MSc
Applied Animal Behaviour and Animal Welfare

Stress in captivity and the role of environmental enrichment in reintroduction programmes
&
The effect of three different foraging devices on the behaviour of long-tailed macaques (*Macaca fascicularis*) in rehabilitation for reintroduction.

by

Wendy Gomez Rubio

2013

A dissertation submitted in part fulfilment for the Degree of Master of Science in Applied Animal Behaviour and Animal Welfare at the University of Edinburgh

Royal (Dick) School of Veterinary Studies
Easter Bush Veterinary Centre
Easter Bush
Roslin EH25 9RG

TABLE OF CONTENTS

Stress in captivity and the role of environmental enrichment in reintroduction programmes

Wendy Gomez Rubio

The Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin EH25 9RG, UK

Abstract

As animals housed in captivity are usually exposed to a variety of artificial stimuli that are often detrimental to their physical and mental health, it is important to provide these animals with a complex environment where they can express their natural behaviour, and where outlets that can help them cope better with captive conditions. Environmental Enrichment (EE) Techniques are a valuable tool to improve the welfare of captive species, and to promote the expression and maintenance of species-typical behaviours, which are also considered a key-step in rehabilitation and reintroduction programmes. Implementation of EE techniques not only reduce boredom and the expression of abnormal behaviours (e.g. stereotypies, self-harming) that are often the result of long term exposure to stressful situations, but can also be used to enhance natural behavioural traits or skills that will not only result in improved welfare, but also in a more fit individual either for long-term captivity or for reintroduction.

Key words: environmental enrichment, welfare, stress responses, reintroduction.

1. Introduction

One of the greatest challenges of keeping animals in captivity is ensuring adequate welfare, especially when housing species with large home ranges and complex social structures and hierarchies, as the deprivation of spaces that provide them with opportunities to behave like their conspecifics in the wild can adversely affect their physical and mental health (Coleman *et al.* 2013).

Life in captivity differs from life in the wild not only because the broad range and nature of stimuli that continuously stimulate an animal's senses in nature (e.g. smells, sounds, temperature, humidity, substrate, light, etc.) (Morgan and Tromborg, 2006) are reduced when housed in a captive environment, but also because captive individuals may have to constantly deal with artificial surroundings and conditions that are potentially detrimental to their welfare (e.g. artificial lighting, restricted movement, reduced retreat space, restricted diet, reduced feeding opportunities, anthropogenic noises, etc.). Therefore, it is of great importance to try to diminish any negative effect of these potential stressors by providing the animals with opportunities to adjust or to better cope with captive conditions.

One of the most powerful tools to do this is Environmental Enrichment (EE) techniques (Shepherdson, 1998; Boere, 2001; Shyne, 2006), which are widely implemented in laboratories, zoos, aquariums and other facilities where wild and domestic species are kept their entire lives, either as experimental, entertainment, educational or companion subjects. In most captive situations, since one of the important threats to welfare is the development of high levels of boredom –as this results in obesity (Herbert and Bard, 2000) and in the expression of abnormal behaviours- EE focuses on: 1. Engaging the animals in activities that keep them busy for long periods of time; and 2. Providing them with a captive environment where they are less prone to face stressful situations.

However, in reintroduction programmes, animals are not housed permanently in captivity, thus the implementation of rehabilitation process prior to release that contributes to the development and enhancement of specific skills for survival in the wild is important (e.g. how to forage, hunt, avoid predators and identify prey etc.). In some of these programmes EE is not only used to improve and maintain the welfare of the individuals whilst in captivity, but it is also the most useful tool to enhance these key survival skills (Reading *et al.*, 2013). If properly designed, EE will result in a more fit individual with higher chances of survival (Miller et al., 1990; Shepherdson, 1994; Reading *et al.*, 2013).

It is necessary to understand that in rehabilitation facilities, EE techniques cannot be based on the mere intention to keep animals busy and provide them with a life free of stressors, but on the contrary, must be based on the main threats and challenges the species faces in the wild.

Here, a description of potential sources of stress in captivity and how these can affect welfare will be given. EE techniques will then be introduced as a mechanism to counteract stress effects and improve welfare, including a brief outline of the case of captive non-human primates, that due to their high levels of intelligence and complex social structures (Reading *et al.* 2013) benefit from strictly structured enrichment plans. Finally, the value of EE as a potential useful tool in reintroduction programmes will be discussed.

2. Welfare and Stress in Captivity

Life in captivity is known to produce stress responses, both physiological and behavioural. Physiologically, stress has a deleterious effect on the function of the central nervous system (Olivares *et al.,* 2008; Soto-Moyano *et al.,* 1999) and produces a wide range of changes in the system that can lead to immunosuppression, hormonal alterations, cardiovascular problems (Rupp, 1999), and reproductive problems (Carlstead *et al.*, 1993b) among others. Behaviourally, although most widely accepted indicators of stress are pronounced changes in affiliative and aggressive behaviours, which are of special concern when assessing stress and welfare states, qualitative changes in the animal's overall behaviour repertoire can also occur (Hones *et al.,* 2004).

These behavioural responses to stress include: increased vigilance behaviour (Carlstead *et al.* 1993b), reduced exploratory or foraging behaviour (Carlstead and Brown, 2005; Carlstead *et al.*, 1993b), increased aggression (Hones and Marin, 2006), increased expression of stereotypies, and increased expression of self-injurious behaviours such as self-biting, hair-pulling, self-mutilation and rocking, these last being indicatives of high levels of stress in primates (Hones and Marin, 2006).

Although a holistic assessment of welfare would include physiological and neurological measures such as immune response changes, hypothalamic-pituitary-adrenal [HPA] axis activation and autonomous nervous system responses (Moberg and Mench, 2000; Palm, 2012), behavioural changes alone are of a great importance, as the expression of behavioural abnormalities are direct indicators of poor welfare, especially if exhibited for long periods of time (Broom, 2010).

2.1 Sources of stress in a captive environment

Potential stressors that present themselves as environmental challenges to animals in captivity, and are responsible for the abnormal behavioural responses noted above, include: aversive sounds, restriction in movement and space, temporally limited food availability, reduced opportunities for species-specific social interactions, artificial lighting and odours, uncomfortable temperatures and substrates, as well as reduced or zero opportunity to express natural behaviours (Morgan and Tromborg, 2006). Following are the descriptions of some of the most stress-related factors in captivity:

2.1.1 Sound

In captivity, animals are routinely exposed to several sources of biotic and abiotic sounds that greatly differ from the ones in their natural habitat. Ambient noise levels in rain forest, riverine and savannah habitats ranges from 20 to 40 decibels (dB) throughout the day (Waser and Brown, 1986) and arise from weather, insects stridulations and birds vocalizations, whereas noises arising from human activities can exceed 85dB, and other anthropogenic noises can exceed 100dB (e.g. commercial trucks, heavy machinery, etc.).

Noise is a stressor that has deleterious behavioural and physiological effects on animals. Noise exposure can trigger cardiac arrhythmia, increase in blood pressure and heart rate, changes in body movements (Berglund and Lindvall, 1995 in Bigert *et al.,* 2005), immunosuppression, intestinal problems and insulin resistance (Spreng, 2000). In humans, major effects of noise include hearing loss, sleeping patterns alterations, pain, vertigo and startle reflexes (Chanaud, 2006).

Detrimental behavioural changes induced by noise have been reported in different species in zoos and aquariums: cage cleaning and human noises (e.g. shouting, metal clanging) increase heart rate in laboratory animals and cattle (Waynert *et al.* 1999); noise from visitors increases vigilance behaviour in harbour seals, capuchin monkeys, orangutans (Birke, 2002) and cotton-top tamarins (Tromborg, 1993); loud noises also increase vigilance behaviour in pandas (Owen *et al.,* 2004), and a change in overall behaviour patterns have been observed in laboratory rabbits when placed in non-sound proof housing (Jildge, 1991).

There is a lack of detailed knowledge about the olfactory ranges of most species (Morgan and Tromborg, 2006), which makes it difficult to accurately evaluate the effect of noise or various types of sound on welfare. However, it is known that the hearing ability of mammals (Fay, 1988) is superior to that of humans, enabling them to perceive and experience sounds inaudible to us. This increased ability of hearing therefore makes them more vulnerable to anthropogenic sounds in their immediate environment.

2.1.2 Odours

Olfactory sense plays a key role in wild survival, as one of the most important and reliable ways to identify the presence of a predator is by its odour (Apfelbach *et al.*, 2005).

Identifying the presence of a predator by its smell or by the chemical cues they produce can have several immediate behavioural effects on the prey, including the suppression of certain non-defensive activities such as grooming and feeding. It can also change behaviour in the medium-term, decreasing locomotion activity, reducing reproduction and litter size (Vasilieva *et al.* 2001), and even reducing levels of social interactions including grooming and playing (Apfelbach *et al.*, 2005).

But the olfactory system is not only related to predator recognition: In humans, odours can play an important role in traumatic memories, which can contribute or even trigger panic attacks associated with Post-Traumatic Stress Disorder (PTSD) and other psychopathologies (see Hinton *et al.* 2004). In other animals, as presentation of cues associated not only with predators, but also with presence of conspecific from other social groups or removal of mark scents- does not only increase stress hormones levels, but also activates the autonomic nervous system (Blanchard *et al.*, 1998) and produces anxiety-related behaviours. Thus, it is important to consider odours –and odour alterations- presented in an captive environment as potential stressors to animals.

2.1.3 Artificial Light

Light is known to play an important role in immunological mechanisms, neuroendocrine responses and regulation of biochemical processes in the body (van Hoof *et al.*, 2009). Whereas each species' vision has evolved to help the individuals to function in their specific habitat in the wild, lighting conditions in captivity are usually designed for human convenience or do not take into consideration its possible influence on welfare. In captive indoor facilities like laboratories, light periods may be subjected to personnel work schedules, forcing the animals to live under a fixed light-exposure period. In zoos around the world, exotic tropical species that would usually have 12-hours of light exposure throughout the year, are forced to live under seasonal changes in light, and species from seasonal areas would also have to live under a continuous 12 hour light cycle when housed in facilities in tropical regions.

Alterations in light exposure are associated to restlessness, altered circadian rhythm and anxiety; and have also been linked to mental disorders in humans (see van Hoof *et al.*, 2009). Studies in non-human primates have shown that some species may prefer some type of lighting or intensity to others, and that changes in light patterns (i.e. intensity, direction) can reduce or increase activity and reproductive behaviour (see Hampton *et al.*, 1966). In broiler chickens, a reduction in exploratory and comfort behaviours have been observed when altering light intensity (see Deep *et al.*, 2012). Overall, deprivation of specific wavelengths and exposure to non-optimal light intensities can trigger stress-related responses, such as increased expression of abnormal behaviour and stereotypies (Morgan and Tromborg, 2006), making improper light exposure a potential cause of stress in animals.

2.1.4 Confinement Size

Compared to wild conditions, life in captivity almost inevitably imposes strong space restrictions, since even a large enclosure could not provide animals the same area they would usually cover or use in the wild. For example, the home range for Bengal tigers (*Panthera tigris tigris*) has been reported to be 55.1km ^{2} (Majumder *et al.*, 2012), Sun bears' (*Helarctos malayanus*) home range are over 15 km ^{2} (Wong *et al.*, 2004), Tuffed capuchins' (*Cebus apella*) average home range has been estimated as of 8-9 km ^{2} (Spironello, 2001), Bottlenose dolphins' home range (*Tursiops truncatus*) can reach up to 190.83 km^2, (Gibson *et al.*, 2013) and similarly, most wild species have large home ranges that can obviously not be replicated in a captive environment.

Since each species' home range is directly related to its ecological, morphological, physiological and behavioural traits (reproduction, body size, metabolism, social behaviour, foraging patterns etc.) (Stan *et al.*, 1986) a captive environment that does not offer animals the opportunity to move and travel as their wild conspecifics do, is expected to have an impact on the quality of life of the individuals. Clubb and Mason (2007) found a positive correlation between expression of stereotypic behaviour in captive animals and the distance they would usually travel if living in the wild (i.e. the larger a specie's home range, the more stereotypic behaviours it will show in captivity); Forthman-Quick (1984) found that pacing behaviour was exhibited more in captive species with larger home ranges (see Clubb and Mason, 2007). Moreover, space limitation is associated with increased agonistic behaviours, pacing and infant mortality (Morgan and Tromborg, 2006).

Even though these studies imply that wild species should not be kept in captivity, since they remain to be kept in these environments, either for the purposes of human entertainment or research, it is important to consider room size as one of the main priorities with housing animals.

3. Environmental Enrichment (EE)

Prior to the development the concept of EE, captive environments were not designed to provide the animals with surroundings that promoted either their well-being or enhanced their cognitive and sensory skills (Morgan and Tromborg, 2006). It was only in the last three decades where EE became an integral part in the maintenance of captive animals (Mellen and Ellis, 1996).

Environmental enrichment (EE) techniques have been shown to improve the living conditions (both physical and cognitive) of animals used in laboratories, zoos and other captive scenarios. EE can help ameliorate the effect of stressors associated with captivity (Coleman *et al.* 2013) either by providing the animals with opportunities to perform a more natural behaviour (i.e. more species-specific behavioural repertoire), or by providing other stimuli that enhance their physical and psychological well-being, even if their effects (i.e. of EE) may not promote the expression of natural conducts (e.g. problem-solving devices that reduce boredom by increasing the time an animal spends working on the device, but that logically differs from the challenges they would typically find in nature) (Baumans and Van Loo, 2013).

Although different definitions of EE can be found in literature, the most suitable definition that outlines the needs of wild animals under rehabilitation for subsequent reintroduction, as well as animals in permanent captivity is offered by Carlstead and Sherpherdson (1994). They described EE as "practices that aims to procure environments of greater social, physical and temporal complexity that affords animals more of the behavioural opportunities found in the wild" (Hones and Marin, 2006).

In order to design an apply EE –both for reintroduction or to ensure welfare in a captive environment- it is necessary to define which behaviours need to be addressed or changed, and how the introduction of a specific EE technique will favour these changes.

3.1 Types of enrichment

As described by Coleman and collaborators (2013), enrichment can be classified into five basic categories: social, physical, sensory, feeding, and occupational. Depending on the species and behavioural needs of the individuals, these types of enrichment can be used in different ways. Following are the descriptions of these five categories, and how they can be used to direct abnormal behaviours or provide welfare for animals in permanent captivity and non-human primates.

3.1.1 Social enrichment

Social enrichment is referred to placing animals in pairs or groups. This is of great importance in species with social hierarchies that do not have complex structures, such as rabbits (Chu *et al.* 2004) and dogs (Hubrecht *et al.* 1992), whose abnormal behaviours decrease when housed with a partner or in groups. However, when forming these groups or pairs, or when adding one or more individuals to an already structured social group, special attention needs to be paid to the rearing history of the animal, its personality, age, sex and physical condition, in order to determine how compatible the individuals would be and to also determine by what method the group will be formed (AWIC, 2009).

In social primates, being a part of a group provides the animals with opportunities to express a broad range of social species-specific behaviours such as huddling, grooming, contact and play, which are important to maintain the welfare of the individuals as these social interactions give them a sense of security, and also reduce the expression of abnormal behaviours (Buchanan-Smith, 2010). When housing primates, individuals should be placed in groups where they are accepted by their conspecifics and can be an active part of the hierarchy. Therefore, groups should be formed trying to mimic those found in nature, regarding composition, size and kinship if possible.

Environmental structure and complexity of the enclosure where groups of animals are housed are crucial to obtain a well-structured group as they can increase compatibility between individuals (Chance *et al.* 1983). Consequently, proper adjustments and/or changes need to be considered and taken care of before presenting the animals (see recommendation section below) and during habituation process -if needed.

However, it is also important to note that since forming groups of more than three individuals could be more difficult than maintaining pairs (i.e. high levels of aggression when group-housed may be produced; see Bernstein 1991, Reinhardt, 1991; Reinhardt *et. al.* 1995; Reinhardt 1997), if animals are not candidates for reintroduction or are used as experimental subjects, forming pairs may be the best option, even if that is not the species-typical social arrangement.

3.1.2 Physical enrichment

This type of enrichment refers to items that can be introduced in the enclosure and can promote species-typical behaviour, as well as improve the physical condition of the animals. These items include climbing structures (e.g. nets, vertical branches), visual barriers, nesting materials, platforms, hiding places (boxes, hollowed branches), toys, and other objects that animals can choose to use or not to use, offering a certain degree of control over their environment (Coleman, 2013), and also a good opportunity to exercise and even increase positive social interactions such as grooming or play behaviours (personal observation).

Even though the size of the enclosure does not necessarily reflect its quality, outdoor housing can usually offer a more stimulating and complex environment than indoor housing. In rhesus macaques, moving animals from an indoor to an outdoor environment has reduced the expression of self-injurious behaviours, and rhesus macaques have also shown to spend more time in positive activities, such as play and foraging rather than in potentially negative behaviours (e.g. aggression). On the other hand, taking into consideration enclosure size, a larger enclosure may be more beneficial than a small one, even if both well structured: in macaques, those individuals housed in small cages may exhibit lower corporal weight and size as well as higher muscle atrophy, compared to the individuals housed in big cages. In the same species, larger cages –compared to small sized ones- individuals showed significantly higher successful pregnancy rates (Boot *et al.* 1985), and along with a proper enrichment programme, levels of aggression as well as stereotypies can decrease when animals have access to a larger area (Kitchen and Martin, 1996).

In the case of non-human primates, although the introduction of physical enrichment can redirect stereotypies and reduce abnormal behaviours (Coleman, 2013), its benefit may only be seen when the device is present in the enclosure (Line *et al.* 1991; Jorgensen and Hazen, 1998 in Coleman, 2013). Therefore, it is necessary to assess the long-term effectiveness of these devices, especially when working with candidates for reintroduction, or when trying to reduce self-injurious behaviours, where the condition of the animal may deteriorate and lead to death if the behaviour is not successfully readdressed.

3.1.3 Feeding enrichment

In nature, some species may spend more or less time either foraging, looking for, manipulating or consuming food, not only because the resource may not be easily accessible (i.e. availability of food may vary greatly depending on season), but also because home-range sizes may influence the amount of food available in a certain area (i.e. presence of other species may decrease the resource, or some areas may count with more food than others) (Redpath, 1995 in Santangeli *et al.*, 2012). However, time spent on these feeding-related behaviours decreases greatly in captive environments, where food is usually provided at specific times and it is also of easy access to the animals. Therefore, providing them with the opportunity to increase the amount of time spent in foraging is of high importance in captivity, especially in those species that naturally spend most of their active time foraging, like some species of primates that can spend up to 85% of their time on feeding-related behaviours (Clutton-Brock and Harvy, 1977)

For non-human primates in captivity, commercial devices that have to be manipulated to obtain food and represent a challenge to the animals (i.e. food is not easily accessible), are often used to increase time spent foraging, and sometimes to also increase physical activity, depending on the design of the device. The use of such devices can decrease the occurrence of abnormal behaviours in primates (Novak *et al.* 1998), although these behaviours seem likely to return after the device is taken or when the novelty of the enrichment is gone (Coleman, 2013).

3.1.4. Sensory enrichment

In the wild, animals are continuously exposed to a variety of olfactory, tactile, auditory and visual stimuli (e.g. biotic and abiotic smells, sounds and substrates) that play crucial roles in survival, as they help animals interpret and understand their environment (i.e. smells that allow them to identify where to find food, detect presence of predators, prey etc.). In a captive environment these stimuli are not present anymore (i.e. they come from living organisms and from abiotic elements that can only be found in the wild), and animals are also exposed to a whole different spectrum of new stimuli (anthropogenic noises, odours, lighting etc.).

The term of sensory enrichment is applied to enrichment techniques, which maintain and/or enhance the animals' senses of smell, hearing, vision, touch and taste. Such techniques aim to produce species-specific responses (i.e. reproductive, hunting, social and others) which may help the animals express a more natural behavioural repertoire. (Coleman *et al.*, 2013)

As olfactory enrichment, animals can be offered scents of prey, predator or novel scents such as objects impregnated with perfumes or spices. Tactile enrichment can include different types of substrates (i.e. different textures); visual enrichment may include the introduction of videos or mirrors, but usually consists of offering animals an environment with different colours or moving objects. Auditory stimuli usually include recordings of nature sounds (e.g. rainforest sounds, vocalizations). Finally, as some species feed on a broad range of food in the wild, when housing them in captivity, would be important to also use gustatory enrichment, by offering them distinct types of food with specific flavours the individuals may enjoy.

3.1.5. Occupational enrichment

The main goal of this type of enrichment is to physically and mentally stimulate the animals, by offering ways to perform physical exercise in order to maintain not only physical fitness, but overall good welfare. Excursive not only reduces stress levels, but is beneficial to overall health, has a positive impact on cognitive skills and memory and is also used to reduce anxiety and depression in humans, non-human primates and other animals (Hötting and Röder, 2013) (e.g. in mice, running wheels can mitigate anxiety, and in dogs, sessions of 25 minutes of exercise a day can reduce cortisol levels as well as aggressive behaviours (Menor-Campos *et al.* 2011).

Although further research on the effects of exercise on animals is needed, physical activity has been proved to improve immune responses, strengthen the muscular system –resulting in lower risk of cancer, diabetes and even digestive disorders- (Sullivan, 2008)

3.2. Considerations for non-human primates

The following considerations should be taken when providing enrichment for non-human primates:

- In arboreal species, enclosures should enable animals to utilize vertical dimensions, and should be designed to provide the animals with both an upper and a lower substrate.
- Active forms of enrichment devices should be placed in the enclosure in order to allow the animals to maintain physical condition through encouraging movement (e.g. perches, branches, car tyres, chains) (Varela, 2007; Wolfensohn, 2010)
- For species that do not sleep in groups in the wild, nest boxes should be placed in the enclosures, as they provide security and control to the animals (Buchanan-Smith, 2010).
- If offering toys as enrichment, a rotational changing scheme should be implemented as these devices may quickly lose novelty (Lutz and Novak, 2005; Wolfensohn, 2010).

- As wild primates species spend between a 25% to a 85% of their active time searching and foraging for food (Clutton-Brock and Harvy, 1977), food items should be provided in a way that increases the time spent foraging (e.g. entire fruits instead of chopped, frozen items, puzzle feeders etc.)
- When housing two or more animals, an appropriate enclosure size and structure (i.e. larger than usual housing) can decrease chances of aggression (Howell *et. al.* 1993).
- If animals cannot be housed socially (e.g. for experimental procedures or in situations of wound healing etc.), grooming panels can be used to allow tactile and olfactory contact between subjects (Coleman *et al.* 2013).

4. Reintroduction

Reintroduction is defined by the International Union for the Conservation of Nature [IUCN] as the "attempt to establish a species in an area which was once part of its historical range, but from which it has become extinct or has been extirpated". It has become an important tool in conservation programmes (Seddon *et al.,* 2007) not only because it can help re-establish populations and save endangered species from extinction (Robert, 2009), but also because it can also help balance an entire ecosystem.

A successful reintroduction programme is that which results in a self-sustaining population (Griffith *et al.,* 1989) (i.e. settlement into the area of release, successful reproduction and survival of the individuals). However, many of these programmes fail as a consequence of releasing animals with poor behavioural skills (Stoinski *et al., 2003;* McPhee and Silverman, 2004; Reading *et al,* 2013) so that they cannot overcome challenges faced in the wild, especially those associated with predation avoidance and identification of environmental cues (Miller *et al.,* 1990; Reading *et al.,* 2013). Therefore, and even though reintroduction success is also influenced by socio-economical, genetics, demographical and other factors, releasing animals that have the physiological and cognitive capacity to respond efficiently to these new environmental challenges is essential.

4.1 The role of enrichment in reintroduction success

As candidates for reintroduction are usually kept captive for a period of time before release, during this period the rehabilitation process should act as a training period to reinforce those behavioural traits that will help the animals cope better with the challenges, which will be faced upon release into the wild (Shepherdson, 1994). Thus, an animal to be released needs to be able to move in complex environments, construct nests or find sleeping sites, efficiently recognise and avoid predators, identify prey, be able to forage, identify, acquire and handle food, be part of the social structure of its own group, and be able to identify humans as a threat and avoid human contact (Derrickson and Snyder, 1992; Miller et al., 1996; Snyder et al., 1996; Griffin et al., 2000; McPhee, 2003; Stoinski *et al.*, 2003; Utt *et al.*, 2008).

Even though EE can be a powerful tool to improve the welfare of captive animals and to increase the expression of species-typical behaviour, not all EE techniques can be applied for reintroduction: most of them have only been designed to engage the animals in problem-solving challenges or activities, that even if could decrease boredom, stress levels, increase activity and reduce abnormal behaviours are not directed towards the improvement of specific skills needed to survive in the wild. Also, these EE do not work on improving the physical condition of the animals, nor promote the expression of species-typical behaviour (Shepherdson, 1994; McLean *et al.*, 1990).

If well applied, EE can generate opportunities to develop and/or increase survival skills, resulting in a great impact on the survival rates of the group or population reintroduced into the wild (Reading *et al.* 2013). An enrichment plan for animals that await reintroduction should not only be a way to provide welfare whilst in captivity, but also as a mechanism to maintain it after release (Swaisgood, 2010). Enrichment should enhance and develop skills that will result in higher survival rates, thus guaranteeing a good quality of life once returned to the wild. However, aspects of reintroduction, such as enhancement of foraging skills, social interactions, predation avoidance and physical fitness, are usually overlooked or seem difficult to provide when managing reintroduction candidates.

Predation avoidance. Predation represents one of the most important factors associated with high mortality rates after reintroduction (Reading *et al.*, 2013). Thus, avoiding predators is a basic skill any reintroduced individual should be able to perform efficiently (Miller *et al.*, 1990; Griffin *et al.*, 2000, Banks *et al.*, 2002). Sensory enrichment should be used in the form of presenting models of predators (e.g. predator‘s skin, smell, vocalization recordings etc.) at the same time as an aversive experience is presented to the animals. This has been widely used in enrichment programmes to increase predator avoidance skills prior to release (Griffin *et al.*, 2000; Varela, 2007). However, it is also important to consider humans as one of the greatest threats to most species, which is why rehabilitation programmes that work with populations usually involved in human-animal conflict, should also: 1. Include the presentation of aversive anthropocentric stimuli; and 2. Work out a plan with the least human interaction to avoid habituation (Miller *et al.*, 1996; Snyder and Snyder, 2000).

Foraging abilities. In reintroduction programmes, food should be provided in a form that would impose obstacles as similar as possible the those faced in nature to obtain resources (e.g. for species that forage on canopy, food should be provided on trees; rather than chopped, entire fruits/vegetables/prey should be used, etc.).

Although introducing EE devices such as puzzle feeders -which are commonly used in laboratories and other captive environments- can increase dexterity skills (Reading *et al.*, 2013), their use in a rehabilitation process should be accompanied by other EE techniques or devices that promote the species typical foraging behaviour in the wild (e.g. foraging boxes, climbing structures to obtain food in higher substrates for arboreal species that forage at canopy levels, etc.).

Physical fitness. While animals could be provided with EE that promotes the expression of natural behaviours and enhances survival skills, physical fitness also needs to be a goal, as some species may have to travel long distances in search of food and may require high muscle strength to climb or to efficiently forage or move through certain types of substrate, and even be fast enough to avoid predation or compete for resources. Therefore, EE presented to animals should be designed to encourage physical activity (see occupational enrichment section above), and prior to release, assessment of physical condition should be performed (Reading *et al.*, 2013).

Social interactions. One main problem in rehabilitation-reintroduction programmes is the difficulty of forming stable social groups, especially when working with a species with linear or complex hierarchical structures, which contribute to survival in the wild. As animals in reintroduction programmes do not have the option to choose their group members (i.e. there is typically a forced-group composition) it is essential to assess social interactions and provide social enrichment directed to facilitate the formation of cohesive social groups. However, in order to do so, the history of each animal, its temperament, sex and age need to be considered before selecting it as a member of a new group (see section 3.1.1. social enrichment).

5. Conclusion

Although the application of environmental enrichment techniques is a valuable tool for increasing the welfare of captive animals, there are many factors with detrimental effects on the quality of life in captivity, which need to be addressed altogether if welfare if to be maximised. Moreover, if EE techniques are to be used in reintroduction programmes, special attention needs to be paid to improving survival skills and physical condition, rather than simply providing animals with stress-free environments. By improving key skills through enrichment, would not only improve post-release welfare, but would also increase survival rates.

REFERENCES

Alberts, A.C., 2007. Behavioral considerations of headstarting as a conservation strategy for endangered Caribbean rock iguanas. Appl Animal Behav Sci 102, 380–391.

Apfelbacha, R., Blanchardbm C. D., Blanchardc, R. J., Hayesd, R.A., McGregor, I.S., 2005. The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci Biobehav R. 29, 1123–1144

Baker, K. C. (1996). Chimpanzees in single cages and small social groups: Effects of housing on behavior. Contemp Top Lab Anim Sci. 35, 71-74.

Banks, P.B., Norrdahl, K., Kropimäki, E., 2002. Mobility decisions and the predation risks of reintroduction. Biol Conserv 103, 133–138.

Bauman, V., Van Loo, P.L.P., 2012. How to improve housing conditions of lab animals. Vet J Press

Bernstein, I. S., 1991. Social housing of monkeys and apes: group formations. Lab Anim Sci. 41, 329-333.

Bigert, C., Bluhm, G., Theorell, T., 2005. Saliva Cortisol – a new approach in noise research to study stress effects.Int J Hyg Environ Health 208, 227-230.

Blanchard, R.J., Nikulina, J.N., Sakai, R.R., McKittrick, C., McEwen, B., Blanchard D.C., 1998. Behavioral and endocrine change following chronic predatory stress. Physiol Behav. 63, 561–9.

Bloomsmith, M.A., Brent, L.Y., Schapiro, S.J., 1991. Guidelines for developing and managing an environmental enrichment program for nonhuman primates. Lab Anim Sci. 41, 372–7.

Boere, V., 2001. Environmental enrichment for neotropical primates in captivity. *Cienc. Rural*. 3, 543-551

Boot, R., Leussink, A.B., Vlug, R.F., 1985. Influence of housing condition on pregnancy outcomes in cynomolgous monkeys (*Macaca fascicularis*). Lab Anim Sci 19, 42-47.

Broom, D.M., 2010. Welfare of animals: Behaviour as a Basis for Decisions. 2010. Encyclopedia of Animal Behaviour. pp. 580-584.

Buchanan-Smith, H.M., 2010. Marmosets and tamarins. In: UFAW Handbook on the Care and Management of Laboratory Animals, Eighth Ed. Wiley-Blackwell, Oxford, UK, pp. 543–563.

Carlstead, K., Shepherdson, D.J., 1994. Effects of environmental enrichment on reproduction. Zoo Biol 13, 447–458.

Carlstead, K.E., Brown, J.L., 2005. Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Zoo Biol. 24, 215–232.

Chance, M. R. A., Byrne, B., Jones, E., 1983. A tandem cage for handling group-living monkeys. Lab Anim. 17, 129-132.

Chu, L., Garner, J.P., Mench, J.A. 2004. A behavioral comparison of New Zealand white rabbits (Oryctolagus cuniculus) housed individually or in pairs in conventional laboratory cages. Appl Anim Behav Sci. 85, 121–39.

Clubb, R., Mason, G., 2003. Animal welfare: captivity effects on wide-ranging carnivores. Nature. 425, 473-474

Clubb, R., Mason, G., 2007. Natural behavioural biology as a risk factor in carnivore welfare: How analysing species differences could help zoos improve enclosures. Appl Anim Behav Sci. 102, 303-328.

Clutton-Brock, T. H., Harvey, P.H., 1977. Species differences in feeding and ranging behaviors in primates, In Primate Ecology: Studies of feeding and ranging behavior in lemurs, monkeys, and apes, T. H. Clutton-Brock, ed., Academic Press: New York, pp. 557-584.

Deep, A., Schwean-Lardner, K., Crowe, T.G., Classen, H.L., 2012. Effect of light intensity on broiler behaviour and diurnal rhythms. Appl Animal Behav Sci. 136, 50-56.

Derrickson SR, Snyder NFR., 1992. Potential and limits of captive breeding in parrot conservation. In: Beissinger SR, Snyder NFR, editors. New World parrots in crisis: solutions from conservation biology. Washington, DC: Smithsonian Institution Press. p 133–163.

Fray, R., 1998. Comparative psychoacoustics. Hearing Research. 34,295-306.

Forthman - Quick, D.L.,1984. An integrative approach to environmental engineering in zoos., Zoo Biol. 3, 65 -77

Gibson, Q. A., Howells, E. M., Lambert, J.D., Mazzoil, M. M., Richmond, J. P., 2013. The ranging patterns of female bottlenose dolphins with respect to reproductive status: Testing the concepto of nursery areas. J Exp Mar Biol Ecol. 445, 53-60.

Griffin, A.S., Blumstein, D.T., Evans, C.S., 2000. Training captive- bred or translocated animals to avoid predators. Conserv Biol. 14,1317–1326.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., 1989. Translocations as a species conservation tool: status and strategy. Science. 245, 477-480.

Hinton, D.E., Pich, V., Chhean, D., Pollack, M.H., barlow, D.H., 2004. Olfactory-triggered panic attacks among Cambodian refugees attending a psychiatric clinic. Gen Hosp Psychiatry. 26, 390–397.

Honess, P.E., Marin, C.M., 2006. Enrichment and aggression in primates. Neurosci Biobehav R. 30, 413–436.

Hötting, K., Röder, B., 2013. Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci Biobehav R. In press.

Howell, S., Matevia, M., Fritz, J., Nash, L., Maki, S., 1993. Pre-feeding agonism and seasonality in captive groups of chimpanzees *(Pan troglodytes).* Anim Welfare. 2, 153-163.

Hubrecht, R.C., Serpell, J.A., Poole, T.B., 1992. Correlates of pen size and housing conditions on the behaviour of kenneled dogs. Appl Anim Behav Sci. 34, 365–383.

Jildge, B., 1991. The rabbit - a diurnal or a nocturnal animal. J. Exp Anim Sci. 34,

170–183.

Jorgensen, M.J., Hazen TJ., 1998. Effects of puzzle feeders on pathological behavior in individually housed rhesus monkeys. Am J Primatol. 46, 213–27.

Kitchen, A.M., Martin, A.A., 1996. The effects of cage size and complexity on the behaviour of captive common marmosets, Callithrix jacchus jacchus. Lab Anim 30, 317-326.

Line, S.W., Morgan, Kathleen N, Markowitz H, Markowitz H., 1991. Simple toys do not alter the behavior of aged rhesus monkeys. Zoo Biol. 10, 473-484.

Lutz, C.K., Novak, M.A., 2005. Environmental enrichment for non-human primates: theory and application. ILAR Journal 46, 178-191.

Maclean, J.M., Phippard, A.F., Garner, M.G., Ouggin, G.G., Howath, J.S., Tiller, D.J., 1987. Group housing of hamadryas baboons: a new cage design based upon field studies of social organization. Lab Anim Sci. 37, 89-93.

Majumder, A., Basu, S., Qureshi, Q., Jhala, Y., Nigam, P., Gopal, R., 2012. Home ranges of the radio-collared Bengal tigers (*Panthera tigris tigris*) in Pench tiger Reserve, madhya pradesh, Central India. Wildl Biol Pract. 8, 36-49.

McPhee, M.E., 2003. Generations in captivity increases behavioural variance: considerations for captive breeding and reintroduction programs. Conserv Biol. 115, 71-77.

McPhee ME, Silverman ED. 2004. Increased behavioral variation and the calculation of release numbers for reintroduction programs. Conserv Biol 18, 705–715.

Mellen, J.D., Ellis, S., 1996. Animal learning and husbandry training. In: Kleinman,

D.G., Allen, M.E., Thompson, K.V ., Lumpkin, S., and Harris, H. (eds). 1996. Wild Mammals in Captivity: Principles and Techniques. Chicago: Univ. of Chicago Press. Pp. 88-99.

Menor-Campos, D.J., Molleda-Carbonell, J.M., Lopez-Rodríguez, R., 2011. Effects of exercise and human contact on animal welfare in a dog shelter. Vet Rec. 169, 388.

Miller, B., Biggins, D., Wemmer, C., *et al*., 1990. Development of survival skills in captive raised Siberian polecats (*Mustela eversmanni*) I. Locating Prey. J Ethol. 8, 89–94.

Miller, B.J., Reading, R.P., Forrest, S., 1996. Prairie night: black- footed ferrets and the recovery of endangered species. Washington, DC: Smithsonian Press.

Moberg, G.P., 2000. Biological response to stress: implications for Animal Welfare. In: Moberg GP and Mench JA (eds) The Biology of Animal Stress. 3-8.

Morgan, K.N., Tromborg, C.T., 2006. Sources of stress in captivity. Appl Anim Behav Sci. 102 , 262-302.

Novak, C.M., Burghardt, P.R., Levine, J.A., 2012. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci. Biobehav. Rev. 36, 1001–1014.

Owen, M.A., Swaisgood, R.R., Czekala, N.M., Stein-man, K., Lindburg, D.G., 2004. Monitoring stress in captive giant pandas (Ailuropoda melanoleuca): behavioral and hormonal responses to ambient noise. Zoo Biol. 23, 147–64

Palm, R., 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim Welfare. 21, 331-337.

Redpath., S.M., 1995. Habitat fragmentation and the individual: tawny owls *Strix aluco* in woodland patches. J Anim Ecol. 64, 652–661.

Robert, A., *2009*. Captive breeding genetics and reintroduction success. Biol Conserv.142, 2915-2922

Santangeli, A., Hakkarainen, H., Laaksonen, T., Korpimaki, E., 2012. Home range size is determined by habitat composition but feeding rate by food availability in male Tengmalm’s owls. Anim Behav. 83, 115-1123.

Seddon, P., Armstrong, D.G., Maloney, R.F., 2007. Developing the Science of Reintroduction Biology. Conserv Biol. 21, 303–312

Sha, M., Hanya, G., 2013. Diet, Activity, Habitat Use, and Ranging of Two Neighboring Groups of Food Enhanced LongTailed Macaques (*Macaca fascicularis*).

Am J Primatol. 75, 581–592.

Shepherdson, D.J., 1994. The role of environmental enrichment in the captive breeding and reintroduction of endangered species. In: Mace GM, Onley PJS, Feistner ATC, editors. Creative conservation. London, UK: Chapman and Hall. p 167–177.

Shepherdson, D.J., 1998. Tracing the path of environmental enrichment in zoos. In: Shepherdson, D.J., Mellen, J.D., Hutchins, M. (Eds.), Second Nature: Environmental Enrichment for Captive Animals. Smithsonian University Press, Washington, DC, pp. 1–12.

Shier DM, Owings, D.H., 2006. Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). Biol Conserv. 132, 126–135.

Shyne, A., 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biol. 25, 317–337.

Snyder, N., Snyder, H., 2000. The California condor: a saga of natural history and conservation. San Diego, CA: Academic Press.

Snyder, N.R.F., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D., Miller, B., 1996. Limitations of captive breeding in endangered species recovery. Conserv Biol 10, 338–348.

Soto-Moyano, R., Fernandez, V., Sanhueza, M., Belmar, J., Kusch, C., Perez, H., Ruiz, S., Hernandez, A., 1999. Effects of mild protein prenatal malnutrition and subsequent postnatal nutritional rehabilitation on noradrenaline reléase and neuronal density in the rat occipital cortex. Dev Brain Res. 116, 51-58.

Spironello, W.R., 2001. The brown capuchin monkey (*Cebus apella*): ecology and home range requirements in central Amazonia. In: Bierregaard RO, Gascon C, Lovejoy TE, Mesquita CG, editors. Lessons from Amazonia: the ecology and conservation of a fragmented forest. New Haven:Yale U Press. p 271-83.

Spreng, M., 2000. Possible health effects of noise induced cortisol increase. Noise Health 7, 59-63.

Stan, L., Lindstedt, B., Miller, J., Steven, W., 1986. Home Range, Time, and Body Size in Mammals. Ecology. 67, 413-418.

Stoinski, T.S., Beck, B.B., Bloomsmith, M.A., Maple, T.L., 2003. A behavioral comparison of captive- born, reintroduced golden lion tamarins and their wild- born offspring. Behavior. 140, 137–160.

Sullivan K. Get fit with your dog. Ivy Press Limited, 2008. Vol. 1.

Swaisgood, R.R., 2010. The conservation- welfare nexus in reintroduction programmes: a role for sensory ecology. Anim Welf. 19, 125– 137.

Tromborg, C.T., 1993. Behavioral effects of exposing captive cotton top tamarins to controlled auditory stimuli. Unpublished Master's Thesis, California State University, San Franscisco.

Reading, R.P., Miller, B., Shepherdson, D., 2013. The Value of Enrichment to Reintroduction Success. Zoo Biol. 32, 332–341.

Reinhardt, V., 1997. Refining the traditional housing and handling of laboratory rhesus macaques improves scientific methodology. Primate Report. 49:93–112.

Reinhardt, V., C. Liss, and C. Stevens, 1995. Social housing of previously single-caged macaques: What are the options and the risks? Anim Welfare. 4, 307-328.

Reinhardt, V., 1991. Group formation of previously single-caged adult rhesus macaqes for the purpose of environmental enrichment. J Exp Anim Sci. 34, 110-115.

Rupp, H., 1999. Excess catecholamine syndrome. Pathophysiology and therapy. Ann NY Acad Sci. 881, 430–444.

Utt, A.C., Harvey, N.C., Hayes, W.K., Carter, R.L., 2008. The effects of rearing method on social behaviors of mentored, captive- reared juvenile California condors. Zoo Biol. 27, 1–18.

van Hoof, J., Aarts, M.P.J., Rense, C.G., Schoutens, A.M.C., 2008. Ambient bright light in dementia: Effects on behaviour and circadian rhythmicity. Build Environ. In press.

Varela, N., 2007. Bases para el Manejo y Atención Médico Veterinaria y Rehabilitación de Pequeños Primates Neotropicales. 2da Ed. Corporación Autónoma Regional de Caldas – Asociación de Veterinarios de Vida Silvestre. 56.

Vasilieva, N.Y., Cherepanova, E.V., von Holst, D., Apfelbach, R., 2000. Predator odour and its impact on male fertility and reproduction in Phodopus campbelli hamsters. Naturwissenschaften. 87, 312-314.

Waser, P.M., Brown, C.H., 1986. Habitat acoustics and primate communication. Am. J. Primatol. 10, 135–154.

Waynert, D.F., Stookey, J.M., Schwartzkopf-Genswein, K.S., Watts, J.M., Waltz, C.S.,

1999. The response of beef cattle to noise during handling. Appl Anim behav Sci. 62, 27-42.

Wolfensohn, S., 2010. Old World primates. In: UFAW Handbook on the Care and Management of Laboratory Animals, Eighth Ed. Wiley-Blackwell, Oxford, UK, pp. 593–617.

Wong, S.T., Servheen, C.W., Ambu, L., 2004. Home range, movement and activity patterns, and bedding sites of Malayan sun bears *Helarctos malayanus* in the Rainforest of Borneo. Biol Conserv. 119, 169-181.

WEB REFERENCES

Chanaud, R.C., 2006. Noise Ordinances. Tools for Enactment, Modification and Enforcement of a Community Noise Ordinance. On-line. Retrieved on 8/12/13 from http://www.noisefree.org/Noise-Ordinance-Manual.pdf

IUCN. 1987. Translocation of living organisms: introductions, reintroductions, and restocking. IUCN Position Statement. Gland, Switzerland. On-line. Retrieved on 8/13/13 from http://intranet.iucn.org/webfiles/doc/SSC/SSCwebsite/Policy_statements/IUCN_Position_Statement_on_Translocation_of_Living_Organisms.pdf

The effect of three different foraging devices on the behaviour of long-tailed macaques (*Macaca fascicularis*) in rehabilitation for reintroduction.

Wendy Gomez Rubio

The Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin EH25 9RG, UK

Abstract

Although extensive literature on how environmental enrichment improves the welfare of captive animals can be found, its potential key role to increase reintroduction success rates by promoting species-typical behaviours and enhance specific skills needed to survive in the wild is still poor documented. This study investigated the effect of three different feeding-enrichment devices (one puzzle feeder and two different foraging boxes: Foraging Box1 and Foraging Box2) on the activity budgets of thirteen long-tailed macaques (*Macaca fascicularis*) undergoing rehabilitation for reintroduction. It also aimed to identify whether or not there was an effect of age and sex on these behavioural responses to enrichment.

An A-B-A experimental design (Pre, During and Post enrichment) was followed, and all three enrichments were low-cost, made of natural materials and designed to target foraging behaviour. Although different individuals exhibited different preferences between treatments, during the enrichment phase the most manipulated device for all thirteen subjects was the Foraging Box 1 ($p<0.001$), and higher levels of foraging and lower levels of inactivity were observed with the introduction of the Foraging Box2 ($p<0.001$). Significantly higher levels of locomotion ($p=0.028$) and foraging behaviour ($p=0.002$), as well as decreased inactivity levels ($p=0.007$) were seen in the Post-enrichment phase compared to baseline observations (Pre-enrichment phase). Also -compared with juveniles- adult individuals showed higher levels of inactivity during the entire period of the study, and higher levels of negative interactions as well. This study shows that low-cost environmental enrichment can be used in rehabilitation programmes for reintroduction to promote foraging and to promote a more species-typical behaviour; and also that individual differences need to be taken into

consideration in order to properly design and implement an enrichment programme that can achieve behavioural goals for all the individuals before release.

Kew words: Reintroduction, rehabilitation, environmental enrichment, feeding enrichment, long-tailed macaques.

1. Introduction

The expression and maintenance of species-typical behaviours is considered an indicative of welfare in captive animals, and a key-step in rehabilitation and reintroduction programmes (Redshaw and Mallinson, 1991; Shepherdson, 1994; Little and Sommer, 2002; Hosey 2005; Seddon *et al.,* 2007; Laule and Whittaker, 2007). The use of Environmental Enrichment (EE) reduces the amount of time in which animals are inactive (Hones and Marine, 2006) and promotes the development of these natural behaviours, also reducing and/or eliminating abnormal ones, such as stereotypies and self-harming. It is also of great importance in rehabilitation programmes for reintroduction since these techniques can be directed towards the enhancement of specific behavioural traits or skills that influence reintroduction success (e.g. locomotion, home construction, predator avoidance, social group interactions and foraging behaviours) (Stoinski *et al*., 2003; Alberts, 2007; Utt *et al*., 2008; Miller *et al.*, 1996). Since reintroducing individuals that do not have these behavioural skills, high mortality rates can be expected (Griffin *et al*., 2000; Shier and Owings, 2006), it is of great importance to not only provide the animals in rehabilitation programmes with proper enrichment devices that promote the expression of a more species-specific behavioural repertoire, but also with devices that improve those natural skills. However, in order to achieve this, the implementation of EE must be accompanied by extensive knowledge of the habitat, ecology and social interactions of the species to be rehabilitated (Little and Sommer, 2002; Hosey, 2005), as well as of the main threats their populations face in the wild.

In the case of non-human primates, since some species can spend up to 85% of their active time on feeding-related behaviours (Clutton-Brock and Harvy, 1977), the enhancement of foraging skills should be of great importance when designing an enrichment programme for reintroduction. This is especially important if working with subjects that have spent years in captivity and have never had the chance to forage, as is the case of many primates victims of the pet trade and that end up in rehabilitation programmes.

The objective of this study was to analyse the effects of the introduction of 3 different low-cost feeding-enrichment devices on the activity budgets of 3 groups of long-tailed macaques undergoing rehabilitation prior to release. Three hypotheses were investigated:

H_1: the introduction of feeding-enrichment devices induces a change in Foraging behaviour towards a wild-type activity budget.

H_2: the introduction of different foraging-enrichment devices induces different changes in behavioural activity budgets.

H_3: sex and age of individuals have an effect on behavioural responses to enrichment devices.

2. Methods

2. 1 Ethical statement

The present study was approved by the Veterinary Ethical Review Committee of the University of Edinburgh. The study was non-invasive and required minimal animal contact and no animal-handling. Permission to carry out the research was granted by the Government of Indonesia, with Dr. Rondang Srinagar as scientific counterpart.

2.2 Location and Period of Study

Research was conducted at the International Animal Rescue Centre in Curug Nangka, Indonesia between 15th March 2013 and 31st May 2013.

2.3 Subjects of study

Thirteen (13) long-tailed macaques (*Macaca fascicularis*) belonging to 3 social groups were used in this study. Six of the thirteen animals were males and seven were females. All animals were assessed as physically healthy by the IAR Centre Medical staff, and age of each individual was determined based on body size, fur colour and dentition.

Group No. 1 consisted of 1 male adult, 1 female adult, 1 female juvenile and 1 male juvenile.

Group No. 2 consisted of 2 male adults, 1 adult female, 1 female juvenile and 1 male juvenile.

Group No. 3 consisted of 2 male adults and 2 female adults.

2.4 Housing

Macaques were housed in a block of 6 cages, each one measuring approximately 3m x 2.5m x 3m and allowing tactile, olfactory and visual contact with the contiguous section. Each cage had some form of environmental enrichment that consisted of branches, bamboo feeders, tyre swings, multilevel perches and hessian sacks. Every group was transferred to an adjacent enclosure every morning between 8am and 10am, taking a total of 6 days for all the groups to go through all the sections/cages of the block.

Diet consisted of a variety of vegetables, fruit and seeds, and food was provided 7 times a day between 7am and 4pm. For every single feeding event, food was scattered on the roof of the cage, and animals were provided with *ad libitum* access to water throughout the day.

2.5 Pilot study and development of ethogram

A pilot study was conducted for 10 days before the main study in order to identify all individual subjects, allow them to become accustomed to the presence of the observer, test sampling procedures and qualitatively determine the type of enrichment devices that would be introduced and tested in the main study. One person recorded all observations for this and the main study in order to avoid observer-biased results.

Table 1. Ethogram of behaviours recorded in this study.

Behaviour	Description
Play	Non-aggressive wrestling, chasing, jumping.
Grooming	Engaging in bout of touches focussed on another animal's coat to remove debris.
Self-grooming	Engaging in bout of touches focussed on own coat to remove debris.
Resting	Staying immobile in absence of other behaviours listed here. Includes sleeping, laying down.
Aggression	Aggressive signals or displays towards a member of the group or towards a member of other group. Includes chasing, slapping, fighting, biting,
Aggression avoidance	Moving away from another individual following an threat or aggressive signal
Vigilance	Scanning surroundings or contiguous cage with the head up.
Locomotion	Running, walking, climbing
Feeding	Consuming food with or without manipulation of it. Includes swallowing food from pouch and chewing.
Foraging	Manipulating substrate or objects in the enclosure, resulting in obtainment of food. Includes periodic transfer of food from substrate to mouth and locomotion of the animals around an area of the enclosure while manipulating substrate.
Masturbation	Rubbing genitals with parts of its own body (e.g. hands, fingers, mouth)
Stereotyping	Repetition of movements, postures, vocalizations. Includes finger sucking, head swing, rocking, pacing, head swing and head circling.
Use of Previous Enrichment	Manipulating any of the three feeding-enrichment devices permanently available in the enclosures (i.e. prior to beginning of study; e.g. puzzle feeder, bamboo feeder, bamboo feeder 2)
Use of Enrichment	Directly manipulating the device or any item that had been previously place inside. It also included foraging behaviour at an arm's length away from the enrichment.

In order to facilitate data analysis, Masturbation, Stereotyping, Self-grooming, Aggression Avoidance and Aggressive behaviours were grouped as Negative Interactions. Play and Grooming behaviours were grouped as Positive Interactions.

2.6 Design and description of enrichment devices (treatments)

Design of two out of the three treatments evaluated in this study was based on already existing devices used at IAR. Whilst these previous enrichments were already being used, their efficacy had not been assessed yet, and also some room for refinement was noticeable. The two selected enrichment devices to modify were a puzzle feeder and a foraging box:

Previous Foraging Box: Fig. 1. the box was made of wood and was filled with leaves, seeds and/or insects. One box per enclosure was provided.

Fig. 1. Previous Foraging Box used at IAR.

Foraging Box used for study (Foraging Box 1 – FB1): Fig 2, Fig 3. In order to make the device big enough to allow its use to more than one animal and to also increase the foraging area, a new wider box was designed. Dimensions of the box were 1m x 0.7 x 0.3m. Also, in order to increase complexity of foraging and increase foraging time, the bottom of the box was covered with a layer of soil, a layer of dry and green leaves. Rocks, coconut shells, chopped fruit, insects and seeds were put inside in between the layers, and wooden bars were also attached on the surface of the box so manipulation of the items inside could not be so easy for the animals.

Fig. 2. Foraging Box 1 designed for study. Images of FB1 already prepared to be used for the animals.

Fig. 3. Left: crickets, seeds, and chopped fruit before being placed inside the box. Right: a group of long-tailed macaques using the device.

Previous Puzzle Feeder: Fig. 4. Made of 3 bamboo branches that were put together. Each branch had a hole along its side that was then covered with wire. Seeds were placed inside each branch so the animals would have to shake and/or manipulate the puzzle feeder in order to obtain food.

Fig. 4. Previous Puzzle Feeder used at IAR

Puzzle Feeder suggested for study: Fig. 5 a,b. Green and dry leaves were put inside the device along with thin sticks and small stones. Chopped fruit would be put inside the feeder and would be visible to the animal. However, in order to promote more complex manipulation of the device and increase time spent on this manipulation, the animal had to insert its fingers and move the items inside to obtain the food. For this, the wire used to cover the compartments had big holes on some sections.

Fig. 5a. Puzzle Feeder designed for study. Left: device before being filled with food items and leaves. Right: Chopped fruit, seeds and leaves being introduced inside the device.

Fig. 5b. Long-tailed macaque using the Puzzle Feeder

The third enrichment device was a second Foraging Box *(<u>Foraging Box 2 – FB2)</u>:*

The box consisted of approximately 40 sections/compartments, filled with different amounts of dry and green leaves, seeds, chopped fruit and insects. The most important difference with the FB1 was the position at which it was placed: whereas FB1 was put on the ground inside the enclosure -and would presumably not require major strength for its manipulation-, FB2 was placed on the outer side of the cage and at a height not less than 1.5 meters from the ground, implicating that the animals would have to invest energy to hold onto the cage whilst manipulating the items inside the box in order to obtain food.

Fig. 6. Foraging Box 2 designed for the study. Left: FB2 already filled with foliage, seeds, chopped fruit and insects. Right: IAR keepers placing the device on the outer side of the enclosure.

Fig. 7. Long-tailed macaque using the Foraging Box 2. Animals had to climb the side of the enclosure to have access to the device.

In order to allow all the animals in each group to use the enrichment devices and reduce the probability of aggressive displays due to competition, when provided, each enclosure would count with two Foraging Boxes2, two Foraging Boxes1, and 5 Puzzle Feeders. This number of devices per cage was established during the pilot study.

2.7 Experimental design and data collection

The study was conducted employing an A-B-A experimental design, having 3 phases:

Phase 1: pre enrichment. No modifications of the housing enclosures were made. Behavioural data were collected over 12 days, and observations were randomized daily between 9am and 4pm, using instantaneous scan-sampling at 30-seconds intervals for periods of time of 30 to 50 minutes, depending on weather conditions. The aim of this phase was to identify time-activity budgets.

Phase 2: introduction of feeding enrichment devices. Diet and amount of food given to the animals remained unchanged, but once a day (out of 7 feeding events) food was offered using one of the three enrichment devices: 1. Foraging Box placed on the ground, 2. Foraging Box placed on the side of the cage, or 3. Puzzle Feeder. Data were collected over 20 days, 4 hours per day for all the groups, using the same sampling method of Phase 1. A randomized design was used to test the 3 different enrichment devices per day (see Appendix A). Observations were made 50 minutes right after the introduction of the devices (between 9 am and 2 pm), and then again 1 to 3 hours later for another 50 minutes.

Phase 3: post-enrichment stage. In this stage, enrichment was not provided, and no modifications to the cages or routine of the animals were made. Data collection took approximately 10 days under same conditions of Phase 1.

2.8 Statistical analysis

Data were analysed using MINITAB Statistical software. As descriptive statistics showed not-normally distributed data, a log10 transformation was used so statistical tests could be applied thereafter. Once normalized, effects of age and sex on behaviour were analysed using a General Linear Model. Paired t-test was used to analyse changes in activity budgets between Phase 1 and Phase 3 of the Study.

For Phase 2 of the study (Enrichment Phase), One-way ANOVA analysis were applied to ascertain differences in treatments and to determine effects of timing on behaviour for each device. Pearson test was performed to identify whether or not there was a correlation between the use of enrichment and other sets of behaviours recorded, and Two-way ANOVA analysis were performed to identify behavioural differences between individuals.

Threshold levels to establish statistical significance was set at $p<0.05$ for all analyses.

3. Results

3.1 Time activity budgets Pre, During and Post enrichment.

Overall activity budgets for all 13 individuals are shown in figure 8.

For the Enrichment Phase, Inactivity was reduced in a 16%, Negative behaviours reduced in a 5%, Foraging and Locomotion behaviours increased in an 11% and a 5% respectively, whereas Positive behaviours increased only by 1%.

For the Post-enrichment phase, time spent on Foraging and Feeding behaviours decreased in a 5% and a 6%, but still were double those of baseline observations. Time spent inactive increased 4% compared to the Enrichment Phase, but was lower than time observed in Phase 1 (12% and 34% respectively). A sample t-test analysis showed a significant increase in Foraging behaviour between Pre and Post enrichment phases ($T=4.00$, d.f.= 1; $p=0.002$), a significant reduction in Inactivity ($T= 3.21$,d.f.= 1; $p=0.007$), and a significant increase in Locomotion as well ($T=2.51$; d.f.=1; $p=0.028$). Changes in negative interactions, positive interactions, feeding and vigilance behaviours were not significantly different between Phase 1 and Phase 3 (i.e. Pre and Post enrichment) of the study.

Between Phase 1 and 2, one-way Anova analysis showed significantly higher foraging activity in Phase 2 ($F=4.3$; d.f.=1; $p=0.041$) as well as a significant decrease in Inactivity ($F=19.67$; d.f.=1; $p<0.001$). For all other behaviours (see **Fig. 9**), these changes were not statistically significant.

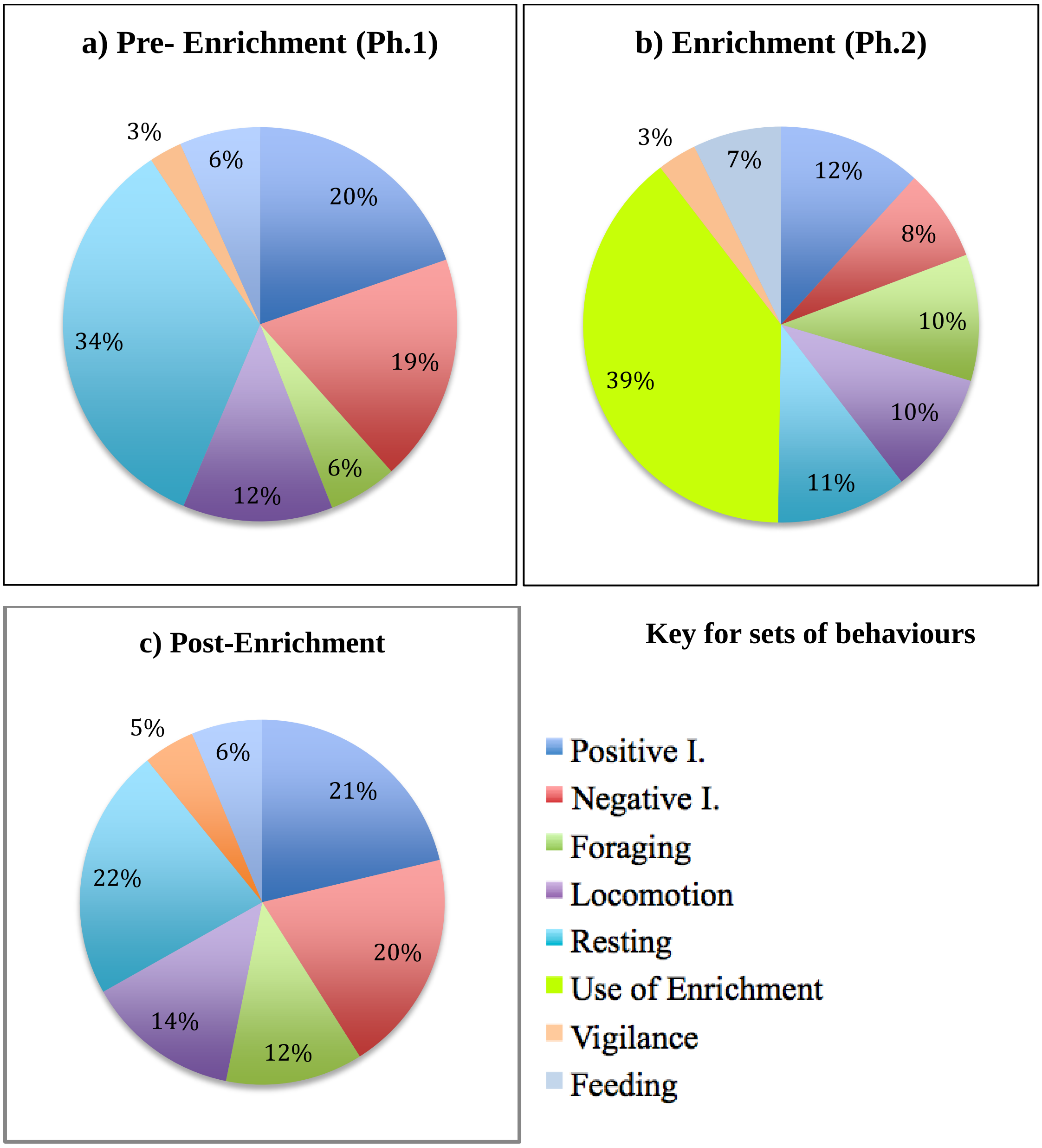

Fig. 8. Average Activity Budget during study phases. a) Phase 1 = Pre-Enrichment; b) Phase 2 = During Enrichment; c) Phase 3 = Post-Enrichment.

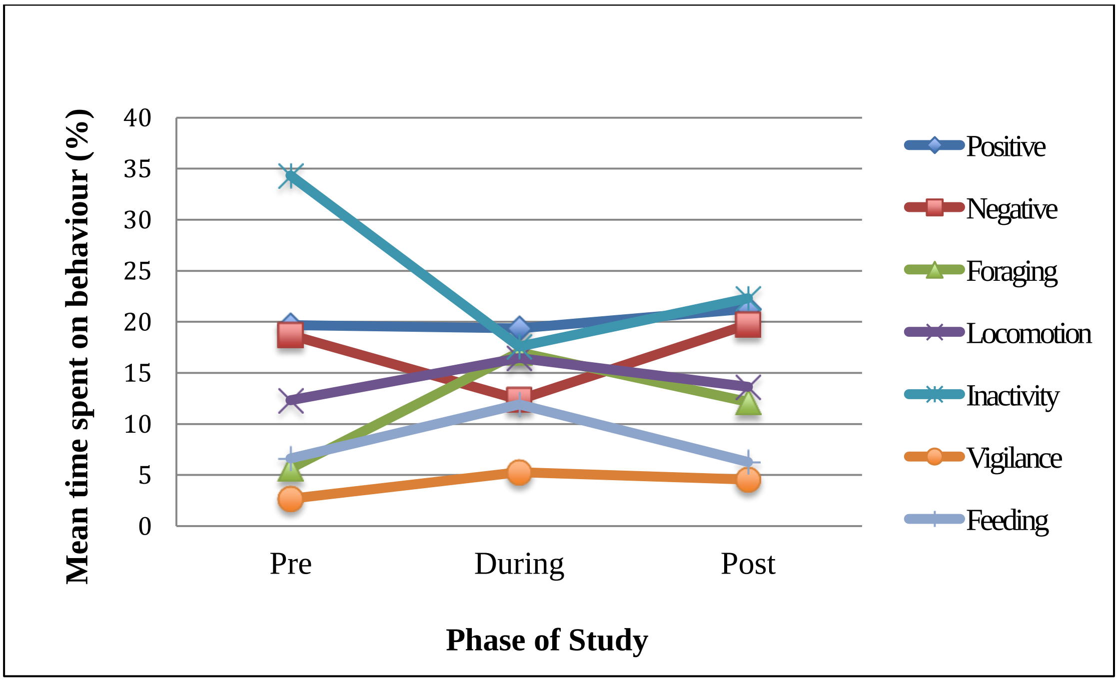

Fig. 9. Changes in Percentage time spent on each behaviour across phases (Pre-enrichment, During enrichment, After enrichment)

3.2 Effects of age and sex on behavioural changes across phases

As described in table 2, Age had a significant effect on all sets of behaviour, except on Foraging and Vigilance. Juveniles showed higher levels of Positive Interactions and Locomotion for all three phases and higher levels of Feeding behaviour in Phase 1 and 3 of the study. On the other hand, adults exhibited higher levels of inactivity for all three phases and also higher levels of Negative Interactions in all three phases as well (Fig10).

Significant effects of gender (i.e. female or male) were only found in locomotion.

Table 2. Results of GLM tests for effect of Age on behaviour changes across all three phases. Bold indicates significant result. Direction of effect can be observed in Figures 10 and 11.

Behaviour	F- test	Degrees of Freedom	Significance
Positive	11.73	1	**0.001**
Negative	12.70	1	**0.001**
Foraging	0.02	1	0.899
Locomotion	13.79	1	**<0.001**
Resting	9.20	1	**0.003**
Vigilance	5.53	1	0.818
Feeding	4.24	1	**0.042**

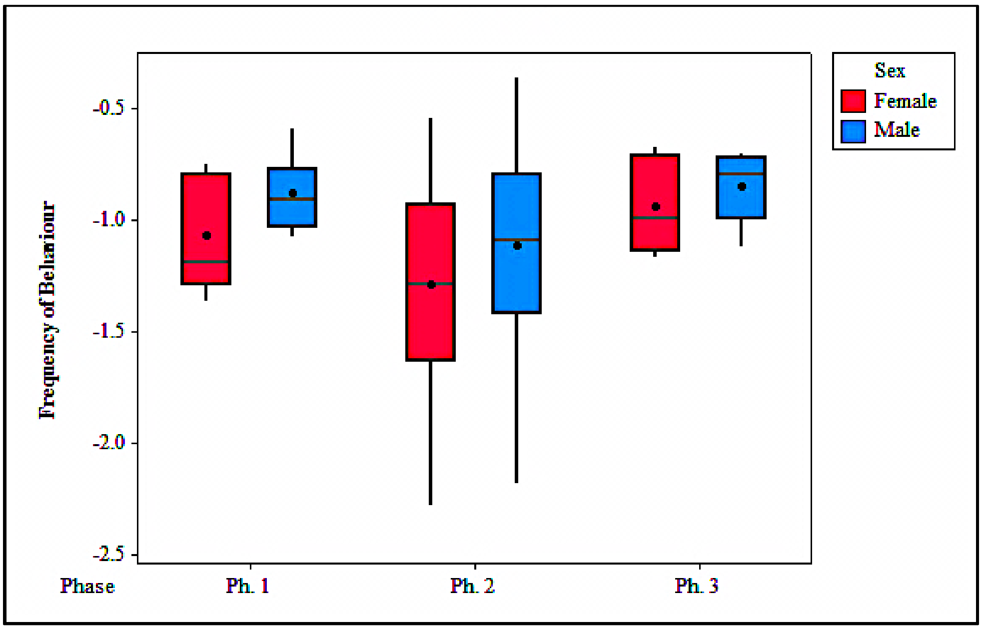

Fig. 10. Differences between female and male individuals in levels of locomotion for the three phases of the study. Ph. 1 = Pre- enrichment; Ph.2= Enrichment; Ph.3 = Post-enrichment.

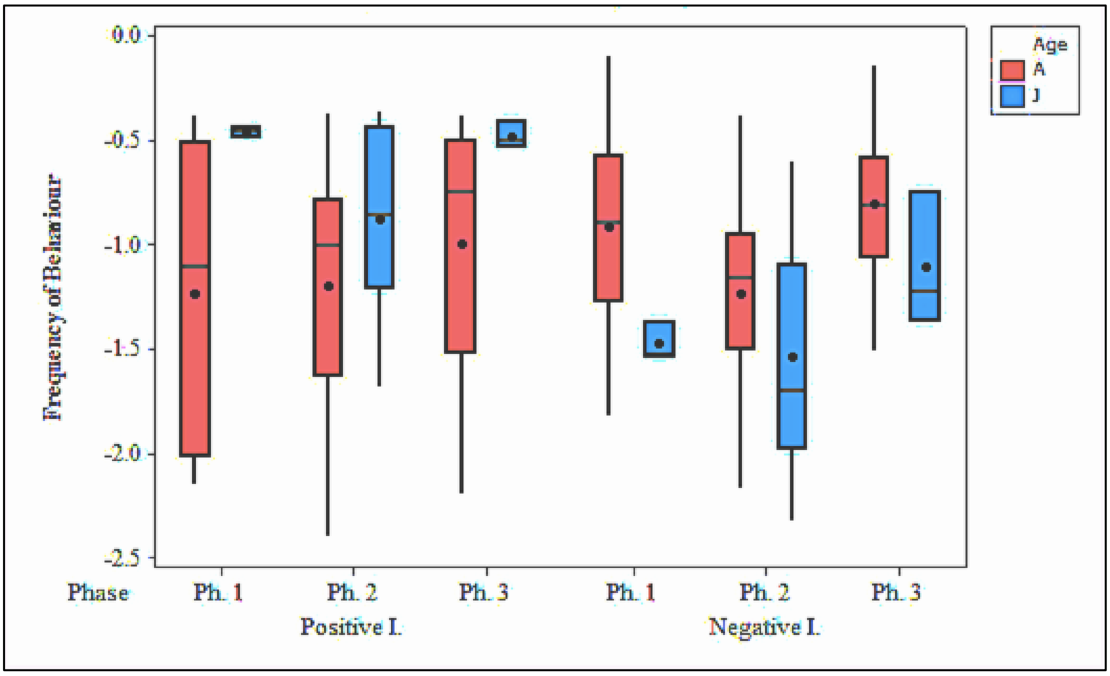

Fig. 11. Differences observed between juvenile and adult individuals on Positive and Negative Interactions for the three different phases of the study. A=Adults; J= Juveniles.

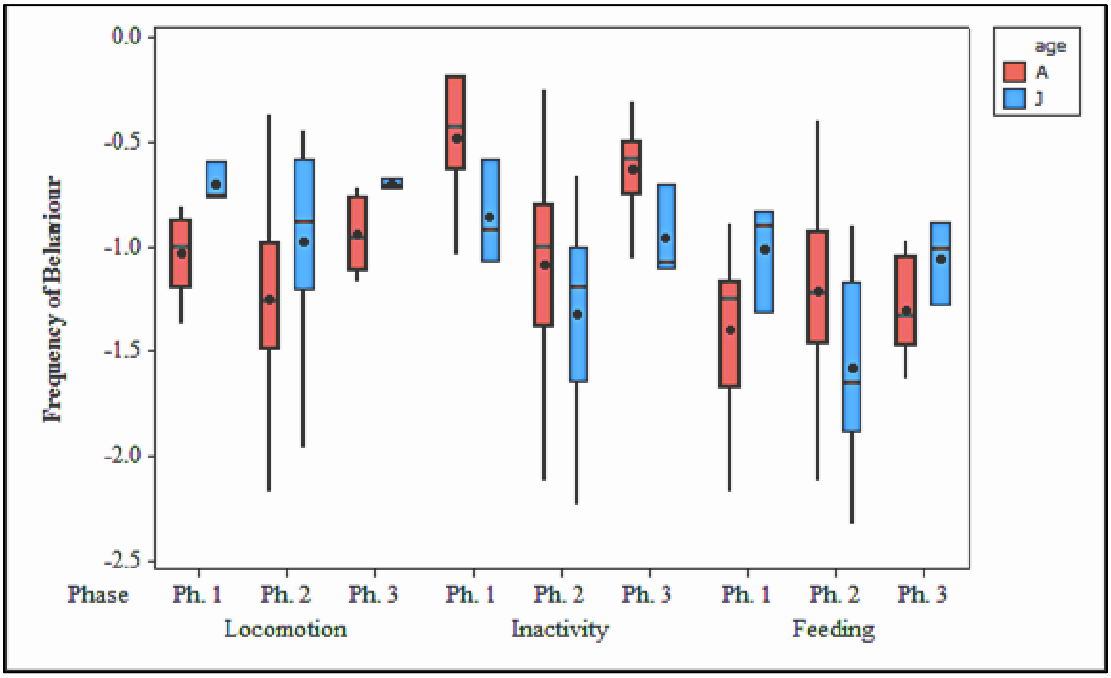

Fig. 12. Differences observed between Juvenile and Adult individuals in Locomotion, Inactivity and Feeding behaviours during the three different phases of the study. A=Adults, J= Juveniles

3.3 Behavioural responses to different enrichment types (Phase 2)

For this phase of the study, analysis for all sets of behaviours showed statistically significant differences between treatments and timing (i.e. a first observation right after the introduction of the device and a second observation 1-3 hours later. See methods section, Phase 2 of Study), except for Feeding-related behaviours. Direction of the responses of the treatments with Post-Hoc pairwise comparisons (Tukey-Kramer test), as well as a more detailed description of the results are shown as follows and can also be seen in fig. 13**.** :

Foraging behaviour ($F=5.79$; d.f.=5; $p<0.001$)
Time spent Foraging was significantly higher during and after the introduction of the Foraging Box 2, than when the Puzzle Feeder or Foraging Box 1 were present. Foraging time was not significantly different between Puzzle Feeder and Foraging Box 1 (when introduced and after introduction of the devices) (Table 3).

Inactivity, ($F=3.19$; d.f.= 5; $p = 0.012$).
Right after its introduction, Foraging Box2 showed significantly lower levels of inactivity, compared to the ones observed with the other treatments, with the exception of the Puzzle Feeder after its introduction, which has the highest mean of time spent inactive (Table 4).

Positive Interactions, ($F= 4.83$; d.f.=5; $p=0.001$).
Positive interactions were higher 1-3 hours after the introduction of the Foraging Box1, the Puzzle Feeder and the Foraging Box2, compared to the levels right after the introduction of all three treatments. However, significantly lower levels of Positive Interactions happened during the introduction of the Foraging Box2, compared to the other treatments and timings. (table 5)

Negative Interactions, ($F=5.51$; d.f.=5; $p<0.001$).
These interactions increased the most 1-3 hours after the introduction of the Foraging Box2, followed by the other two treatments, also 1-3 hours after their introduction.

However, a statistically significant difference was only seen between the Foraging Box2 and the other three treatments right after introduction (table 6).

Locomotion, ($F=5.97$; d.f.=5; $p<0.001$).

Individuals showed major locomotion 1-3 hours after the introduction of the Foraging Box2. These levels of locomotion were not significantly different from the ones observed after the introduction of the Foraging Box1 and the Puzzle Feeder (1-3 hours after as well), but were significantly higher than the ones observed for the other treatments right after introduction. (table 7)

Vigilance Behaviour, ($F=7.29$, d.f.=5; $p<0.001$).

Vigilance behaviour increased the most 1-3 hours after the introduction of the three enrichments and during the introduction of the Foraging Box2, compared to the increase right after the introduction of the other 2 treatments (Puzzle Feeder and Foraging Box1). (table 8)

Use of Enrichment ($F=4.95$; d.f.=5; $p=0.001$)

Right after its introduction, Foraging Box1 showed the highest manipulation by the animals, followed by the Foraging Box2 and Puzzle Feeder, also right after their introduction. The least manipulated enrichment device was the Puzzle Feeder, and the Foraging Box –both 1-3 hours after their introduction. (table 9)

Tables of Post-Hoc (Tukey-Kramer procedure) comparisons (*D* for all the enrichments refers to the first hour of observation right after the introduction of the enrichment. *A* refers to observations made 1 to 3 hours after the introduction of the device):

Table 3. Multiple pairwise comparisons using the Tukey–Kramer procedure for Foraging Behaviour. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box2 - During	-0.8153	A
Foraging Box2 – After	-0.9959	AB
Puzzle Feeder – After	-1.1501	ABC
Puzzle Feeder – During	-1.2948	BC
Foraging Box1 – After	-1.2958	BC
Foraging Box1 – During	-1.4971	C

Table 4. Multiple pairwise comparisons using the Tukey–Kramer procedure for time spent Inactive. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Puzzle Feeder – After	-0.8711	A
Foraging Box1 – After	-1.0469	AB
Puzzle Feeder – During	-1.0793	AB
Foraging Box2 – After	-1.0948	AB
Foraging Box1 – During	-1.3116	AB
Foraging Box2 - During	-1.4579	B

Table 5. Multiple pairwise comparisons using the Tukey–Kramer procedure for Positive Interactions. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box1 – After	-0.8522	A
Puzzle Feeder – After	-0.8737	A
Foraging Box2 – After	-0.8898	A
Puzzle Feeder – During	-1.2784	AB

Puzzle Feeder – During	-1.3041	AB
Foraging Box2 – During	-1.5585	B

Table 6. Multiple pairwise comparisons using the Tukey–Kramer procedure for Negative Interactions. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box2 – After	-0.8347	A
Foraging Box1 – After	-1-2320	AB
Puzzle Feeder – After	-1.2597	AB
Foraging Box1 – During	-1.3957	B
Foraging Box2 – During	-1.5038	B
Puzzle Feeder – During	-1.6115	B

Table 7. Multiple pairwise comparisons using the Tukey–Kramer procedure for Locomotion. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box2 - After	-0.9024	A
Foraging Box1 – After	-0.9510	A
Puzzle Feeder – After	-1.0296	AB
Puzzle Feeder – During	-1.3106	ABC
Foraging Box2 – During	-1.4561	BC
Foraging Box1 – During	-1.4614	C

Table 8. Multiple pairwise comparisons using the Tukey–Kramer procedure for Vigilance Behaviour. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box2 - After	-1.1928	A
Puzzle Feeder – After	-1.2768	AB
Foraging Box1 – After	-1.3132	AB
Foraging Box2 – During	-1.6316	ABC
Puzzle Feeder – During	-1.6495	BC

Foraging Box1 – During	-1.9630	C

Table 9. Multiple pairwise comparisons using the Tukey–Kramer procedure for Use of Enrichment. Means that do not share a letter are significantly different.

Treatment	Mean	Grouping
Foraging Box1 – During	-0.3105	A
Foraging Box1 – After	-0.6382	A
Foraging Box2 – After	-0.8223	AB
Foraging Box2 – During	-0.3404	AB
Puzzle Feeder – After	-0.8259	B
Puzzle Feeder – During	-0.3975	B

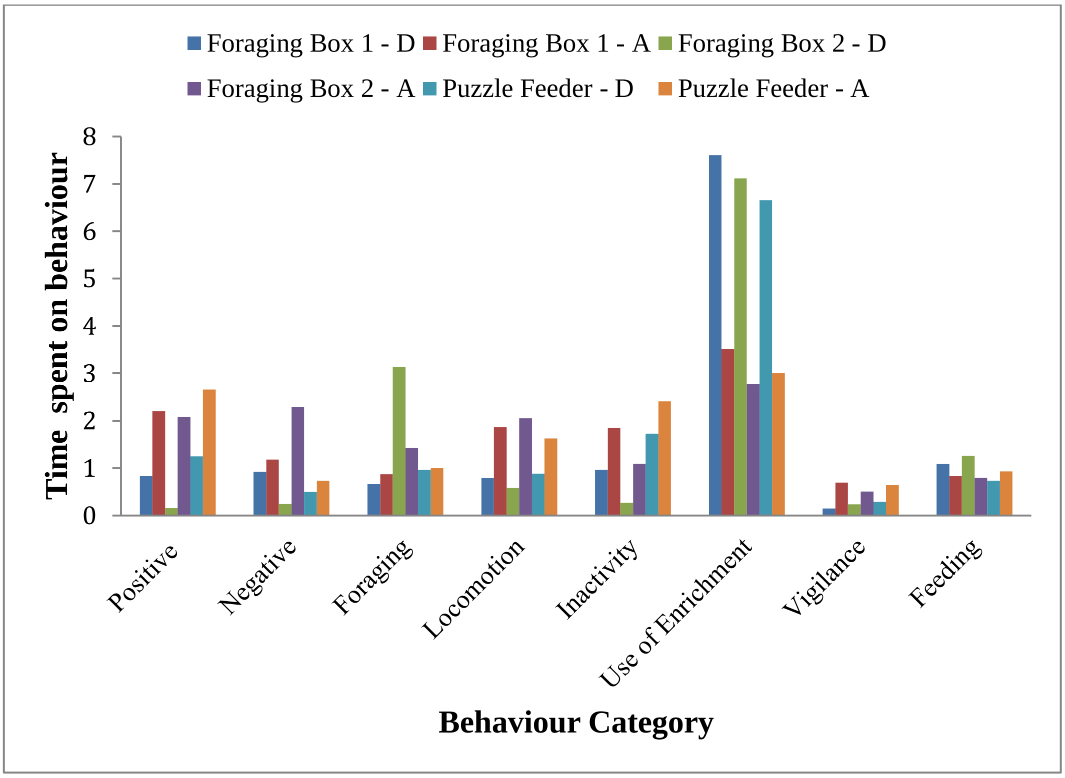

Fig. 13. Behavioural responses to enrichments and timing during Phase 2.

3.4 Correlation between Introduction of Enrichment and other behaviours

Pearson correlation tests showed a significant positive correlation between the use of the enrichment devices and Foraging Behaviour (E=0.320; p=0.004), a significant negative correlation between use of enrichment and Feeding Behaviour (E=-0.323; p=0.004), and a significant negative correlation between the use of enrichment and Inactivity (E=-0.380; p=0.001), all of them with a low level of association. Correlations with the other sets of behaviours did not show significant results.

3.5 Individual preferences for Enrichment and differences in foraging activity during Phase 2

Use of enrichment significantly changed between individuals (i.e. some individuals used the enrichment more than others) (F=2.25; d.f.=12; p=0.028) (see fig. 14, 15, 16). However, there was not a significant difference on the type of enrichment the animals preferred, even though the most manipulated device was Foraging Box 1, followed by Foraging Box 2, and the Puzzle Feeder.

With regard to the overall foraging activity presented during Phase 2 (i.e. the sum of data recorded as “Use of Enrichment” plus data recorded as “Foraging behaviour” *per se*), a significant difference between these two behaviours was found (T-value= -7.93; d.f.=1; p<0.001), being levels of Use of Enrichment higher than Foraging behaviour.

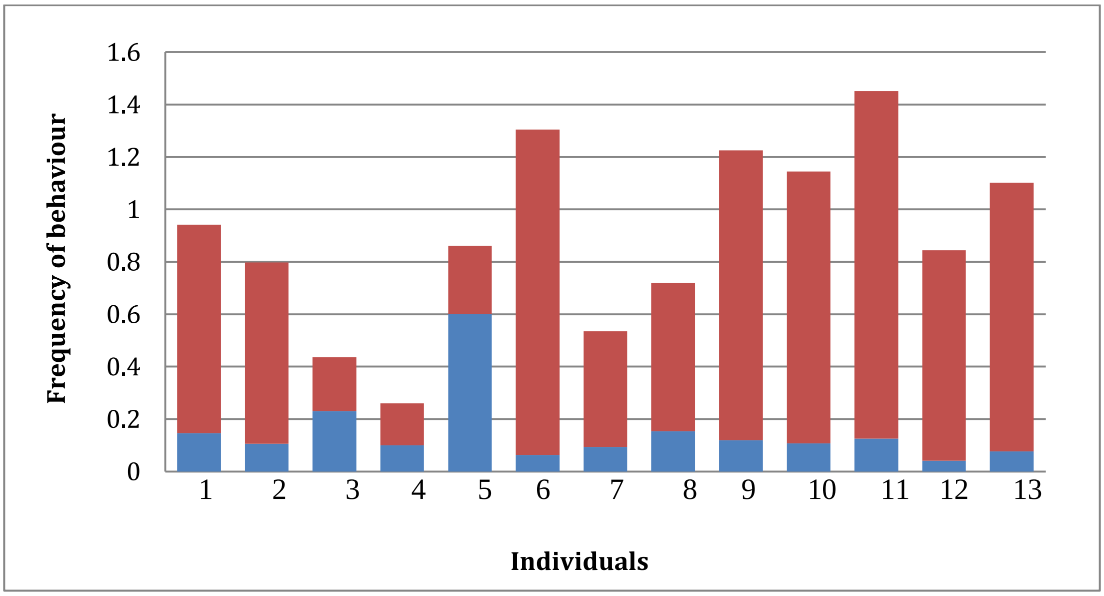

Fig. 14. Description (frequency) per animal of overall foraging behaviour due to use of Puzzle Feeder (Red) Vs. foraging behaviour *per se* (Blue).

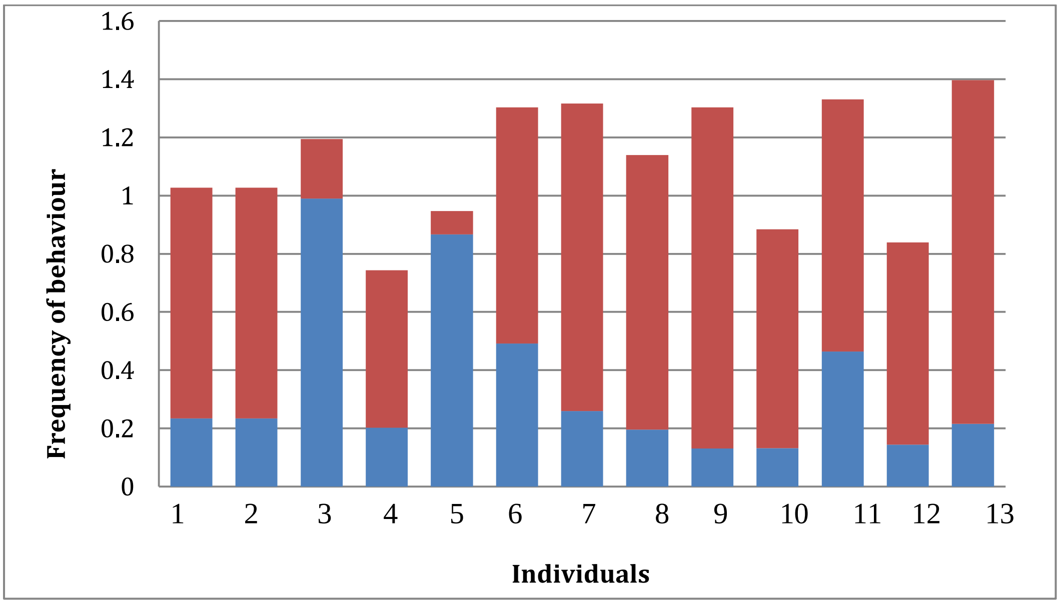

Fig. 15. Description (frequency) per animal of overall foraging behaviour due to use of Foraging Box2 (Red) Vs. foraging behaviour *per se* (Blue).

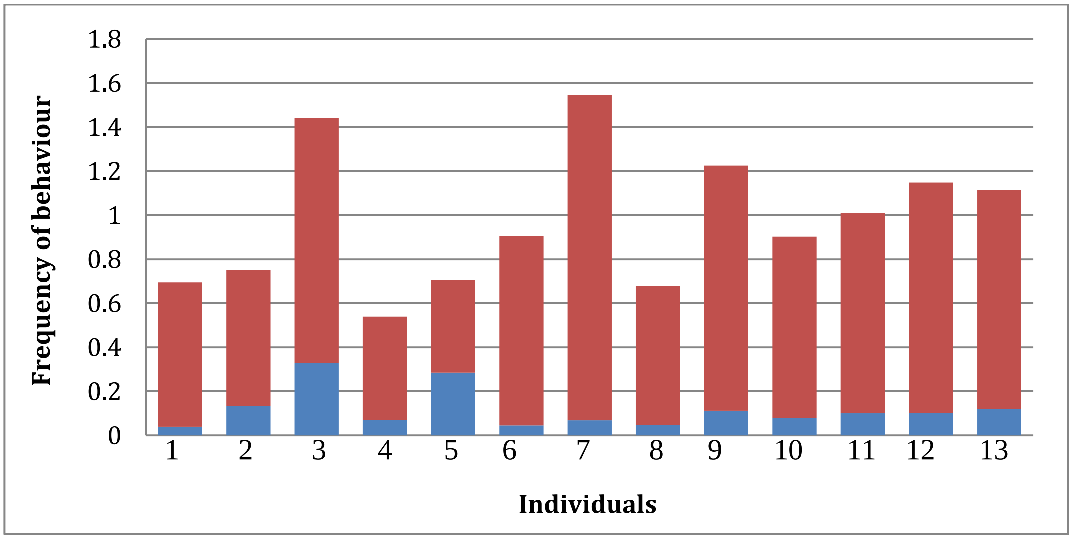

Fig. 16. Description (frequency) per animal of overall foraging behaviour due to use of Foraging Box1 (Red) Vs. foraging behaviour *per se*. (Blue)

4. Discussion

4.1 Time activity budgets Pre, During and Post enrichment

As the expression of a behavioural repertoire that resembles the one seen in the wild is an indicator of good welfare in captive animals (Redshaw and Mallinson, 1991; Shepherdson, 1994; Mellen and Sevenich 2001; Little and Sommer, 2002; Hosey 2005; Seddon *et al.*, 2007; Laule and Whittaker, 2007), assessing time activity budgets in captivity is of great importance. These estimates can help elucidate whether or not the conditions the animals are being provided with are good enough to ensure their welfare, and also because these estimates can be used to determine the progress in a rehabilitation process, as one of the main objectives in these programmes is to release animals that exhibit similar behaviours shown by their conspecifics in the wild.

In the case of long-tailed macaques, however, estimates of these budgets for non-captive populations differ greatly depending on the home range of the group selected for study, and on their proximity to human settlements, as this species can be found in their wild/natural habitat, or in close proximity to people: whereas some authors (see Hambali *et al.*, 2012) report activity budgets that refer to 19% of the time dedicated to foraging activities, and almost an equal amount of time of inactivity (i.e. 17%; see Hambali *et al.*, 2012), other studies report 40% to 45% of the animals’ time invested on foraging behaviours, both in proximity to or far from human settlements (see Sha and Hanya, 2013). Also, higher periods of inactivity have been reported for those groups of *M. fascicularis* living in areas with little human–animal interaction compared with the ones found in groups living in urban settings (see Sha and Hanya, 2013). Taking this into consideration, along with the fact that primates are known to spend considerable high time on foraging behaviours (Clutton-Brock and Harvy, 1977), and also that subjects used in this study will be released in areas far from human settlements, a foraging time of more than a 17% of the total amount of time of activity should be expected.

As results for the Pre-enrichment Phase showed very low Foraging Behaviour (i.e. 6%) and high levels of Inactivity (34%), the need to encourage foraging skills in the animals was evident. Compared with these baseline results, post-enrichment phase showed significantly higher levels of Foraging and Feeding behaviours and significantly lower levels of Inactivity, which indicates that the introduction of the enrichment did change the activity budgets in a species-typical behaviour direction. However, as the data collection period was a total of 42 days and a rehabilitation programme may take years counting with post-release monitoring (Reading *et al.* 2013) - only a mid-term effect can be assessed in this study, and it is not possible to determine whether or not these effects could be maintained in the long-term.

The fact that changes between Phase 1 and Phase 3 for the other sets of behaviours (i.e. negative interactions, positive interactions, feeding and vigilance) were non-significant, along with the fact that no significant correlation was found between Use of Enrichment and these behaviours, could suggest that other types of enrichment (e.g. social, physical, occupational etc.) may be needed to obtain a significant change in these conducts, as the three treatments were designed only towards food-related behaviours. Also, in order to obtain stronger effects on locomotion –since its change was not so marked, other type of enrichment strictly directed towards these set of behaviours is needed.

4.2 Effects of age and sex on behavioural changes across phases

Macaques that are brought to the IAR for reintroduction are individuals that come either from the pet trade industry or that were involved in human-animal conflict due to habitat loss. These individuals usually have a history of neglect and/or abuse (e.g. some of them were street beggars or were used for entertainment in urban settings, chained-up etc.). As early–life experiences and also amount, intensity and exposure to different events throughout life can have several effects on an individual, including its cognitive abilities and social behaviour (Leca *et al.*, 2007; Novak *et al.,* 2012), individual variation could be expected when exposing different animals to a same environment and/or challenges.

In the particular case of the individuals used for this study, higher inactivity and less positive interactions in adults could be the result of more or higher exposure to noxious stimuli during their period of life (e.g. physical abuse and exposure to anthropogenic stressors since they are used for street entertainment), compared to the juveniles, that even if could have been exposed to stressful situations, may have not accumulated as many. However, since Positive Interactions were the sum of play and grooming behaviour, and play is more frequently observed in juveniles (Tartabini, 1991), that may have contributed to a higher percentage of positive interactions in juveniles as compared to that in adults. Still, play behaviour and grooming were not individually analysed, therefore it cannot be conclusively determined whether these two behaviours had a direct effect on the increase in Positive Interactions observed.

4.3 Behavioural responses to enrichment (Phase 2)

As results from one-way ANOVA analysis showed significant behavioural changes between treatments and timing, it can be said that introduction of different treatments do produce different changes in activity budgets, and also that these changes continue and vary between treatments and throughout time (i.e. hours after the enrichment has been introduced).

The difference in timing may be explained by two reasons: 1. The novelty factor, as all three enrichments were not given to the animals every day, and therefore right after the introduction of the devices the animals would feel more “attracted” to them, but would lose interest in time (i.e. a few hours after the introduction); and 2. Availability of food: as devices were designed in order for the animals to look and work for food, and right after the introduction it would be easier to find it, but chances would decrease in time, this could also decrease the interest in the device.

These different responses throughout time are important, considering that it is better to provide the animals with devices that would keep a moderate-interest the longest time possible, rather than provide them with a device that would highly promote behaviour for a short period of time, but that would not interest the individuals later in time. Also, identifying these type of effects on each set of behaviour is of great value, as depending on the group or individuals in rehabilitation, it may be better to use one type of enrichment over others (e.g. some individuals or groups of individuals may need reinforcement in positive behaviours rather than feeding, whereas others may need more reinforcement in vigilance).

Regarding Inactivity, since both Foraging Boxes resulted in lower time spent inactive 1-3 hours after their introduction, it could be suggested that in order to keep the animals more active, these may be better types of enrichment than a puzzle feeder. This can be supported by the fact that these two same enrichments were the ones that were more manipulated by the animals right after their introduction (see fig. 13, Use of Enrichment), and that the Foraging Box 2 produced the highest levels of Foraging Behaviour also during and after introduction. In other words, a trade-off between Inactivity and Foraging+Use of Enrichment behaviours was seen.

One of the reasons why both foraging boxes resulted in higher levels of foraging behaviour 1 to 3 hours after their introduction, was probably that when the animals first started to manipulate these two enrichments, and as food was presented in small pieces of chopped fruit and seeds hidden in a considerable larger amount of foraging substrate, compared to the amount of substrate provided in the Puzzle Feeder- they would unconsciously throw this “foraging substrate + food” on the ground of the enclosure, which they would notice and use to forage only after the main source of food (i.e. the device) was empty.

In regard to Locomotion, its higher levels after the presentation of the three treatments (1-3 hours) and also its significantly increased levels for Phase 3 compared to Phase 1, indicate that the introduction of the enrichment can increase activity levels in the animals -a result that has been previously reported in other studies (Biggins *et al*., 1999; Mathews *et al*., 2005). This is a very interesting finding, since physical activity has shown to benefit not only cognitive function, but also improve physical fitness in both animals and humans (Hötting and Röder, 2013), which is also important in a rehabilitation programme taking in consideration that along with a change in activity budgets (if needed), these programmes may also seek to 1. Engage animals in learning new skills (e.g. predator detection, foraging strategies, locomotive patterns etc.) and 2. Release fit individuals.

4.4 Individual preferences for enrichment and differences in foraging activity during Phase 2

The fact that the time spent by the animals foraging on the enrichment devices was significantly higher than the time they foraged on other substrates, along with an increase in foraging behaviour between Phase 2 and 3 compared with Phase 1, suggests that the individuals did show an interest in the devices and that this interest made them work more to obtain food.

Significant differences in the time spent on a specific device (i.e. treatment) between individuals is an important finding since this not only tells that different subjects show different preferences on enrichment and on the way they obtain food, but also that the behaviour of different individuals may need to be addressed or reinforced in a different way (i.e. some individuals may need more foraging abilities than others and the same may happen with other behaviours) in order to provide them with better environments that would make them more suitable for release.

4.5 Suitability of enrichment and welfare impact

Design and materials of the enrichment devices were thought aiming to: 1. Provide low-cost devices that could be used at IAR for the long-term (i.e. did not represent a high monetary investment); and 2. Increase foraging behaviour, enhance foraging skills and reduce inactivity.

Although long-tailed macaques (*Macaca fascicularis*) are primarily arboreal (Rowe, 1996) and most of the feeding-related behaviours are observed around 12 meters of height, the design of the enclosures (3m x 2.5m x 3m) only allowed to introduce the devices on the ground and at a low height. However, since they feed on fruit (Wich *et al.*, 2002) insects, seeds, leaves, grass etc., and the they also forage on the ground (Ungar, 1996), the three enrichment options were expected to be appropriate to enhance

their natural foraging skills. However, although a general increase in foraging behaviour was seen with all three treatments, since the foraging boxes were the devices that reduced the most the time spent inactive and also resulted in significantly higher foraging behaviour, it could be said that these are more suitable enrichments for the animals than the puzzle feeder.
This can be explained due to the fact that the puzzle feeder requires more complex manipulation of items/higher cognitive skills (i.e. manipulation of stones and sticks to access food items) that macaques do not usually exhibit (i.e. although stone-use has been reported in this species, they are not considered tool users) (see Gumert *et al.*, 2011). Although it might be argued that a puzzle feeder could enhance cognitive skills, if it is too complex for the animals, it may result in frustration by making acquisition of feed more difficult, and therefore having a negative effect on the welfare of the animals.

Contrary, foraging boxes offer a substrate more similar to the one the animals would find in the wild, also promoting a more species-typical foraging behaviour and species typical foraging skills.
Overall, foraging boxes showed to be the most suitable enrichment types to enhance natural foraging skills and to positively impact welfare of the animals.

5. Conclusion

This study confirms that the use of environmental enrichment is a powerful tool that can modify an individual's behaviour in the short-to-middle term, and could therefore be used as a potential useful tool in rehabilitation programmes for reintroduction. Results indicate that low-cost enrichment cannot only change activity budgets towards a more species-specific type, but can also have a positive impact on welfare.
However, in order to accentuate these results and to properly design and apply an enrichment programme that increases probability of survival after reintroduction, factors that were not measured in this study (e.g. history of each individual and individual variation among other) and that could possibly expand the outcome of this research would need to be integrated in further studies.

REFERENCES

Alberts, A.C., 2007. Behavioral considerations of headstarting as a conservation strategy for endangered Caribbean rock iguanas. Appl Animal Behav Sci. 102, 380–391.

Biggins, D. E., Vargas, A., Godbey, J., Anderson, S. H., 1999. Influence of prerelease experience on reintroduced black-footed ferrets (*Mustela nigripes*). Biological Conservation. 89 , 121-129

Clutton-Brock, T. H., Harvey, P.H., 1977. Species differences in feeding and ranging behaviors in primates, In Primate Ecology: Studies of feeding and ranging behavior in lemurs, monkeys, and apes, T. H. Clutton-Brock, ed., Academic Press: New York, pp. 557-584.

Griffin, A.S., Blumstein, D.T., Evans, C.S., 2000. Training captive- bred or translocated animals to avoid predators. Conserv Biol. 14,1317–1326.

Gumert, M.D., Kuan Hoong, L., Malaivijitnond, S., 2001. Sex Differences in the Stone Tool-Use Behavior of a Wild Population of Burmese Long-Tailed Macaques (*Macaca fascicularis aurea).* American Journal of Primatology. 73,1239–1249

Hambali, K., Ismail, A., Md-Zain, B. M., 2012. Daily Activity Budget of Long-tailed Macaques (*Macaca fascicularis*) in Kuala Selangor Nature Park. IJBAS. 12, 47-52.

Hones, P.E., Marin, C.M., 2006. Enrichment and aggression in primates. Neurosci Biobehav R. 30, 413–436.

Hosey, G., 2005. How does the zoo environment affect the behaviour of captive primates? Appl. Anim. Behav. Sci. 90, 107-129.

Hötting, K., Röder, B., 2013. Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci Biobehav R. In press

Laule, G., Whittaker, M., 2007. Enhancing Nonhuman Primate Care and Welfare Through the Use of Positive Reinforcement Training. J Appl Anim Welfare Sci. 10, 31-38

Leca, J.B., Gunst, N., Huffman, M. A., 2007. Age-related differences in the performance, diffusion, and maintenance of stone handling, a behavioral tradition in Japanese macaques. J Hum Evol. 53, 691–708.

Little, K.A., Sommer, V., 2002. Change of enclosure in langur monkeys: implications for the evaliation of environmental enrichment. Zoo. Biol. 21, 549-559.

Lutz, C.K., Novak, M.A., 2005. Environmental enrichment for non-human primates: theory and application. ILAR Journal 46, 178-191.

Mathews, F., Orros, M., McLaren, G., Gelling, M., Foster, R., 2005. Keeping Fiton the ark: assessing the suitability of captive-bred animals for release. Biol Conserv. 121 , 569-577.

McPhee, M.E., 2003. Generations in captivity increases behavioural variance: considerations for captive breeding and reintroduction programs. Biol. Conserv. 115, 71-77.

Mellen, J., Sevenich MacPhee, M.S., 2001. Philosophy of environmental enrichment: past, present and future. Zoo Biol. 20, 211–226.

Miller, B.J., Reading, R.P., Forrest, S., 1996. Prairie night: black- footed ferrets and the recovery of endangered species. Washington, DC: Smithsonian Press.

Novak, C.M., Burghardt, P.R., Levine, J.A., 2012. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci Biobehav R. 36, 1001–1014.

Reading, R.P., Miller, B., Shepherdson, D., 2013. The Value of Enrichment to Reintroduction Success. Zoo Biol. 32, 332–341.

Redshaw, M.J., Mallinson, J.J.C., 1991. Learning from the wild: improving psychological and physical well-being of captive primates. Dodo, Jour. Jersey. Wildl. Preserv. Trust. 27, 18-26.

Rowe, N., 1996. The pictorial guide to the living primates. East Hampton (NY): Pogonias Pr. p 263.

Seddon, P., Armstrong, D.G., Maloney, R.F., 2007. Developing the Science of Reintroduction Biology. Conserv Biol. 21, 303–312.

Sha, M., Hanya, G., 2013. Diet, Activity, Habitat Use, and Ranging of Two Neighboring Groups of Food Enhanced LongTailed Macaques (*Macaca fascicularis*). Am J Primatol. 75, 581–592.

Shepherdson, D.J., 1994. The role of environmental enrichment in the captive breeding and reintroduction of endangered species. In: Mace GM, Onley PJS, Feistner ATC, editors. Creative conservation. London, UK: Chapman and Hall. p 167–177.

Shier DM, Owings DH. 2006. Effects of predator training on behavior and post- release survival of captive prairie dogs (*Cynomys ludovicianus*). Biol Conserv. 132, 126–135.

Son, V.D., 2003. Diet of *Macaca fascicularis* in a mangrove forest, Vietnam. Lab Prim News 42, 1-5.

Stoinski, T.S., Beck, B.B., Bloomsmith, M.A., Maple, T.L., 2003. A behavioral comparison of captive- born, reintroduced golden lion tamarins and their wild- born offspring. Behavior. 140,137–160.

Tartabini, A., 1991. Social play behaviour in young rhesus monkeys *Macaca mulatta* at three different ages: from the 3rd to the 6th month of life. Behav Processes. 24, 185-192.

Ungar, P.S., 1996. Feeding height and niche separation in sympatric Sumatran monkeys and apes. Folia Primatol. 67, 163-8.

Utt, A.C., Harvey, N.C., Hayes, W.K., Carter, R.L., 2008. The effects of rearing method on social behaviors of mentored, captive- reared juvenile California condors. Zoo Biol. 27, 1–18.

Wich, S.A., Fredriksson, G., Sterck, E.H.M., 2002. Measuring fruit patch size for three sympatric Indonesian primate species. Primates. 43, 19-27.

CRITICISM OF METHODOLOGY

Constraints at the site of study

Although enrichment devices (i.e. type, material and aims) and also data collection schedules had been discussed prior to arrival, husbandry and cage design did not allow the use of these treatments or schedules of observation. Therefore, enrichment devices and data collection scheme had to be re-designed and adjusted to the main objectives that had been set before for the study.

Sample size

Initial sample size was reduced as some groups of animals were suddenly selected for release, and also upcoming socialisation of individuals was scheduled right at the beginning of the study. Regarding this, although a smaller sample size was still enough to observe significant results, since macaques at IAR come from different backgrounds and this may strongly affect the way they respond to environment, a larger number of individuals could have allowed to increase the generalizability of the results.

Time

Even though significant results were obtained and treatments proved to increase the expression of some behaviours associated with good welfare, constraints at site of the study (e.g. change of experimental design, treatments and weather conditions) as well as illness of the observer considerably resulted in a shorter period of observation than expected. This influenced the total number of hours of observation for each phase of the study, which resulted in inconsistent number of observations between phases (i.e. total number of observations for phases 1 and 3 were lower than for phase 2). However, this inconsistency was solved by calculating an average into the percentage of time observed for each phase.

PERSONAL CONTRIBUTION

The experimental design of the study, data collection, analysis and interpretation of results were performed by Wendy Gomez Rubio under supervision of Dr. Phyllis Lee and Dr. Susan Jarvis.

Design of treatments and introduction of the devices to the enclosures were done in collaboration with the IAR macaques keepers, and under supervision of Dr. Richard Moore.

ACKNOWLEDGMENTS

Firstly I would like to thank my parents for supporting my dreams and ambitions.

I want to thank all the macaques at IAR because they kept me motivated throughout the study and not a single day stopped to amaze me with their incredible nature and manners. I feel honored and blessed to have had the chance to observe and study these magnificent creatures.

My experience in Indonesia would have not been as enjoyable without Brownie, Blacky, Moni and Lana, who always kept me company and greeted me with excitement and wagging their tails no matter the weather conditions. I also want to thank my five Indonesian cats and the other dogs at the Centre.

Special thanks to Alan Night for giving me the opportunity to conduct this study at IAR and for being so nice and friendly all the time. Christine Rattel and Richard Moore were also a big support during my time in Indonesia, and I would also like to thank Dr. Rondang Sinegar for inspiring me to keep doing what I do. It was a real privilege to meet her.

I want to thank Dr. Phyllis Lee for supervising my work, and I want to thank my course director Dr. Susan Jarvis for being so patient and helpful all the time.

Thanks to all the macaques keepers for teaching me Indonesian, for their constant willingness to help me, and for making me laugh all the time. Thanks to all the staff at IAR for making me feel so comfortable, and a huge thanks to Ayut for being such an incredible friend.
Thanks to my closest friends who supported me and encouraged me from different parts of the world no matter the distance and our very different time zones. Thanks for everything Namu.

Finally, a huge thanks to my Colombian capuchin monkeys, for touching my heart and for being the greatest inspiration in my life. I will continue to learn and work so I can one day make a difference in your lives.

APPENDIX

Appendix A. The tables represent the randomized structure of the application of three treatments in phase 2 of the Study.

D denotes Day of treatment; GP denotes Group; FB1 denotes Foraging Box 1; FB2 denotes Foraging Box 2; PF denotes Puzzle Feeder.

Numbers 1,2 and 3 correspond to the three social groups used for the study.

GP \ D	1	2	3	4	5	6
1	FB1	FB2	PF	FB2	FB1	PF
2	PF	FB1	FB2	PF	FB2	FB1
3	FB2	PF	FB1	FB1	PF	FB2

GP \ D	7	8	9	10	11	12
3	FB1	FB2	PF	FB2	FB1	PF
1	PF	FB1	FB2	PF	FB2	FB1
2	FB2	PF	FB1	FB1	PF	FB2

GP \ D	13	14	15	16	17	18
2	FB1	FB2	PF	FB2	FB1	PF
3	PF	FB1	FB2	PF	FB2	FB1
1	FB2	PF	FB1	FB1	PF	FB2