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Assessing animal welfare in a captive primate: an analysis of stress related behaviour in Mandrillus sphinx

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Assessing Animal Welfare in a Captive Primate: an analysis of stress related behaviour in *Mandrillus sphinx*

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Submitted for the degree of Master of Science

University of Durham

Department of Anthropology

2006

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ABSTRACT

The study of stress in captive animals maintained in zoos allows to consider elements that play a main role for the welfare of these species. Among these elements there are the zoo visitors, recently found in the scientific literature as negatively influencing the animals especially when these are primates. At Chester Zoo (UK), a group of six mandrills was investigated after having shown signs of stereotypical behaviour comprised of hair plucking. Through the application of ethology, notably the procedure of all-animal scan sampling, a series of variables was collected. From the data it was tested whether the public's density and the noise produced were responsible for the occurrence of visitor-directed aggression and stereotypies. These data were statistically analysed through mixed effects linear models. This statistical treatment provides a powerful way of testing of data with a degree of interdependency (such as scan samples of a particular individual). Such data are often yielded in studies with low numbers of individuals, as is often the case under captive conditions. The results collected from the zoo visitors found that they used to stay for longer in proximity of the enclosure producing a louder noise when the animals were found in the in-door enclosure and in proximity of the glass window dividing the public. The analysis of the animal behaviour showed that a correlation exists between the visitors' density and noise and the occurrence of aggressive related displays towards the public by the animals. The visitors' density was also found to be responsible for the higher occurrence of stereotypical bouts of hair plucking. It was hypothesised that the latter was an indirect relationship and resulted from the fact that aggressive behaviours towards visitors did not have the effect anticipated by the mandrills leading to frustration and subsequent hair plucking.

INTRODUCTION

In the study of stress in applied animal welfare sciences, both the use of physiological techniques and behavioural analyses have served in the attempt to quantify the well-being of captive animals. Captive animals include domestic livestock in farms, wildlife maintained in circuses and zoos, and animals used for biomedical research housed in laboratories. Through the analysis of stress-related hormone concentrations in the blood stream as well as in other body secretions, it is now possible to detect the physiological status of an organism subjected to stress. Nevertheless, there is some dispute on the reliability of such techniques when used to compare different species or even just different individuals within a group. In fact, the hormone production in response to stress varies from individual to individual. In the study of animal behaviour, researchers are facing less criticism, as it has been long accepted that certain behavioural responses are the clear consequence of a stressful status. The measure of such behaviours (such as stereotypies or increased aggressive behaviour) is therefore helping to ascertain whether the welfare of captive animals is at risk through a pre-clinical assessment of distress and disease. Moreover, the use of behaviour could help evaluating animal well-being yet more thoroughly when used in combination with the application of powerful statistical analyses that are still not widely applied in the context of ethology.

The purpose of this research is to apply a conventional behavioural methodology to study sequences of behaviours in a captive primate model maintained in a zoo, and to analyse these sequences with a novel statistical analysis relying on multilevel modelling of mixed-effects linear regression. The study will reveal causation agents which affect behavioural responses of the captive primates depending on external cues typically found in a zoo context.

1 Stress from a proximate and from an evolutionary perspective

The widespread acceptance of the term "stress" in the everyday language has served to focus debate about its definition as applied to different scientific contexts. Practically all the medical and some biological disciplines contend for the prerogative of the study of stress. Each ascribes a particular meaning to stress, a meaning specific

to that particular field of expertise. This section will present an introduction of the term in the medical literature to classify an unbalanced physiological status and how this gradually has become of everyday use. At the same time it will be considered how stress was and still is seen as a driving mechanism shaping the evolution of organisms which become adapted to environmental and social changes after being subjected to stress.

With this perspective, an attempt will be made to depict (i) some of the proximate components of stress, describing its mechanisms and the organs involved, and (ii) some evolutionary aspects which in turn look at the function of stress in different conditions.

1.1 Defining the term stress: founding concepts and a proposed new definition

The introduction of the term stress in biology has posed a series of questions regarding its actual meaning. Trying to find an inclusive definition to be adapted to all the disciplines which endeavour to analyse stress is a rather controversial operation, the term being open to wide interpretation. In attempting to reconstruct its acquisition in everyday language and its use in science, it is necessary to briefly mention the etymology of the term, and to historically reconstruct the context in which the term originated.

The word derives from the Latin "*strictus*", meaning tight or narrow. There is a lack of documentation referencing the change in use of the term from being an adjective to being used also as a noun (Oxford English on CD-ROM, 2005).

The term was first used in mechanics to refer to a physical strain or pressure exerted upon a material object force. In the context of mechanics stress is thus *a force acting upon an object* (Oxford English on CD-ROM, 2005).

It is thanks to the physiologist Walter Cannon that the term was introduced in biology just after the Second World War with a completely new significance as applied to a physiological status (Cannon, 1935). Due to the exhaustive research that followed thereafter, the term stress has acquired a high relevance in the medical study of physiology and psychology. It is common to ascribe to stress the sensation of constraint and the state of increased alertness from for example the pressure of everyday deadlines and a number of other tasks to absolve daily.

In relation to a physiological definition of stress, the Oxford English dictionary reports:

"Stress: an adverse circumstance that disturbs, or is likely to disturb the normal physiological functioning of an individual; such circumstances collectively. Also, the disturbed state that results". (Oxford English on CD-ROM,2005)

With this definition stress is used to refer both to the event causing a physiological response and to the physiological response itself. It lacks therefore a rigorous terminology and it can be applied in a broad context.

The more specialised Oxford dictionary of Zoology similarly gives a broad definition, stating:

"Stress: a physiological condition, usually affecting behaviour, produced by excessive environmental or psychological pressures". (Oxford. A dictionary of Zoology.1999)

Although this second definition seems to add some more information, mentioning also a behavioural change as a result of stress, it is still not a very exhaustive definition for the purpose of studying stress from the animal welfare perspective of this research.

Although these definitions are reported from dictionaries written recently, it seems that the meaning of the term is still rather controversial. An open debate already existed back in the 1960s when an eminent figure in field of physiological stress, Richard Lazarus, wrote that even trying to ascribe a specific meaning to the term stress is somewhat restrictive. In relation to this, he asserted that stress is not a stimulus, nor a response, but rather a collective term for an area of study (Lazarus, 1966).

The disquisition on the use of the term in the scientific literature has not stopped, and still today there are attempts to substitute stress with other more specific terms. Contribution from biomedical work has allowed the replacement of the general term "stress" with **allostatic overload**. Allostatic overload can be defined as a disruption of an organism's homeostatic equilibrium because of an excessive energetic demand (McEwen & Wingfield, 2003). More precisely, it occurs when normal life-history tasks, such as breeding, are impaired because of an incapacity of physiological and/or behavioural adaptation to emerging environmental changes. Regardless of the

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correctness of using allostatic overload rather than stress, from the point of view of the present research, it is more advisable to maintain the term stress. In fact, the focus here is not on the physiological consequences of stress but it is rather on the behavioural changes of the animals subjected to stress.

Moreover, looking at different definitions of the term in modern scientific literature, stress has also been defined as:

> *"the prolonged inability to remove a source of potential danger, leading to activation of systems for coping with danger beyond their range of maximal efficiency"* (Archer, 1979).

This definition becomes useful when looking at the inability of an individual to oppose or to escape from a pressure. This state has been pictured in biology with the expression of "fight or flight", but it shall be seen later how this expression is specifically used in the context of physiology.

More recently the definition of stress has been reworded as:

> *"an environmental effect on an individual which overtaxes its control system and reduce its fitness or appear likely to do so"* (Broom & Johnson, 1993).

This second meaning is also functional because it mentions a reduced fitness of the individual subjected to stress, especially when this is chronic and cause long-term consequences.

For the purpose of this study it is useful to connect these last two definitions together and mould a **new more comprehensive meaning** more appropriate for the purpose of studying physiological and above all behavioural stress in animals. Hence stress is defined here as:

> *the prolonged inability to cope with an environmental or social influencing factor, which overexerts the homeostatic control of an individual consequently leading to a decrease in its fitness.*

1.2 History of the introduction of stress

1.2.1 Cannon's concept of "fight or flight"

The first usage of the term stress in physiology is credited to one of the founding fathers of the study of physiology and homeostasis, **Walter Cannon**, a Harvard Medical School professor. In 1930s the term started to be associated with a

physiological state of an organism. The tendency to maintain a status of constant internal equilibrium and to safeguard internal stability (at the level of organs, tissues and cells) is called homeostasis. Biological systems on average tend to be resistant to changes and external cues. For instance, this happens constantly in homoeothermic organisms which thermoregulate to maintain a temperature dependant homeostasis at around 37°C for primate species.

Cannon recognised this as a fundamentally important characteristic and called "**fight or flight**" the condition of exchange of inputs and outputs from the external environment to the body which reacts accordingly (Cannon, 1935). Regardless of its exact temporal correctness, for which we now know it is more appropriate to reverse the expression and say "flight or fight" given its actual onset (Bracha et al., 2004), "fight or flight" was (and still is) the elegant attempt to describe a reaction to threats to which the body prepares to oppose or escape from potentially dangerous situations. This reaction is controlled by the Autonomic Nervous System which in turn activates hormone glands. To function properly the system must maintain its constant homeostatic conditions which ensure physiological equilibrium for the body to remain stable. Cannon was concerned with the effects of environmental stressors such as sudden temperature changes and haemorrhagic shocks. On average, adaptation to the environment should allow its inhabitants to respond effectively to environmental alterations unless disruptive changes occur. When homeostasis is maintained for most of the overall events, the "fight or flight" response should function so to produce an effective ultimate response in the organism which will preserve its fitness.

1.2.2 The physiological mechanisms: Selyes' General Adaptation Syndrome

The German **Hans Selye** devoted all his research to the intimate study of neuroendocrine activity resulting from stress in the 1940s and 1950s. As a result of Hans Selye's researches, the term "stress" has earned the attention of the general public and the word has become part of everyday use; a use to signify a general adverse condition for the physiological equilibrium of cells, tissues and organs. The definition for the term at issue became the biological consequence of exposure to adverse environments (Selye, 1973).

Inspired by the work of Cannon, Selye formulated the stress-response theory as being characterised by a three-step process which he called **General Adaptation Syndrome**. As a reaction to stressors, a general non-specific response is triggered by

the organism and allows it to fight or flee as Cannon had pictured. The start of the reaction (1) commences with the perceiving of the stressor agent acting upon the body – this is the *alarm* phase; when the homeostatic condition is brought back at constant levels (2) the body has responded with the so called *adaptation*, or *resistance*; the third phase (3) comes with the term *exhaustion* when the equilibrium cannot be maintained anymore and a pathology occurs. Thanks to Selye's (1973) physiological experiments, it was found that there is an hormonal flow triggered by the response to stressors and that it is coincident with the three phases. During the first phase (1) the anterior pituitary gland secretes adenocorticotropic hormone (ACTH) which activates the adrenal cortex to secrete additional hormones (cortical steroids). During adaptation (2) the hormonal discharge remains constant. It is during exhaustion (3) that the glands lose their secretion capacity and organs become vulnerable to illness.

The General Adaptation Syndrome allows an organism to stand the reaction to stress by diverting the energy from non-vital functions (such as reproduction or growth) to, for example, prompt accelerated heart beat or a change of behaviour. The stress response is mediated by increasing activity of the adrenal cortex and the thymus. From the adrenal gland, glucocorticoid hormones (corticosterone in rodents, cortisol in primates) are released activating the reaction of many tissues to employ energy for responding to the stress-related stimulus.

Because of Selye's (1973) work, after the Second World War, the word stress gained conceptual consistency in science. Although the researcher significantly contributed towards the understanding of the stress syndrome, criticisms were addressed towards his definition of the third phase called adaptation. Selye (1973) thought that the homeostatic equilibrium could not be maintained when the hormone supplies were depleted. In fact this is never the case; due to the prolonged releasing of the stress-related hormones, the body becomes sick (Möstl & Palme, 2002). Therefore, it is the prolonged status of stress response mediated by the release of hormones from the pituitary and the adrenal glands that induces the organism out of balance (Möstl & Palme, 2002).

As research continued after the 1950s, additional criticisms of the concept of the General Adaptation Syndrome arose (Mason, 1975). The increased adrenal cortex activity could also take place as a result of emotionally arousing situations. Physiological responses similar to those driven by exposure to adverse environments can be triggered by a variety of external stimuli, and the endocrinological response

may be less general than that detailed by the General Adaptation Syndrome. Different stressors may result in different patterns of response, and different animals respond in their own way according to the acting stressor (but see further below in *A current model for stress*).

Moreover, Selye's (1973) empirical work was based on laboratory experiments with little insights into the psychological consequences of stress. Nevertheless his work is still important for having depicted the stress response as emerging from the Autonomic Nervous System. He showed that this defensive response, or as Selye called it, syndrome, was not dependent upon the type of stressor, because the alarm phase was automatically triggered and the perception of the stressor had no influence on it. To cause the syndrome to be activated, there were not only harmful agents but a wide variety of demands including positive factors such as novel stimuli.

1.2.3 The role of psychology: Mason and Lazarus

In the 1970s **John Mason** was the first to highlight the dynamics for adaptation through the analysis of the intermediate phase between the stressor and the response. The author identified the role of emotions in the response to stress and pointed out that the individual will go through an emotional arousal as a consequence of a stressor (Mason, 1975). In fact, the body response depends also on the activation of structures dealing with an emotional response at physiological level. With this perspective, the response to stressor is not indistinct as Selye (1973) theorized, but it becomes personalised and specific.

As a consequence of **Richard Lazarus'** work (1966) the scientific literature started to ascribe a significant role to the psyche as causal factor in determining the stress syndrome, supplanting the simplistic archetype of a process based on *stimulus-response* action. Lazarus pictured a model of stress in which a stressor can be called as such only when the individual is aware of it and an assessment process has taken place *a priori*. Subsequently, the *stimulus-response* process was updated and modified in *stimulus-appraisal-response*. The appraisal step can be divided in *primary appraisal*, in which the individual assesses whether the environmental cues exceed a certain gravity threshold, and *secondary appraisal* which occurs when the danger has exceeded the threshold and a decision making process will determine what coping options or behaviours are available to deal with the threat. The appraisal step within the stress syndrome ascribes a psychological definition to stress in terms of being

subjectively unable to cope with stressors and this may depend on the individual's psychological make-up.

In summary, the role of psychology in defining the term stress has allowed a much deeper understating of the causal factors ruling stress responses that are by no means universal, and in which the individual's perception of threats plays an important role.

1.3 A current model for stress

As represented in Figure 1, at present there is general consensus about how the stress response is controlled at the perception of a proximate agent interfering with the homeotic status of an animal (Moberg, 2000). The stress response begins (1) when the organism perceives the presence of a stressor: at this stage the nervous system mobilises a neuroendocrine reaction regardless of the actual danger of the stressful stimulus. Once the nervous system is mobilised, (2) it activates a cascade of four different responses involving: (a) behavioural changes; (b) autonomic nervous system responses; (c) neuroendocrine responses; and (d) immune responses. The biological response should cause the stressor (3) to be fought and possibly to be overcome. If this is not the case, (4) the stress will become chronic after an extended period of time, causing the continuous biological mobilisation of resources to become noxious to the organism. The four types of responses may occur altogether, or only some of them may manifest. This is the main criticism to Selye's general adaptation syndrome system in which the physiologist theorised a general and universal response to stress (Selye, 1973). As Mason had correctly supposed, the responses are of different types; they may also be of different intensities depending on the type of stressor, and only some of them may occur at a time.

The **behavioural response** is the most evident reaction to overcoming a stressor. The animal could move away from the source of strain or modify it to make it neutral or favourable. On the other hand a behavioural reaction may often not be possible when the source of stress is psychological. The response triggered by the **autonomic nervous system** involves modifications in functioning of other systems such as the cardiovascular system and the gastrointestinal and exocrine glands. This will involve an accelerated heart beat rate, suspension of digestion and possibly perspiration. These effects tend to be of difficult interpretation for the measuring of chronic stress. In contrast, the **neuroendocrine system** is that biological channel to be stimulated

which gives a long-lasting effect. Hormones produced by the hypothalamus, pituitary and adrenal glands (HPA axis) are released into the metabolism causing the body to function in an altered way. Reproduction, immune response, cognitive abilities, growth, and behaviour can all be affected.
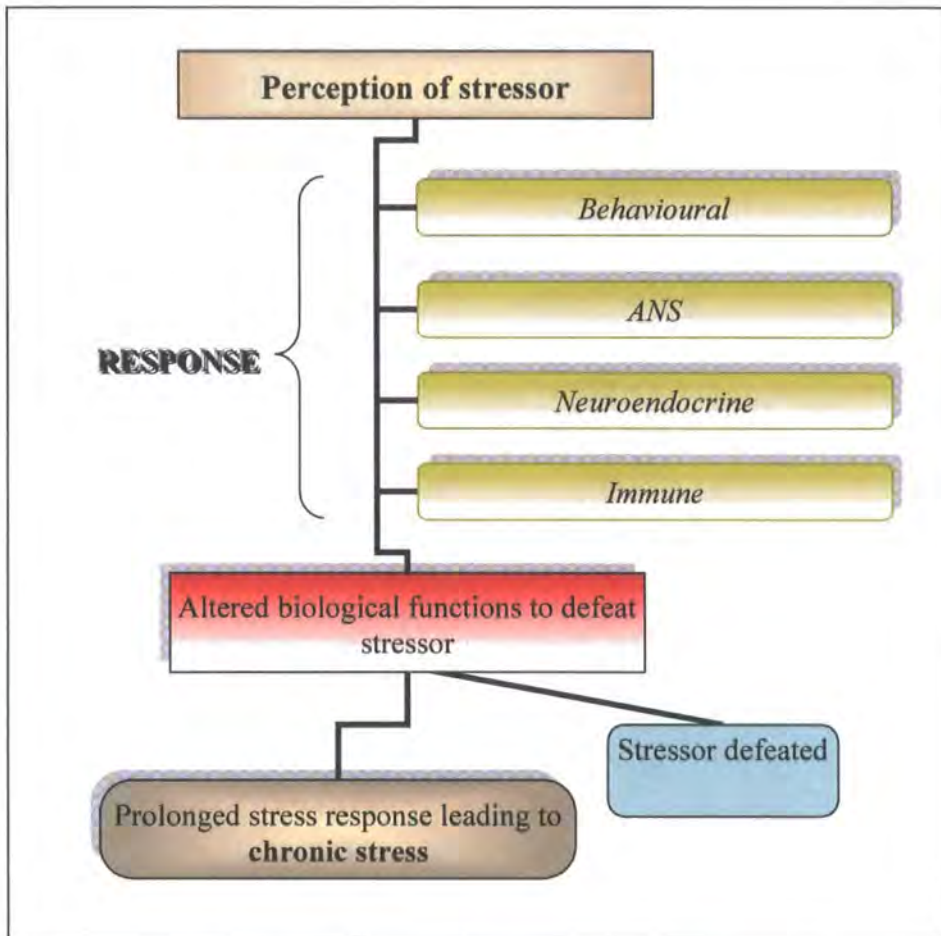
Figure 1 The stress response model. The perception of a stressor triggers four types of different responses: (a) behavioural, (b) from the Autonomic Nervous System, (c) neuroendocrine, and (d) immune. The biological system is subsequently altered, and this will cause a chronic affection if the system does not return to a balanced status.

The result of shifting some of the energy in the system from the normal activities to the extraordinary action required to confront the condition of strain is that some regular activities, such as reproduction, become impaired. The **immune response** was once thought to be a consequence of the neuroendocrine response, but in fact this is not always the case (Moberg, 2000). Immune competence can be harmed allowing the organism to develop infections and other pathologies (Moberg, 2000).

Physiological reactions such as secretion of glucocorticoids, or behavioural changes such as stereotypies have evolved to be systems to cope with stress, leading to an altered biological reaction to fight or flight stress.

There is a psychological sphere of influence in the reaction to stress, and the role of coping is based upon the awareness of the individual that resources for coping effectively are available to fight the stressor.

For the purpose of this study, the proposed definition of stress is "*the prolonged inability to cope with an environmental or social influencing factor which overexerts the homeostatic control of an individual consequently leading to a decrease in its fitness*". This definition implies that stress can be environmentally driven as well as socially driven. The context of a stressor, that is its environment, is important in predicting how an animal will respond to the stressor.

1.4 Abiotic factors: Environmental stress

The role that the **environment** has on stress has recently been researched through molecular techniques. The purpose of this type of research was to see whether the endemic species inhabiting a certain territory are less stressed as a consequence of having adapted a phenotype more suitable to that environment. Conversely, a population is expected to increase its stress levels the more alien an environment becomes (Bijlsma & Loeschcke, 2005). In general terms, organisms are expected to be better adapted to daily and seasonal changes in factors such as temperature, humidity, circadian fluctuations, than to extreme weather conditions occurring infrequently (Bijlsma & Loeschcke, 2005), because their metabolic potential should be more adapted to stand everyday environmental fluctuations than exceptional ones. This suggests that the survival of a species in its environment depends upon possessing the metabolic potential to survive the energetic costs necessary to sustain environmental changes (Parsons, 1996).

The concept of **fitness** can now be introduced as being directly involved in the consequences of stress. Darwinian fitness can be defined as the extent to which an organism is well adapted to its environment to reproduce and pass on genes to future generations. Therefore stress is the impairment of an individual from attaining optimal fitness within its environment. To overcome reductions in fitness, selective pressure is expected to favour those genotypes and their expressed forms of phenotypes that have

better responded to the detrimental impact of stress (Bijlsma & Loeschcke, 2005). In these terms stressors are to be seen as the causation agents for any reduction in fitness of populations. As a consequence, organisms would be continually prone to stressors no matter how small they are. Hence, it is customary to use the term stress for those conditions in which the intensity of the stressors cross a level beyond which the survival or reproductive success of the individual is potentially harmed.

1.5 Biotic factors: Social stress

As presented above, stress can be caused by abiotic environmental factors, yet also **biotic factors** can play a role in causing animals to be out of balance. Biotic factors can be for instance ecological conflicts of predation, competition or parasitism. Even in the absence of environmental threats, an animal may mobilise the stress response due to psychological threats or social conflicts, and this is especially true for primates and other cognitively sophisticated species.

The gathering of individuals belonging to the same or similar species is one of the most widespread phenomena occurring in nature. This gives rise to sociality. Living in a group involves many benefits, but also some costs. These costs are usually related to the limited resources available in the environment as well as in the group itself, and they give rise to forms of competitions for food intake and mating success.

Based on the work of Witter (1995), Parsons (1996) reports that in the European starling (*Sturnus vulgaris*) food intake (a measure of fitness) decreases when the level of competition is experimentally augmented at feeding sites. The energy spent in tackling the stress resulting from competition is therefore lost at the expense of food intake. There is also some evidence that the combination of abiotic and biotic factors triggering stress can entail a much higher physiological demand upon the organisms that can abiotic stress alone. Based on the work of Peterson and Black, Parsons (1996) reports how bivalves are pushed close to a lethal physiological status when a history of crowding is added to stress caused by sedimentation.

Living in group causes the animals to be established in a hierarchy determined by genetic and environmental factors as well as experience. Therefore it is assumed that the dominance rank of an animal will influence the methods the animal adopts to tackle the stressor.

Although most of the research in psychology has focussed on the role of social divergences in human society as a causing factor of stress, it has been pointed out that animals which live in groups are also subjected to psychological pressures that often result in a stress-induced response (Beehner et al., 2005). **Social stress** can be defined as that induced by conspecifics, and results in aggressive behaviour and/or psychological pressure. Potentially dangerous conflicts and disruption of physiological balances are often the consequences of establishing and maintaining **dominance rank**. Social conflicts of group-living animals may often arise because low ranking individuals try to prevail over higher ranking individuals. In baboon species (*Papio spp.*) the immigration of an adult male in a stable group causes more frequent aggressive encounters and an elevation in glucocorticoids in both males and females especially when the immigrant male challenges the resident alpha male (Beehner et al., 2005). Moreover, in chacma baboons (*Papio hamadryas ursinus*), the replacement of the alpha male by an immigrant adult is followed by an increase in female faecal glucocorticoids of lactating and pregnant females (Beehner et al., 2005). It has been shown that this may be the result of a society in which infanticide often occurs when high-ranking immigrants take over the group.

There is no general evidence about whether dominant animals are more stressed than subordinate or vice versa. In the 1950s, research pointed to dominant animals as the more stressed, but a decade after the view was the opposite and subordinate animals were seen as the most stressed (Sapolsky, 2005). The current view is that it is unrealistic to group all the animal species and try to conclude a general pattern, but it is rather more justifiable to look at a general pattern within species. Robert Sapolsky (2005) in a recent paper reviewing social conflicts in non-human primates, divides primate species by those with egalitarian hierarchies and others more despotic. In the egalitarian species the distribution of resources is equal and the dominance is maintained with assistance from subordinate individuals. At the other extreme, in the despotic species, high-ranking individuals recurrently strengthen their status through aggressive behaviour towards subordinates (e.g. ring-tailed lemurs). In the latter, the dominant individuals tend to show higher stress levels as sign of the physical demand employed by the frequent fighting. When psychological coercion is used as the sole or major means of attaining the social class, then the subordinates turn out to be the class of individuals with higher levels of stress (e.g. savannah baboon). A long-term study on olive baboons (*Papio hamadryas anubis*) has found the low-ranking individuals to

have higher basal level of glucocorticoids associated with stress, but these levels were dependant on the individual's rank and personality (Virgin & Sapolsky, 1997).

In a meta-analysis study comprising four Old World and three New World species of monkeys (Abbott et al., 2003), it was found that stress (as measured through the glucocorticoid concentrations of cortisol in blood and urine) occurs among subordinates only in species in which subordinate status carries the highest rates of physical and/or psychological stressors. Further, lack of social support and unavailability of close kin are also prerequisites to higher stress levels among subordinates (Abbott et al., 2003). When the subordinate status implies not being able to attain reproductive success, then the stress imposed by the dominant individuals on the low-ranking individuals is so high that the fitness of these individuals is markedly impaired.

1.6 Chronic stress and the emergence of stress-related disease

It is useful to make a distinction between acute stress, which occurs as soon as a single stressor impinges, and **chronic stress**, the persisting of an out-of-balance homeotic status. In the former case, the acute response is adaptive in many ways as it is implicated in the mobilisation of energy needed for the behavioural response. In the latter case, when a more permanent stress response is triggered, the energy spent to react is shifted away from normal activities. The frequent occurrence of this response may often become maladaptive for the organism which can develop pathological changes detrimental to its health.

When an animal is subjected to chronic stress, it is frequently the case that the accumulation of many acute stressors causes the animal to go into a typical pre-pathological status. With an experiment involving two restrained mice, the combination of two stressors (restraint and lipopolysaccharide injection) caused the animals to employ more metabolic resources and inhibited growth more than either stressor acting alone (Moberg, 2000). The period of distress caused by the two stressors continued for a period of time longer than the cessation of the stressors, equal to the restoring of the normal metabolic functions present before the stress occurrence. This experiment gives strong indication that the persistence of chronic stress is noxious for the metabolic balance of the organisms.

As reviewed by Sapolsky (2004), there is evidence that in particular the neural tissue can be impaired by the presence of chronic stress. Although short-term stress usually enhances cognition, chronic stress disrupts synaptic plasticity and it hinders the neuronal regeneration. Furthermore, at the level of the encephalon tissues, if glucocorticoid hormone levels are chronically elevated, the brain's sensitivity to the hormone becomes blunt, causing a deceptive mechanism of the brain to knock down the hormone concentrations when the stress response should be terminated (Sapolsky, 2004). This is what happens in psychiatric pathologies of **depression** in which there is basal hypersecretion of glucocorticoids. Among wild baboons, subordinate individuals subjected to long-term stress caused by their low-ranking status have been found bearing high levels of glucocorticoids and a blunted response of the brain to knock down circulating stress-related hormones (Sapolsky, 2004). This point is particularly relevant in the context of this study, as it will be shown that the primates under investigation have been screened for the presence of chronic stress.

Occurrence of **cardiovascular-related diseases** is also more frequent in individuals who experience chronic stress. Evidence has been brought forward to show how chronic stress is responsible of atherosclerosis, high blood pressure, suppression of the HDL cholesterol, and suppression of oestrogens. HDL and oestrogens contribute in preventing cholesterol plaque formation and in protecting blood vessels from damage (Sapolsky, 2004). In macaques, the low-ranking individuals of both sexes, which more often are subjected to stress, have low levels of HDL cholesterol and higher chances of atherosclerosis of the coronary vessels (Sapolsky, 2004). Furthermore, females manifest suppression of oestrogens, a process that contributes to the condition of cardiovascular diseases.

1.7 An evolutionary perspective: the functional stress response

As already mentioned, the first insight into the functional response of stress was given by Cannon by depicting the 'fight or flight' response. Under an evolutionary perspective the stress response should have an advantage in the organisms that respond to the many stressors present in the environment. To be such, this advantage should outweigh the costs of the pathological consequences that stress may involve. Arousal of the HPA system increases gluconeogenesis, converting proteins and lipids into carbohydrate, causing more essential energy-mobilisation processes to take over

non-vital ones (e.g. Möstl & Palme, 2002). This process of coping is useful in the short-term period, and stress *per se* is not innately bad (Moberg, 2000). What it is puzzling, however, is not the proximate causation of stress, which is, as we have seen, to trigger a biological change to react to stress. It is rather the understanding of why such a biological response, although so well developed, causes tissue damage and ultimately chronic pathologies. Many components of the stress response are likely held in reserve, instead of being expressed continuously, specifically because they cause tissue damage (Nesse, 1999). This state of affairs is useful only in those situations when the benefits are likely to outweigh the costs. If a more permanent hormonal shift takes place driven by the stress response, the important processes of reparation (immune system), growth and reproduction will be seriously affected causing the stress response to become maladaptive.

Nevertheless, in general, if the term stress has to be referred to in a less negative way, so to consider it as any novel stimuli, then the stress response has evolved so to be within the range of the coping abilities of an animal. Therefore the preservation of homeostasis is usually attained with the mobilisation of the Cannon's fight or flight response.

In order to differentiate between noxious stimuli and others that can be more easily endured, it is useful introducing the concept of **tolerance**, that is, the ability of an individual to accept an external stimulus. The stress stimulus in Broom and Johnson (1993) is divided as (a) tolerable with ease of coping, (b) tolerable but with difficulty in coping, and (c) intolerable with collapse and death. In the first group there are for instance moderate temperature changes, moderate parasitism or social conflicts which are tolerable indefinitely in time and do not cause any loss in fitness. Also those greater stressors in the second group may be tolerable indefinitely, but coping during the time of stress affection is difficult. These are for example body injuries, severe parasitism or, for humans, the condition of being emarginated if not derided at the work place, for which stomach ulcers may result as a consequence (Broom & Johnson, 1993). In the third group of noxious stimuli there are for instance extreme temperature changes or even harsh psychological stressors such as social antagonism. These latter, if they last for too long, will very likely cause the individual to die prematurely or to reproduce not as successfully as without the presence of the stressor.

2. Animal Welfare

The introduction of the concept of Animal Welfare into the scientific literature is a relatively new practice. The first scientific publications about the well-being of farm animals date back to 1980s. Earlier is the establishment in the UK of a Farm Animal Welfare Committee at the end of the 1960s that later became the Farm Animal Welfare Council (Webster, 1998). The remit of the Committee was to give guidance on how to rear farm animals. Later, the Council would establish a code of standards which would have a status in law. This code is of importance as it is the first attempt to recognise the ethical value of maintaining a high standard of physical and psychological health in captive animals. The code was based on five general criteria called the 'Five Freedoms'. These norms were updated in 1993 and they are: (1) Freedom from malnutrition; (2) Freedom from discomfort by providing a suitable environment; (3) Freedom from pain, injury and disease by prevention and prompt treatment; (4) Freedom to express normal behaviour by providing an appropriate environment and company of the animal's own kind; and (5) Freedom from fear and distress by avoiding mental suffering. With the introduction of these 'Five Freedoms' the idea of Animal Welfare overtook the simple equation of good welfare equal to proper nutrition and freedom from disease, pertaining to livestock producers. Gonyou (1994) and Webster (1998) point out that freedoms 1-3 have traditionally been accepted and applied by stockmen, but the latter two freedoms reflect the current concerns raised by the public ethical perception. They divide the freedoms into production traits (1-3) and ethological issues (4-5). The latter division emphasises the importance of ethology as an important discipline in detecting animal welfare issues.

The acceptance of the 'Five Freedoms' as a code of standards to be followed by the animal-husbandry workers meant accepting that animals can experience stress-causing sensations of pain and suffering, and also feelings such as rage, fear, and frustration (Gonyou, 1994). This was a big step forward in accepting animals as conscious beings capable of suffering and feelings in contrast with Descartes' conception of animals as automata.

At the end of the 1980s, the first studies of welfare-related issues such as malnutrition, environmental discomforts and stereotypies appeared. They allowed one of the first reviews on the topic of farm Animal Welfare to be published by Donald Broom (1991). Welfare was defined there as the state of an individual as regards its attempts

to **cope** with its environment (Broom, 1991). When an individual cannot cope effectively, it will be subjected to stress and possible related suffering. Suffering and poor welfare often occur together, and an individual which is suffering usually has more difficulties in coping than an individual not subjected to suffering (Broom & Johnson, 1993). Considering chronic stress as a pathology which causes reduced reproductive success and reduced life expectancy, it implies that when such a state occurs, animal welfare will also be highly affected causing a great reduction in animal well-being. But a poor animal welfare condition may also be present in a condition of lack of chronic stress. For instance, a temporary adrenal super-activity in production of corticosteroid stress-related hormones caused by an attempt of escaping, or an injury-related pain, are likely not to cause any reduced life expectancy, and are therefore not to be classified as chronic stressors. Nevertheless they still cause welfare conditions to decrease.

Suffering was defined by Marian Dawkins (another pioneer in the field of animal welfare) in 1990 as occurring when unpleasant subjective feelings are acute or persist for long because an animal is unable to carry out the actions that would normally reduce risks to life and reproduction in those circumstances. We now possess the tools to measure whether an animal is feeling pain, such as the screening of indicators of vocalisation, body rigidity, and the physiological release of hormones related to pain (Bateson, 1991; Rutherford, 2002). Although suffering is one of the main aspects of animal welfare, it is not useful to equate suffering with poor welfare, as the term welfare is something wider and encompasses the notion of suffering but not solely this. There are in fact instances in which poor welfare can be found with an absence of suffering, as the animals can be for instance not even consciously aware of the presence of a stressor, but still be subjected to stress. An example is when analgesic drugs are dispensed to overcome pain due to an injury provoked in an environment not suitable for the animal. During the medical treatment the animal will not suffer any pain, but the welfare will nevertheless be low.

The **psychological well-being** of the animal is obviously attained when the welfare conditions are at a high standard. Novak and Suomi (1988) proposed a set of criteria to measure the psychological well-being of confined primates. Captive primates should: (1) be in good physical health; (2) display a range of species-specific behaviours and no aberrant ones; (3) not be subjected to chronic distress; and (4) display the ability to respond effectively to environmental challenges. Although

admitting the difficulty of this definition, the authors propose that if two of these four standards are respected, there would be evidence for psychological well-being. Now the standard in animal welfare is of a relatively higher profile and a good welfare should be considered as attained if all of these four criteria are met.

Much more recently, Dawkins (2004) identified two components of good welfare, in the attempt of concisely capturing both the physical and mental aspects of welfare in a general compendium. These two components answer respectively the following two questions: "Are the animals healthy?", and "Do they have what they want?" In order to test whether the environment and the surrounding conditions in which captive animals live satisfy their needs by positively answering the two questions, animal behaviour is imputed as the principal discipline to revise. Behaviour is already used widely in the assessment of health (Gonyou, 1994; Moberg, 2000; Rutherford, 2002). In the assessment of what the animals want, Dawkins (2004) proposes four direct measurements: (1) the judgement of choice and preference, including the use of demand analysis; (2) ecological or in-the-wild measures of preferences; and (3) spatial distribution in the wild. Additionally, (4) the indirect measure of assessing the behaviours related to a positive or negative well-being in the laboratory informs about the same repertoire of behaviours as displayed in the captive environment.

A series of **measures** (see Table 1) can be taken into consideration when assessing for animal welfare. These measures can be divided in poor conditions and good conditions of welfare depending on their attainment. Some of them will be treated and analysed separately in the following sections.

Measures of welfare
Life expectancy
Ability to grow
Reproductive success
Health status of the body
Endocrine system activity
Immune system activity
Presence of disease
Behavioural activities

Table 1 Measures of welfare used to assess animals in captivity.

2.1 Reduced life expectancy

Usually when an animal does not live as long as it would in better conditions, than the animal is said to suffer from bad welfare. The reduced life expectancy of an animal is often caused by poor welfare, but this may not always be the case when animals are maintained in a condition such as in captivity in which they are artificially protected from infections or predation. Because of this, meaningful comparisons of life expectancy of captive and wild animals are difficult because of the external aversive conditions of natural environments not found in captivity. An estimate of life expectancy in the wild worth comparing to the expectancy in captivity would be, for example, the one considering only those individuals not eaten or injured by predators, or not affected by parasites and other life threatening pathologies. That is, free from all the noxious agents found in the wild and not found in captivity.

Reduced life expectancy is found with animals that are not prone to a captive life. For instance, several small species of tortoise which can live for longer than 20 years in the wild, do not go over 2 years in zoos or other captive environment (Warwick, 1989). Similarly certain types of birds captured in the wild have short life expectancy, and cetaceans enclosed in places no larger than human swimming pools live for very short periods. Nevertheless, some primates and other species live in zoos as pets for as long as in the wild, if not for longer.

2.2 Reduced reproductive success

Relative reproductive performance can be used as a parameter to compare the welfare of animals maintained under different conditions. In very poor welfare conditions in which malnutrition is a variable, the lack of **food resources** will at some point cause the animal's body to respond setting off a safeguard mechanism to protect itself from investing resources into non-vital mechanisms such as the reproduction system. The reproductive system can be temporarily arrested in functioning by shutting down some of the many physiological mechanisms which constitute it, such as delays in the onset of puberty (leading to a delay in growth as well), failure or delay in ovulation, failure of embryo implantation, suppression of spermatogenesis, and spontaneous abortion (Pottinger, 1999).

In those animal managements in which **crowding** plays a role in the low-level welfare, reproductive success will be restrained, so to regulate population growth. At

all stages of the reproductive cycle, the number of offspring will be reduced leading to a gradual decrease in population size. It has been found for example that in crowded conditions the size of the testes, seminal vesicles and other reproductive glands are decreased in most farm animals (Archer, 1979). In females fewer fertilised eggs complete their development, leading to a deceptive oestrus that will impede the individual to be receptive (Archer, 1979). An increase in spontaneous abortions can be witnessed in overcrowding conditions, and even when the females succeed in delivering, they will tend to be poor mothers because of being physically prevented to care for the offspring compared to a less crowded environment and also because of a decrease in levels of hormone necessary for efficient maternal behaviour (Archer, 1979).

Even when there is no starvation and the effect of overcrowding is not found, captive animals may be so unsettled in their artificial environment and by their living conditions that may not reproduce when the opportunity is presented to them. Many species of animals maintained in zoos do not breed and some others do only when the conditions are more favourable (Broom & Johnson, 1993). A comparative analysis study using independent contrasts on wide-ranging carnivores that would roam over a large territory in the wild, has recently found that natural home-range size predict captive-infant mortality; when maintained in zoos, carnivores with larger home-range size have on average a higher infant mortality rate (Clubb & Mason, 2003).

2.3 Endocrine system activity

In most vertebrates, the production of the glucocorticoid hormone cortisol is regulated by the **Hypothalamic-Pituitary-Adrenal (HPA) axis**. The adrenal cortex has a central role in the response of an unbalanced homeotic status. Following any stressful event, the hypothalamus increases the release of the Corticotropin-Releasing Hormone (CRH). CRH stimulates the adenohypophysis (anterior pituitary) in the production and release of Adrenocorticotropin Hormone (ACTH) which in turn triggers the release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex. Glucocorticoids have multiple functions in the regulation of the body functions, such as modification of the cardiovascular tone and alerted muscular dynamism. In principle they mobilise energy in the body to stimulate a behavioural response. The physiology of an individual has been designed to make use of

glucocorticoids both for routine functions and also for the less frequent stress-induced response. Because of this, glucocorticoids have two principle effects (reviewed in Munck et al., 1984): (1) the permissive effect of glucocorticoids that allows other hormones to perform their function, and (2) the regulatory effect that is active only during the response to a stressor. The name regulatory was given due to protective function that such response elicits for the organism, preventing the defence reaction to stress from overshooting. Elevated levels of glucocorticoids during the acute phase of the stress response are adaptive, as they effectively set up a cascade of necessary reactions resulting in the regulation of the homeostatic control. Nevertheless, when there is a chronic surge of these hormones, the individual's fitness will be affected, and the individual will possibly manifest pathologies.

The role acted by the **immune system** during the stress response is very relevant, as this is the system involved in resistance to disease. Nevertheless, when the stress response becomes chronic, the system will be affected. Generally acute stress enhances specific immune responses such as increase in phagocytic activity in the blood which mobilises a primary defence against pathogens (Munck et al., 1984). On the other hand, other immune functions are suppressed: this is the case for lymphocytes and antibodies that are initially controlled to avoid an over-activation of defence mechanisms (Munck et al., 1984). But what hormones are responsible for the activation or suppression of the immune response? It has been shown that catecholamines enhance the immune response, whereas corticosteroids have an anti-inflammatory and immunosuppressive impact (Munck et al., 1984).

To focus now on the role of catecholamines, during an emergency response the autonomic nervous system leads to the release of the principal products of the **adrenal medullary**. These are the **catecholamines** adrenaline (epinephrine) and noradrenaline (norepinephrine). In humans, it has been shown how the adrenaline release is triggered by those situations which involve a more passive psychological response. Conversely, for those situations in which a more active physical response is needed, the noradrenaline hormone is activated in turn. Epinephrine and norepinephrine are needed for the regulation of blood pressure, heart beat rate and cardiovascular tone. If catecholamine hormones are to taken into account as measures of stress response, these have to be quantified in a very short period of time, as the metabolism of these hormones is very rapid. The release of these catecholamines from the adrenal medulla occurs within 1 or 2 second of the perception of the initiating

stimulus. Therefore the use of plasma epinephrine and norepinephrine as a measure of welfare in conditions lasting for a short period of time is of value, but only when samples can be taken very rapidly after the treatment (quantified in a very few minutes after the onset of stress response) (Broom & Johnson, 1993).

However, both systems of the HPA and the adrenal medullary production of catecholamines can be activated in beneficial as well as detrimental conditions. So an extra care has to be taken when assessing welfare only considering the hormones involved in the possible stress response. As it shall be seen in the following section of *Assessment of stress*, there is individual variation in the release of stress-related hormones, even when basal level of the same hormones are taken into consideration as covariables.

2.4 Disease incidence

The tenet that the welfare of diseased animals is poorer than the welfare of healthy individuals is self-evident. In relation to this, three variables are responsible for the assessment of how much disease infection is influencing the welfare of animals: (1) the ability of the animal to fight back against the causing agents of the pathology; (2) the extent of the body damage; and (3) the degree of suffering caused by the pathogen (Broom & Johnson, 1993).

2.5 Abnormal behaviour

Conflict behaviours arise as a result of a poor welfare condition due to artificial social condition or an artificial environment that does not allow the enclosed animals to behave as they would in more natural conditions. The introduction of uncommon behaviours in the display range of an animal is a consequence of an attempt to cope with the unpleasant feeling derived from the inability of the animal to solve the conflict.

Abnormal behaviours are those that differ in pattern, frequency or context from that shown by most members of the species in a context which allows the animals to show a full behavioural display range (Broom, 1991).

Usually it is appropriate to ascribe a meaning to these abnormal behaviours so to justify the new behaviour in a functional context. In relation to this, an abnormal behaviour may help the individual to cope with a stressful situation. However, for

some of these abnormal behaviours there is no evidence that performing the behaviour actually helps the individual to cope better. In harsh conditions, the individual can undergo a behavioural pathology when the behaviour by itself causes the animal to worsen its condition by, for example, causing damage to its body or by causing adverse social attrition. For instance, scratching behaviour can cause skin lesions when it is excessive (Fraser & Broom, 1997).

There are behaviours which are associated with lack of resources such as abnormal feeding behaviours caused by an unbalanced diet provided to the animals in captive conditions. Chewing and eating wood, soil, nails, feathers and so on could be the consequence of lack of phosphorous in the feeding regime (Broom & Johnson, 1993). But other more serious abnormal feeding behaviours such as eating faeces or hair is usually the result of marked low welfare conditions. Such behaviours may become "stereotypical" when repeated over time.

Other abnormal behaviours may be the consequence of isolation and lack of social contact with conspecifics. The presence of other animals in the group is a natural source of enrichment that is based on the principle that living in a group is usually adaptive. Companions provide themselves coalitions for finding food or hunting, for rearing offspring in species where helpers supply assistance to parents, and generally for establishing social bounds that constitute the essence of sharing the same environment.

3 Assessment of stress

3.1 Physiological responses

3.1.1 HPA-axis activity

As it has been seen in previous sections, upon the emergence of a stressor, the physiological mechanisms which have adapted to react to the stressors change their activity both endocrinologically and metabolically. During situations of chronic stress, changes at various levels of the HPA-axis may occur, and a production of the glucocorticoid hormone cortisol in primates and other mammals is the direct consequence of arousal of the adrenal gland as a response to stress. Hence, the measurement of concentrations of cortisol excreted is a widely used indicator of stress (Broom & Johnson, 1993; Toates, 1995). Nevertheless, caution should be taken when

measuring such hormones as an index of stress, since it has been noted that a series of confounding factors can play a role in determining the release of this hormone. First of all glucocorticoids are produced in various activities that are not necessarily stressful, such as courtship, copulation and hunting (Broom & Johnson, 1993). It is therefore necessary to measure basal levels of the cortisol that will constitute the covariables in the hormone analysis. Moreover, measurements should take place at constant times of day, as glucocorticoids have a circadian rhythm in many mammal species, but when prolonged stress occurs those rhythms may be pushed out of phase (Möstl & Palme, 2002). Usually it is advisable to survey cortisol level in conjunction with sexual hormones, as the former tend to vary according to oestrus season. Also, using only corticosteroid as an index of stress, it could be concluded that an electric shock is as stressful to a rat as moving its cage in position (Toates, 1995). It is therefore advisable to flank other hormone measurements to cortisol (or corticosterone in rats). In this instance, measuring catecholamines it would be found that an electric shock is indeed more stressful (Toates, 1995). As discussed, catecholamines are a product of the adrenal gland and the main hormone produced in this category is the adrenaline. It must be noted though that catecholamines should also be taken cautiously as indexes of stress. For instance, hard physical exercise in humans induces hypersecretion of these hormones, and so do pathological affections leading to hypoglycaemia (Toates, 1995). Last but not least, when looking at both glucocorticoids and catecholamines, a series of studies have shown how some stressors provoke the production of one or the other, and some species show a bias towards the production of one category of hormones than the other (acknowledged in Broom & Johnson, 1993; Möstl & Palme, 2002; Toates, 1995).

The media to collect hormone concentration measurement has traditionally been the blood, but the tendency of using non-invasive sampling methods has pushed physiologists to develop hormone extraction techniques from urine, saliva, milk and faeces. In fact, sampling from blood often involves the confinement and handling of animals that can cause stress. On the other hand, the use of urine, saliva and milk does not involve breaking the skin, but handling is still necessary. In particular, sampling from faeces reveals itself as the least invasive option, and the use of cortisol metabolites excreted through faeces is gaining more importance both in captivity and in the wild (Möstl & Palme, 2002).

In summary, the time course (see also previous section) and the type of physiological response through hormones differ between various kinds of stressors. As a consequence, the assessment of chronic stress when the welfare of animals is at risk should be done considering multiple hormone indices and for a long period of time, to circumvent circadian and seasonal effects. More generally, it is advisable to contextualise hormone indices in a more comprehensive framework of indicators comprising behavioural indicators.

3.1.2 Heart beat rate

In medicine and farm veterinary sciences, the monitoring of the heart frequencies can give an estimate to judge whether an individual is under the influence of a stress response. Stressors may be the cause of a decrease of the beat rate (bradycardia) or conversely they can cause an increase of the frequency (tachycardia). The two phenomena may be justified in evolutionary terms, as the former could elicit avoidance from being attacked by a predator (in this case seen as the source of stress) discarding a not responding prey, and the latter will instead induce an increase in the metabolic rate for the fight or flight response (Broom & Johnson, 1993).

Change in heart frequency may however be caused by many different factors, other than stress, both physical and psychological, and it may be difficult to discern whether a change in heart frequency is a result of stress solely (unless experimental conditions are used). For instance, if a stressor gives rise to a flight response, such as moving away from a danger, it would be problematic distinguishing between an increased heart beat due to the psychological and emotional pressure acting on the receiver or the rise in metabolic activity to escape from the predator. In addition, the means to measure cardiac activity require the use of heart rate monitors that involve a degree of invasiveness and they can therefore bias the heart response if not carefully tested in advance. Because of this, and because of the issues of interpreting the causes that induce the increase or decrease of frequency, using this index may involve too many inconveniences in a captive environment such as that one provided by a zoo.

3.2 Behavioural responses

In the field of animal welfare the applied animal behaviour sciences are a very important mean to measure animal well-being in captivity. On the other hand,

physiology with the study of hormone flows, heart rates and body temperatures is competing for the scientific explanation of an animal under stress and low welfare status. Why, then, may applied animal behaviour sciences contribute ultimately to unravel this field beyond other disciplines? Likely because animal welfare is not just about pathological statuses deriving from faulty coping techniques confirmed by a measurable unbalanced homeotic status. Animal welfare is above all about consciousness, self-awareness and cognitive processes to date successfully shown by ethological studies particularly. This argument is reinforced also when animal welfare is studied at the level of primates when these are constrained by poor welfare. Although the physiology of primates will have many differences from that of say mice, it is possible that most of the coping mechanisms at the level of body fluids and their constituents are shared among the class of mammals and therefore primates should not deviate too much from mice. On the other hand, it is expected that behavioural mechanisms to cope adopted by cognitively highly evolved species will show a high degree of variability, depending on sex, age, rank position, and so forth. When the diagnosis of psychic disorders driven by difficult environmental or social conditions in human beings is done through consultation with a psychological practitioner, in animal welfare it is the study of behaviour that helps to establish whether the mental status of an animal is at risk.

Analyses of behaviour that suggest poor welfare can be divided into three categories (Broom & Johnson, 1993): (1) difficulty in displaying normal body movements; (2) lack of environmental or social resources leading to frustration; and (3) the presence of artificial and undesired noxious element which cannot be avoided leading as well to frustration. The occurrence of one or more of these conditions inevitably brings the animals to experience chronic stress and the animals exposed to these conditions are likely to experience "behavioural pathologies" (Broom & Johnson, 1993).

The first types of behavioural pathologies are those associated with lack of sufficient space for the animal to move and train its locomotory muscles. These are more frequent in laboratory conditions or circuses, so not very pertinent to the arguments of this research.

The zoo environment may often lack of those necessary environmental and social conditions so to allow the animals, to behave in such a way similar to the one in the wild. With limitation to primates, these animals not only require a large space to allow full locomotory behaviour comprised of climbing, but they also need a complex

environment to induce behaviours such as looking for food (Hosey, 2005). Quite an extensive research has been carried out to design environmental enrichment devices for nonhuman primates, but as it has been observed, no device can substitute the variety of behaviours the primates experience when in close contact with their similar (for a review Lutz & Novak, 2005).

Among the most important elements that can be found in a zoo context and that may become undesired to the captive animals, there are the zoo keepers' presence and the zoo visitor presence. The zoo keepers rarely represent a problem for the animals, since they provide the animals with care and food. On the other hand, the visitors can provide distress to the animals (see later for further considerations).

3.3 Coping styles

Coping styles can be defined as behavioural and physiological stress responses to attain homeostasis typical of a certain group of individuals which are consistent over time (Koolhaas et al., 1999). As reported by Broom and Johnson (1993), it was found that an apparently docile group of hens showing little response to the presence of humans approaching the animals were in fact reacting with a heart response greater than a more proactive group of hens.

Various studies have found that different coping styles exist in rodents (Koolhaas et al., 1999) but also for example in teleost fish (Schjolden et al., 2005). There is therefore now the tendency to ascribe at least two different personality types shown by individuals to tackle stressors. **Proactive** individuals tend to respond to stressors by actively facing the problem with a tendency of being aggressive; **passive** individuals respond to the same stressors by lack of initiative and a tendency to immobilise their bodies when a major stressor impinges (Schjolden et al., 2005). Physiologically, proactive individuals display a sympathetic activation, that is the fight or flight response; whereas passive individuals show a higher HPA axis reactivity with larger glucocorticoids released into the blood plasma (Koolhaas et al., 1999).

One form of copying behaviour usually employed by dominant individuals is **aggression**. The use of hostile and offensive behaviour to attain the control over the social environment can be seen as an instrument utilised by those individuals using proactive coping strategies to reach homeostasis (Koolhaas et al., 1997) and repress

the frustration feeling arising for the unsuccessful control of the stressor (Broom & Johnson, 1993).

The recognition of the existence of different coping mechanisms and the different behavioural and physiological responses to stressors depending on the type of individual shows that extra care must be taken when assessing welfare merely using a singular measure such as activity levels or glucocorticoids.

4 The zoo environment: the role of the zoo visitors

A researcher who tries to collect behavioural data from captive animals kept in a zoo environment faces the challenge to extrapolate and critically evaluate the knowledge on animal behaviour studies conducted in the wild to the captive situation. For instance, concerning the inferring of whether a behavioural response is functional to the animal that performs the action, it shall be noted that the meaning of 'functional' in a zoo environment could be different from that of a more natural environment such as that found in the wild. If the behaviour is functional, this means that it contributes to the animal's survival and reproduction in the environment where it has evolved. Flexibility and adaptability to different environments may lead to a functional response in an unnatural environment also. Increased survival and reproduction cannot be properly measured under captive conditions, due to the lack of predation, food provision, health care, and other factors.

Despite the improved conditions with respect to food, predation and disease, under which species in zoos live, and which might be expected to lead to increased survival and reproduction, often it can be found a reduction in animal welfare conditions leading to incapability of reproduction, stereotypical behaviours and other conditions. These are clear signs of a response that appears to be maladaptive.

The **zoo visitors** are an important reason why the zoo environment may be an unsuitable environment for some animal species to live in and a major reason for their inability to adapt. Looking at the literature published which investigates the role that the zoo visitors have on the captive animals' life, three major trends can be found: (1) the visitors represent a form of enrichment for the animals that live better in their presence (Creel & Johnson K.G., 1995); (2) the zoo visitors do not affect the animal activity, so their presence does not exert influence to the animals (Snyder, 1975); and (3) the zoo visitors are a noxious element for the animals which would live better in

their absence (Birke, 2002; Chamove et al., 1988; Davis et al., 2005; Hosey, 2000; and 2005; Mallapur et al., 2005; Mallapur & Choudury, 2003; Wells, 2005). Simply looking at the number of publications available, on balance, it would appear that the hypothesis most supported is the third one (visitors as a noxious element for the zoo animals), in particular in the recent literature on primates (Birke, 2002; Chamove et al., 1988; Davis et al., 2005; Hosey, 2005; Mallapur et al., 2005; Wells, 2005). Among one of the first studies that sought the influence of the public on the activity of a group of captive mandrills (Chamove et al., 1988), it was found that the zoo visitors acted as a negative form of excitement for the animals which showed stereotyped locomotion, masturbation and hair pulling during times of high visitor density. Similarly, a group of captive orang utans displayed hiding behaviour consisting of putting on paper bags given by the zoo keepers as an enrichments device (Birke, 2002). Again, a group of captive gorillas showed an increased intragroup aggression, signs of stereotypical behaviours given by teeth clenching and body rocking, and increase in frequency of autogrooming behaviour at higher visitor densities at Belfast Zoo (Wells, 2005).

5 Mandrillus sphinx

In the present study, the mandrill (*Mandrillus sphinx*) serves as the non-human primate model species to be analysed to shed some light on possibly occurring welfare issues present in captivity conditions such as those found in a modern zoo. Below the main characters of this species are described. Measuring behaviour to ascertain stress needs to be mindful of the general patterns of behaviour of the species under analysis and it is thus useful to give an overview of the characteristics of the species.

5.1 Phylogeny and Range

In the past, mandrills, drills and baboons were classified as belonging to the genus *Papio* (Wolfheim, 1983). Because of more recent morphological and genetic evidence that has shown a more marked distance to the baboon, the mandrill (*Mandrillus sphinx*) belongs today to the genus *Mandrillus* (Groves, 1993). The drill also belongs

to this genus (*Mandrillus leucophaeus*), but not the baboon (*Papio spp.*). In captivity the mandrill and the drill can hybridise (Painter et al., 1993), but there has never been evidence of such an occurrence in the wild.

A study looking at the cytochrome *b* gene of 70 mandrills in the wild has recently revealed that there are two distinct phylogroups of this species (Telfer et al., 2003). The river Ogooué divides the range of the mandrills from the Cameron and Northern Gabon to those of southern Gabon which have followed different evolutionary trajectories because of geographical separation (Telfer et al., 2003). Additional territories inhabited by mandrills are Equatorial Guinea and Congo (Grubb, 1973).

The mandrills' natural habitat has gone through considerably high alteration due to human settlement and climatic change (Brugiere, 2005). Although there are still patches of almost untouched environment, there has been a gradual impoverishment in the environment composed by the forest-savannah mosaic, old forest growth and the canopy forest of Marantaceae (Brugiere, 2005).

5.2 Morphology

The average body mass of the adult mandrill is about 21-28 kg for males and 11-12 kg for females (Hill, 1970). This size places the animal at the top of the list as the largest monkeys in the world. Because of the remarkable difference in body mass between the two sexes as well as other distinguishing characteristics, the species is highly sexually dimorphic. The extent of body mass dimorphism is extreme when compared not just to other primates, but also to other terrestrial mammals such as the elephant (Setchell et al., 2001). Similarly, also the dimorphism in body ornaments and colours due to sex and the rank status is very pronounced. The adult male mandrill is the most brightly coloured species of mammals and was already recognised as such by Darwin in 1874. Both male and female mandrills have a white and blue snout and an orange yellow beard. In general dominant males present secondary sexual characteristics that are more pronounced than other individuals (Wickings & Dixson, 1992). Dominant males are more colourful than juveniles, subordinates and females. Dominant males present a red nose, whereas subordinate males and females have a less bright and brown nose. The dominant males' rump skin has the same red and blue colours of the snout while the rump is fattened. Similarly, also the mandrill physiology displays

extremes. Serum testosterone levels are five- to ten-fold higher in those males who live in a group than in those who live solitarily (Whitten, 2000).

The signal function of the mandrill body colouration has been recently researched by Setchell (2005). Sexual behaviour of female mandrills does not show preference for males with a higher level of dominance, but it is rather the brightness in colour of the males which is positively correlated with sexual behavioural display. In relation to this, it has been speculated that it may be due to the female's preference for colourful males that male mandrills have evolved their appearance so as to bear colourful characters to attract females (Setchell, 2005).

5.3 Ecology

The social organisation of mandrill societies in the wild is partly unknown, as this primate is extremely elusive in its endemic territories. It is nevertheless known that groups of mandrills can be found in the wild from 15 individuals to hundreds, with more than one dominant male living in group (e.g. Abernethy et al., 2002; Rogers et al., 1996). From studies carried out in semi-free ranging populations in the Lope reserve in Gabon (Setchell et al., 2001; Setchell & Dixson, 2001; Setchell & Wickings, 2005) mandrills are known to have a distinct social hierarchy with young males leaving the group when they mature and living by themselves until they find another group to join (Harrison, 1988). In fact, in mandrill societies, the intra-group male-to-female ratio is about 1:9 (Caldecott et al., 1996). This is good indication that the highest ranking male individuals monopolise sexual access to females.

5.4 Behaviour and Communication

Male mandrills use a range of facial and gestural signals to communicate their status to other conspecifics. As already mentioned, Setchell and Wickings (2005) found that male mandrills possess rank-dependant red coloration on the snout, rump and genitalia that function as a advertisement signal of the fighting abilities of the animals. A mate-guarding behaviour has been found in alpha males to protect their exclusive control over mating (Caldecott et al., 1996). The male behaviour towards females is dependent upon their oestrus status. When the female's rump is swollen the alpha male will near the other sex showing grooming behaviour and mounting (Caldecott et al., 1996). The mounting is usually sudden and rapid.

Recently it has been possible to understand the significance of two elusive signals that were considered by some as occurring during aggressive encounters and by some others as occurring across different contexts. The silent bared-teeth face and the crest-raise signals are now believed to have a conciliatory meaning and they tend to occur in non-hostile contexts (Laidre & Yorzinski, 2005).

Threat behaviour is displayed by jerking the head forward and down sharply in situations when other species use the open mouth gesture protrusion.

These are just some of the behaviours displayed by *Mandrillus sphinx*, others will be analysed subsequently showing an ethogram used in this study.

6 Aims and objectives of this research

The study of animal behaviour for researching stress in captive animals represents one of the goals of the current research sector of animal welfare which is still developing to date. In addition, animal welfare has recently received increased interest from both researchers and the general public that are raising the issue of tackling the discomfort shown by some animal species kept in captivity that display, stereotypical behaviour and other signs of distress (Clubb & Mason, 2003; Dawkins, 2004; Rutherford, 2002). This issue becomes even more timely when it concerns primates, particularly known for being affected by environmental and social conditions at which they are maintained (for example Hosey, 2005; Mallapur & Choudury, 2003).

From observations by the zoo keepers and the research officer at Chester Zoo, a group consisting of six (adult and young) mandrills had shown signs of stereotypical behaviour consisting of hair plucking behaviour. Specifically, the two older males showed alopecia resulting from the hair plucking.

The zoo's research officer and the keepers hypothesised that the most likely cause of this behaviour was the presence of the zoo visitors and, in particular, the behaviour of the young members of the public staring at the animals, shouting at them and also banging on the glass window that separated the public and the animals.

The purpose of this research was, therefore, to investigate whether the zoo visitors truly acted as the stressor agent and could thus be identified to be responsible for the stereotypical behaviour of hair plucking displayed by two of the six animals of the

group. This hypothesis had already been tested and confirmed in other studies that looked at captive primates kept in zoos (Birke, 2002; Chamove et al., 1988; Davis et al., 2005; Hosey, 2000; and 2005; Mallapur et al., 2005; Mallapur & Choudury, 2003; Wells, 2005). However, other studies had also found that the zoo public did not represent a disturbing factor for the primates (Creel & Johnson K.G., 1995; Snyder, 1975).

In particular, the hypotheses in relation to the relationship animal-visitor that were tested have been the following:

1) the zoo visitors' densities cause aggression in the animals;
2) the visitors' noise cause aggression;
3) the zoo visitors' densities cause stereotypical behaviours in the animals.

To overcome the statistical problem arising from the low number of individuals available for observation (only six mandrills are maintained in Chester), mixed linear models were used for the analysis of the data collected through the scan sampling procedure of all the animals. These tests allowed also to take into account the partial dependency of the collected observations that were only 5 minutes apart in time. To the researcher's knowledge, it is the first time that the mixed linear models are utilised for the analysis of animal behaviour.

METHODS

1 Subjects and Housing

The research took place between March and May 2005. It involved six individuals of mandrills (*Mandrillus sphinx*) housed at Chester Zoo (UK). The location of Chester Zoo was chosen according to multiple factors: (a) the high availability of this zoo and its research staff to welcome researchers, (b) the general attitude of the zoo staff for research into welfare issues of the maintained animals, and (c) the large size of this zoo which receives among the highest number of visitors in Europe. The *Mandrillus sphinx* species was chosen as a model to look into stress related behaviour since the animals housed at Chester had shown stereotypical behaviours for quite a long time prior the research, namely comprised of trichotillomania (hair plucking) behaviour. The time spanning from March to May was chosen to allow the observations to take place during visitor-quiet and visitor-busy times of the year (including the very quiet March month and very busy Easter season). This same time period allowed also the collection of data during the cold temperatures of March and the warmer time of May. Depending upon the temperature the animals were expected to range from the inside to the outside enclosures. Of the six individuals monitored, there were three males and three females. One male was adult and identified as the leading alpha male (pilot study with sociometric matrixes not reported in this thesis), the two other males were juveniles. Of the three females there were two adults and one infant (Table 2).

Name	Sex	Date of birth	Age at sampling	Comments
JC	M	25/12/1992	12	Alpha male
Junior	M	31/07/2000	4	Sire: JC Dam: Cabinda
Becks	M	24/05/2002	2	Sire: JC Dam: Cabinda
Cabinda	F	01/02/1991	14	Partially injured left hand precluding agile climbing
Marjorie	F	09/09/1996	8	Under contraceptive treatment
Cath	F	03/11/2003	1	Sire: JC Dam: Cabinda

Table 2 Short life history of the mandrill subjects sampled in this experiment at Chester Zoo (UK).

The animals were housed in an indoor enclosure which had access to an outdoor enclosure constituted by an artificial island built in 1997. The indoor part measuring 19 m. x 10 m. x 5 m. (Figure 2) was furnished with tree branches, ropes and strings hanging from the ceiling, logs and various enrichment devices to encourage complex feeding behaviour and climbing. The floor of this section was covered by wood bark. The temperature of the building was controlled by three warm air heaters and maintained at a minimum of 22°C. The outdoor part of the cage (Figure 3), measuring 40.6 m. in length and 27.8 m. in width (1128.68 m^2), was occupied with young trees, bushes and shrubs. The animals were usually allowed to go in and out between the two cage parts which were connected through two tunnels (3 m. x 0.8 m. x 0.8 m.) which passed through the zoo keepers' area used for the cage maintenance and animal feeding purposes. During the cold winter days the animals were not used to go outside often at all. When the temperature increased the animals did go out more often, but they were used to stay into the building for most of the time during which the zoo was open to visitors.
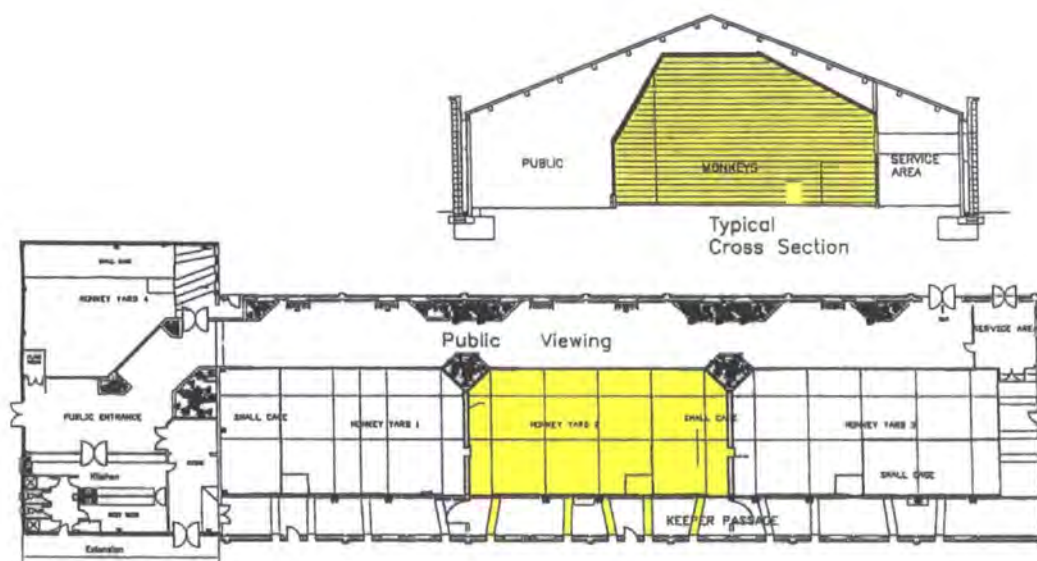

Figure 2 Diagrammatic representation of the indoor enclosure used by the sampled individuals at Chester Zoo (UK). The yellow areas represent the zones occupied by the mandrills. Scale 1:540.
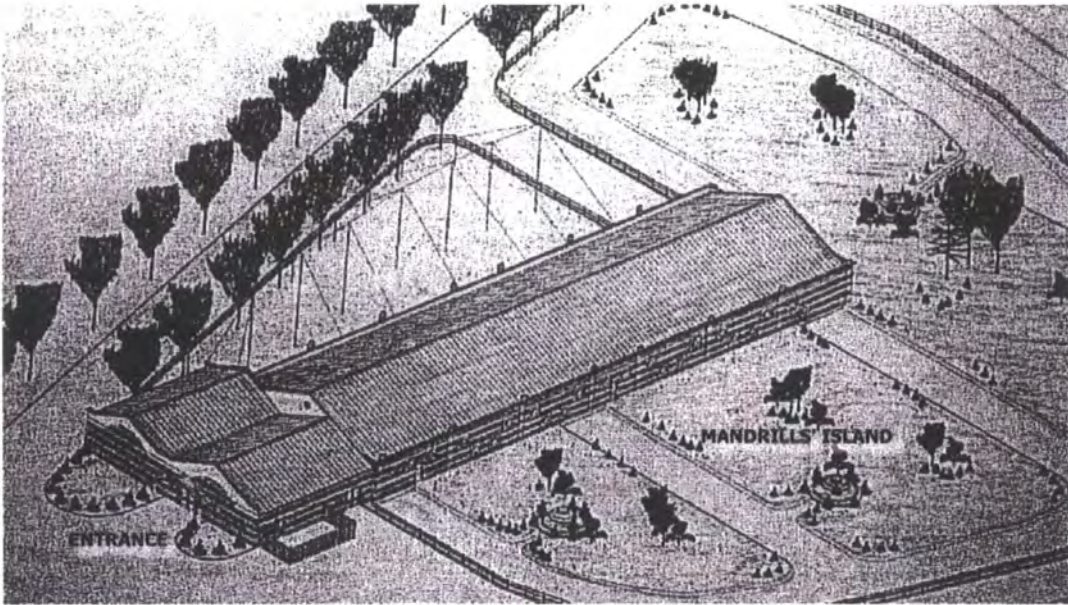
Figure 3 Diagrammatic representation of the outdoor enclosure used by the mandrill group at Chester Zoo (UK). Scale 1:900.

In the inside enclosure, the mandrills and visitors were separated by large laminated glass panels (Picture 1). Here, the areas where it was allowed to the animals escaping from the zoo visitors' view were comprised by the tunnels connecting outside. In the outer area, on the artificial island, most of the time the animals were not accessible to the visitors' view because of the numerous hidden places behind bushes and trees.

Picture 1 Photo illustrating the area where the visitors can observe the mandrills at Chester.

The feeding regime at which the monkeys were kept varied in time from three to four per day. This difference, including the feeding time of the day which was scattered, was done to avoid habituation of the animals according to an enrichment hypothesis. The food was positioned either inside or more often outside in the island especially during the warm season to encourage the animals to use their outside enclosure. Seldom the food was sited at the top of the inside cage above which the keepers could access through a sliding runner. The food consisted of various fruit and vegetables, sometimes enriched by supplemented vitamins and proteins. Water was available in a pool at a corner of the inside enclosure which was used also for hygienic purposes by the animals.

2 Procedure

2.1 Data sampling

The data collected in this study have been taken accordingly to two different methodologies for a total of: (1) focal animal sampling of 35 minutes of continuous behavioural recording of one mandrill at a time for a total of 37 sections of behavioural sampling during 18 days randomly spread through March, April and May 2005; and (2) all-animals scan sampling of activity budget (see later for a definition) of all six mandrill individuals, recorded every 5 minutes during 6 hours of zoo opening time for 42 days during March, April and May 2005. The total hours of observation for the actual study here analysed excluding the time employed for pilot studies amounted to over 250.

The former procedure of focal animal sampling (1) was adopted to collect a continuous stream of data especially from those individuals a priori hypothesised to be mostly affected by stress related behaviours. This continuous stream of data collected through a video camera would have served to be analysed with mathematical software to detect a fractal-like trend. From this procedure it would have been possible to quantify the amount of behavioural complexity per session to be compared among different individuals and between two different scenarios of zoo visitor busy and visitor quiet times. This procedure to date has still to be applied to the collected data since the mathematical software has not been fully developed yet.

Scan samples (2) have been taken to quantify the activity budget of the six mandrills under study. Activity budget can be defined as the way the animal breaks down its activity period during the day, mainly characterised by feeding, resting, and locomotory behaviours. Activity budget data have been usually recorded in the field of behavioural ecology because they reflect differences of how determined species interact with their environment and with conspecifics. In this research these types of data have been recorded to give a broad analysis of the repertoire of behaviours the mandrills display during the time in which they are on display to visitors at zoo opening times. This type of data collection may reveal itself as very useful since it provides recording of dense data points for all the individuals included in the group of subjects under analysis.

A sample check sheet used for the collection of scan-sampled observations is reported in Appendix A.

At each scan sample observation the behaviour performed by the all the animals was collected (Table 4) together with a number of other indexes. These comprises: (1) the receiver of a certain behaviour when this was of a social kind; (2) the nearest neighbour distance measured in metres; (3) the distance to the glass window; (4) the height from the ground in metres; (5) the zoo keeper presence. At each scan sample observation the density of the zoo public was also recorded. In addition, also other sets of data characterising the visitors were taken (Table 3). These other parameters were collected: (1) the type of visitors (whether adults, children or both of them), and (2) the noise produced by the visitors. The noise was indexed as: (a) 0, if the visitors where totally quiet, as when no visitors were found; (b) 1, when the visitors where talking at a quiet volume level; (c) 2, when at least one visitor was talking at a loud level or screaming; and (d) 3, when at least two visitors were found shouting.

Activity (see Table 4)	*code*
Receiver Behaviour with individual # or visitor (RC)	**I#**
Nearest Neighbour Identity (NNI)	**I#**
Nearest Neighbour Distance (NND)	**m.**
Distance to Glass (GL)	**m.**
Height from ground (HT)	**m.**
Zoo keepers' presence (ZK)	☑
Visitor number (Vis.)	**#**
Visitor type (Ty.): A. Adults	**α (1)**
B. Adults and children	**ß (2)**
C. Children or kids	**γ (3)**
Noise Index (dB): A. Silence	**0**
B. People talking quietly	**1**
C. At least one visitor shouting	**2**
D. Two shouting or loud noise	**3**

Table 3 This table gives a further explanation of the check sheet in Appendix A with some of the parameters expanded according to the different categories they belong to.

2.2 Behavioural categories

Twenty-eight behavioural categories were recorded in total and then some of these were grouped into broader categories for statistical analysis. For an ethogram of these behaviours, Table 4 should be consulted.

Caption on the next page

	Behaviour (definition)	*Abbre-viation*	*Bud-get*
1	**Vigilance behaviour** (eye-scanning horizontally rotating the head, guarding from visitors)	**VG**	1
2	**Vigilance hiding from visitors** (eye-scanning the visitors horizontally rotating the head usually from the back of the cage, or hidden by e.g. branches in the cage)	**VB**	1
3	**Staring** (short- long-time eye contact between a mandrill and a visitor. The body is immobilised during these encounters)	**ST**	1
4	**Crest raise** (raising the fur placed around the forehead area. This behaviour takes place during aggressive related encounters with the public)	**CR**	1
5	**Head protrusion** (moving forward the mandrill's head quickly with a challenge meaning)	**HP**	1
6	**Bare teeth** (uncovering the teeth to show them to the zoo visitors. A horizontal and repeated movement of the head may take place concurrently)	**BT**	1
7	**Chewing** (only occurring when next to the glass window towards the visitors with a significance possibly similar to BT)	**CW**	1
8	**Yawning** (with obvious aggressive significance and not for boredom; i.e. next to the visitors. After a long open-mouth act the lips are retracted so to show the teeth)	**YW**	1
9	**Banging window** (using arm and hand against the glass. Usually all the body and the head follow the movement towards the visitors)	**BG**	1
10	**Pacing** (nervous walking back and forth next to visitors with no obvious significance a part from displaying frustration)	**PC**	2

11	**Trichotillomania** (hair plucking using one hand by either carefully selecting one hair to pluck or grabbing a tuft. Trichophagy could follow)	**TR**	**2**
12	**Being protected** (hiding the body behind another's one when a third individual behave aggressively. This behaviour was always noticed by Cath (individual number 6) being protected by Cabinda (4)	**PR**	**3**
13	**Self grooming** (process consisting of carefully brushing and cleaning the body fur with the fingers)	**SG**	**4**
14	**Allogrooming** (as SG but cleaning somebody else's fur)	**AG**	**4**
15	**Playing** (being engaged in activities aimed to enjoyment)	**PL**	**4**
16	**Submissive behaviour** (showing rump, running away being willing to yield to the will of another mandrill)	**SB**	**3**
17	**Resting** (sitting, lying, sleeping in order to relax or recover strength)	**RS**	**8**
18	**Resting while awake** (as RS but with eyes open)	**RA**	**8**
19	**Sexual behaviour** (behaviour aimed to reproduction such as anogenital inspection and copulation)	**SX**	**4**
20	**Looking for food** (digging into the substrate litter, getting food from the top of the cage in order to access food)	**LF**	**5**
21	**Feeding** (eating or drinking)	**FE**	**5**
22	**Handling items** (usually sticks or ropes with the use of one or two hands)	**HN**	**7**
23	**Walking** (moving at a regular but slow pace by lifting and setting down each foot in turn)	**WK**	**6**
24	**Running** (as WK but at a fast pace)	**RN**	**6**
25	**Climbing** (ascending in height steadily)	**CL**	**6**
26	**Waiting for zoo keepers** (staying in the same position looking towards the zoo keepers' direction)	**WZ**	**5**
27	**Aggressive behaviour between two mandrills** (comprising of HP, chasing, etc.)	**GR**	**3**
28	**Out of sight** (behaviour taking place in the outer enclosure)	**OU**	**9**

Table 4 A total of 28 different behavioural categories have been collected in this study, coded for collection purposes in a two-letters key. These behaviours have been sometimes grouped into broader categories (last column identified as activity budget behaviours) for the purpose of analysing broader budget behaviours. These broader categories have been identified in (1) aggressive behaviour towards the zoo visitors, (2) stereotypical behaviours, (3) aggressive behaviours towards conspecifics, (4) sociopositive behaviours towards conspecifics, (5) feeding related behaviours, (6) locomotory behaviours, (7) handling behaviours, (8) resting behaviours, and (9) behaviours happening in the outside enclosure.

2.3 Data analysis

2.3.1 Levels of analysis: observation between and within individuals

The behavioural sampling of this study has been applied in order to collect multiple observations in time from each individual of the sampled group. Through this, it is possible to compare the amount of behavioural variation in place *between* individuals relative to the amount of variation observed *within* each individual. A simple way for the analysis of repeated observations would be the comparison of the total number of occurrences of given behaviours per observational session to estimate a reliable ranked ethogram of behaviours depending on the frequency of their occurrences.

However analysing total numbers of occurrences removes information about within-individual variation which, if used during the analysis, could improve the statistical power of analysis allowing insights into interaction across levels of analysis. By levels of analysis it is intended the level of between and the level of within individual behavioural variation.

2.3.2 The data independency issue

Having collected behavioural scan samples every 5 minutes for each individual poses the dilemma of considering these data points either independent or dependent among each other. A simple way to circumvent the problem would be to consider every behavioural bout as a very specific one which can easily be interrupted by e.g. a glance toward another animal or toward the zoo visitors' space. In such a way during a pilot study conducted prior the actual study it was found that except for the sleeping/resting behaviour, no other behaviour could have usually lasted any longer that a few seconds in a row and never longer than the scan sampled period of 5 minutes (statistics not reported). Considering such a specifically defined behavioural bout definition it could be concluded that every behaviours is independent among each other since a number of other behaviours will have occurred between two different scan samples.

The above conclusion is nevertheless too simplistic, as it would imply the impossibility of having a behaviour never influenced by its 5 minutes preceding one. This is likely to be wrong since behaviour is usually driven by a continuous stream of information and emotional cues which may well last longer than 5 minutes.

Following the same argument it would be erroneous considering all the behaviours found every 5 minutes as dependent among each other. It is self-evident that this is not the case when, for instance, unpredictable events influence the change from a behavioural status to another one.

It has therefore to be concluded that behavioural bouts sampled every 5 minutes are dependent *to a certain degree* among each other. The degree of dependency between the analysed behavioural bouts has to be taken into account and calculated while analysing the data so to adjust for the dependency resulting from the clustering of the data in time.

2.3.3 Mixed-effects models

Both the above discussed problems consisting of estimating variation at different statistical levels and estimating the degree of dependency among the data points may be solved by using the so called mixed-effects models. They are based upon the General Linear Models but here the data are allowed to have non-constant variability and to be correlated.

Mixed-effects models statistical tests have also been called multilevel or hierarchical linear models to point out their utilisation for studies involving different levels or hierarchies of data collected between and within individuals or groups of individuals. In fact mixed-effects models offer a powerful solution for the estimation of variation at the temporal level, within an individual and between different individuals (Hruschka et al., 2005). In order to do so, they offer an index of correlation among observations coming from the same individual. This index is called Intra-class Correlation Coefficient (ICC) is a measure of the proportion of a variance (McGraw & Wong, 1996) and it gives an estimate of correlation within individuals but at the same time also between individuals.

Regarding the matter of partially dependent data collected through scan sampling procedure of 5 min distant data points, mixed-effect models assumes that data are dependent to some degree. The models estimate the degree of dependency together with the usual model parameters and then adjusting for the dependency. For longitudinal data, those repeated in time, the mixed-effect model estimates individual varying intercepts and slopes across time (Goldstein, 2003).

Nevertheless the method does not deal with possible differences within individuals.

In addition the data are not required to be evenly distributed among the individuals, and this becomes useful in situation in which it is not always possible to collect the same amount of data for all the individuals, if e.g. some of them are out of sight. In this case though also the out of sight data have been calculated, as they can provide a useful indication (Goldstein, 2003).

2.3.4 Statistical models used

The model used to analyse the data have been ANOVAs, Mixed Linear Models and ultimately a comprehensive General Mixed Linear Model was conducted.

Confidence intervals at a 95% level for the standard deviation were adopted in all the analyses. All *p*-values reported for significant terms (and non-significant ones, when biologically relevant) originate from the appropriate minimal models.

2.3.5 Statistical software used

The graphical representation of summaries of data here reported has been carried out using MINITAB 14 Statistical Software (MINITAB Inc.), SPSS 13 for Windows (SPSS Inc.), and Excel 2003 (Microsoft Corp.).

It has been possible to statistically analyse the data through SPSS 13 for Windows and MIXOR 2.02, a program for mixed-effects ordinal regression analysis (Hedeker & Gibbons, 1996).

2.4 Permits

Prior the start of the project the necessary permits have been approved by the Ethical board of University of Durham and Chester Zoo. See Appendix B for a copy of the permit from University of Durham.

RESULTS

The results reported in this section are based on a study conducted at Chester Zoo (UK) to assess whether a group of six mandrills was subjected to stress due to the presence of zoo visitors within the proximity of the animals' enclosure. The results have been statistically analysed from a pool of 2699 behavioural samples per each individual (for a total of 16194 observations of six individuals) collected via all-animal scan sampling at 5 minute intervals (all animals scan sampling technique) over 42 observation days in March, April and May 2005.

This results section is divided in two parts: a first part gives a description of the collected data with no statistical analysis; the second part is more analytical and makes use of parametric statistics in order to advance arguments on the role that zoo visitors have on the animals' behaviour. To avoid Bonferroni errors and to guard against Type 1 errors with a confidence interval of 95%, statistical analysis was performed only when it was important to establish significant correlations.

1 Daily activity period

1.1 Activity period

These data are representative of the zoo opening times, and are based on an average of 6 hours per day of sampling period. During the research the zoo used to be open to the public most of the days for 7 hours a day.

The activity period is defined as the time in which the animals were seen performing behaviours different from "Resting Awake" and "Resting Asleep" (see ethogram of Table 4 – Methods – for the associated definitions).

Figure 4 reports in six pie charts the percentage of cases in which the animals have been found active or non-active.

These differences in time management can be interpreted depending upon the rank position, sex and age of the animals. It can be noted how the alpha male (individual no. 1) was the one devoting the least time to resting. It can also be noted how on average the females (individuals 4-6) and the young animals (individuals 2, 3 and 6)

devolved more time to resting when compared to the males and older animals of the group respectively.
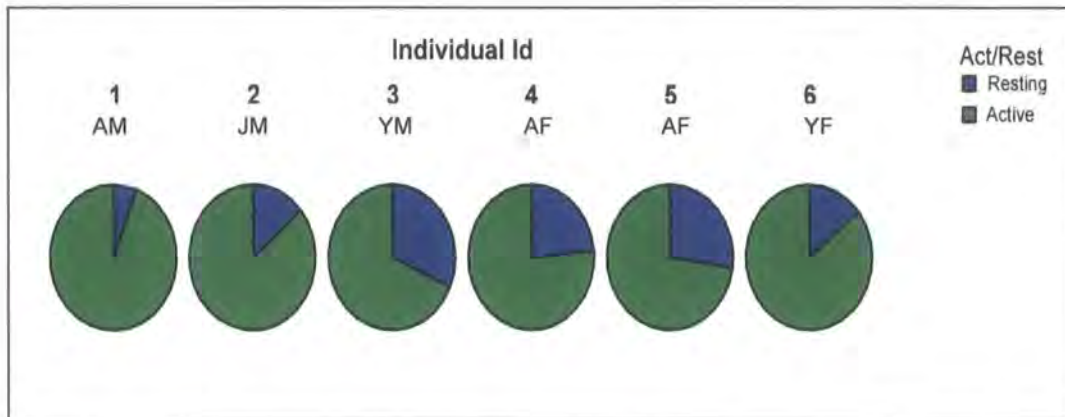

Figure 4 Pie charts of cumulative daily activity period of the six mandrills (1-6). From the data it can be acknowledged the animals had a different activity budget possibly depending upon the rank position, sex and age. Slices represent percentage of cases. AM means Adult Male, JM mean Juvenile Male, YM means Young Male. AF means Adult Female and YF is Young Female.

1.2 Activity budget

The activity budget behaviours are groups of behaviours (see Table 4 in Methods to identify these groups) that can be distinguished as distinct from each others depending on the different functions they acquire for the performing animals. In this research the group of behaviours have been subdivided into: (1) aggressive related behaviours towards the zoo visitors, (2) stereotypical behaviours, (3) agonistic behaviours between two mandrill individuals such as chasing, and threat behaviours, (4) sociopositive behaviours such as allogrooming, (5) feeding related behaviours, (6) locomotory related behaviours, (7) handling related behaviours, (8) resting related behaviours, and (9) all the behaviours performed in the external part of the enclosure on the artificial island. Also the behaviours taking place in the outer cage, although being out of sight, have been considered since, for the purposes of this study, they play a role. In fact these behaviours cannot be related to the presence of the visitors in any way, as the public is not in contact with the animals when outside.

From Figure 5 it can be seen how it is the alpha male of the group and the second oldest male to be the ones performing most of the aggressive related behaviours

towards the zoo visitors (47.5% and 23.9% of their respective total activity budget). Similarly, they are also those above the others performing stereotypical related behaviours mostly represented by the hair plucking behaviour (shown in green in the graph). The alpha male was engaged in hair stereotypical behaviours for 3.8% of its time, whereas the young male for 9.2% (the other mandrills showed much lower percentages). Agonistic behaviours among the animals are little performed by all the animals of the group. Sociopositive behaviours are very seldom seen performed by the alpha male (for only 1.1% of its time), but are frequently performed by the oldest female and her little daughter (for an average of 20% of their time). From the graph there appears to be very small difference among the individuals in the amount of feeding behaviours performed.

The locomotory behaviours appear to be mostly performed by the young individuals of the group (number 2, 3 and 6). At the same way, handling related behaviours comprising manipulation of objects such as trunks and sticks seem to be mostly performed by the young individuals of the group. Resting behaviours have already been shown in the previous section. Finally, all the animals go in the outer part of the enclosure regularly.
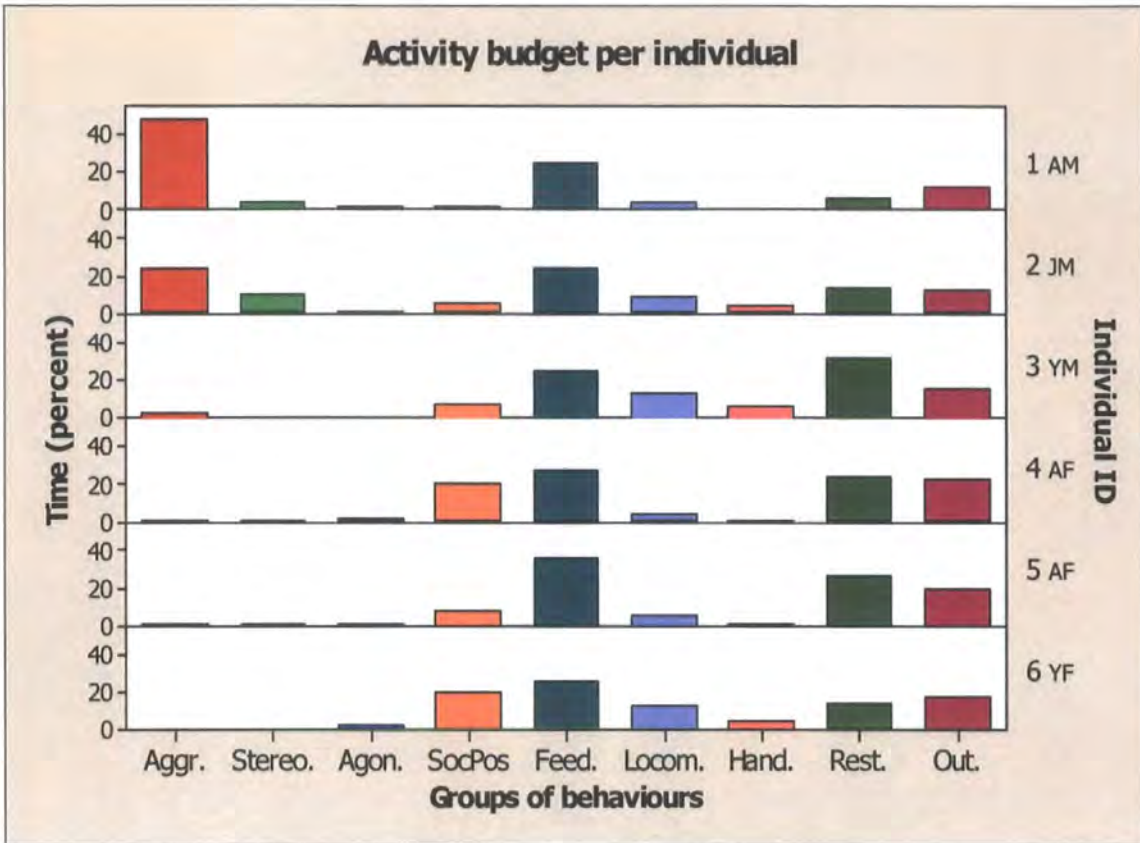
Figure 5 Histogram bar chart of activity budget per mandrill individual. From top to bottom (from the alpha male to the baby female of the group), the figure shows six different charts highlighting the different repartition of time in group of behaviours employed by each animal. Aggr. means aggressive related behaviours, Stereo. is stereotypical behaviours, Agon. means agonistic behaviours, SocPos means sociopositive behaviours, Feed. means feeding related behaviours, Hand. means handling related behaviours, Rest. is resting related behaviours, and Out. are behaviours performed in the external part of the enclosure on the artificial island. AM means Adult Male, JM mean Juvenile Male, YM means Young Male. AF means Adult Female and YF is Young Female.

2 Social behaviours between pairs of mandrills

In order to study the internal dynamics of sociality found in the mandrill group, a histogram chart matrix has been produced to show the frequencies in which the animals where found performing a social behaviour (Figure 6). By plotting this chart matrix with an initiator and a receiver represented in the graphic's axes, it is possible to detect those animals mostly involved in social behaviours and with whom these behaviours took place.

The alpha male was very rarely found involved in social behaviours with the other mandrills. Similarly, this animal was rarely seen performing sexual behaviour with the two adult females of the group, more often with individual number 5.

The second oldest male was never found performing sexual behaviour with any of his mates, given his juvenile age. The social behaviours he was observed performing were exclusively of playing type with his other younger brother (number 3) and younger sister (number 6).

The younger male was also found performing play behaviour with his brother and sister, and very rarely allogrooming behaviour with his mother (number 4).

Individual number 4, the oldest female of the group and mother of 2, 3 and 6 was often found performing allogrooming behaviour with her sons and daughter, especially with the latter of these.

The other adult female was rarely found performing allogrooming behaviour, only seldom with the other adult female. Rare bouts of sexual behaviour took place with the dominant male.

The baby female of the group was seen performing social behaviour with her two brothers and especially with her mother.

To summarise, the two animals mostly found performing social acts were the baby female and her mother together. These social acts were mostly comprised of allogrooming behaviour. The other two animals found performing social behaviour were the two male brothers, who used to play frequently among each others.
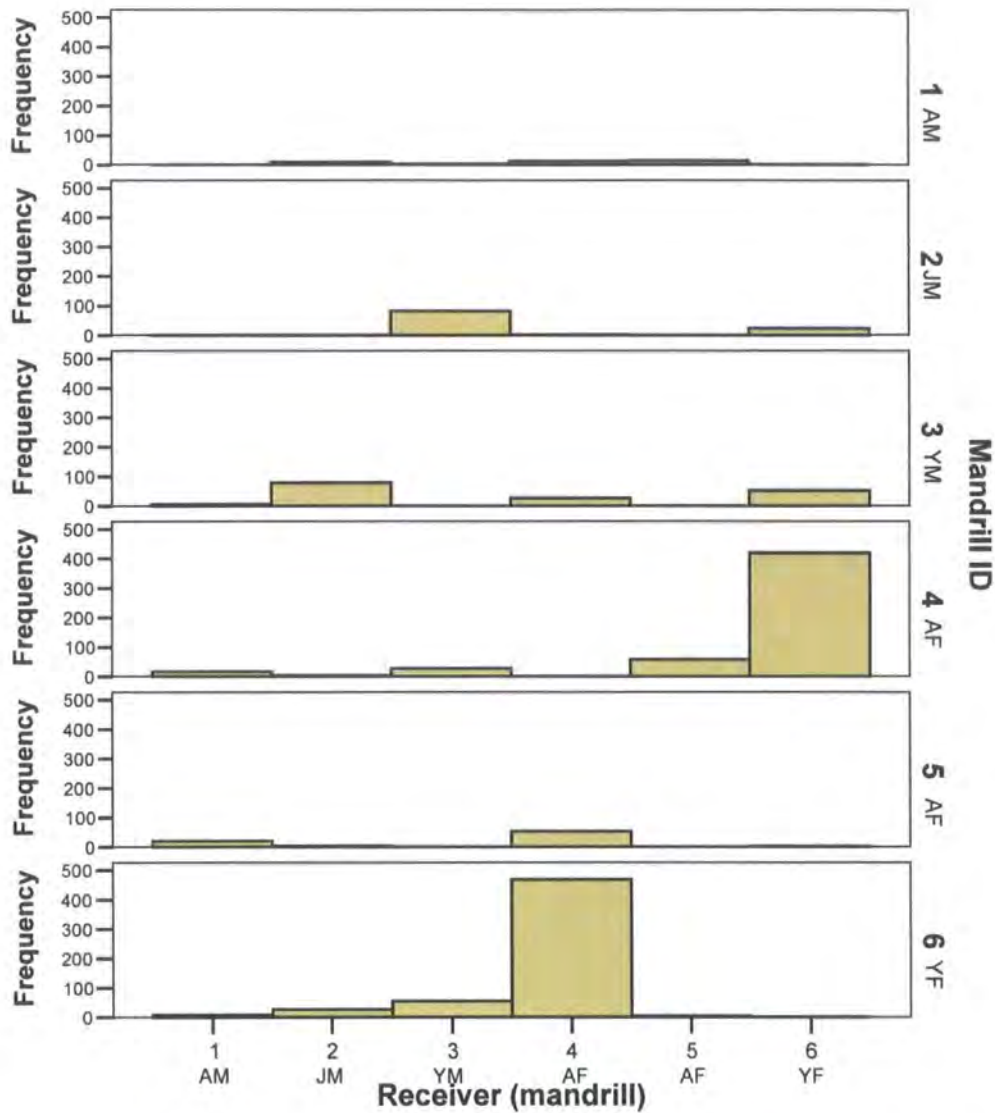
Figure 6 Histogram bar charts showing interrelated social behaviours performed jointly by two mandrills at a time. The *y* axis represents the frequency (the accumulated number of times) at which an animal was found performing a social behaviour involving also another animal. From top to bottom the graphs represents the six mandrills as the initiators of the action. The *x* axis conversely represents the mandrill receivers of the social action. AM means Adult Male, JM mean Juvenile Male, YM means Young Male. AF means Adult Female and YF is Young Female.

3 Position in the cage per individual

This section has the purpose to analyse the way the animals utilise the space provided in the inside enclosure. The graphs showing the results are not intended to give any indication about stress. The aim is to provide a picture on the way depending upon rank, age and sex, the animals move differently and are more likely to be found in different part of the cage at a time.

Individual **number 1**, the alpha male, is mostly found at 0 m. and 2 m. from the ground (Figure 7). At 2 m. from the ground the animals are provided with tunnels which give access to the outer enclosure. Here the animals were often found resting or hiding from the zoo visitors, since these parts of the cage where not very much lighted and accessible to the public view. In addition, the animal was also frequently found right next to the glass window. In this position the animal could perform most of the aggressive related behaviours against the zoo visitors.

Quite differently from individual number 1, individual **number 2**, the second oldest male, utilised the space of the cage in a different manner (Figure 7). Although similar to the alpha male, individual number 2 was mostly found on the ground. The animal was found also at heights from the ground different than just 2 m. above. This is an indication that this much younger animal, if compared to his father, the alpha male, was much more prone to climbing and moving at different heights from the ground. Similarly to individual number 1, individual number 2 was also found right next to the glass window where he could perform most of the aggressive related behaviours towards the zoo public.
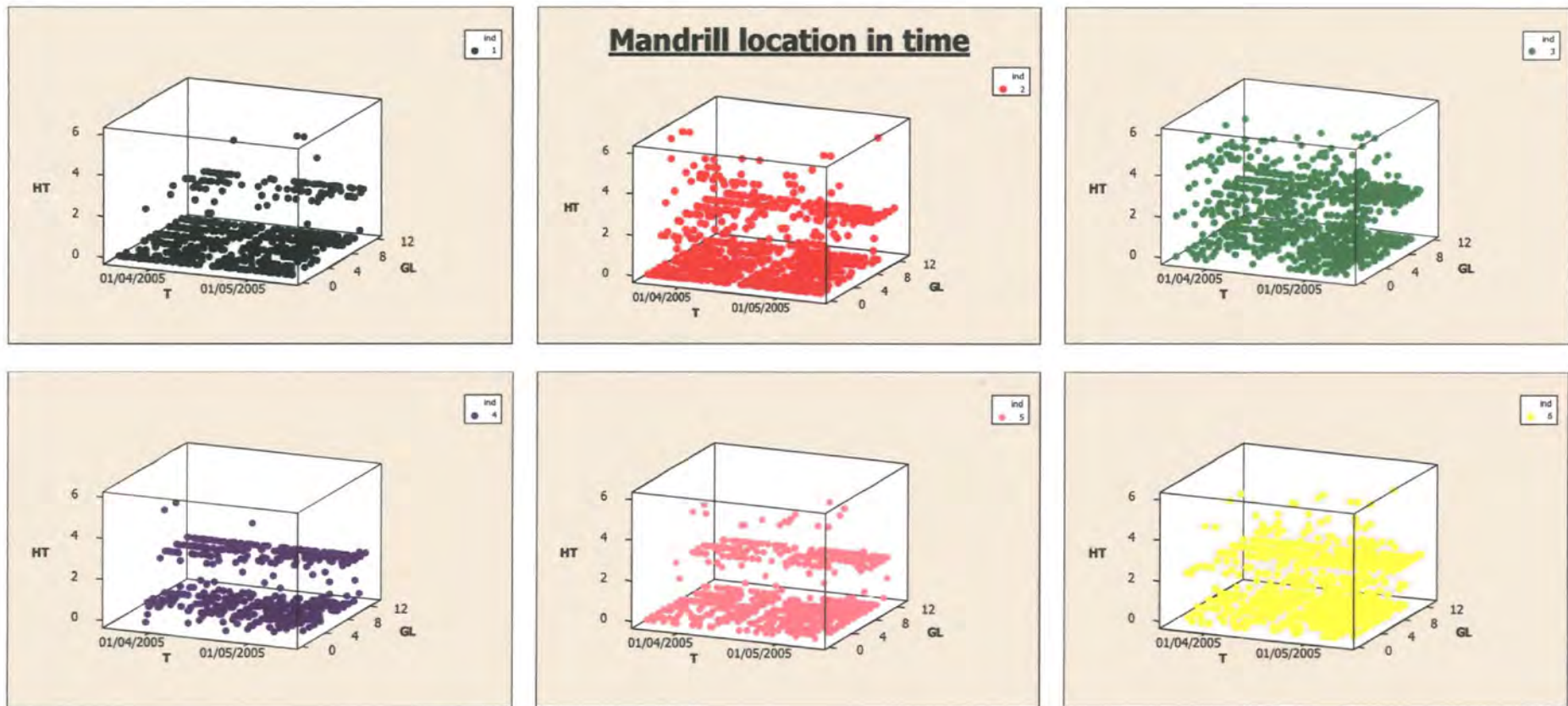
Figure 7 Three-dimensional scatterplot of the position in the cage shown by the six mandrill individuals. The *x* axis represents "time". Each dot represents an observation: since thousands of observations have been collected, it shall be assumed that the graph is dense of multiple observations which overlap. HT means height from the ground, T is time, and GL is distance from the glass window which is at 0 m. Measures are in meters, time is in days.

Individual **number 3** was the youngest male of the group. His larger activity range has well been pictured in Figure 7. From the scatterplot it is clear how the animal fully used the whole space of the cage. This spatial difference is therefore explained given his young age. Quite differently by the two previous individuals, this young male was not found very often at all very close to the glass window. In fact it appeared like the animal did not like the zoo visitors' presence, possibly due to his young age. It is therefore proposed that the animal did not prefer this position, where the close contact with the zoo visitors was most likely.

Individual **number 4**, the oldest female of the group, had a movement pattern much more repetitive and constant over time. About this animal, it has to be noted that full movement was not possible due to a handicap of her left forelimb which caused an impairment of climbing and running. In addition this animal was in a pregnant status: she gave birth to a son in July 2005.
This animal was extremely rarely found next to the glass window of the cage. Similarly, she appeared to prefer the position at 2 m. above the ground, hidden inside the cubicles provided for the connection with the outer island. In this position the animal was often seen allogrooming other mandrills, especially her young daughter.

The pattern of movements of individual **number 5** was quite similar to the previous animal, with the quite evident difference that the animal was also found in the position very close to the glass window. In fact this animal did not appear to be influenced at all by the presence of the zoo public. At times the animal was even seen showing her rump to the visitors, sometimes rubbing it against the glass.

The youngest female of the group, **number 6**, was found in essence in every place of the cage with a movement pattern much more similar to individual number 3, her youngest brother. She was often seen playing with her two brothers and also often allogrooming with her mother. In conclusion the distance from the glass did not appear to have an influence on her movements.

4. The hair plucking behaviour (trichotillomania)

The stereotypical behaviour displayed by some animals was found to be trichotillomania, a condition that raises questions about the welfare standards in which the animals were maintained and the main reason for which this group of mandrill was chosen for the analysis of stress rather than other animals.

Picture 2 shows the condition in which the alpha male was found during the sampling period. This animal presented bald patches of skin on his limbs. Especially his right forearm lacked fur for the majority of its length. The animal in fact was mostly found using his left hand for the purpose of plucking hair off. This male was observed several times tearing off tufts of hair at once, rather than carefully selecting hair by hair (Picture 3). It is probably because of this that this individual was exhibiting this relatively severe condition. After having extracted the hair, the mandrill used to ingest the fur (trichophagy) in the same way it is sometimes seen in human beings with the same form of disorder classified as obsessive and compulsive (for a review Diefenbach et al., 2000).
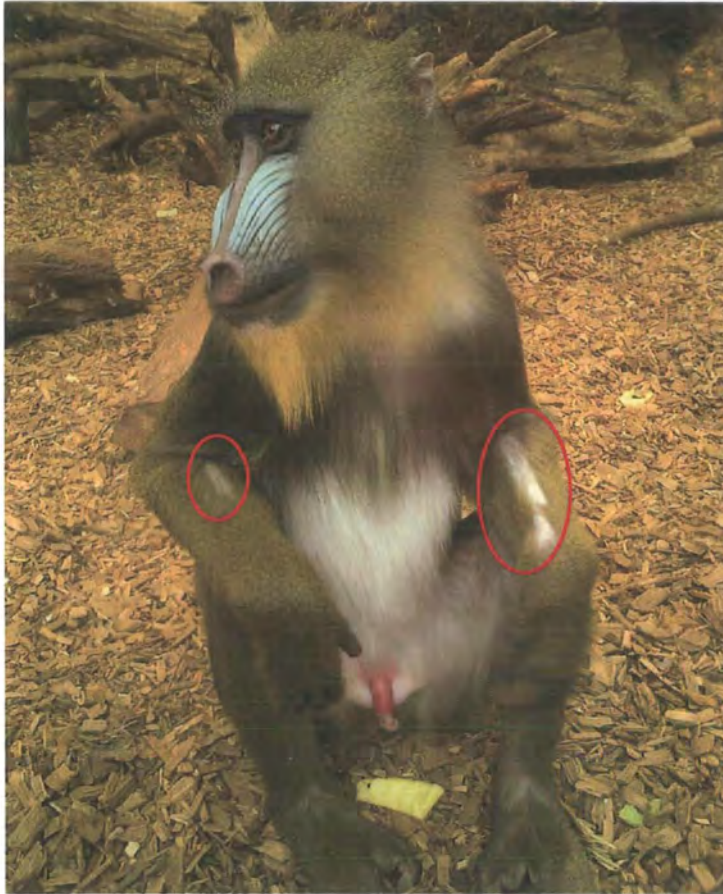

Picture 2 In this photo, individual number 1 (named JC) shows white bald patches of skin on his limbs due to trichotillomania behaviour. The white area of his abdomen is not bald but with a white coloured fur.

Picture 3 The alpha male, individual number 1, is performing hair plucking behaviour on his right forearm which shows consequent alopecia. In this shot the animal has torn a tuft of hair off and he is ready to ingest it (leading to trichophagy).

The other individual often seen performing hair plucking behaviour was the second oldest male (Picture 4). Although this animal did not present lack of fur of the same extent as the dominant male, it was seen performing this aberrant behaviour more often than his father (Figure 5). This animal did not show lack of fur on his legs, and only a small bald patch and a lager one was found respectively on his right and left forearms. The animal was nevertheless noted plucking his hair from other parts of his body, such as from the area around his mouth (Picture 5). As with his father, this mandrill was frequently observed eating the plucked hair.

The two males were not the only animals found performing this type of behaviour. All the mandrills were observed engaged in this activity at least once. Female number 5, for instance, was seen more than once hair plucking from her arms just after denied sexual approaches by the dominant male. Nevertheless, the frequency of these encounters was very low, and the other four animals did not present noticeable bald areas lacking fur.

Picture 4 In this photo, individual number 2 (named Junior) shows bald patches of skin in his forearms. The left arm was the most affected by alopecia.

Picture 5 Individual number 2 is pulling a hair off the area around his mouth. Differently from the dominant male, this animal was more often observed carefully selecting one by one the hair to pluck.

4.1 Location of hair plucking behaviour compared to aggression

In this section the first two graphs of the previous six (Figure 7) have been repeated removing all the behaviours not related to stereotypes. The purpose of these two graphs is therefore to identify whether there are preferred locations where the two oldest males were observed performing maladapted behaviours of hair plucking. If preferred locations are found, the zoo keepers could be advised of encouraging the animals to use other spaces, so to avoid the repetition of stereotypical behaviour. In addition, if these areas overlap with those in which the animals are seen displaying aggressive related behaviours towards the visitors, some conclusion could be drawn about phenomena of causation.

The alpha male was observed hair plucking more frequently very close to the window and at 8 m. from the glass, where the animal was often seen performing eye scanning behaviour towards the zoo visitors from an inner cage used to isolate an animal from the others (Figure 8). This inner cage was usually left open when not used for its primary purpose of isolation, and the alpha male appeared to like this place staying still in there, possibly because the bars between the animal and the visitors allowed the animal to feel in a place out of sight.

At 0 m. from the glass this male was also seen performing most of the aggressive related behaviours trying to frighten the zoo public. So, it looks like there may be a correlation between the position in which the animal displays aggressive related behaviours towards the public and the position in which the animal is found performing trichotillomania. As already mentioned, if the behaviour associated with performing eye scanning behaviour to guard from the visitors entering into the space closest to mandrills' enclosure is considered as an aggressive related behaviour, also the position at 8 m. from the glass is then a location in which the animal performs aggression. This position is as well one in which the animal is seen performing stereotypical behaviour (Figure 8).

As a first preliminary conclusion, some sort of relation is therefore hypothesised between the two types of behaviours of visitor-directed aggression and stereotypies performed by the dominant male of the mandrill group.
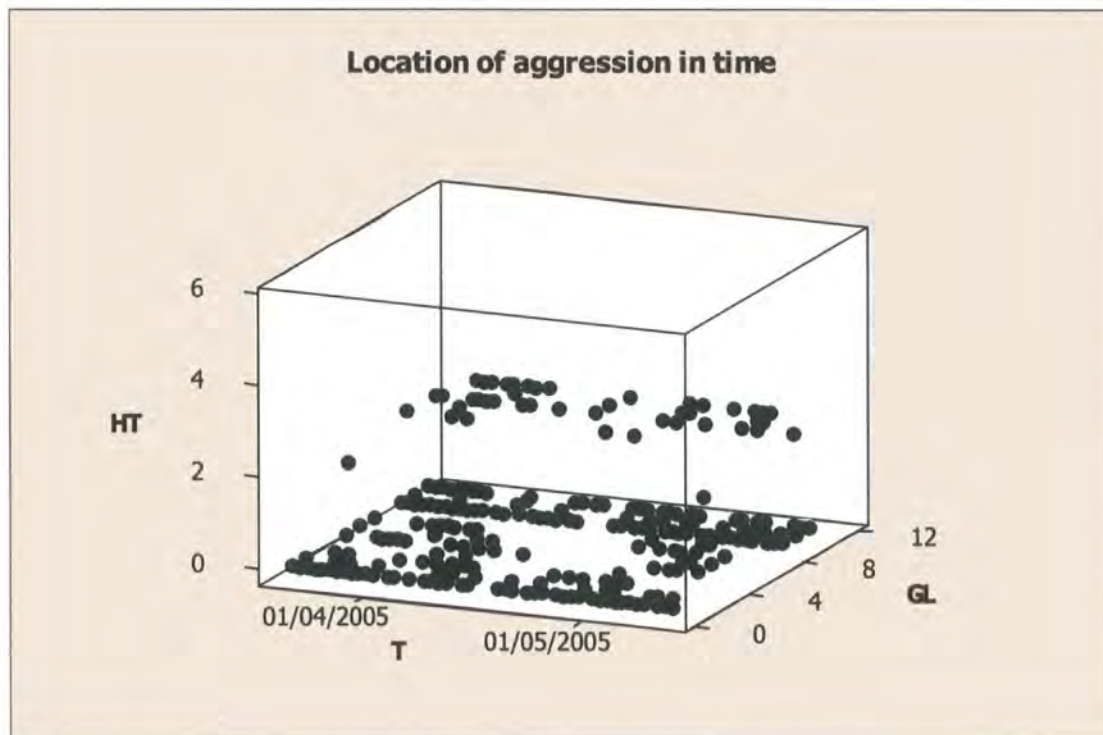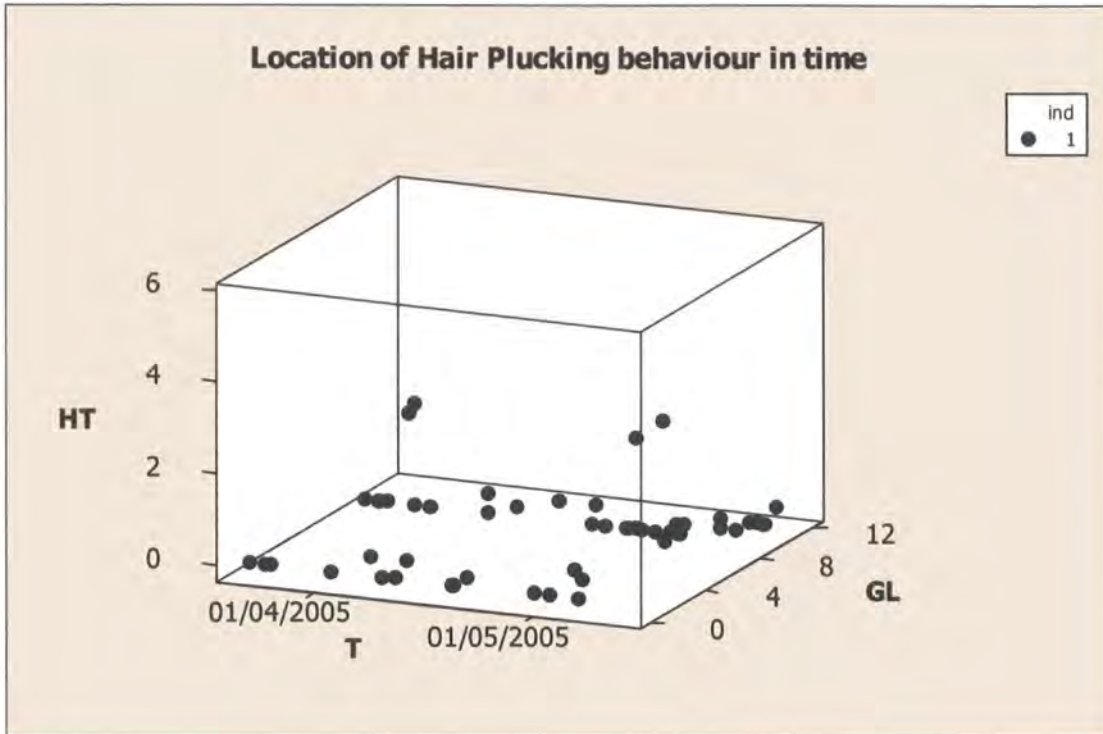
Figure 8 Three-dimensional scatterplot of the position in the cage shown by individual number 1 (the alpha male) while showing trichotillomania (top graph) and aggression towards the visitors (bottom graph). The x axis represents "time". Each dot represents an observation; since thousands of observations have been collected, it shall be assumed that the graph is dense of multiple observations which overlap. HT means height from the ground, T is time, and GL is distance from the glass window which is at 0 m. Measures are in meters, time is in days.

The location of hair plucking behaviour performed by the second oldest male of the group appears to present a different character than the alpha male's one (Figure 9). The only common point is the preferred location at 0 m from the glass window. Indeed and similarly to his father, also this animal was found performing trichotillomania in this position of the cage, close to the zoo public. Moreover, the animal was noticed performing the aberrant behaviour ubiquitously in the cage, with no particular preference in depth or height. Nevertheless, an association in the location of the stereotypical behaviours can be found with the occurrence of aggression towards the public. Although the stereotypical data is less dense than the aggression which happened more frequently, from the two graphs of Figure 9 it appears that a correlation in place took place between the two behaviours.

In conclusion it can be inferred that the position very close to the glass window of the cage is a preferred one for both of the two mandrills for aggression and hair plucking. The other positions are probably a consequence of the different general movement patterns of the two animals: the alpha male more repetitive and constant over time, and the other male more scattered across the enclosure.

Further analysis is required in order to establish if aggression (together with the presence of visitors) was causing stereotypical behaviours.
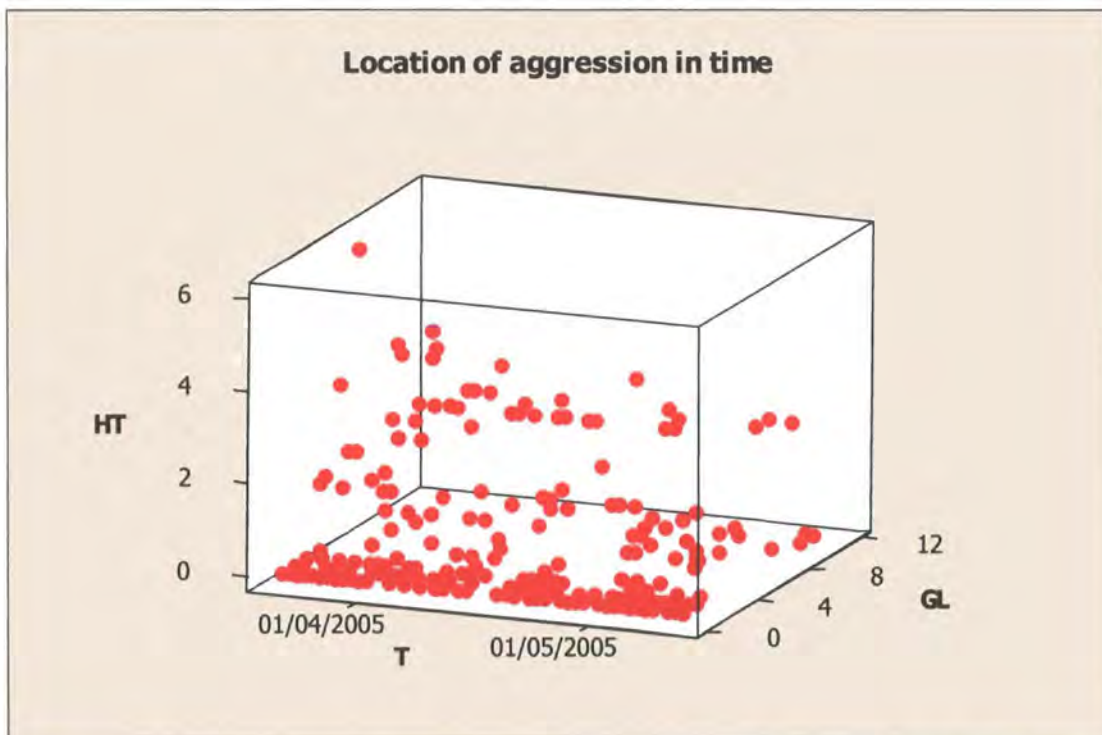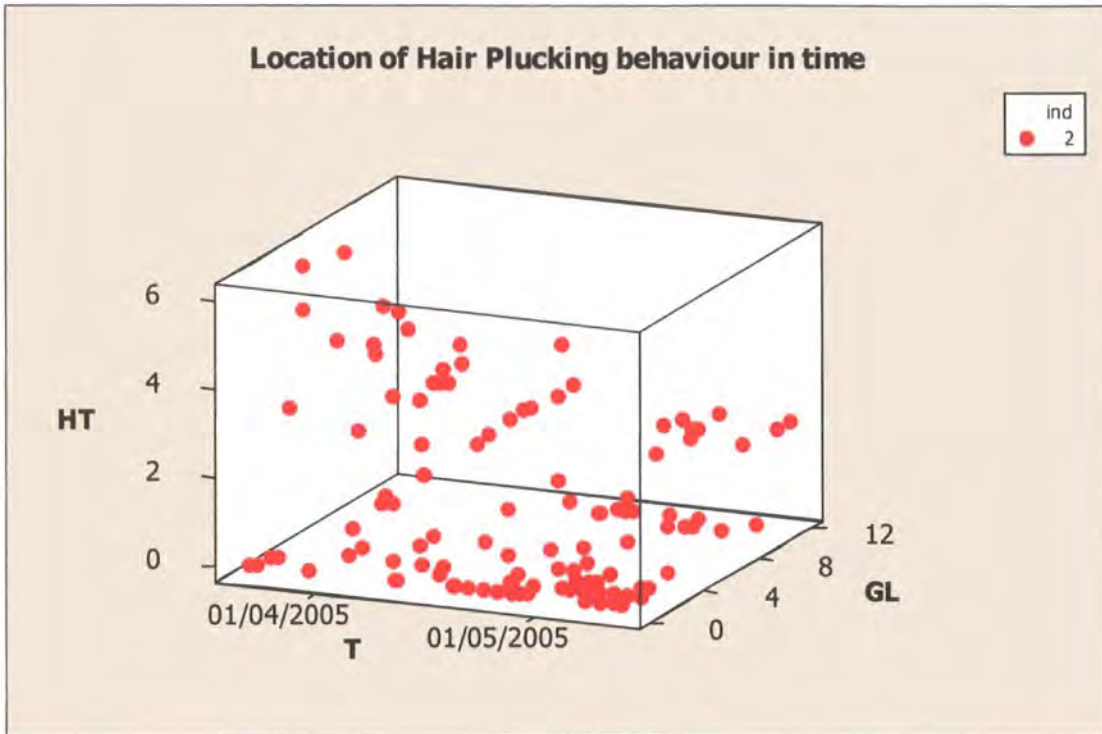
Figure 9 Three-dimensional scatterplot of the position in the cage shown by individual number 2 (the second oldest male) while showing trichotillomania (top graph) and aggression towards the visitors (bottom graph). The x axis represents "time". Each dot represents an observation; since thousands of observations have been collected, it shall be assumed that the graph is dense of multiple observations which overlap. HT means height from the ground, T is time, and GL is distance from the glass window which is at 0 m. Measures are in meters, time is in days.

5 Zoo Visitors

During a sample study, the zoo visitors have been analysed in order to detect differences in the duration of their visit in front of the mandrills' enclosure depending on the animals' behaviour. It was hypothesised that the visitors were going to stop in front of the cage for longer when the animals where found in the inside enclosure, rather than when the animals were in the outer enclosure, not reachable to the visitors' view. In addition, it was also hypothesised that the zoo visitors would have stayed for longer in proximity of the cage when the animals were found very close to the glass window. A number of data samples were taken in order to assess this and a descriptive analysis is reported.

A total number of 48 samples were taken when the animals were found in the outer enclosure. In these instances, the zoo visitors were found stationary in front of the inner cage for a mean time of 24 seconds (± 1 sec. std. error) (Figure 10). Conversely, for a total number of 42 samples, when the animals were found inside, the average time was longer: 1 min and 33 seconds (±10 sec. std. error). When at least two animals where found right next to the glass window, for 41 times the visitors were found spending 2 minutes and 33 seconds (±16 sec. std. error) in proximity of the enclosure. These three sets of data, when analysed statistically (one-way ANOVA), have been found significantly different among each other ($F_{3,127} = 36.03$, $p < 0.001$). Subsequent Tukey post hoc tests were performed to see whether all of the sets of data were different among each other, and this was confirmed (statistics not reported). This figure suggests how the presence of the animals in the inside cage and their location were of a determinant influence in the duration of the visit.

Looking at the other parameters collected, that is the number and the type of visitors and the noise produced by them, the following was found.

The number of visitors in front of the enclosure, that is whether the visitor groups would have been larger, have not been found statistically different depending on the animals' location (one-way ANOVA, $F_{2,127} = 1.72$, $p = 0.18$).

The type of visitors (whether adults, children, or both) have not been found changing depending on the animals' presence (one-way ANOVA, $F_{2,127} = 0.515$, $p = 0.59$).

The results have been found with a significance in the variation of the noise index produced by the visitors (Figure 11): the visitors were noisier when the animals were

inside the cage and even more when the animals were next to the glass window (one-way ANOVA, $F_{2,127} = 11.61$, $p < 0.001$).

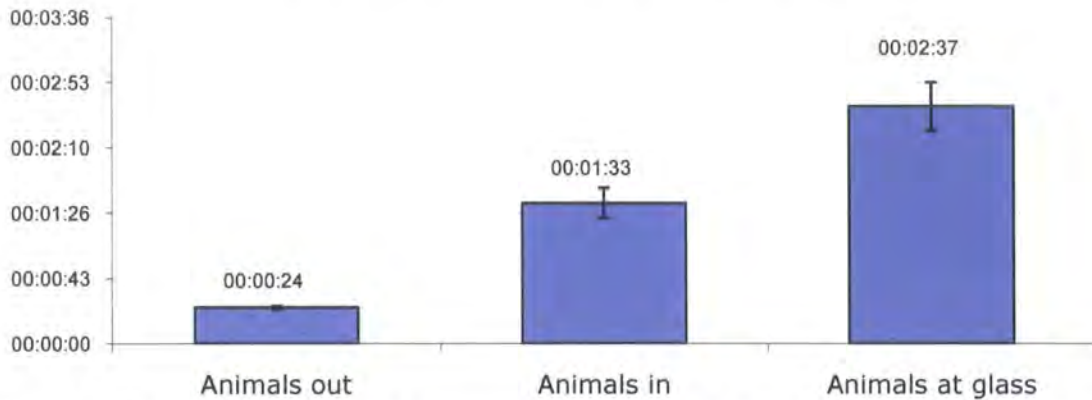## Average public's visit (hh:mm:ss)

Figure 10 Histogram bar chart of the average duration in time a visitor would spend in front of the enclosure depending on the location of the mandrills. The visitors tended to spend longer time watching the animals when these were found in the inside cage, and even longer when at least two animals were found very close to the glass window. The error bars represent ± the standard errors.
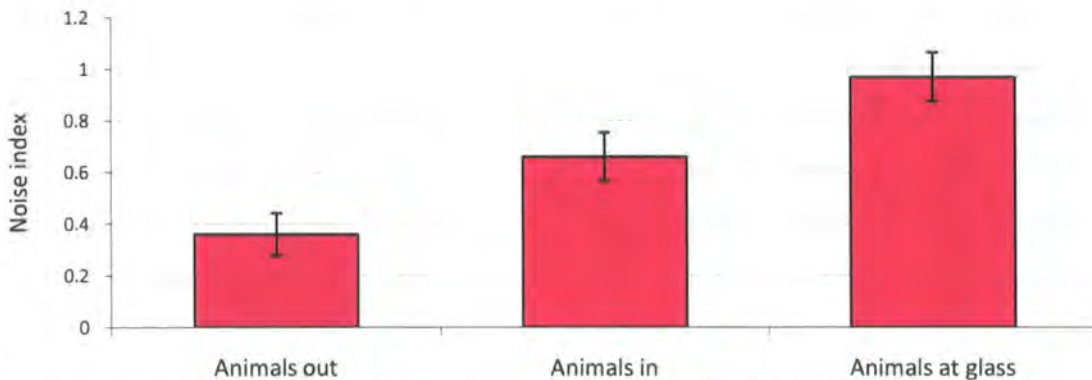
Index of visitor-produced noise

Figure 11 Histogram bar chart of the average noise produced by the public in front of the enclosure depending on the location of the mandrills. The visitors tended to produce louder noise when the animals were found in the inside cage, and even louder when at least two animals were found very close to the glass window. The noise was measured with an index consisting of: 0, if the visitors where totally quiet, as when no visitors were found; 1, when the visitors where talking at a quiet volume level; 2, when at least one visitor was talking at a loud level or screaming; and 3, when at least two visitors were found shouting. The error bars represent ± the standard errors.

6 The influence of the zoo visitors on the mandrills' behaviour

6.1 The visitors' presence and their density influence mandrills' aggression

In order to test whether the zoo visitors had an influence on the mandrills' behaviour, and more in particular on the aggressive related behaviours displayed by the animals, the Mixed Linear models were used to analyse interdependency between the visitor number data and the frequency of these behaviours. The aggressive related behaviours towards the visitors were grouped together and were assigned a value of 1 (see budget behaviour number 1 of Table 4); all the other behaviours were assigned a value of 0. Also the behaviour classified as out-of-sight was considered as 0, since it was assumed impossible for the animals to display aggression towards the visitors while on their island because of lack of eye-contact with them. These data were therefore maintained so to describe the totality of the budget behaviours collected from the animals.

Although it appeared clear that only the first two males were the ones performing most of the aggression, as a first analysis all the animals were analysed for this character so to outline a general trend. Under the Multilevel Linear model wording (a synonymous for Mixed Linear models), the first level of analysis was constituted by 2698 repeated observations per each animal (within each animal) and the second level was constituted by the 6 individuals of the group (between animals).

This preliminary analysis was performed using the statistical software called MIXOR, which did not give any problems of overinterpreting the results, since the assumptions of the test were fully met. The data were therefore of a dichotomous type analysable by the ordinal assumption used by the software. It shall be noted, for methodological purposes, that a logistic function model was used as response.

Through a total number of 1701 iterations used to compare the stream of data with its random equivalent, the analysis detected a significative correlation between the number of visitors and the occurrence of aggressive related behaviours in the mandrills ($Z_{6,16067} = 3.45$, $p < 0.001$).

Figure 12 reports the correlation between the incidence of aggression and the natural logarithm of the number of visitors. The mandrill individuals have been panelled to stress the difference in behaviour depending on the rank of the individuals. The visitor number data was transformed into its natural logarithm only for graphical purposes.
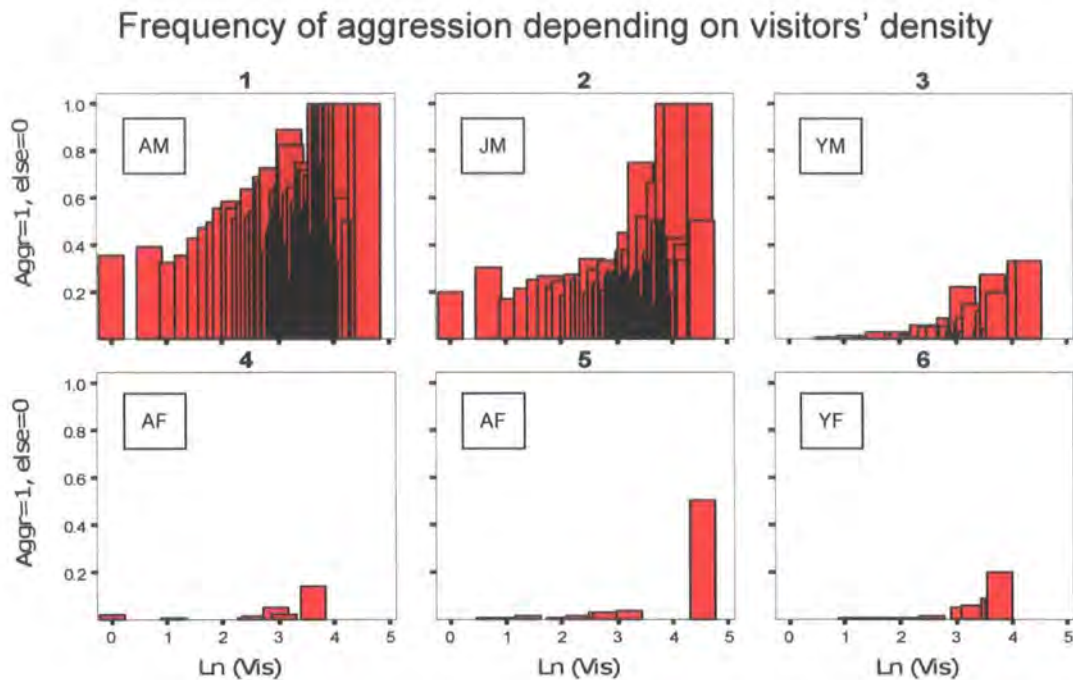
Frequency of aggression depending on visitors' density

Figure 12 The six panelled bar charts represent the increasing in frequency of aggressive related behaviours (budget behaviour number 1, see Table 4) that the six animals (panels) performed depending on the number of visitors present in proximity of their cage. For graphical purposes, the visitor number variable (the *x* axis) has been transformed in its natural logarithm in order to group to fewer classes the more disperse occurrences of higher numbers of visitors (those towards the right side of the *x* axes). In fact, very high numbers of visitors have been encountered only rarely (from above 40 visitors). Since the *x* axis is scaled at a logarithm scale, the bars at the right sides of the panels tend to gradually overlap. The y axis represents means of the probability of encountering aggression towards the visitors by the animals (0 = null probability).

The Intra-class Correlation Coefficient (ICC), which gives an estimate of correlation between individuals, gave a value of 0.091. This value in the multilevel models provides an indication for the fact that there is likely to be a high variance between the individuals. This argument was also supported by the fact that all the individuals except number 1 and 2 were seen performing very little aggression towards the zoo public (Figure 5 and Figure 12).

Since it was hypothesised the presence of a significative difference in the way different individuals behave depending on the visitors' presence, it was considered appropriate to analyse further the data with a different statistical package. For this purpose the SPSS software was chosen, since it gives a more powerful output for the Mixed Linear model (in SPSS called MIXED). It thus provides a table with the estimates of the fixed effects for the dependent variable (in this case occurrence of

aggression towards the zoo visitors) which shows the calculations of a p-value which indicates whether each individual meets the null-hypothesis. Nevertheless the assumptions that SPSS calls for were not fully met by the type of data that were collected: the assumption of normality of the visitor number data was not met. In fact this type of data is skewed and it is more frequent at lower visitors' densities (it happened more rarely to encounter high number of visitors, on average).

Although caution should therefore be taken while looking at these results, the following is a report of the statistics coming from the estimates of the fixed effects. (1) Individual 1, the alpha male, was found being highly influenced by the visitors' density in performing aggression towards the public ($t_{6,2698} = 61.68$, $p < 0.001$); (2) individual 2, the second oldest male, was as well influenced by the public ($t_{6,2698} = 30.67$, $p < 0.001$); (3) individual 3, the 1 year old male, was unexpectedly found also performing aggression depending on the visitors ($t_{6,2698} = 2.71$, $p = 0.007$); (4) individual 4, the oldest female, was found acting regardless the presence of visitors ($t_{6,2698} = 0.24$, $p = 0.8$); (5) individual 5 was also found not influenced by the visitors ($t_{6,2698} = 0.19$, $p = 0.84$); and (6) individual 6, the baby female, was also found not depending on the visitors' presence (statistics not available, as lacking from the output because found redundant. Being the young female the last term of one of the degree of freedom levels it was excluded from the analysis. Nevertheless, from the little aggression shown – Figure 5 – and its very low frequency at high values of visitor number – Figure 12 – it is concluded that this female's behaviour does not depend on the visitors' presence).

6.2 The visitors' noise correlates with mandrills' aggression

An analysis was also performed in order to test whether the noise produced by the visitors (see Table 3 for the definition of noise) was another factor playing a role in the mandrills' aggression. Although it is expected to be a variable strictly depending on the number of visitors present in the proximity of the mandrills' area, since higher numbers of visitors are expected to produce louder noise, an analysis was yet performed to detect the role of this index (Figure 13).

Although caution should be taken while looking at these results (as mentioned above, the test's assumptions were not fully met), the alpha male was found being highly

influenced by the visitors' noise in showing aggression ($t_{6,2698}$ = 46.19, p < 0.001). Individual 2 was also influenced by the public's noise ($t_{6,2698}$ = 23.99, p < 0.001). Individual 3 was found to be at the borderline: ($t_{6,2698}$ = 1.92, p = 0.054). All the other mandrills (the females) did not show influence to noise (statistics not reported).

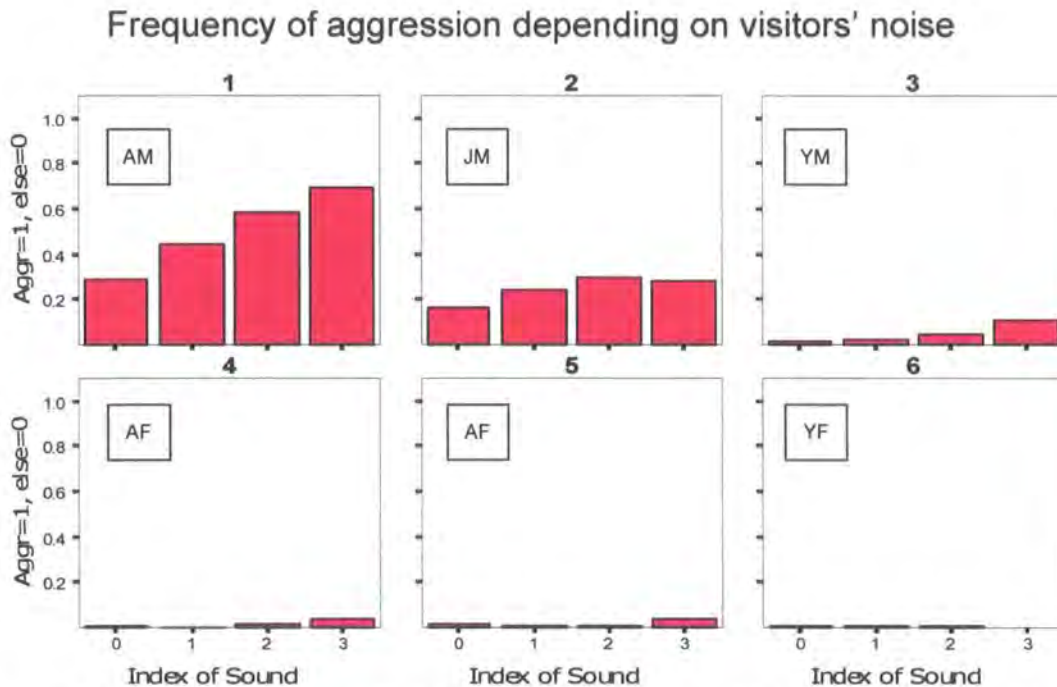## Frequency of aggression depending on visitors' noise

Figure 13 The six panelled bar charts represent the increasing in frequency of aggressive related behaviours (budget behaviour number 1, see Table 4) that the six animals (panels) performed depending on the noise produced by the visitors present in proximity of their cage. The noise was indexed as follows: 0, if the visitors where totally quiet, as when no visitors were found; 1, when the visitors where talking at a quiet volume level; 2, when at least one visitor was talking at a loud level or screaming; and 3, when at least two visitors were found shouting. The y axis represents means of the probability of encountering aggression towards the visitors by the animals.

6.3 The visitors' influence on the animals' aggression: a comprehensive model

In order to achieve the most accurate statistics from the data collected, a General Linear Mixed model was conducted taking into account the visitors' number, the noise produced and the interaction between the these two terms.

Once again, caution should be taken while looking at these results because the test's assumptions were not fully met. The oldest male was found being highly influenced by the visitors ($t_{6,2698}$ = 46.5, p < 0.001). The second oldest male was also found behaving aggressively depending on the visitors ($t_{6,2698}$ = 24.17, p < 0.001). The

youngest male showed a behaviour with questionable interpretation: ($t_{6,2698} = 1.96$, $p = 0.049$). The other mandrill behaviours were not found depending to the noise (statistics not reported).

6.4 The visitors' density correlates with the trichotillomania behaviour

As well as analysing the aggressive related behaviour depending on the zoo visitors, also the stereotypical behaviour of hair plucking shown by the two oldest males (Figure 5) was analysed so as to test whether also these stereotypies were under the influence of the visitors. However, while collecting these types of data, it was noticed the animals were not used to display the aberrant behaviour during times in which the public's turnout was highest; rather, these abnormal displays were taken place more frequently after an aggressive encounter in a quieter atmosphere. To show this, the visitor number data was manipulated: the average values per day were calculated, and these latter values were then compared to the incidence of trichotillomania bouts. By doing this, it was tested whether encounters of hair plucking actions were happening more often in days in which the public's turnout was higher.

Overall a total of 369 trichotillomania bouts were observed throughout the sampling period. To give an estimate of this figure, it shall be noted that a total of 2698 behavioural bouts (regardless of their type) were recorded. Considering that hair plucking behaviour was shown predominantly by the two oldest males (Figure 5), it can be calculated that this stereotypy could be observed for an average of 6.8% of the activity budget for these two animals.

The analysis was carried out both through one-way ANOVA and Mixed Linear models. In fact ANOVA was performed since it was hypothesised that the collected observations of stereotypical behaviour would have been largely independent among each other, since they used to occur only rarely in succession and for no longer than 5 minutes. Nevertheless the analysis through Linear Mixed models is presented so to include a second statistical estimation. Moreover, the latter analysis provides a p-value for each individual, which is doubtless useful for the purpose of underpinning the differences in behaviour of the mandrill individuals.

Through the one-way ANOVA analysis, with the assumption of independency of observations, the majority of trichotillomania bouts were found depending on the (higher) average visitor number per day ($F_{1,41} = 3.9$, $p < 0.001$). This value represents

the high probability of finding this stereotypy only at high visitors' densities per day, with no distinction among the animals.

Assuming a degree of dependency between the aberrant bouts, the Linear Mixed model gave differentiates p-values per individual. As expected, the statistics reported relevant results only for those individuals who showed themselves employed in hair plucking for a relevant amount of time during the sampling period (Figure 5). As a result, the alpha male was found performing trichotillomania at higher visitor numbers ($t_{6,2698}$ = 8.21, p < 0.001), at the same way of the juvenile male ($t_{6,2698}$ = 22.147 p < 0.001). Surprisingly also female number 5 gave a value on the borderline to reject the null hypothesis ($t_{6,2698}$ = 1.95, p = 0.051). Nevertheless, given the very low occurrence of hair plucking behaviour displayed by this animal, this result should not be overinterpreted but considered with caution. The other animals did not report a significative trend (statistics not shown).

The animals displaying hair plucking behaviour showed a more or less constant frequency of this activity between about 1 and 3.5 natural logarithm measures of visitor densities which are equivalent to about 0-33 total number of visitors present in proximity of the enclosure (Figure 14). It is at higher densities, above 35 visitors, that the animals showed higher frequencies of hair plucking.

In conclusion, considering both the tests, the animals affected by trichotillomania reported a significative trend which denotes that there is a correlation between the occurrences of hair plucking behaviour and the visitor number in proximity of the cage.
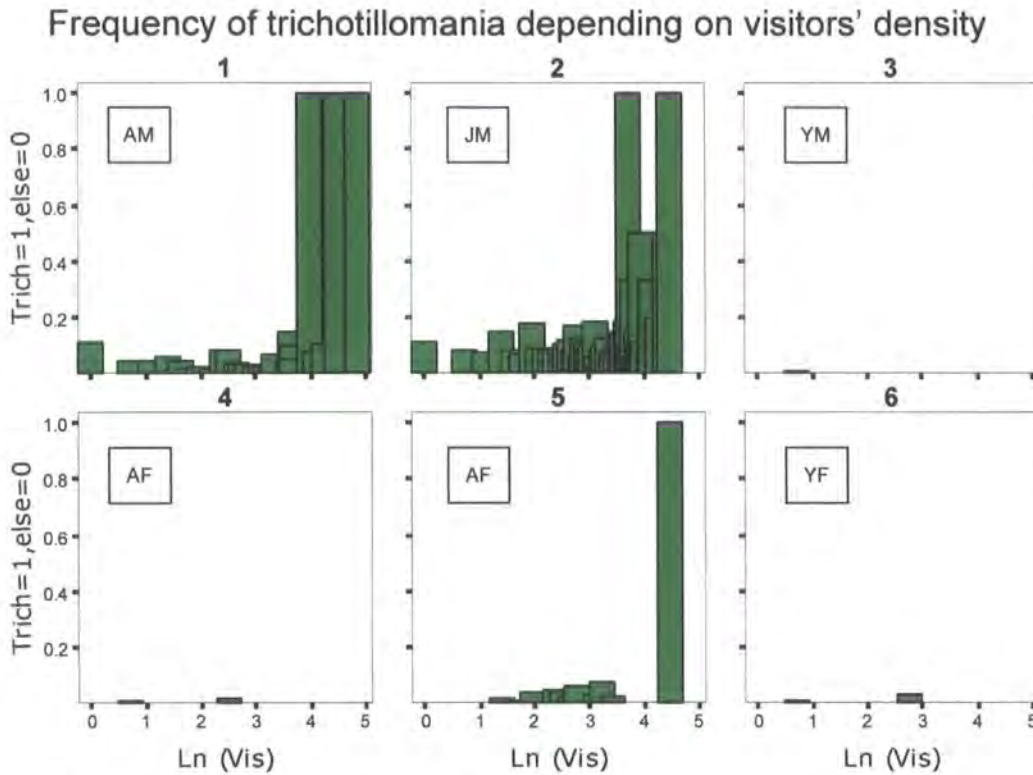
Frequency of trichotillomania depending on visitors' density

Figure 14 The six panelled bar charts represent the increase in frequency of the stereotypical behaviour of hair plucking that the six animals (panels) performed with increasing visitor density. For graphical purposes, the visitor number variable (the x axis) has been transformed in its natural logarithm in order to group to fewer classes the more disperse occurrences of higher numbers of visitors (those towards the right side of the x axes). The y axis represents means of the probability that the animals showed hair plucking behaviour.

6.5 The visitors' noise correlates with the animals' trichotillomania behaviour

The last analysis was performed to test if the hair plucking behaviour was driven by the visitor-produced noise. At the same way as the other analysis the two males responded to noise with hair plucking (individual 1 $t_{6,2698} = 6.9$, $p < 0.001$; individual 2 $t_{6,2698} = 21.162$, $p < 0.001$). All the other mandrills (including the youngest male) did not show influence to noise (statistics not reported), but this is an evident observation as these animals displayed very little stereotypies.

7 A comprehensive summary of the statistical results

Table 5 reports a summary of the statistical results coming from the mixed linear models.

Caption on the next page

	F or t value, d.f.	Sum of Square	Mean Square	p-value	Fixed effects	Random effects
Animals: in, out, at glass						
Public's visit time	$F_{2,127} = 36.03$	1306147468	653073734	***		
Public's density	$F_{2,127} = 1.72$	5.631	2.815	n.s.		
Public type	$F_{2,127} = 0.515$	0.264	0.132	n.s.		
Public's noise	$F_{2,127} = 11.61$	8.263	4.132	***		
Public and animals' behaviour						
Public's density and animal's aggression					Ind#, intercept	Public's density
Ind1	$t_{6,2698}=61.68$			***		
Ind2	$t_{6,2698}=30.67$			***		
Ind3	$t_{6,2698}=2.71$			(***)		
Ind4	$t_{6,2698}=0.24$			n.s.		
Ind5	$t_{6,2698}=0.19$			n.s.		
Ind6	redundant					
Public's noise and animal's aggression					Ind#, intercept	Public's noise
Ind1	$t_{6,2698}=46.19$			***		
Ind2	$t_{6,2698}=23.99$			***		
Ind3	$t_{6,2698}=1.91$			(0.054)		
Ind4	$t_{6,2698}=0.3$			n.s.		
Ind5	$t_{6,2698}=0.06$			n.s.		
Ind6	redundant					
Density + noise and animal's aggression					Ind#, intercept, density, noise, density*noise	Public's density, noise
Ind1	$t_{6,2698}=46.5$			***		
Ind2	$t_{6,2698}=24.17$			***		
Ind3	$t_{6,2698}=1.96$			0.049		
Ind4	$t_{6,2698}=0.27$			n.s.		
Ind5	$t_{6,2698}=0.36$			n.s.		
Ind6	redundant					
Public's density and animal's	$F_{1,41} = 3.9$	3.539	0.086	***	Ind#, intercept	Public's density

thricotillomania				
Ind1	$t_{6,2698}=8.21$	***		
Ind2	$t_{6,2698}=22.14$	***		
Ind3	$t_{6,2698}=0.09$	n.s.		
Ind4	$t_{6,2698}=0.11$	n.s.		
Ind5	$t_{6,2698}=1.95$	0.051		
Ind6	redundant			
Public's density and animal's thricotillomania			Ind#, intercept	Public's density
Ind1	$t_{6,2698}=61.68$	***		
Ind2	$t_{6,2698}=30.67$	***		
Ind3	$t_{6,2698}=2.71$	(***)		
Ind4	$t_{6,2698}=0.24$	n.s.		
Ind5	$t_{6,2698}=0.19$	n.s.		
Ind6	redundant			
Public's noise and animal's thricotillomania			Ind#, intercept	Public's noise
Ind1	$t_{6,2698}=6.9$	***		
Ind2	$t_{6,2698}=21.16$	***		
Ind3	$t_{6,2698}=0.11$	n.s.		
Ind4	$t_{6,2698}=0.002$	n.s.		
Ind5	$t_{6,2698}=1.07$	n.s.		
Ind6	redundant			

Table 5 A summary of the statistical analyses performed in this study. n.s. means not significant; *** means $p<0.001$. The parentheses are added when the frequency of the recorded behaviours was so low that a significative p-value is of little meaning.

DISCUSSION

The present study has shed some light on the characterisation of stress through the analysis of stereotypies and visitor-directed aggressive behaviours by a group of six mandrills maintained at Chester Zoo (UK). Through scan sampling procedure which has allowed to collect dense data points for all the animals on a number of coordinates, such as animal behaviour, location, and others, it was possible to finely analyse these data through Mixed Linear models which take into account of the partial dependency of the data points, collected only partly distant in time among each others. It is believed these tests are a powerful means to characterise statistical correlations together with the scan sampling tool. In addition, with these tests, it is feasible to obtain statistical significative results also on a small sample size such as those often offered by primates and other animals maintained in captivity.

Through the use of the zoo visitors' density and noise produced, flanked by the observed animal behaviour, support has been found for a concurrent cause and effect scenario which this section tries to give evidence for.

The result section was divided into a descriptive part, providing results on the activity budget of the animals, their location and other parameters. After this, another section followed providing the results of the statistical tests applied.

1 Daily activity period

From the plotting on pie charts of the time in which the animals were found active and not active, it can be inferred that the mandrills behaved differently among each other (Figure 4). More in particular, these differences in time management can be interpreted depending upon the rank position, sex and age of the animals. From the results it was found that the alpha male was the one devoting the least time to resting. On the other hand, the females and the young animals devoted on average more time to resting when compared to the males and older animals of the group respectively.

Since a substantial part of the activity budget of the alpha male was constituted by the aggressive related behaviour towards the zoo visitors (comprised of vigilance behaviour) (Figure 5), it is concluded that one of the reasons why the alpha male was least engaged in resting has to be justified looking at the aggressive related behaviour

data: this conspicuous data suggests how a substantial part of the activity budget of this animal was occupied by the aggressive related behaviours.

1.1 Activity budget

The ethogram used for the collection of the animal behaviours included all the activities usually represented in these kinds of studies so to give an overall picture of the way the animals employ their time during the day. Undoubtedly, among the most interesting group of behaviours observed and recorded, there are the aggressive related behaviours shown by the mandrills towards the zoo public. It was hypothesised that it was mostly due to these behaviours that the animals were found in a compromised welfare status due to the stereotypical behaviours shown by some of the animals. It shall therefore be stressed the importance given by the frequency of the performance of these behaviours only by some animals of the group.

It could have functional significance that the alpha male is the animal mostly engaged in these aggressive related behaviours in that he is protecting the group from the visitors. In relation to the previous section of activity and resting, the alpha male might have been the animal found least performing resting also because often engaged in this protective activity. In fact in mandrill communities in the wild, the alpha male was found not only leading the group, but also protecting it from outsiders (Abernethy et al., 2002). In a captive situation such as that one offered by a zoo, there is no possibility for the captive animals to meet with conspecific animals trying for example to overtake the group (unless under a specific programme from the zoo managers). It is therefore hypothesised that the alpha male would implement his defensive behaviour in this situation towards the zoo visitors, often relatively close to the animals and separated only from them through a glass window, which gives a little physical separation from the public.

From the activity budget behaviours recorded it is also evident how the alpha male and his oldest son are the animals most affected by hair plucking behaviour. The latter individual is the one who was observed performing most of these behaviours. This result is unexpected, since it is the older animal the one who bears lager areas of alopecia. However it has been observed several times that the phenomenology of this aberrant behaviour was different between the two animals: in fact the older male used to pluck tufts of hair at once, whereas the young male used to carefully select one by

one the hair to be torn out. It is therefore most probably due to this different hair plucking behaviours that the young animal was observed more often engaged in the repetitive action, although eventually the symptoms of this compulsive behaviour were of lower extent.

It is of some interest also looking at the sociopositive behaviours, that is those social behaviours engaged by two mandrills together different from aggression intents, mostly performed on average by the females, and in particular by the oldest female together with her young daughter, often found performing allogrooming (but see following section for more comments on this). The handling behaviours were mostly seen performed by the young animals of the group, most probably engaged in those exploratory behaviours typical of the young age.

2 Social behaviours between pairs of mandrills

Of all the observations collected, it was of some interest looking at the social behaviours to detect those animals mostly engaged in these types of activities. It was hypothesised that especially the young mandrills and possibly the females would have been the animals spending a larger amount of time in these social behaviours (Harrison, 1988; Rogers et al., 1996).

From the graphical results achieved, the hypothesis was supported. In particular, the adult female and her young female baby were the two individuals spending most of the time in this activity when compared to the other animals. In relation to this, these two mandrills were often observed allogrooming: considering the very young age of the baby, it is concluded that a considerably high amount of parental care was still needed. Although all the other animals did not show a comparable frequency of social behaviours to these two females, it is still noticeable that the second oldest male still in his juvenile age and his young brother were 'socially linked' together to a certain extent. In fact these two males were often observed engaged in playing activities.

On the other hand, the animals that did not employ part of their activity budget for social activities were the other adult female and above all the alpha male. It is hypothesised that this adult female was not particularly interested in sociality since this animal was the only one not to have a direct kinship relationship with the other mandrills. Moreover at the time of the research, this animal was also going under contraceptive treatment as she was in the past involved in unsuccessful pregnancy.

Finally the alpha male was the animal that almost completely lacked social behaviours in his budget activities. Some more hypotheses can be drawn when looking, on the other hand, at the conspicuous amount devoted for the display of visitor-directed aggression (see later analyses). Being the dominant male, it is expected that the variety of his budget activities has a higher amount of agonistic displays. It is nevertheless to prove that the lack of social activities (such as also sexual behaviour) has to be justified looking at the visitor-direct behaviours and presence of stereotypies. However, it is speculated that it may due to the presence of this high frequency of aggression and the presence of stereotypies that this animal does not show social behaviours.

3 Position in the cage per individual

The results achieved had the purpose to show the different habits of the mandrill individuals, analysing through the location graphs how the animals utilised the space provided in the inner enclosure, depending on age and sex. Since the zoo visitors were always located in the same position in respect to the animals, it was significant to detect whether the animals near the public at the same manner and with the same frequency, or whether to see that there was a difference, such as depending on the sex. Regardless of the visitor presence, the results showed that it was marked the difference between the young individuals and the adults. While the adults tended to prefer ranging on the ground and at 2 m. height from the ground above the tunnels connecting to the outer enclosure, the young individuals could be found at different heights; this is a sign that the young mandrills were engaged in climbing, chasing and playing among each other.

The position close to the window was preferred by the two oldest males, the dominant animal and his juvenile son. These individuals were also found performing most of the aggressive related behaviours towards the zoo public at this position, comprised of "bared teeth" display, "yawning", "head protrusion", "banging on the glass" with one hand, and other behaviours. It is therefore deduced that the two animals are very often found at this position as they are engaged in visitor-direct aggression.

The scope of these results was also to isolate the locations in which the animals where used to display the two behaviours that have been subsequently analysed statistically: aggression and stereotypies.

4 The hair plucking behaviour and its location compared to aggression

The alpha male and its older son were the animals found affected by alopecia caused by the stereotypical behaviour of trichotillomania. Nevertheless it should be noted that all the individuals were recorded showing hair plucking at least once during the research. Although the younger male was observed more than twice as frequently than the dominant animal, the reason behind his fewer and smaller skin areas showing alopecia was found in the fact that he performs the stereotypy plucking one by one the hair rather than by tufts as his father.

This disorder takes place in human beings usually as a consequence of unconscious conflicts, intrusive thoughts and repetitive behaviours; for this reason it is classified as an obsessive-compulsive disorder which has gained increased attention in medical research especially in this last decade (Diefenbach et al., 2000). It is interesting to see how the aetiology of this condition may be related to grooming behaviour that has become pathological: because of neurological dysfunctions the grooming behaviour signal is released inappropriately and it becomes stereotypically repeated ending up being constituted by the extraction of hair (Diefenbach et al., 2000). In relation to selfgrooming behaviour, Wells (2005) found an increase in frequency of this behaviour performed by captive gorillas as induced by the presence of zoo visitors. In mandrills Chamove (1988) found hair pulling behaviour in similar conditions of overcrowding, so it is interesting to see how different degrees of hair manipulation can be found in captivity as a consequence of the public's presence.

In this study a correlation between the occurrences of visitor-directed aggression and hair plucking was hypothesised since it was observed that the animals used to hair pluck more frequently during days in which frequency of visitor-directed aggression was higher. From the graphical analysis of the locations assumed by the two males performing trichotillomania, it was found that the occurrences of stereotypical behaviour took place in the same locations where the aggressive bouts occur. Further analysis was therefore necessary in order to establish whether an real relationship between these two behaviours existed.

5 The zoo visitors

At this stage the research looked at how the visitors' behaviour is conditioned by the locations of the animals in their enclosure. Analysing the visitors' behaviours allowed to infer that the average duration of the visit possibly depended on whether the animals were present in the cage, and not in the outer enclosure invisible from the visitors' view. Moreover, the visitors used to stay longer in proximity to the enclosure when at least two animals were located very close to the glass window. Concurrently with a longer stay, also louder noise was produced by the humans when the animals were found inside, and even louder noise was recorded when two animals were at the window. It is not certain that the noise produced was higher, since the crowd size remained the same regardless the animals' position. However since the public used to be of a various type, very often comprising of children, it shall be deducted that nearing primates such as the mandrills did provoke an overall excitement in the public (Chamove et al., 1988; Mallapur et al., 2005; Wells, 2005). Disappointingly, the visitor-produced noise was later found partly responsible for the occurrence of aggression in the animals.

6 The influence of the zoo visitors on the mandrills' behaviour

The first analysis which looked at the visitor density as a causing factor for the visitor-directed behaviours was performed in two steps. Firstly, a general trend of all the animals was shown: since statistical significance was found, it was concluded that on average the whole mandrill group was responding to larger visitor crowds displaying more frequent aggression. Nevertheless this trend was investigated further, as from the cumulative figures of the budget behaviours and the inter-individuals correlation coefficient produced by the analysis it was clear that this tendency was mostly due to the two older males that exhibited most of the aggressive bouts. Further analysis showed that the two older males where those causing the null-hypothesis to be rejected and accept the conclusion there was a correlation between visitor-directed aggression and the visitors' presence. Surprisingly, also the third young male denoted this trend (with a highly significant p-value), but the frequency of his aggression was markedly lower than the other two males. This result may well be an indication that this young animal is already showing that behaviour that may increase in frequency if

in the future he will continue to be subjected to the uncontrolled presence of the public.

The same analysis was performed looking at the visitor-produced noise (rather than their density) to speculate about this element as causing agent of aggression. Similarly to density, the higher noise produced was found correlated with the behaviour of the two older males, whereas the less frequent aggression shown by the younger male, this time, gave a borderline significance. Again, if it is safe to infer that a correlation exists between noise and aggression for the two older males, this is not true for the youngest male. On the other hand the females did not show any correlations since they did not displayed visitor-directed aggression frequently enough to conclude so.

An overall analysis for the aggressive behaviours was eventually achieved by combining both the density data and the noise data, so to outline the general influence that public had on the animals. Once again: (1) the two older males responded to the visitors, (2) the youngest male responded only just, and (3) the females did not respond showing aggression.

After having discussed the aggressive related behaviours, it is now the turn of the stereotypies that were as well analysed against the visitors. The statistics reported similar trends to those found for aggression: the first two individuals responded to the visitors for both their density and the noise produced by displaying stereotypies. The other animals did not reported significance as the frequency of the aberrant behaviours was extremely low. This is again an important result as it confirms that stereotypical behaviour occurs when the visitors are at higher densities and when they produce louder noise. In fact, in this case, it is safer to predict the direction of causality for this correlation. The hair plucking behaviour as such displayed by the animals is considerably difficult to notice, unless the observer knows a priori of such occurrences. I would thus be unrealistic to imagine that the visitors were gathering in front of the enclosure and were producing louder noise because of the stereotypical behaviour shown by the animals.

In conclusion, the aberrant behaviours that the animals showed have been put in relation to the presence of the zoo visitors. Under an animal welfare perspective, these findings are considered in the light of the scientific literature that has tried to depict the causes of the chronic stress response.

7 A functional stress response

Most of the behavioural and physiological responses shown by an animal can be interpreted from a functional perspective resulting from selection pressures directly leading to the form and occurrence of these responses in a functional context. An animal is generally considered to be evolutionary adapted to its environment as a direct result of these selection pressures. This adaptiveness, however, is an assumption and remains to be tested, as one means of a theoretical frame work for behavioural analysis. Nevertheless, adaptive models can be developed of various aspects of behaviour, including an adaptive stress response. In the natural environment animals may be assumed to have evolved a response to stressful situations that on average helps them cope better with these situations. In nature, for instance, part of this response is physiological: the endogenous release of opioids for self-narcotisation purposes when the animal feels pain (Broom & Johnson, 1993), and the release of cortisol and behaviourally associated response together forming the so called "fight or flight" response (Broom & Johnson, 1993).

In the case of this study, the behavioural response shown by the animals was a class of behaviours that could be interpreted as visitor-direct aggressive. The two males that most frequently displayed this class of behaviours were demonstrated to be influenced by the stressor agent of the visitors when showing the onset of aggression. Aggressive behaviours are a common component of the stress response employed by those proactive individuals that are actively engaged in facing the stressors (Koolhaas et al., 1997; Schjolden et al., 2005). This aggression showed by the mandrills may well represent an evolutionary adaptive response functioning in the wild to deter predators and aggressive conspecifics (Abernethy et al., 2002; Rogers et al., 1996). When the hypothesised intruder becomes aware of the presence of one or more dominant males performing displays of aggression, another fight or flight reaction will follow by the receiver, and the dominant males will therefore achieve a response to their aggression signal.

More generally, aggressive behaviour is used by animals as an instrument to reach homeostasis by obtaining control over the social situation (Koolhaas et al., 1997). However the captivity condition does not offer the possibility to these mandrills to achieve the desired retreat effect on the zoo visitors. In stead visitors usually keep

watching the animals, or may even show more aggression (such as banging on the glass) following the aggressive displays by the male mandrills. This lack of response from the visitors is likely to causes frustration in the animals and lead to the stereotypical behaviour displayed. Stereotypical behaviour has been widely reported as an outcome of thwarted ability to respond "functionally" as well as an outcome of chronic stress. The here proposed interpretation of the mandrills response to visitors from a functional perspective may prove fruitful, in that the model has the potential to generate specific hypotheses about the mechanisms underlying the response that can be empirically tested, and the results applied in the zoo context. If, for example in response to aggressive displays, visitors would become less visible to the mandrills (e.g. by an automatic darkening of the display window, dimming of the lights, or other technical response) the "frustration hypothesis" could be tested, and, in principle, subsequently implemented in technical improvements of the display.

8 Criticism

The present research has been able to show in a group of captive mandrills a correlation between visitor numbers and the rate of aggressive related behaviour and the stereotypical behaviour and stress that may partly result from the inability of the individuals to control their environment through their (aggressive) behaviour. This correlation could be demonstrated through the application of mixed linear models. The analysis was performed in two steps: first it was shown that the general pattern of the individuals of the mandrill group under screening was affected by the visitor presence. In a second step, different statistics and software has been applied, allowing to investigate individual-specific responses. The drawback of using this second software (SPSS) is that the statistics' assumptions were based on the normal distribution of the original data. This is a limitation, as it is often the case that the data are skewed, such as those of the visitors. Although it is believed that the test used is robust – which implies that if the assumptions of the test are partially not met than the results should still provide with a reliable estimate – it would be preferred that the test used could imply also more diverse types of data than the normally distributed ones.

This research has shown that there is a correlation between the number of visitors and the occurrence of aggressive related behaviours towards the public and the occurrence of stereotypical behaviour. During the data sampling it suggested to the zoo

management, on a number of occasions, to modify the display and thereby provide an experimental set-up to test the influence of the adjusted zoo display. It was suggested by the researcher, with the support of the primate zoo keepers and the research officer to set up a barrier to avoid that zoo visitors could get close to the animals and were able to touch the glass window dividing the animals to the people. Unfortunately, the processes involved within the zoo in modifying displays proved to be extremely slow so that it was not possible to study the effect of the barrier on the mandrill behaviour within the context of this thesis. Another student has been able to compare behaviours before and after a few tall planters connected through a fence were positioned as a barrier in front of the glass windows and found that both aggression and stereotypical behaviours were significantly reduced under all conditions (high visitor numbers/low visitor numbers and noisy visitors (Hargreaves, 2006).

9 Future directions

This study has shown the influence of the zoo visitors on the mandrills' behaviour through the analysis of scan sampled results taking into account the partial correlation of these data in time with the mixed effects models. The true novelty of this ethological study has therefore been analysing the dense scan sampled data from all the individuals so to go beyond the simplification assumed by other ethological studies which applied scan sample methodologies and granted for true that these data where all either dependent or independent of each other (Lehner, 1996).

Also continuous sampled behavioural data were collected with the purpose of analysing them through analysis of fractal patterns, which to date represents a very interesting and promising technique to detect whether the animals under analysis undergo subtle stress responses (Alados et al., 1996; Maria et al., 2004). It is therefore proposed, as soon as the software will be available and fully developed, to widen this study taking into account also this alternative methodology so to confront these latter results with those coming from the mixed effects analyses. To summarise, the fractal methodology assumes that an animal is likely to be affected by a stressors by showing a less complex sequence of behaviours at a given time (Alados et al., 1996; Maria et al., 2004). Looking at the fractal pattern and in particular at the complexity of a stream of behavioural data, it is therefore possible to conclude whether an animal is responding to stress, even when not showing a specific stress related behaviour (such

as, looking at this research, the stereotypical behaviours and aggressive related behaviours towards the zoo public). A possible scenario could therefore be, in the case of this research, that also the young male and the females were responding to the presence of the zoo visitors in a more subtle way that the more conventional analysis presented in this manuscript has not been able to detect.

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ACKNOWLEDGMENTS

Appendix A – A sample check sheet used to collect the data in the research

In the next page it is attached a sample check sheet used for collecting scan-sampled observations from each of the six mandrills under analysis every 5 minutes for sessions of 6 hours a day. In the first column there is the time (hh:mm) at which every scan observation was performed. The group of columns from the second to the seventh are repeated by six, that is the number of individuals screened. The second column Id. represents the Behaviour performed by each of the individuals. In the RC column it goes the individual towards which the behaviour was performed when this belongs to the social behaviour category. NNI means Nearest Neighbour Individual. NND means Nearest Neighbour Distance and it is measured in metres. GL is the distance from the glass window dividing the mandrills from the visitors; this is measured in metres. HT is the distance for the ground floor which increases when the mandrill climbs the cage. In the Vis. column it was reported the number of visitors present in the area in front of the mandrills' enclosure. Ty. was the type of visitor (see below table). dB was a noise index. ZK represented the zoo keeper presence and it was a box to be ticked. In Notes, it was reported anything relevant as a biasing or worth mentioning situation to take into account while analysing the data.

date: _____

T	1	RC	NNI	NND	GL	HT	2	RC	NNI	NND	GL	HT	3	RC	NNI	NND	GL	HT	4	RC	NNI	NND	GL	HT	5	RC	NNI	NND	GL	HT	6	RC	NNI	NND	GL	HT	T	Vis.	Ty	dB	ZK	Notes
:00	RA		2	2	8	0	RA		1	2	9	2	RA		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	0	0		0		
5	RA		2	2	8	0	RA		1	2	9	2	RA		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	5	0		1		
10	RA		2	2	8	0	RS		1	2	9	2	RA		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	10	2	1	0		
15	RA		2	2	8	0	RS		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RA		3	3	10	2	15	5	3	2		
20	RA		2	2	8	0	RA		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RA		2	3	12	2	RS		3	3	10	2	20	8	3	2		
25	RS		2	2	8	0	RA		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	25	0		1		
30	RA		2	2	8	0	RS		1	2	9	2	RS		4	1	11	2	RA		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	30	0		1		
35	VB		2	2	8	0	RS		1	2	9	2	RS		4	1	11	2	RA		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	35	4	2	1		
40	RS		2	2	8	0	RS		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RA		2	3	12	2	RA		3	3	10	2	40	3	2	1		
45	VB		2	2	8	0	RA		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	45	2	2	1		
50	VB		2	2	8	0	TR		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RS		3	3	10	2	50	0		2		
55	VB		2	2	8	0	TR		1	2	9	2	RS		4	1	11	2	RS		3	1	12	2	RS		2	3	12	2	RA		3	3	10	2	55	19	2	2		
:00	VB		2	2	8	0	TR		1	2	9	2	RA		6	2	10	2	RS		3	1	12	2	RA		2	3	12	2	HN		4	1	10	2	0	5	2	2		
5	VB		2	2	8	0	TR		1	2	9	2	RA		6	2	10	2	WK		1	2	10	0	RA		2	3	12	2	CL		4	1	10	1	5	6	2	1		
10	VB		6	2	8	0	VG		6	4	0	0	RS		4	2	10	2	SG		3	2	12	2	RA		1	3	12	2	LF		6	2	6	0	10	12	2	2		
15	VB		3	3	8	0	TR		6	2	2	0	AG	4	4	0	12	2	AG	3	3	0	12	2	RA		1	4	12	2	LF		2	2	4	0	15	7	2	2		
20	VB		6	2	8	0	TR		6	4	2	0	AG	4	4	0	12	2	AG	3	3	0	12	2	FE		1	4	12	2	LF		1	2	6	0	20	7	2	2		
25	RA		5	3	10	0	TR		1	7	2	0	SG		5	3	12	2	AG	6	6	0	12	2	RA		1	3	10	0	AG		4	0	12	2	25	15	2	2		
30	WZ		5	2	10	0	WZ		1	2	10	2	WZ		2	2	12	2	AG	3	6	0	12	2	WK		1	2	10	0	AG		4	0	12	2	30	20	2	2		
35	WK		5	4	4	0	TR		3	3	10	0	WK		2	3	12	2	AG		6	0	12	2	RA		1	4	0	0	AG		4	0	12	2	35	4	3	2		
40	VG		5	4	8	0	TR		3	2	10	0	RA		2	2	10	2	AG	6	6	0	12	2	TR		1	4	12	2	AG		4	0	12	2	40	7	3	1		
45	VG		5	4	0	0	WK		6	1	10	0	WK		6	2	6	0	RA		6	5	12	2	SG		2	2	8	2	CL		2	1	8	0	45	12	3	1		
50	VG		2	2	8	0	PL	3	3	1	10	0	PL	2	2	1	10	0	SG		2	3	12	2	RS		1	5	12	2	LF		2	1	9	0	50	9	2	1		
55	VB		3	1	8	0	VG		6	2	2	0	SB		1	1	8	0	SG		1	3	11	2	RS		1	5	12	2	LF		2	2	4	0	55	26	2	2		
:00	VB		2	8	8	0	VG		1	8	0	0	PL	6	6	1	8	3	AG	5	5	0	12	2	AG		4	0	12	2	PL	3	3	1	9	3	0	6	2	1		
5	VG		3	3	10	0	HN		1	9	0	0	RA		1	3	10	2	AG	5	5	0	12	2	AG		4	0	12	0	RA		3	3	13	2	5	12	2	1		
10	WZ		2	2	10	0	WZ		6	2	10	0	WZ		6	2	10	2	LF		5	2	8	0	WK		1	4	6	0	CL		3	2	10	1	10	3	3	1		
15	WZ		2	2	10	2	CL		6	2	10	1	WZ		4	2	10	0	WZ		3	1	8	0	WZ		4	2	8		WZ		2	2	10	0	15	14	3	1	X	
20	OU						OU						OU						OU						OU						OU						20	0		0		F.O.I.
25	OU						FE				11	2	OU						OU						OU						OU						25	4	2	1		
30	OU						FE		3	2	10	2	FE		2	2	12	2	OU						OU						OU						30	12	2	1		
35	WK		6	2	9	0	WK		3	2	12	2	WK		2	1	11	2	FE		6	2	12	2	OU						CL		1	2	10	2	35	2	3	0		
40	VB		6	2	8	0	FE		3	3	10	0	FE		6	2	11	2	FE		6	2	12	2	OU						FE		3	2	11	2	40	11	2	1		
45	FE		6	2	10	0	FE		1	4	10	0	CL		6	3	10	2	OU						OU						WK		1	2	12	2	45	5	2	1		
50	WK		6	6	9	0	FE		6	2	10	0	OU						OU						OU						WK		2	2	10	0	50	3	2	1		
55	SG		4	2	10	0	FE		6	3	10	0	OU						FE		1	2	11	2	OU						FE		2	3	12	2	55	4	2	1		
	1	RC	NNI	NND	DG	HT	2	RC	NNI	NND	DG	HT	3	RC	NNI	NND	DG	HT	4	RC	NNI	NND	DG	HT	5	RC	NNI	NND	DG	HT	6	RC	NNI	NND	DG	HT	T	Vis.	Ty	dB	ZK	Notes

Research and Economic Development Support Service

Memorandum

TO: Mr R Pansini, Department of Anthropology ✓

FROM: Rob Statham, Director of REDSS/Secretary to the LSERP Committee

CC: Dr K J Orford, Dean of Science
Dr R A Hill, Department of Anthropology
Dr J de Ruiter, Department of Anthropology

DATE: 21 April 2005

SUBJECT: THE APPLICATION OF A NEW BEHAVIOURAL ANALYSIS TOOL TO ASSESS PRIMATE HEALTH AND STRESS IN CAPTIVITY

Dear Mr Pansini

I refer to your application for ethical review of the above project. I am pleased to inform you that having considered your application "The application of a new behavioural analysis tool to assess primate health and stress in captivity", the LSERP Committee have a favourable ethical opinion regarding your research project.

With best wishes

Robin

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