposes world-wide. Significant variation in reproductive output among females has been found compared to other anthropoid primates. The present study explores this reproductive variation, focusing on potential predictors of dam longevity and litter size, as well as changes over time. Back-record analysis was conducted, yielding litter information and reproductive summaries of 360 dams housed at three UK marmoset colonies over four decades (1970s–2000s). Results revealed differences among the colonies, as well as within colonies over decades, suggesting environment may play an important role. Cox proportional hazards regression analyses revealed significant effects of mean litter size and yearly production on dam longevity. Decade, mean inter-birth interval and mean dam weight were found to be significant factors explaining dam longevity when looking at colonies individually. The most commonly recorded cause of death was "poor condition." Linear regression models found that no reproductive variable was useful in explaining mean litter size, except dam weight at conception, data which was only consistently recorded at one colony. While triplets were common at all three colonies, these larger litters were consistently associated with higher infant mortality, despite human intervention to improve survival. This study increases our understanding of marmoset reproduction, and possible improvements to practical aspects of colony management to enhance survival and welfare are discussed. Am. J. Primatol. © 2014 Wiley Periodicals, Inc.

Key words: reproduction; dam longevity; litter size; colony management; marmosets

INTRODUCTION

Reproduction in the Common Marmoset

The common marmoset (*Callithrix jacchus*) is widely used as a non-human primate model in biomedical research [Buchanan-Smith, 2010; Hart et al., 2012]. Combined with their small body size (usually <400 g), relative ease of handling, and absence of many zoonoses [Tardif et al., 2011], marmosets are inexpensive to keep compared to the larger macaques (*Macaca* spp.). They also have the highest potential fecundity of any anthropoid primate [Smucny et al., 2004; Tardif et al., 2003], and can be bred in sufficient numbers to meet research requirements [Poole & Evans, 1982]. These factors make them one of the most frequently used New World primates in research and testing [Council of Europe, 2010; Home Office, 2011; USDA, 2007]. Many more are also currently housed for breeding purposes.

Callitrichidae (i.e. marmosets and tamarins) produce more offspring per delivery, with more variation in litter size, than any other anthropoid primate [Smucny et al., 2004]. There are routinely multiple ovulations per cycle. Twins are the norm, although triplet litters are also common. Inter-birth intervals (IBIs) are also often short (approximately 5 months), with females able to conceive again shortly after birth [Smucny et al., 2004]. This means they can produce two litters a year [Tardif et al., 2008]. However, their high fertility is accompanied by high rates of pregnancy losses and infant mortality [Jaquish et al., 1991]. There can therefore be significant variation in reproductive output per year, as well as over a female's lifetime [Smucny et al., 2004].

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An overview, combining data from published literature and a large American multi-colony database, reported that breeding females had an average longevity of 5–7 years and a maximum of 16.5 years [Tardif et al., 2011]. Animals had a reproductive life span in captivity of around 2 years [Smucny et al., 2004]. However, in a report of another colony, maintained at the University of Cambridge [Ridley et al., 2006], 80% of breeders (males and females) were alive at 10 years of age. These animals were allowed to live out their optimum captive lifespan, only being euthanized for welfare reasons. Due to difficulties acquiring data, there is little known about longevity in wild common marmosets. Results from a wild population, followed for 10 years at a field site in Northeastern Brazil, suggest that early life mortality is relatively high compared to other age groups (66.7% infant survival). Females began reproducing around 4.5–5 years, and continued until they were 8–9 years old. Tenure therefore averaged 3.5 years. Females can breed until relatively close to their maximum life span, with a rather abrupt reproductive decline, associated with follicular depletion, or inability to maintain behavioral dominance [Tardif et al., 2008]. Whilst longevity and infant survival may be expected to be higher in captivity than in the wild, as captive marmosets are protected from predators and dominance competition, as well as have ample food provided, this may not be true for some common marmoset breeding colonies.

Litter Size and Dam Longevity in Captivity

Few studies have looked at variables that can influence the number of infants born per reproductive attempt in callitrichids [Bales et al., 2001]. Jaquish et al. [1997] found that there was low heritability of litter size, with only husbandry changes significant in the common marmoset. Increased cage volume and complexity, combined with increased protein content in the diet, were associated with a greater number of triplets. A good quantity of usable space has also been found to maximize well-being and breeding success in cotton-top tamarins [Savage, 1995]. Maternal body weight is also known to be important in marmosets, influencing ovulation number, losses during gestation and born litter size [Tardif & Jaquish, 1997; Tardif et al., 2005]. Bales et al. [2001] also found that higher pre-pregnancy body mass was associated with a greater number of live births (wild golden tamarins of known age, for 162.5 female-seasons).

However, the most important factor in infant survival is litter size [Tardif et al., 2003]. Several studies following the production of a single captive breeding colony over a number of years report that litter sizes have increased since establishment [Box & Hubrecht, 1987; Poole & Evans, 1982]. However, larger litters generally result in higher infant mortality [Jaquish et al., 1991]. The likelihood of all triplet infants surviving is greatly increased if one or all infants are partially or completely hand-reared [Hearn & Burden, 1979]. However, the welfare consequences and effect on subsequent scientific output of these rearing practices have been questioned [Buchanan-Smith, 2010].

It is also important to examine factors affecting dam longevity in captive colonies. Longevity in the current study is defined as the animals' life span in the colony, which often involves decisions to euthanize due to health or breeding management. In previous studies, Cox proportional hazards regression analysis revealed dam longevity to be significantly affected by number of litters, age at first parturition and site [Smucny et al., 2004]. Dams first reproducing later in life (4 years and over) tended to live longer than those first reproducing at younger ages. Although it may be expected that larger litters would be associated with high energetic cost [Tardif et al., 1993] and reductions in life span, there is no evidence that this is the case [Jaquish et al., 1991; Smucny et al., 2004]. Changes in longevity over time have however been found at an American captive colony. Average life span extended from 4.82 years during colony establishment, to 7.07 years when the colony was stable. Mortality however increased with associated changes to the colony, including new animals and housing conditions [Tardif et al., 2011]. With greater experience of colony management and husbandry practices, as well as increases in basic biological knowledge and cage sizes, one might expect improved welfare and less infant mortality from colony establishment to present day.

Aim

The present study examined reproductive information from three large well-established UK captive *Callithrix jacchus* colonies, each using different infant-rearing practices, over a period of four decades. Patterns of change between establishments and over time in litter size, infant mortality, and dam longevity were determined to increase our understanding of reproductive variation, particularly factors affecting dam longevity and born litter size. This has the potential to aid in the management of captive common marmoset colonies [Smucny et al., 2004], many of which are housed for breeding purposes to provide models for biomedical research [Hart et al., 2012].

METHOD

Population Description

Reproductive information was obtained from records of marmoset dams used for breeding or in reproductive studies at three UK colonies. One colony was a commercial breeder, the other two bred marmosets primarily for use on site. The first dams in the records, which began breeding early in each decade, were selected. Data were collected from

120 dams at each site. At Colony A (CA), 30 dams in each of four decades (1970s, 1980s, 1990s, and 2000s) were selected. As there were no data available from the 1970s at Colony B (CB) and Colony C (CC), data from 40 dams in each of three decades (1980s, 1990s, and 2000s) were collected from these sites. This yielded information from 360 dams. Fifteen wild-caught and 15 in-house bred animals were sampled in the 1970s at CA (no difference was found between the two in number of litters ($t = 0.00$ (28), $P = 1.00$) and litter size born in captivity ($t = 1.14$ (134), $P = 0.256$)). All other animals were bred in-house. This produced data from 2,712 litters (CA 527; CB 1237; CC 967 litters). Loss of archived data at CB meant that born litter size was lost from all files in the 1980s, although weaned litter size could still be extracted. The data therefore consisted of dam information for 5588 born infants (CA 1287; CB 2004; CC 2297 infants). Lack of records during the early 1980s at CC also meant that survived litter size could not be extracted. Data were collected between February 2011 and February 2013, and were approved after review by the Stirling University Psychology Ethics Committee and by each facility involved. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates.

Two sets of back-record data were examined for each colony. The breeding file contained litter information for each dam, and the stock file contained individual dam life histories (including dates of birth and death, and manipulations for experimental or management purposes). These data sets were cross-referenced to provide a full account of each female's life in the colony. Dams euthanized at the end of an experiment were not included, although many sampled at CA were manipulated for non-terminal studies (e.g. given implants, injected with hormones and bled periodically).

Litter Information

Litter information consisted of data from each particular dam, regarding dates of birth for each litter, litter size, sex ratio, and inter-birth intervals. Survival of each infant at birth (CA, CB, and CC) and to weaning age (6 months; CB and CC) was recorded. Data for the first litter following intentionally aborted pregnancies, contraception administration or a mate change were excluded when calculating mean IBI. Contraception was generally only used once or twice towards the end of a female's breeding life, usually if there was a health problem. If contraception was stopped, females did occasionally become pregnant again.

Reproductive Summaries

Reproductive history was also summarized for each female. Reproductive output variables included mean litter size born, mean litter size survived, number of litters produced and mean IBI. Longevity, age at first parturition, reproductive life span (calculated as the years between a dam's first and last birth), lifetime production, lifetime survived production, production per reproductive year and survived production per reproductive year (calculated by dividing lifetime production or survived production by (reproductive life span + 0.67)). The figure 0.67 years represents the average in utero investment in the first litter (5 months), plus the lactation investment in the last litter (time until weaning (3 months)) [Smucny et al., 2004]. Table I shows the number of dams sampled for each variable at each colony.

Infant-Rearing Practices

At CA, one infant from each triplet litter was either fostered or hand-reared in the 1970s. In later years, no intervention was carried out when triplets were born. At CB, infants from triplet litters were rotationally hand-reared (one was removed for 8 hr/day from the family and given supplementary food), in an attempt to improve survival. Triplets were also fostered if an appropriate dam was available, or completely hand-reared if the family rejected or abused their young. At CC, triplets were supplementary fed, in which all infants were removed from the family for 2 hr twice a day for hand feeding. Very light infants (<27 g) were routinely euthanized at day 1.

Maternal Body Weight and Number in Dam Litter

As all animals are weighed every month at CC, this information was available on individual records. Weights at likely conception dates or early in pregnancy, approximately 5 months prior to the birth date, before significant gain from the fetuses [Bales et al., 2001; Tardif & Jaquish, 1997], were recorded and used in analysis. Mean dam weight ranged from 366.06 g ± 49.39 for singleton litters ($N = 47$) and 373.80 g ± 41.57 for twins ($N = 489$), to 396.49 g ± 45.74 ($N = 376$) for triplets and 391.20 g ± 40.16 for quadruplets ($N = 10$). The number of infants in the dam's litter at her birth was also recorded at CC, and so this was included to look at any potential genetic influence in mean litter size. Neither weights nor dam's own litter size was recorded consistently at CA or CB.

Statistical Analysis

Data were summarized and analyzed using SPSS statistical software. Descriptive statistics were carried out to summarize the reproductive output of the 120 dams at each colony. The percentages of each born litter size and their associated losses, as well as

TABLE I. Number of Dams Included for Each Variable in Each Colony

Variable	Colony A	Colony B	Colony C
Dam longevity	105 (Ex 15 wild-caught in 70s)	120	115 (Ex 4 ex breeders still alive in 2000s and 1 purchased in 80s)
IBI	93 (Ex 27 primiparous)	115 (Ex 5 primiparous)	108 (Ex 12 primiparous)
Age at 1st parturition	105 (Ex 15 wild-caught may have had previous litters)	120	119 (Ex 1 purchased in 80s)
Lifetime production	105 (Ex 15 wild-caught)	80 (Ex 40 in 80s- no record of born litters)	119 (Ex 1 purchased in 80s)
Survived production	105 (Ex 15 wild-caught)	120	80 (Ex 40 in 80s- no record of losses)
Production/year	120	80 (Ex 40 in 80s)	120
Survived production/ year	120	120	80 (Ex 40 in 80s)
Reproductive life span	80 (Ex 40 wild-caught and primiparous)	115 (Ex primiparous)	107 (Ex primiparous and 1 purchased in 80s)
Litter size	120	80 (Ex 40 in 80s)	120
Survived litter size	120	120	80 (Ex 40 in 80s with missing data)
Number of litters	105 (Ex 15 wild-caught)	120	119 (Ex 1 purchased in 80s)
Maternal body weight at conception	0	0	118 (Ex 2 in 80s with missing data)
Number in dam litter	0	0	118 (Ex 2 in 80s with missing data)

Ex, excluding.

changes in litter size and dam longevity over time were also examined.

Descriptive statistics were also conducted to summarize cause of death over all three colonies ($N = 356$). These were divided into "euthanized," "died naturally," or "not stated" (some within this category gave a cause of death, but did not specify whether the animal was euthanized or died naturally of the problem). This was further divided into "health" or "breeding management" reasons for death, as well as if this was "not stated" (in some cases it was recorded that the animal was euthanized or died naturally, but the reason was unknown).

Mean Litter Size

Multi-linear regression procedures using the Enter method were performed on 258 dams for whom we had complete data on all independent variables (IVs), to describe the amount of variation in the dependent variable (DV) mean litter size. Preliminary Spearman's Rank correlations were first used to look for potential multicollinearity between variables. Number of litters was not included in the analyses, due to the strong correlation with dam longevity ($r = 0.89$, $P < 0.001$), although no other variable was highly correlated ($r > 0.60$) with another. R^2 change values for each additional variable entered in the regression model were used to describe the variance explained by each IV. The criterion for entry into the model was $P < 0.05$. Although DVs were not normally distributed, models can still be used to make valid conclusions from this sample [Field, 2009]. Colony and decade were regression control variables. Independent variables of longevity, mean IBI, age at first parturition and yearly production [following Smucny et al., 2004] were entered into the model.

It became clear from comparions that the colonies showed different patterns. There were also different issues that arose, including data from wild-caught animals in the 70s at CA, missing data in the 80s at CB and CC, and no weights or dam litter size recorded at CA and CB. Each colony was therefore also analyzed separately, to prevent important information being lost. An ANOVA was conducted to look at differences in weight between litter sizes at Colony C.

Survival Analysis

Cox proportional hazards regression was performed to investigate which reproductive output variables could affect dam longevity. This is appropriate as it can be used to evaluate the effect of two or more continuous or categorical variables on whole-life survivorship. It also handles censored cases, so animals without a completed lifespan can be included [Jaquish et al., 1991].

Survival analysis was conducted for 262 dams of known birthdates, using the Enter method, with covariates of mean litter size, mean IBI, age at first parturition and yearly production. Site and decade were included as control variables. Each colony was also analyzed separately, with decade as a control variable. Additional covariates of number of dam litter and dam weight at likely conception were included for CC. For dams with known date of death, longevity was the time of death. For dams still alive in the colonies ($N = 4$), longevity was the age at censor date. This was defined as the date of the last update in the colony records.

RESULTS

Variation in Reproductive Output

Reproductive output variables for the dams of the three colonies (combined decades) are summarized in Table II. The values represent grand mean and medians calculated from the mean values of all dams. For CA data, no measured parameter was normally distributed (>0.05) and so median values are most appropriate. For CB data, "yearly production" and "yearly survived production" were normally distributed, and for CC, "dam longevity" and "dam weight at conception" were normally distributed, and so mean values are most appropriate for these.

Changes in Mean Litter Size and Dam Longevity

Figures 1 and 2 display median dam longevity and median of the mean litter size, for each colony over the decades. These graphs reveal the different patterns of change over the decades between the sites.

Litter Sizes and Associated Losses

Figure 3 displays the percentage of births at Colonies A, B, and C. Compared to twins, triplet births were equally as common at CA, more common at CB and a little less common at CC, when data from all four decades were combined. Table III shows the total percentage of mortality (number of infants) associated with each litter size at each colony at birth, within 6 months and in total. In the majority of cases, these were by natural causes or euthanasia due to poor growth. Infant mortality was highest in quadruplet and quintuplet litters.

Dam Cause of Death

Descriptive statistical analysis was carried out on 356 dams from all three colonies. Table IV shows the number of animals that were euthanized or died naturally, as well as when this was not stated, and the associated percentages of each cause of death (health, breeding management, or unknown). Where

TABLE II. Variation in Dam Reproductive Variables (Colonies A, B, and C, Combined Decades)

	Mean and SD			Median, min–max		
Variable	A	B	C	A	B	C
Dam longevity (years)	5.31 ± 2.06	7.39 ± 2.60	6.04 ± 2.47	4.98 (1.31–11.34)	6.99 (2.80–16.20)	5.76 (1.88–13.59)
Inter-birth interval (days)	229.17 ± 81.71	190.87 ± 39.22	192.05 ± 81.85	206.00 (151.00–669.00)	180.00 (151.00–337.00)	170.20 (149.67–754.00)
Age at first parturition (years)	2.68 ± 0.82	2.32 ± 0.68	2.30 ± 0.63	2.49 (1.19–5.17)	2.19 (1.14–6.69)	2.13 (1.33–5.62)
Lifetime production (no. of infants born)	10.77 ± 9.16	25.05 ± 17.10	18.88 ± 13.72	9.00 (1.00–42.00)	21.00 (1.00–59.00)	16.00 (1.00–59.00)
Survived production (no. of infants)	9.74 ± 8.61	19.05 ± .68	13.45 ± 11.54	8.00 (0.00–42.00)	16.00 (1.00–53.00)	10.50 (0.00–46.00)
Production/year (infants born/year of RL)	3.84 ± 1.19	4.67 ± 1.31	4.32 ± 1.05	3.62 (1.49–7.71)	4.60 (1.49–7.74)	4.42 (1.49–6.48)
Survived production/year (infants/year of RL)	3.34 ± 1.29	3.39 ± 0.88	2.99 ± 1.47	3.24 (0.00–6.58)	3.39 (0.76–5.49)	3.43 (0.00–5.44)
Reproductive lifespan (years)	2.61 ± 1.91	4.75 ± 2.26	3.77 ± 2.39	2.15 (0.42–9.06)	4.61 (0.63–13.36)	3.58 (0.41–11.68)
Litter size (no. of infants born)	2.37 ± 0.53	2.55 ± 0.55	2.32 ± 0.43	2.33 (1.00–4.00)	3.00 (1.00–4.00)	2.33 (1.00–3.50)
Survived litter size (no. of infants)	2.06 ± 0.65	1.87 ± 0.37	1.56 ± 0.71	2.00 (0.00–3.23)	2.00 (1.00–3.00)	1.75 (0.00–3.00)
Number of litters (litters/dam)	4.37 ± 3.37	10.31 ± 6.15	7.93 ± 5.49	3.00 (1.00–14.00)	9.00 (1.00–30.00)	7.00 (1.00–23.00)
Dam weight at conception (g)			373.39 ± 43.44			369.13 (283.00–503.00)
Number in dam litter			2.42 ± 0.53			2.00 (1.00–4.00)

Reproductive life span (RL) is summarized for multiparous females only. Survival age and age at first birth were calculated for dams born into the colony, and so exclude wild caught animals. Inter-birth intervals were calculated excluding abortions and after a change of mate. For CA, medians are most appropriate for all values. For CB, "production/year" and "survived production/year" and CC "dam longevity" and "dam weight at conception" mean values are most appropriate.

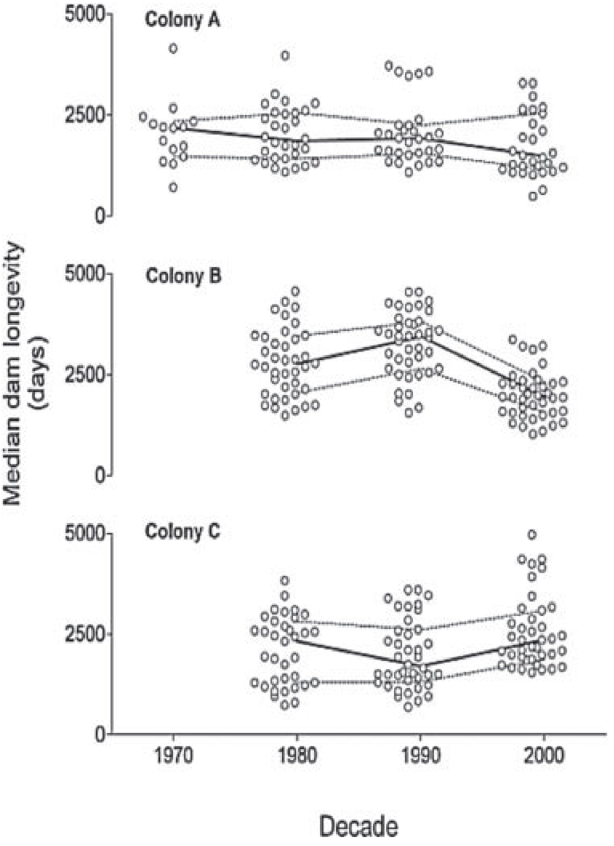

Fig. 1. Median dam longevity ($N = 105$ CA; 120 CB; 115 CC) for each colony over four decades. Median: solid line; 25 and 75 percentiles: dotted line; individual dams: open circles.

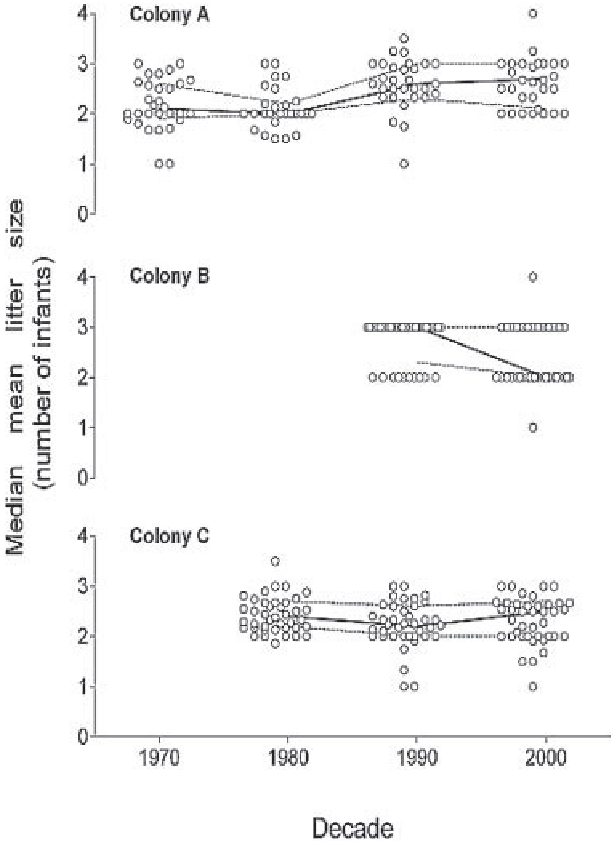

Fig. 2. Median of mean litter size ($N = 120$ CA; 80 CB; 120 CC) for each colony over four decades. Mean litter size calculated as sum of number of infants in each litter, divided by total number of litters, for each dam. Median: solid line; 25 and 75 percentiles: dotted line; individual dams: open circles.

this information was recorded, the most common cause of death was euthanasia due to poor condition.

Mean Litter Size

A linear regression model of mean litter size was estimated ($R^2 = 0.45$), explaining 44.8% of the variance in mean litter size for the combined colonies. Two hundred and fifty eight cases were included in the analysis. Control variables for decade, and colony were included in the model. Significant differences in mean litter size were found between colonies (explaining 45%), with CC having significantly lower mean litter size than CA and CB. CA and CB were not significantly different. A significant difference was also found between decades (explaining 42%). Mean litter size in the 90s was significantly higher than in the 80s. No other comparisons were significant. Net of the control variables, yearly production had the highest explanatory value (44.7%, positive effect) followed by longevity (9.8%, positive), with all being significant.

A linear regression model of mean litter size was estimated for each colony. For CA, 80 cases were included, and 45.9% of the variance was explained. Control variables for decade were included in the model (explaining 23.6%). Mean litter size in the 70s and 80s were significantly lower than in both the 90s and 2000s. Net of the control variables only yearly production was significant (22.3%, positive effect).

For CB, 75 cases were included, and 47.8% of variance was explained for mean litter size. As all cases in the 1980s were incomplete, only those in the 1990s and 2000s were included. Mean litter size was significantly higher in the 90s than the 2000s. Net of the control variables (explaining 13.1%), only yearly production was significant (34.6%, positive effect).

For CC, 102 cases were included, and 55.7% of the variance in mean litter size was explained. No decade was significantly different to another. Net of the control variables yearly production had the highest explanatory value (51.3% positive effect), followed by mean dam weight (21.7%, positive effect), with both significant. A one-way ANOVA revealed a significant difference in dam weight at likely conception between born litter sizes (F $(3, 918) = 21.61$, $P < 0.001$), with post hoc tests showing dam weight to be higher in triplet births than twin ($P < 0.001$) and singleton births ($P < 0.001$). No difference was however found in quad births. While dam's own litter size was included in analysis, this was not found to contribute

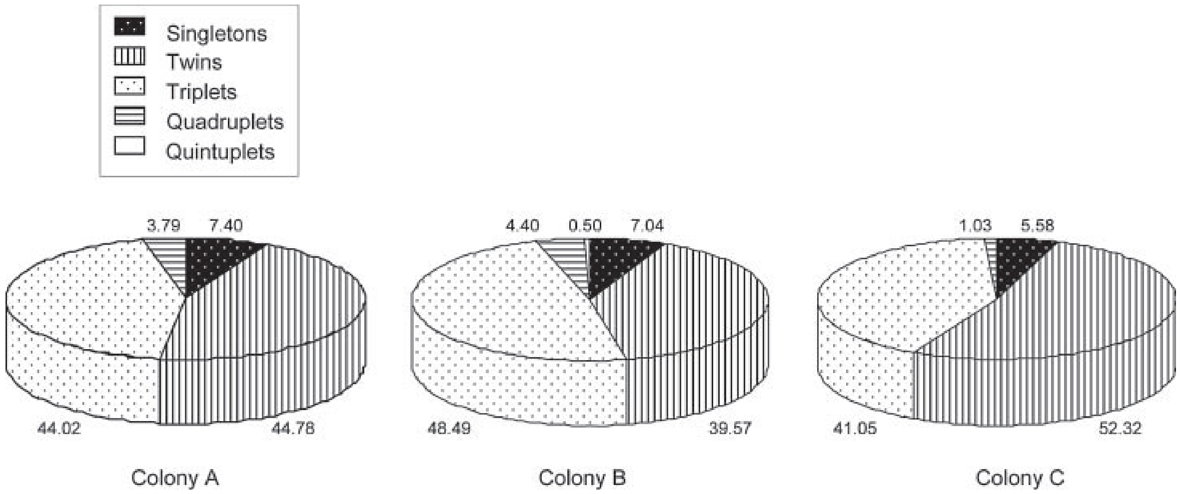

Fig. 3. Percentage of litter sizes at birth at Colonies A ($N = 527$), B ($N = 796$), and C ($N = 967$).

significantly to the model. Table V summarizes the results of the Multiple Linear Regression from combined and separate colony analysis.

Survival Analysis

A whole-life survivorship analysis revealed that colony, mean litter size and yearly production were significant ($P < 0.05$) factors affecting dam longevity. CA had significantly lower survival than CB and CC, although CB and CC were not significantly different. Decades 80 and 90 were significantly higher than in the 2000s, although no other comparison was significant. Increases in mean litter size and yearly production were both significantly associated with higher longevity.

Analysis of individual colonies revealed that only mean IBI had a significant relationship (positive) with dam longevity at CA. Dams with longer mean IBI demonstrated higher longevity than those with shorter mean IBI. There were no significant differences in longevity between the decades at CA. Only decade was significant at CB. Females breeding in the 90s lived for longer than those breeding in the 2000s. At CC, mean litter size (positive), yearly production (positive), mean IBI (negative), and mean weight (positive) were all significant factors affecting dam longevity. Females with higher mean litter size, higher yearly production, shorter mean IBI and higher weight showed greater longevity. No significant differences in longevity were found between decades at CC. While dam's own litter size was included in analysis, this was not found to contribute significantly to the model. Table VI summarizes the results of the Cox Proportional Hazards Regression from combined and separate colony analysis.

DISCUSSION

Reproductive Output and Dam Longevity

The present study summarized the reproductive output of captive marmosets housed at three UK colonies over four decades. Overall, many values are similar to those previously described [Box & Hubrecht, 1987; Smucny et al., 2004; Tardif et al.,

TABLE III. Percentage of Each Litter Size, Together With Their Associated Mortality (Colonies A, B and C)

	Singletons			Twins			Triplets			Quadruplets			Quintuplets		
Colony	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Number of litters born	38	56	54	235	315	506	228	386	397	20	35	10	0	4	0
Number of infants born	38	56	54	470	630	1,012	684	1,158	1,188	80	140	40	0	20	0
Number of infant losses at birth	3	3	5	38	17	45	82	54	104	6	20	8	0	0	0
% losses at birth	7.89	5.36	9.25	8.09	2.70	4.45	11.99	4.66	8.75	7.50	14.29	20.00	0.00	0.00	0.00
Number of infant losses at 6 months	N/A	9	3		84	44		302	205		43	6		13	0
% losses at 6 months	N/A	16.07	5.55		13.33	4.34		26.08	17.26		30.71	15.00		65.00	0.00
Total number of infant losses	N/A	12	8		101	89		356	309		63	14		13	0
% total losses	N/A	21.43	14.81		16.03	8.79		30.74	26.01		45.00	35.00		65.00	0.00

N/A, no data on infant mortality after the day of birth.

TABLE IV. Percentages of Each Cause of Death When Animals Were Either Euthanised, Died Naturally or When This Was Not Recorded ($N = 356$)

	Euthanised ($N = 274$)	Natural death ($N = 22$)	Not stated ($N = 60$)
% Health	65.69	27.27	48.33
Gastrointestinal	1.45	4.55	1.67
Injury	1.82	0	0
Neurological	2.19	0	1.67
Poor condition	44.90	13.64	33.33
Reproductive	7.30	9.09	10
Respiratory	3.28	0	1.67
Surgical complications	1.09	0	0
Tumor	3.28	0	0
Optic	0.36	0	0
% Breeding management	1.82	0	0
Removed from breeding	1.09	0	0
Not breeding	0.36	0	0
Infanticide	0.36	0	0
% Unknown	32.48	72.72	51.67

2003], although several are greater in the UK colonies. These higher UK values appear to be due to the lifetime production and number of litters at CB in particular, where there was also the highest reproductive lifespan and shortest IBIs. While some females had a reproductive life span of only one or two litters, others had consistently high production over many years. There was therefore considerable variation between female common marmosets. Table VII provides comparative data from previous research.

Over all three colonies, average longevity was approximately 6 years in the UK. This is similar to other establishments from the 1980s [Box & Hubrecht, 1987] to the 2000s [Smucny et al., 2004]. It appears that while the majority of animals was euthanized, rather than died naturally, this was due to health and welfare reasons, most commonly “poor condition.” More detailed records would however be beneficial, including a more specific cause of death. Management decisions can also be made regarding which animals are most suitable to keep in breeding, and so longevity could be related to production [Essl, 1998]. However, only a very small portion, of those with adequate records, were euthanized due to breeding management. Dam health and longevity is therefore a concern. While one may expect increased longevity in captivity compared to the wild, as predators and food shortage are not constraints, this does not appear to be the case at some colonies.

Factors Affecting Dam Longevity

A whole-life survivorship analysis, combining data from all three UK colonies, found that site,

TABLE V. Summary of Regression Results for Mean Litter Size Born Age (Combined and Separate Colony Analysis)

Model variables	R^2	Adjusted R^2	R^2 change	Significance of added variable
Combined colonies ($N = 258$ complete cases)				
Whole model r2 = 0.448, adjusted 0.432				
Site	0.045	0.037	0.045	$P < 0.01$
Site AvC				$P < 0.05$
Site BvC				$P = 0.001$
Decade	0.042	0.030	0.042	$P < 0.05$
Decade 90v80				$P < 0.01$
Yearly production	0.447	0.434	0.373	$P < 0.001$
Dam longevity	0.098	0.077	0.024	$P < 0.01$
Colony A ($N = 80$ complete cases)				
Whole model r2 = 0.459, adjusted 0.43				
Decade	0.236	0.206	0.236	$P < 0.001$
Decade 70v100				$P = 0.01$
Decade 80v100				$P < 0.001$
Decade 90v70				$P < 0.05$
Decade 90v80				$P < 0.001$
Yearly production	0.459	0.430	0.223	$P < 0.001$
Colony B ($N = 75$ complete cases)				
Whole model r2 = 0.478, adjusted 0.463				
Decade	0.131	0.120	0.131	$P = 0.001$
Decade 90v100				$P = 0.001$
Yearly production	0.478	0.463	0.346	$P < 0.001$
Colony C ($N = 102$ complete cases)				
Whole model r2 = 0.557, adjusted 0.539				
Mean dam weight	0.255	0.232	0.217	$P < 0.001$
Yearly production	0.551	0.537	0.513	$P < 0.001$

TABLE VI. Cox Proportional Hazard Regression Coefficients for Whole-Life Survivorship Analysis of Dams (Combined and Separate Colony Analysis)

Covariate	Estimate	SE	Wald statistic	df	P	Relative risk	Lower 95% CI for relative risk	Upper 95% CI for relative risk
Combined colonies ($N = 262$)								
Whole model ($X^2 = 43.923$)								
Site			18.289	2	<0.001			
BvA	−0.696	0.165	17.854	1	<0.001	0.499	0.361	0.689
CvA	−0.425	0.151	7.899	1	=0.005	1.530	1.137	2.057
Decade			11.938	3	<0.01			
80v100	−0.356	0.174	4.178	1	<0.05	0.700	0.498	0.985
90v100	−0.512	0.512	11.417	1	=0.001	0.599	0.445	0.806
Mean litter size	−0.444	0.153	8.426	1	<0.005	0.641	0.475	0.866
Yearly production	−0.231	−0.062	13.812	1	<0.001	0.794	0.703	0.896
Colony A ($N = 80$)								
Whole model ($X^2 = 5.15$)								
Mean IBI	−0.004	0.002	4.555	1	<0.05	0.996	0.992	1.000
Colony B ($N = 75$)								
Whole model ($X^2 = 38.216$)								
Decade 90v100	−1.823	0.304	36.072	1	<0.001	0.161	0.089	0.293
Colony C ($N = 106$)								
Whole model ($X^2 = 30.172$)								
Mean litter size	−0.688	0.334	4.250	1	<0.05	0.502	0.261	0.967
Mean IBI	0.003	0.001	6.154	1	<0.05	1.003	1.001	1.005
Yearly production	−0.584	0.125	21.841	1	<0.001	0.557	0.436	0.712
Mean weight	−0.011	0.003	12.167	1	<0.001	0.989	0.984	0.995

decade, yearly production, and mean litter size were all significant predictors of dam longevity. Dam longevity and lifetime productivity at CA, where experimental manipulations were often carried out for reproductive studies, was the lowest of the three colonies, and very similar to those obtained by Smucny et al. [2004]. Average longevity was 5.31 years, which was relatively similar in each decade. However, many animals were placed on terminal experiments in the 2000s, which did limit the available sample in this decade. Dam longevity and lifetime productivity at CB, a commercial facility in which breeding pairs were rarely disturbed, was the highest. Average longevity was 9.58 years in the 1990s, which is similar to the University of Cambridge [Ridley et al., 2006]. However, this significantly decreased in the 2000s, after a change in diet and moves between buildings. Differences in housing and husbandry could therefore be important factors in dam longevity between colonies. Results from CC, an establishment that bred for purpose, fell between those obtained at the other two sites. Longevity remained at around 6 years over the decades, which is similar to data published by Tardif et al. [2003]. This suggests that longevity in captivity does not appear to have improved significantly, despite increased understanding of the species' biological and psychological needs and concurrent improvements in their care. While there were insufficient details to investigate which specific environmental factors are most important, it appears that appropriate housing and particularly a diet that meets nutritional needs is necessary, as is a stable, closed colony with minimal disturbance [Tardif et al., 2011].

Although the costs of high reproduction might be expected to reduce condition and longevity [Tardif et al., 2008], there was no evidence that this was the case. In fact, dams with larger mean litter sizes, producing more infants per year, tended to have higher longevity. Previous research [Jaquish et al., 1991; Smucny et al., 2004] has found no relationship between litter size and dam longevity. Although larger litters did not appear to be detrimental to physical health, there is evidence that they may be stressful for parents. Tardif et al. [2002] found that dams spent less time carrying and nursing triplet infants, compared to twin infants. There was also a higher frequency of triplet-infant initiated interactions, associated with increased harassment by mothers, than for twins. These findings suggest that dams could only tolerate a limited amount of time with their young, and that larger litters seem to disrupt maternal behavior [Tardif et al., 2002].

Only mean IBI was significant in explaining dam longevity at CA, with dams experiencing longer interbirth intervals surviving longer. Mean IBI was also significant at CC, although a negative association was found at this colony. Instead, heavier dams survived for longer at CC, where weight was recorded. This may be because lactation is relatively costly for marmosets, with small mothers experiencing substantial mass loss and high risk of mortality following twin litters [Tardif et al., 2001]. While, it is

TABLE VII. A Summary of Results From Previous Studies of Captive colonies, Including Combined Results From All Three Sites in the Present Study

Variable	Current study ($N = 302^{1}$; 304^{2}; 305^{3}; 316^{4}; 320^{5}; 340^{6}; 344^{7} dams)	Smucny et al. [2004] ($N = 272^{1}$; 287^{2}; 400^{3} dams)	Tardif et al. [2003] ($N = 479$ dams)	Box & Hubrecht [1987] ($N = 543$ infants)
Dam longevity (years)				
Mean	$6.29^{6} \pm 2.55$	$5.74^{3} \pm 2.46$	5.99 ± 2.31	6.00
Median	5.94^{6}			
IBI (days)				
Mean	$202.54^{4} \pm 71.27$	$216.7^{1} \pm 98.53$		
Median	181.21^{4}		162.00	158.00
Age at 1st parturition (years)				
Mean	$2.42^{7} \pm 0.73$	$2.91^{3} \pm 1.16$		
Median	2.25^{6}			
Lifetime production (number of infants born)				
Mean	$17.70^{2} \pm 14.48$	$8.03^{3} \pm 7.15$	7.75	
Median	14.00^{2}		6.00	
Survived production (number of infants)				
Mean	$14.38^{3} \pm 11.80^{a}$	$4.37^{3} \pm 4.36^{b}$		
Median	11.00^{3a}			
Production/year (infants born/year of RL)				
Mean	$4.23^{5} \pm 1.21$	$3.66^{3} \pm 1.57$	2.30	
Median	4.23^{5}			
Survived production/year (infants/year of RL)				
Mean	$3.27^{5} \pm 1.21$	$1.87^{3} \pm 1.29^{b}$		
Median	3.37^{5}			4.00^{b}
Reproductive life span (years)				
Mean	$3.84^{1} \pm 2.51$	$2.08^{2} \pm 1.55$		
Median	3.33^{1}			
Litter size (number of infants born)				
Mean	$2.40^{5} \pm 0.50$	$2.22^{3} \pm 0.56$		
Median	2.33^{5}			
Mode	2.00		2.00	3.00
Survived litter size (number of infants)				
Mean	$1.86^{5} \pm 0.61$	$1.87^{3} \pm 0.68^{b}$		
Median	2.00^{5a}			
Number of litters (litters/dam)				
Mean	$7.67^{7} \pm 5.72$	$3.54^{3} \pm 2.84$	3.45	
Median	6.00^{7}		4.00	

± SD.

[a] Survived the day of birth and up to 6 months.

[b] Survived up to 1 month after birth.

possible that the constant high energetic demand of pregnancy and lactation could reduce longevity, and so increasing time between births may give females time to recover body condition, this effect does not span all three colonies and so no robust conclusions can be made. However, this could be an interesting area for future research to explore.

Although results from previous studies suggest that delaying the onset of breeding in captivity may increase longevity [Jaquish et al., 1991], with early age at first reproduction having detrimental health consequences, no association was found between age at first parturition and dam longevity in the present study. However, age at first parturition was generally around 2.0 years, with very few after this time. Perhaps if more females had begun breeding after 4 years, a similar result to Smucny et al. [2004] would be found. This may be another interesting area of future research, and a possible consideration in the management of breeding marmosets. While it is important to consider age-related pathologies, marmosets could be managed to survive for longer before degeneration occurs [Tardif et al., 2011].

Litter Size and Infant Mortality

Litters larger than two accounted for approximately half of the births examined in each colony. However, these larger litters did have considerably greater perinatal mortality than in twins, ranging from 30% of infants from triplet litters to 65% from quintuplets. High infant mortality has been reported previously in captive colonies [Jaquish et al., 1991], primarily due to the large proportions of triplets born.

As marmoset families are rarely able to rear more than two infants at a time [Poole & Evans, 1982], these young are unlikely to survive without some form of human intervention. While CA did not intervene when triplet litters were born in later decades, CB and CC both consistently carried out supplementary feeding of triplet infants. Despite hand-rearing, large litters still resulted in higher mortality than twins. While it was rare for all three triplets in a litter to die, there was often one infant loss within the first few weeks. These rearing practices also involve removal from the family for extended periods of time, which has been associated with adverse developmental outcomes [Dettling et al., 2002; Pryce et al., 2004]. Although triplet losses at birth were higher at CC than CB, due to routine euthanasia of very light infants, losses at 6 months were lower. This suggests that their practice of rotational hand-rearing may have been more successful, as litter mates remained together and were separated from the family for shorter periods of time. Due to our ethical obligation to ensure good welfare, as well as the importance of raising animals that are "fit for purpose," potential factors affecting mean litter size were also studied.

Factors Affecting Born Litter Size

A linear regression model, combining data from all three UK colonies, found that 44.8% of variance in mean litter size born was explained by site, decade, yearly production and dam longevity. CC had the lowest mean litter size of the three colonies. Differences over time were also found at CA, where births changed from predominantly twins in the 70s and 80s to predominantly triplets in the 90s and 2000s. Litter size fell significantly in CB, although remained similar at CC.

Inspection of colonies separately showed that only yearly production was significant at CA and CB. However, these findings are somewhat obvious or unavoidable, and so are not useful predictors. They are therefore of little interest, as they will not contribute to Refinements. Mean dam weight at likely conception was a significant predictor of mean litter size at CC, with heavier dams producing larger litter sizes. Dam weight was also significantly higher prior to triplet births compared to twin or singleton births. Tardif & Jaquish [1997] also showed that higher weight was associated with higher number of ovulations. However, mothers that lose mass during pregnancy can reabsorb fetal material, leading to litter size reduction in utero [Tardif & Jaquish, 1997]. Litter size could therefore change from date of conception, which may explain why this factor did not explain more of the variation.

The dam's own litter size was not significant in predicting litter size, a finding reported by previous authors [Jaquish et al., 1991; Tardif & Jaquish, 1997], and so genetic variance does not appear to play a major role. Tardif & Jaquish [1997] found that much variation in number of ovulations was seen within, rather than between, females. Low repeatability of final litter size per dam has also been discovered [Jaquish et al., 1991]. It is therefore unlikely that selecting breeding females who were born to twin litters themselves would be a successful way of promoting twin births in captive colonies. Litter size instead appears to be flexible [Jaquish et al., 1996], determined by environmental variables affecting energy availability, such as diet or physical activity. Captive animals can weigh as much as 600 g [Poole & Evans, 1982], compared to their wild counterparts weighing around 330 g, which may account for captive females producing more larger litters than their wild counterparts. Maintaining dams at lower weights, may help to reduce larger litters, which are associated with higher infant mortality. This must be applied carefully, as heavier dams also seem to have greater longevity.

CONCLUSION

The present study provides interesting information on reproduction and life history in female marmosets housed at UK breeding colonies, in comparison to similar international establishments. Areas of concern include high rates of infant deaths and dam health. Potential predictors of mean litter size and dam longevity were therefore examined, and possible ways of aiding with practical aspects of managing these animals discussed. Maintaining a colony of experienced breeders, with longer healthy life spans and an increased incidence of twin births could have far-reaching implications to improve the quality of life for marmosets in breeding facilities. This is especially important given the considerable number bred for use in a wide range of biomedical research around the world.

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