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Environmental Enrichment of Captive Primates: a Research for Welfare at Maia's Zoo

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Evolução e Biologia Humana, realizada sob a orientação científica da Professora Doutora Cláudia Sousa (Universidade Nova de Lisboa), do Professor Doutor Miquel Llorente (Universidade de Girona) e da Professora Doutora Eugénia Cunha (Universidade de Coimbra)

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Abstract

Environment enrichment (EE) is a recent concept. However its recognition had been considerably increasing and its practice has been branching out, adapting it for each particular species. We can define environment enrichment as a practice aiming to grant improved conditions to captive animals, as it develops behavioural opportunities to mimic wild life. Implementation of many varieties of enrichment is now a standard routine worldwide in recovery centers, zoos and laboratories, as also public opinion demand better conditions for animals and law stipulate its practice. The aim of this study is to test if individuals of three non-human primate species at Maia's Zoo (gibbons, N=2; Mona monkeys, N=2; brown lemurs, N=2) need environmental enrichment ad if the devices implemented reduce boredom and apathy, symptoms that captive animals are more prom to. The apparatus here presented acts as a cognitive stimulus and feeding enrichment. Also, to prove its applicability the type of enrichment device chosen must be easy, simple and inexpensive to build. With this in mind, the feeding devices given to the subjects of this study consist in food-filed small pieces of bamboo canes and a wire box filled with fruits and straw. The results of the present study show that the subjects did indeed need EE intervention, as inactivity was reduced in the three groups and abnormal behaviours was decreased in brown lemurs. It is clear that the effect of an enriching foraging strategy depends on the species and its individuals' personalities, which are important aspects that should be taken into account when designed and maintained EE programs. EE technique should be planned according to the expected effect and at the same time, we must provide opportunities for the animals to manipulate the devices and choose when to do that. Enrichment effect may not be immediate so we must be 'patient' with its use. Future projects should include a larger number of individuals.

Key words: zoo, enrichment, feeding apparatus, behaviour opportunity, individuality

Resumo

O enriquecimento ambiental é um conceito recente. No entanto, o seu reconhecimento foi aumentando consideravelmente e sua prática foi ramificando-se, adaptando-se para cada espécie em particular. O enriquecimento ambiental tem como objetivo conceder melhores condições para animais em cativeiro, desenvolvendo oportunidades para aproximar o comportamento destes ao comportamento natural das espécies em habitat natural. Muitas variedades de enriquecimento são agora uma rotina em centros de recuperação, zoológicos e laboratórios a nível mundial, à medida que a opinião pública exige melhores condições para os animais e a lei estipula a sua prática. O objetivo deste estudo é testar se os indivíduos de três espécies de primatas não-humanos no Zoo da Maia (Gibões (N = 2), macacos Mona (N = 2) e lémures castanhos (N = 2)) precisam de enriquecimento ambiental, assim como se os dispositivos implementados pelo estudo reduziram o tédio e apatia, sintomas que aos quais os animais de cativeiro são expostos. Os dispositivos apresentados aqui atuam como um estímulo cognitivo e enriquecimento alimentar. Além disso, para provar a sua aplicabilidade, o tipo de dispositivo de enriquecimento escolhido deve ser fácil, simples e barato de construir. Com isto em mente, o dispositivo de alimentação dado aos indivíduos neste estudo consiste pequenos pedaços de canas de bambu enchidos com pasta alimentar e uma caixa de arame com frutas e palha. Os resultados do presente estudo mostram que os sujeitos necessitam de intervenção de enriquecimento ambiental, sendo que a inatividade foi reduzida nos três grupos e os comportamentos anormais diminuíram nos lémures castanhos. Torna-se claro que o efeito de uma estratégia enriquecedora de alimentação depende da espécie e da personalidades de seus indivíduos, aspetos importantes que devem ser tomados em conta quando concebidos e mantidos este género de programas. A técnica deve ser planejada de acordo com o efeito esperado mas mesmo tempo, devemos dar oportunidades para os animais de escolherem quando e como manipular os dispositivos. Efeito de enriquecimento pode não ser imediato, por isso devemos ser "pacientes" com a sua utilização. Projetos futuros deverão incluir um número maior de indivíduos.

Palavras-chave: zoo, enriquecimento, aparatos alimentar, oportunidade comportamental, individualidade.

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1. Introduction

1.1. *Zoo's "goods" and "bads"*

"The presence of human visitors has been shown to affect the behaviour of several different mammalian species in a number of different zoos, but the behavioural changes observed are not always consistent with a simple 'stressful influence' explanation" (Hosey, 2000:343).

Captive environments impose limitations to the behavioural opportunities available to their inhabitants (Hosey, 2005). Wild animals have the freedom to choose friends, mates and ranging area, they chose when and what to feed as well as the process of acquiring and processing such food. On the opposite spectrum, captive animals have for such decisions inhibited as their life is managed by humans and a place where they didn´t choose to live. Environmental enrichment, social housing, naturalistic enclosures and unpredictable feeding may enhance such limitations and decrease the development of abnormal behaviours so often observed in captive animals. Design and implementation of such requirements in order to maximize welfare, predict a proper knowledge and understanding of animals' use of such environmental features, behaviour and use of space (Estevez and Christman, 2006).

Animals in captivity are only acceptable if animal's welfare is assured and if public awareness and education is the main goal of such condition. If we think about it, these two assumptions are inter-dependent as naturalistic well-designed cages and suitable environment for animals are the best way for the public to observe their active natural behaviours, learn about them and became more aware and sensible about their conservation. However, if public presence can have a negative influence on animals' behaviour and in fact, some visitor's behaviours (e.g. aggressive of teasing) had been proven to instigate the same responses by the captive animals and cause them stress (Hosey, 2000). In addition, Hosey (2005) defend that responses towards public is not uniform across primates species in captivity, but instead it diverges between specie. Furthermore, this author suggest that both captive and non-captive primates, may become familiarized to humans and human public when interaction between the two is frequent, although such habituation depends on species-typical response and on the extent of contact (Hosey, 2005).

As with human audiences, restricted place as a negative feature of captivity may also diverge between species which have singular response patterns to constrained space and which also have different home ranges in the wild, also inhibited by borders of some sort. For example, and concerning overcrowding, primates in large groups are likely to appeal to comforting behaviours as reconciliation, consolation and avoidance of moderate aggression and cope peacefully in a constrained set (de Wall et al., 2000). Also, the complexity of the space beyond it physical dimension, plays an important role in captive animal welfare. For example, Perkins (1992) in her research with orangutans (29 individuals housed in zoological parks), found that large enclosures with high amounts of movable objects, which make possible social interactions, increased levels of activity. This assumption, however, depends on the species (Wilson, 1982 *in* Perkins, 1992).

Introducing structures to the captive setting often provides a naturalistic environment, in an attempt to reconstruct surroundings complexity similar to that seen in the wild, having “measurable effects on their (primates) behaviour consistent with both improved welfare and a more naturalistic profile of behaviour” (Hosey, 2005:116).

Related to husbandry, zoo captive animals are managed by their keepers in many aspects: feeding, enclosure cleaning, veterinary procedures and separation and introduction of individuals to the group. Feeding for example, may represent an excellent opportunity to implement some kind of enrichment related to it, in order to increase activity budget of the individuals (e.g. Vick et al., 2000) who otherwise will not need any type of foraging behaviours and will bring close together wild and captive behavioural profile (Britt, 1998). This assumption, however, bring us to another one: primates are generalist species, they show great flexibility and adaptability to their surroundings and so, zoo environments should take into account such individuals requirement (Poole, 1991 *in* Hosey, 2005). With this in mind, Hosey (2005: 110) suggest that maybe zoos are not “extreme environments for primates” and so, species should be tested separately. Taking Melfi and Feistner (2002) work as an example: these authors found that activity budgets of Sulawesi macaques (*Macaca nigra*) diverge between zoos but, overall they were not significantly different from activity budgets reported in the wild. Note that activity budget represent a helpful information about animal welfare and therefore should be monitored. Therefore, and for the species of Melfi and Feistner (2002) study, and considering that activity budget seen in wild is the natural and representative behavioural repertoire of the species, captivity did not influence negatively the life of the individuals in this study, as both captive and wild presented similar activity budget pattern.

Another difficulty concerning feeding is the pre-feeding agonism. Mason and Mendl (1997) suggest that locomotor stereotypies may be linked to appetitive of the foraging phase. Pre-feeding stress can be overpass by positive reinforcement training (oral commands and food reward for males chimpanzees: Bloomsmith et al., 1994) and by unpredictable feeding schedules (Bloomsmith and Lambeth, 1995).

Concerning behavioural research, a benefit of studying zoo primates for example is that the researcher can identify subtle behaviours rarely seen in the wild, taking an important role in developing theories and testing them (Hosey, 2005). However, we must take to account that such captive studies' results may be influenced probably by several variables relating to zoo environment and therefore, should be interpreted in context (Hosey, 2005).

1.2. *Environmental enrichment for captive primates*

"Captive conservation breeding programs should not be wholly concerned with maintaining a diverse gene pool – they should also be concerned with conserving species-typical behaviors, especially if they are to produce behaviorally intact captive animals that can be reintroduced to the wild with minimal training, financial resources, and loss of individuals" (Kerridge, 2005: 71).

In the wild, primates are very energetic animals. They spend most of their time looking for food, processing and ingesting it (Doran, 1997) in an active social community (Honess and Marina, 2006). In Zoos, for example, non-human primates and carnivores are the first animals to inspect new objects (Glickman and Sroges, 1966). However, as mentioned before, in captivity environments faced by animals can be significantly distinct from the wild habitat and different species may have different adjustments toward these settings (Gottlieb et al., 2012). Such adaptations depend on the resemblance of the captive condition to the species natural habitat (Carlstead and Shepherdson, 1994 *in* Mallapur and Choudhury, 2003). Therefore, animals in captivity should have the chance to exercise, react and play, being their best possibility for those behaviours to occur, the design and introduction of apparatus and objects used by the individuals to work or play (Yerkes, 1925) and also, being that an attempt for humans to control animal's behaviour in captivity (Morimura, 2003). Otherwise monotony routine can cause several abnormal behaviours, particularly stereotypies, an usual indicator of poor welfare (Broom, 1983). The Committee on Well-Being of Nonhuman Primates of the United States Department of Agriculture (2009: 83), in the National Research Council's Institute for Laboratory Animal Research (ILAR) originated in 1985 a revision to the Animal Welfare Act, Code of Federal Regulations obligating zoos and medical research laboratories to "an appropriate plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates". Environmental enrichment is now an essential piece of Refinement, one of the 3 Rs for acceptable animal experimentation (Buchanan-Smith, 2010).

Hence, environmental enrichment' techniques have been undertaken, selected and adapted for each species to prevent monotony and subsequent abnormal behaviours by giving animals challenges and opportunities to deal with and to be entertained, promoting physical and psychological well-being and eliciting species typical behaviours. Márquez-Arias and colleagues (2010: 32) define environmental enrichment as "a program designed to enhance the welfare of confined animals by providing them with a more stimulating background". However, environmental enrichment concept is still discussed in the scientific area, as its function is decidedly context specific (Meehan and Mench, 2007). Nevertheless it is undoubtedly the best resource to promote and maintain captive animal welfare, indispensable for animal husbandry, under limited conditions. For example, chimpanzees housed individually show a significant decrement of abnormal behaviour when given toys (Brent et al., 1989; Kessel and Brent, 1998). Also, Bayne and colleges (1992b) reported that singly-housed rhesus monkeys increased stereotypes when enrichment objects were removed of the cage. Conversely, aggression and stereotypic behaviours decreased as exploration increased, after implementation of an enrichment program to a colony of stumptail macaques (*Macaca arctoides*) (Márquez-Arias et al., 2010).

Enrichment must be carefully revised before being given to animals to avoid any type of danger to the animals. The choice of the enrichment device to be used must take into account the skills of the animal. The same way the number of objects provided must be accurately thought to prevent differential individual's access to the enrichment and consequents fights (Honess and Marina, 2006; Clark and Melfi, 2011). Moreover enrichment needs to be evaluated to insure it is beneficial (Lutz and Novak, 2005; Buchanan-Smith, 2010). Although this evaluation is difficult to make, some studies had incorporated experimental design that allows this. For example, Schapiro and Bushong (1994) measured the amount of veterinary attention required by animals to access the effect of the enrichment. Also, cortisol concentrations (indicated of stress) (eg. Suzuki et al., 2002) and behaviour (Boinski et al.,1999) have been measured to access animals' welfare.

In order to evaluate viability of an enrichment program, it is required both previous and subsequent assessment of activity budget of subjects toward such enrichment implementation (Chamove, 1989a, b *in* Celli et al., 2003). For example, Ross and colleagues (2009) found that open spaces were not chosen by both gorillas and chimpanzees and instead individuals preferred areas densely furnished within the enclosures. Knowing this, enclosur designers should include such findings into their projects and so, incorporate solidly structures such as mesh walls, water sources, corners, shotcrete trees and others, to provide climbing, hiding and shade opportunities. Also, care staff must take into account that a certain enrichment device might be more effective for one individual than to other (if effective at all) and, in the case of enrichment directed to the positive exploitation of the environment, some areas are just preferred by animals and is not acceptable to turn those areas less desirable in order to appeal animals to explore others but to make the "undesirable" ones more attractive (Buchanan-Smith, 2010).

Because habituation to a device can decrease activity towards it, it might be effective only for short periods of time (Bloomsmith et al., 1990; Line, 1987; Novak et al., 1993; Paquette and Prescott, 1988; Pruetz and Bloomsmith, 1992), an outcome which can be reduced by a superior variety of objects or alternating items (Bayne, 1989; Paquette and Prescott, 1988). Also, removal of a successful enrichment device should be carefully planned so it would not cause reappearance of abnormal or aggressive behaviour (Bayne et al., 1992a). As van Hooff (1967: 35) observed, "only those devices seem to occupy them most fully that (1) have an inherent amount of complexity or variability, (2) permit them to perform some natural activity (e.g. nesting or climbing) or (3) which render a no devaluating reward".

Feeding has a vital positive impact in captive animal routine (Bassett and Buchanan-Smith, 2007). Foraging process can includes tool-use (Goodall, 1986; Morimura, 2003; Yamakoshi, 1998), which can provide an excellent enrichment *per se* and provides the acquisition of species-typical behaviour (Celli et al., 2003). For instance, chimpanzees when presented with a artificial device with food or drink, they prefer to use tools (and they select them) rather than their hands to get to it (Morimura, 2003).

Although foraging enrichment devices are not the most used ones (de Azevedo et al., 2007) it is considered by several authors to be the most effective enrichment hitherto used (eg. Knott, 2002). This was proved to be true to Javan Gibbons by the study of Gronqvist and colleagues (2013) in which the four subject groups respond to the three types of enrichment (foraging, olfactory and novel object enrichment) but showed a stronger reaction to the foraging box. Moreover, feeding enrichment has several other advantages. First, it's unlikely to cause habituation to it (Bayne et al., 1991; Bloomsmith et al., 1988; Bloomstrand et al., 1986; Brent et al., 1991; Lutz and Farrow, 1996; Maki et al., 1989). Second, it can easily mimic natural habits and habitats by natural exploration and foraging behaviour (Swaisgood and Shepherdson, 2005; Britt, 1998) and it make possible multifaceted problems' solving activity (Meehan and Mench, 2007). Third it can " provide an healthy and varied diet in a creative manner" (Sanz et al., 1999: 2). Also, as pointed out by Clark and Melfi (2011), it may have vital secondary utility as it might endorse appetitive behaviours in zoo animals. For that matter, we have chosen foraging devices to implement in this experiment.

1.3. *Abnormal behaviour*

"We should be beyond attempts to repairing, or limiting the damage, and proactively be providing a life worth living (...) Permanently removing ingrained abnormal stereotypic behaviours is rare so prevention is far better than cure." (Buchanan-Smith, 2010: 45)

Life experiences may have a profound effect in an individual brain and as a result, a profound effect in an individual behaviour (Knudsen, 2004; Hellemans et al., 2004; Veenema, 2009).

Abnormal behaviours are frequently observed in zoos or other captive settings, due to deficient environmental and social stimuli. Captive animals in zoos have mainly three backgrounds:

1. Born in the zoo,
2. Confiscated from illegal situations, and
3. Trade with other zoos.

As Buchanan-Smith (2011: 44) points out, preventing abnormal behaviour "is proactive, and not reactive" in the way that our work implies not only animals who present already these type of behaviours but also healthy-psychological individuals. But once again, it is essential to understand what may cause abnormal behaviours. Social deprivation, mostly maternal separation often seen in zoo/captive environments, may dictate the abnormal behaviour development. Even in African Sanctuaries, some animals rescued from illegal captivity (e.g. pets and tourist attraction) also exhibit stress related behaviours which persist for many years after rescue even when integrated in the social and more natural-wild like environments of the sanctuary (Lopresti-Goodman et al., 2013). In research laboratories on the other hand, factors like restraining, handling and single housing are also main reasons for the development of stress related behaviours.

Several studies have investigated disturbances of the normal neurobehavioural development in animal models after stress's occurrences in early infancy, such as maternal separation and deprivation (see Parker and Maestripieri, 2011 for a review), whereas supportive-enriching conditions can smooth the adaptation's progress of the developing brain to its environment and also, the opportunity of overcome previous disturbances (Buchanan-Smith, 2010; Marco et al., 2011; Hellemans et al., 2004). Hence, the animals' development and early infancy do indeed play an important role and may even dictate the adulthood. As Marco and colleagues stated (2011: 286), "development represents a critical moment for shaping adult behaviour and may set the stage to disease vulnerability later in life".

Abnormal behaviour, mainly self-aggression can become severely ingrained in animal's behavioural repertoire but can decline by subsequent peer or group housing, also considered as enrichment, though it may prove hard (e.g. Reinhardt, 2004) or may be unfeasible to eliminate (e.g.Weed et al., 2003). For example, after presenting western captive gorillas (*Gorilla gorilla gorilla*) with four enrichment devices, Rooney and

Sleeman (2008) did not noticed abnormal behaviours declining (maybe because they were deeply rooted), although it did enhanced species-typical behaviours and reduced boredom. Birkett and Nweton-Fisher (2011) suggests that the insertion of human-reared chimpanzees into a social group can ameliorate these effects of maternal deprivation, being such "social enrichment" the most effective to decrease and stop abnormal behaviours in primates (Lutz and Novak, 2005). A special case was the one of Endoo, a wild born gibbon, captured and kept as a pet in Thailand. When rescued, Endoo has profoundly traumatized as she displayed disturbing self-harming and lack of appetite. Fortunately, her behaviour and health improved due to the implementation of both specific environmental enrichment devices (feeding puzzles, which improved significantly her appetite) and social companion (Gray, 2012). Buchnan-Smith (2011) also points out that environmental enrichment in early phases is beneficial and can decrease future abnormal behaviour of primates in research facilities deprived from a maternal rearing. For example, Francis (2002) study with corticotrophin-releasing factor and glucocorticoid receptors in rats showed that environmental enrichment reverses the effect of maternal separation on behaviour responses to stress.

Despite the small amount of quantitative studies of abnormal behaviour conducted in zoos, several authors predict the high occurrence of captive animal's abnormal behaviour. For example, Birkett and Newton-Fisher (2011) found that every individuals in their study of 40 chimpanzees, socially-housed in zoo environment in the United States of America, presented at least some abnormal behaviour but they couldn't found an explanation for the differences observed between individuals concerning their frequency and duration. Birkett and Newton-Fisher calculated that an hypothetical average individual of their sample exhibited five types of abnormal behaviours, one at least once every forty minutes, occupying 4-5% of their activity time, concluding that "abnormal behaviour is endemic in captivity" despite the enriched and social environment where their sample was integrated, testing the animals capacity to cope with captivity (Birkett and Newton-Fisher, 2011: 5).

1.4. *Captive primates' welfare*

"To make informed management decisions about psychological well-being of captive animals, it is important to objectively quantify and examine the influences on their behavior" (Rooney and Sleeman, 2008: 339)

Barnard and Hurst (1996) suggest that welfare should be measured and evaluated according to individual species evolution. That is welfare depends of the coping mechanisms developed by species to survive and flourish in the environment to which it is adapted. In other words, animals' needs and desires should be taken into consideration in order to improve welfare. In that order, we should be acquainted with the fact that wild animals are also prone to stress (injury, malnutrition, disease, etc.) and therefore, could also have compromised welfare (Buchanan-Smith, 2010; Hosey, 2005). For that matter, habitat conservation and human impact in such habitats are also a welfare concerns (Sainsbury et al., 1995). On the other hand, adaptation to captive environment may not inevitably be a sign of improved welfare (Buchanan-Smith, 2010), but a reflection of the past life conditions and routine of the animals. For instance, in their study, Mallapur and Choudhury (2003: 281) found that confiscated animals "exhibited higher levels of undesirable behaviour than did animals whom were reared in recognized zoos".

In this study, we use Márquez-Arias and colleagues (2010: 33) definition of welfare: "animal welfare is defined as a reduction in abnormal behaviours – stereotypes, agonistic behaviour and coprophilia – together with an increment in those common in animals in the wild and, therefore, considered normal, such as exploration and play".

The relationship between abnormal behaviour and welfare is not fully understood (Mason and Latham, 2004) and some authors consider it an anthropocentric concept (Chamove, 1989 *in* Márquez-Arias et al., 2010). However it is a vital aspect of management of captive animals and ethic logic related to it. As Newberry (1995: 232) suggest it is necessary to "quantify the costs and benefits to the individual of performing the behavior" in order to conclude causes and consequences of it as well as efficiency of the enrichment. Some authors observed that animals suffer if incapable to carry out the behaviour even if it is not necessary to meet instant physiological needs (Hughes and Duncan, 1988; Jensen and Toates, 1993). Animals that do not interact with new items in great extent as other members of their species may have endured poor conditions to the extent that their curiosity has been diminished (Wemelsfelder et al., 2000).

Some studies show that more cognitive complex animals (big brained) need more stimulation and undergo boredom more severely (Maple and Perkins, 1996). This can be explained by the fact that generally, cognitively sophisticated species tend to be generalists, meaning that in wild they occupy complex environments (e.g.Kreger et al., 1998) which require continuous activity and stimulus. On the other hand, Broom (2010) supports that higher cognition may in fact help individuals to cope with poor conditions (reason for what he believes that cognition must be a parameter for the design of environmental enrichment for captive animals). However, an important fact is that primate life expectancy is larger than other laboratory animals and so, their requirements for well being change throughout their lives, being such continuous alteration a challenge for the care staff in the exercise of providing enough conditions to assure individuals' welfare (Buchanan-Smith, 2010). For instance, young individuals may require distinct conditions in order to develop a healthy future behaviour. Social experiences may control gene expression and behaviour, and conversely these pressures early in life may predict a particular intense outcome (Champagne and Curley, 2005). Accordingly, during the sensitive or critical period for the individual development, these experiences are essential for the individual environmental adaptation and survival. To maximize survival, developmental rate and rhythm can be adjusted by the individuals (Janson and van Schaik, 1993 *in* Hutchinson and Fletcher, 2010; Pereira and Leigh, 2003 *in* Hutchinson and Fletcher, 2010). A sensitive period is coupled with neural plasticity throughout brain development, during which environmental pressures (either positive or negative stimulatory demands) are greater (Marco et al., 2011). On the other hand, plasticity is essential for brain development through different paths which will, in adulthood, consist in diverse adaptations, depending upon the individual's ecological niche: "During the early post-natal phases the brain is experience-seeking and provided by a considerable plasticity which allows a fine tuning between the external environment and the developing organism" (Cirulli et al., 2010).

Here we define boredom as a state of inactivity and apathy due to lack of stimulation from the animal's surrounding environment. Thus, activities such as environmental enrichment in form of a more complex and social environment, novelty and controllability, are crucial to maintain the (almost) natural behavioural repertoire of the species and reduce self-directed abnormal behaviours and stereotypies (e.g. Buchanan-Smith, 2010; Honess and Marina, 2006). Controllability for instance, has shown to be particularly relevant by the study of Vick and colleagues (2000), where they used three potential enrichment objects for zoo-housed Barbary and stump-tailed macaques, being “the exploration of response potential during interactions with objects (…) more rewarding than the responses *per se*” (Vick et al., 2000: 182)

In conclusion, enrichment promotes natural actions which are necessary to display an ecologically valid behaviour and psychological welfare and enrichment programs must adapt to particular individuals and their requirements (e.g. Videan et al., 2005). Note that physiological well-being and environmental enrichment are not commutable: physiological well-being is a dependent variable, measure after manipulation of environment enrichment, while environmental enrichment is an independent variable, a change to the cage environment (Lutz and Novak, 2005) providing animals with the possibility of recreating their own typical behaviour. Nevertheless, natural behaviour is often inaccurate because there is no single standard for this term (Newberry, 1995). For this study we define natural behaviour as the behavioural repertoire frequently exhibited by wild animals, physical and psychologically healthy, of the same species, with no previous traumas.

Conversely to the assumptions of many authors that abnormal behaviours indicate beyond question a “bad” welfare, Mason and Latham (2004), based on a survey of previous studies, link individual stereotypy routine with enhanced welfare. These authors divide stereotypic behaviour in two categories: “do-it yourself enrichment” and “mantra effect” (calm down through repetition). These ‘habit-like’ behaviours, centrally controlled, are for that matter, unreliable to measure welfare and enrichments that do not instantly decrease them, and they should not be considered to failures:

“Thus environments that induce or increase stereotypy are indeed typically worse than those that do not, but within a stereotypy-inducing environment, the most stereotypic animals are likely to be the least welfare-compromised individuals. However, even this distinction is clearly not the whole story.” (Mason and Latham, 2004: S60).

Bearing in mind this, we must also take in account that such stereotypic behaviours, while a ‘bad sign’, may also represent a linkage to frustration and energy accumulation and therefore caution must be taken when deciding to implement drugs, structures and materials (eg. substances with an unpleasant taste barred in surfaces to prevent biting).

2. Main research goal

"To confirm enrichment actually occurred, evaluation is required to determine whether the well-being of the animals improved and thus whether the strategy was actually effective enrichment" (Hoy et al., 2010: 304).

In order to guarantee captive animal welfare as well as a proper zoo public education service, behaviour research must be undertaken and implemented in daily routine in these institutions.

The study here presented intend to test and evaluate the need for environmental enrichment for three non-human primate species in Maia's Zoo, gibbons (*Hylobates lar*), Mona monkeys (*Cercopithecus mona*) and brown lemur (*Eulemur fulvus*), using time budget to monitor alterations in animal behaviour, which is a widespread method to assess enrichment in literature (de Azevedo et al., 2007).

2.1. *Enrichment devices*

As already mentioned, primates are very energetic animals as they spend most of their time feeding in an active social community. For example, wild moloch gibbons (*Hylobates moloch*) were reported to spend up to 70% of their time foraging (Kappeler, 1984 *in* Wells and Irwin, 2009). Hence, the welfare of these animals depends in our ability to improve captivity conditions in order to mimic life in natural habitat. Apparatus here described acts as a cognitive stimulus and food enrichment, in an attempt to reduce boredom and apathy, symptoms that captive animals are more prom to. Also, to prove its applicability the type of enrichment device chosen must be easy, simple and inexpensive to build. Kerridge (2005: 72) notes that "In practice, zoos must select an enrichment strategy based on their budget, and the reliability and ease of constructing and maintaining the environment". For example, in her survey, Hoy and colleagues (2010) found that regardless of the importance given by the staff to environmental enrichment, providing it is often less frequent than the desired due to limitations on time and other husbandry tasks.

Individual enrichment items which can be lifted up and carried are more prone to stimulate than heavy permanent equipment (Newberry et al., 1988). Also, enrichment devices which had been reported success elsewhere are more easily accepted and implemented (Hoy et al., 20010). With this in mind, one of the feeding devices given to gibbons, Mona monkeys and brown lemurs in this experiment consists in food-filed small pieces of bamboo canes, an alternative to the polyvinyl chloride tubes of the Wells and Irwin (2009) study, designed to promote foraging and activity towards food-processing in moloch gibbons. A similar form, Pvc pipes, were also given by Reinhardt (1990) and Holmes and colleagues (1995) to rhesus (*Macaca mullata*) and cynomolgus monkeys (*Macaca fasicularis*), respectively. Moreover, Maloney and colleagues (2006) prepared a similar apparatus made of paper towel tubes for black (*Eulemur macaco*) and ringtail lemurs (*Lemur catta*). All four studies proved its efficiency as enrichment and encouraged natural habits. In Wells and Irwin study, three feeding devices (food-filled baskets, polyvinyl chloride tubes, frozen ice pops) were given to 4 zoo-housed moloch gibbons that interacted with all three devices in equal number of times but were slower to approach the food-filled tubes, perhaps due to the animal's weak capacity of visualizing the food within. However, the design of the bamboo tube presented to animals in this study differs from the one developed by Wells and Irwin (2009) given it is made of natural material and filled with crushed fruits (activating scent), in an attempt to optimize this enrichment device.

In addition to the bamboo pieces, a wire box filled with fruit and straw is also used in our study, similar to the one used in Maloney and colleagues study (2006). These researchers used three designs (also including a food-filed tubes as mentioned before), but the wire box was the primary device to increase activity budget, decreasing rest and increasing play, although there was no significant difference in feeding behaviour (Maloney et al., 2006). One possible explanation for this result is that the wire box is the device which causes the most handling by the lemurs (Maloney et al., 2006). In addition, grooming and play (social cohesion behaviours) increased during the period in which the researchers presented animals to this item.

Another justification for the use of these enrichment devices is the natural postural activity resulting from manipulation of the devices hanging from trees, recreating a natural arboreal food source (Britt, 1998; Maloney et al., 2006). For example, in a study of several lemurs species (*Varecia variegata variegata* and *Varecia v. rubra*), agonism decreased as the time spent in the tree increased (Zimmermann and Feistner, 1996 *in* Maloney et al., 2006) when forced by the new disposition of the food items into baskets placed in trees, to sit or be suspended from a nearby branch. Also, such postural activity presents an accurate illustration of species physical and locomotor adaptations' to its natural habitat (Britt, 1993 *in* Britt, 1998) and may serve as education purpose for the public (Kerridge, 2005).

The reason why two different enrichment devices are used in this experiment is to prevent animal's habituation towards those device and consequently to take most advantage of each enrichment. This matter is a primary issue of importance to several authors (Bayne, 1989; Paquette and Prescott, 1988; Maloney et al., 2006) as it can avoid animal boredom to a constant contact to an item, which intention was to create change and diversity in the environment. For example, Maloney and colleagues suggested that, for lemurs, the arbitrary granting of enrichment items presents stimulus without being over stimulating.

2.2. Species subject

The present study species subjects were chosen for several reasons:

1. Observation *ad libitum* to all primates' residents in the Zoo suggested that gibbons, lemurs and Mona monkeys were the individuals most inactive.
2. Both Mona monkeys and the female brown lemur, presents stereotypic behaviour. Note that such behaviour (among others) is frequently a sign of poor welfare, although this assumption is not fully understood (Mason and Latham, 2004). This may be a consequence of the amplified handiness of spare time (Britt, 1998) and which may decrease by environmental enrichment (e.g. Kerridge, 2005; Márquez-Arias et al., 2010).
3. All three groups have equal number of individuals.
4. Gibbons form a pair as well as brown lemurs, a similar group composition to the one we can observe in natural habitat. On the other hand, Mona monkeys group's is composed by two males. However, this species frequently forms closely bonded all-male groups, unlike no other Cercopithecus species (Glenn et al., 2003). Also, several authors support that the sex of the individuals is not related to their response to enrichment programs (lemurs: Maloney et al., 2006; stumptail macaques: Márquez-Arias et al., 2010).

5. Three different species from three different taxonomics groups, a prossimion (brown lemur) and two anthropoid species, a cercopithecus (Mona) and an ape (gibbon), will allow comparisons, in terms of welfare and enrichment influence.

2.3. *General characterization of the three study species*

Hylobatids

"The pristine image of an infant clutching its mother's chest as she soars through the canopy is fading. It is more likely for one to catch a glimpse of the infant gibbon clad in baby clothes, scampering through the streets of Phuket for the exploitative tourism market" (Gray, 2012: 45)

Hylobatids are known to be the smallest apes, native of Indonesia (Sumatra), Peninsular Malaysia, Thailand, Myanmar and Lao People's Democratic Republic rainforest (see illustration 1).

Accordingly to IUCN, Hylobatidae has 4 genus (Hoolock, Hylobates, Nomascus and Symphalangus). Hylobates has 7 species, including the white handed gibbon, *Hylobates lar*, one of our species' study (see Figure 2). This species distribution can be observed in Figure 1.

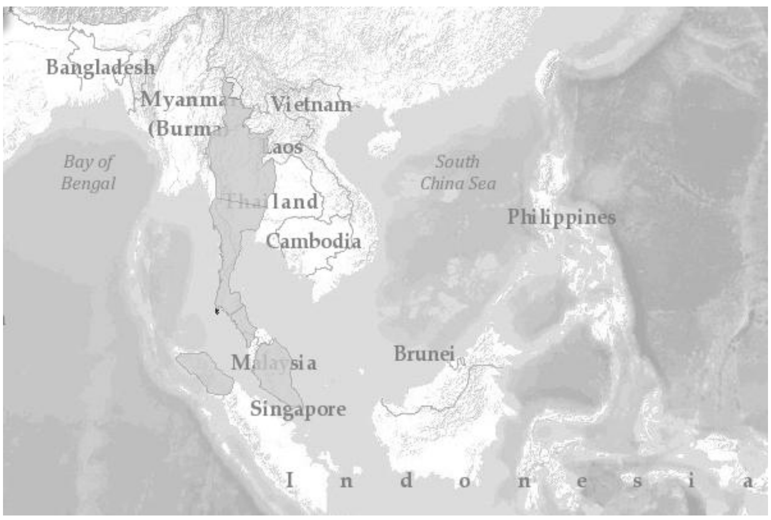

Figure 1. Geographic distribution of *Hylobates lar* (adapted from IUCN 2008). IUCN (International Union for Conservation of Nature) 2008. *Hylobates lar*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Accessed 21/03/2013.

Recent studies on molecular data show that genetic differences between the hylobatid species are superior to the ones seen between chimpanzees and humans (Roos and Geissmann, 2001).

Figure. 2. Male gibbon, Ágil.

Anatomically, white handed gibbon is characterized by long upper limbs, a physiological specialization which denounces them as brachiators (see Figure 3), the perfect locomotion for highly arboreal species such as this species. In addition to the longest forelimb in living primates, gibbons have elongated muscular pollex and hallux, and long and curved fingers and toes that act like a “hook” to impel the animal throughout the branches (Gray, 2012).

Figure 3. Female gibbon, Maya.

Concerning behaviour, gibbons are frugivorous, they spend most of their time foraging for leaves, stems, shoots, insects and birds’ eggs.

Social behaviour (mostly grooming, play and physical contact) may sometimes occupy of one fifth of the activity budget in gibbons (Bartlett, 2009 *in* Gray, 2012), an important aspect of daily life that promotes bonding between the individuals. As well, their famous melodious song bouts and duets reinforce pair bonding and uphold territory (Gray, 2012). However, this characteristic is not common in all Hylobates species (Whittaker et al., 2007).

With a life span of 20- 40 years (Palombit, 1995), gibbons reach maturity by the age of 8, in the wild, or earlier (5 years) in the case of captive individuals who grew with an older mate (Geissmann, 1991).

Data on dispersal in wild groups is scarce but Barrelli and colleagues (2007) reported two sub adult female *Hylobates lar* who leaved their group at the age of 9 and Brockelman and partners (1998) also aknowlege the dispersal of six sub-adult males, also *Hylobates lar*, with approximated 10.5 years of age. This may suggest that the dispersal take place two years following sexual maturity (Burns et al., 2011).

Hylobates are mostly monogamous, but their social and matting patterns may present significant plasticity and other social organization than monogamy may occur (*Symphalangus syndactylus*, Lappan 2007; *Hylobates lar*, Barelli et al. 2008) generally achieved by retention of the maturing offspring, with reciprocal benefits (e.g. Brockelman et al, 1998) indicating that social structure may be influenced by selective pressures and resources, and besides to improve parent investment (Lappan, 2007). Females give birth to a single offspring every 4-5 years (Palombit, 1995). However, population is decreasing rapidly. Brockelman's and Geissmann's Red List review (2008) state that white-handed gibbon population has declined 50% within the past three generations (45 years) and hunting replaced habitat loss as the main threat to the survival of this species and placing this species as Critically Endangered. Moreover, gibbons are commonly used as pets, removed from their family at a young age, and abandoned or confiscated by authorities once they reach sexual maturity. At this time, they are too strong and aggressive to be kept as pet (Gray, 2012). Large profits, even with legal protection to this species, delay the effective action to this trade.

Mona monkey

Accordingly to the IUCN Red List, there are 18 species of the genus Cercopithecus: *C. ascanius, C. campbelli, C. cephus, C. diana, C. dryas, C. erythrogaster, C. erythrotis, C. hamlyni, C. lhoesti, C. mitis, C. mona, C. neglectus, C.*

nictitans, *C. petaurista*, *C. pogonias*, *C. preussi*, *C. sclateri* and *C. solatus*.

Our species subject, *Cercopithecus mona,* is a Old World guenon monkey ranging 2 to 6 kg (between 32 to 53cm), with males slightly larger than females. They have a long tail, up to 90 cm (Napier, 1981 *in* Rowe, 1996). They've "a brown agouti back with a white rump. The face is bluish gray, with a pink muzzle and a dark stripe from each ye to each ear. The cheek hair is yellowish, and the forehead is a yellowish white. The underparts and the inside of the leg are white. The outside of the leg is black, as the tail (MacDonald, 1985 *in* Rowe, 1996) (see Figure 4).

Figure 4. Male Mona monkey, Longa.

This is a natural widespread species in southwest Africa, in rainforest of Benin, Cameroon, Ghana, Nigeria and Togo and was also introduced in Grenada, Saint Kitts and Nevis and São Tomé and Principe (see Figure 5).

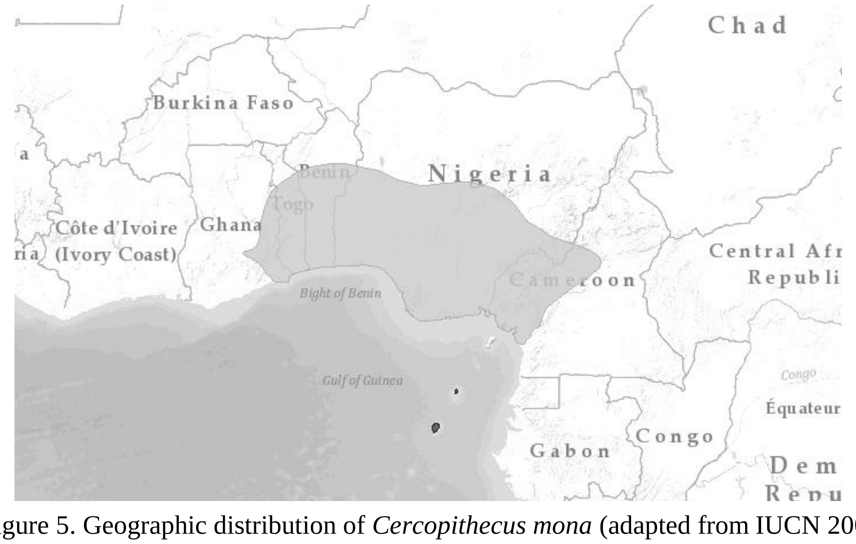

Figure 5. Geographic distribution of *Cercopithecus mona* (adapted from IUCN 2008). IUCN (International Union for Conservation of Nature) 2008. *Cercopithecus mona*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Accessed 02/05/2013.

Mona is most thriving in gallery and river zones, with groups with a territory ranging from 5 to 50 acres. However, this species is characterized by its great resilience, being subjected to a significant habitat loss and hunting, Mona monkey has the capacity to adapt to less appropriate habitat (Oates et al., 2008). Such flexibility is probably the main factor for its conservation status, Least Concern, although the population trend is unknown (Oates et al., 2008).

Mona monkeys are omnivorous, mainly fruits, sprouts, leaves and vertebrates. Interesting, monas monkey cheek pouches' capacity to store food is almost as large as their stomach, giving these animals of a significant role on seed dispersal. Their predators are leopards, golden cats, crested eagles and pythons (Liu, 2000).

Concerning behaviour, Monas monkeys are arboreal and very active and agile, travelling in troops on average of 12 individuals, but may range to 50. Such large groups may be the result of temporary arrangements of small groups combined. They are known to be very loud, with males vocalizing to show territory and rank (Gautier, 1988 *in* Rowe, 1996). Aggression among male guenons developed as a result of competition for access to females and that it inhibits male-male social bonding. However, males Monas have been observed to form all-male groups. "No other Cercopithecus species regularly forms as closely bonded all-male groups as those of *C. mona*" (Glenn et al., 2002: 140).

They are polygynous although not much is known about their mating behaviour. Generally, females give birth to one young after 5 to 6 months of gestation, every 2 years (Liu, 2000). They have no prominent sexual swelling. Weaning come about around one year of age and sexual maturation around 2 to 5 years in both sexes (lifespan in approximately 22 years) (Ross, 1998 in Rowe, 1996).

Brown lemur

Malagasy prossimians diverge from anthropoids by several physical and social features such as their lack sexual dimorphism, cyclic breeding and female dominance but have also some similar particularities concerning social behaviour such as female philopatry, organized societies and individual identification (e.g. Palagi and Dapporto, 2006) which is believed to be a requirement for reconciliation (Aurelli et al., 2002).

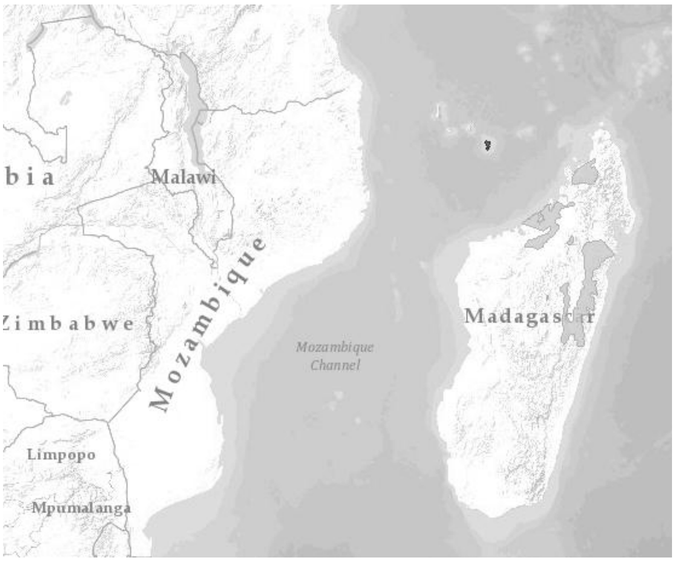

Figure 6. Geographic distribution of *Eulemur fulvus* (adapted from IUCN 2008). IUCN (International Union for Conservation of Nature) 2008. *Eulemur fulvus*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Accessed 09/04/2013.

According to the IUCN Red List, the genus Eulemur has 6 species (*E. albifrons, E. cinereiceps, E. collaris, E. fulvus, E. rufus* and *E. sanfordi*). Eulemur fulvus habits tropical forest of Madagascar in three populations (see Figure 6) of which, one is isolated in the Ambohitantely Special Reserve. In sum, E. fulvus is reported in four national parks (Ankarafantsika, Mantadia, Andringitra and Zahamena) and nine reserves (Tsaratanana, Zahamena, Ambatovaky, Ambohitantely, Analamazaotra, Bora, Mangerivola, Manongarivo and Tampoketsa-Analamaitso) (Mittermeier et al., 2008). Additionally, they were introduced in Mayotte (Comores) (Mittermeier et al., 2008).

Eulemur species are characterized by their small-sized body (1.2-2.5 kg), cathemeral activity, quadrupedal and arboreal locomotion and sexual dichromatism, with the exception of *Eulemur fulvus* (Mittemeier et al., 1994 in Mittemeier et al., 2008) a species with both male and female with brown to dark-gray bodies and dark faces and light beards (see Figure 7).

Eulemur species reach sexual maturation in about 1-2 years of age, breeding season occurs in June and July (late May in the case of Sanford's lemurs), and after a gestation period of 120 days, female give birth to a single infant which is weaned 4-6 months after. Their life expectancy is 36 years in captivity and 20-25 in the wild (e.g. Mittermeier et al., 1994 *in* Rowe, 1996).

Eulemur fulvus can weigh 2.6 kgs and their body and tail length are 50 cm each.

They occur in groups of 3-12 individuals (multi-male and multi-female) in territories of 7.20 ha in tropical and montane forest in Madagascar (Mittermeier et al., 2008). They eat mainly fruit (major role as seed' dispersals), flowers, leaves, but they also predate insects and bird nests and are prey for others species, affecting animals' population as well (Overdorff, 1993). However, Madagascar may present seasonal resource's constrains, which results in unpredictable food supplies, possible raison d'être for the atypical social hierarchy of these primates, the female dominance (front burner in a feeding situation) (Simmen et al., 2010).

Concerning behaviour, *E. fulvus* exploit grooming (to ascertain social bonds) with a distinctive technique, a dental comb (their 6 lower procumbent teeth) (Bharti, 2000). Also important for communication in these animals is olfact, possible by scent glands situated at the wrist throat, which may communicate physical condition, place and individual recognition (Bharti, 2000).. Brown lemurs scent-mark during sexual behaviour, alarm response to human observers, territorial defense and travel (Harington, 1979 *in* Rowe, 1996). Males have strong smelling secretions from their scrotum. Brown lemur scent has been experimentally shown to identify of the marker but not its subspecies" (Harington, 1979 *in* Rowe, 1996). On the other hand, vocal communication is not fully understood but territorial and alarm calls are already known (Sussman, 1977).

Figure 7. Male and female brown lemur at Maia's Zoo.

Population densities range from 40-60 individuals/Km (Mittermeier et al., 2008) and has a decreasing trend (Near Threatened) due to human pressures as agriculture (slash-and-burn practices), logging and charcoal production. Moreover, hunting is increasing and in some cases, capture of entire groups occurred (Andrainarivo et al., 2008).

Madagascar had become an eco-tourist destination with great benefits four local community and yet, lemurs are still hunted for their meat.

2.4. *Predicted results*

With the proposed implementation of enrichment devices we had the following predictions for the three species of non-human primates:

- Foraging behaviour increase (attempt to mimic the time spent in foraging by wild animals) as well as interaction with the enrichment devices. Previous studies showing that generally enrichment reduce inactivity level's (Honess and Marina, 2006; Maki et al., 1989) along with others that confirm increased activity levels with enrichment devices (Bloomsmith et al., 1988; Brent et al., 1991).
- Inactivity and locomotor activity decreases, due to the enrichment manipulation,
- Diminished attention to public, due to the enrichment manipulation,
- Social interactions:
    - affiliation and grooming may increase during the enrichment period with a single wire box - hypothesis of proximity between individuals through tasks as an important factor for immediately and positive social relations;
    - on the other hand, agonism may also increase when given to animals a single wire box_ increased level of agonistic dominance behaviours explained by competition over access to food's device (Bloomstrand et al., 1986).

We had also predicted some changes at the species/group level:

- Gibbons are the one group who interact more with enrichment devices, following Mona monkeys and then lemurs – gradual difference due to cognitive capacities (to test if interaction with enrichment objects and devices increases with cognition).

- Decrease in Mona monkeys's stereotypic behaviour_ previous studies suggest that enrichment programs implementations can reduce abnormal behaviours (Márquez-Arias et al., 2010) and, more specifically, addition of food items to the enrichment as a foraging device can reduce abnormal behaviour frequency in chimpanzees (e.g. Bloomsmith et al., 1988).

3. Methodology

3.1. *Location and study sample*

The three species chosen to be subjects for this study are housed at the Maia's Zoo, north of Portugal. The Zoo also has five other non-human primate species, in a total of eight species of primates.

3.1.1. *Enclosures descriptions*

Gibbons (*Hylobates lar*) are housed in an island' type enclosure (Figure 8), having a border with the marine lion's pull (which get most of the public attention especially during shows and demonstrations) and being near the Zoo's bar esplanade (Figure 9). Evergreen vegetation inside the enclosure offer several hiding places at numerous levels of arboreal substrate, for the two existing animals. In addition, it also has many artificial structures (ropes, wood vertical ladder and small huts as shelters) promoting vertical locomotion, brachiation, hiding places, play and rest. Sun light is direct and it doesn't depend on the time of the day.

Figure 8: Gibbons habitat (showing vegetation and structural enrichment).

Figure 9: Surrounding of the gibbons habitat (border with the bar esplanade and the sea lion pull).

Mona monkeys (*Cercopithecus mona*) are housed in a more traditional enclosure (room with three concrete walls and one glass for exhibition). In Mona monkeys' enclosure, few branches and trunks are present and a small shelter function as a dorm for the animals, soil substrate, little direct sun at the end of the day (Figure 12).

Brown lemurs (*Eulemur fulvus*) are housed in an enclosure similar to the Mona ones (figure 10 and 11) although brown lemurs enclosure is smaller with no shelter, soil substrate and with some evergreen vegetation, although may not be enough to guarantee hiding. Sun light (indirect) reaches this enclosure at the afternoon so a lamp heater is provided to the animals.

Figure 10. Brown lemur habitat.

Figure 11. Brown lemur habitat's vegetation.

Figure 12. Mona monkey habitat.

3.1.2. *Individuals descriptions (name, age, sex)*

Each group/species has two individuals, a pair of gibbons, a pair of brown lemurs and two male Mona monkeys. Individuals' information (sex, names and backgrounds) are described in table 1. Ages of all six individuals are unknown but gibbons and brown lemurs are more than 20 and Mona monkeys are around 10 years old.

Table 1. Individuals distribution by species, name, sex and background.

Species	Name/sex	Background
Hylobatides lar	Ágil ♂	Other zoo (without documents)
	Maya ♀	Other zoo (without documents)
Cercopithecus mona	Cauda curta ♂	Private house
	Cauda longa ♂	Private house
Eulemur fulvus	Mr. Piggy ♂	Other zoo (without documents)
	Mrs. Piggy ♀	Other zoo (without documents)

3.1.3. *Routine descriptions*

Daily routine in Maia's Zoo is quite simple for animals. Primates are given meals twice a day, once in the morning (around 11 a.m.) and another in the afternoon (around 4 p.m.). Food (fruits and vegetables) is thrown directly in the gibbon's enclosure and placed on the ground of the other two groups. Monas' enclosure is cleaned every day, before the first meal. Gibbons' and brown lemurs' areas are cleaned once or twice a week, with no fixed schedule. Individual's routine can also be disrupted by public attendance, although, in Mona monkeys and brown lemurs case, individuals generally ignore people, other than the zoo keepers.

Enrichment devices implemented by this study were given to individuals every experimental day at the usual feeding time for several reasons: 1) because "food related events temporally predictable is an effective way to decrease the stress associated with both waiting for and experiencing these events" (Gottlieb et al., 2012: 7); 2) to ease keepers work and therefore convince them to implement in future such husbandry practices. Enrichment devices stayed in subjects' enclosures all day long.

3.2. *Materials and task*

3.2.1. *Schedule*

The details of the chronogram of this study are described in Table 2, as well as the percentage of the time spent in each period in Chart 1 and the organization of the study in phases, their duration and the number of hours of observation is in Table 3. Observations and registration of the behaviour started on December, 26th with a three-week Baseline phase (40 observation sessions - animals are not provided with any type of enrichment). A three-week treatment phase followed, in which animals received Bamboo canes filled with food paste as enrichment device (40 observation sessions), described in section 3.2.2. Following this period, there was in a one-week period (5 days) of interval, before the next enrichment phases, in which individuals were also observed and behaviours recorded to access immediate effect of the previous enrichment (First Immediate Effect Baseline, 15 observation sessions). After this, a second three-week enrichment treatment was implemented in animal's enclosure (Wire Boxes with straw and fruits, 40 sessions, described in section 3.2.2). Again, immediate effect of this device was recorder during a one-week period after the last enrichment phase (Second Immediate Effect Baseline, 15 observation sessions). To finalize experimental observations, a three-week post-treatment phase was also accomplished to perceive, or not, subject's new behaviours tendencies after several weeks of enriched environment.

Table 2. Chronogram, indicating study dates appointments and the correspondent percentages of time spent in each one.

Topic	Starting	Ending	Days	% of time
Volunteer work	October, 8th	October, 20th	7	2,89
Searching for literature/writing	October, 21st	December, 24th	60	24,79
Observation	December, 26th	April, 29th	123	50,83
Results analyzes and thesis writing	May, 1st	June, 10th	40	16,53

Total	September, 20th	June, 10th	242	100

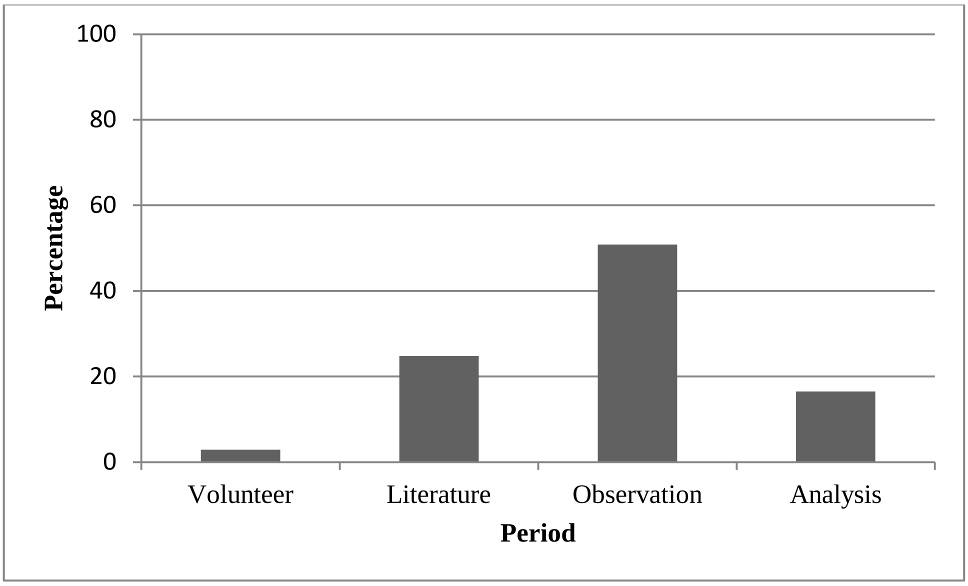

Chart 1. Graphic representation of time (percentage) correspondent to each study's phase.

The differences in observation's dates for each species were due to zoo routine management (see Table 3).

Table 3. Observation's distribution concerning the phase of the study and the number of hour of observation.

Topic	Hylobates lar	Cercopithecus mona	Eulemur fulvus
Observation (Baseline, 40 sessions)	December, 26th	February, 5th	December, 26th
Observation (Bamboo, 40 sessions)	January, 30th	February, 6th	January, 30th
Observation (First Immediate Effect Baseline, 15 sessions)	March, 3rd	February, 27th	February, 27th
Observation (Wire Box, 40 sessions)	March, 20th		March, 13th
Observation (Second Immediate Effect Baseline, 15 sessions)	April, 14th	April, 10th	April, 10th
Observation (Post-Baseline, 40 sessions)	April, 19th	April, 16th	April, 16th
Results analysis	April, 30th	April, 30th	May, 1st
End	June, 10th	June, 10th	June, 10th
Total (hours)	95	95	95

3.2.2. *Apparatus, Descriptions and Procedure*

As mentioned above, the present experiment used pieces of bamboo canes (Figure 13) filed with food-paste as a feeding device for enrichment. Unlike the Wells and Irwin (2009) experiment with the polyvinyl chloride tubes, the bamboo tubes used in this experiment were not perforated with circular holes. Bamboo canes were collected from grown bamboo plants in the Zoo installation's and were cut in pieces (10 to 15 cm long) for this study. It took approximately 10-15 minutes to clean and refill an assemblage of 6 pieces (one piece per subject for a total of 6 subjects). Therefore, the cost and the time spent planning and preparing these enrichment devices were minimal.

The food paste was composed by fruit and vegetables and the amount of the food used to fille the tubes was taken from the daily normal food rations for the animals to prevent overfeeding. Hence, the same amount of alimentary items was provided to the animals in control and experimental conditions.

Figure 13. Bamboo canes pieces used in the first enrichment treatment.

In addition to the bamboo canes, the present experiment used a wire box (Figure 14) filled with fruits and straw as well. This device was made using net wire available in the Zoo for several other purposes besides enrichment. The food placed in the device consisted in an entire meal (the first portion of fruit and vegetables given to the animals in the morning).

Figure 14. Enrichment devices consisting in wire boxes which will be filled with straw and fruit and positioned inside subjects' habitat (one per group).

As a final note, subjects in this study have not experimented before enrichment devices such as the ones described above, constructed and implemented by this study for the first time.

3.3. *Data collection*

Behavioural assessment is the most used method by researchers to evaluate environmental enrichment, because it requires no scientific licenses, little apparatus are necessary and it's not an evasive procedure (de Azevedo et al., 2007). Also, behaviours grants knowledge on an individual's needs, desires and internal condition (Mallapur, 2008). For that matter, this project used exclusively observation and the recording of subjects' behaviour with a stop watch.

Variables were selected and defined in an ethogram shown in Table 4. A data field sheet' (with behaviours as variables) was also used to support registration notes. During each session, the two subjects were observed and scanned simultaneously and their behaviour recorder during each 30-minute period, with scan sample (with 30 seconds' intervals). Atypical happenings were also noted as written comments.

Table 4. Ethogram.

Behaviour	**Definition**
Feeding	Search for (foraging), collecting, manipulating, chewing and ingesting food and liquids.
Locomotion	Walk, run, jump, climb or brachiating from a point A to a point B, without other action performed (ground or on climbing apparatus).
Inactivity	Stay still, sleeping or resting, alone or closer to other individual. Eyes may be open or closed. Maintaining dorsal, ventral or lateral contact with the surface (Wells and Irwin, 2009).
Vocalization	Producing vocal sounds. Individual may also be inactive, resting, feeding, grooming, etc.
Affiliation	Can occur play, chasing, tumbling and sharing food.
Agonistic	Aggressive interaction: a) Dominance: exhibition of a higher status by charge, threat, intimidation and/or aggression (standing straight, pilo erection). b) Submission: exhibition of a lower status by defense, avoidance, pullout and/or hide (crouching, bowing, presenting back) and beg for food. c) Others: neutral exhibition like pacification, intermediation, consolation and frustration.
Grooming	Social bounding behaviour: a) Self-grooming (Self-groom): an individual picks his own dirt and ticks and/or brushes his hair or skin with the mouth and/or hand(s). b) Social grooming: cleaning another individual's hair or skin with mouth and/or hand(s) and request for grooming.
Sexual behaviour (Sex)	Genitalia presentation, inspection, solicitation, masturbation and/or copulation.
Abnormal behaviour	Non-typical behaviour of the specie a) Stereotypic: repetitive movement without any objective or purpose (pacing, rocking, head shaking). b) Self-injurious behaviour: self-inflicted wounding (hair pulling, mutilation). c) Not stereotypic: a non-typical behaviour of the specie besides stereotypes and self-injurious behaviour (chewing food).
Enrichment object's manipulation	Manipulation (touching, holding, pulling) of the objects introduced by this study.
Other object's manipulation (Object)	Manipulation of objects that were not introduced by this study, i.e., branches, leaves and ropes.
Human interaction	Visual contact and communication with humans: a) Vocal (Hum vocal): an individual follow and try to communicate with humans by vocalizing or asking for food.

	b) Negative (Hum -): an individual throw stones and dust to humans; displays; c) Neutral (Hum 0): an individual follow and steers at humans.
Not visible (N. V.)	Out of the observer's sight.
Others (“Others”)	Any behaviour (social or individual) not described by this catalogue.

Husbandry management was consistent across experimental days of observation. Behaviour registrations begin around 9.30 a.m. Attention to the observer was minimal due to habituation.

Three 10-minute videos (one for each group) were recorder in order to access within observer reliability. These video clips were coded and analyzed. Behaviours were registered twice for each video and differences between the two time were used to calculate the intra-observer reliability test (Martin and Bateson, 2007) by using the index of concordance with at least 85% intra-observer reliability (98% for gibbons and brown lemurs and 93% for Mona monkeys). Moreover, to ensure continuous viability during the entire study, this procedure was repeated regularly throughout enrichment and baseline baselines’ phases.

3.4. *Data analysis*

To verify or disconfirm significant differences between the two groups’ and individual’s behaviours throughout the experiment, the software SPSS version 20 was used for the statistical analysis.

Not only behaviours toward enrichment devices are to be summed but also each behaviour on the ethogram, providing a general rate of recurrence per animal per behaviour. We look for significant changes in each behaviour. Data normality was tested with the Shapiro-Wilk test. As data is non-normally distributed we used non-parametric methods of analysis. Session by session was analyzed using a Kruskal-Wallis test (comparing multiple independent samples). The significant differences (P< 0,005) given by this test were then explored by Mann-Whitney U test (comparing two conditions to time) in order to verify enrichment influence over animals’ behaviour.

4. Result

Since we are dealing with three different species, that frequently exhibited different behavioural repertoires and frequencies, and the total number of sessions in each condition is not the same, we calculated rates and mean dispersion (*per* session). These results are the values being presented in the following sections (4.1 and 4.2).

The number of behavioural occurrences in each condition for each individual is shown on Table 14 (Appendix).

4.1. *Results by species*

4.1.1. *Hylobates lar*

Table 6 and Chart 2 show the total sum per phase of relative frequency per session of the behaviour exibitted by the two gibbons. Chart 2 shows only the behaiours with significant alteration during the experiment. It shows that Inactivity is the most frequent behavioural patterns in all the six phases of the project. However, it decreased in Bamboo and in the following stages and reached its lower value during Post Baseline. Feeding behaviour is the second more frequent behaviour in Gibbons' routine. It slightly increased during in the Wire box condition. "Non-visible" behaviour increased evenly throughout the experiment, with the least value in Baseline and the highest in Pos-baseline. Neutral Human Interaction had also a consistently increase during the project. On the other hand, Locomotion had an increase during Wire Box but declined again in the Post-Baseline phase.

Grooming decreased during the Bamboo phase, comparing to the Baseline. Nevertheless it increased again in the Wire box, to a value close but higher than to the baseline, again in the 2CEI and then it decrease in the Post-Baseline. "Others" behaviour also increased during the experiment, especially in the Wire box phase, weigh against Baseline. On the other hand, Vocalization behaviour had a decline throughout the project, with a pick value during the Baseline and a decrease in the following phases. Oppositely with seen in the wild and characteristic to the species, our subjects did not engage in sing duets. The female was the only one to sing while the male only made calls, which stop immediately when the female starts to sing. This usually happens in the morning, but sometimes also in the afternoon.

The remaining behaviours did not have an important change and their frequency was low.

Table 5. Relative frequency and average per minute in Gibbons' behaviour observed in the experiment (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity*	105,20	2,63	89,97	2,25	35,63	2,38	71,97	1,80	27,10	1,81	69,37	1,73
Human 0*	1,67	0,04	9,13	0,23	3,67	0,24	11,00	0,28	7,07	0,47	15,77	0,39
Grooming	2,07	0,05	0,40	0,01	0,67	0,04	2,67	0,07	1,27	0,08	1,40	0,04
Others*	0,37	0,01	1,40	0,04	0,60	0,04	2,57	0,06	0,80	0,05	2,17	0,05
Affiliation	0,40	0,01	0,10	0,00	0,07	0,00	0,20	0,01	0,07	0,00	0,23	0,01
Dominance	0,10	0,00	0,20	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,00
Submission	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding	16,77	0,42	16,67	0,42	6,10	0,41	21,63	0,54	6,80	0,45	19,23	0,48
Ab. Behaviour	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Human -	0,33	0,01	0,00	0,00	0,00	0,00	0,40	0,01	0,07	0,00	0,70	0,02
Human vocal	0,93	0,02	2,10	0,05	0,43	0,03	2,73	0,07	1,13	0,08	2,37	0,06
Objects	1,40	0,04	2,20	0,06	0,37	0,02	1,27	0,03	0,93	0,06	1,80	0,05
Locomotion	9,33	0,23	10,93	0,27	3,13	0,21	12,60	0,32	3,53	0,24	7,57	0,19
N. V.	10,47	0,26	12,23	0,31	8,40	0,56	15,50	0,39	10,47	0,70	35,00	0,88
Self-grooming	0,93	0,02	2,27	0,06	0,77	0,05	2,30	0,06	0,73	0,05	2,50	0,06
Sex	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalization	12,67	0,32	8,40	0,21	1,17	0,08	5,07	0,13	1,03	0,07	4,47	0,11

Gibbons together presented significant alterations (*) between phases in Inactivity (x^2= 50,44; p= 0,000), Neutral Human Interaction (x^2= 69,60; p= 0,000) and "Other" behaviour (x^2= 25,00; p= 0,000).

Gibbons' inactivity had a significant decrease during both Bamboo (U=2344,00; p=0,003) and Wire Box condition (U=1687,00; p=0,000), comparing to the Baseline period. This effect was maintained during the Post-Baseline phase (U=2165,000; p=0,000) comparing to the Bamboo phase.

Gibbons had no significative alteration on feeding behaviour during the entire study and they never presented stereotypic behaviour or another abnormal behaviour, except the high inactivity rate.

On the other hand, gibbons had a significant increase of Neutral Human Interaction during the enriched periods of Bamboo (U=1602,000; p=0,000) and Wire box (U=1525,00; p=0,000) and even in Post-Baseline phase (U=228,43: p=0,000).

This species showed also a significant increase in "other" behaviour during Wire Box period (U=2304,500; p=0,000) comparing to the Baseline.

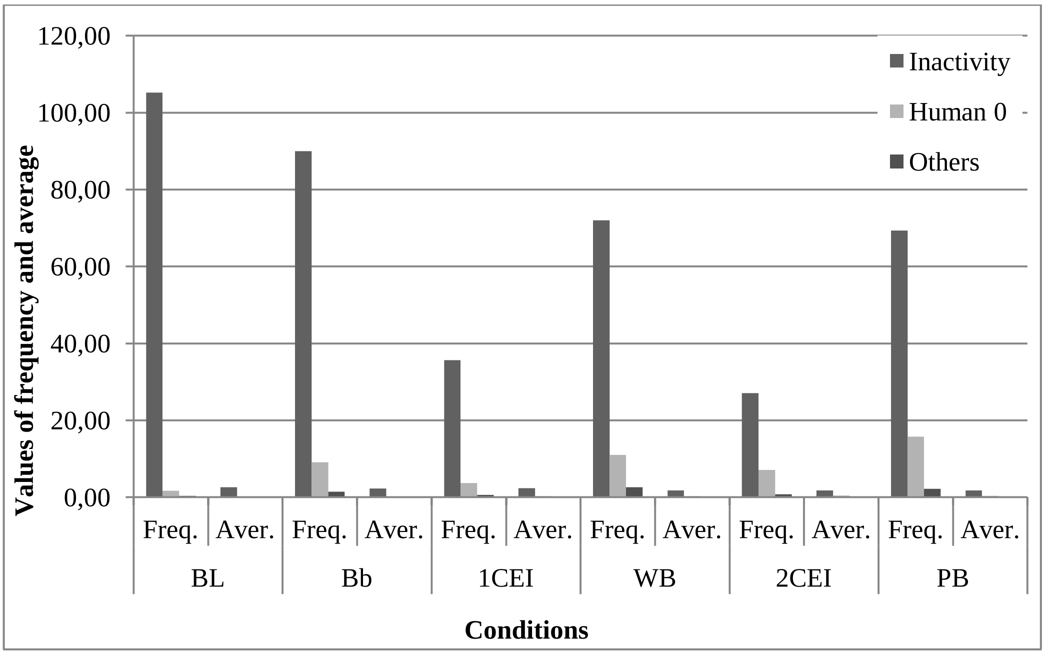

Chart 2. Behaviours with a significant alteration during the experiment.

4.1.2. *Eulemur fulvus*

Table 7 and Chart 3 show the total sum per phase of relative frequency per session of the behaviours exibitted by the two lemurs. Chart 3 shows only the behaviours with significant alteration during the experiment. Inactivity was undoubtedly the most frequent behavioural pattern in every phase of the project. It was higher in the Baseline, declined in Bamboo and stabilized during the following stages and slightly increased again in the Post-Baseline phase.

The second most frequent behaviour, although in must less frequency than Inactivity was Locomotion which had not a significant alteration during earliest phases, but had a noteworthy decrease in the Wire box and subsequent periods. Next, we see that Grooming was stable during the entire study, with similar frequencies in all conditions. Feeding behaviour was lower in Baseline and 2CEI, but a little higher in the following stages, with a pick in Wire Box. Stereotypic behaviour was greater in the Baseline, but had an important decline in the following phases particularly in the Wire box stage. On the other hand, other abnormal behaviours were not registered in the entire study. Non-visible behaviour, like stereotypic behaviour, was higher during the Baseline but it did reduced significantly in the succeeding conditions particularly in the three last phases of the project. Self-grooming on the contrary, was lower in the Baseline and increased in remain periods. Vocalization followed the same pattern, with the lower frequency in the Baseline and increased values during the in the subsequent phases. Neutral Human Interaction had also a minor frequency in the Baseline and increased in Bamboo and 1CEI, declined again in the Wire Box and increased in the latest stages. Conversely, Vocal Human Interaction and “Others” behaviours maintained low frequency during all experiment.

The remaining behaviours did not have an important change or if they had (Submission and Sex) their frequency was low.

Table 6. Relative frequency and average per minute in Brown lemurs’ behaviour observed during the experiment (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity	121,90	3,05	108,90	2,72	40,57	2,70	107,70	2,69	39,60	2,64	114,40	2,86
Human 0*	1,03	0,03	9,17	0,23	3,53	0,24	5,27	0,13	6,27	0,42	9,77	0,24
Grooming	10,80	0,27	10,70	0,27	3,83	0,26	10,53	0,26	4,50	0,30	10,83	0,27
Others	0,57	0,01	1,03	0,03	0,47	0,03	0,67	0,02	0,33	0,02	1,70	0,04
Affiliation	1,60	0,04	1,00	0,03	0,47	0,03	1,40	0,04	0,73	0,05	0,67	0,02
Dominance	0,03	0,00	0,10	0,00	0,00	0,00	0,07	0,00	0,03	0,00	0,00	0,00
Submission	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00
Feeding	3,40	0,09	5,90	0,15	3,70	0,25	12,50	0,31	2,60	0,17	7,87	0,20
Ab. Behav.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy*	4,20	0,11	0,90	0,02	0,40	0,03	0,07	0,00	0,20	0,01	0,93	0,02
Human -	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
H. vocal*	0,20	0,01	0,17	0,00	0,23	0,02	0,20	0,01	0,53	0,04	1,20	0,03
Objects	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Locomotion	11,37	0,28	11,47	0,29	4,90	0,33	7,90	0,20	2,70	0,18	8,37	0,21
N. V.*	3,17	0,08	1,33	0,03	0,50	0,03	0,20	0,01	0,10	0,01	0,13	0,00
Self-groom*	2,77	0,07	4,73	0,12	1,63	0,11	5,87	0,15	1,90	0,13	4,13	0,10
Sex*	0,77	0,02	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalization*	0,83	0,02	2,40	0,06	0,77	0,05	3,53	0,09	1,47	0,10	2,67	0,07

Analyzing both Brown lemurs individuals, we see significant changes (*) in Stereotypic behaviour (x^2= 27,24; p= 0,000), Neutral Human Interaction (x^2= 92,817; p= 0,000), Vocal Human Interaction (x^2= 21,04; p=0,001), Non-visible behaviour (x^2=81,00; p= 0,000), Self-grooming (x^2= 21,35; p= 0,001), Sexual behaviour (x^2= 26,53; p= 0,000) and Vocalization (x^2= 53,22; p= 0,000).

Brown lemurs only showed an important decrease on inactivity during Bamboo phase (U=2360,500; p=0,004) comparing to Baseline period and also a significant decrease in stereotypies in Wire Box period, both comparing to Baseline (U=2437,00; p=0,000) and Pos-Baseline (U=2801,50; p=0,003).

They had a significant increase in Feeding behaviour during Wire Box (U=2100,500; p=0,000) comparing to Baseline.

In Neutral Human Interaction they showed a increase in Bamboo (U=1093,50; p=0,000) in the Wire Box condition (1538,00; p=0,000), both comparing to the Baseline. Moreover, we see that lemurs had a larger increase during the first enriched period, Bamboo, than the second, Wire box (U=2396,50; p=0,005).

This group showed a significant increase in Vocalization during both Bamboo (U=1760,00; p=0,000) and Wire Box (U=1720,00; p=0,000) comparing to the Baseline period.

Brown lemurs were also the only group who presented a significant increase in self-grooming during the Wire Box condition (U=2024,00; p=0,000) comparing to the Baseline, and a significant decrease in Sexual behaviour during the Wire Box stage (U=2840,00; p=0,002) comparing to the Baseline also.

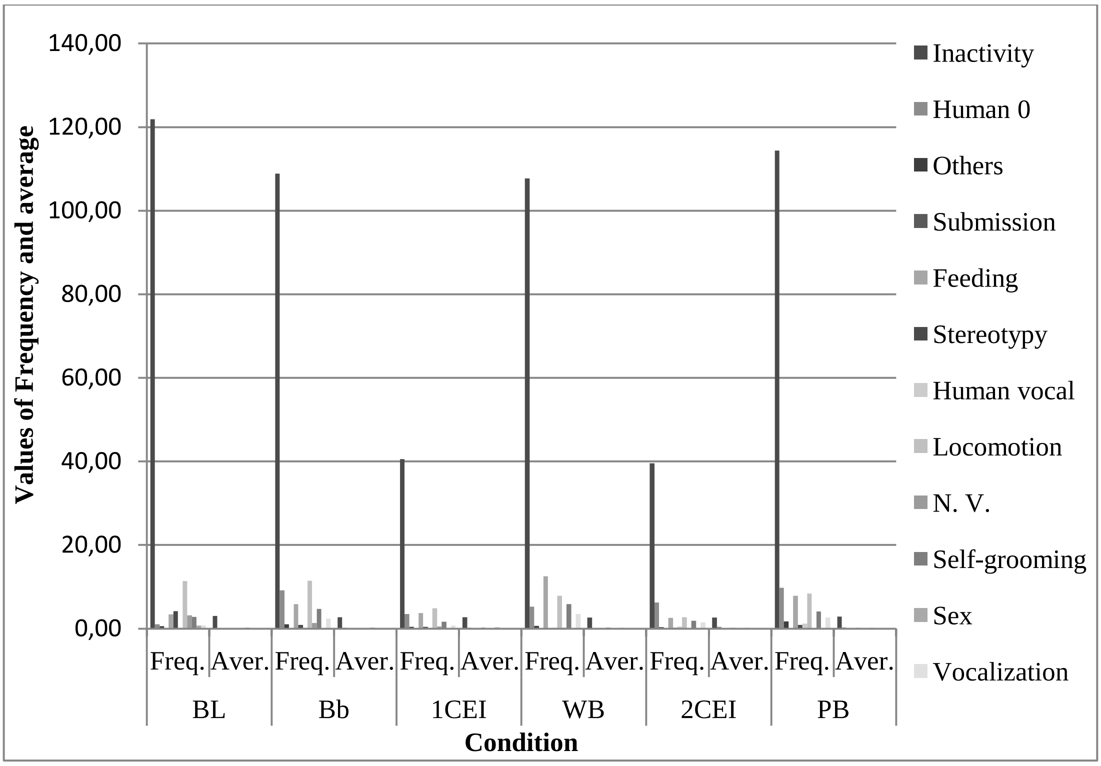

Chart 3. Behaviours with significant alteration during the experiment.

4.1.3. *Cercopithecus mona*

Table 8 and Chart 4 show the total sum per phase of relative frequency per session of the behaviours exhibited by the two Mona monkeys. Chart 4 shows only the behaviours with significant alteration during the experiment. As seen for the other species, also in Mona monkeys, Inactivity is the most frequent behaviour. It decreased in enriched periods, particularly in Wire box, comparing to non-enriched periods. Concerning Stereotypic behaviour, it also declined in the enriched periods of Bamboo and Wire Box and even in the first intermediate non-enriched period, 1CEI, comparing to the non-enriched periods of Baseline, 2CEI and Post-Baseline. Other abnormal behaviours also decreased in subsequent stages of Baseline, especially during Wire Box.

Feeding behaviour on the other hand, presented an increase in the enriched periods and in the first intermediate non-enriched periods and decreased in the tow last non-enriched periods.

Non-visible behaviour had a strong decrease in Wire Box and 2CEI, while Grooming declined in 1CEI and increase again in the following periods with a pick in the Wire Box stage.

Locomotion fluctuated, rising in Bamboo, 1CEI and 2CEI comparing to the Baseline and falling again in Wire box and Post-Baseline.

As the other groups in this project, Monas monkeys' Neutral Human Interaction, raised throughout the experiment (except in the 1CEI period) especially in the last condition, Post-Baseline.

The remaining behaviours did not have an important change and their frequency was low.

Table 7. Relative frequency and average per minute in Mona monkey's group during the study (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		Bb		1CEI		WB		2CEI		PB	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity*	58,83	1,47	47,97	1,20	21,23	1,42	30,90	0,77	19,00	1,27	57,90	1,45
Human 0*	6,43	0,16	15,67	0,39	2,47	0,16	11,00	0,28	6,83	0,46	16,20	0,41
Grooming	18,93	0,47	15,93	0,40	3,37	0,22	23,20	0,58	5,33	0,36	14,83	0,37
Others*	0,20	0,01	0,53	0,01	0,40	0,03	0,27	0,01	0,10	0,01	0,43	0,01
Affiliation	0,13	0,00	0,23	0,01	0,07	0,00	0,03	0,00	0,13	0,01	0,13	0,00
Dominance	0,07	0,00	0,13	0,00	0,13	0,01	0,07	0,00	0,00	0,00	0,07	0,00
Submission	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding	23,43	0,59	45,40	1,14	20,07	1,34	51,70	1,29	12,90	0,86	38,37	0,96
Ab. Behav.	2,90	0,07	1,10	0,03	0,10	0,01	0,10	0,00	1,07	0,07	1,13	0,03
Stereotypy	16,33	0,41	6,20	0,16	2,33	0,16	7,57	0,19	7,93	0,53	10,03	0,25
Human -	0,10	0,00	0,30	0,01	0,07	0,00	0,47	0,01	0,03	0,00	0,33	0,01
Hum. vocal	0,07	0,00	0,17	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,10	0,00
Objects	0,43	0,01	0,43	0,01	0,07	0,00	0,37	0,01	0,00	0,00	0,33	0,01
Locomotion	8,57	0,21	12,47	0,31	5,13	0,34	8,97	0,22	5,67	0,38	9,83	0,25
N. V.*	22,90	0,57	5,00	0,13	3,90	0,26	0,90	0,02	0,20	0,01	4,03	0,10
Self-groom	2,73	0,07	1,90	0,05	1,60	0,11	3,17	0,08	1,70	0,11	4,37	0,11
Sex	0,57	0,01	0,93	0,02	0,07	0,00	1,60	0,04	0,10	0,01	0,27	0,01
Vocalization	0,00	0,00	0,27	0,01	0,00	0,00	0,07	0,00	0,00	0,00	0,23	0,01

Monas monkeys (together) showed significant differences (*) in Inactivity (x^2= 32,29; p= 0,000), Neutral Human Interaction (x^2= 22,07; p= 0,001), Non-visible behaviour (x^2= 60,57; p= 0,005) and “Other” behaviour (x^2= 16,74; p= 0,005).

They showed a significant decrease of Inactivity in Wire Box condition comparing both to the Baseline (U= 1814,00; p= 0,000) and Post-Baseline (U=3162,00; p=0,000). Moreover, they had a significant increase in Feeding behaviour also during Wire Box comparing to Baseline (U=1505,00; p= 0,000) and Post-Baseline (U=2250,00; p=0,005).

Monas monkeys presented stereotypic behaviour throughout the experiment with no significant alteration.

As for the other groups, Mona also presented a significant increase of Neutral Human Interaction in Bamboo (U=2272,50; p=0,003) comparing to the Baseline.

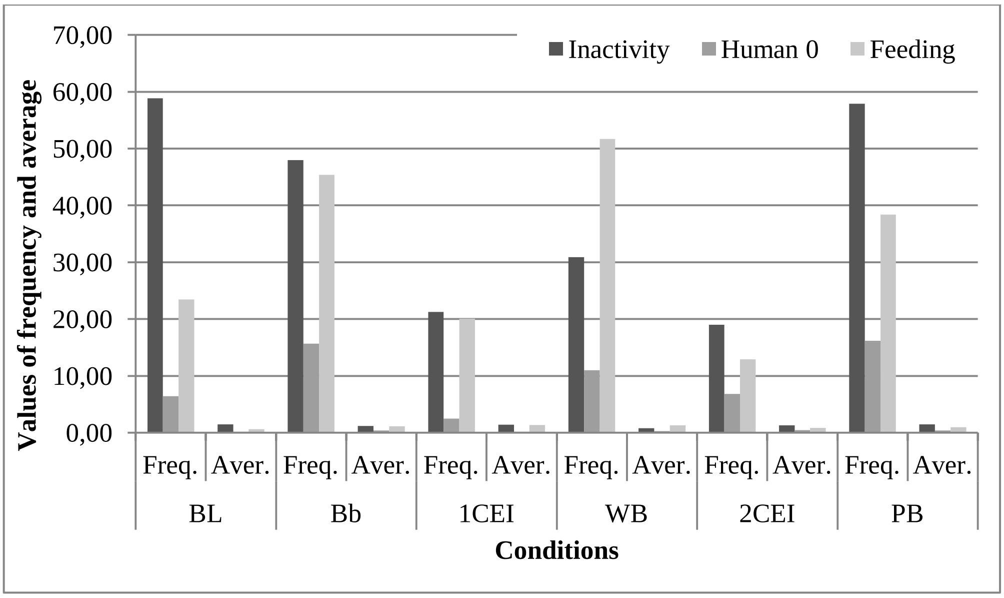

Chart 4. Behaviours with a significant alteration during the experiment.

4.2. *Results by individual*

4.2.1. *Hylobates lar*

4.2.1.1. *Ágil*

Ágil was inactive for most of the time in all conditions (see table 8 and chart 5). However, he was less inactive during the enriched periods but slightly more in the last two non-enriched periods. The second behaviour most performed by Ágil was

Vocalization, which was higher during Baseline and declined in the following stages. Feeding behaviour on the other hand, was lower in Baseline and it rose in the subsequent conditions, especially in Wire Box and Post Baseline. As well, Neutral Human Interaction also increased throughout the study and also this increase was higher during the two last conditions.

Locomotion and Non-visible behaiour fluctuated through the experiment. “Other” types of behaviour increased mainly in Wire Box condition.

The remaining behaviours did not have an important change and their frequency was low.

Table 8. Relative frequency and average per minute in Àgil behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity*	51,40	1,29	39,77	0,99	18,37	1,22	28,87	0,72	13,47	0,90	36,07	0,90
Hum O*	1,50	0,04	7,33	0,18	3,17	0,21	9,50	0,24	5,87	0,39	14,50	0,36
Grooming	1,03	0,03	0,20	0,01	0,33	0,02	1,33	0,03	0,67	0,04	0,73	0,02
Others*	0,23	0,01	1,07	0,03	0,33	0,02	2,17	0,05	0,73	0,05	1,73	0,04
Afiliation	0,17	0,00	0,00	0,00	0,03	0,00	0,10	0,00	0,03	0,00	0,13	0,00
Dominance	0,07	0,00	0,20	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,07	0,00
Submission	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding*	6,83	0,17	9,07	0,23	4,83	0,32	13,97	0,35	3,90	0,26	13,93	0,35
Abn. Behav.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum -	0,33	0,01	0,00	0,00	0,00	0,00	0,40	0,01	0,07	0,00	0,70	0,02
Hum vocal	0,73	0,02	2,00	0,05	0,43	0,03	2,73	0,07	1,10	0,07	2,37	0,06
Objects	1,00	0,03	1,27	0,03	0,30	0,02	0,80	0,02	0,80	0,05	1,07	0,03
Locomotion	3,30	0,08	4,80	0,12	1,07	0,07	7,83	0,20	2,30	0,15	4,37	0,11
N. V.	2,93	0,07	3,13	0,08	0,33	0,02	1,90	0,05	0,10	0,01	0,60	0,02
Self-groom	0,40	0,01	0,87	0,02	0,37	0,02	0,60	0,02	0,47	0,03	1,27	0,03
Sex	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalization	11,37	0,28	7,23	0,18	0,93	0,06	4,63	0,12	1,00	0,07	3,80	0,10

Ágil presented significant alterations in Feeding (x^2=19,69; p=0,001), Inactivity (x^2=37,60; p=0,000), Neutral Human Interaction (x^2=75,72; p=0,000) and “Others” behaviour (x^2=21,37; p=0,001).

Feeding behaviour increased during the Wire Box condition (U=450,00; p=0,000) comparing to the Baseline. Inactivity decreased in both enriched periods, Bamboo (U=489,50; p=0,003) and especially in Wire Box (U= 294,50; p=0,000) comparing to the Baseline. As well, Neutral Human Interaction also raised both in Bamboo (U=277,00; P=0,000) and Wire Box (U=210,50; p=0,000) comparing to the Baseline. In addition, Neutral Human Interaction by Ágil, had a strong enhance in Post-Baseline (U=393,00; p=0,000) comparing to Bamboo condition.

Concerning “Other” behaviour, it increased in the Wire Box phase (U=480,50; p= 0,000) comparing to the Baseline.

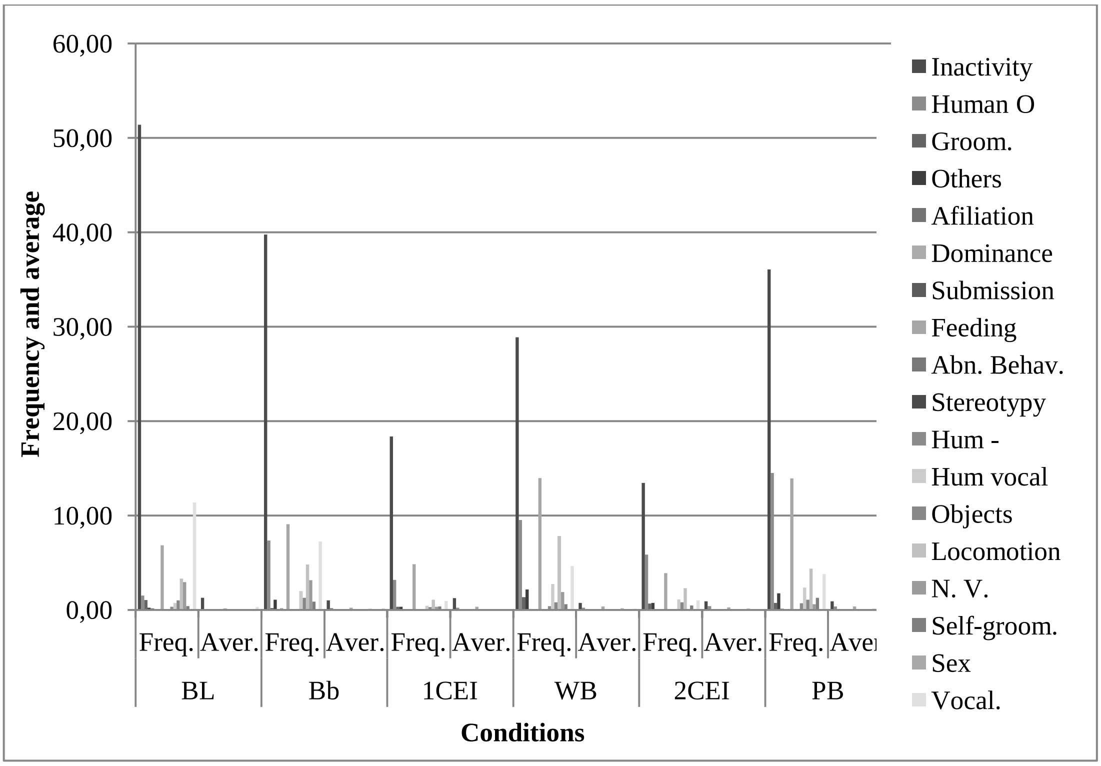

Chart 5. Comparison of the total frequency and average per minute in Àgil’s behaviour.

4.2.1.2. *Maya*

Maya was also very inactive in all study stages. However, inactivity performance by this individual declined consistently through the experiment (see table 9 and Chart 6). On the other hand, Maya was getting more non-visible through the study while locomotion declined from the second enriched period. Neutral Human interaction increased in both enriched and in the Immediate Effect Baseline periods.

The remaining behaviours did not have an important change and their frequency was low.

Table 9. Relative frequency and average per minute in Maya's behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		Bb		1CEI		WB		2CEI		PB	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity	53,80	1,35	50,20	1,26	17,27	1,15	43,10	1,08	13,63	0,91	33,30	0,83
Hum O	0,17	0,00	1,80	0,05	0,50	0,03	1,50	0,04	1,20	0,08	1,27	0,03
Grooming	1,03	0,03	0,20	0,01	0,33	0,02	1,33	0,03	0,60	0,04	0,67	0,02
Others	0,13	0,00	0,33	0,01	0,27	0,02	0,40	0,01	0,07	0,00	0,43	0,01
Afiliation	0,23	0,01	0,10	0,00	0,03	0,00	0,10	0,00	0,03	0,00	0,10	0,00
Dominance	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00
Submission	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding	9,93	0,25	7,60	0,19	1,27	0,08	7,67	0,19	2,90	0,19	5,30	0,13
Abn. Behav	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum -	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum vocal	0,20	0,01	0,10	0,00	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00
Objects	0,40	0,01	0,93	0,02	0,07	0,00	0,47	0,01	0,13	0,01	0,73	0,02
Locomotion	6,03	0,15	6,13	0,15	2,07	0,14	4,77	0,12	1,23	0,08	3,20	0,08
N. V.	7,53	0,19	9,10	0,23	8,07	0,54	13,60	0,34	10,37	0,69	34,40	0,86
Self-groom	0,53	0,01	1,40	0,04	0,40	0,03	1,70	0,04	0,27	0,02	1,23	0,03
Sex	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalization	1,30	0,03	1,17	0,03	0,23	0,02	0,43	0,01	0,03	0,00	0,67	0,02

Maya showed alteration in Inactivity (x^2=20,73; p= 0,001), Neutral Human Interaction (x^2=22,01; p=0,001) and in Non-visible behaviour (x^2=40,48; p=0,000).

Inactivity suffer a decrease in Post-Baseline (U=723,00; p=0,001) comparing to the first enriched period, Bamboo. On the other hand, Neutral Human Interaction in both enriched periods, Bamboo (U=503,00; p=0,000) and Wire Box (U=521,00; p= 0,001), comparing to the Baseline.

Regarding Non-visible behaviour, it increased in Post-Baseline, comparing to both Bamboo (U=297,00; p= 0,000) and Wire Box (U=424,50; p=0,000).

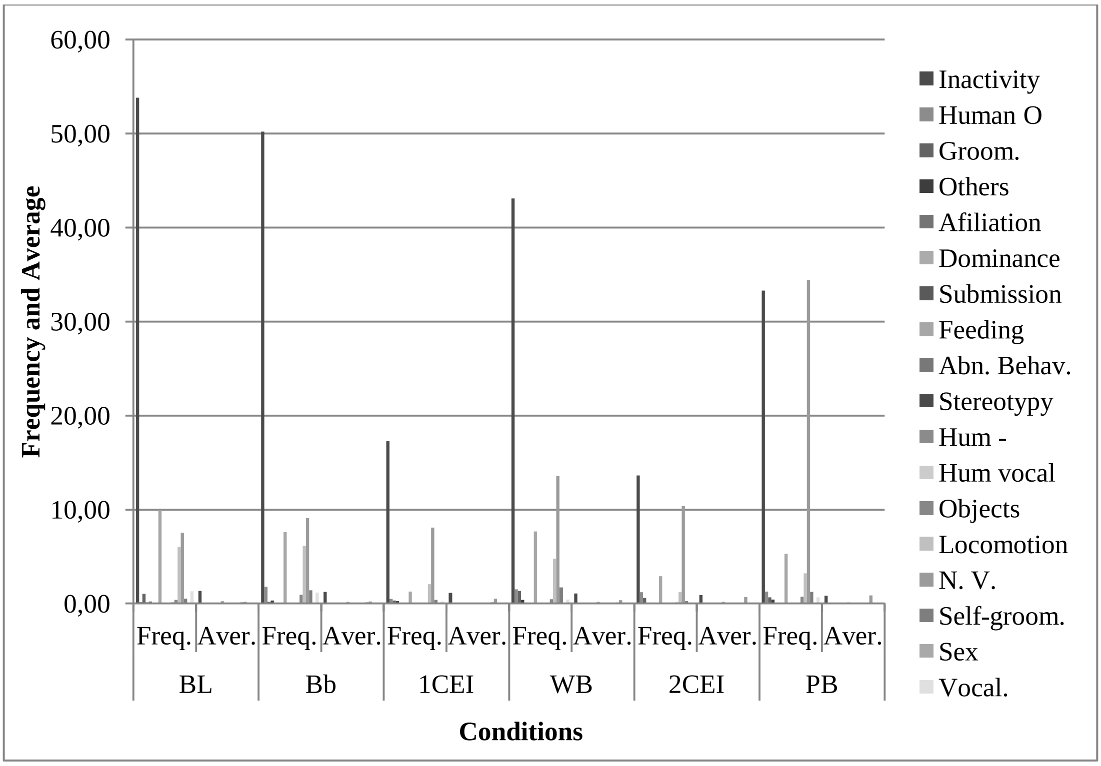

Chart 6. Comparison of the total frequency and average per minute in Maya's behaviour.

4.2.2. *Eulemur fulvus*

4.2.2.1. *Mrs. Piggy*

Inactivity was the predominant behaviour performed by Mrs. Piggy in all stages of the project. Moreover, Inactivity decreased a little following Baseline (see table 10 and Chart 7). Next in line, Locomotion slightly declined during Wire Box and second Immediate Effect Baseline. With similar values to the previous behaviour category, Grooming was stable throughout the study. Stereotypy on the other hand, had a strong decline in Mrs. Piggy's routine, especially during Wire Box stage as well as Non-visible behaviour which also declined, mainly in this stage. On the contrary, Neutral Human Interaction rose mainly in the second Immediate Effect Baseline.

Mrs. Piggy vocalized more in the enriched periods and in the two last non-enriched periods.

The remaining behaviours did not have an important change and their frequency was low.

Table 10. Relative frequency and average per minute in Mrs. Piggy's behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity	57,67	1,44	51,97	1,30	19,80	1,32	52,53	1,31	19,60	1,31	55,47	1,39
Human O*	0,50	0,01	4,00	0,10	1,97	0,13	2,23	0,06	2,53	0,17	4,47	0,11
Grooming	5,40	0,14	5,33	0,13	1,87	0,12	5,27	0,13	2,27	0,15	5,43	0,14
Others	0,27	0,01	0,60	0,02	0,37	0,02	0,50	0,01	0,23	0,02	1,07	0,03
Afiliation	0,83	0,02	0,50	0,01	0,23	0,02	0,70	0,02	0,37	0,02	0,33	0,01
Dominance	0,00	0,00	0,07	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,00	0,00
Submission	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00	0,00	0,00
Feeding	1,83	0,05	3,67	0,09	2,07	0,14	6,90	0,17	1,57	0,10	4,23	0,11
Abn. Behav.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy*	4,20	0,11	0,90	0,02	0,40	0,03	0,07	0,00	0,20	0,01	0,93	0,02
Hum -	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum vocal	0,17	0,00	0,07	0,00	0,13	0,01	0,13	0,00	0,20	0,01	0,63	0,02
Objects	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Locomotion	6,67	0,17	6,40	0,16	2,33	0,16	4,03	0,10	1,57	0,10	4,77	0,12
N. V.*	1,47	0,04	0,90	0,02	0,23	0,02	0,13	0,00	0,03	0,00	0,10	0,00
Self-groom.	1,47	0,04	2,50	0,06	0,63	0,04	3,33	0,08	1,17	0,08	2,50	0,06
Sex	0,23	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalizat.*	0,63	0,02	1,50	0,04	0,47	0,03	1,77	0,04	0,73	0,05	1,40	0,04

Mrs Piggy suffered a change (*) in the following behaviours: Stereotypy (x^2=31,63; p=0,000), Neutral Human Interaction (x^2= 49,20; p=0,000), Non-visible behaviour (x^2=39,50; p=0,000) and Vocalization (x^2=30,36; p=0,000).

Mrs. Piggy demonstrated an increase in feeding behaviour during the Wire Box period (U=493,00; p=0,002) comparing to Baseline. Concerning Neutral Human Interaction, Mrs. Piggy presented a strong increase in this behaviour in both enriched periods, Bamboo (U=322,00; p=0,000) and Wire Box (U=461,00; p=0,000) comparing to Baseline. On the other hand, she had a decline in Non-visible behaviour during Wire Box (U=427,00; p=0,000) comparing to Baseline and also in Pos-Baseline period (U=549,50; p=0,001) in comparison to Bamboo. Moreover, comparing this behaviour in both enrichment conditions, we see that it was lower in Wire Box (U=527,00; p=0,000) than in Bamboo.

Finally, Mrs. Piggy vocalized more in the enriched periods, Bamboo (U=380,00; p=0,000) and Wire Box (U=440,000; p=0,00) than in Baseline period.

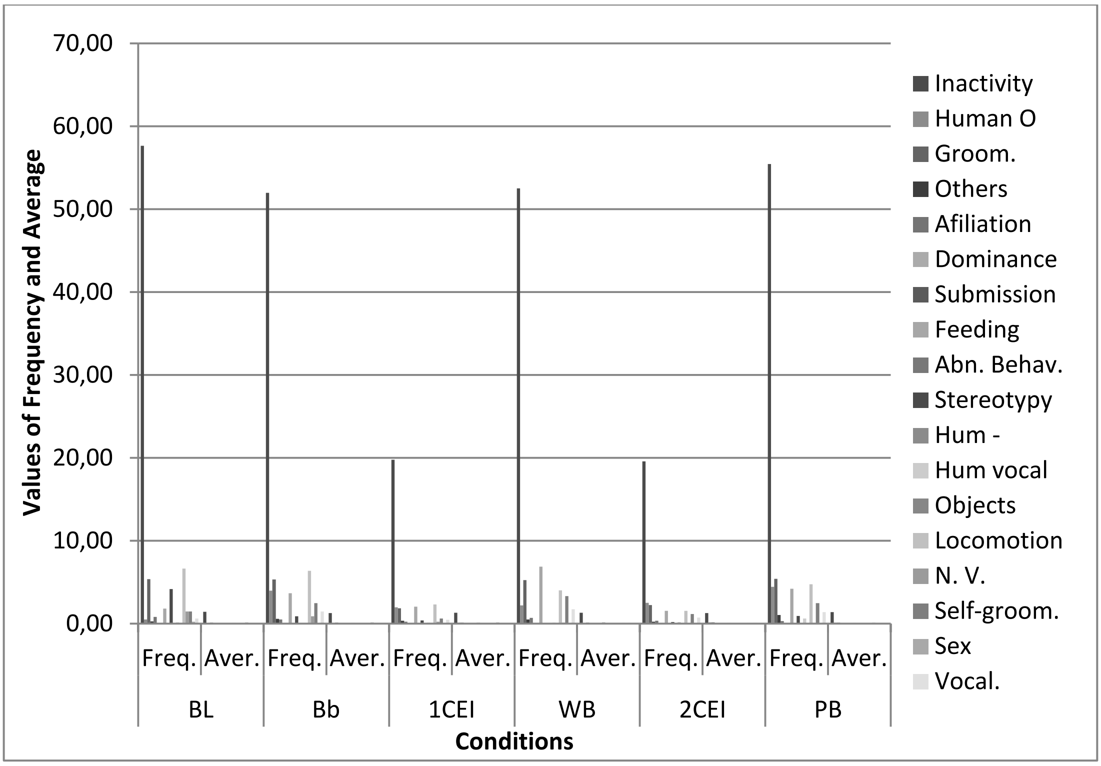

Chart 7. Comparison of the total frequency and average per minute in Mrs. Piggy's behaviour.

4.2.2.2. Mr. Piggy

Mr. Piggy was inactive for the most time in all study conditions with no significant changes all over the experiment, but somewhat more during Baseline period (see Table 11 and Chart 8). The next behaviour most performed by this individual was grooming, which was stable, also with no important alteration. On the other hand, Locomotion had its pick in the first Immediate Effect Baseline but decreased again in the following stages. In this same condition plus Wire Box, the continuous increase of Neutral Human Interaction was interrupted but it returns to increase in the following conditions.

Mr. Piggy was less visible in Baseline but more in the subsequent stages. Conversely, this individual was more sexually active during the two initial conditions and has never observed to perform any kind of sexual activity in the following ones.

Mt. Piggy vocalized more in Wire Box and in the second Immediate Effect Baseline.

The remaining behaviours did not have an important change and their frequency was low.

Table 11. Relative frequency and average per minute in Mr. Piggy's behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity	64,23	1,61	56,93	1,42	20,77	1,38	55,17	1,38	20,00	1,33	58,93	1,47
Human O*	0,53	0,01	5,17	0,13	1,57	0,10	3,03	0,08	3,73	0,25	5,30	0,13
Grooming	5,40	0,14	5,37	0,13	1,97	0,13	5,27	0,13	2,23	0,15	5,40	0,14
Others	0,30	0,01	0,43	0,01	0,10	0,01	0,17	0,00	0,10	0,01	0,63	0,02
Afiliation	0,77	0,02	0,50	0,01	0,23	0,02	0,70	0,02	0,37	0,02	0,33	0,01
Dominance	0,03	0,00	0,03	0,00	0,00	0,00	0,03	0,00	0,03	0,00	0,00	0,00
Submission	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding	1,57	0,04	2,23	0,06	1,63	0,11	5,60	0,14	1,03	0,07	3,63	0,09
Abn. Behav.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Stereotypy	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum -	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Hum vocal	0,03	0,00	0,10	0,00	0,10	0,01	0,07	0,00	0,33	0,02	0,57	0,01
Objects	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Locomotion	4,70	0,12	5,07	0,13	2,57	0,17	3,87	0,10	1,13	0,08	3,60	0,09
N. V.*	1,70	0,04	0,43	0,01	0,27	0,02	0,07	0,00	0,07	0,00	0,03	0,00
Self-groom.	1,30	0,03	2,23	0,06	1,00	0,07	2,53	0,06	0,73	0,05	1,63	0,04
Sex*	0,53	0,01	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vocalizat.*	0,20	0,01	0,90	0,02	0,30	0,02	1,77	0,04	0,73	0,05	1,27	0,03

Mr. Piggy presented a significant change in Neutral Human Behaviour (x^2=47,14; p=0,000), Non-visible behaviour (x^2=44,06; p=0,000), Sexual behaviour (x^2= 19,98; p=0,001) and Vocalization (x^2=26,32; p=0,000).

Mr. Piggy demonstrated a strong increase in Neutral Human Interaction in both Bamboo (U=226,00; p=0,000) and Wire Box (U=310,00; p=0,000) comparing to Baseline.

Mr. Piggy was least Non-visible in both enriched periods, during Bamboo (U=536,00; p=0,003) and Wire Box periods (U= 418,50; p=0,000) comparing to Baseline. On the other hand, Mr. Piggy vocalized more in these enriched stages, Bamboo (U= 5000,00; p=0,000) and Wire Box (U=420,00; p=0,000) comparing to Baseline.

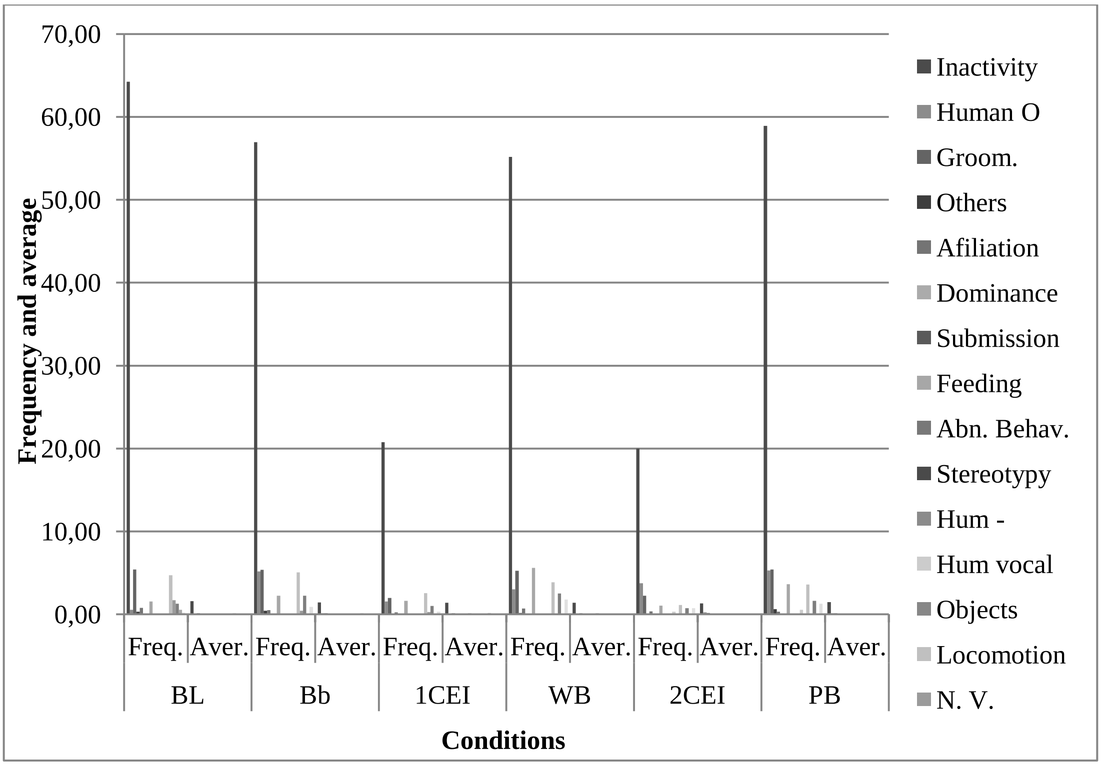

Chart 8. Comparison of the relative frequency and average per minute in Mr. Piggy's behaviour.

4.2.3. Cercopithecus mona

4.2.3.1. Longa

Although not so severe as in the other species subjects', Inactivity was also the predominant behaviour of Longa's routine, but this decreased during the enriched periods, especially during Wire Box (see table 12 and Chart 9). Conversely, stereotypic behaviour declined during both enriched periods and first Immediate Effect Baseline, but it increased again in the last couple of conditions, but still it was lower on those periods than in Baseline. Oppositely, feeding behaviour was higher in those stages, where stereotypy was lower and inferior where stereotypy increased. On the other hand, grooming declined in the first Immediate Effect Baseline.

Longa began to interacted increasingly (neutral interaction) with audiences, during the project as he became more visible (Non-visible behaviour decreased throughout the study).

The remaining behaviours did not have an important change and their frequency was low.

Table 12. Relative frequency and average per minute in Longa's behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		Bb		1CEI		WB		2CEI		PB	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity*	29,13	0,73	19,10	0,48	10,27	0,68	10,60	0,27	10,30	0,69	22,27	0,56
Human O	3,83	0,10	8,27	0,21	2,00	0,13	6,47	0,16	4,30	0,29	8,20	0,21
Grooming	8,70	0,22	7,30	0,18	1,67	0,11	11,43	0,29	2,67	0,18	7,60	0,19
Others	0,20	0,01	0,37	0,01	0,20	0,01	0,13	0,00	0,07	0,00	0,17	0,00
Afiliation	0,07	0,00	0,13	0,00	0,03	0,00	0,00	0,00	0,07	0,00	0,07	0,00
Dominance	0,03	0,00	0,10	0,00	0,07	0,00	0,07	0,00	0,00	0,00	0,00	0,00
Submission	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding*	12,90	0,32	25,20	0,63	11,10	0,74	26,00	0,65	6,47	0,43	22,97	0,57
Abn. Behav.	0,97	0,02	0,20	0,01	0,03	0,00	0,00	0,00	1,00	0,07	0,13	0,00
Stereotypy	6,03	0,15	1,07	0,03	0,20	0,01	0,83	0,02	1,33	0,09	2,43	0,06
Hum -	0,03	0,00	0,07	0,00	0,03	0,00	0,03	0,00	0,00	0,00	0,00	0,00
Hum vocal	0,07	0,00	0,03	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,07	0,00
Objects	0,33	0,01	0,33	0,01	0,00	0,00	0,30	0,01	0,00	0,00	0,30	0,01
Locomotion	5,63	0,14	8,37	0,21	3,37	0,22	4,63	0,12	3,10	0,21	7,00	0,18
N. V.*	10,53	0,26	1,73	0,04	0,20	0,01	0,20	0,01	0,10	0,01	2,07	0,05
Self-groom.	2,40	0,06	1,53	0,04	1,27	0,08	2,50	0,06	1,00	0,07	3,60	0,09
Sex	0,47	0,01	0,73	0,02	0,07	0,00	1,37	0,03	0,10	0,01	0,17	0,00
Vocalization	0,00	0,00	0,20	0,01	0,00	0,00	0,07	0,00	0,00	0,00	0,23	0,01

Longa had a significant change (*) in Feeding behaviour (x^2= 18,32; p= 0,003), Inactivity (x^2= 29,12; p=0,000) and Non-visible behaviour (x^2=38,58; p=0,000).

Longa showed a raise in feeding activity during Wire Box condition (U= 368,50; p=0,000) comparing to Baseline. Inactivity by this individual had a strong decline also in this second enriched phase (U= 314,00; p=0,000) but it increased again in the last non-enriched condition, Post-Baseline (U=322,00; p=0,000).

Longa's Non-visible behaviour oscillated. It declined during Bamboo (U=440,50; p=0,001) and Wire Box (U=453,50; p=0,000) comparing to Baseline. However, it enhanced during Post-Baseline period (U=521,50; p=0,005) comparing to the last enriched condition.

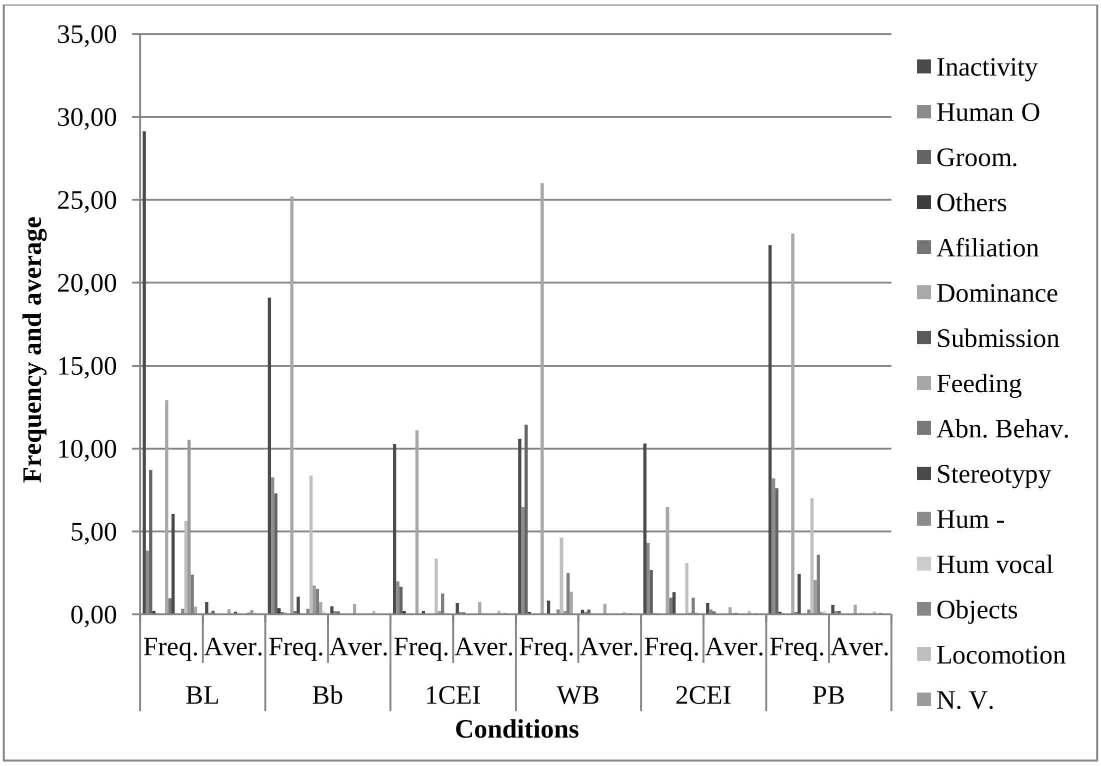

Chart.9 Comparison of the total frequency and average per minute in Longa's behaviour.

4.2.3.2. *Curta*

Curta was also very inactive but inactivity in this individual's routine had a slightly decline during Wire Box and second Immediate Effect Baseline conditions (see Table 13 and Chart 10). Curta was also very Non-visible during Baseline, but he got more visible in the following stages, except in the first Immediate Effect Baseline where he was very Non-visible. Moreover, Curta increasingly spent more time feeding until Wire Box, while Grooming and Neutral Human Interaction fluctuated but with a lower common pick in the first Immediate Effect Baseline. Stereotypic behaviour declined in both enriched periods and in the first Immediate Effect Baseline.

The remaining behaviours did not have an important change and their frequency was low.

Table 13. Relative frequency and average per minute in Curta's behaviour (BL – Baseline; Bb – Bamboo; 1CEI – First Immediate Effect Baseline; WB – Wire Box; 2CEI – Second Immediate Effect Baseline; PB – Post-Baseline). The behaviours which presented significant alteration are marked with (*).

	BL		**Bb**		**1CEI**		**WB**		**2CEI**		**PB**	
	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.	Freq.	Aver.
Inactivity	29,70	0,74	28,87	0,72	10,97	0,73	20,30	0,51	8,70	0,58	35,63	0,89
Human O	2,60	0,07	7,40	0,19	0,47	0,03	4,53	0,11	2,53	0,17	8,00	0,20
Grooming	10,23	0,26	8,63	0,22	1,70	0,11	11,77	0,29	2,67	0,18	7,23	0,18
Others	0,00	0,00	0,17	0,00	0,20	0,01	0,13	0,00	0,03	0,00	0,27	0,01
Afiliation	0,07	0,00	0,10	0,00	0,03	0,00	0,03	0,00	0,07	0,00	0,07	0,00
Dominance	0,03	0,00	0,03	0,00	0,07	0,00	0,00	0,00	0,00	0,00	0,07	0,00
Submission	0,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Feeding*	10,53	0,26	20,20	0,51	8,97	0,60	25,70	0,64	6,43	0,43	15,40	0,39
Abn. Behav.	1,93	0,05	0,90	0,02	0,07	0,00	0,10	0,00	0,07	0,00	1,00	0,03
Stereotypy	10,30	0,26	5,13	0,13	2,13	0,14	6,73	0,17	6,60	0,44	7,60	0,19
Hum -	0,07	0,00	0,23	0,01	0,03	0,00	0,43	0,01	0,03	0,00	0,33	0,01
Hum vocal	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,03	0,00
Objects	0,10	0,00	0,10	0,00	0,07	0,00	0,07	0,00	0,00	0,00	0,03	0,00
Locomotion	2,93	0,07	4,10	0,10	1,77	0,12	4,33	0,11	2,57	0,17	2,83	0,07
N. V.*	12,37	0,31	3,27	0,08	3,70	0,25	0,70	0,02	0,10	0,01	1,97	0,05
Self-groom.	0,33	0,01	0,37	0,01	0,33	0,02	0,67	0,02	0,70	0,05	0,77	0,02
Sex	0,10	0,00	0,20	0,01	0,00	0,00	0,23	0,01	0,00	0,00	0,10	0,00
Vocalization	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Curta showed change (*) in Feeding behaviour (x^2= 18,70; p=0,003) and in Non-visible behaviour (x^2=25,34; p= 0,000).

This individual had a raise in feeding activity in Wire Box (U=368,00; p=0,000) comparing to Baseline. He was also more active in Post-Baseline (U=403,50; p=0,000) in comparison to Wire Box enriched period.

Concerning Non-visible behaviour, Curta showed a decline in both Bamboo (U=483,00; p=0,001) and Wire Box (U=453,50; p=0,000) conditions, in comparison to Baseline.

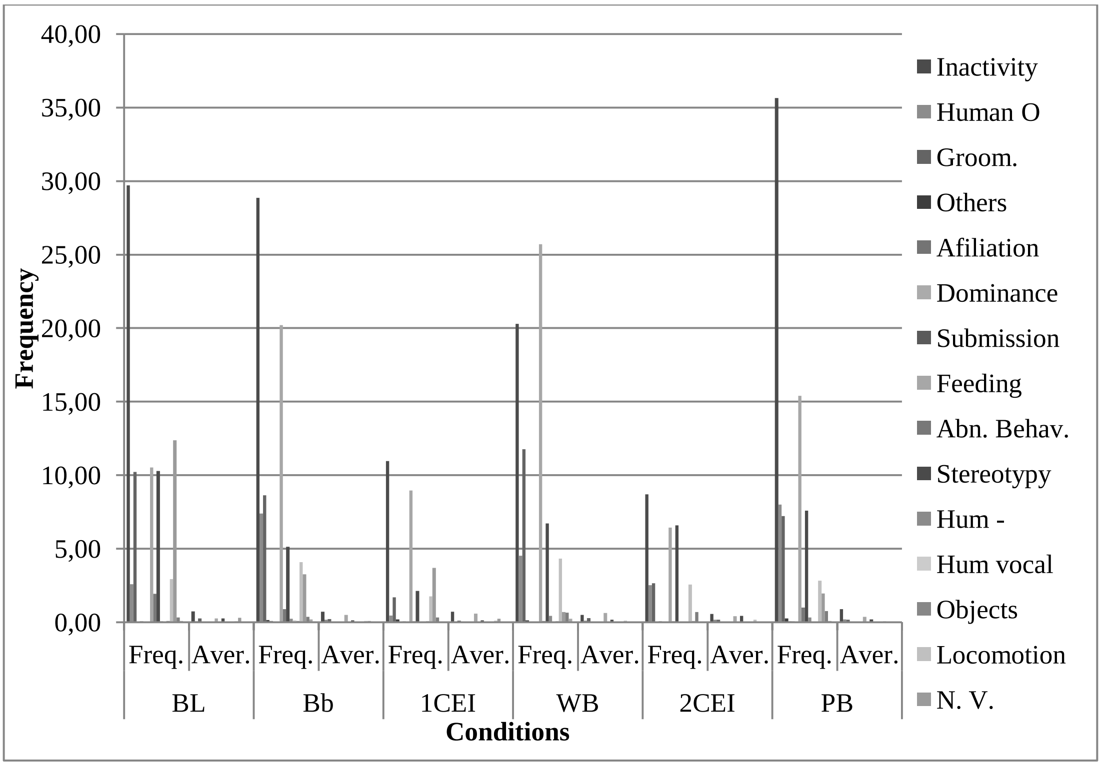

Chart 10. Comparison of the total frequency and average per minute in Curta's behaviour.

4.3. Main behaviours comparision

Here, we present behaviours which had significant modifications influenced by the different conditions, comparing one to another (average frequency per session). For that reason, we will only consider the main conditions (Baseline, Bamboo, Wire Box and Post-Baseline), ignoring the intermediate ones (first and second Immediate Effect Baseline).

4.3.3. Inactivity

During Baseline period, *Eulemur fulvus* group was the most inactive group ($\bar{x}$= 3,05), followed by *Hylobates lar* group ($\bar{x}$= 2,63), while *Cercopithecus mona* was the least inactive ($\bar{x}$= 1,47).

The same pattern was observed in all conditions (see Chart 11). *E fulvus* group maintained equal inactivity level in all project's phases, Bamboo ($\bar{x}$= 2,72) and Wire

Box ($\bar{x}$= 2,69), but with a slight increase in the last non-enriched period, Post Baseline ($\bar{x}$= 2,86). On the other hand, *H. lar* presented a low heighten continuous decline on inactivity, Bamboo ($\bar{x}$= 2,25), Wire Box ($\bar{x}$= 1,80) and Post Baseline ($\bar{x}$= 1,73). *C. mona*, on the other hand, were more active during the enriched periods, Bamboo ($\bar{x}$= 1,20) and mainly in Wire Box ($\bar{x}$= 0,77) and more inactive (similar to Baseline) in the non-enriched period Post Baseline ($\bar{x}$= 1,45).

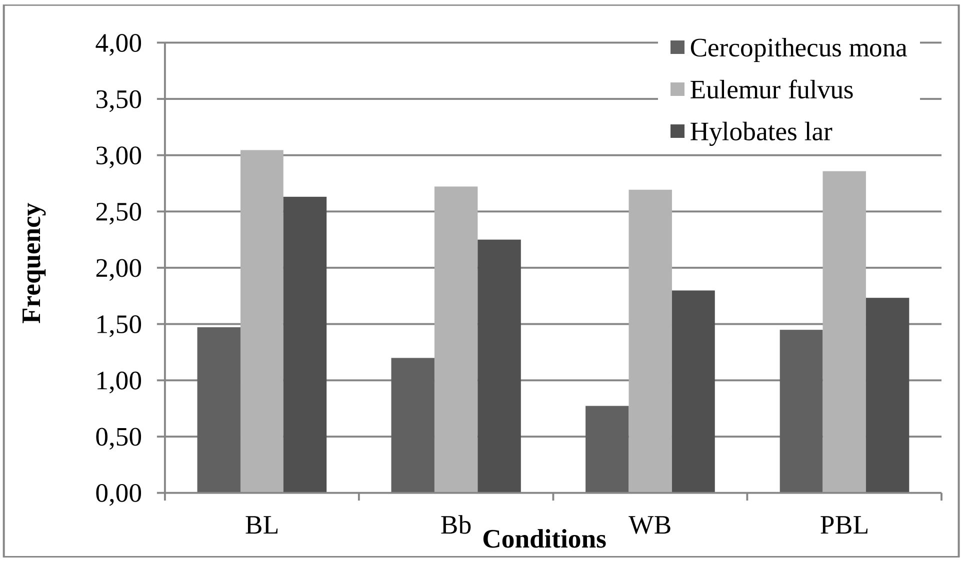

Chart 11. Comparison of Inactivity behaviour (average frequency per session) in all stages of the project.

4.3.4. Feeding

Concerning feeding behaviour (see Chart 12), we see that *C. mona* were, by far and in all conditions, the group who dispended more time in this activity, which had an increase during enrichments, Bamboo ($\bar{x}$= 1,14) and Wire Box ($\bar{x}$= 1,29), comparing to the remain phases, Baseline ($\bar{x}$= 0,59) and Post Baseline ($\bar{x}$= 0,96). On the other hand, *E. fulvus* was the group least occupied by this activity in comparison to the other two groups Baseline ($\bar{x}$= 0,09), Bamboo ($\bar{x}$= 0,15) and Post Baseline ($\bar{x}$= 0,20), although they also presented an important increase during Wire Box period ($\bar{x}$= 0,31).

H. lar maintained similar levels on feeding behaviour throughout the study, but also with a pick in Wire Box ($\bar{x}$= 0,54) (like the other groups) and lower and constant levels in the remain stages, Baseline, Bamboo and Post-Baseline ($\bar{x}$= 0,42; $\bar{x}$= 0,42; $\bar{x}$= 0,48, respectively).

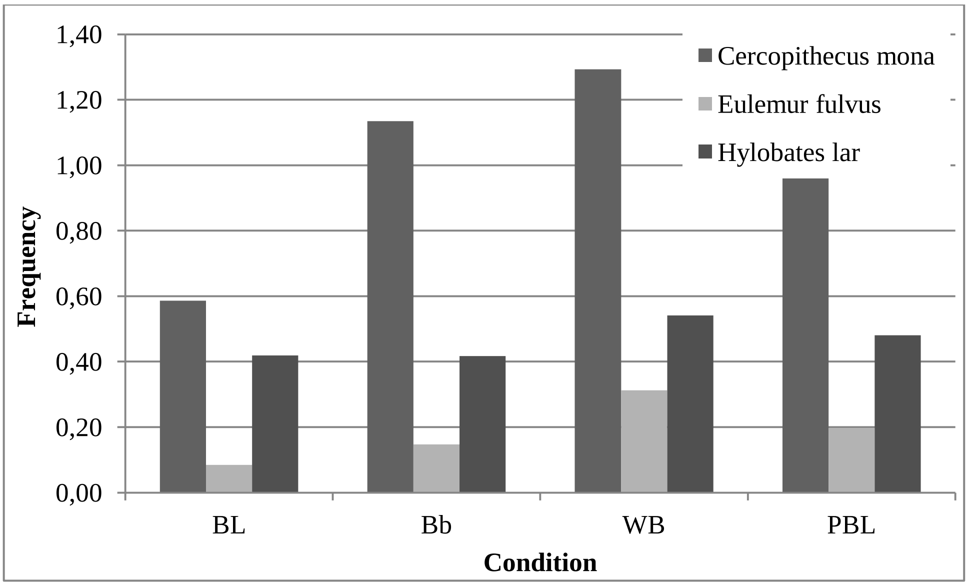

Chart 12. Comparison of Feeding behaviour (average frequency per session) in all stages of the project.

4.3.5. Stereotypy

H. lar had never exhibited stereotypic or other abnormal behaviours. On the other hand, in Chart 13 we see that *C. mona* exhibited an important level of stereotypic behaviour in Baseline ($\bar{x}$= 0,41) and an important decline too of such behaviour, in Bamboo ($\bar{x}$= 0,16), Wire Box ($\bar{x}$= 0,19) and Post Baseline ($\bar{x}$= 0,25).

While least frequent comparing to *C. mona*, also *E. fulvus* presented stereotypic behaviour, especially during the Baseline ($\bar{x}$= 0,11), followed by a decrease in the subsequently conditions, Bamboo ($\bar{x}$= 0,02), Wire Box ($\bar{x}$= 0,00) and Post Baseline ($\bar{x}$= 0,02).

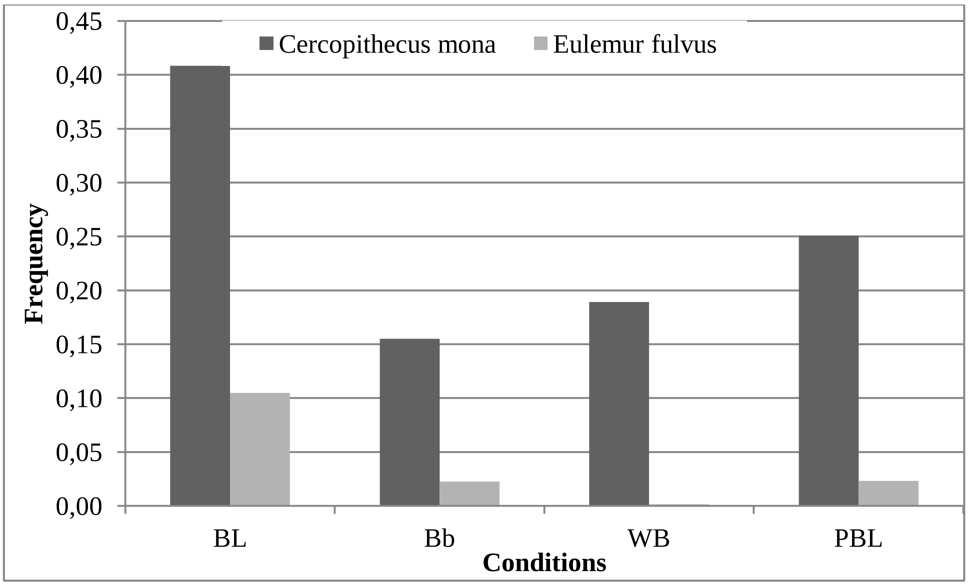

Chart 13. Comparison of Stereotypic behaviour (average frequency per session) in all stages of the project.

4.3.6. Human interaction

Neutral Human Interaction must be carefully analyzed as it represents captive animals' behaviour but also audiences' behaviour and affluence. Looking at Chart 14, we see that *C. mona* for example, had higher neutral human interaction during Bamboo ($\bar{x}$= 0,39), Post Baseline ($\bar{x}$= 0,41), and lower in Baseline ($\bar{x}$= 0,16) and Wire Box ($\bar{x}$= 0,28). This group was whom interacted more with the public (in this sense), except in Wire Box and Post Baseline, when also *H. lar* showed similar levels of Neutral Human Interaction ($\bar{x}$=,28; $\bar{x}$= 0,39, respectively). *H. lar* neutral human interaction in the previous phases, Baseline and Bamboo were lower ($\bar{x}$= 0,04; $\bar{x}$= 0,23, in that order), compared to the *C. mona*, but similar to those values of E. *fulvus* in those same periods ($\bar{x}$= 0,03; $\bar{x}$=,23, in that order). Alternatively, *E. fulvus* also showed a decrease in this behaviour in Wire Box ($\bar{x}$= 0,13) and again a increase in Post Baseline ($\bar{x}$= 0,24) as the other two groups but with lower incidence.

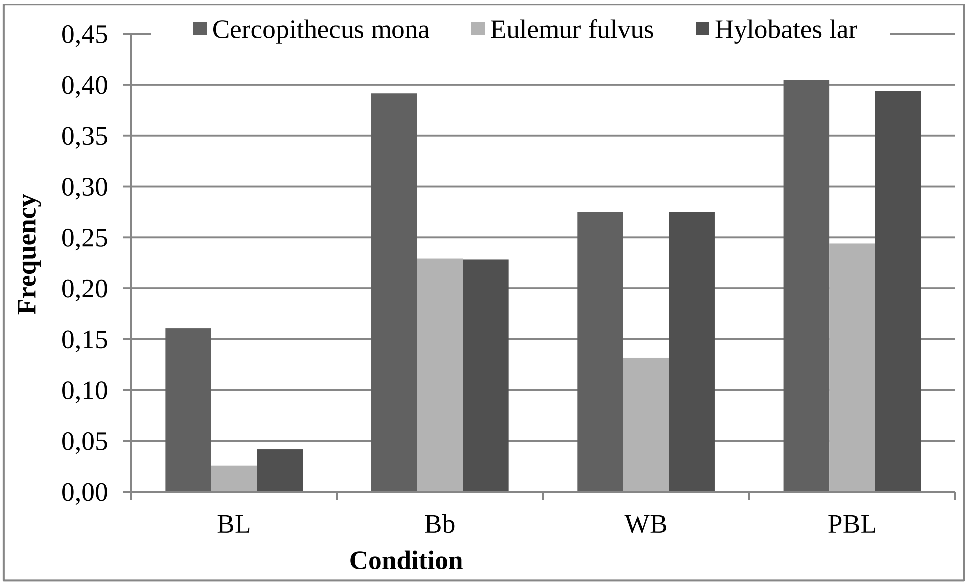

Chart. 14. Comparison of Neutral Human Interaction (average frequency per session) in all stages of the project.

5. Discussion

Animal's daily routine depends on physical and environmental factors. But for captive animals for example, trouble with predators and competition with others, for scarce resources such as food and shelter, among others, is not to be considered as affecting their activity budgets. On the other hand, life in captivity may present difficulties that animals are not able to cope with. A boring routine in which animals have nothing to do, to reach, or escape from, in which social interactions are constrained by the number of individuals and the individuals themselves who stay together 24 hours a day, 7 days a week and the human interaction that is not in their control, are still factors with which captive animals are not able to cope and so can cause low welfare.

However, the consequences of lack of "natural stresses" can also have negative effects on the life of captive animals, which evolved to cope with such pressures and even when limited by captivity, are still highly-motivated to properly perform such activities (Broom, 2011). Pomerantz and colleagues (2013) suggest that this motivation and coping ability are also dependent on the species and therefore, under equal captive environment, animals of different species may present different types of motivation and consequently of incapability to express various behaviours. "When needs are not satisfied, welfare will be poorer than when they are satisfied" (Broom, 2010: 4). For example, wide rangers species (as Mona monkeys in this study) are more prom to a negative welfare due to the small area of the habitat and motivation to range, and may produce stereotypic pacing (Pomerantz et al., 203) as our subjects have presented. However, a single score such as stereotypy should not be used as exclusive indicator of welfare because they can simply be an indicator of past worse welfare and the becoming of an "habit" (Mason and Latham, 2004). For that matter, enrichment effect may not be immediate so we must be 'patient' with its use (Mason and Latham, 2004) as it explains why some stereotypies can take many months to reduce when the environment is enriched (Meehan et al., 2001 *in* Mason and Latham, 2004). For example, in our study, only brown lemurs presented a decline in abnormal behaviours and this was only achieved during the second enrichment phase. It's possible that for Mona monkeys, the process needed more time or maybe it's a "habit-like" stereotypy which may not be possible to remove. Also, the stereotypic pacing exhibited by Mona monkeys' was mainly pre-feeding. Bloomsmith and Lambeth (1995) suggests that unpredictable feeding may prevent this but, as defended by Wait and Buchana-Smith (2001), this change of routine must be carefully considered before implemented as the feeding delay may induce self-directed behaviour, vocalizations, inactivity and abnormal behaviours.

Looking at the levels of inactivity and abnormal behaviours at the three species studied, during the baseline period, we see that our subjects have indeed endured poor welfare. However, as suggested by previous authors, we've proven that both inactivity and abnormal behaviours may be reduced by introducing devices (see Figure 15 and 16) which animals can manipulate and therefore be "entertained" with (e.g. Birke, 2002). Still, brown lemurs presented high rates of inactivity behaviour throughout the study which may be explained by the low basal metabolic rates of Malagasian primates, perhaps a response to hard and volatile environments (Simmen et al, 2010) and perpetuated here in captivity as a species feature. Also, the age of these individuals may take an important role on their activity. Nevertheless, brown lemurs presented the larger number of significant behavioural changes: both individuals showed increase in Neutral Human Interaction and Vocalization, as well as decline in Non-visible behaviour, during the enriched periods, indicating perhaps that those individuals were more active and attentive to surroundings during these conditions. On the other hand, both gibbons presented significant differences in Inactivity. Ágil was less inactive during the enriched periods and feeding more in Wire Box stage, indicating effective enrichment, while Maya only showed a significant decrease in Inactivity in the last non-enriched period, which can indicate the lack of interest towards the enrichment devices, suggesting individuality. Moreover these individuals presented opposite directions concerning Neutral Human Interaction: Ágil interacted more with audiences in both enriched periods and Post Baseline, while Maya interacted less during these same periods, except in Post Baseline (with no alteration). In fact, Maya tended to be more isolated and hide from large crowds, going up in the trees or inside shelters, while Ágil seemed comfortable with human presence

Figure 15. Male Mona monkey using the bamboo cane.

Figure 16. Female brown lemur trying to take food from the bamboo cane.

and sometimes even juggled to call for attention. Again, it's clear that these two individuals of the same species, with the same background and used to the same habitat, show important and distinct personality traits. This suggests that not only enrichment strategies should be adapted to the species and to its needs but also to the individual's personalities, with implications the enrichment design.

Figure 17. Male Mona monkey feeding from the Wire Box.

Generally, when the environmental enrichment devices were available for the individuals to manipulate, their daily time budget spent in sleeping or resting declined. This suggests that an increase in the general activity thus presented to be a significant improvement in welfare and perhaps even on their health (Clay et al., 2011), particularly during the Wire box period, in which the first meal of the day was integrated in the enrichment (see Figure 17). Also, feeding behaviour (that includes foraging and processing food items) which is one of the major fractions of the activity budget of primates (Mallapur, 2008) had risen during the Wire box phase in both Mona monkeys and brown lemurs (however we must consider that feeding is related to body mass and nutritional requirement). On the other hand, the bamboo device had a weak effect probably due to the fact that there was no urgency to extract the food from it because it was just a small reward (without a need to exercise or process, see Figure 18), in comparison to the daily feeding portion which was plentiful and easy to obtain. Futhermore, we've also to consider that these devices (opaques) were possibly too complex for untrained subjects (Clark and Smith, 2013). For example, some challenges may be initially frustrating for animals (Meehan and Mench, 2007). On the other hand, enrichment must be challenging and solvable to counteract habituation and so the equilibrium between these two factors must be achieved (Gronqvist et al., 2013;

Figure 18. Male gibbon using the bamboo cane.

Meehan and Mench, 2007). In fact both our enrichment had a positive effect although used differently by the individuals. Challenge is essential to individuals' fitness and may also be successful in reducing stereotypic activity (Meehan and Mench, 2007). Nevertheless, enrichment even when not fully used by the individuals may prove itself positive for the behaviour of the individuals. Clark and Smith (2003) analyzed a group of captive chimpanzees and the implemented two types of EE apparatus, concluding that although the device use was low (2.5% of the observation time), it increased social play and decreased rough-scratching (self-directed behaviour) with the device exploitation.

Cognitive skills are considered to be more developed in wide-range species (Pomerantz et al., 2013) such as our subject species, Mona monkeys. Indeed, with the introduction of the Wire Box (a device that requires cognitive skills to be handle and to retrieve the food within), Mona monkeys began to spend considerable time with this task. They were actually the group who demonstrated the most significant reduction in inactivity (Longa) and increase in feeding behaviour (both individuals). Also, they were more visible in this condition. Accordingly, following the suggestion of Pomerantz and colleagues (2013), it is probably more important the social stimuli and the environmental complexity for captive animals' welfare than enlarging the enclosure which has been revealed to be unsuccessful in reducing stereotypic abnormal behaviour, as opposed to the addition of enriched space (environmentally complex playgrounds) which promotes species typical behaviour and raises activity (Honess and Marin, 2006). Encouraging individuals to present their species behaviour repertoire is believed for a long time now, to improve welfare by easing stress levels (Novak and Suomi, 1988 *in* Gronqvist et al., 2013) as an enriched environment may promote cognition development (Turnbull et al., 2011). Also, the reform of the enclosure is often much more easy for zoos to achieve than to construct new ones, as construction of new enclosure may prove to be very expensive and long-drawn-out.

Complex environments are indeed related to expression of the natural behaviour repertoire and to the decrease of abnormal behaviour (Mallapur, 2008) and so a great variety of and the opportunity to use objects are important. Moreover, it presents a better educational ground for the public, whom may thus observe species-specific behaviours patterns and therefore, become more alert and educated about species conservation. For example, an enclosure with abundant vegetation and structures, similar to those seen in the wild (eg. termite mounds), give the public the opportunity to understand in a much clear way the animal's habitat. In fact, Gusset and Dick (2011) estimate that annually near 700 million people visit zoos and aquariums. Zoo animals seem to get used to the presence of audiences, but the loud noise of some audiences is still a stress factor for these animals, a stimulus often out of control (Birke, 2002), and that often results in abnormal behaviours (eg. brown lemur female of this study tended show pacing behaviour when confronted by large and noisy crowds). Hence, complex environments for the captive animals should undoubtedly include hiding places for animals to escape this disturbance and, once more, take the opportunity to give the public the better view on the natural behaviour repertoire. On the other hand, as discussed before, an enriched life for the captive individuals will prevent and reduce abnormal behaviours which do not contribute to the public education and even may made audiences turn away from zoos. In the present case, adding more vegetation and natural structures are not possible for logistic and financial constraints. The same limitation is present in the majority of zoos. Even when these limits are exceeded, captive life still doesn't satisfy animal's needs it does not present all the conditions and opportunities of a natural habitat. It is now time to turn to environmental enrichment to fill gaps in animals' routine. The present study, although with significant positive improvements in the subjects' welfare, is still constrained by the same factors common to research in zoos: small group sizes and habituation towards the enrichment object (Clark and Smith, 2013). Both gibbons and brown lemurs have not been subject of much enrichment research as have been great apes, probably due to their restricted arboreal lifestyle. Moreover, it may prove unfeasible to apply to gibbons and brown lemurs, enrichment strategies generally used with great apes because of their singular behaviour, social system, ecology and morphology (e.g. for gibbons: Wells and Irwin, 2009) and so further research is require to determine appropriate

Figure 19. Male brown lemur trying to take food from the Wire Box.

Figure 20. Female gibbon extracting food items from the Wire Box.

environment enrichment devices for these species. In the present case, both devices were projected to be suitable to these animals and in fact, both gibbons and brown lemurs were positively affected by enrichment devices but in different ways (reduction of inactivity during Bamboo phase in both gibbon and brown lemurs, but also in Wire Box and Post-baseline in respect of gibbons (Figure 20); decrease in stereotypy during Wire box in brown lemurs (Figure 19). On the other hand, Mona monkeys are more resilient and adaptive species. In this group however, only Inactivity, Feeding and Neutral Human interaction had a significant alteration. Inactivity reduced during the Wire box and Feeding activity increase also during Wire box. Human interaction increased during Bamboo, compared to the baseline and the posterior phases. Nevertheless, the affluence of the public in a zoo was not identical during the study period (greater during the last month, April) and also enclosure design and location had a vital role on Neutral Human Interaction. Visitor group size and noise level have already be related to affect *H. lar* self-directed behaviour (Cooke and Schillaci, 2007), but besides Vocalization (by the male, possible to atract or call attention to the public), *H. lar* group did not presented abnormal behaviour related to Human Interaction.On the other hand, this pressure was often followed by stereotypic pacing in both Mona monkeys (both individuals) and brown lemurs (female), particularly when the audience were zookeepers passing by or were loud crowds (personal observation). Such anxiety-related behaviour may be overpast by signal this event (Rimpley and Buchanan-Smith, 2013). Also, creating enclosures with hiding opportunities, soundproof and one-way glass may smooth the stress influence by visitors. On the other hand, Negative Human Interaction towards the public was recorder several times, always by the same Mona monkey and almost always subsequent to a dominat display by the other monkey. Nevertheless, these episodes were too infrequent to be quantified.

For the above reason, it is urgent to evaluate the enrichment effect in order to develop and adapt the enrichment to the individuals and to species. For example, from the results of this study, we suggest that the Wire Box should be introduced possibly every day while Bamboo cane should only be used from time to time as a complement, inside which it is possible to add cereals and honey to the diet of the animals and by this way, also preventing both habituation and poor dietary diversity which in turn may also lead to abnormal behavioural development (Mallapur, 2008). Moreover, more enrichment devices should be put into practice and frequently restructured to add cognitive challenge to enrichment (Meehan and Mench, 2007). But this may prove to be unpractical for staff and zoo keepers.

6. Conclusion

There is no published data showing link involving well-being and the probability of a species to obtain positive results from enrichment (de Azevedo et al., 2007). In fact, although environmental enrichment had been subject of extensive study in the past 30 years, and most zoos, laboratories, sanctuaries and farms use at least some type of enrichment, staff still misses time to conduct serious and appropriate enrichment. Therefore, the great research done in this field has little effect especially in captive animals' life or at least, should have much more impact. This is probably due to the lack of workers and their knowledge about enrichment and so, zoos should adopt serious plans to instruct their staff and even hire people specifically for that work. Moreover, establish proper kinds of enrichment to particular species and individuals as the evaluation of appropriate levels of challenge are, in our days, still an early ongoing research (Meehan and Mench, 2007).

Environmental research in zoos is also often hampered by the low number of individuals, a small sample. Despite these limitations, usual to zoo environment, we've recognized prospective improvements on the environmental enrichment design. And, most above all, "it is why the enrichment was applied that is most important" (de Azevedo et al, 2007: 342).

It's vital to include applied animal behaviour and welfare research to the draw and uphold of model captive environments, in order to proceed with the conservation programs in zoos, although in the case of primates this may prove to be more complex and tricky (Mallapur, 2008). In fact, there is still little degree of research which sustain zoos' actual role in educate visitors for conservation actions and most results show that the impact from a lone experience at a zoo is limited and short lasting as visitors may resent or refuse to accept to learn about appropriate behaviours or may be already committed to conservation action (Smith et al., 2008).

Conservation *in situ* should be the primer and most important approach to protect wildlife, while zoological parks must focus on public education and awareness. Also, for that reason, it's vital to achieve a good welfare so the visitors could observe the animals' natural behaviour repertoire, learning about it. On the other hand, abnormal behaviour could pass on a wrong notion to visitors (Mallapur, 2008), whom will not gain knowledge about animals (and consequently, will not be responsive for wildlife conservation) (Clay et al., 2011) or can retract from zoos, with the financial consequences for both animals and institution.

The results of the present study show that the subjects did indeed need environmental enrichment's intervention. It's clear that the effect of an enriching foraging strategy depends on the species and its individual's personalities, important aspects which should be taken into account when designed and maintained environmental enrichment programs. Enrichment technique was to be projected according to the desired effect and at the same time, we must ensure that animals choose if, what and when to manipulate environmental enrichment, providing a superior "control" and certainty over their environment, vital to develop well-being in captive individuals (Shepherdson, 1994). Moreover, cognitive capacity must be reflected and integrated in the design of enrichment methods (Broom, 2010).

To improve the success of breeding programs and consequently, wildlife preservation, we need a better perception of appropriate enrichment for different species, to support welfare of the captive populations (Gronqvist et al., 2013). Hence, is urgent to instruct zoos staff for the implementation and innovation of environmental enrichment and that the enrichment effect may not be immediate so we must be 'patient' with its use (Mason and Latham, 2004). It is also vital for researchers to exercitise in communication theory and work with zoo staff to fashion an interactive presentation to spread detailed conservation actions (Smith et al., 2008).

Future projects should include a larger number of individuals and species.

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Appendix

Table 14. Behaviour distribution in all conditions by individual (Ágil - A; Maya - M; Mrs. Piggy - Mrs.; Mr. Piggy - Mr.; Longa - L; Curta - C).

	Baseline						**Bamboo**						**1CEI**					
	A	C	L	M	Mr.	Mrs.	A	C	L	M	Mr.	Mrs.	A	C	L	M	Mr.	Mrs.
Afiliation	5	2	2	7	23	25		3	4	3	15	15	1	1	1	1	7	7
Dominance	2	1	1	1	1		6	1	2		1	2		2	2			
Submission	1	1																
Feeding	205	316	387	298	47	55	272	606	782	228	67	110	145	269	333	38	49	62
Bamboo							132	43	76	58	56	91						
Wire Box																		
Abn. Behav.		58	29					27	6					2	1			
Stereotypy		309	181			126		154	32			27		64	6			12
Grooming	31	307	261	31	162	162	6	259	239	6	161	160	10	51	50	10	59	56
Inactivity	1542	891	874	1614	1927	1730	1193	866	602	1506	1708	1559	551	329	308	518	623	594
Hum (--)	10	2	1					7	2					1	1			
Human 0	45	78	115	5	16	15	220	222	272	54	155	120	95	14	60	15	47	59
Human vocal	22		2	6	1	5	60	4	1	3	3	2	13				3	4
Object	30	3	10	12	1		38	3	10	28			8	2		2		
Locomotion	99	88	169	181	141	200	144	123	256	184	152	192	33	53	101	62	77	70
N. V.	88	371	316	226	51	44	94	98	56	283	13	27	10	111	6	242	7	7
Other	7		6	4	9	8	32	5	11	10	11	18	10	6	6	8	3	11
Self-groom	12	10	72	16	39	44	26	11	50	42	69	75	11	10	38	12	31	19
Sexual		3	14		16	7		6	34		2				2			
Vocal	341			39	6	19	217	2	5	35	27	42	28			7	9	14
Total	2440	2440	2440	2440	2440	2440	2440	2440	2440	2440	2440	2440	915	915	915	915	915	915

Table 14. Behaviour distribution in all conditions by individual (Ágil - A; Maya - M; Mrs. Piggy - Mrs.; Mr. Piggy - Mr.; Longa - L; Curta - C) (continued).

	Wire Box						**2CEI**						**Pos Baseline**						**Total**
	A	C	L	M	Mr.	Mrs.	A	C	L	M	Mr.	Mrs.	A	C	L	M	Mr.	Mrs.	
Afiliation	3	1	1	3	21	21	1	2	2	1	11	11	4	2	2	3	10	10	231
Dominance			2		1	1					1		2	2	1	1			33
Submission												1							3
Feeding	419	762	820	230	168	207	117	181	194	87	31	51	418	450	720	159	109	127	9519
Bamboo																			456
Wire Box	195	162	400	127	92	111													1087
Abn. Behav.								2	30					30	4				189
Stereotypy		196	25			2		229	40			8		228	91			28	1758
Grooming	40	356	344	40	158	158	20	80	80	18	67	68	22	229	228	20	162	163	4274
Inactivity	866	624	350	1293	1655	1576	404	252	309	409	600	563	1082	1079	695	999	1768	1664	35123
Hum (--)	12	13	1				2	1					21	10					84
Human 0	285	135	199	45	91	67	176	72	129	36	112	92	435	229	262	38	159	134	4303
Human vocal	82		1		2	4	31				10	4	71	1	2		17	19	373
Object	24	2	9	14			26			5			32	1	13	22			295
Locomotion	235	130	152	143	116	121	69	72	93	37	34	49	131	86	225	96	110	143	4367
N. V.	57	27	9	469	2	4	3	2	3	311	2	1	18	59	69	1032		2	4120
Other	65	5	6	12	5	15	22	1	2	2	3	7	52	6	6	13	19	32	438
Self-groom	18	20	77	51	76	100	14	21	30	8	22	40	38	25	110	37	48	76	1398
Sexual		7	42						3					3	5				144
Vocal	139		2	13	53	53	30			1	22	20	114		7	20	38	42	1345
Total	2440	2440	2440	2440	2440	2440	915	915	915	915	915	915	2440	2440	2440	2440	2440	2440	69540