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Expedients for the ethical use of Rattus norvegicus as live prey in Wildlife Sanctuaries. A case study.

Relatore/ Supervisor
Prof. Gianfranco
Gabai

Laureanda/ Submitted by
Lucia Mondin

Matricola n./ Student n.
2033612

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Introduction

The study presented in this thesis is situated at the intersection of two fields of research: the improvement of the wellbeing of the species *Rattus norvegicus* in captivity and the analysis of the wellbeing of live prey. The first area of study is frequently conducted in laboratories **(11)**, where this species is used for scientific research, ranging from drug testing to behavioural studies. In this framework, it is important to consider another point of view, derived from the care practices in a domestic context. The second field is primarily bioethical and involves the regulatory aspect of using live prey in zoos, rehabilitation centres, sanctuaries, and domestic environments. The thesis seeks to propose concrete improvements for the rats used as live prey in the context of the Alturas Wildlife Sanctuary, a non-profit organization whose goal is rescuing, rehabilitating, and reintroducing injured Costa Rican wildlife. The novelty of this research lies in the fact that it does not have a purely utilitarian purpose, in terms of management benefits, but also focuses on the wellbeing of the individual animal. The case study explores various improvement strategies, starting with modifications to the initial practices in place at the centre, followed by the implementation of policies related to health, nutrition, and the enclosure. The effectiveness of the actions taken was verified through direct observations and the use of camera traps, comparing the situation prior to these modifications with that following the improvements. The work was conducted during the internship carried out in the academic year 2023/2024.

The structure of the thesis is the following:

Chapter 1: The first chapter addresses the ethical aspect related to the use of animals as live prey.

Chapter 2: The second chapter provides the opportunity to gain a more detailed understanding of the species *Rattus norvegicus* from a biological, ecological, and physiological perspective.

Chapter 3: The third chapter will focus on the experimental study, in which certain improvements were implemented for three rats housed in the same cage, with the aim of enhancing their wellbeing.

1. An overview of live prey debate

1.1 Live prey and ethical debate

Within the realm of exotic species, including birds, amphibians, reptiles, and mammals, in the context of captive animal management, the feeding of carnivorous and omnivores predators presents a multifaceted and frequently debated issues.

This chapter will analyse and evaluate the problems related to a particularly sensitive topic within this domain, which is the use of live prey. Indeed, this practice raises numerous ethical, biological, and managerial concerns, sparking significant discussion both in the scientific community and in society. In the second part of the chapter, an in-depth examination of a specific type of live prey, the *Rattus norvegicus*, will be conducted. This species, commonly known as the Norway rat, serves as the focal point of the research presented in this work. The analysis will encompass various aspects of the species, highlighting its significance and relevance to the study. Before delving into the particulars, it may be appropriate to provide a definition of "live prey" which represents the subject of the study. Based on the literature and the experience gained as intern at the Alturas Wildlife Sanctuary[1], it has been possible to identify the following definition: live prey refers to those vertebrates and invertebrates that are offered as food for predators that are found in captive conditions. The justification for using live prey, frequently rests on the argument that it promotes natural behaviours in predators, thereby supporting their mental and physical health, and enhancing the post-release survival of releasable animals. Indeed, predators can acclimate to the conditions they will face in their natural habitats. Moreover, containing sometimes important essential amino acids, vitamins, and other nutrients it is considered indispensable from a nutritional perspective **(13)**.

However, the employment of "live feeding" prompts apprehensions about the welfare of both the predators and the prey involved. Regarding the predator, despite of the advantages shown before, it is essential to acknowledge that live prey feeding could potentially harm the wellbeing of the hunter. This risk arises from the possibility of injury during hunting and killing, as well as the energy expended in an unnatural and confined setting such as an enclosure, cage, or tank. Furthermore, from

[1] Alturas Wildlife Sanctuay is a non-profit organization dedicated to the rescue, rehabilitation, and release of injured, sick, and orphaned wildlife native to Costa Rica.

a behavioural perspective, the presence of live prey could exacerbate territorial and aggressive behaviours in those predators that are less agile and have slower reflexes during hunting, thereby hindering their ability to achieve their objective **(8)**.

In relation to the prey, this procedure faces criticism due to the pain and distress experienced by these sentient beings before and during their consumption by predators. The potential for stress exists at various stages. From a managerial point of view, during their production or collection, live food items are often found themselves in unfamiliar and unnatural environments, exposed to stressors such as high temperatures or intense lighting, making them more vulnerable to the predators they are fed to. The most critical welfare issue arises during the consumption process. In some instances, the prey is killed almost immediately by the predator, through physical trauma or envenomation, leading to rapid immobilization and unconsciousness, thus minimizing suffering.

However, in many other cases, death is prolonged. For example, a rodent may be constricted and suffocated by a snake, or a cockroach may be dismembered while still alive; some preys are swallowed whole and remain alive and possibly conscious until they die from asphyxiation or the predator's digestive process. If the live prey is not immediately consumed, it may be eaten later or may remain uneaten. In this scenario, the prey must survive in an alien environment, often lacking water, food, or appropriate shelter. In some cases, uneaten prey may die from starvation, dehydration, or exposure to extreme temperatures within the predator's enclosure. Alternatively, the prey may adapt to the enclosure or escape into the broader environment, such as rats or crickets in a zoo setting which represents a management issue **(9)**. Thus, the welfare of live food items is a significant concern that necessities careful consideration and human practices to minimize suffering and ensure ethical treatments of all animals involved.

1.2 Pain and human empathy

Given the limited research on the use of live prey, this study aims to focus primarily on examining practices within the United Kingdom, where the majority of pertinent research has been undertaken. Furthermore, the studies conducted in the UK closely mirror conditions and practices observed throughout Europe, providing a comprehensive understanding of the subject in both contexts.

This investigation is situated within a framework of increasing attention and sensitivity towards animals. By focusing on the use of live prey, it aims to provide a deeper understanding of the dynamics involving animals, both in terms of their protection and in relation to the empathy that humans feel towards these practices.

Efforts and campaigns in UK aimed at prohibiting the practice of using vertebrate feeding live prey to reptiles, exemplified by the London Zoo's implementation of a formal ban approximately a century ago. Moreover, a debate emerged in the 1970s among animal welfare groups and society regarding the illegality of live feeding. Indeed, awareness and importance attributed to animal welfare have grown significantly due to the campaigns carried out by environmental and animal rights groups. These groups have not only played a fundamental role in shaping human thought on this topic but also in exerting political pressure that has led to the creation of legislation to protect animals. Public and institutional scrutiny of the treatment of animals has increased, with recognition of their rights and dignity, resulting in the establishment of laws to protect as much as possible living beings that do not have their own voice. This has led to the establishment of regulations based on different contexts and species, which will be discussed more thoroughly in the following subsection.

Indeed, the London Zoo trained reptiles housed in captivity to accept freshly killed prey which could be manipulated to mimic life or placed in unconventional settings, like hollow tubes, to engage the interest of hungry predators. This approach gained popularity in other zoos in Britain and among British herpetologists, leading to widespread adoption of using dead prey by the late 1980s.

However, at that time, it was merely considered "good practice" without any specific laws governing it, apart from the Protection of Animal Act[2]. This lack of clear legislation led to ambiguous interpretations. Consequently, those who persisted with live feeding were advised to minimize suffering by not leaving live prey in vivarium for prolonged periods and providing shelter and water for the prey species.

Despite efforts to dissuade the practice of live feeding, evidence form online video clips indicates that certain reptile keepers persist in employing live vertebrate prey as feeding method **(9)**.

[2] An Act to consolidate, amend, and extend certain enactments relating to Animals and to Knackers; and to make further provision with respect thereto. (https://www.legislation.gov.uk/ukpga/Geo5/1-2/27).

The heightened awareness of both the public and political spheres regarding these issues is intricately linked to the advancement of scientific knowledge concerning animal suffering and the attendant cultural shifts.

A crucial aspect is the concept of pain and nociception. Pain, delineated as a sensory, physiological, and psychological phenomenon, manifests as a shared experience between humans and animals alike. Indeed, as reported by the IASP[3] “the inability to communicate verbally does not negate the possibility, that an individual is experiencing pain” **(18)**. On the other hand, nociception is a biological process corresponding to a simple detection through nociceptors and reflex response to noxious stimulus. Nociceptors, serving as the primary mediators in the detection of high mechanical pressure, extreme temperatures, or exposure to noxious chemicals stimuli, are distributed throughout the organism, prominently within regions such as the skin, muscle tissue, joints, and internal organs. When stimulated by adverse stimuli, nociceptors initiate a complex cascade of signals that propagate along nerve fibres to the central nervous system (CNS). Within the CNS, these signals undergo integration with higher-order sensations, ultimately contributing to the perception of pain. Over the course of history, disparate viewpoints have emerged, leading to a dichotomy between contrasting ideologies that concern the correlation between nociceptors and the experience of pain. On one hand, there is the perspective asserting that all those animals which possess specific anatomical structures such as neocortex, opioid receptors, nociceptors, can detect and instinctively react to various detrimental stimuli. This viewpoint extends to a myriad of vertebrate species, including but not limited to rats, mice, rabbits, squirrels, and birds. Conversely, an opposing viewpoint posits that certain animal species as insects lack the requisite neural structures to experience pain and, by extension, the psychological and psychological facets associated with it.

Human perception of the pain experienced by animals varies significantly depending on the category of people being asked for their perspective on this issue. The understanding and interpretation of animal pain are influenced by various factors, including professional background, personal beliefs, cultural context, and level of education. Moreover, the perception varies depending on the specific animal in question. Certain animals such as rabbit, elicit greater empathy compared to others

[3] International Association for the Study of Pain.

as rats, invertebrate's species or fishes. Indeed, even if invertebrates constitute more than 90% of all species on the earth, the practice of feeding live invertebrates to captive animals has received less scrutiny historically. Despite the vital ecological and societal roles played by those animals, there exists a notable lack of public concern regarding their welfare.

An illustrative case arises from a study conducted in the summer of 2017, wherein data were collected via questionnaire responses from 248 participants. The questionnaire was examined by specialists at the Bristol Zoological Society and obtained ethical approval from the BIAZA Research Committee. It also received endorsement from the Research Ethics Panel of the Anglia Ruskin University Biology Department. The study has been performed to investigate the perception of the general public, aquarium professionals and terrestrial zoo animal experts, regarding the practice of feeding live fishes to one another. Previous investigations have indicated that women feel a higher emotional attachment to animals, leading to decreased acceptance of live prey feeding. However, this feeling was not evident when considering aquatic creatures, perhaps due to their lesser familiarity and distinct phylogenetic and behavioural traits compared to terrestrial animals, resulting in a lower level of compassion. As such, the analysis focused on discerning the perceptual differences between visitors and those actively engaged with animals, revealing that the majority of the former were opposed to the use of live prey. In contrast, workers found this practice acceptable, likely stemming from a background that facilitates reflection on the use of live prey not solely based on emotional grounds **(8)**.

Another research conducted in UK has consistently shown that the general public harbours negative sentiments towards most invertebrates, often manifesting feelings of aversion and fear towards these species. Invertebrates are commonly perceived as lacking the capacity to experience pain or possess cognitive faculties, with few exceptions such as butterflies or bees, which leads to a prevailing view of them as pests to be eradicated **(13)**. It is important to highlight that concerns regarding the neglect and misunderstanding of invertebrates have been raised for a significant period. Notably, such issues were already being reported as early as the 1980s. Indeed, Scottish animal rights group began advocating for greater awareness of invertebrate welfare, including their use as food for captive mammals, birds, and reptiles **(9)**. Over the past two decades, interest in the health and welfare of invertebrateshas increased, leading to discussions and research on whether they

experience pain and the ethical considerations of using them as live prey.
Invertebrates span around 30 distinct phyla, each with varying capabilities to respond to noxious stimuli. Literature evidence suggest that many invertebrates, such as arthropods, molluscs, and annelids, produce endorphins and may have an awareness of pain because they possess nociceptors, opioid receptors, and pain response behaviours, indicating a potential awareness of pain. Investigation on species like *Caenorhabditis elegans* has shown behaviours resembling chronic pain responses in vertebrates. The notion of suffering encompasses not only physical pain but also involves evaluating cognitive abilities, when considering the effects of behavioural deprivation **(8)**.
Thanks to the efforts of animal rights activists and environmentalists, whose initiatives date back decades and continue vigorously to this day, laws concerning animal protection, especially in the context of live feeding, have been enacted.

1.3 Live feeding legislation

Depending on the country under consideration, legislation may either support or oppose the use of live animals presented as food to predators. Unfortunately, this is not the first nor the last context in which laws, formulated in a less specific manner, are interpreted based on what is most convenient for humans. An example can be provided through the "minimise suffering" law under the Animal Welfare Act (UK), which leaves room for interpretation of this meaning. Indeed, some interpret this as providing normal stimulation through the use of live prey, thereby improving the welfare of the predator; on the other hand, this law may instead take on a completely opposite meaning, leading to the denial of the use of live prey in order to eliminate their suffering from being eaten alive. Currently in the UK, there are certain circumstances in which live prey feeding can be practiced, provided that a written justification and an ethical review are presented, and that a veterinary surgeon has advised this practice. Subsequently, the feeding process must be carefully monitored by trained staff, the public cannot participate, and if the prey is not eaten, it must not be left inside the enclosure.
In EU/UK countries, the EU Directive 98/58/EC can be found as a general line guide.

However, often it is up to member states and DEFRA[4]. As for the relevant acts, we have the "Animal Welfare Act and Zoo Licensing Act 2006", where the use of live vertebrate prey is discouraged, unless there are exceptional cases where veterinary advice is necessary. In these rare cases, there is an obligation to ensure as much as possible that the prey is killed before being offered to the predator. It should be noted that this law applies exclusively to vertebrates, which is why live invertebrate prey are not protected and can therefore be used as live prey. "The Welfare of Farmed Animals" stipulates that animals may not be fed anything that could cause them harm. "European Conventions of the Protection of Animals Kept for Farming Purposes" (Article 3, 6, 9, and 14) applies only to farmed, vertebrate fish. Fish feeding must be appropriate for species, and health must be optimal. Prey may cause harm and can be avoided if the diet is otherwise suitable. Animals' food must be appropriate for their physiological and ethological needs in accordance with scientific knowledge, however, no food may be given that could cause unnecessary harm. "Directive 2010/63/EU" establishes measures for the protection of animals used for scientific or educational purposes and requests to "avoid inflicting undue distress, pain and suffering on an animal" **(9, 13, 8)**.

[4] Department for Environment, Food and Rural Affairs.

2. *The Rattus norvegicus*

2.1 Domestic history

The *Rattus norvegicus,* commonly known as the brown rat, sewer rat, wharf rat, or street rat, originates from Asia, where it was predominantly found in Mongolia and northern China. It spread to Europe in the 18th century, traveling on cargo ships from Norway. Consequently, the naturalist John Berkenhout mistakenly assigned the species the name *Rattus norvegicus* or Norway rat.
The earliest concrete evidence of rats being kept as pets can be traced back to Japan during Edo period (1603-1868). During this era, it became common practice to keep rats and mice as domestic animals and to selectively breeding them to develop distinctive colorations and markings. In Europe, during the Victorian era, rats began to be regarded as pets. England's global trade led to a rapid increase in the rat population, prompting Queen Victoria to hire "Rat Catchers" to control them. These catchers made money by capturing and eliminating rats and selling them for rat-baiting shows, where terriers hunted rats in an enclosure while audiences placed bets. Joe Black, known as "The Queen Victoria's Royal Rat Catcher", began breeding rats as pets, sparking interest in albino rats among noblewomen. Mary Douglas further promoted rats as pets, writing articles and organizing exhibitions. In 1913, the National Mouse Club became the National Mouse and Rat Club with Douglas as President. Although the popularity of pet rats declined after her death in 1921, this type of habit still persisted and it noticeable that in 1976 the National Fancy Rat Society was founded to promote them **(16)**. In the 70's there has been a significant increase in the presence of domestic rats in English households. It is currently estimated that around 0.1 million rats are kept as pets in the United Kingdom. This growth not only pertains to the population of rats as companion animals, but also it extends to the domestic use of these rodents in the homes of reptile enthusiasts, where rats are frequently used as live prey **(7)**. Beyond the domestic sphere, rats are also employed in wildlife rehabilitation centres, where current legislation does not impose significant restrictions on the use of these animals as a food source. Two prominent examples of such centres are the Alturas Wildlife Sanctuary, a rehabilitation centre, and Reptilandia, a reptilarium, both located in Costa Rica. In these places, rats are utilized as an integral part of feeding programs for predators in rehabilitation and reptiles on display, thus demonstrating the versatility of this rodent in various usage contexts. This increase in the use of rats, both as pets and as live prey, underscores the need

for careful consideration of their ethological and physiological needs to ensure their welfare, both in domestic environments and in rehabilitation centres and reptilariums.

2.2 Biology

2.2.1 Taxonomic classification

Domestic rats, scientifically known as *Rattus norvegicus,* belong to the mammalian class Mammalia, order Rodentia, and family Muridae. It is known also as brown rat (*Rattus norvegicus Berkenhout*) and classified as being of Least Concern (IUCN 2016) **(6)**.

2.2.2 Lifespan

Rattus norvegicus can live up to 4 years in captivity. However, in the wild, their lifespan is generally estimated to be up to 2 years **(2)**.

2.2.3 Morphology

From a morphological point of view, Norway rats can attain a weight of up to 0.5 kg and a body length exceeding 12 cm with a maximum of 40 cm (which includes a tail of 15-18cm), with males typically outweighing females. The cranium displays a squared structure, curved temporal arches, and two bony crests on its upper surface, setting it apart from the black rat. Its incisors are notably prominent and hypsodont. The ears, which are shorter than those of relates species, and the tail are bald. The limbs exhibit a glabrous texture, displaying a pink hue; notably, the hind limbs surpass the forelimbs in length. The latter are primarily utilized for grasping food items and conveying them towards the mouth. The fur is most commonly bristly and grey or brown with a lighter face and underside. However, domesticated specimens may exhibit white or mottled coloration. Their long tails, often mistakenly perceived as hairless due to sparse fur, usually approach the length of the body **(6, 7, 17)**.

2.2.4 Reproduction

Their mating system is polygynandrous, with both males and females engaging with multiple partners. While *Rattus norvegicus* does not follow a strict seasonal breeding pattern, there is an increase in mating activity during the warmer months. Females typically give birth approximately seven times annually, with litter comprising up to 14 pups. Approximately 18 hours after giving birth, females experience postpartum oestrus and can mate again almost immediately. This reproductive trait contributes to the high birth rates of Norway rats, with each female potentially producing up to 60 young annually, usually continuing for up two years. The gestation period is short, lasting 22 to 24 days. The newborns are altricial with their eyes opening after 14 to 17 days. Postnatal development can be categorized into five distinct stages, each with its own characteristics. The stages include the neonatal period, where the rat is newly born and highly dependent; the infantile period, marked by rapid growth and initial exploration, indeed it represents an advancing sensory period; the juvenile period, characterized by increased activity and social interactions, is related to leaving of the nest and so it concludes the weaning period; the peripubertal period, where significant physical and hormonal changes occur; and the adolescent period, during which the rat approaches full sexual maturity and adult behaviours. Males typically reach sexual maturity at three months, while females mature at around four months. However, females usually mate earlier because competition among males often prevents the immediate success of smaller, less dominant males. Each stage plays a crucial role in the overall growth and development of the rat. Multiple females often share nests, and all offspring are cared for by the adult rats, regardless of biological percentage. This communal care behaviour qualifies the species as cooperative breeders. Parental care is provided exclusively by females. Due to the communal nesting habits, the offspring of different females often share the same nest. In cases where the mother is not able to care her pinkies or is killed, other foster mums will nurse the newborns **(6, 7)**.

2.2.5 Behaviour

Social by nature, brown rats form colonies that inhabit defined territories. Typically, these rats live in large, male-dominated social groups, with hierarchies based on individual size. In circumstances where resources are scarce or widely dispersed, rats typically form small social groups consisting of a single male, several females, and their offspring, often centred around as underground burrow. Conversely, when resources are abundant and easily accessible, these animals can establish large, interconnected colonies, showcasing a more intricate social structure and increased levels of cooperation. Members within a group primarily engage in communication through a diverse array of squeaks, with variations observed based on factors such as sex, age, and hierarchical status within the group. It is widely theorized that beyond vocalizations, body posture, and particularly chemical cues, usually in urine or faeces, play significant roles in facilitating communication among these rodents, allowing them both to find food and to distinguish between individuals of a group. The *Rattus norvegicus* possesses an exceptionally acute sense of smell, enabling it to interpret chemical signals with great precision. Furthermore, despite their relatively limited visual acuity, rats have the remarkable ability to detect ultrasonic frequencies, their hearing range is around 0.2-80 kHz, and, to a certain extent, perceive certain wavelengths within the ultraviolet spectrum. The rats rely on their sensitive whiskers for discerning objects, detecting obstacles, maintaining balance, and navigating through complex environments. Additionally, their footpads and toe tips exhibit heightened sensitivity to tactile stimuli, allowing them to perceive and interact with the surrounding in a detail manner **(6, 7)**.

In the realm of normal behaviours observed in rats, social interaction plays a significant role, often characterized by grooming activities, both self-directed and directed towards fellow rats. Chewing or gnawing is another prevalent behaviour, essential for various aspects of their daily activities such as the maintenance of their dental health. Regarding the locomotion, rats engage in climbing, walking, and occasionally standing on their hind legs, reaching a maximum height of approximately 26-30 cm. Playful interactions are also common, involving leaping, chasing, and playful scuffling, with distinctive behaviours such as snout or oral contact aimed at the opponent's nape rather than the rump, as seen in more serious confrontations. Furthermore, resting and hiding are integral parts of their routine, along with the habitual building of nests. Foraging behaviours involve the manipulation, carrying, and hoarding of food and objects, contributing to their survival strategies. Additionally, rats exhibit caecotrophy, a digestive process essential for nutrient absorption and overall health. These behaviours collectively constitute the repertoire of normal activities observed in rats, reflecting their adaptive strategies and social dynamics within their environment **(3)**.

Considering negative emotional states, signs of anxiety in rats include hesitancy to leave familiar environments, heightened tendency to hug walls, increased fear of unfamiliar objects, and exaggerated efforts to escape or hide. Persistent anxiety may suggest ongoing stress rather than mere fear of new experiences. Signs of pain or infections, include a hunched posture, reduced activity, decreased grooming, loss of appetite, abnormal gait, poor coordination, guarding of body parts, or heightened aggression. Distinguishing aggression from playful behaviour is crucial, with aggression typically being unidirectional and undesired by the victim, whereas play involves mutual engagement and smooth fur.

Audible vocalizations may indicate discomfort or distress, especially if a usually quiet rat vocalizes strongly during handling. Ultrasonic vocalizations at around 50 kHz may signify pleasure, while those at around 22 kHz may indicate unpleasant emotional states, such as aggression, predator presence, startle response, or pain. Detecting these sounds requires specialized equipment like bat detectors. Barbering, whether self-inflicted or directed towards others, may signal social incompatibility or a lack of enrichment. Tail manipulation might suggest insufficient social interaction, while bar biting or chewing may reflect frustration or an attempt to escape from the cage, highlighting underlying social or environmental issues. These behaviours are typically observed during nighttime. Importantly, the absence of abnormal behaviour does not necessarily indicate satisfactory health and welfare for the rat **(7)**.

2.3 Ecological requirements

2.3.1 Natural habitat

In Asia, *Rattus norvegicus* was originally indigenous to forests and regions abundant in dense vegetation. Nowadays, it inhabits a wide array of environments, including garbage dumps, sewers, open fields, woodlands, basements, and virtually any locale providing sustenance and refuge. This widespread distribution is closely linked to human habitations and the associated dispersal mechanisms. However, there are notable exceptions to their presence. They are absent from Antarctica, the province of Alberta in Canada, and certain designated conservation reserves in New Zealand **(10)**. These exceptions are due to stringent control measures and environmental conditions that prevent their establishment and proliferation **(3)**.

2.3.2 Diet

Renowned for its adaptability, the brown rat is primarily omnivorous but demonstrates a preference for a carnivorous diet. It is an excellent forager, and aggressively pursues a diverse array of prey, including shrimp, snails, mussels, insects, bird eggs, and chicks, amphibians, eels, fish, pheasants, pigeons, poultry, rabbits, and carrion. Additionally, they consume seeds, various plant material, and human food waste. In captivity they can be fed with pelleted complete rat diets with an implementation of small amounts of unprocessed food types such as seeds, grains, fresh fruit and vegetables, and of cooked egg or meat, in order to promote the manipulation of these items. Food can be hidden around the enclosure and treats placed in challenging spots, like buried in sand or hidden in cardboard tubes, to encourage natural foraging behaviour. The amount of food rats consume depends on their social rank and age, with younger, growing rats requiring more food **(7)**.

2.3.3 Activity patterns

Norway rats are primarily nocturnal or active at dusk, engaging in activities such as digging burrows, foraging for food, and constructing nests during these hours. They often inhabit areas near water, which provide essential resources and suitable conditions for their burrows. In captivity, this digging, burrowing and foraging behaviour can be facilitated with deep bedding made of large particles like cellulose or canvas that allowing the manipulation of these materials. Their foraging trips can be extensive, as they navigate well-established routes to food-rich areas. Norway rats exhibit impressive learning abilities, allowing them to memorize and traverse complex sewer and burrow networks efficiently. New colonies form when a pair establishes a nest in unoccupied territory. Nest construction involves gathering materials such as leaves, garbage, and twigs, resulting in complex structures with multiple chambers for food storage, nesting, and emergency exits. In captivity nesting boxes such as “Sputnik” or disposable options like cardboard boxes can be offered, along with nesting materials such as paper. In the wild, their intricate burrows provide not only shelter but also secure habitats, featuring specialized chambers for different functions. In captivity, shelters should be provided for various reasons. They allow withdrawal from light, offer a choice of microclimates that aid in thermoregulation, provide a means of escape from aggressive social interactions, and give rats a degree of control over their environment. Shelters also satisfy the thigmotactic aspects of rat behaviour and provide additional structures for climbing, enhancing their use of vertical space. The activity levels of rats are significantly influenced by environmental variables such as temperature and humidity. Indeed, in less favourable conditions, such as extreme temperatures or high humidity, rats tend to become more lethargic. Therefore, managing environmental conditions is crucial for the wellbeing and daily activity of rats, both in the wild and in captivity **(7)**.

2.4 Physiological needs

2.4.1 Environmental variables

Rats are highly sensitive to various environmental factors that can significantly influence their health and behaviour. Understanding the impact of light, temperature, humidity, and air quality is essential for ensuring their wellbeing in captivity settings.

Exposure to bright light negatively affects rats by reducing their activity levels, which disrupts normal locomotor and play behaviours, particularly in juveniles. This exposure can induce fear behaviours, such as wall-hugging, where rats stay close to walls for security. Since rats' retinas are only partially developed at birth, bright light can inhibit the rods in their eyes, further impacting their vision. Moreover, bright light can impair reproductive performance, decreasing the number of litters, the number of pups per litter, and gestational weight gain. Regarding the temperature, the thermos-neutral zone for rats is between 27°C and 30°C, allowing them to maintain normothermia in ambient ranging from 10°C to 30°C. While rats handle lower temperatures better, they struggle to disperse heat efficiently. Environmental temperature variations outside this range can adversely affect their reproductive performance, leading to smaller litter sizes, increased embryonic deaths, and stunted growth. Such temperature fluctuations also cause significant changes in food and water intake and affect haematological and biochemical parameters. Gestating and lactating females have reduced thermoregulatory capabilities, making them particularly susceptible to temperature extremes. Generally, being nocturnal creatures, rats primarily rest and sleep during the day and become active at night. For their resting period, they prefer ambient temperatures between 25°C and 30°C. During their active nighttime hours, they choose cooler temperatures ranging from 17°C to 25°C **(14, 3)**. Lastly, a relative humidity of 40-70% is ideal for their wellbeing. Low humidity can cause "tail ring", characterized by a circular lesion around the tail, which may result in the distal portion of the tail sloughing off. On the other hand, high humidity can increase bacterial growth and ammonia production in cages, thereby putting animals at higher risk of infection **(14, 3)**.

2.4.2 Hydration and Nutrition

Rats require ad libitum food and water. It is essential to consider that their consumption of food and water can be influenced by their social environment, as previously seen **(7)**. Food and water must be uncontaminated and provided in adequate quantity and quality to meet the nutritional needs of rats, including those that are pregnant, lactating, or growing. Where possible, it is beneficial to offer a variety of food types. Water can be offered using bottle drinkers and should be accessible at the level that allows rats to sit while drinking to avoid bony and cartilaginous damage. It is also suggested to provide food to them in an accessible way, such as from an open bowl. In addition, scattering food can help reduce competition, while offering whole food items like nuts and eggs in shells encourages manipulation with their paws. Providing wood blocks and low-calorie dog biscuits can promote gnawing, aiding in natural tooth wear and preventing overgrowth **(14, 3)**.

2.4.3 Environmental enrichment

Creating an enriched environment is vital for the wellbeing and behavioural development of rats. They naturally prefer complex environments that stimulate their cognitive abilities and natural behaviours. Without adequate enrichment, their behaviours may be constrained, potentially leading to abnormal brain development. When choosing enrichment items, it is crucial to consider potential health risks. Materials such as plastics, galvanized metals, or painted objects can be toxic if chewed, so it is important to prioritize non-toxic options. For chewing and gnawing, suitable items include small non-toxic wooden blocks drilled with holes, branches, and softwood sticks like tongue depressors. Cardboard toys that hide food inside can also engage their chewing instincts and provide mental stimulation. To encourage burrowing behaviour, a digging box with appropriate substrate and other materials for hiding food or objects can be provided. Regular monitoring ensures it does not become a toilet area, and options for burrowing can be offered intermittently or permanently. For locomotion, rats benefit from branches, running wheels, ledges, ample three-dimensional space, and regular introduction of new objects like plastic toys to encourage exploration and play. Resting and hiding are essential behaviours, supported by nesting boxes filled with materials such as non-fraying fabric strips, strips of paper, jute, straw, hay, and leaves. These materials can be placed strategically throughout the enclosure, such as attached to cage bars, inside egg boxes, or spread on hammocks and shelves. Rats also enjoy carrying and hoarding food and objects. Providing food items like sunflower seeds that can be picked up, held, and hoarded within the cage encourages these natural behaviours. In summary, enriching the environment with a variety of safe and stimulating materials not only meets rats' physical and cognitive needs but also enhances their overall wellbeing and encourages natural behaviours **(3)**.

2.4.4 Health and disease management

Common diseases in rats include respiratory problems, often caused by mycoplasma or multi-bacterial infections, and diarrhoea resulting from bacteria, internal parasites, severe stress, or diet. These issues can lead to rapid dehydration and require veterinary attention. Overgrown or misaligned incisors due to damage, poor nutrition, bar chewing, or genetics can cause feeding difficulties and weight loss. Tumours, particularly in the mammary glands, are frequent in older rats and are associated with high dietary fat intake and obesity. Moreover, pathophysiological signs in rats include chromodacryorrhea, or 'red tears', which can indicate stress or illness, with nasal secretions appearing almost immediately and ocular secretions within 15-30 minutes of a stressful event. This condition may also occur during normal social interactions or due to illnesses like respiratory infections, so further monitoring is essential if detected. Other signs of poor health include a rough, clumped coat, diarrhoea, bald patches from barbering or mange, weight loss, slow growth, and reduced food intake.

Regular health checks for rats are essential, as they often do not exhibit signs of illness until the condition becomes severe. Key areas to monitor include weight and temperature: adult females should weigh between 225 and 400 grams, and adult males between 250 and 550 grams. The normal body temperature for rats ranges from 27.5 to 38 °C. Physical examinations should encompass checks for abnormal behaviours such as lethargy, agitation, or seizures. Eyes should be inspected for discolouration, cloudiness, or swelling, while ears should be examined for discharge, odour, or crusting. The mouth and teeth should be checked for misalignment, swelling, or drooling, and the nose for excessive porphyrin staining and noisy breathing. Cheeks and neck should be inspected for swelling. Additionally, signs of respiratory distress such as wheezing, congestion, or laboured breathing should be observed. Other indicators of poor health include eye and nose discharge, poor appetite, hunched posture, or puffed-up fur. The body should be checked for injuries, bleeding, cuts, and bruises, and the tail and feet for bumblefoot and ringtail. For the genital area, females should be checked for vaginal discharge or blood, and males for penile discharge. Lastly, extremities should be examined for unusual colour, and the pinch test should be performed to assess hydration. Regularly covering these areas is crucial for maintaining the health and wellbeing of rats **(3, 7)**.

3. *Case study*

3.1 Introduction

This chapter provides a detailed presentation of a case study conducted over a nine-week internship at the Alturas Wildlife Sanctuary during the 2023/2024 academic year. The primary objective of this project was to propose concrete improvements to the quality of life for *Rattus norvegicus* used as live prey at the Sanctuary and to field-test their effectiveness. The study specifically compared the behaviour of three adult subjects, housed in the same animal facility, before and after the implementation of measures proposed by a protocol that was personally drafted, based on the information collected and described in Section 2.2 and 2.3. Although certain behaviours of the rats were observed, it is important to emphasize that the primary objective was not to conduct a behavioural study, but rather to assess whether the rats' performance indicated an improvement in their wellbeing following the introduction of new elements. A reduction in activity, for example, can be an indicator of a situation where the rat is not in good health, as noted in Section 2.2.5, as well as progressive social isolation **(7)**. Research consistently shows that environmental enrichment interventions - such as physical, social, cognitive enhancements - significantly improve the welfare of laboratory animals. These interventions help reduce stress, encourage natural behaviours, and boost cognitive function. Studies such as those conducted by Makowska, Arduini, Silva, and Yamamoto **(1, 4, 12)** offer strong evidence supporting the benefits of environmental enrichment, demonstrating its positive impact on the quality of life for laboratory animals. It is noticeable that they also use behavioural observations to conduct their research. However, it is necessary to consider the limitations that hindered the development of a rigorous and accurate study in this case. These limitations include, on one hand, the economic constraints of the Alturas centre, which prevented the use of adequate equipment that would allow for more substantial changes in the rats' living conditions. On the other hand, the challenging environmental conditions, as the rats were not housed in a controlled laboratory setting but in enclosures exposed to various climatic conditions, posed additional difficulties. Moreover, the presence of predators such as pumas and other species potentially caused anxiety and discomfort among the rats. Despite these numerous limitations, it was still possible to extract significant data in this relatively unexplored scenario.

a) b)

***Figure n.3.1**: Photo of the initial rats' enclosure on the left and photo of the pinkies found on the ground due to the gaps in the wire mesh of the enclosure on the right.*

3.2 Material and methods

3.2.1 Enclosure and diet situation before the intervention

The enclosure before the modifications is shown in Figure n.3.1a. The three adult rats, two females and one male, were housed in a cage measuring 59x59x91 cm (depth, height, and width), made of wire mesh with a metal roof to protect them from rain and sunlight. The cage did not have a solid floor, which led to the loss of pinkies (Figure n.3.1b) that fell through the holes and landed on the ground, as the enclosures were elevated about 1 m from the ground. As a base, bedding and hiding place, there were only a few sheets and a bamboo tunnel, which was too small to accommodate more than one rat at a time, causing conflict among the three rats. Inside, there was also a bottle and bowl waterer, along with a bowl containing dry pellets, which were the only type of food provided. Dry foliage was also provided weekly, with the idea of encouraging the rats to engage in gnawing behaviour and build nests.

3.2.2 Interventions made

The first intervention was to increase the cage height the rats' enclosure, as can be seen in Figure n.3.2a, with dimensions of 59x91x59 cm, thereby offering a significantly larger area for climbing, one of their essential natural behaviours. A solid PVC plastic floor was added as a base for the bedding, made of wood shavings, providing a more hygienic and comfortable environment, offering benefits such as dissipating body heat. The sheets previously used as a base were removed, leaving only one or two small cloths to maintain a degree of familiarity and comfort for the rats. Three levels were created, also made of PVC plastic, allowing the rats to explore and spend their time on the higher floors, thus stimulating their curiosity and physical activity. In the centre of the three levels, a sturdy stick was fixed, which could be used as a ramp to facilitate access to the upper shelves, as can be seen in Figure n.3.2b, further promoting their climbing behaviour and providing a form of environmental enrichment. Additionally, three shelters were introduced within the enclosure which, as seen in Section 2.3.3, serves various functions such as providing protection from light, a refuge in case of need or reduce stress **(7, 3)**. These included a coconut shell with a central hole for the rats to pass through and a plastic container previously used for disinfectant, repurposed as a safe refuge. Each hiding spot was strategically placed to offer each of the three rats the opportunity to retreat alone when necessary, allowing them to exhibit safety-seeking behaviours and reducing potential social conflicts.

From a feeding perspective, in addition to the dried pellets provided daily, various types of unprocessed food were offered in small quantities. These included fresh vegetables and fruits, often presented in the form of ice cubes. This measure was essential not only to ensure freshness of the food, given the extreme temperatures of the rats' environment, but also to stimulate their interest in the food and encourage exploration. Additionally, this solution guaranteed freshness while also keeping the rats hydrated and cool during the hottest parts of the day. In addition to the diet, various types of environmental enrichments were provided to stimulate the rats mentally and physically. These enrichments were replaced approximately every three days to maintain a high level of interest and stimulation. Common enrichments included toilet paper rolls and egg cartons, which the rats could explore, manipulate, and destroy, thereby satisfying their natural instincts for gnawing and nest-building. A particularly appreciated and consistently present type of enrichment was a bowl used as a small pool. This bowl allowed the rats to cool off by immersing themselves in the water, providing immediate relief from the heat, helping to regulate the rats' body temperature.

a) b)

Figure n.3.2: *Photo of the new enclosure from outside (on the left) and of the interior (on the right).*

3.2.3 Methods of observation

The research focused on three adult subjects, consisting of two females and one male, all housed within the same enclosure and lasted 28 days. This period was subdivided in two subgroups, before and after the implementation of the enrichments. The study of the activity patterns was done with both daily direct observations, which were conducted at 4:00 PM for a duration of 20 minutes, supplemented with the use of camera traps, which were activated every half hour for 20 seconds, as the rats' area was frequented by large feline, such as pumas, during nighttime hours. This presence made it impossible to obtain data through direct observations during the hours when the rats were most active, as they are nocturnal animals. Direct observation, often referred to as an observational study, is a method for gathering evaluative data where the observer monitors the subject in its natural environment without making any alterations. This approach was particularly common in earlier research, before the development of automated techniques like data loggers. In this study, direct observations were utilized due to economic constraints, as the Alturas centre did not have the resources to support monitoring with more advanced, state-of-the-art software. It is possible to identify advantages and disadvantages in the use of direct observations. Among the advantages, the observer was able to distinguish the three subjects, which was not possible with the use of camera traps, and the method can be implemented without significant financial expense. Among the disadvantages, the observer's presence influenced the behaviour of the rats, making them more elusive and less inclined to explore; additionally, it is likely that not all behaviours of interest were recorded. Finally, the method is time-consuming. Furthermore, the physiological analysis required to detect the presence of specific hormones associated with relaxation states, such as dopamine and endorphins, or stress states, such as adrenaline and cortisol, was not feasible. This was due not only to economic constraints but also to the fact that the rats, not being accustomed to handling, would have reacted with a stress response **(5)**. The direct observations were conducted using the Ad libitum sampling method, where all relevant data were systematically recorded. Additionally, for recording purposes, the one zero sampling recording method was employed throughout the entire 20-minute session, divided in four sessions of five minutes each one. This methodological choice enabled comprehensive data collection, facilitating a thorough analysis of behavioural patterns and responses during the observation period.

Regarding the observations made with the camera traps ("Browning BTC-8E-HPS" models, Figure n.3.3), they were placed within the enclosure, and they allow to obtain data both during day and night, thanks to their high-resolution images and videos recording capability in visible and infrared range. Although, as previously said, the inability to uniquely and accurately identify individual subjects within the observed group emerged. The sampling method adopted was behavioural recording. This choice was motivated by the difficulty in positioning the camera traps inside the cage, which made it impossible to obtain a complete view of the events occurring within. The events that were not directly observable are classified as "out of sight". Concerning the recording rules, one zero sampling recording was always used. During the initial fourteen-day period the focus was on observing the presence or absence of lethargic behaviour in the rats. Whereas after the enclosure modifications, attention shifted to observing the use of a tunnel as a hiding place and a stick placed inside the enclosure as a ramp to facilitate movement between different levels.

Figure n.3.3*: Photo of the camera trap used to monitor rats (Model: Browining BTC-8E- HPS).*

3.2.4 Data analysis

The proportions (frequencies) of the behaviours observed before and after enrichment were analysed by the chi-square test. The chi-square test for independence, also called Pearson's chi-square test or the chi-square test of association, is used to discover if there is a relationship between two categorical variables. The frequencies between different behaviours were analysed by the McNemar test. The McNemar test is used to determine if there are differences on a dichotomous dependent variable between two related groups. The McNemar test is used to analyse pre-test/post-test study designs, as well as being commonly employed in analysing matched pairs and case-control studies. The variations in environmental temperature were analysed by Oneway ANOVA using the experimental phase (Phase 1: before enrichment, 12-16 April; Phase 2: after enrichment, 18-21 April; Phase 3: after enrichment, 23-26 April) as fixed effect, and differences among means were studied by Duncan’s test.

3.3 Results and discussion

3.3.1 Analysis of observational data from direct observation

The data collected from direct observations are presented in Table n.3.1, organised by observation period (before and after enrichment), by individual, and by observed

Table n.3.1: *Percentage frequencies of behaviours observed by direct observations of the three subjects of the study, during the period before and after the implementation of environmental enrichments. The behaviours monitored are indicated with the following abbreviations: Fe=Feeding; Dr= Drinking; Cli w.me=Climb wire mesh; Gr=Grooming; S Gr=Self grooming; Hi Sh=Hiding sheets; Leth = Lethargy; Cli cam=climb camera; Cli wo=Climb wood; Po=Pool; Shel=Shelves. The acronym "nd" stands for "not detected".*

		Observed Behaviours (Frequency)										
Experimental Phase		Fe	Dr	Cli w.me	Gr	S Gr	Hi Sh	Leth	Cli cam	Cli wo	Po	Shel
Before enrichment	Rat 1	0,00	0,21	0,29	0,07	0,29	0,57	0,29	0,29	nd	nd	nd
	Rat 2	0,14	0,43	0,36	0,07	0,29	0,50	0,29	0,36	nd	nd	nd
	Rat 3	0,21	0,07	0,29	0,14	0,29	0,43	0,14	0,36	nd	nd	nd
After enrichment	Rat 1	0,29	0,50	0,14	0,00	0,43	0,57	0,50	nd	0,57	0,36	0,64
	Rat 2	0,14	0,36	0,21	0,14	0,29	0,43	0,21	nd	0,57	0,50	0,57
	Rat 3	0,50	0,21	0,21	0,14	0,50	0,29	0,14	nd	0,57	0,21	0,57

behaviours. The behaviours of interest were feeding, drinking, climbing on wire mesh, grooming, self-grooming, hiding under covers, lethargy, climbing on the camera trap, climbing on wood, using the pool, and interacting with shelves. Within each experimental phase, the frequencies of observed behaviours did not significantly differ between rats (Chi-square test; IBM SPSS 29.0). Thus, the frequencies of individual behaviours can be compared across the two study periods, using the average frequency observed across the three individuals. This comparison is illustrated in the graph in Figure n.3.4 which shows the behaviours recorded both before and after the change. It can be seen that for feeding, there were significantly different frequencies between observations made before and after enrichment ($P<0.05$; Chi-square test; IBM SPSS 29.0), whereas this was not the case for the other behaviours. The percentage frequencies of four specific behaviours not present in both sub-periods are reported in Figure n.3.5. As highlighted, the frequency of "climbing on the camera trap" after the implementation of enrichments is 0%, due to the new position of the camera trap, which prevented the rats from climbing. Conversely, for the three subsequent not behaviours, the frequencies recorded before the implementation of the enrichments were 0%, as the enrichments, such as the wood stick, pool, and shelves, were present at the beginning of data collection.

It is important to note that no statistical analyses were performed for these cases, as the behaviours observed "after" were not present "before" the enrichments were introduced. Furthermore, the percentage frequencies of the final three behaviours are particularly

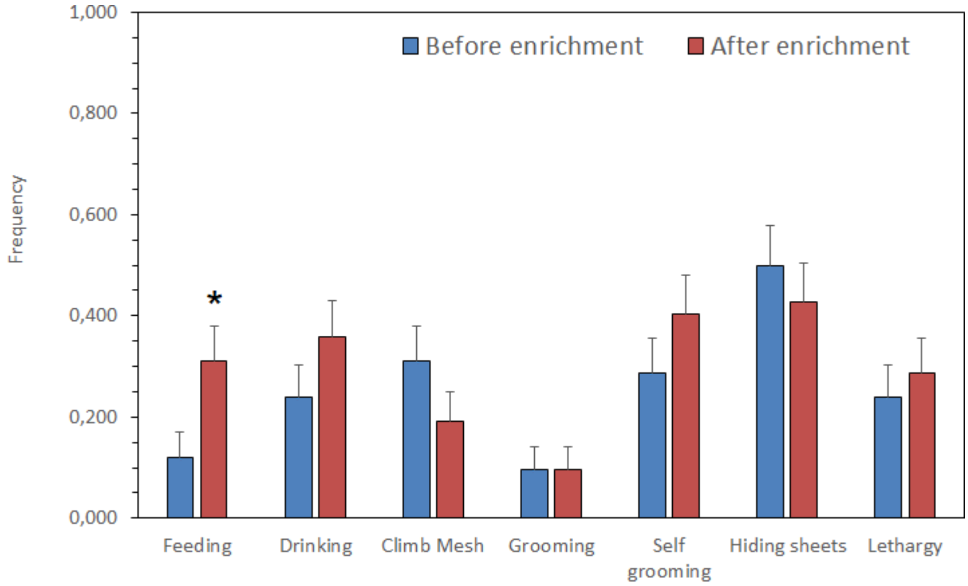

Figure n.3.4*: Comparison of the percentage frequencies of averaged behaviours observed in the three subjects through direct observations, before (blue) and after (red) the implementation of environmental enrichments. Standard error bars are reported. (*) indicates a significantly different frequency between the observations made before and after enrichment (P<0.05; Chi-square test; IBM SPSS 29.0).*

high. Figure n.3.6 compares specific averaged behaviours to "lethargy" and is divided into two sections: "before enrichment" and "after enrichment". In the "before enrichment" section, two behaviours - "grooming" and "hiding in sheets" - are marked with an asterisk (*). This indicates a significant difference in their frequencies compared to "lethargy" (P<0.05; McNemar test; IBM SPSS 29.0). Specifically, "grooming" is observed at a lower frequency than "lethargy", whereas "hiding in sheets" occurs at a higher frequency. In the "after enrichment" section, the frequencies of "grooming" and "hiding in sheets" continue to differ significantly from "lethargy", mirroring the patterns observed before enrichment. Additionally, "climbing on wood" and "interacting with shelves" are also marked with an asterisk, reflecting significant differences in their frequencies compared to "lethargy" following the implementation of enrichment.

3.3.2 Analysis of observational data from camera traps

The analysis of observational data obtained with camera traps is presented here. Figure n.3.7 (top) shows that the occurrence of lethargic behaviour is significantly higher than that of behaviours classified as "out of sight" (P<0.001; McNemar test; IBM SPSS 29.0). It is also noted that the frequency of "out of sight" behaviours was lower compared to the post-enrichment period, despite changes in the field of view due to the repositioning of the camera for spatial reasons ($P<0.05$; McNemar test; IBM SPSS 29.0). The lower panel of Figure n.3.7 examines the use of two new objects introduced into the enclosure: the tunnel and the wood stick, which facilitates climbing up or down from the shelves now present. Notably, the use of these objects shows a statistically significant difference compared to the lethargic behaviour observed before enrichment ($P<0.05$; McNemar test; IBM SPSS 29.0). Finally, an investigation was conducted to determine whether ambient temperature had any impact on the subjects' behaviours, given the rats' sensitivity to high temperatures. As depicted in Figure n.3.8, there is no apparent relationship between temperature trends and the percentage frequencies of "out of sight" behaviours, which present an increment in the last period of observation relative of the use of tunnel.

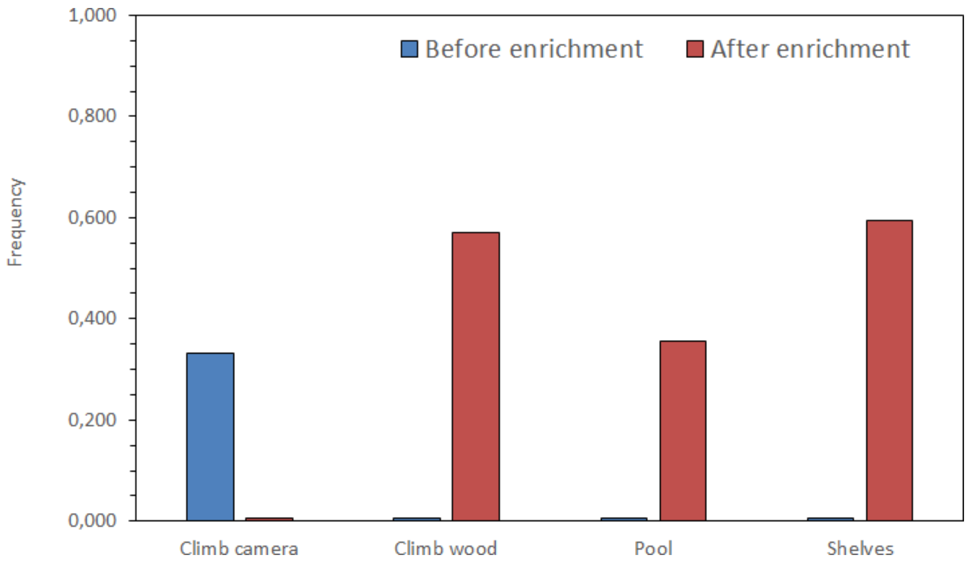

Figure n.3.5: *Representation of the percentage frequencies of specific averaged behaviours observed in the three subjects through direct observations, which have not correspondence before (blue) and after (red) the implementation of environmental enrichments: climbing on the camera trap, climbing on wood, using the pool, and interacting with shelves.*

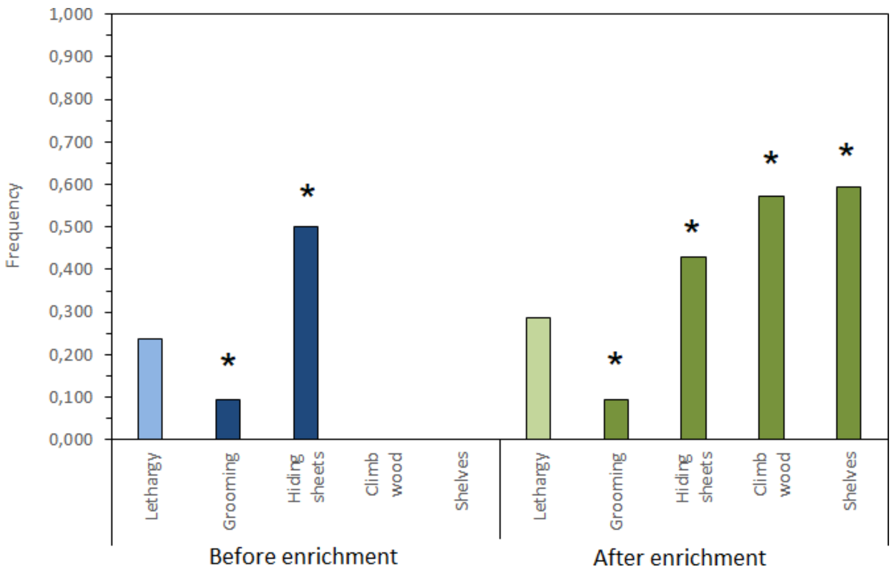

Figure n.3.6: *Comparison of specific averaged behaviours directly observed in the three subjects with "lethargy." The colour legend is as follows: blue for observations before enrichment, green for observations after enrichment. An asterisk (*) over the column corresponding to a specific behaviour indicates a significantly different frequency compared to "lethargy." (P<0.05; Mc Nemar test; IBM SPSS 29.0).*

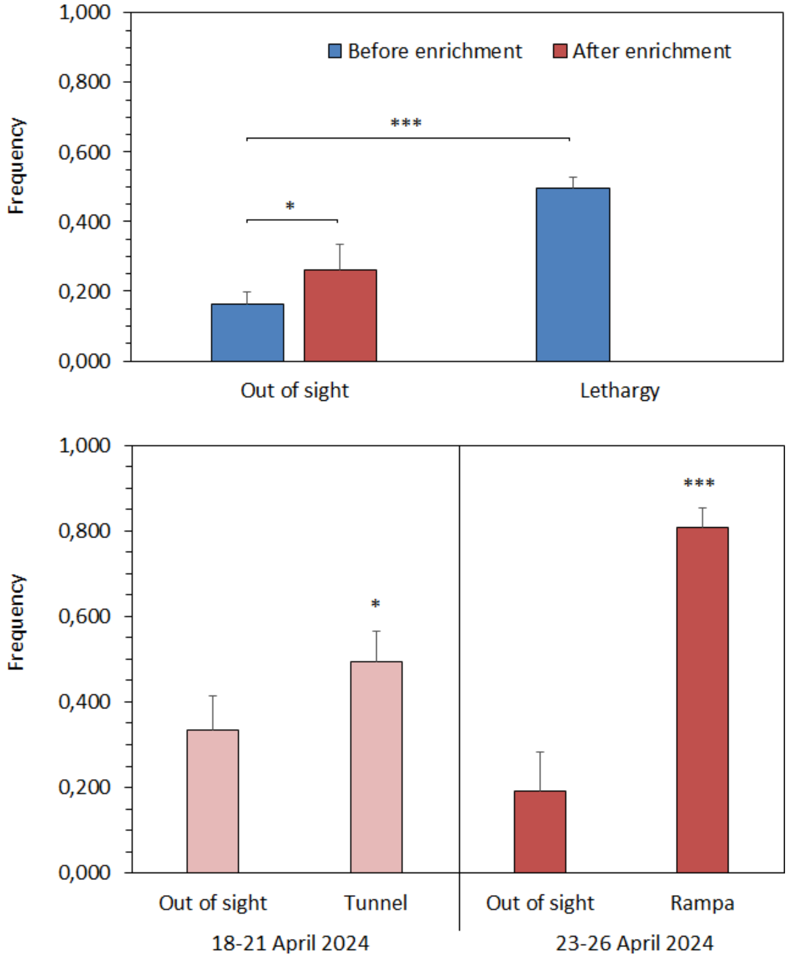

***Figure n.3.7**: (Top) Representation of the observation frequencies of lethargy and out of sight behaviours recorded with camera traps before enrichment, compared to their corresponding frequencies after enrichment. The asterisks indicate a positive result from the McNemar test between data points connected by a line (*P<0.05; ***P<0.001; McNemar test, IBM SPSS 29.0). (Bottom) Plot of the percentage frequencies based on observations made with camera traps regarding the use of the tunnel and ramp, labelled as "tunnel" and "ramp" respectively, along with the percentage frequency of "out of sight". An asterisk over the column corresponding to a specific behaviour indicates a significantly different frequency compared to "lethargy" (*P<0.05; ***P<0.001; McNemar test, IBM SPSS 29.0).*

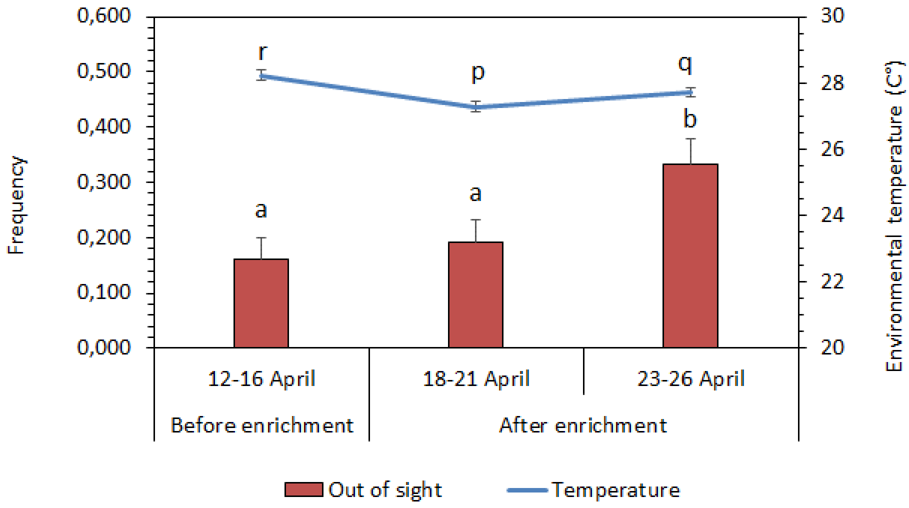

Figure n.3.8: *Comparison of the frequency trends of "out of sight" behaviours observed with the camera trap in relation to ambient temperature. Different superscripts indicates significantly different frequencies (a,b P<0.05; Mc Nemar test; IBM SPSS 29.0) or means of environmental temperature (p,q,r P<0.001; Duncan's test; IBM SPSS 29.0).*

The statistical analysis of data obtained through direct observation and video footage collected from camera traps reveals the effects of introducing new elements into the enclosure. Furthermore, it is evident that increasing the availability of various foods, such as fruits and vegetables, likely led to both a rise in the frequency of feeding behaviours, as shown in Figure n.3.4, and a stimulation of their interest in food. From the same plot, it can be inferred that these changes did not lead to any negative variations in the behaviours observed before and after the enrichment. Indeed, behaviours such as "hiding" and "lethargy", which would significantly increase as indicators of stress or fear **(3)**, remained unchanged. Perhaps, no change is observed in certain behaviours, such as those just mentioned, since the interventions were limited due to the constraints already outlined and probably also because of the scorching environmental conditions.

However, the main focus of the research is on the increase in active behaviours among the three subjects, which is an indicator of wellbeing **(3)**, as observed in Figures n.3.5, 3.6, and 3.7. The subjects' preference for using the tunnel, shelves, and the wooden ramp leading to these areas, as well as the coconut used as a hiding place, over lethargic behaviours, suggests that without adequate enrichment, their behaviours could be restricted **(4, 7)**. In captivity, shelters serve multiple purposes: they enable rats to retreat from light, offer a range of microclimates to help with thermoregulation, provide escape routes from aggressive social interactions, and give rats a degree of control over their surroundings. Additionally, shelters add climbing structures, encouraging the use of vertical space **(7)**. Environmental conditions, particularly high temperatures and humidity can be sources of stress **(7)**. However, analysis of data obtained from camera traps, as presented in Figure n.3.8, shows that the trend of high-temperature values is not correlated with the behaviours of the three subjects. It is important to note that temperatures detected remain relatively stable within the range of 27.5-30 °C during the observation period, and there are not significant thermal fluctuations between daytime and nighttime hours (maximum 5 °C) **(19).** This range is compatible with the thermoneutral zone for rats, which is around 27-30 °C **(14, 3)**. In conclusion, it is not a decisive factor for the conclusions drawn so far.

One of the main indicators of improved wellbeing in rats is an increase in reproduction. During the first weeks of residence, including the entire observation period, the number of pups seen was 3, including the one fallen in the Figure n.3.1. However, in the last week, approximately twenty days after the implementation of the enrichments, a significant increase in births was observed, totalling 24 pups. This renewed sexual interest in these adult subjects is an indicator of improvement in the living conditions. Indeed, reproductive behaviour is often used as a key parameter to assess animal welfare, as it is closely linked to various physiological and psychological factors. When animals are stressed or live in unfavourable conditions, they tend to reduce or even suspend their reproductive activity. Moreover, the production of such a large number of offspring can be interpreted as a sign that the rats perceive their environment as safe.

In conclusion, the application of relatively few and inexpensive enrichment items, even simple handmade structures, led to an overall improvement in the living conditions of the three rats, enhancing their natural tendency to explore their surroundings and use the available environmental elements, while reducing stress and passive behaviours. These findings are consistent with the existing literature, even though the subjects were in a laboratory setting **(1, 4, 12, 15)**. These results highlight the profound impact that simple environmental enhancements can have on animal welfare, even in not fully controlled environments.

Appendix A. Interventions suggested

For completeness, unexplored intervention possibilities and policies to adopt to enhance the wellbeing of rats used as live prey are also reported in an application protocol personally drafted, based on information gathered from literature **(3, 7)** and discussions with Loyal Clarke, manager of Reptilandia, and two volunteers from the "Rat Rescue Centre". These are summarised schematically in Table A.3.2. Optimal cage dimensions suitable for hosting at least two rats simultaneously and recommended construction materials are outlined. In addition to the implemented furnishings, additional items such as hammocks, nests, and toys are suggested. Various materials and designs for bedding and shelters are also explored, with a preference highlighted for materials like canvas and cellulose, featuring particles large enough to facilitate easy manipulation. An entire section is dedicated to cleaning the enclosure with suggestions on the products to use, such as unscented or natural products like bicarbonate solutions, and more aggressive products like diluted bleach to be used occasionally. A weekly cleaning routine is also proposed to ensure a hygienic and healthy environment. Environmental variables such as light, temperature, humidity, and sounds and vibrations are discussed to ensure the rats' maximum wellbeing and some proposal of monitoring technique, such as bats' detector, is described. Remedies against heat are also proposed, such as the use of fresh fabrics or thermal insulating materials like wood, cardboard, ceramics, and pottery, to cope with extreme equatorial temperatures and maintain a comfortable environment. A crucial point of the protocol concerns environmental enrichment, essential for meeting the needs of these active and curious animals. The necessity for complex environments and enrichments that promote natural behaviours such as chewing, burrowing, locomotion, resting, manipulating, carrying, and hoarding food and objects are discussed. The technique of "gentling" or habituation to human contact and "rat tickling" to reduce fearful reactions to humans is also reported **(11)**, improving interaction with caregivers. The protocol also addresses the social environment, analysing how social interaction is influenced by spatial and social density. The optimal number of individuals according to different life stages within a group and the recommended method for integrating unfamiliar adults if necessary are presented, maintaining a harmonious environment and reducing conflicts. The aspect of reproduction is examined to understand how to manage basic aspects such as sex determination and more complex but fundamental aspects like the timing of when males and females reach the anatomical and physiological development suitable for safe reproduction, thus ensuring the health and wellbeing of both breeders and offspring. Finally, the protocol pays particular attention to health monitoring, a crucial aspect for all species but especially important for rats, which tend not to show signs of illness until in extreme and emergency situations. Monitoring aspects such as weight, temperature, a general physical check-up, and behaviour is recommended. Regular monitoring of these parameters is essential for timely intervention in case of health issues, thus ensuring the well-being and longevity of the animals **(7)**.

Table A.3.2: *a synthetic view of the aspects investigated by the protocol.*

Variables	**Characteristics**	**Details**
Cage	➢ Design	○ Size ○ Material ○ Floor ○ Furniture
	➢ Shelter	○ Material
	➢ Bedding	○ Material
	➢ Cleaning of the enclosure	○ Frequency ○ Products
Diet	➢ Food	○ Processed food ○ Unprocessed food
	➢ Water	○ Administration
Environmental variables	➢ Light ➢ Temperature ➢ Humidity ➢ Air quality and ventilation ➢ Sound and vibrations	
Enrichments	➢ Based on normal behaviour	○ Chewing/ Gnawing ○ Burrowing ○ Locomotion ○ Resting/ Hiding ○ Manipulation
	➢ Gentling ➢ Rat tickling	
Management	➢ Composition of the group ➢ Introduction of a new member ➢ Sex determination	
Health check	➢ Vital signs	○ Weight ○ Temperature ○ Breathing
	➢ Life's history ➢ Injuries and wounds	
	➢ Anatomy	○ Head ○ Tail and feet ○ Fur and skin ○ Genital area

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