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Development of an RFID based monitoring system for group-housed mice and rats

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Mestrado em Bioengenharia

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Abstract

Animal experimentation, particularly with mice and rats, is crucial for biomedical research. Since there are many associated ethical challenges, it is important to reduce the suffering caused to the animals, as well as to produce results as reliable as possible, minimising subjective bias and the effect of the experimenters themselves.

Home-Cage Monitoring systems record data 24/7, and need less intervention of the experimenter. They can also help detecting clinical signs earlier, so that measures can be taken to prevent or reduce animal suffering.

Looking at what is available on the market and reported in literature, there are several monitoring systems available, measuring different parameters and operating using different principles. A particular type of monitoring system relevant for this work is the use of implantable Radio Frequency Identification (RFID) tags. Although the tags need to be implanted in the animal, this procedure usually has negligible effects. Since each tag has a unique ID, it is possible to know where each animal is at any given time, provided there is an array of antennas of known location. If thermosensitive tags are used, the body temperature can also be monitored.

With all this in mind, an automatic monitoring system for mice or rats based on RFID technology was developed. This system not only monitors each individual mouse/rat in a cage, but also the environment of each cage. The monitoring of each animal is done by implanting the animals with thermosensitive RFID tags and then placing six RFID reader antennas below the cage, so that by knowing which antenna detects each tag at each time-point, the position of each animal can be estimated, alongside its body temperature. Using this information, it is also possible to estimate if each animal has a preference for certain zone of the cage, and whether it is more or less active. Since an RFID reader can only be connected to one antenna at a time, each antenna was connected to a reed relay. By quickly closing and opening the switch of each relay it was possible to read tags in all the antennas sequentially. The cage environment was monitored by placing temperature, humidity, and light sensors inside the cage, above the cage grid. A box for the hardware components was also designed and 3D printed.

Two commercially available antennas and an antenna built in the lab were compared in terms of reading range, so that the most adequate antenna for this application could be chosen.

All the measured information by the system was then sent by Wi-Fi to the user interface. This user interface shows all the measurements in real-time and has several additional functionalities like setting alarms and exporting the results to Excel.

Finally, the system was tested at the i3S animal facility using two mice during 2 hours. This final test showed that the system was capable of continuously monitoring the mice temperature, as well as the cage temperature, humidity, and light. However, the tags were not always detected by the antennas, making the activity and zone preference measurements less reliable.

Keywords: Mice, Rats, Home-Cage Monitoring, RFID

Resumo

Experimentação animal, especialmente com murganhos e ratos, é essencial para a investigação biomédica. Tendo em conta que há vários desafios éticos associados, é importante evitar ou reduzir o sofrimento causado aos animais, bem como produzir resultados o mais confiáveis possíveis, minimizando a subjetividade e os efeitos causados pela presença do próprio investigador.

Sistemas de monitorização automatizada dos animais nas suas gaiolas permitem gravar dados 24/7 sem intervenção direta do investigador. Também podem ajudar a detetar sinais clínicos mais cedo, podendo-se agir imediatamente para prevenir ou reduzir sofrimento animal evitável.

Olhando para o que existe no mercado e na literatura, é possível perceber que há grande variedade de sistemas de monitorização que medem parâmetros diferentes usando abordagens diferentes. Um tipo de sistema particularmente interessante são as tags implantáveis de Identificação por Radio Frequência (RFID). Embora as tags tenham de ser implantadas no animal, este procedimento geralmente tem efeitos negligenciáveis. Como cada tag tem um ID único, é possível saber onde está cada animal em cada instante de tempo, se hover uma matriz de antenas de posição conhecida. Se tags termossensíveis forem usadas, também é possível medir a temperatura corporal.

Com base nesta informação, foi construído um sistema de monitorização automático para murganhos ou ratos baseado na tecnologia de RFID. Este sistema faz a monitorização de cada murganho/rato numa gaiola, bem como do ambiente da gaiola. A monitorização de cada animal é feita implantando cada animal com tags de RFID termossensíveis e depois colocando seis antenas de leitores RFID debaixo da gaiola, de tal modo que sabendo quando e qual antena deteta cada tag, a posição de cada animal pode ser estimada, assim como a sua temperatura corporal. Também é possível estimar assim se cada animal tem preferência por certa zona da gaiola ou se estão mais ou menos ativos. Como cada leitor de RFID só se pode conectar a uma antena de cada vez, cada antena foi conectada a um relé reed, de tal maneira que fechando e abrindo o interruptor de cada relé, é possível ler todas as antenas sequencialmente. O ambiente da gaiola foi monitorizado colocando sensores de temperatura, humidade e intensidade luminosa dentro da gaiola, acima da grelha. Uma caixa para os componentes de hardware foi desenhada e impressa a 3D.

Duas antenas comerciais e uma antena criada no laboratório foram comparadas em termos de distância de leitura, de modo que a melhor antena para esta aplicação pudesse ser escolhida.

Os dados medidos pelo sistema foram depois enviados por Wi-Fi para a interface do utilizador. Esta interface mostra as várias medições em tempo real e tem outras funcionalidades adicionais, tais como definir alarmes e exportar os resultados para Excel.

Finalmente, o sistema foi testado no biotério do i3S numa gaiola com dois murganhos durante 2 horas. Este teste mostrou que o sistema era capaz de monitorizar a temperatura dos murganhos, bem como a temperatura, humidade e luz da gaiola. No entanto, as tags nem sempre foram detetadas pelas antenas, tornando as medições de atividade e preferência menos fiáveis.

Keywords: Murganhos, Ratos, Monitorização da gaiola, RFID

Agradecimentos

E assim termina mais uma longa etapa...

Gostaria de começar por agradecer aos meus orientadores. Ao professor Joaquim Gabriel por todas as ideias e por aquilo que me ensinou e também ao Nuno Franco pela disponibilidade, descontração e apoio que sempre mostrou.

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"Pro ano não há mais!!!"

Diogo Espinheira Moutinho

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"Success consists of going from failure to failure without loss of enthusiasm."

Winston Churchill

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Abbreviations

BAT	Brown Adipose Tissue
EEG	Electroencephalography
EMG	Electromyography
FDX	Full Duplex
HCM	Home-Cage Monitoring
HDX	Half Duplex
HF	High Frequency
I^2C	Inter-Integrated Circuit
ID	Identifier
ISO	International Organization for Standardization
IVC	Individually Ventilated Cage
LCT	Lower Critical Temperature
LDR	Light Dependent Resistor
LF	Low Frequency
LPS	Lipopolysaccharide
PIT	Passive Integrated Transponder
PWM	Pulse-Width Modulation
RF	Radio Frequency
RFID	Radio Frequency Identification
RSPCA	Royal Society for the Prevention of Cruelty to Animals
SHF	Super High Frequency
SPI	Serial Peripheral Interface
TNZ	Thermoneutral Zone
TTL	Transistor–Transistor Logic
UART	Universal Asynchronous Receiver-Transmitter
UHF	Ultra High Frequency

Chapter 1

Introduction

Animal experimentation (also referred as animal testing or animal research) can be defined as "the use of live nonhuman animals for scientific purposes" [1]. These purposes can include testing drugs and devices, as well as obtaining new biological knowledge and developing therapeutic approaches [2–4].

Although animals have been used as proxies of human anatomy and physiology since antiquity, with the earliest known experiments referring to the 4th, 3rd and 2nd centuries BC, only with the advent of the scientific method and reasoning – in the 18th and 19th centuries – animal experiments started contributing to important scientific and medical discoveries [3, 5–7]

The 19th century was also characterised by a larger importance given to animal welfare and ethics, namely with the emergence of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in England. This new concern with animal well-being was particularly motivated by Charles Darwin's observations, which made the difference between humans and animals less clear in people minds [2, 3, 8].

In 1959, William Russell and Rex Burch proposed the Three Rs principles of "humane experimental technique": Replacement, Reduction, and Refinement. In their own words, “Replacement means the substitution of conscious living higher animals for insentient material. Reduction means reduction in the number of animals used to obtain information of a given amount and precision. Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used”. Today, the Three Rs are the most widely accepted principles guiding ethically acceptable animal research, and form the basis of many laws and codes regulating laboratory animal use, including EU directive 2010/63/EU [9, 10].

Given the ethical challenge put forth by animal research, it is important to obtain as reliable results as possible, by minimising subjective biases, as well as the effect caused by the presence of the experimenters themselves [11–13]. Autonomous monitoring systems for laboratory animals can contribute to objectively obtaining large datasets on behaviour and physiology of animals in their home-cage, contributing to both data quality and the 3Rs. These systems remove observer bias, measure data 24/7, and remove the possible influence of the presence of the experimenter on the animals' behaviour, in addition to other advantages [11, 14, 15]. Automated Home-Cage Monitoring (HCM) can also help detecting clinical signs earlier, so that action can be taken as soon as possible (treatment or euthanasia), reducing or avoiding animal suffering [12].

Another important requirement of scientific research – with animals or otherwise – is that results are reproducible, which is aided by environmental standardisation [16, 17]. Having a monitoring system that can measure environmental conditions – such as ambient temperature or relative humidity – can help in that regard, and even more so if this is done at the individual home-cage level.

1.1 Context

1.1.1 Animal Experimentation in Numbers

The 2010/63/EU directive establishes that statistical data related to the use of animals for scientific purposes must be available, describing the species used, for what purpose, and the severity of the procedures [18].

The most recent report covers the data from 2019 in the 27 Member States of the European Union, United Kingdom and Norway [19]. According to this report, 10 401 673 animals were used for the first time in 2019. The three most popular species were mice (52.5%), fish (24.6%), and rats (9.4%) (Figure 1.1).

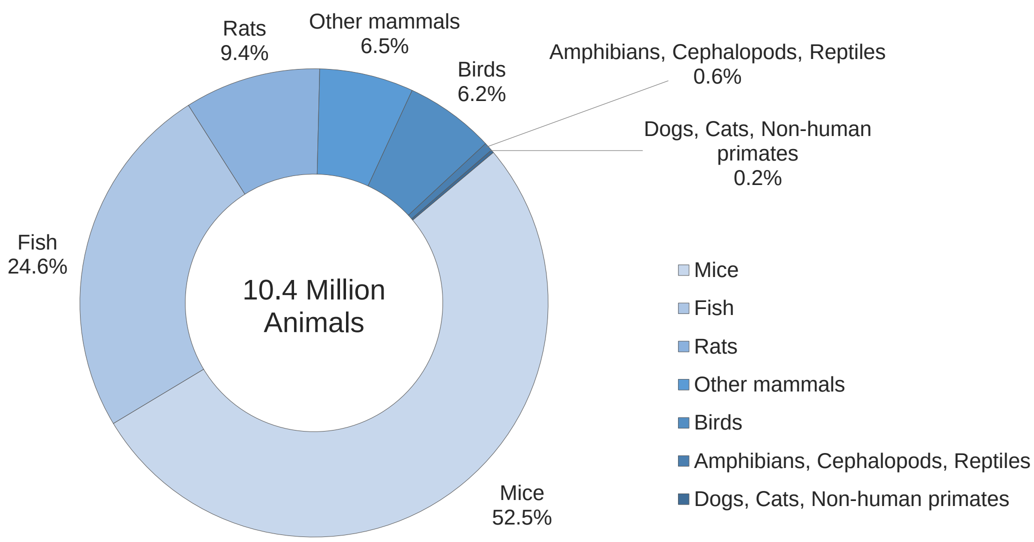


Figure 1.1: Statistics on the number of animals used for the first time, by main class of species, in the European Union, United Kingdom and Norway in 2019 (adapted from [19])

If we look at the numbers in Portugal, a total of 73 334 animals were used in 2019, where 68.9% were mice, 21.3% were fish, 8.6% were rats, and the remaining 1.2% were other type of mammals, birds, or amphibians.

For this project, we are particularly interested in mice and rats, hence covering 61.9% of the animals used. Reasons for the popularity of mice and rats include their anatomical and physiological similarity to humans (given they are mammals), as well as their small size, short gestation times, rapid development and well-known genetics, making it easier to manipulate their genes [20]. Smaller size, lower maintenance cost, and superiority as genetic models explain why mice are usually preferred over rats, in most fields [20, 21].

Another important information is the use given to the animals used. The previously mentioned report divides the uses in five main categories: basic research, translational and applied research, regulatory use, routine production, and others. Each of these categories can be further divided into sub-categories. Figure 1.2 illustrates the six main uses for mice and rats, where it should be noted that rats are particularly used in toxicity and other safety testing as, in order to interpret them, control data is required, which historical has been done in this species [22].

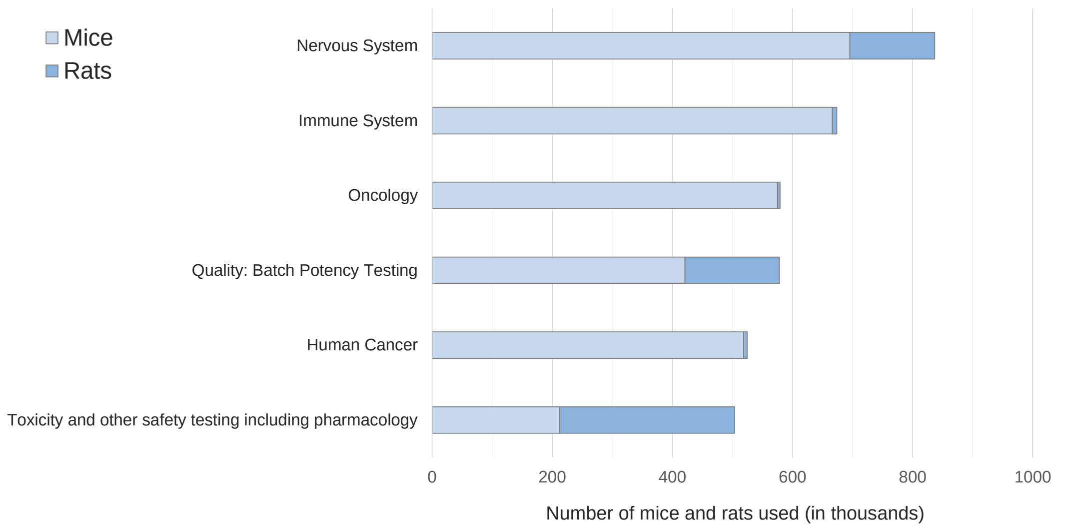


Figure 1.2: Statistics on the number of mice and rats used for the first time, by application, in the European Union, United Kingdom and Norway in 2019 [19]

1.1.2 Automatic monitoring systems for laboratory mice and rats

Since mice and rats are commonly used animal models in experimentation, there is an increasing interest in automated monitoring systems for these species [14], with many different types of monitoring systems available on the market and reported in the literature. These automated monitoring systems can use different measurement approaches, as well as measure different parameters. Thus, and depending on the application, a different type of monitoring system may be of interest.

In spite of such variability, these systems have in common that they measure data continuously without the intervention of the experimenter, presenting the data in a user friendly interface. Additional functionalities, such as setting alarms, exporting data to different file formats or allowing to make annotations can sometimes be present. There is an interest for these systems to be non-invasive and to allow studying group-housed animals, since separating them from their cage mates causes distress, affecting both animal welfare and the measurements [4, 11, 14].

1.2 Motivation

Since rodents are usually housed in groups, it is important to identify and monitor each animal individually [11]. One of the most popular solutions to achieve this is Radio Frequency Identification (RFID). RFID is a short-range communication technology that is frequently used nowadays in many different applications, including security, commerce and, of course, animal monitoring [23]. Although there are active RFID systems (chip with battery that emits its own signals), passive RFID systems are the most relevant in animal monitoring [24].

Passive RFID systems are composed of transponder tags and reading units. Each reading unit emits electromagnetic signals (usually radio waves). If a transponder tag receives that signal, it responds to the reading unit with the unique identification number of the tag [23, 24]. Hence, this technology allows knowing which tag is close to each reading unit, serving as a way to detect where tagged animals are, and when. Some more advanced transponder tags can measure certain physiological signals, like temperature, also sending that information to the reading units [11]. Since the tags only respond to the signals emitted by the reading units, they do not require a battery, which enables them to, in theory, work forever, which is a very important feature for tags implanted in animals, as replacing tags or batteries would cause unwanted disturbance.

One problem of HCM, is that they can be very expensive. Systems able to measure temperature and activity can reach values of 5000 €/cage. More complex systems with additional functionalities can reach up to 20 000 €/cage.

Another disadvantage is related to their size. Many monitoring systems require adding bulky equipment to the cages or even buying an entirely new caging system.

Finally, when only certain parameters like temperature and/or activity are of interest, buying complex monitoring systems with additional features may not be appropriate. Although some systems are already modular, for example the TSE Systems PhenoWorld [25], enabling the experimenter to only buy the modules that interest them, the additional modules can increase significantly the size of the caging system.

With these considerations in mind, it is of great interest to create an affordable, small, modular HCM system, able to measure continuously several important parameters of group-housed animals. Measuring environmental parameters is also important to interpret animal data – for example body temperature and activity – in the context of their microenvironment. Additional features, like setting alarms, adding annotations, and exporting the measurements to other file formats are also of interest.

1.3 Main Objectives

The main objective of this work is the development of an affordable RFID based monitoring system, capable of autonomously and continuously measure different parameters of mice or rats, mainly the temperature and activity. Since mice and rats are usually group-housed, this system should be able to individually monitor each animal. Not being invasive is also a plus, since it reduces the stress caused to the animals.

The system should also be able to monitor the environment of the cage where the animals are housed.

Creating a user-friendly interface that enables the user to check the results, set alarms, export the results to Excel, and mark when a certain treatment is applied is also important.

1.4 Main Contributions

The main contribution of this work is the creation of a monitoring system capable of measuring temperature and activity of group-housed mice or rats in a much more affordable way than other similar products on the market.

This work also contributes with the methodology that enables the experimenter to create its own monitoring system, using commercially available implantable RFID tags.

Finally, this is also the first system that combines the measurement of the temperature and activity of each animal with the monitoring of the cage environment, leading to a more complete characterisation of the experiment.

1.5 Document Structure

Chapter 2 addresses the most important physiological parameters of mice and rats and shows how they can be used to assess their well-being. Environmental factors that affect these animals are also addressed.

Chapter 3 introduces the RFID technology, giving a special attention to the use of this technology in animal identification and monitoring.

Chapter 4 shows several examples of mice and rats monitoring systems available on the market and reported in the literature, demonstrating the characteristics that a good monitoring system should have.

Chapter 5 provides an overview over the architecture of the built system, including detailed information about the hardware components and the tests that were done when choosing the components to use. A comparison of the built system with the products currently available on the market is also present.

Chapter 6 describes the software that was developed, including the code for the microcontroller and for the user interface. The tests that were done to decode the information present in the RFID tags that were used are also presented.

Chapter 7 presents the test done in mice at the i3S animal facility to validate the built system, as well as the interpretation of the obtained results.

Chapter 8 addresses the main conclusions that can be taken from this work and discusses the improvements that can be done to the system in the future.

Chapter 2

Mice and Rats Physiology

Rodents, like mice and rats, are active animals, spending much of their time eating, drinking, grooming and interacting with their cage mates (especially during the dark periods, as they are nocturnal animals) [26]. Although mice and rats have many physiological and behavioural similarities, they can easily be distinguished by their size and morphology (especially in regard to the proportions of the head, ears and tail), with adult rats weighing about ten times as much as adult mice [27]. These represent surface/volume ratio differences that lead to thermoregulatory differences [28, 29].

This chapter describes some important physiological parameters of mice and rats and shows how they can be used to assess their well-being. Environmental factors that affect these animals are also addressed.

2.1 Important physiological parameters and housing conditions

2.1.1 Activity

Rodents are very active and social animals. Some of their major activities include eating, drinking, grooming, breeding, nest building, and interacting with their cage mates [21, 26].

Although mice and rats are active throughout the entire day, they tend to be more active during the dark period, particularly during dawn and dusk [21, 30, 31]. Figure 2.1 shows the distance travelled by mice of the C57BL/6J strain during 24 hour periods, over 7 consecutive days, showing patterns of increased and decreased activity throughout the 24 hours, being in general more active during the dark phases. This activity pattern may differ, however, with rodent species and strain [11].

Locomotive activity can be used to evaluate the well-being of animals, including the evaluation of recovery following injury [32] or the prediction of death in certain diseases, such as infections [33] or cancer [34].

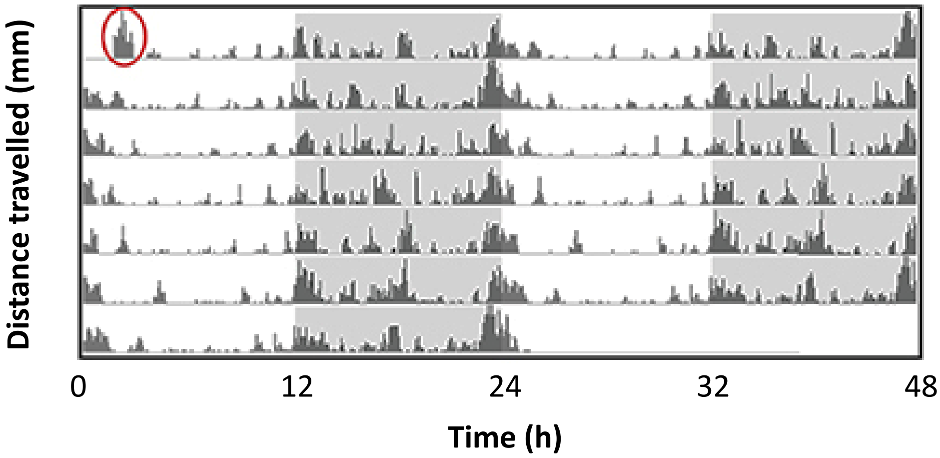


Figure 2.1: Double raster plot of the sum of the distance travelled by three mice of the strain C57BL/6J (scale 20 000 mm) divided in time bins of 6 minutes, over 7 consecutive days. The shaded area represents the dark phase and the red circle highlights the burst of activity that resulted from moving the cage into the experimental rack (adapted from [11])

2.1.2 Social Behaviour

Mice and rats are social animals showing behaviours such as playing, social grooming, huddling, and following [35]. They are also able to communicate with each other using diverse visual, auditory, olfactory, and tactile signals [21]. This means that, in general, these rodents should be housed in groups, to enable social interaction [36]. The characteristics of their social structures depend on several factors, including the strain and species, number of animals and even the housing conditions [35, 36]. Although it may be needed for some studies, single housing of mice or rats is known to cause them high levels of stress [35].

Another interesting factor is that the social interactions also depend on the circadian cycle, since during the light phase mice and rats are huddled together and during the dark phase they become more active and increase the distance between each other [11].

Changes in the social behaviour, like isolation from cage mates, can be a sign of certain diseases like cancers [34] or infections [37]. Changes in social behaviour are particularly important when studying neuro-behavioural disorders [35].

2.1.3 Food and Water Intake

Similarly to other mammals, regular food and water intake are important for mice and rats to compensate their daily energy expenditure and water losses [38].

The intake of food and water in healthy animals depends on several physical and physiological characteristics, as well as the housing conditions [21, 39]. It also depends on the circadian cycle of the animals, being higher in the dark phase for mice and rats since they are more active in that phase. [12, 38].

Food and water intake are also important factors to evaluate the well-being of mice and rats [38]. Drugs [38], stress [40], infections [33], and cancer development [34] are all known to affect food and water intake of both mice and rats. Moreover, food intake is of particularly importance in studies on metabolism [41]. An example of the food intake throughout the circadian cycle and after Lipopolysaccharide (LPS) injection (component of the outer membrane of many bacteria that induces sickness behaviour in mice [12]) can be seen in Figure 2.2. As it is possible to see, the food intake increases during the dark phase, when the mice are healthy, and drops after LPS injection.

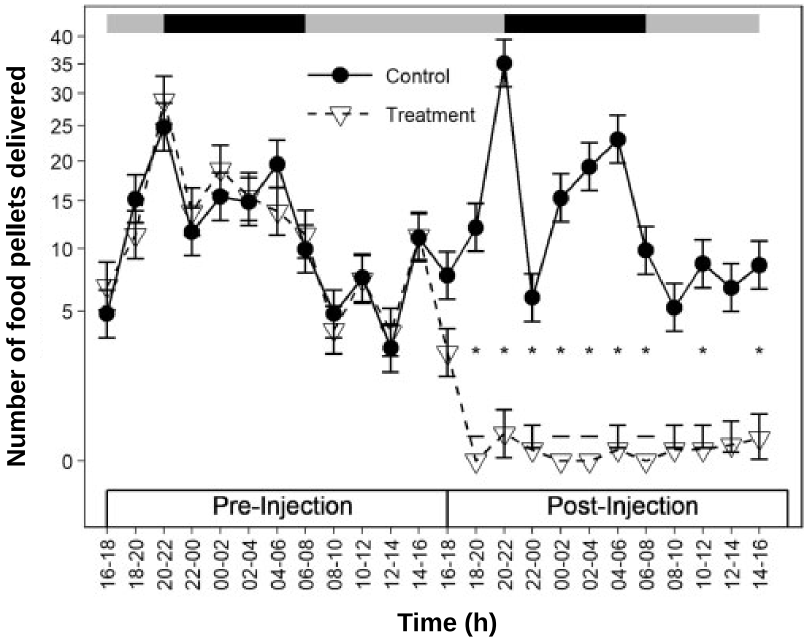


Figure 2.2: Comparison between the food intake in healthy mice (Control) and mice after LPS injection (Treatment). The grey and black bars represent the light and dark phase, respectively. The asterisk indicate that the treatment mice fed themselves significantly less than the control mice in that 2-hour bin (adapted from [12])

The type of diet is another important factor, since it also affects the response of the animal to certain treatments, which can affect the consistency of the results [21, 42].

2.1.4 Temperature

Mice and rats are endotherm mammals, which means that they use their metabolic heat production to maintain their body temperature [28]. In order to better understand their behaviour, a Thermoneutral Zone (TNZ) can be defined. The TNZ corresponds to the range of ambient temperatures for which the heat produced at a resting metabolic rate is at equilibrium with the heat that is lost by evaporation to the surrounding environment [28]. The temperature at which the TNZ starts is known as the Lower Critical Temperature (LCT). Since mice and rats have a large surface area-to-volume ratio and low body insulation, especially on their extremities - legs, ears and tail

- they have a narrow TNZ. This results on a oscillation of body temperature with amplitudes of approximately 1 ºC, even within the TNZ, although the body temperature is stable when averaged over a long period of time [28, 43]. The relation between the metabolic rate and the ambient temperature in both mice and rats is illustrated in Figure 2.3.

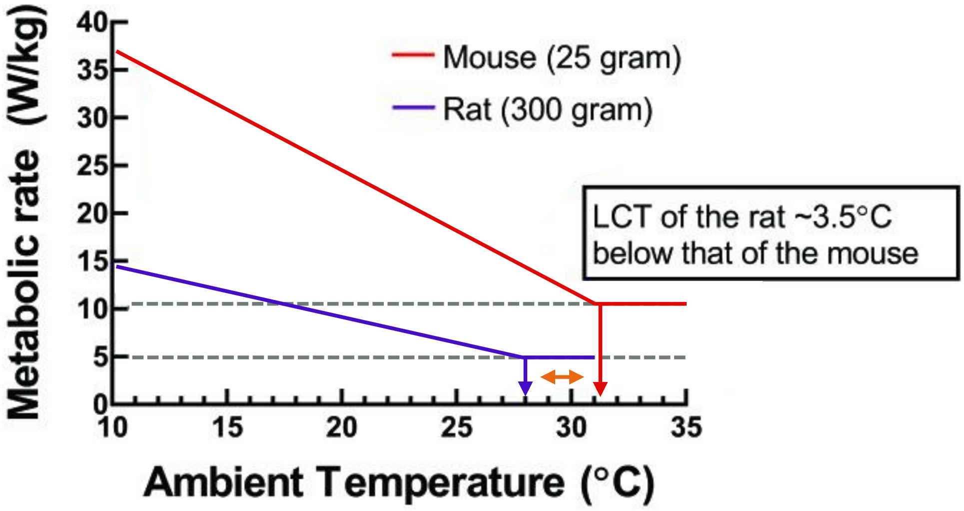


Figure 2.3: Comparison of the relation between the ambient temperature and metabolic rate in the typical laboratory mouse and rat. It is possible to see that mice have a LCT around 31.5 ºC, while rats have a LCT around 28 ºC (adapted from [28])

Mice and rats maintain their body temperature at around 37 ºC through physiological and behavioural adaptations, although this temperature varies with several factors like the time of the day or the nesting material of the cage [21, 28, 44].

The preferred way of mice and rats to regulate their temperature is behavioural since it is less energy-consuming. Some of these behaviours include retreating into the burrow, nest building, thermotaxis (moving to a warmer environment), posture change to reduce the exposed body surface, and huddling with cage mates [21, 28, 43].

When it comes to the physiologic adaptations to temperature changes, mice and rats can reduce heat loss by decreasing the peripheral blood flow to the tail and paws (vasoconstriction), since these extremities are the main places for heat loss [28, 45]. They can also do thermogenesis (production of heat), which is divided in two types: shivering and non-shivering. Shivering thermogenesis corresponds to the repeated contraction of skeletal muscle, but is relatively ineffective in mice and rats because of their small size. On the other hand, non-shivering thermogenesis usually relates to the Brown Adipose Tissue (BAT), since BAT fat cells have a high number of mitochondria with a high capacity for oxidative metabolism, resulting in the generation of heat. If necessary, white adipocytes can manifest a phenotype similar to BAT, being referred as beige adipose tissue [28, 44, 45].

Body temperature is a relevant parameter for assessing an animal's physiological state and well-being. Hyperthermia – either localised (e.g. due to swelling) or generalised (e.g. fever) –

is an indicator of an immune response to a foreign body or pathological agent [46]. Variations in temperature, particularly hypothermia, can also indicate disease progression or even predict death in several diseases like acute illnesses [47], cancers [48], and infections [49–52]. One factor that can also affect temperature is acute stress. Similarly to other mammals, subjecting mice and rats to stress leads to stress-induced hyperthermia, where the core body temperature of the animal increases and the temperature at extremities decreases, due to the redirection of blood-flow from the extremities to the vital organs [53].

It is important to take in consideration the method used for temperature measurement. The two main types of approaches include body surface temperature assessments, where non-contact infrared thermometers are used, and the core body temperature measurement, either by rectal probes or implantable RFID tags [47,53]. Although both approaches can be used to assess the well-being of the animals, surface temperature depends on the place of the measurement and is more affected by ambient temperature, while core temperature can in theory be more reliably measured, as long as the thermoregulatory mechanisms are working [47]. If the used method requires restraining animals, then the measurements are compromised by stress-induced hyperthermia [53].

Finally, it is important to mention that although the ambient temperature of the rodents colony rooms (macroenvironment) is usually monitored, it is also relevant to monitor the ambient temperature in each cage (microenvironment), since it can differ from room temperature [17,54].

2.1.5 Relative Humidity

In general, a relative humidity between 30% and 70% is acceptable for mice and rats [55,56]. The relative humidity can affect several factors, like the activity, thermoregulation, water loss, food intake, propagation of diseases, and even the immune system of the animals [16,36,57,58].

Similarly to the ambient temperature, the relative humidity of the rodents colony rooms is usually monitored, but it is also important to monitor the relative humidity in each cage since it is usually higher than the room humidity [17,36,54]. A comparison between the relative humidity in the cage and the room can be seen in Figure 2.4. As it is possible to see, the relative humidity in the cages tends to be higher than in the room, as expected due to water output from the animals [36].

2.1.6 Light

Light intensity and its wavelength are also factors that can affect the physiology and behaviour of mice and rats [56]. In general, it is recommended that the light intensity at the cage is between 130 and 325 lux, since higher values can cause retinopathy in mice and rats, especially for albinos [36,55,56]. The light intensity also depends on the properties of the cage, such as transparency, and on the position of the cage on the rack, since the light intensity decreases with the distance from the light source and the top cages and shelves prevent some light from reaching the lower cages [36,56].

When it comes to the wavelength of the light in the visible spectrum (i.e. its color), it can also have some physiologic effects, including on the duration of the oestrous cycle or on the

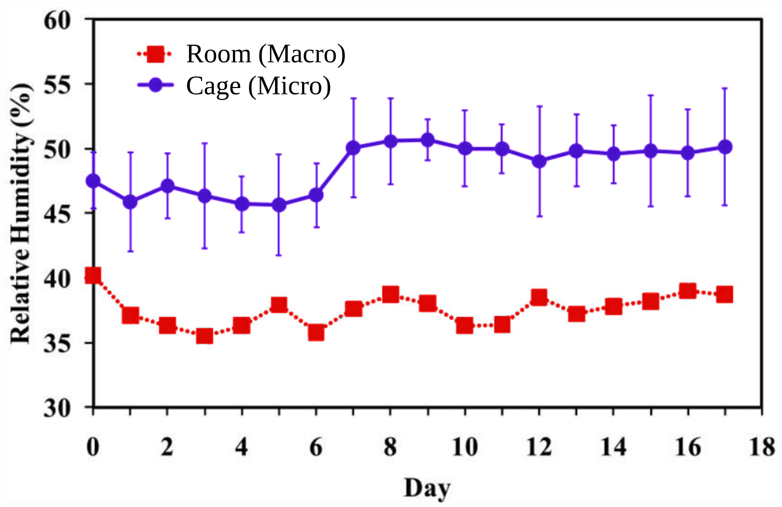


Figure 2.4: Comparison between the average daily relative humidity in all cages (microenvironment) and the room (macroenvironment) during a study. The error bars represent 1 standard deviation of all the cages averaged (adapted from [17])

organ weight, with most facilities using warm-white or cool-white fluorescent lamps, which are considered adequate for the animals [59].

Finally, the photoperiod (period of the day when light is available) is also important, since it affects several factors, like the metabolism and reproductive behaviour of the animals [56, 60]. A cyclic 12 hours of light and 12 hours of darkness are typically used in animal facilities [59], usually inverted in regard to the external daylight cycle (e.g. lights out in the animal room from 8.00 to 20.00, with lights on when scientists and caretakers are away). Short periods of light during the dark phase can affect the physiology of the animals, so turning on the light during this phase should be avoided [36].

2.1.7 Noise

Another important factor is noise, since it can cause stress to the animals [55]. Both mice and rats hearing range can reach frequencies close to 100 kHz, so ultrasounds are also of concern [36, 56, 59]. On the other side of the spectrum, rats and specially mice have difficulty hearing low frequency sounds, below the frequencies of about 1 kHz [36, 59, 61].

Repetitive and/or loud noises are known to cause several effects in mice and rats, including reduced fertility, changes in the immune system components, impaired wound healing, altered activity, reduced food intake, interference with foetal development, and audiogenic seizures [36, 56, 59].

With that in mind, any activity or equipment that generates noise – even if inaudible for humans – should be kept in areas separated from the colony rooms [56].

2.2 Summary

Laboratory rodents are very active animals, particularly during the dark period. Since they are also social animals, it is important to house them in groups.

Variations in parameters such as activity, social behaviour, food and water intake, and temperature can be used to assess the health conditions of these animals - which is important when studying certain diseases like cancers or infections - but also their welfare.

There are several environmental factors - including ambient temperature, relative humidity, light and noise - that should be monitored and controlled, since they can affect the physiology of mice and rats, which in turn will affect the results of any study being carried out.

The aforementioned suggests that an automatic monitoring system able to measure these physiological and environmental parameters would be valuable for animal studies, by helping researchers and caretakers to objectively evaluate disease progression or treatment effects, while also helping make the results obtained more reproducible and standardised.

Chapter 3

Radio Frequency Identification

Radio Frequency Identification (RFID) is a short-range communication technology that is frequently used nowadays in many different areas, including security, manufacturing, commerce, and, of course, animal tracking or monitoring [23, 62].

This chapter addresses many fundamental aspects regarding RFID, including the main components of RFID communication, their principle of operation, some factors that should be taken in consideration when dealing with this technology, and also the standards that should be followed when using RFID for the identification and monitoring of animals.

3.1 Components of RFID systems

RFID systems are composed of three main components [24, 63], as can be seen in Figure 3.1:

- **RFID Tag/Transponders** — item that contains information, like a unique ID, and is usually attached to certain object or animal [24, 63]. Certain tags can also contain sensors [63];

- **RFID Reader/Interrogator** — electronic component that sends and receives modulated Radio Frequency (RF) waves to communicate with the RFID tags [24, 62];

- **Back-end System** — system like a computer, that matches the information received by the reader with a database [24, 63]. In some cases, it can also send commands to the RFID reader [64].

Figure 3.1: Main components of the RFID Technology (adapted from [24])

3.1.1 RFID Tags/Transponders

RFID tags are essentially constituted of an integrated circuit and an antenna [62, 65].

- **Integrated Circuit** — responsible for implementing the transmission algorithms and control any additional sensor that the tag may have [62];
- **Antenna** — receives and transmits the RF waves that enable the communication with the reader [62]. The design of this antenna should be carefully considered in order to have efficient communication with the reader [64].

Depending on the application, RFID tags can have several different morphologies. Some examples can be seen in Figure 3.2.

Figure 3.2: Different types of RFID tags (reproduced from [66])

In terms of the principle of operation, RFID tags can be divided in two main categories: passive tags and active tags. Some authors also consider that there are semi-passive and/or semi-active tags, which have simultaneously active and passive components [63].

3.1.1.1 Passive Tags

Passive tags do not have any built-in power source, being powered by the RF waves sent by the RFID reader [24]. So, when the tag is in the range of the RF wave field generated by the RFID reader, a small current is induced in the antenna of the tag, powering its internal components and enabling it to communicate with the reader [62].

These tags have several advantages, such as being inexpensive to produce, small, and having an unlimited lifetime in terms of power, since they do not have a power source [23, 62–64]. The main disadvantage is having a short reading range [62, 64].

3.1.1.2 Active Tags

Active tags have a built-in power source, like a battery, which allows them to send the signal to the reader [63, 64].

The built-in power source brings some advantages, like having a longer reading range and being less affected by interference, but on the other hand, results in disadvantages like being larger than passive tags and also more expensive to build and maintain [62–64].

3.1.2 RFID Readers/Interrogators

RFID readers work as the interface between the tags and the back-end system [65]. So, the RFID reader needs to receive and decode the information sent by the RFID tag, and then send that information to the back-end system [62, 64]. If passive tags are being used, the reader is also responsible for sending the RF waves that will activate the tags [62]. Some readers are also able to send commands to the tags, changing their behaviour [64].

The RFID readers can be divided in three main components: RF electronics module, control module, and antenna [65].

- **RF electronics module** — responsible for the communication with the tags, including sending and receiving the RF signals [64];

- **Control module** — controls the operation of the reader according to the commands of the back-end system [65]. It also decodes and converts the information sent by the tag to the appropriate communication protocol, before sending them to the back-end system [64];

- **Antenna** — receives the RF waves that enable the communication with the tags. They are particularly important when dealing with passive tags, since they need to generate a RF wave with the appropriate frequency and then detect the small changes made by a detected tag in that RF wave [62]. Just like with the tag antennas, the design of the reader antenna should be carefully considered [64].

3.2 Principle of Operation

The characteristics of RFID depend on the frequency of the RF waves used. The main frequency ranges considered and some of their properties are summarised in Table 3.1. In general, with the increase of the frequency, there is also the increase of the reading range and the reading/writing data rate [65]. On the other hand, the performance worsens near metals and liquids [65,67]. Metal is specially problematic in RFID, since it not only reflects and blocks RF waves, but also can change the characteristics of nearby antennas [65]. For this work, LF waves are of special interest, as they can travel through water with minimal attenuation, making them adequate for tagging animals, which have high water content [64].

There are two main regions with different properties where the reader and the tag can interact: near field and far field [67], as can be seen in Figure 3.3.

- **Near Field Region** — interaction happens by inductive coupling between the reader and the tag [68];
- **Far Field Region** — interaction is based on the reflection of the electromagnetic waves [69].

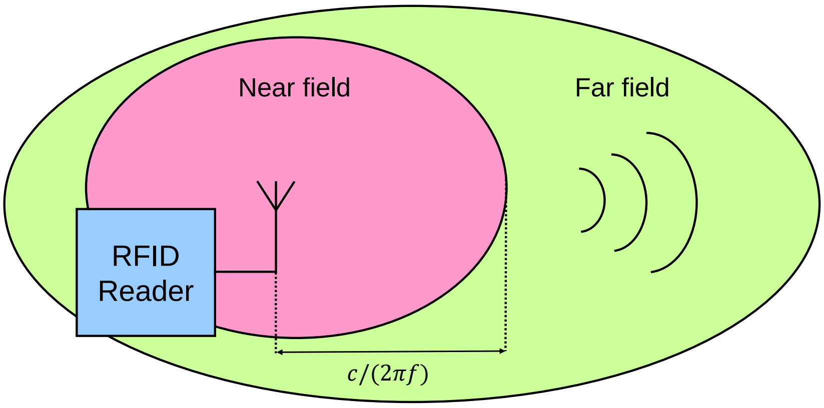


Figure 3.3: Representation of the near field and far field regions (adapted from [68]).

In general, the length of the near field region can be given by $c/(2\pi f)$, where c is the speed of light and f is the frequency of the RF wave [69]. Since the length of the near field is inversely proportional with the frequency of the RF wave, the communication between the reader and the tag happens in the near field for lower frequency signals, like LF and HF, and in the far field for UHF and SHF [67,69]. Since LF is of particular interest in this work, the communication in near field will be further described in the following section.

3.2.1 Near Field Region

According to the Ampere's law, current flowing in a conductor produces a magnetic field around that conductor [71]. Then, according to Faraday's law, a change in the magnetic flux in a surface bounded by a closed path induces a voltage in that loop [71,72].

Table 3.1: Different RFID frequency ranges and respective properties [65, 67, 70]

Name	Frequency Range	Communication Method	Limitations	Typical Applications
Low Frequency (LF)	125-134 kHz	Near Field	Metal	Animal Tracking and Monitoring
High Frequency (HF)	13.56 MHz	Near Field	Metal	Smart cards, item level tracking (libraries, supply chains, pharmacies, baggage)
Ultra High Frequency (UHF)	860-960 MHz	Far Field	Metal, liquid	Pallet and case tracking (supply chain), electronic toll collection
Super High Frequency (SHF)	2.4 GHz and 5.8 GHz	Far Field	Metal, liquid	Electronic toll collection

When working with passive tags, the reader passes an alternating current through its antenna coil, generating an alternating magnetic field (Ampere's law) [69]. If a tag is placed near that magnetic field, a voltage is induced in the tag antenna coil (Faraday's law) [69, 71]. This voltage will simultaneously power the tag circuit, but also generate a current in the tag which will generate a magnetic field that will oppose the magnetic field produced by the reader [64, 69, 71].

By modulating the load of its antenna, the tag can change the current that is being produced and, consequently, the magnetic field that is being generated [69]. Thus, by changing the load of its antenna over time, the tag can cause specific variations in the magnetic field produced by the reader, which will encode certain information in that field, for example, the ID of the tag [62, 69]. Depending on the application, several different communication protocols can be used [69].

This principle is demonstrated in Figure 3.4.

3.3 Factors that affect the reading range

The reading range can vary between just a few millimetres to more than 15 metres [73]. Some properties that affect the reading range include:

- **Operating frequency** — higher frequency waves lead to a wider reading range since they are more energetic and result in a larger antenna gain since the wavelength is closer to the antenna dimension [62, 65];
- **Type of tag** — active tags tend to have a wider reading range, since they are not limited by the energy that is provided by the reader [73];
- **Size of the antennas** — larger antennas usually results in longer reading ranges [64];

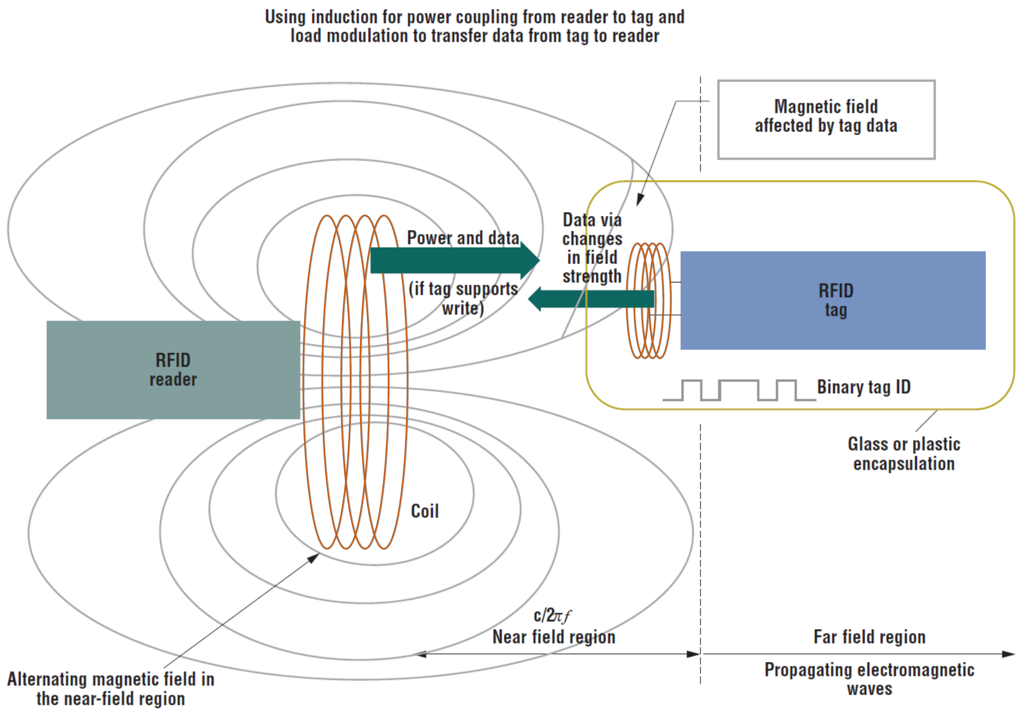


Figure 3.4: Communication between the reader and the tag in the near field (adapted from [69])

- **Quality factor (Q factor) of the antennas** — the Q factor indicates how selective the antenna is to frequencies above and below the operating frequency (resonant frequency). The Q factor should not be too low, since it can result in more interference, nor should be too high, in order to not reject part of the bandwidth that is being used in the RFID signal [64];

- **Interference of other materials** — like previously mentioned, if materials such as metal are nearby an antenna, they can decrease the efficiency of the antenna at the operating frequency, decreasing the reading range [64];

- **Orientation of the antennas** — the reading range depends on the orientation between the antennas of the reader and the tag. The maximum reading range is obtained when these are parallel and the minimum reading range is obtained when they are perpendicular [71].

It is also important to mention that depending on the application, a smaller or wider reading range may be of interest.

3.4 Standardisation in RFID

In order to enable RFID devices from different vendors and countries to communicate with each other, facilitating the evolution of the technology, there was the need to create several standards [62, 65]. These standards can regulate several factors, like communication protocols, operating frequencies, hardware specifications, data formats or commands between the reader and the back

Table 3.2: Structure of the ID code of tags used for the identification of animals, according to ISO 11784 [74, 76, 77]

Name	**N.º of Bits**	**Description**
Country Code or Manufacturer Code	10	Represents the country where the tag was issued or the manufacturer of the tag
National Identification Code	38	Distinguishes between tags with the same country or manufacturer code
Animal Application Indicator	1	This bit is 1 if the tag is being used in an animal; 0, otherwise
Additional Data Block Indicator	1	This bit is 1 if the tag contains additional information besides the ID; 0, otherwise
Reserved bits	14	Bits without meaning that are reserved for future applications

end system [62,67,70]. One of the main institutions responsible for the industry standards in RFID is the International Organization for Standardization (ISO) [62,67] which, for animal identification and monitoring, defines two main ISO standards:

- **ISO 11784 (Radio frequency identification of animals — Code structure)** — responsible for the ID code structure in animals [74];
- **ISO 11785 (Radio frequency identification of animals — Technical concept)** — describes how the tag is activated and how it communicates with the reader [75].

3.4.1 ISO 11784 (Radio frequency identification of animals — Code structure)

According to ISO 11784, the ID of the animal should be represented in 64 bits and contain the information present in Table 3.2.

3.4.2 ISO 11785 (Radio frequency identification of animals — Technical concept)

According to ISO 11785, there are two communication protocols that are used for the identification of animals: Half Duplex (HDX) and Full Duplex (FDX) [75]. The main difference between these two protocols resides in the fact that while in HDX the tag waits for the antenna of the reader to stop transmitting the magnetic field, before sending its information to the reader, in FDX the tag transmits its information while the antenna of the reader is transmitting the magnetic field [78]. FDX can be further divided in FDX-A and FDX-B, depending on several characteristics, like operating frequency or number of bits used in the communication [79].

Since the FDX-B protocol is more widely used than HDX [80] and will be used in this work, it will be further described in the following section.

3.4.2.1 FDX-B Protocol

As usual in animal identification, this protocol defines that the frequency of the RFID is 134.2 kHz [75, 76]. This communication protocol can be summarised as follows (Figure 3.5):

1. The reader transmits the activation signal (magnetic field) with 134.2 kHz [81];

2. The tag receives the activation signal, powering its circuits and generating a clock with a frequency of 4194 Hz (1/32 of 134.2 kHz), where for each cycle of that clock a bit is encoded in the 134.2 kHz wave, by changing the load of the tag antenna [81]. If a 1 is to be encoded, the load is constant through a cycle of the clock. If a 0 is to be encoded, the load changes during the cycle [81, 82];

3. The changes in the load of the antenna generate changes in the amplitude of the 134.2 kHz activation signal [75, 81]. This results in changes in the sidebands (frequencies 129 to 133.2 kHz and 135.2 to 139.4 kHz) of the activation signal [83];

4. The reader decodes the information that was encoded in the sidebands, receiving the code that the tag wanted to transmit [81, 83]. Since the information was encoded in the sidebands, the reader does not have to stop transmitting its magnetic field, for the tags to send its information [81].

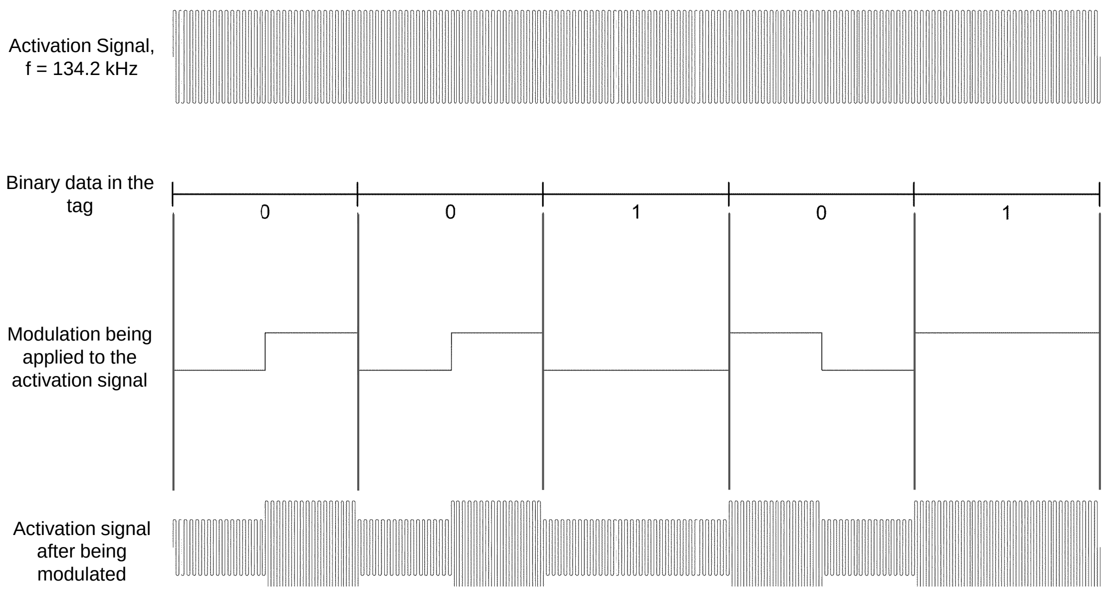


Figure 3.5: Summary of the FDX-B protocol (adapted from [81])

When it comes to the structure of the tag information, the tags that follow the FDX-B protocol contain 128 bits [76]. The information that is present in those bits is summarized in Table 3.3.

Table 3.3: Tag data structure according to the FDX-B protocol [76]

Name	**N.º of Bits**	**Description**
Header bits	11	Indicate the start of the data block
Identification bits	64	Bits characterized in Table 3.2
Checksum	16	Used to verify if there was any mistake in the transmission of the data
Extended data	24	Additional information, for example, the temperature measured by the tag
Control bits	13	Differentiate the data from the header bits

3.5 Summary

RFID is a technology used for short-range communication that found use in many different areas, including animal identification and monitoring.

An RFID system is usually composed of a tag, a reader and a back-end system. Tags can be divided in passive tags and active tags. Passive tags are powered by the RF signals sent by the reader, while active tags are powered by a battery. Since passive tags do not have a battery, they can, in theory, work forever, which makes them useful for animal applications.

Depending on the application, several different frequency ranges can be used. For the case of animal monitoring, LF waves are used since those frequencies are less affected by water and a long reading range is usually not required.

In order to build a system with the adequate reading range, it is important to take into consideration several factors which include the operating frequency, type of tag, size and quality factor of the antennas, interference of other materials, and orientation between the tag and reader antennas.

There are also standards that define the data structure and the communication protocols for animal applications. In general, animal applications use RF waves with 134.2 kHz and follow the FDX-B or HDX communication protocols.

Chapter 4

State of the art

This chapter describes the different types of rodent monitoring systems available on the market and reported in the literature. The advantages and disadvantages of each type of monitoring system are also described. Furthermore, other aspects - such as user interface - are addressed.

4.1 Different types of monitoring solutions

4.1.1 Devices added to the cages

To study the physiology of animals, it can be useful to add different types of devices to the cage that can measure different parameters when the animal interacts with them.

The In-Cage Running Wheels commercialised by STARR Life Sciences is a small and durable running wheel that measures the number of rotations, distance travelled, and time that the animal spends on the wheel (Figure 4.1). With this system, the experimenter can evaluate the activity of the animal throughout the circadian cycle and after a certain treatment [84].

Figure 4.1: In-cage Running Wheels attached to a cage (reproduced from [84])

The Grip Strength Meter by Panlab contains a bar or grid where mice or rats can grab, so that the muscular strength is measured (Figure 4.2). This type of device can be important when studying the neuromuscular function of the animal, but has the disadvantages of being invasive, depending on the experimenter handling, and also having highly variable results [85].

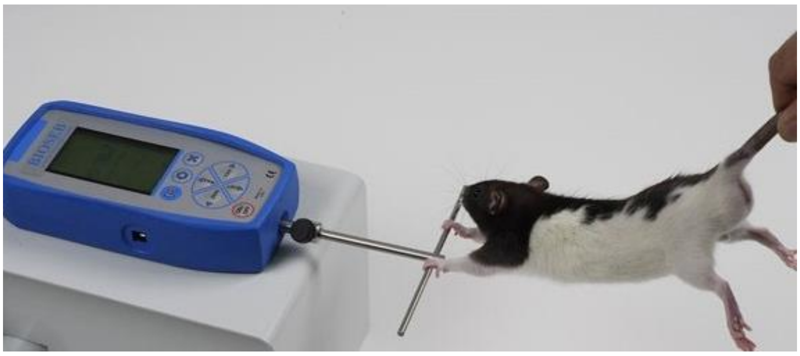


Figure 4.2: Test being done to a rat using the Grip Strength Meter (reproduced from [85])

There are also some creative ways in literature that try to do measurements with sensors added to the cage without the animals noticing. One example is the work of González-Sánchez et al. [4] where the authors wanted to evaluate the stress of mice during cage transportation. To do that, electrodes were placed in a platform, so that the heart rate and breathing rate could be obtained if the animals placed their paws on the electrodes. This system, however, had the problem of the animals rarely placing their paws correctly in the electrodes and the signals having low amplitudes. Additionally, the authors also measured some environmental parameters of the cage using a camera and acceleration, temperature, humidity, light and pressure sensors. The configuration of the cage can be seen in Figure 4.3.

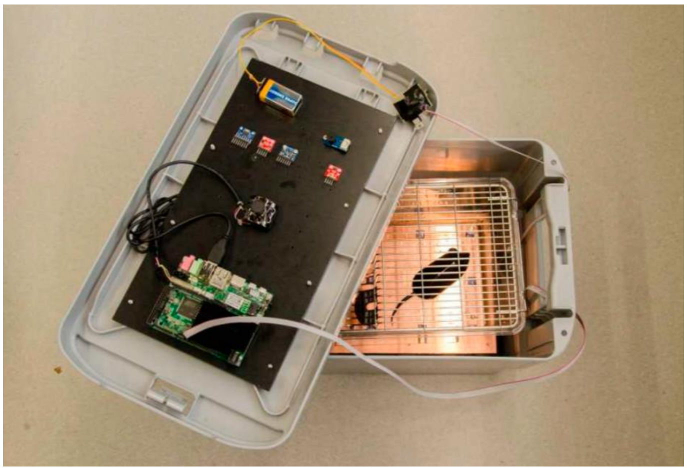


Figure 4.3: System built by González-Sánchez et al. On the backside of the cage cover it is possible to see several environmental sensors and a CPU board. On the bottom of the cage, it is possible to see the matrix of electrodes that measure the heart rate and breathing rate (reproduced from [4])

In summary, these devices can be useful, since they measure different parameters depending on the study specificities, yet they require animals to interact with the devices or the handling by the experimenter. Also, most of the times, these devices are not able to distinguish the measurements of the different animals in the same cage.

4.1.2 Video Cameras / Motion Detectors

Another interesting type of devices are video cameras or other types of motion detectors. These devices are not in direct contact with the cage, using different signal and image processing algorithms to provide information on the activity and interactions of the animals.

In regard to motion detectors, one example is the Home Cage Infrared Motion Detector commercialised by STARR Life Sciences, which uses infrared light to detect the activity of the animals, namely informing on whether they are moving or resting (Figure 4.4) [86].

Figure 4.4: Home Cage Infrared Motion Detector attached to the top of a cage (reproduced from [86])

For a more detailed activity analysis, there is the SMART Video Tracking System from Panlab, which uses a camera and advanced image analysis to record not only the activity, but also trajectories, events, and social interactions. Another advantage is that this system is modular, so that the user can add different modules with different features, depending on the objective of the study. Some of these modules include tracking an animal in a personalised enclosure, recognise certain behaviours like rotating and stretching, and even study multiple animals on the same cage at the same time, including their interactions [87]. One possible interface of the SMART Video Tracking System can be seen in Figure 4.5.

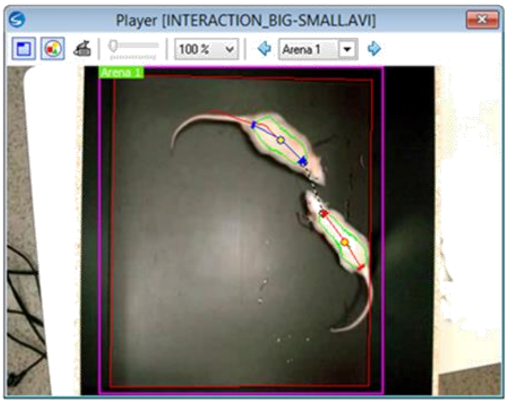
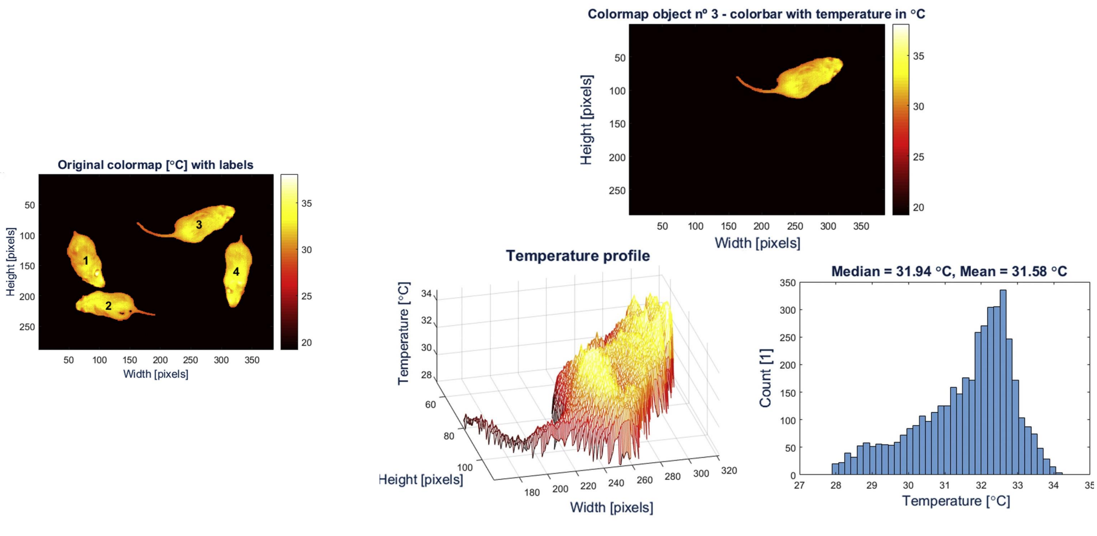


Figure 4.5: Possible interface of the SMART Video Tracking System. The module to detect the head, centre of mass and base of the tail, and the module to track multiple animals are being used. The simultaneous use of both these modules enables the software to better recognize the type of interaction that is happening between the animals (reproduced from [87])

There are also systems that try to use cameras to measure other physiological parameters, like temperature. One example is the *ThermoLabAnimal* software (Figure 4.6), which is able to automatically analyse thermal images, separating the background from the body of freely-moving mice, and then presenting the mean body surface temperature as well as the temperature profile of each mouse. One of the main advantages of this system is enabling the experimenter to obtain the surface temperature of mice, without any stress induced by the handling of the animals. This system, however, has the limitation of not being able to identify the same animal in different images [46]. An improved version of this software was latter presented in the work of Blenkuš et al. [53].



(a) (b)

Figure 4.6: Interface of the *ThermoLabAnimal* when analysing (a) the temperature of multiple mice and (b) the temperature profile and mean temperature in an individual mouse (reproduced from [46])

In short, video cameras and motion detectors can be used to measure certain parameters in a totally non-invasive way, mainly activity and interactions. Some more advanced software can even distinguish between different animals. The main problem of these systems is that they cannot measure many physiological parameters, like heart rate.

4.1.3 Implantable Sensors

4.1.3.1 Passive Integrated Transponder (PIT) tags

Another typical approach to monitor laboratory mice and rats is to implant them with certain types of sensors. A popular approach is to use the RFID technology addressed in Section 3.

By implanting a mouse or rat with a small passive RFID tag, typically known as Passive Integrated Transponder (PIT) tag, it is possible to know which tag is close to each RFID reader antenna, serving as a way to detect where the animals are in the cage. These tags usually have a standardised size of 12 mm x 2.1 mm [88], but the size may vary slightly depending on the tag model. The appearance of a typical PIT tag can be seen in Figure 4.7. Since passive tags are powered by the signals emitted by the reading units, they do not need a battery, which enables them to, in theory, work forever, which is very important since the tag is being implanted in the animals [23].

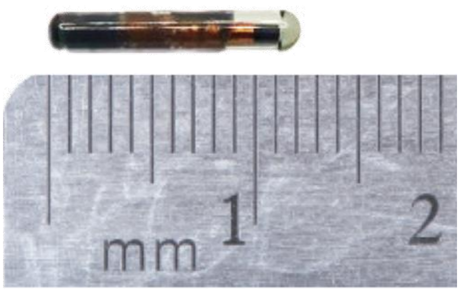


Figure 4.7: Typical appearance of an animal implantable RFID tag (reproduced from [89])

One application of this technology is the UID Mouse Matrix by the company Unified Information Devices (Figure 4.8). This device is composed of a matrix of antennas that can be placed below the cage. So, by implanting each mouse with an RFID tag, it is possible to know where each mouse is at all times, depending on which antenna detects which tag. With this information, the experimenter can assess activity by tracking movement (although it should be born in mind that not all activity requires moving in the cage). If the mice are implanted with thermosensitive RFID tags, the experimenter can also monitor body temperature [90].

(a) (b)

Figure 4.8: (a) Antenna matrix of the UID Mouse Matrix and (b) antenna matrix placed below the cage so that the measurements can be done (reproduced from [90])

A similar product that also uses a matrix of RFID antennas is the Home Cage Analyser by Actual Analytics (Figure 4.9). In addition to measuring the activity, temperature, and preference of each mouse or rat, this system also has an infrared camera that can help detect certain behaviours from the animals, like eating, drinking or climbing [91].

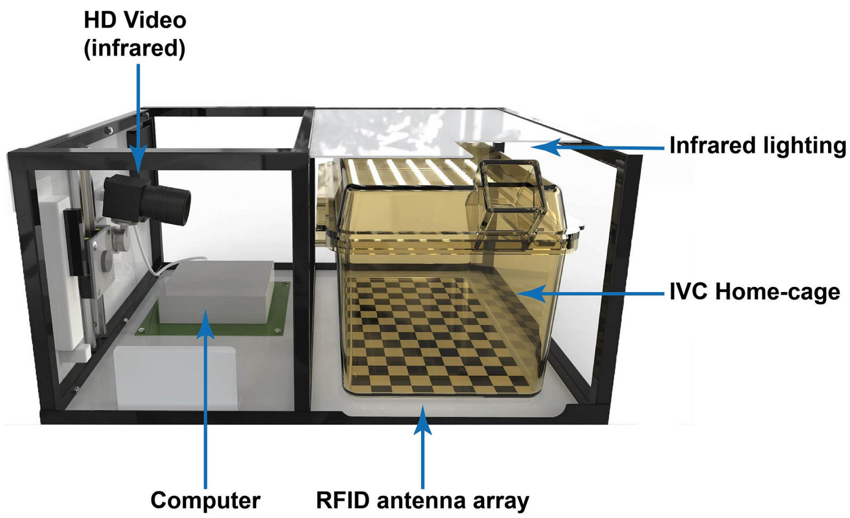


Figure 4.9: Home Cage Analyser system. On the right side there is an Individually Ventilated Cage (IVC) above an array of RFID antennas. The system also has an infrared video camera on the left side to analyse certain behaviours of the animals, like eating, drinking, climbing, etc. (reproduced from [11])

Another possible application of RFID tags is the system developed by Ahloy-Dallaire et al., which monitored the body weight and food consumption of each mouse, by attaching a tunnel with a food dispenser to the cage (Figure 4.10). This tunnel was above a body weight sensor and next to a camera. When the mouse entered the tube, the RFID reader would detect the tag of the animal and dispense some food, while also annotating how much food was dispensed, the body weight of the animal, and taking a photo (during the light periods) [12].

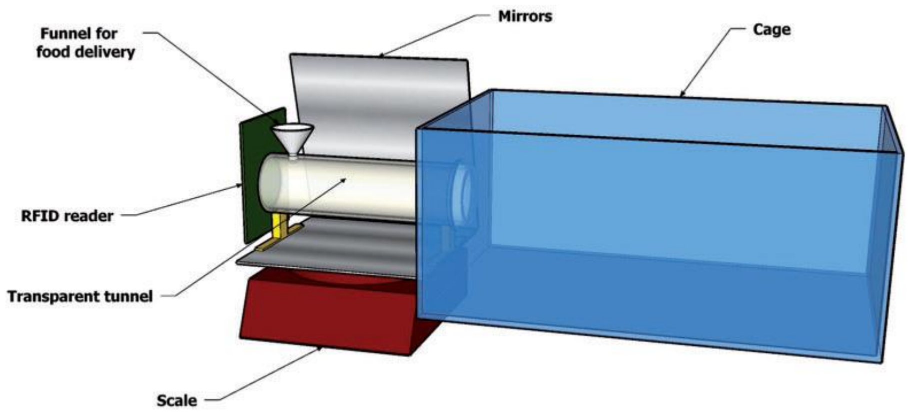


Figure 4.10: System built by Ahloy-Dallaire et al. A tunnel with a food dispenser and an RFID reader were added to a normal cage. The tunnel is above a scale that measures the body weight of each animal when it is inside the tunnel. There is also a camera, but it is not represented in this image (reproduced from [12])

In conclusion, implantable PIT tags are simple and powerful at the same time. Although tag implantation is required, this procedure is relatively simple and usually has negligible effects [23], thus being a popular means of measuring several parameters of individual group-housed laboratory animals.

4.1.3.2 Other implantable sensors

Depending on the application, the experimenter may be interested in obtaining more detailed physiological signals, which can require larger battery-operated sensors, a more invasive procedure to implant the device, or both.

One example of this is the E-Mitter Telemetry Implants from STARR Life Sciences (Figure 4.11). This product consists of a cylindrical implant, with a size ranging from 15.5 mm x 6.5 mm to 26 mm x 8 mm depending on the model, that can measure temperature, motor activity, and heart rate. Like the RFID implants previously mentioned, these implants do not have a battery, so a device that works as an energiser, but also as a receiver of the information sent by the implants, is also needed. Since these implants are larger than the typical RFID tags previously mentioned, a small surgery is needed to implant them on a mouse or rat [92].

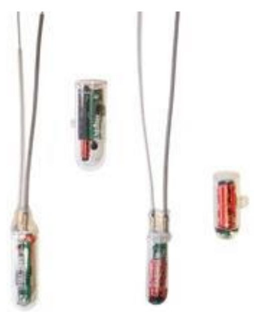


Figure 4.11: Different available E-Mitter Telemetry Implants with different sizes. The first and third implants have wires to measure the heart rate (reproduced from [92])

Another example is the MouseOx Plus by STARR Life Sciences, which consists of sensors placed on the thigh, paw, foot, collar, or throat of the animal, that measure a wide variety of signals, like arterial saturation, heart rate, breathing rate, temperature, activity, etc. (Figure 4.12). This device is invasive and can be used in awake or anaesthetised mice or rats [93].

Figure 4.12: MouseOx Plus being used to monitor two mice (reproduced from [93])

Neurologgers, such as the one from TSE Systems, can measure Electroencephalography (EEG) and Electromyography (EMG) in freely moving animals (Figure 4.13). This device can continuously record up to 90 hours and the batteries can be easily replaced [94]. The main problem is that surgery is needed to implant the device on the head of the animal [94, 95].

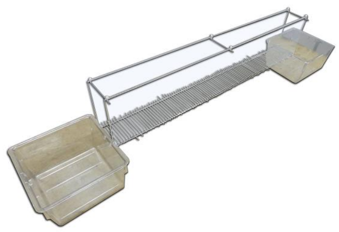
(a) (b)

Figure 4.13: (a) Neurologger upper side and (b) neurologger attached to the head of mice (reproduced from [94])

In summary, this type of implantable sensors can be used to obtain individual measurements of important physiological parameters in group-housed mice and rats. However, they usually need surgery and have an impact on animal welfare.

4.1.4 Behavioural tests

On some occasions, the experimenter is interested in analysing animal behaviour in different situations, rather than measuring a specific physiological signal. With that in mind, there are several products on the market designed to test different behavioural paradigms, with the objective of assessing motor coordination [96, 97], attention performance [98], fatigue [99, 100], social dominance [101], among others. Some of these behavioural tests can be seen in Figure 4.14.



(a) Manual Foot Misplacement Corridor [96]

(b) Body Reversal Corridor [97]

(c) Five/Nine Holes Box [98]

(d) Treadmill [99]

(e) Touchscreen Rota Rod [100]

(f) Tube Dominance Test [101]

Figure 4.14: Examples of behavioural tests from the company Panlab

Some more advanced and automated products to test the animal's behaviour include the TSE Systems IntelliCage (Figure 4.15). This system is composed of a cage with four similar corners, composed of two nose poke holes that give access to water bottles. The experimenter can then configure when each nose poke hole gives water or a negative reinforcement (air puff). There are also LEDs that can be personalised to distinguish between different nose poke holes. The system then stores how much water each animal drinks using a "lickometer" and distinguishes between each animal using RFID tags [102].

Figure 4.15: Version of the TSE Systems IntelliCage that is used for mice (reproduced from [102])

Although behavioural tests can be interesting when studying how certain diseases - such as Alzheimer - affect the behavioural phenotype, many of them still require manual observation by the experimenter.

4.2 Interface of the system

When building a monitoring system, it is also important to create a user interface that interacts with the sensors that are being used to acquire the signals. By looking at what already exists on the market, there are several characteristics that need to be present on the user interface like being user-friendly, customisable, and easy to configure. It is also important to enable the user to make annotations at specific time instants, set alarms if certain parameters reach abnormal values, zoom in or out on continuous signals to enable a more detailed analysis, and even export the results to other types of files, like Excel or MATLAB [25,87,103,104]. Some examples of interfaces can be seen in Fig. 4.16.

4.3 Putting it all together

Although there are several different approaches and technologies, they can all be combined into a single system. One example is the TSE Systems PhenoWorld, which consists of a modular system where the experimenter can add different modules to a main cage, to test the desired parameters. All these modules are connected to a single software, which facilitates the analysis of the data. There are modules related to feeding and drinking, learning and memory, activity, exercise, video tracking and others. Since each module is separated by animal gates and the animals are implanted with an RFID tag, the system knows which animal is in each module, so that the measurements can be attributed to each animal [25]. A possible system built with this product can be seen in Figure 4.17. So, by looking at this system, it is possible to see that by combining different types of technologies, it is possible to create a single and more robust system.

Table 4.1: Different categories of mice and rats monitoring systems and respective advantages and disadvantages

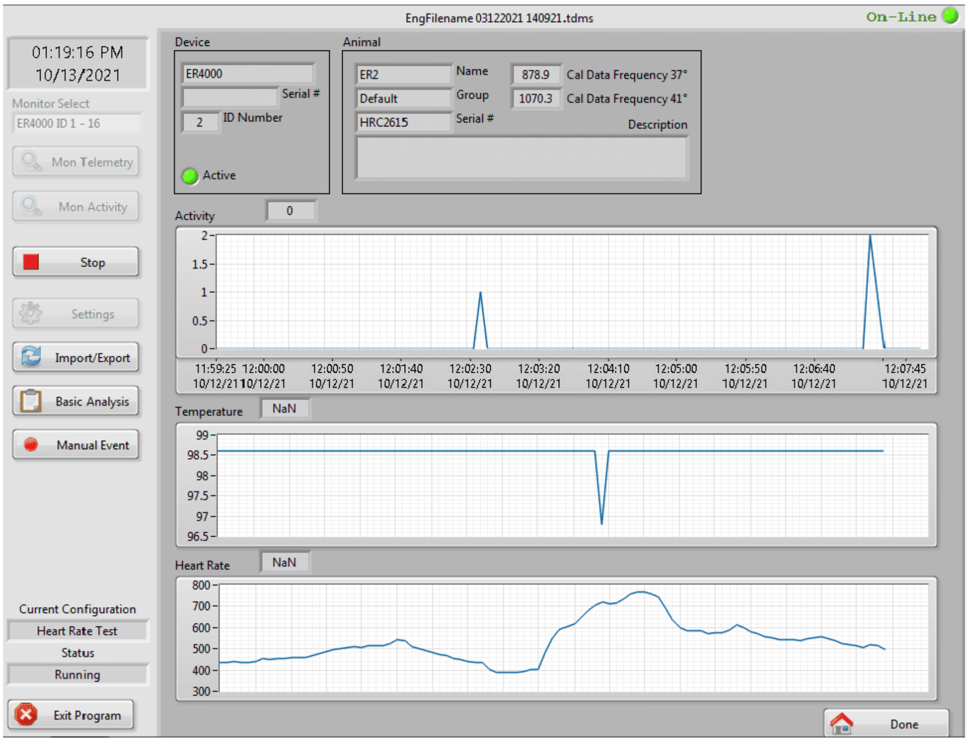
Category	Advantages	Disadvantages	Examples
Devices added to the cages	- Typically Non-invasive	- Animals need to interact with the device - Cannot distinguish between different animals in the same cage	[4, 84, 85]
Video cameras / Motion detectors	- Totally Non-invasive - More advanced systems can distinguish between different animals	- Cannot measure some important physiological parameters	[46, 86, 87]
PIT tags	- Can distinguish between different animals on the same cage - Can be combined with other types of devices - Effects of the implant are normally negligible	- Animals need to be implanted with the PIT tags	[12, 90, 91]
Other implantable sensors	- Can distinguish between different animals on the same cage - Can be combined with other types of devices - Can measure several different physiological signals	- Usually require surgery, and have considerable impact on animals - May require the changing of batteries	[92–94]
Behavioural tests	- Useful to characterise the evolution of a given disease or condition - Can evaluate subjective parameters	- Require considerable effort from the experimenter	[96–102]

4.4 Summary

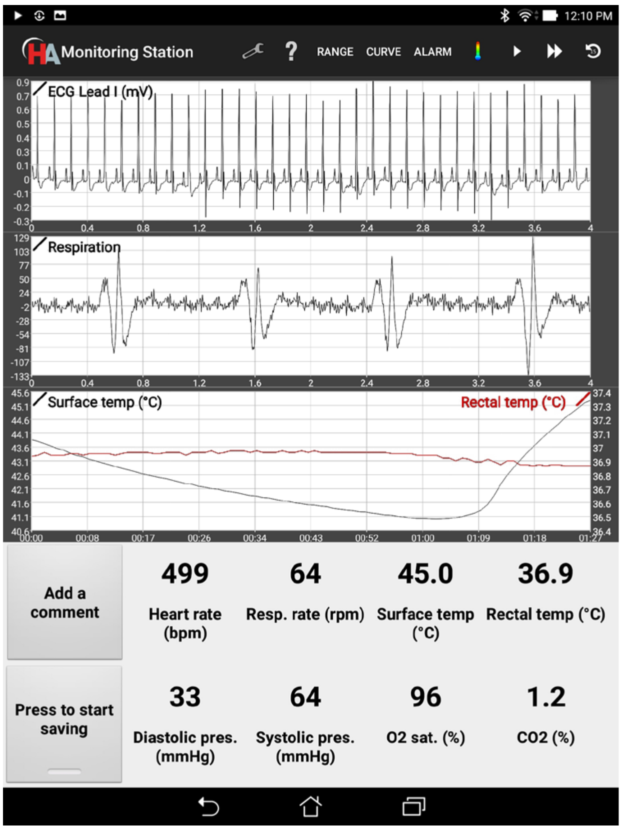
There are many different monitoring systems for mice and rats on the market and reported in the literature, which choice depends on the application. In general, these products can be divided into five different categories: devices added to the cages, video cameras / motion detectors, implantable PIT tags, other (mostly battery-operated and more invasive) implantable sensors, and behavioural tests. There are also more advanced systems that combine different technologies, to create a more robust system.

A summary of the main advantages and disadvantages of each type of product can be seen in Table 4.1. A more detailed summary of all the products mentioned in this work can be seen in Appendix A.

Since mice and rats are usually housed in groups, implantable PIT tags are of particular interest as they enable the experimenter to obtain measurements from different animals in the same cage, without bothering the animals with handling.



(a)



(b)

Figure 4.16: Interface of the (a) E-mitter Telemetry Implants and the (b) Small Animal Physiological Monitoring System (reproduced from [92] and [103])

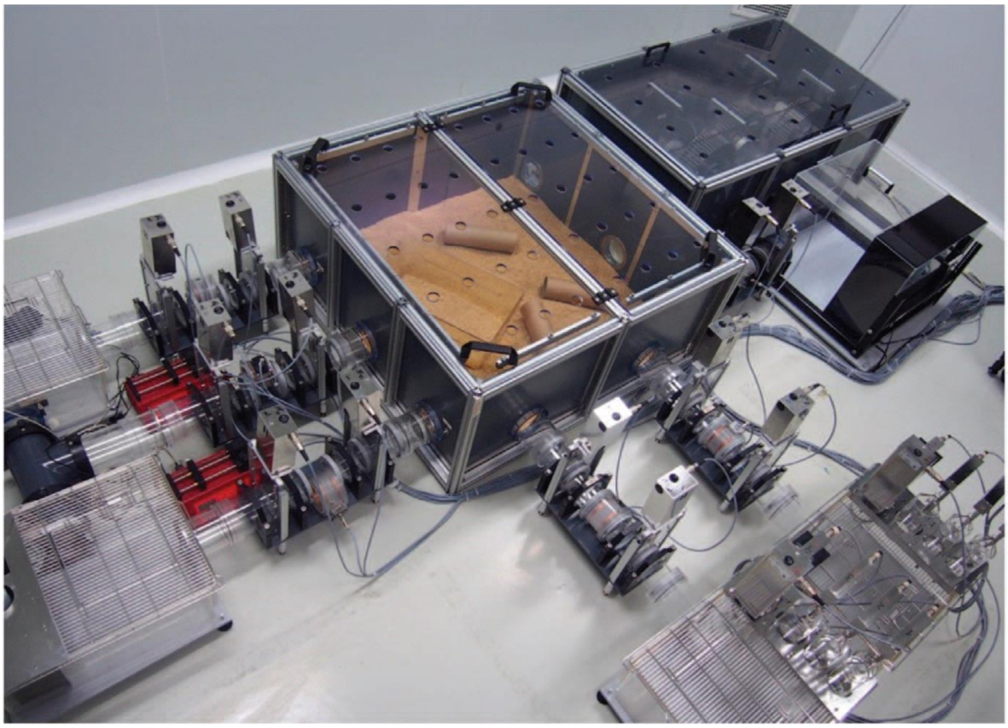


Figure 4.17: Example of a system built with the TSE Systems PhenoWorld. It depicts the several modules connected to each other by animal tunnels that detect the RFID tag of the animals (reproduced from [105])

Chapter 5

Hardware Design and Development

This chapter provides an overview over the architecture of the built system, including detailed information about the hardware components that were chosen and the assembly of the system. The tests that were done when choosing the best components for this application are also included. Finally, a comparison of the built system with the products currently available on the market is present.

5.1 System architecture

The developed monitoring system is focused on monitoring mice at two different levels: monitoring of each individual mouse in a cage, and monitoring the environment within each cage. Although we are mainly focused on monitoring mice, the same principles and technology used in this system would also be applicable to the monitoring of laboratory rats.

The monitoring of each animal is done using an RFID based technology. More precisely, each animal is implanted with a thermosensitive PIT tag and then six RFID reader antennas are placed below the cage, so that by knowing which RFID reader antenna detects each tag at each time-point, the position of each animal can be estimated, alongside its body temperature. Using this information, it is also possible to estimate if each animal has a preference for a certain zone of the cage, and whether it is more or less active.

When it comes to the monitoring of the cage environment, a temperature, a humidity, and a light intensity sensor are placed inside the cage, above the cage grid.

All the measured signals are then sent to a database using Wi-Fi, so that the user can monitor the information in real-time in a computer or mobile device. The user interface also allows the user to do several other actions, like exporting the data to Excel, setting alarms, etc.

The architecture of the system is summarised in Figure 5.1.

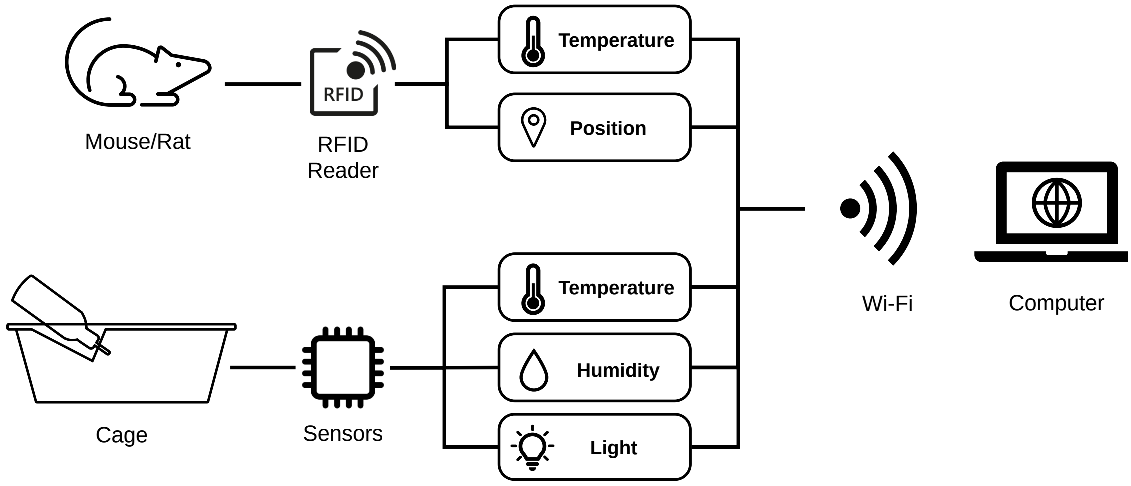


Figure 5.1: Architecture of the built monitoring system

5.2 Hardware Components

To build a working prototype, several different hardware components were used. The following subsections describe the requirements for each component of the prototype and the model of the components that were chosen. The pinout of all the chosen components can be seen in Appendix B.

5.2.1 RFID Tags

Since one of the main objectives of this work is to monitor the position and temperature of mice, it is important to use minimally invasive thermosensitive PIT tags, compliant with the ISO 11784 and 11785 standards, which refer to animal monitoring.

5.2.1.1 Biotherm13 - FDX-B High-Performance PIT Tag

The Biotherm13 PIT tag by Biomark is a 13 mm x 2.12 mm RFID tag with temperature measuring capabilities, similar to the ones mentioned in Section 4.1.3.1. The reported temperature measuring of this tag ranges from 33 ºC to 43 ºC, making it adequate to measure the temperature of mice [89]. This tag is ISO 11784 and 11785 compliant, operating at 134.2 kHz and following the FDX-B protocol, which makes it compatible with any reader that is also compliant with ISO 11784 and 11785 [83, 89].

The company Biomark also sells PIT tags implanters to facilitate the implantation of the RFID tags in the animals [89].

5.2.2 RFID Reader

When it comes to the RFID reader, it is important to choose a reader that is compliant with ISO 11784 and 11785. The reader should also be able to read any additional data that is present in the RFID tag, for example, the temperature.

Having an external antenna is also a plus, since it makes it possible to test antennas with different properties, in order to obtain the desired reading range. It also makes it possible to use multiple antennas in the system, if only one of them is active at a time.

5.2.2.1 RFIDRW-E-TTL - Animal tag and RFID reader writer with external antenna, and TTL output

The RFIDRW-E-TTL by Priority 1 Design is an RFID reader module that is compatible with many different types of RFID tags, including the ones that follow the FDX-B and HDX protocols described in ISO 11784 and 11785 (Figure 5.2) [106].

This module communicates using TTL UART at 3.3 or 5 V, making it compatible with most microcontrollers. This board is also capable of receiving several AT commands through its RX pin, enabling the user to customise the board behaviour for different applications [106].

The operating frequency of this RFID reader can also be tuned by connecting different capacitors between the CT and VC pins. This enables this reader to operate with coil antennas with different properties, as long as the frequency is tuned to the desired value [106].

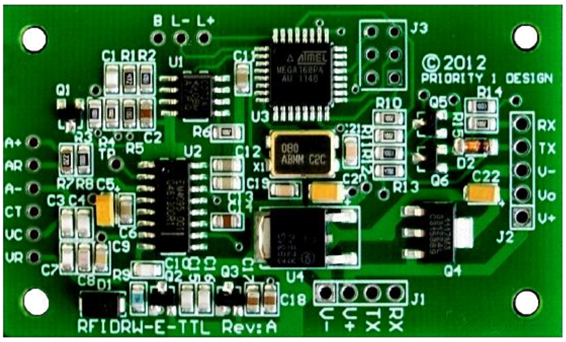


Figure 5.2: RFIDRW-E-TTL board

5.2.3 Multiplexing of the antennas

Since the RFID reader described in section 5.2.2.1 can only connect to one antenna at a time, it is necessary to add another component that switches between different antennas. This can be accomplished by connecting each antenna to a different switch and then only having one of the switches closed at a time. An example on how to connect three antennas is demonstrated in Figure 5.3. By opening and closing each switch in a small fixed time interval, it is possible to make the RFID reader use all the antennas in a short period of time.

It is also important that the switches can be opened and closed by a microcontroller and that they do not produce any significant noise when opening and closing, since noise can have undesirable effects on mice as was seen in Section 2.1.7.

Since RFID antennas need to have very specific resonant frequencies for the system to work correctly, it is important to avoid any interference between the multiple antennas that are connected to the switches.

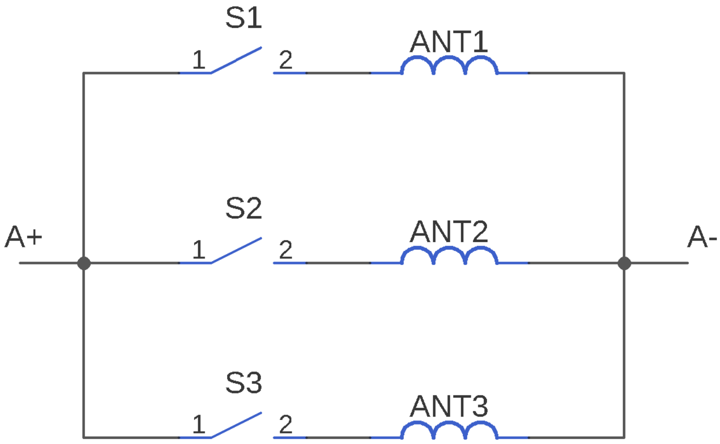


Figure 5.3: Schematic of the connection of multiple antennas to the RFID Reader. The A+ and A- pins correspond to the antenna pins in the RFID reader. By closing one switch at a time, the reader will only be connected to the corresponding antenna

5.2.3.1 HE721A0500 - Miniature Dual In-line Reed Relay

The HE721A0500 by Littelfuse is a small dual in-line reed relay (Figure 5.4) [107].

A reed relay is composed of two ferromagnetic blades (reeds), with a small gap between each other, and a copper coil. By supplying the coil with a certain voltage, a magnetic field will be generated which in turn will bring the two reeds together, closing the circuit [107, 108].

The HE721A0500 in particular closes the circuit when 5 V are supplied to the coil. This model is also Single-Pole (the switch only controls one circuit) and Single-Throw (the switch is only connected to an output in one of the states) [107].

Since this device has small dimensions, it does not produce any significant noise when opening and closing the circuit. Having a physical switch is also important, since it better isolates the antennas that are not active, having a resistance of 10^{10} Ω when the switch is open [107].

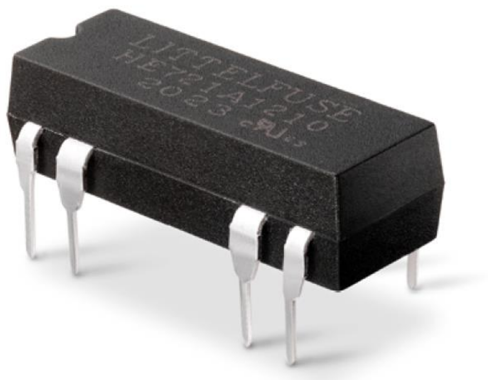


Figure 5.4: HE721A0500 Miniature Dual In-Line Reed Relay

5.2.3.2 HCF4051 - Single 8-channel analog multiplexer/demultiplexer

The HCF4051 by STMicroelectronics is a single 8-channel multiplexer/demultiplexer (Figure 5.5). This device has one common output/input that is connected to one of the eight independent inputs/outputs. The independent input/output that is active is selected by three binary control pins. A supply voltage between 3 and 20 V is recommended and the input voltage can range between 0 V and the supply voltage [109].

Since only one of the reed relays mentioned in Section 5.2.3.1 needs to be active in each time instant, the HCF4051 can be used as a demultiplexer to distribute 5 V between each of the reed relays with a defined time interval. The switch of the reed relay that is receiving the 5 V becomes closed, and consequently, the corresponding antenna is active.

Figure 5.5: HCF4051 multiplexer/demultiplexer

5.2.4 Environmental Sensors

5.2.4.1 DHT22 - Temperature & Humidity Sensor

The DHT22 is a low-cost temperature and humidity sensor that uses a digital data pin to send the measurements to a microcontroller (Figure 5.6). This sensor uses a thermistor (THERMally sensitive resISTOR) to measure the temperature and a capacitive sensor to measure the humidity. It can measure the temperature between - 40 and 80 ºC with an accuracy of ± 0.5 ºC and the relative humidity between 0% and 100% with an accuracy of 2-5%. In terms of supply voltage, a voltage between 3.3 and 5.5 V DC is recommended [110].

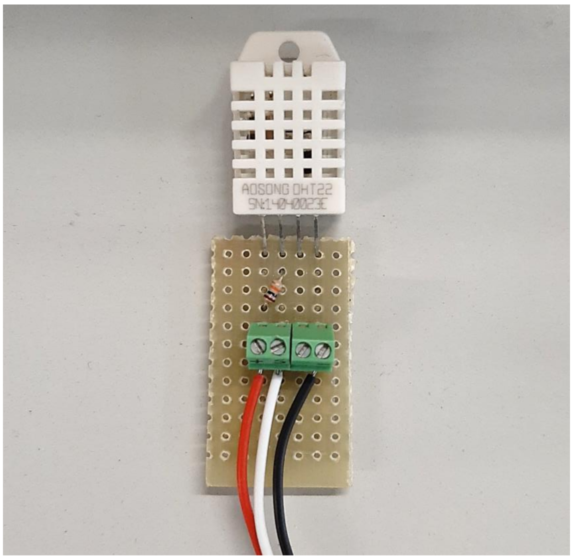


Figure 5.6: DHT22 used in this work. A 10 kΩ pull-up resistor is connected between the voltage source and the digital data pin

5.2.4.2 LDR - Light Dependent Resistor

A Light Dependent Resistor (LDR), also known as photoresistor, is a low-cost component which resistance changes with the light intensity (Figure 5.7). With the increase of the light intensity, the resistance decreases [111].

Figure 5.7: Light Dependent Resistor

By using a simple voltage divider, it is possible to convert the LDR resistance into a voltage value that can be read by a microcontroller. Since there is not a direct relation between the LDR resistance and the light intensity in lux, a calibration process may be needed to associate each resistance value to the respective light intensity.

5.2.5 Wireless Communication

In order for the system to be placed in any cage without needing to be next to a computer, a wireless communication module is needed. This device must send the data that is recorded by the sensors to a database, so that these measurements can be checked in a computer or mobile device.

5.2.5.1 ESP-07 Serial Wi-Fi Module

The ESP-07 by Ai-thinker is a low-cost and low-power wireless communication module based on the ESP8266 microchip. This module has multiple input/output pins and supports several types of communication like UART, I^2C and SPI. This module is also compatible with external Wi-Fi antennas. In terms of supply voltage, a voltage between 3 and 3.6 V is recommended [112].

This module can be used by one of two approaches: use the ESP-07 module as a microcontroller by connecting it to the computer and programming it directly, or only using the module for the wireless communication, while doing all the remaining processing in another microcontroller and then sending the necessary data to the ESP-07 using AT commands.

In order to facilitate the use of this module, an adapter was used, as can be seen in Figure 5.8. This adapter has some useful functionalities like having a 3.3 V voltage regulator, so that the module also works with 5 V, a reset button, and a switch to select between UART communication mode and program mode in case the user wants to change the firmware of the module. This adapter is particularly useful to be operated using AT commands, since it leaves the TX and RX pins free, enabling the module to communicate with a microcontroller, like an Arduino, using TTL UART communication.

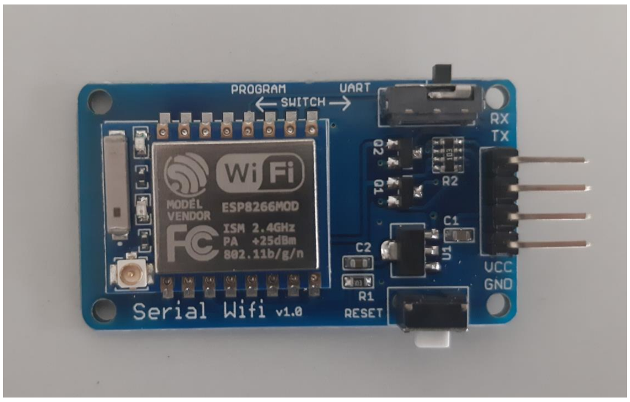


Figure 5.8: ESP-07 with the adapter

5.2.6 Microcontroller

One fundamental part of this project is having a microcontroller that can be programmed to control and receive the data from the various sensors, process that data and then send that data to a database. This microcontroller must also be able to communicate with the ESP-07 Serial Wi-Fi Module and the RFIDRW-E-TTL at the same time using TTL UART.

5.2.6.1 Arduino Leonardo

The Arduino Leonardo is a microcontroller board based on the ATmega32u4 microchip that can be programmed using the Arduino IDE software (Figure 5.9). This board has an operating voltage of 5 V and contains 20 digital input/output pins, 12 of which can be used for analogue inputs and 7 that can be used for PWM output. This microcontroller is also compatible with 5 V TTL UART. Other types of communication like I^2C and SPI are also possible. Finally, this board contains a 3.3 V and a 5 V output pins that can be used to power other components [113].

The main characteristic of this board is the separation between the USB and the hardware serial pins (RX and TX), which enables the Serial monitor of the Arduino IDE to be used for debugging even if the hardware serial pins are connected to a sensor (which is not possible in other models, like the Arduino UNO or Arduino Nano). It is also possible to use the SoftwareSerial library to simulate TX and RX pins on another digital pins of the Arduino [113, 114].

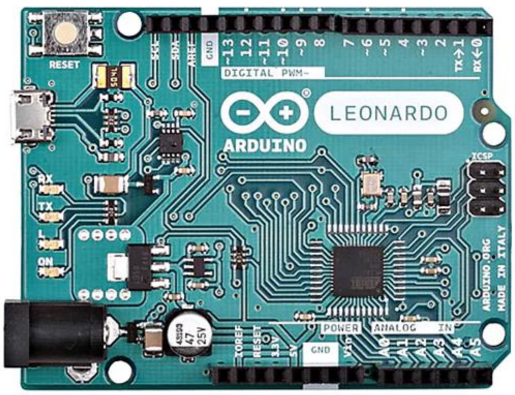


Figure 5.9: Arduino Leonardo microcontroller board

5.3 Calibration of the LDR

To obtain an approximation between the resistance of the LDR and the light intensity in lux, the resistance obtained with the LDR was compared with the light intensity given by a BH1721FVC ambient light sensor in different light conditions. The relationship obtained can be seen in Figure 5.10.

Looking at the graph, it is possible to see that there is a nonlinear relationship between the resistance and the light intensity. By approximating the data to a power trendline, the equation 5.1 was obtained. The coefficient of determination (R^2) for this type of trendline was 0.99767.

$$Light = 23852124.30183 \times (R_{LDR})^{-1.23623} \quad (5.1)$$

Even though the LDR is not capable of giving very accurate values for the light intensity, the R^2 close to 1 shows that after calibration this is a good cheap sensor for applications where a very accurate value of light intensity is not needed.

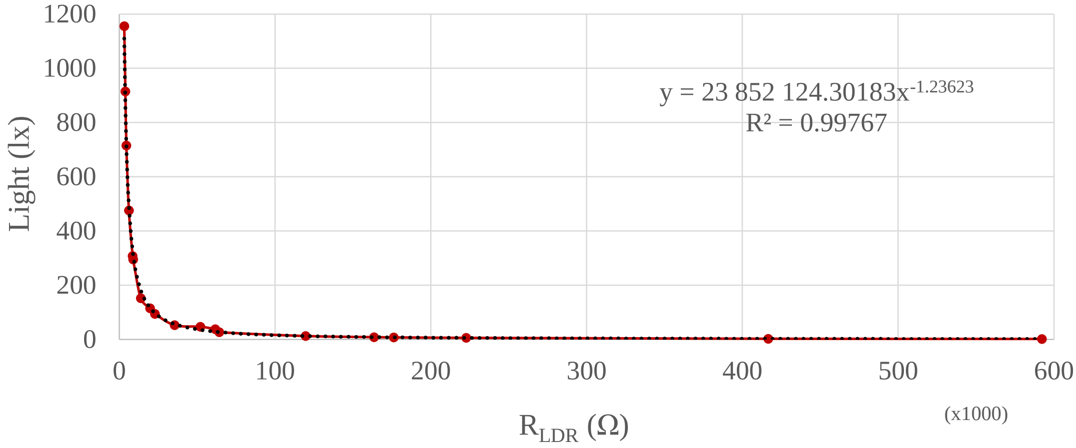


Figure 5.10: Relation between the light intensity and the resistance of the LDR

5.4 Antennas design and position

After deciding the hardware components that were going to be used in the system, it was necessary to decide the position and properties of the antennas that were going to be connected to the RFID reader.

5.4.1 Hand-made antenna

A hand-made coil was built to find if a coil built in the lab would work well as an antenna for the RFIDRW-E-TTL reader. This would enable the experimenter to easily obtain antennas with the desired reading range, without the need of buying them.

To do this, we first started by calculating how much turns the antenna coil should have in order to obtain the desired inductance. The Multilayer air code inductor calculator by Circuits.dk[1] was used for this task. By choosing the desired coil inductance, inner diameter, length (distance between the top and bottom of the coil), and wire gauge (wire diameter), the calculator outputs the number of turns that the coil should have in total, how many turns should have per layer, as well as other values. The output obtained when choosing a coil with 100 µH inductance, 51 mm of inner diameter, 5 mm of length and a wire gauge of 23 AWG (≈ 0.57 mm) can be seen in Figure 5.11.

After seeing the results of the calculations, a 23 AWG enamelled copper wire was winded around a cardboard tube with an outer diameter of 51 mm. Two rubber bands with 5 mm between them were used to limit the top and bottom position of the coil (Figure 5.12). Electrical tape was used at the end of each layer to avoid the unwinding of the coil.

[1] https://www.circuits.dk/calculator_multi_layer_aircore.htm

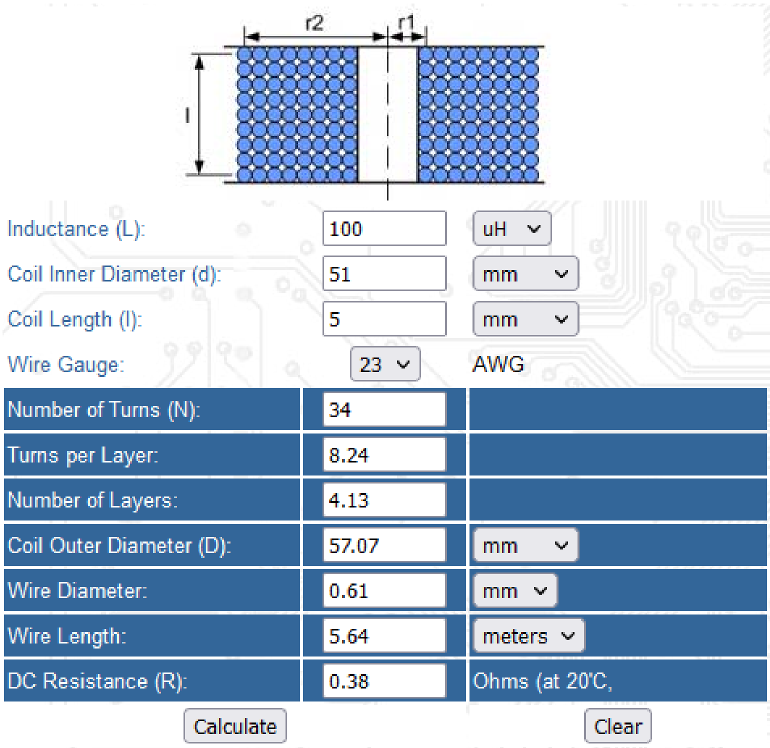


Figure 5.11: Output obtained when using the Multilayer air code inductor calculator. The values with white background correspond to the inserted values, while the values with blue background correspond to the obtained values

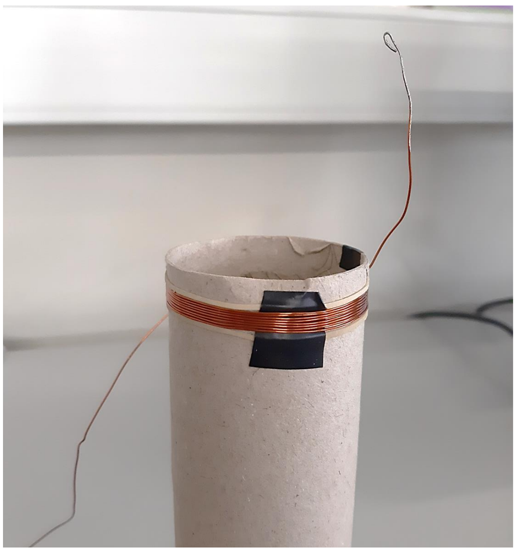


Figure 5.12: Wire being winded in the cardboard tube

After completing the 34 turns around the cardboard tube, the wire was removed from the tube, and was glued with electrical tape for the coil to maintain its shape.

Finally, sandpaper was used to remove the insulating enamel from the two extremities of the coil, so that the coil would conduct electricity when connected to a breadboard.

5.4.1.1 Properties of the hand-made antenna

After creating the hand-made antenna, its properties were compared with the expected properties. This can be seen in Table 5.1.

Table 5.1: Comparison between the expected values and the obtained values for the hand-made coil

	Inductance (H)	Resistance (Ω)	Inner Diameter (mm)	Outer Diameter (mm)	Length (mm)
Expected value	100.0E-6	380.0E-3	51.0E-3	57.1E-3	5.0E-3
Obtained value	97.7E-6	300.0E-3	50.3E-3	55.0E-3	6.0E-3
Relative error (%)	2.3%	21.1%	1.4%	3.6%	20.0%

By looking at the properties of the created antenna it is possible to see that a coil with properties very similar to the expected was obtained. This shows that the calculator and methodology used to create the antenna are valid.

5.4.2 Comparing the different antennas

In order to choose the antennas with the best performance for this application, three models were compared: the RFIDCOIL-49A by Priority 1 Design, a coil by D-Think Inc. and the hand-made coil. (Figure 5.13).

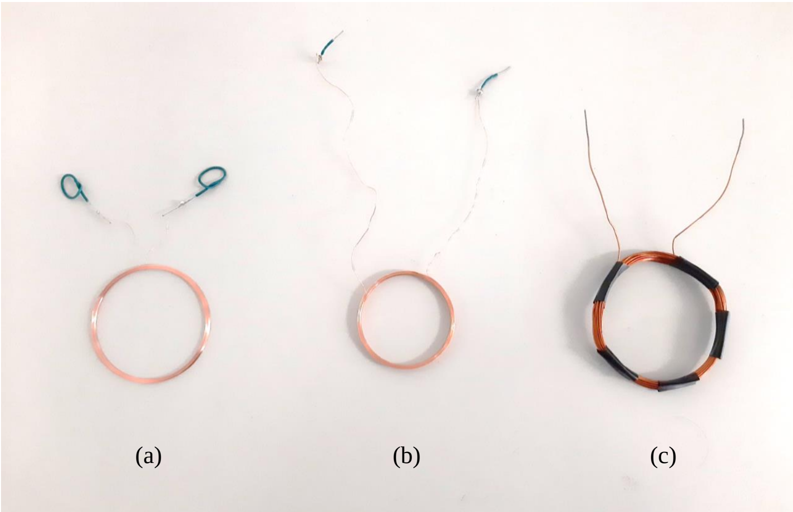


Figure 5.13: (a) RFIDCOIL-49A, (b) coil by D-Think Inc. and (c) hand-made coil

5.4.2.1 Properties of the different antennas tested

We first started by comparing the properties of the there different antennas types as can be seen in Table 5.2.

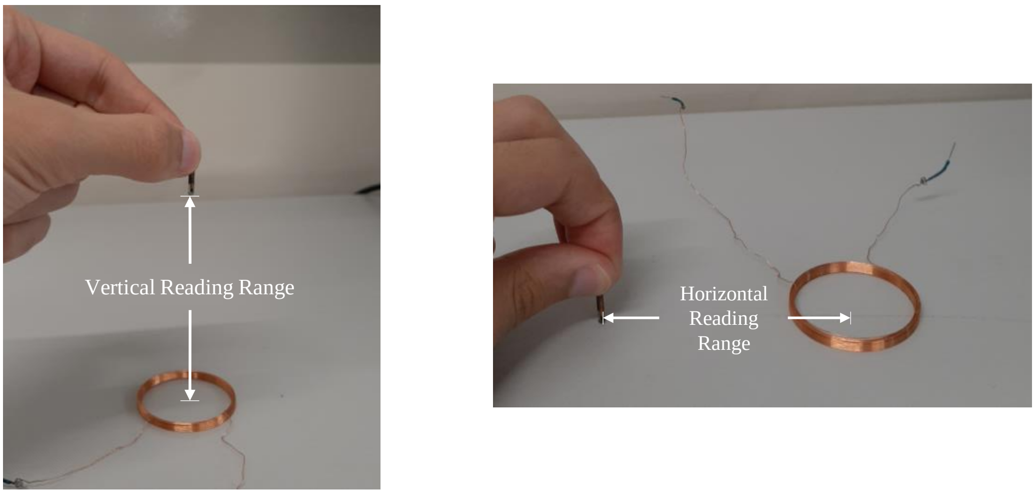
Table 5.2: Properties of the different types of coils used

Coil	**Wire gauge (mm)**	**Inner diameter (mm)**	**Outer diameter (mm)**	**Resistance (Ω)**	**Inductance (H)**
RFIDCOIL-49A	0.10	46.0	49.0	64.3	2.5E-3
D-Think Inc.	0.25	37.8	40.8	10.5	333.8E-6
Hand-made	0.60	50.3	55.0	0.3	97.7E-6

From this table, it is relevant to mention the very small wire gauge of the RFIDCOIL-49A, which made them very fragile. Looking at the wire resistance, the hand-made coil has a very small value, which can be explained by the relatively small length of wire used (about 5.6 meters as was seen in Figure 5.11), and its larger wire gauge, in comparison with the other two types of coils. Finally, it is also possible to notice that the RFIDCOIL-49A has an inductance about ten times higher than the other two types of coils.

5.4.2.2 Reading range for different antennas

After comparing the properties of the three different types of coils, the horizontal and vertical reading range was measured as seen in Figure 5.14 using different coils and connecting different capacitors to the RFIDRW-E-TTL. The formulas used to determine the capacitors and resistors that should be used with the RFIDRW-E-TTL reader can be seen in Appendix C. This appendix also contains an example calculation for the coil by D.Think Inc.



(a) (b)

Figure 5.14: (a) Vertical reading range and (b) horizontal reading range

The readings ranges obtained for each type of antenna and value of capacitors can be seen in Table 5.3. The C_E capacitor was connected between pins CT and VC, while the C_D capacitor was connected between pins VC and VR.

Table 5.3: Reading range for the different types of coils and capacitors

Coil	C_E **(F)**		C_D **(F)**		**Vertical Range (mm)**	**Horizontal Range (mm)**
	Calc.	**Used**	**Calc.**	**Used**		
RFIDCOIL-49A	-	-	-	-	67.0	63.0
D.Think Inc.	3.7E-9	3.3E-9	34.5E-12	33.0E-12	37.0	37.5
		3.9E-9			43.0	42.0
		4.7E-9			26.0	31.0
Hand-made	13.9E-9	10.0E-9	111.8E-12	120.0E-12	20.0	33.0
		12.0E-9*			41.0	46.0
		15.0E-9			5.0	30.5

* A film capacitor was used, while for the other C_E values ceramic capacitors were used

Looking at the table, it is possible to notice that the antenna that produced a greater reading range was the RFIDCOIL-49A. This can be explained by the fact that this coil came with the RFIDRW-E-TTL reader (being already tuned for the used reader) and it was also the coil with the largest inductance. When it comes to the different values of capacitors used with the coil by D-Think Inc. and with the custom-made coil, capacitors with values closer to the calculated capacitance resulted in longer reading ranges, as expected.

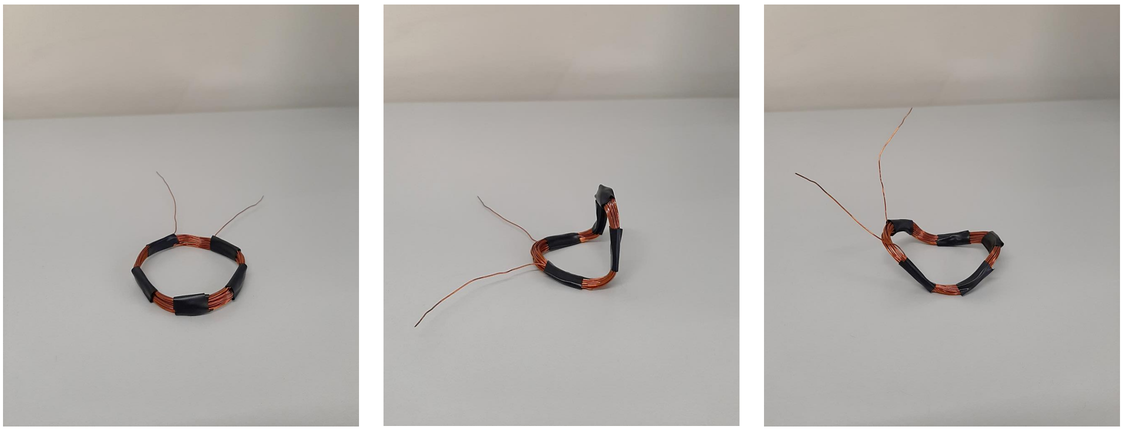
While most C_E capacitors were ceramic capacitors, the capacitor with 12 nF corresponded to a film capacitor. It was noticed during testing that while the capacitance of the ceramic capacitors changed slightly with time, the film capacitor maintained a fixed value. This was further confirmed when using an AT command on the RFIDRW-E-TTL reader that gives the operating frequency of the reader with the antenna and capacitors being used: when using the film capacitor, the operating frequency was stable, while it changed with time when using ceramic capacitors, which could result in changes in the reading range. This can be a possible explanation to why the 12 nF capacitor presented a much higher reading range than the other capacitors used for the hand-made coil.

Another interesting result is that the hand-made coil was more sensitive to different capacitor values. One possible explanation is the fact that since the coil was hand-made, the wires were not as tightly bound together as the wires in the commercial coils, which resulted in small variations in the inductance value depending on the position of the coil.

Only one value of C_D is presented for each type of coil because using capacitors with other capacitance values did not seem to significantly affect the reading range. In the future, more extreme values of C_D could be used to better test its impact.

5.4.2.3 Different coil shapes

Since the orientation between the antenna of the tag and the antenna of the reader affect the reading range, the shape of the hand-made coil was changed to see the effect that it caused. Three shapes were tested, as can be seen in Figure 5.15: *Flat* coil, *L* coil, and *Bowl* coil.



(a) (b) (c)

Figure 5.15: (a) *Flat* coil, (b) *L* coil, and (c) *Bowl* coil

During the tests, the *L* coil performed as a mix between a coil placed in the vertical and in the horizontal: on the vertical side, the tag was better detected if it was in the horizontal and in the horizontal side, the tag was better detected if it was in the vertical.

When it comes to the *Bowl* coil, there was not a significant difference relatively to the *Flat* coil.

In conclusion, the coil shape can be changed depending on the application. In this case, since the coils will be placed in the bottom of the cage, the *Flat* coil ends up being the better shape for this application.

5.4.3 Antennas position

Then, it was necessary to choose how many antennas were going to be placed in the cage. On one hand, by having more antennas, it takes longer for each antenna to activate (since only one antenna is active at each time), but it increases the accuracy of the detected position for each animal. On the other hand, by using a smaller number of antennas, each antenna is activated more frequently, but the precision detecting the position is smaller. Since each antenna can only detect one tag at once, being unable to detect any tag if more than one tag is in its reading range, it is also important to choose a number of antennas that avoids multiple animals being simultaneously in the reading range of the same antenna. Finally, it is also relevant to avoid that an animal is detected by more than one antenna at once, making it important to avoid the intersection between the reading range of the multiple antennas.

With all these issues in mind and by measuring the floor dimensions of the cage to be used, an array with 3 x 2 antennas was chosen. The position of the antennas in the cage floor can be seen in Figure 5.16. As it is possible to see, each antenna is centred in a region with 7.7 cm x 8.5 cm, meaning that each antenna should be able to approximately only detect the tags that are in that region.

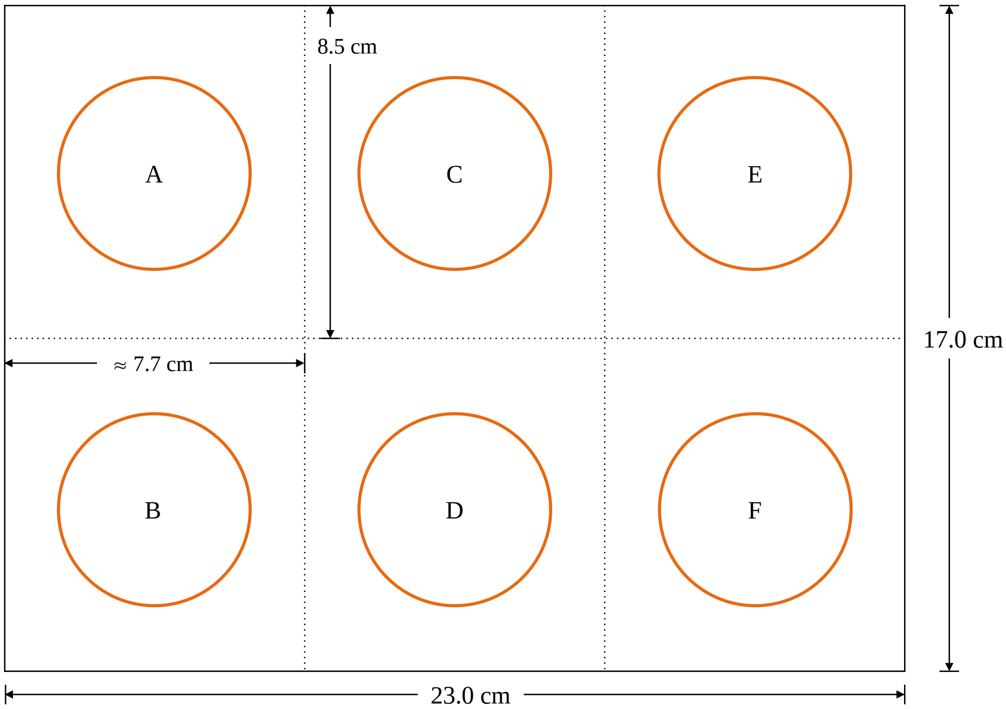


Figure 5.16: Cage base dimensions and position of the antennas. The letters from A to F correspond to the name given to each region

Since there needs to be a layer of corn-cob bedding in the cage floor, increasing the distance between the antennas and the tags implanted in the animals and the other components on the system can cause interference, decreasing the reading range of the antennas, the RFIDCOIL-49A from Priority 1 Design was chosen as the one to be used in the system, since it presented the longest reading range, having a higher probability to detect the tags implanted in the mice.

5.5 Assembly of the system

5.5.1 Overview of the built circuit

Knowing all the components that were used in the system, a schematic of the built circuit was drawn, as can be seen in Figure 5.17. In order to simplify the schematic, only four antennas and four HE721A0500 relays are included, instead of the six that were actually used.

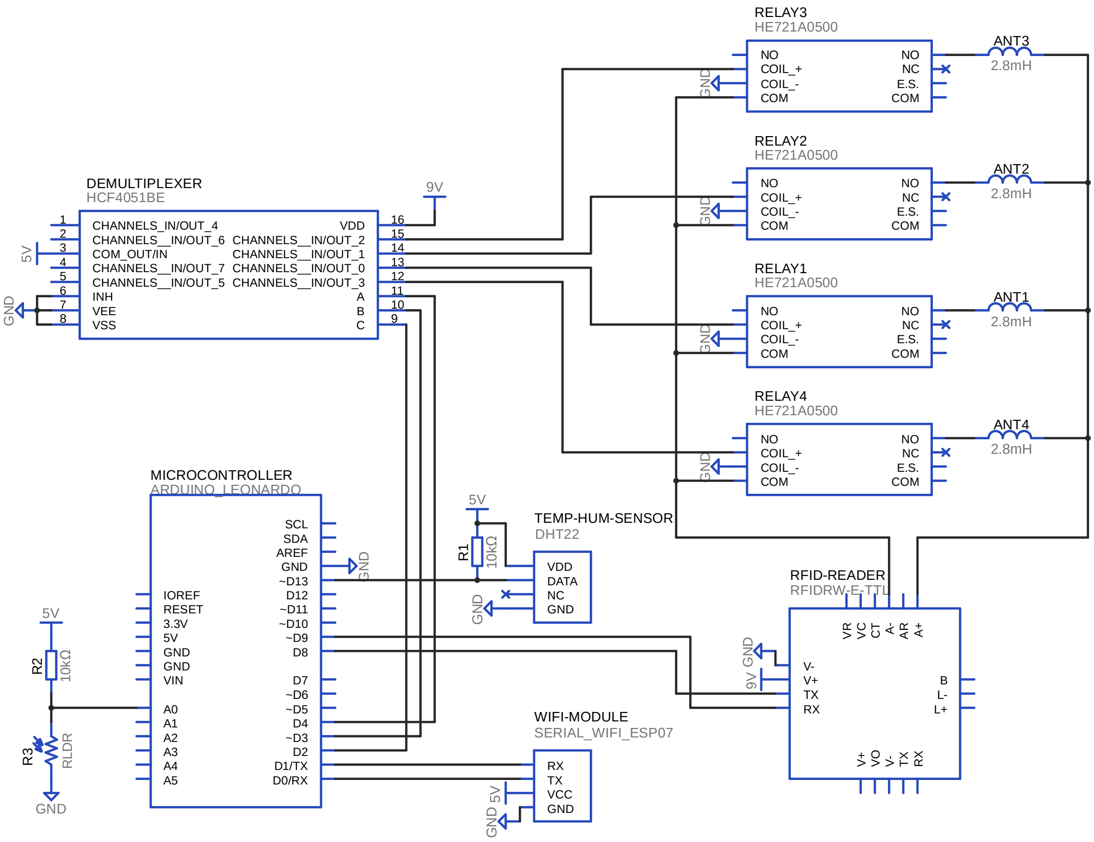


Figure 5.17: Schematic of the built circuit. To simplify the schematic, only four antennas and four relays are represented

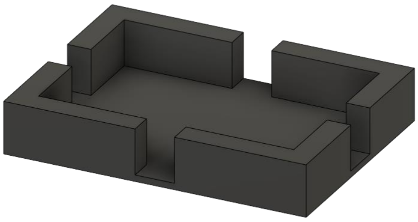
5.5.2 Circuit box

In order to make the system easier to transport and also protect the electrical components, a box was developed in the Autodesk Fusion 360 software. This box is divided in two different parts:

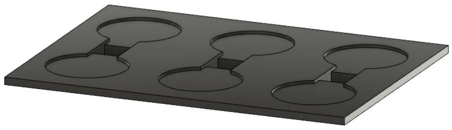
- **Bottom Part (Figure 5.18a)** — box where most of the components are placed. This box has four lateral openings not only to help in the heat dissipation but also to enable connections between the components that are inside and outside of the box;
- **Top Part (Figure 5.18b)** — cover of the box where the animal cage will be placed. This part has six grooves for the antennas. The three holes between the grooves enable the connection between the antennas and the rest of the circuit.

The full dimensions and more views of the two parts of the box can be seen in Appendix D.

After designing the 3D models of both parts, they were then printed in the Flashforge Creator Pro 2 3D Printer. Since the dimensions of both parts of the box were above the printer maximum dimensions, the parts were divided in two smaller parts and then glued together.



(a)



(b)

Figure 5.18: (a) Bottom part and (b) top part of the printed box

It is also important to mention that although most of the hardware components were inside the box, the ESP-07 Serial Wi-Fi Module was placed on the side of the cage to increase the Wi-Fi signal and the DHT22 and LDR were placed inside the cage, above the cage grid, in order to measure the environmental parameters inside the cage.

The system assembled in the 3D printed box can be seen in Figure 5.19.

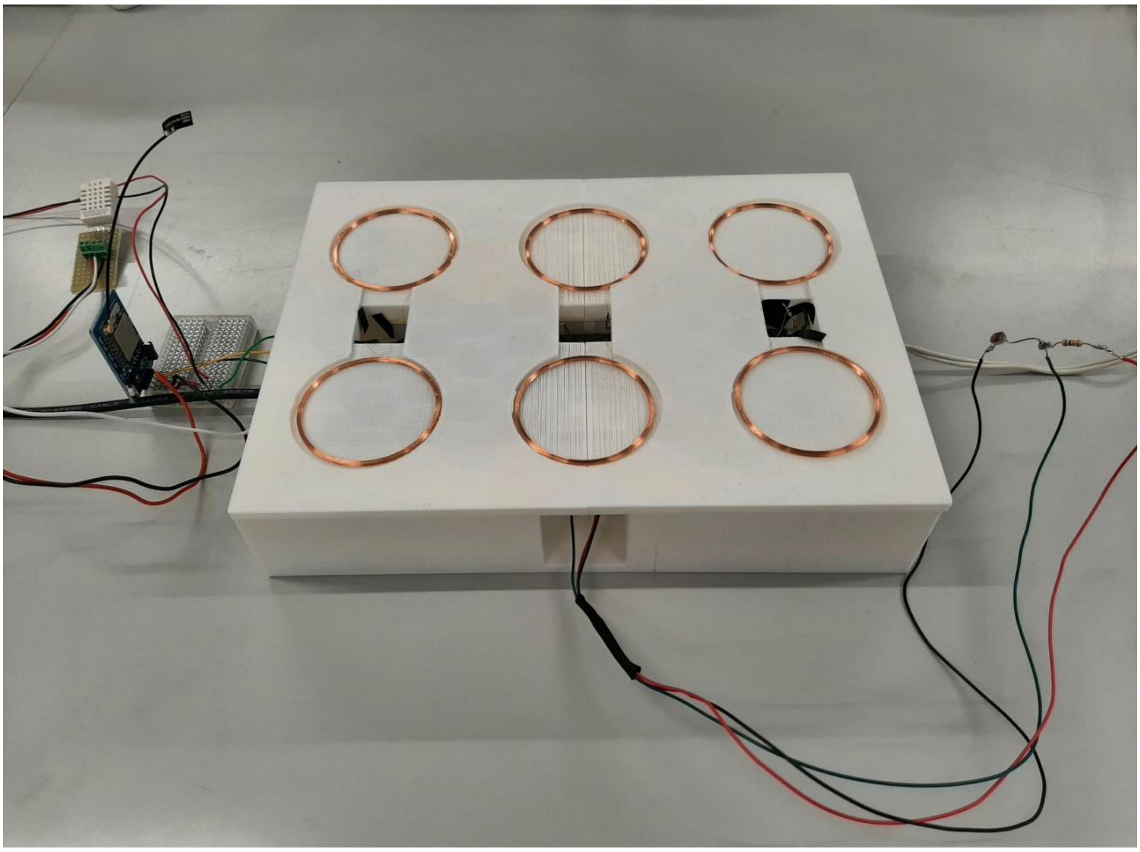


Figure 5.19: System assembled in the 3D printed box. On the top of the box, the six RFIDCOIL-49A can be seen. The ESP-07 Serial Wi-Fi Module, as well as the DHT22 can be seen in the left, and the LDR voltage divider is coming out of the front of the box

Table 5.4: Price of the main components of the built system

Item	Price per unit (€)	Units	Total price (€)
Arduino Leonardo	21.60	1	21.60
RFIDRW-E-TTL (+1 RFIDCOIL-49A)	57.00	1	57.00
RFIDCOIL-49A	4.70	5	23.50
ESP-07 Serial Wi-Fi Module	4.29	1	4.29
DHT22	9.40	1	9.40
LDR	0.47	1	0.47
HE721A0500	2.11	6	12.66
HCF4051	1.48	1	1.48
Total			130.40

5.6 Comparison with the State of the art

Comparing this system to what is already on the market and in literature, this system is similar to the UID Mouse Matrix reported in Section 4.1.3.1, being able to measure each mouse or rat temperature, activity and zone preference. In addition to that, our system also contains environmental sensors to measure the temperature, humidity, and light intensity inside the cage, which enables the experimenter to also know the conditions of the environment where the animals are housed.

Contrary to what happens in commercial products like the UID Mouse Matrix and the Home Cage Analyser, the antennas of our product can be freely moved to any position. This not only makes the system more compatible to cages with different sizes, but also gives the experimenter freedom to do several other tests, including:

- **Count the number of times an animal drinks during a day** — an antenna can be placed next to the water bottle in the mouse or rat cage, counting the number of times that each animal drinks in a day;
- **Preference tests** — by placing antennas next to different food types (or any other possible type of rewards) it is possible to automatically know the animal's preferences.

Another vantage of this system is the environmental sensors also being able to work in a modular way, similar to other products in the market (e.g. TSE Systems PhenoWorld). Although only temperature, humidity, and light sensors were included, other devices like Inertial Measurement Units (IMUs) to evaluate if the cage is being shaken or transported, or microphones to know if the noise being produced is being uncomfortable for the animals, could also be added to the system.

Finally, another advantage of our system is the low price of most components, leading to a total price of approximately 150 € as can be seen in Table 5.4.

5.7 Summary

In summary, the built system is able to simultaneously monitor each mouse individually (even if they are group-housed) using RFID, as well as the cage environment using several sensors. The measurements done are then sent to a database using Wi-Fi.

Each mouse is monitored by implanting them with a thermosensitive PIT tag and then placing multiple antennas below the cage. Each time a PIT tag is detected by an antenna, the experimenter knows the temperature and position of the corresponding mouse at that time-point.

The cage environment conditions are monitored by placing a temperature/humidity sensor and a light intensity sensor inside the cage.

After studying different types of antennas, including a hand-made coil, the RFIDCOIL-49A was chosen as the one to be used in the system, because of its longer reading range. A methodology to develop custom antennas for RFID was also tested.

The various components of the system were then assembled in a box that was designed and then 3D printed.

Comparing to what already exists on the market and in literature, the major advantage of this system is simultaneously combining the monitoring of each individual animal with the monitoring of the cage environment. A higher flexibility and the low price are also key advantages.

Chapter 6

Software Development

This chapter describes the code that was developed for the microcontroller and for the user interface. The tests that were done to decode the information present in the RFID tags are also presented.

6.1 Microcontroller Code

6.1.1 Overview of the code

The Arduino was programmed to manage the system, controlling which antenna is active, receiving and processing the data from the various sensors, and then ordering the Serial Wi-Fi Module to update the database with the measured information. An Activity Diagram summarising the Arduino code can be seen in Figure 6.1.

After initializing the pins and the serial communication, the Serial Wi-Fi Module connects to a wireless network so that the information read by the sensors can be sent to the database.

Then, a loop where each one of the antennas is activated one by one starts. After an antenna is activated, the RFID reader checks if there is any tag in the reading range. If there is, the tag code is read and then sent to the database if it is valid (for example, the code has the correct number of characters). After 400 ms, the activated antenna is deactivated, being all the antennas simultaneously deactivated for 600 ms. This 600 ms interval guarantees that a relay switch is completely open before closing the next relay switch, avoiding more than one antenna being activated at once. Also during this 600 ms interval, the environmental sensors send the temperature, humidity, and light to the microcontroller if 60 seconds passed since the last time that the environmental data was sent to the database. If this environmental data is valid, the Serial Wi-Fi Module publishes them in the database.

Finally, the system starts the antenna cycle again, activating all the antennas one by one, until the system is turned off.

The full Arduino code can be seen in Appendix E.

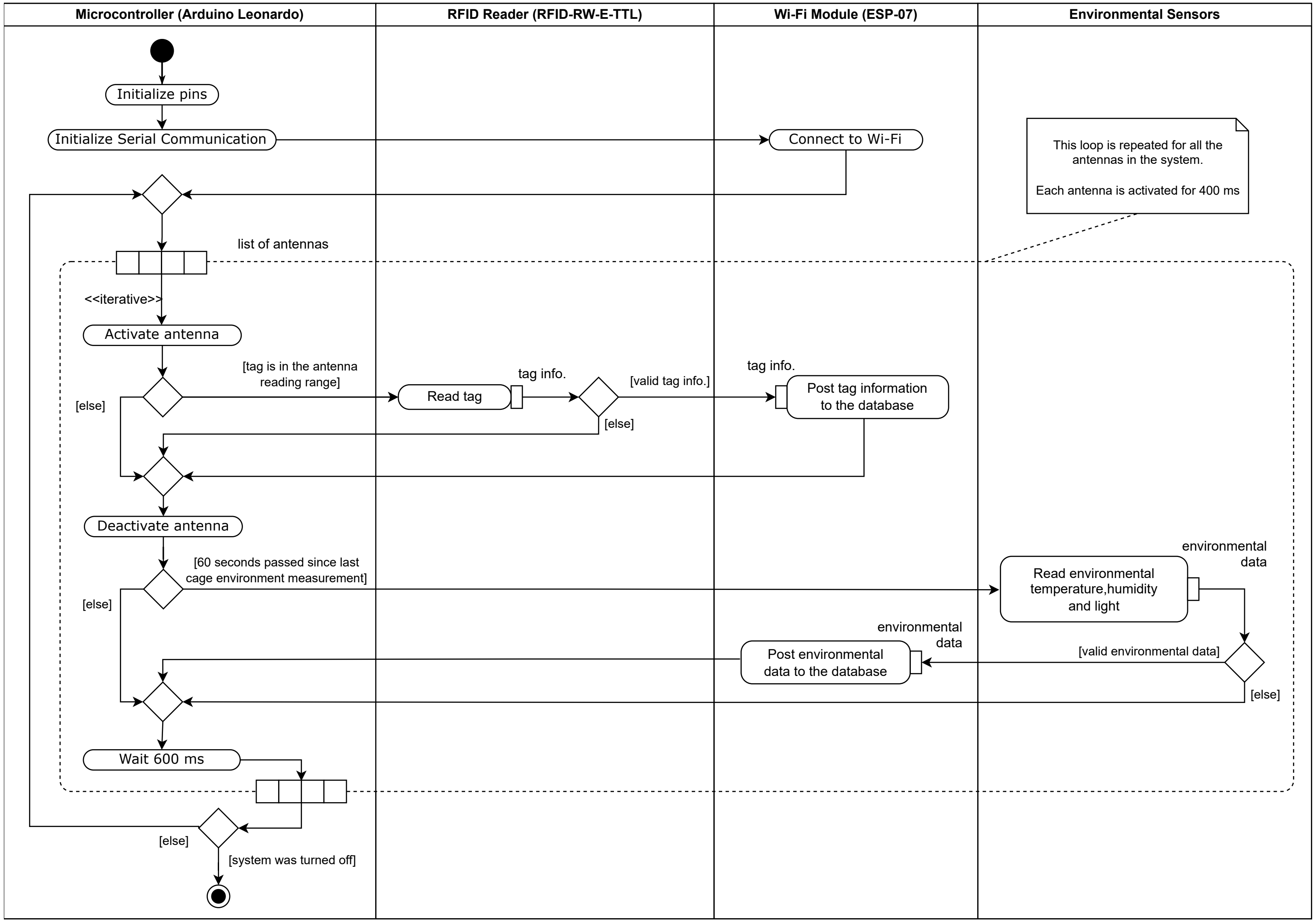


Figure 6.1: UML Activity Diagram of the Arduino code

6.1.2 Used libraries

In order to simplify the programming of the Arduino, several libraries were included, as can be seen in Table 6.1. To facilitate the interpretation of the tag codes that were read by the RFIDRW-E-TTL reader, the RFIDTag library was created.

Table 6.1: Libraries used in the Arduino code

Library	**Description**	**Reference**
WiFiEspAT.h	Implements the standard Arduino Wifi API using ESP8266 or ESP32 AT commands	[115]
DHT.h	Facilitates the communication with the DHT series temperature/humidity sensors	[116]
SoftwareSerial.h	Simulates serial communication on other Arduino digital pins	[114]
RFIDTag.h	Interprets the tag code that is read by the RFIDRW-E-TTL reader	-

6.1.2.1 RFIDTag library

An example code obtained by reading a Biotherm13 PIT tag using the RFIDRW-E-TTL reader can be seen in Figure 6.2. In this figure, each part of the code is described and compared with the code structure described in ISO 11784 and 11785, that was summarised in Tables 3.2 and 3.3.

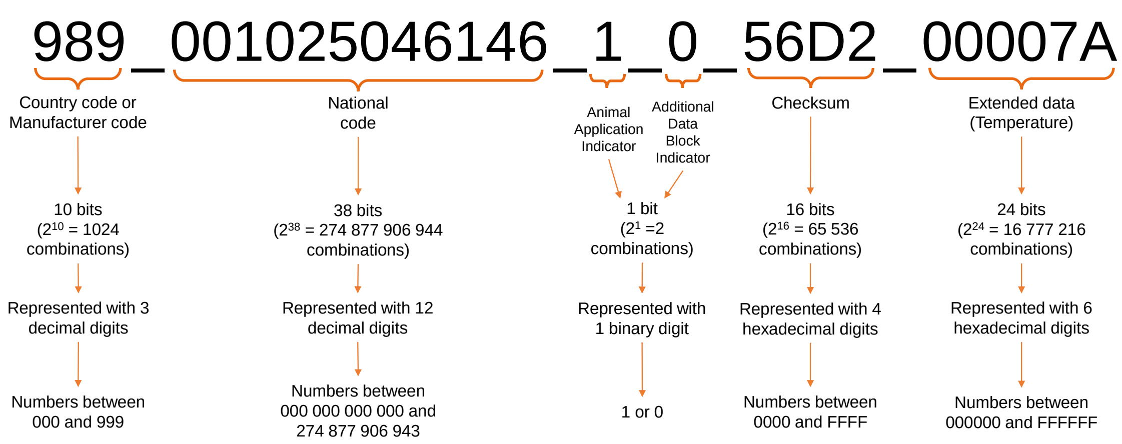


Figure 6.2: Code obtained by reading a tag using the RFIDRW-E-TTL reader, as well as the description of each of the parts of the code

As expected, the manufacturer code is 989 which corresponds to the code of Biomark, the company that sells the Biotherm13 PIT tags [117].

Although the additional data block indicator is set as 0, the tag still sends the extended data containing the temperature.

After understanding each of the components of the obtained code, the RFIDTag library was created. This library contains a class called "Tag", which contains fields with the information that is read in each tag and methods that help the user to use that information. The fields and methods defined for this class are summarised below. The full code of this library is in Appendix F.

```
#ifndef RFIDTAG_H
#define RFIDTAG_H

#include <Arduino.h>

class Tag
{
public:
    Tag(); // Create tag object with empty information
    Tag(String code); // Create tag object using the code obtained
       by the RFIDRW-E-TTL reader
    void print(); // Print the information present in the tag to the
        Serial Monitor
    String getID(); // Return the unique ID of the tag (country/
       manufacturer code + national code)
    float getTemperature(); // Return the temperature that the tag
       measured

private:
    String countryCode; // Country code or Manufacturer code (3
       digits)
    String nationalCode; // National code (12 digits)
    bool extraData; // Boolean that is true if the tag contains
       extra data; false, otherwise
    bool animalApplication; // Boolean that is true if the tag is
       being used in animal applications; false, otherwise
    String checksum; // Checksum (4 hexadecimal digits)
    float temperature; // Temperature read by the tag after being
       converted from six hexadecimal digits to a float

    bool stringToBool(String c); // Auxiliary method that converts
       "0" to False and "1" to True
};
#endif
```

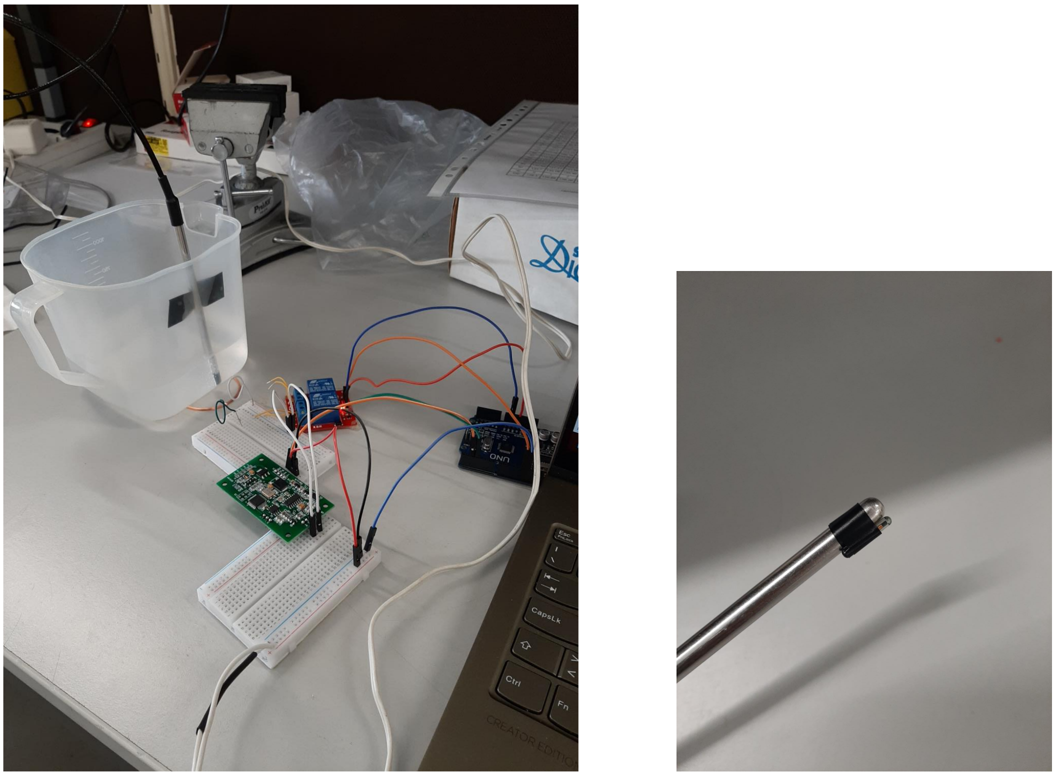
Since the information of the temperature is codified in a hexadecimal number, it was necessary to know how to convert that code into a temperature value. To do that, the obtained hexadecimal number was compared to the temperature obtained by other temperature sensors in different temperature conditions. This procedure is described in more detail below.

6.2 RFID tags temperature measurements validation

6.2.1 Water cooling experiment

As was seen in Figure 6.2, the RFIDRW-E-TTL reader represents the extended data of the tag in six hexadecimal digits, so there needs to be a way to convert those digits into the tag temperature.

To do that, water was heated in a recipient to approximately 46 ºC using a microwave. Then, a Biotherm13 PIT tag was glued to the probe of a pt100 temperature sensor and placed under the water. The code given by the tag as well as the resistance of the pt100 were measured every 30 second until the water cooled to about 28 ºC. The used experimental setup can be seen in Figure 6.3. This water temperature range was chosen because it covers the 33 ºC to 43 ºC range within which the Biotherm13 PIT tags are reported to operate.



(a) (b)

Figure 6.3: (a) Experimental setup used to measure the tag temperature during cooling and (b) close-up of the Biotherm13 PIT tag glued to the pt100 probe. The red module in (a) corresponds to a relay that disconnected and connected the coil to the RFIDRW-E-TTL reader every 30 seconds, controlling the pace at which the tag measured the temperature

The extended data sent by the tag as well as the temperature obtained from the resistance of the pt100 during this experimentation can be seen in full in Appendix G. An excerpt of that table can be seen in Table 6.2.

Table 6.2: Comparison between the temperature given by the pt100 and the code sent by the tag

Time since beginning (min:sec)	Temperature pt100 (ºC)	Extended data	Processed Extended data	Temperature PIT (ºC)
16:00	38.1	000181	181	38.5
16:30	37.9	00007F	17F	38.3
17:00	37.7	00017E	17E	38.2
17:30	37.6	00007C	17C	38.0
18:00	37.4	00007A	17A	37.8
18:30	37.3	000079	179	37.7
19:00	37.1	000177	177	37.5
19:30	36.9	000075	175	37.3
20:00	36.7	000174	174	37.2
20:30	36.6	000172	172	37.0
21:00	36.4	000171	171	36.9
21:30	36.2	000070	170	36.8
22:00	36.1	00006E	16E	36.6

Looking at Table 6.2, it is noticeable that only the three last digits of the extended data change with temperature. It is also possible to notice that the last two digits decrease with temperature, while the third to last digit varies between 0 and 1. If only the three last digits are considered and the third to last digit is converted to 1, a three digit hexadecimal number that decreases with the temperature is obtained, as seen in the column "Processed Extended data". If this number is converted from hexadecimal to decimal and then divided by 10, the value in the column "Temperature PIT (ºC)" is obtained. This value is close to the temperature obtained from the pt100.

Using this conversion, the comparison between the temperature measured by the pt100 and the PIT tag throughout the experiment can be seen in Figure 6.4.

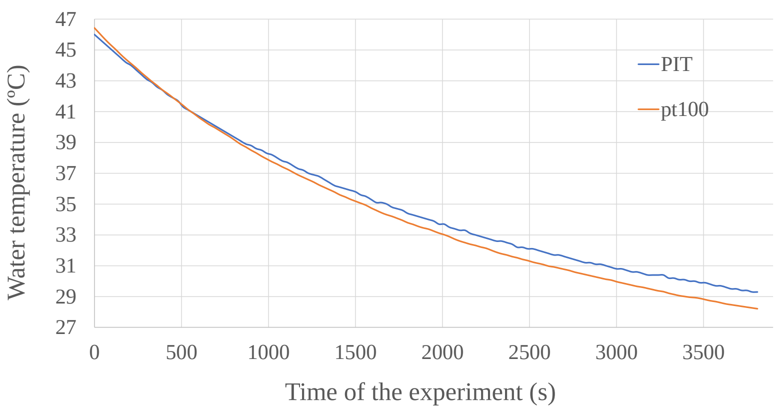


Figure 6.4: Comparison between the temperature measured by the pt100 and the Biotherm13 PIT tag in the water cooling experiment

Looking at this graph, it is possible to see that the temperature measured by the tag and the pt100 both decrease in a similar way while the water cools. For the lower temperatures, the temperature measured by the tag starts being approximately 1 ºC higher than the temperature measured by the pt100. One possible explanation is the fact that the tag is made of glass which has a worse thermal conductivity than the platinum of the pt100, resulting in a slower response to the water cooling. The mechanism used by the Biotherm13 PIT tag to measure the temperature may also have some associated error. These possible explanations are further explored in the next subsection.

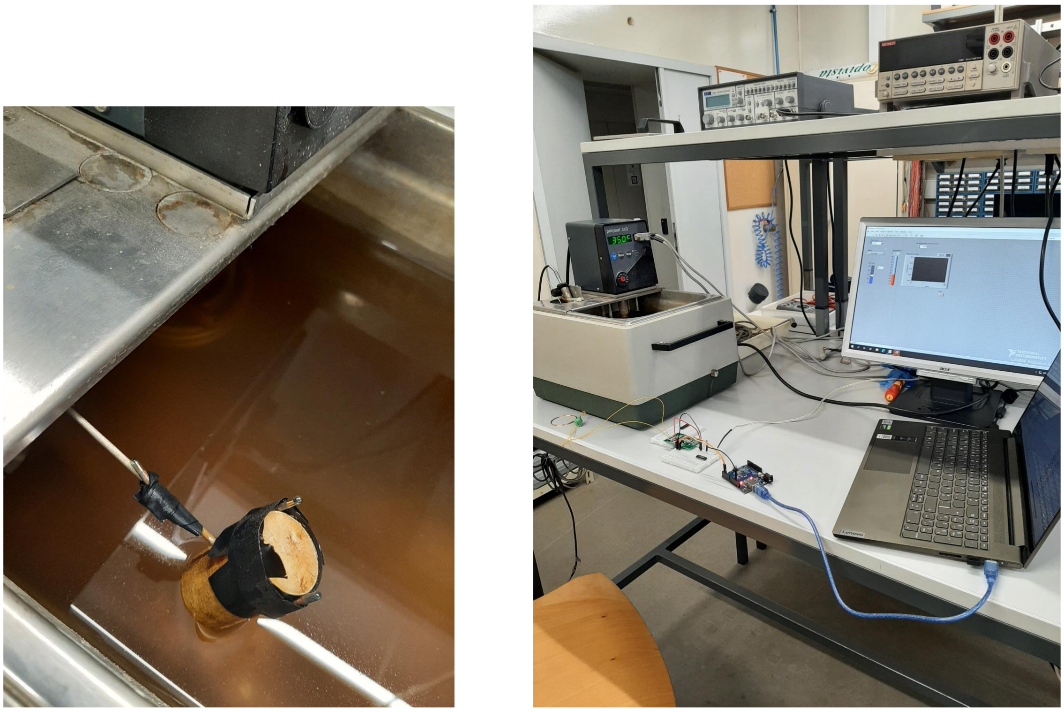
In any case, the temperature measured by the Biotherm13 PIT tag accompanied the temperature of the water, making it adequate to measure changes in temperature in mice or rats after certain procedure.

6.2.2 Circulation thermostat experiment

Instead of measuring the temperature of water during cooling, the circulation thermostat Huber Polystat CC3 was used. With this device, it was possible to set the water temperature to a constant value, enabling the tag to stay in a constant temperature for as long as desired before making the measurement. The temperature of the thermostat was controlled using a LabVIEW program.

Using this thermostat, three different Biotherm13 PIT tags were tested in four temperatures between 35 ºC and 41 ºC using the built system and an RFID reader produced by the same company as the tags (GPR+ Handheld PIT Tag Reader).

To do this experiment, three tags were glued to a cork stopper that was easily emerged and submerged in the water tank. Then, after choosing the water temperature on the LabVIEW program, the tags were submerged. Every two minutes, the cork was quickly emerged and one of the tags was read using the built system or the GPR+ reader, before being submerged again. This experiment was repeated for all the three tags, for the four temperatures, using the built system and the GPR+ reader. Since the GPR+ reader only operates in the reported reading range of the Biotherm13 PIT tags (33 ºC to 43 ºC), only temperatures inside that range were tested. The experimental setup used can be seen in Figure 6.5.



(a) (b)

Figure 6.5: (a) Close-up of the three tags glued to the cork stopper and (b) experimental setup used in the experiment with the thermostat

The full results of this experiment can be seen in Table 6.3.

Table 6.3: Temperature obtained with the built system and the GPR+ reader for three different tags and in four different temperatures

Tag	**Water Temperature (ºC)**	**Tag Temperature (ºC)**	
		Built System	**GPR+ Reader**
1	35.0	35.6	34.6
2	35.0	35.6	34.6
3	35.0	35.7	34.5
1	37.0	37.4	36.4
2	37.0	37.7	36.7
3	37.0	37.7	36.7
1	39.0	39.3	38.1
2	39.0	39.3	38.6
3	39.0	39.5	38.6
1	41.0	40.8	40.3
2	41.0	41.1	40.5
3	41.0	41.0	40.5

6.2.2.1 Comparing different tags using the GPR+ reader

One of the objectives of this experiment was to detect if different Biotherm13 PIT tags gave different temperature readings when subjected to the same temperature. To do that, the difference between the tag temperature measured with the GPR+ reader and the temperature of the water for three different Biotherm13 PIT tags was analysed as seen in Figure 6.6.

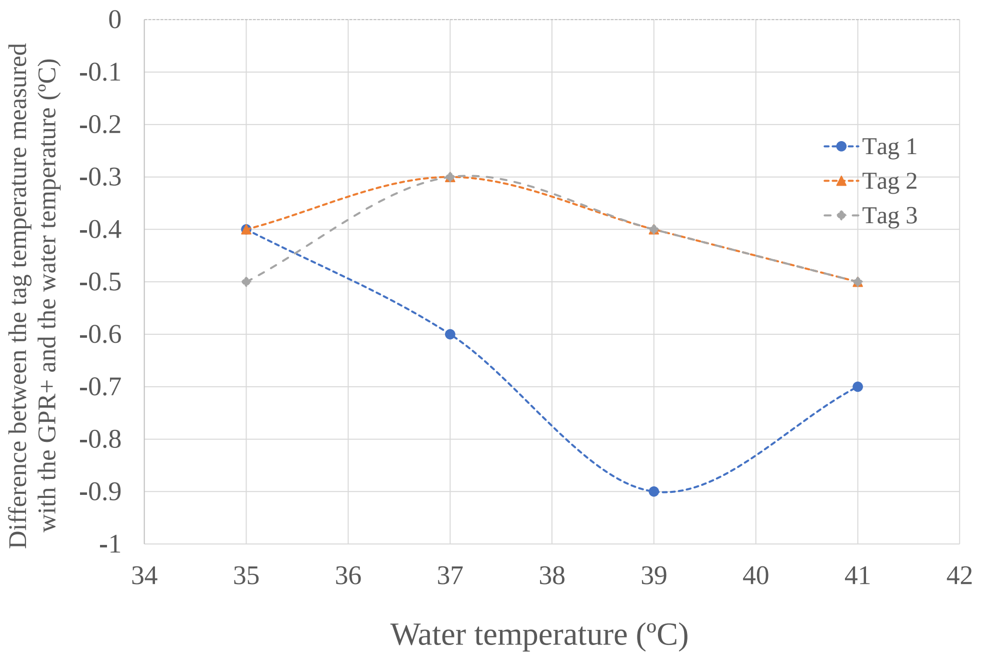


Figure 6.6: Difference between the tag temperature measured with the GPR+ reader and the temperature of the water for three different Biotherm13 PIT tags

By looking at the chart it is possible to see that although tags 2 and 3 measured similar values of temperature, tag 1 measured a temperature below the other two tags, which shows that there are some differences between different tags.

6.2.2.2 Comparing the built system with the GPR+ reader

Another important objective of this experiment was to know whether the built system was reporting the same temperature as the GPR+ reader. Figure 6.7 shows the difference between the tag temperature measured with the built system and the GPR+ reader. For each temperature, a mean of the values given by each tag was calculated.

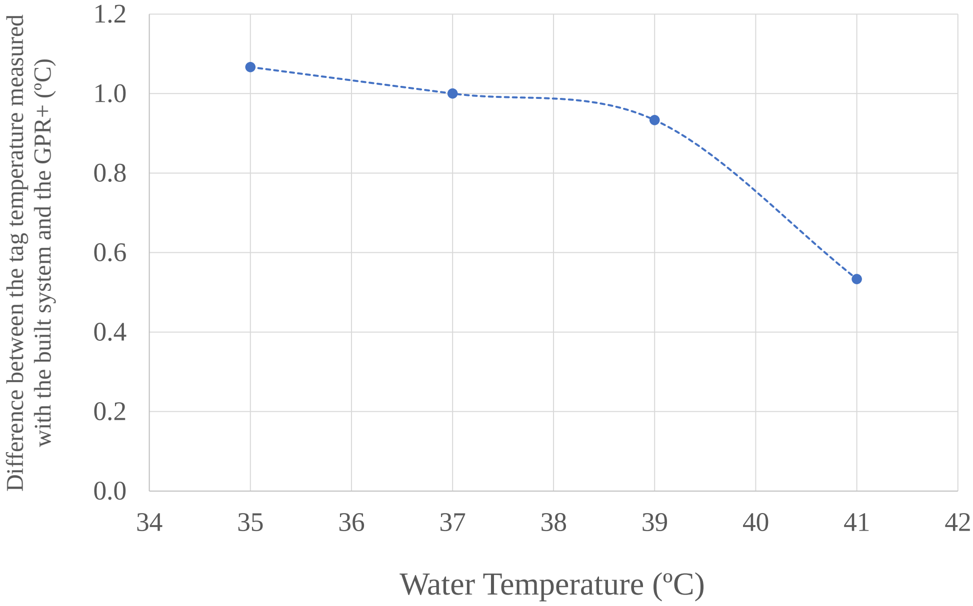


Figure 6.7: Difference between the average tag temperature measured with the built system and the GPR+ reader

This chart shows that the built system measures a temperature approximately 1 ºC above the temperature measured by the GPR+ reader. When the water temperature was 41 ºC this difference was closer to 0.5 ºC. This suggests that the GPR+ reader may have a more advanced algorithm that converts the tag extended data to the temperature.

A close approximation of this algorithm is to subtract 1 ºC from the temperature that was interpreted from the tag extended data.

6.2.2.3 Comparing the temperature of the tag with the water temperature

Finally, the difference between the tag temperature measured with each reader and the water temperature was also calculated, as can be seen in Figure 6.8. Once again, for each temperature, a mean of the values given by each tag was calculated.

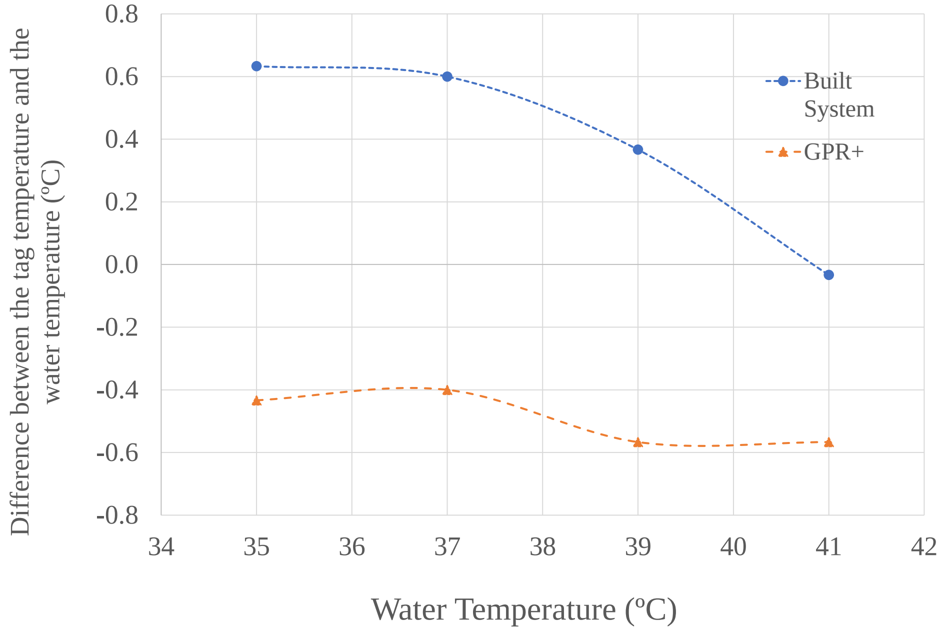


Figure 6.8: Difference between the average tag temperature measured with each reader and the water temperature

When it comes to the GPR+ reader, the error is approximately constant for the tested temperatures. For higher temperatures, the error goes from -0.4 ºC to -0.6 ºC, which may be related to the fact that since the tag is quickly emerged before the measurements, for higher water temperatures the tag temperature may decrease more before being measured.

For the built system, the error changes more significantly with the temperature, once again suggesting that the GPR+ reader may have a more advanced algorithm to convert the tag information into the temperature.

6.2.3 Conversion between the tag extended data and the temperature

In conclusion, the water cooling experiment (Section 6.2.1) showed that a value close to the real temperature is obtained if the three last digits of the tag extended data are converted from hexadecimal to decimal and then divided by 10 (while forcing the third to last digit of the hexadecimal number to be 1). By comparing the temperature measured by the built system and by the GPR+ reader in the thermostat experiment (Section 6.2.2.2) it was shown that the value measured by the built system can be approximated to the value measured by the GPR+ reader by subtracting the temperature by 1 ºC. These conclusions were then implemented in the RFIDTag library previously mentioned in Section 6.1.2.1.

Further experimentation with more values for the water temperature and using a methodology that does not require the tags to be emerged before the measurement could be helpful to better mimic the conversion from the extended data into the temperature that is implemented by the GPR+ reader.

6.3 User Interface

As was mentioned in Section 4.2, an important part of a monitoring system is having a user-friendly interface. With that a mind, a website that works as the user interface was created using MySQL, HTML, CSS, PHP, and JavaScript. The website has several functionalities, like adding, editing, and deleting animals and cages from the database, setting alarms, exporting the results to Excel, adding treatments in specific time instants, and also checking the results in a detailed way. A use case diagram of the website can be found in Figure 6.9.

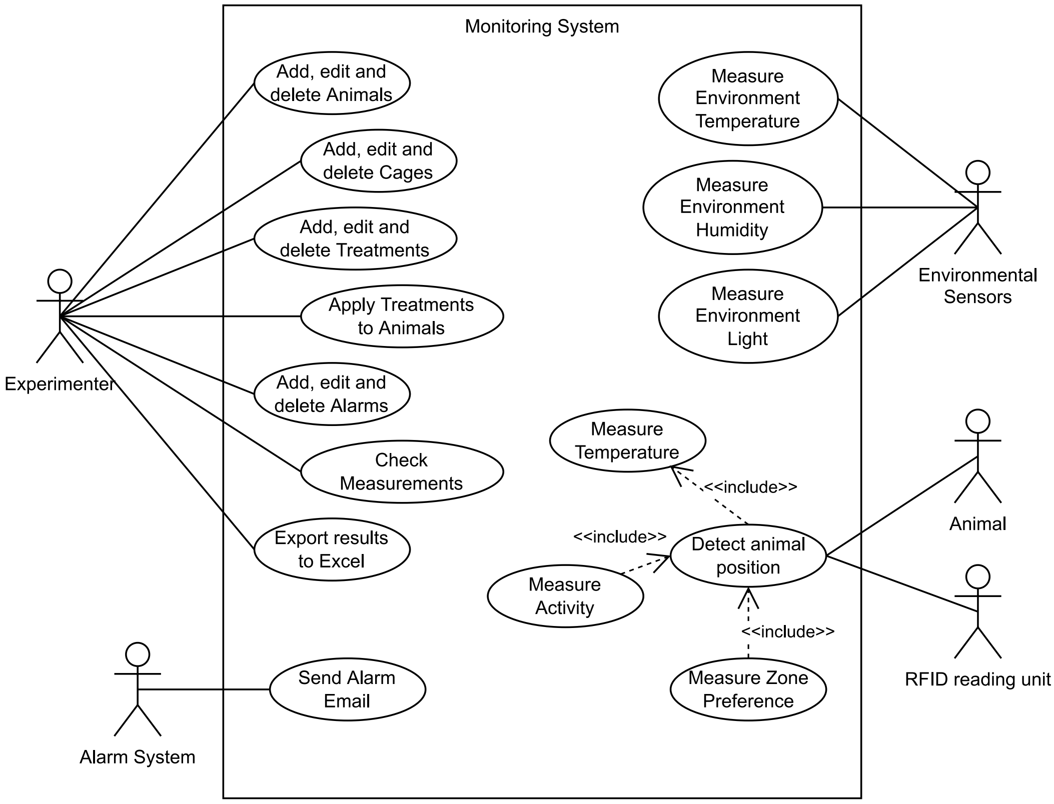


Figure 6.9: UML Use Case Diagram of the user interface

The main advantage of having the user interface in a website is avoiding the need for the user to buy proprietary software to check the results and also make the interface compatible with any type of device, like computers, mobile phones, or tablets.

To avoid buying a website domain for the user interface, the system was instead tested in a local server created by a computer using the XAMPP software. Any device in the same network would have access to the user interface by connecting to the IP of that computer.

6.3.1 Database

One of the most important parts of the website is the database. Since the monitoring system is constantly updating the database, the results can be checked in real time.

A class diagram of this database can be seen in Figure 6.10. The MySQL code that creates this database is in Appendix H.

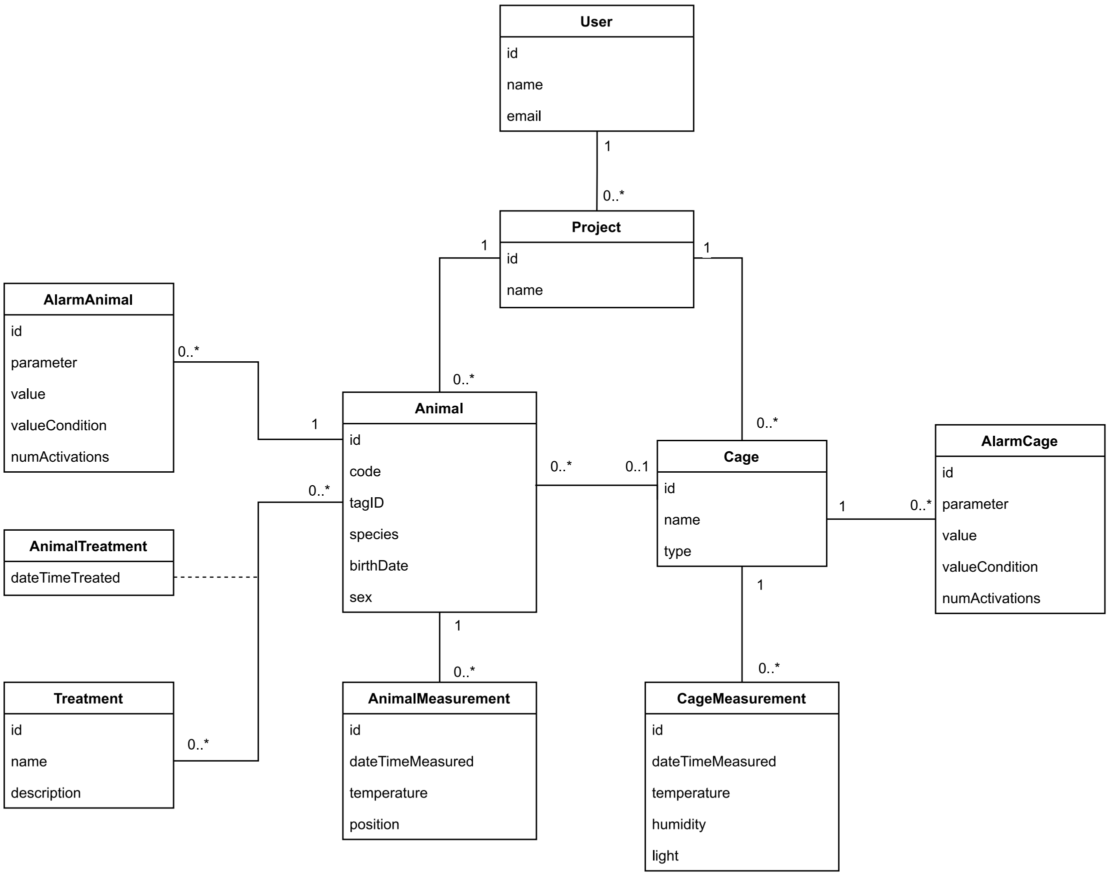


Figure 6.10: UML Class Diagram of the database

This class diagram shows the ten classes that are stored in the database.

In summary, the database contains users, with a name and an email, that can create any number of projects. Each project has a name and its own animals and cages. For each animal, we have a code that describes the animal and the ID of the implanted tag. The species, birth date and sex of the animal are also included. When it comes to the cage, the name and type of cage are stored.

Each animal can have several AnimalMeasurement, that include the date and time of the measurement, and the temperature and position of the animal in that measurement. The CageMeasurement also includes the data and time of the measurement, as well as the cage temperature, humidity, and light.

For each animal or cage, an alarm can be defined. Each alarm has the parameter that is being controlled, the value and valueCondition (above or below) that trigger the alarm, and the number of activations. The number of activations increments each time the condition for the alarm is reached. With this attribute, the alarm can be triggered only if the alarm condition is reached several times in a row, avoiding false positives.

Finally, the database also contains the name and description of the treatments applied. The data and time when each treatment is applied to an animal is also stored in the database.

6.3.2 Demonstration of the user interface

The homepage of the website can be seen in Figure 6.11. In this page, the user can choose the project that they want to check and also set the email that is used for the alarms. In the top of the page, there is the logo of the monitoring system and a navigation menu with several pages of the website.

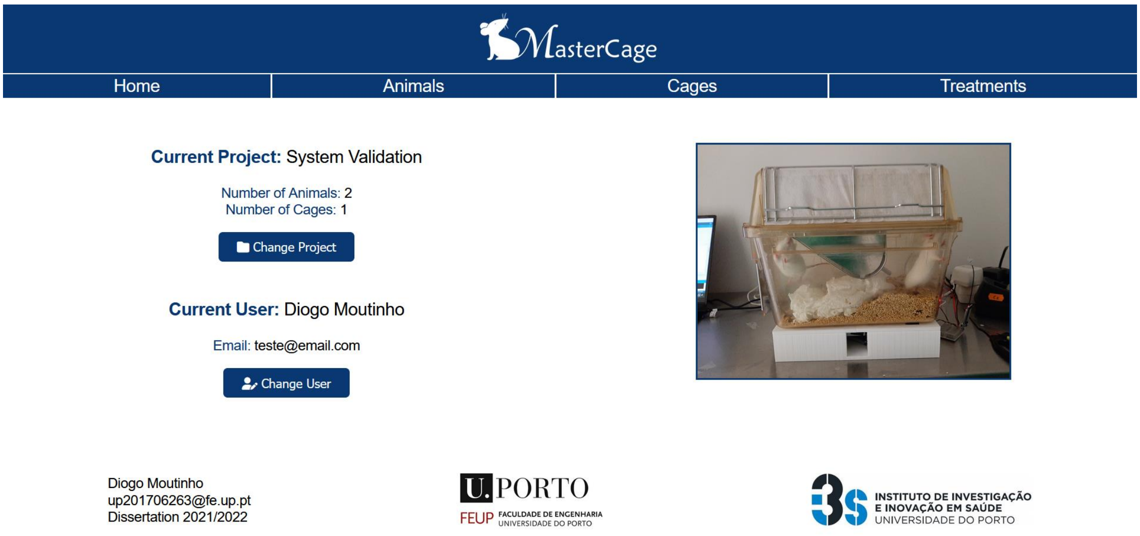


Figure 6.11: Homepage of the user interface

6.3.2.1 Animals Menu

By opening the "Animals" page (Figure 6.12), a table with the information of the various animals in the selected project is shown. The last two columns of the table contain a button to check the information of the animal in more detail and a button to delete the animal. Before deleting any animal, a warning is shown.

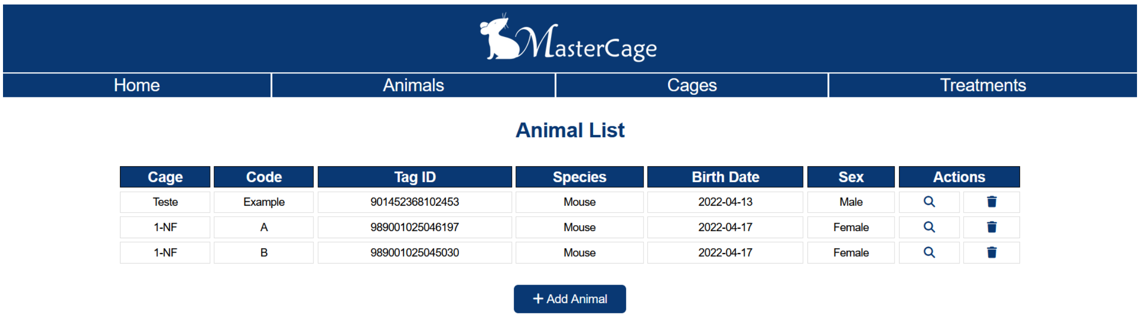


Figure 6.12: Page where the user can check the list of animals in the project

Below the table, there is a button to add new animals to the project (Figure 6.13).

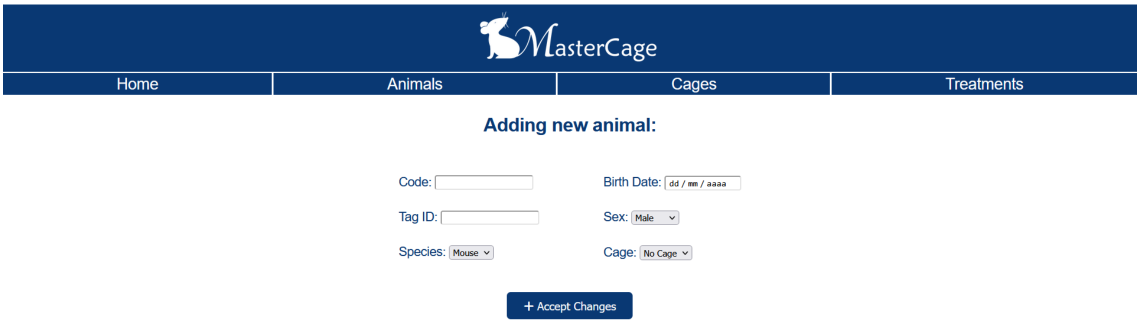
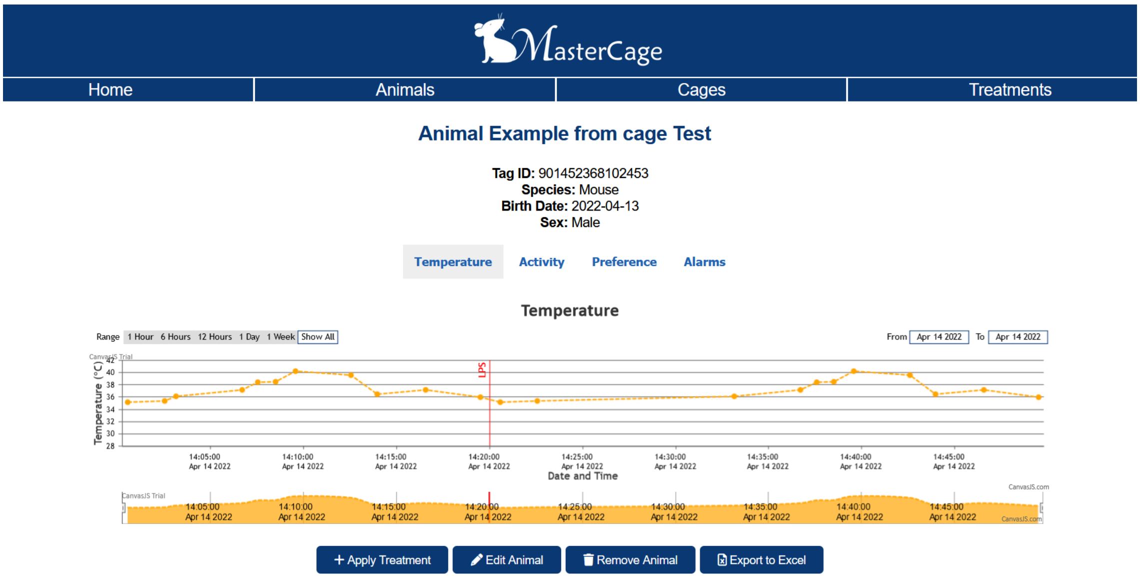


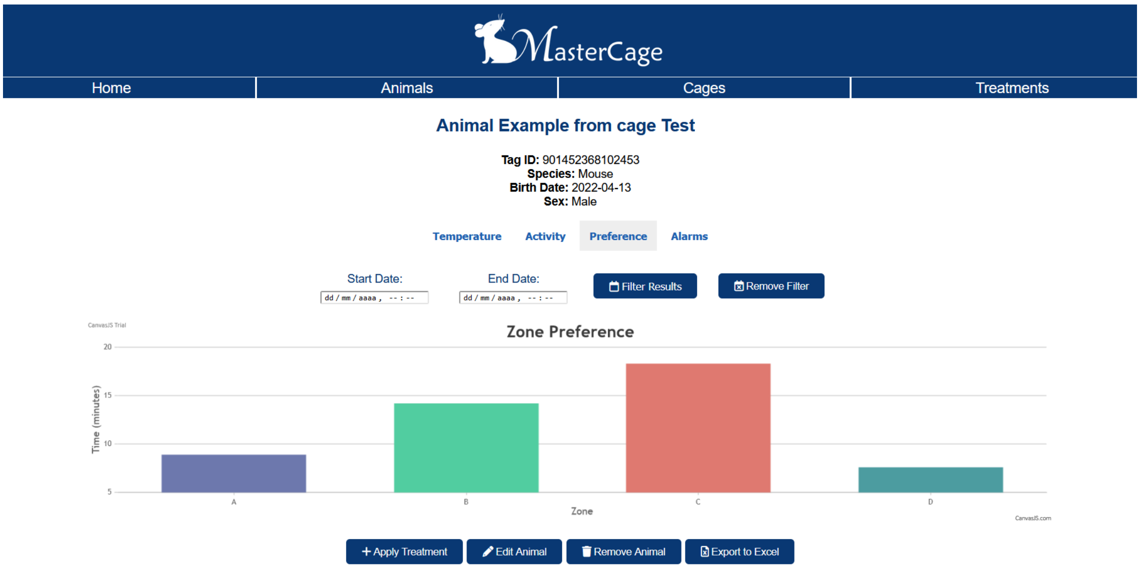
Figure 6.13: Page where the user can add new animals to the database

If the user decides to check the information of an animal in more detail, a page with four tabs is shown (Figure 6.14): Temperature, Activity, Preference and Alarms. Regardless of the tab that is active, the page has buttons to apply a treatment to the animal, edit the animal and delete the animal. There is also a button to export the measurements of that animal to an Excel file.



(a) Temperature tab

(b) Activity Tab



(c) Preference Tab

(d) Alarms Tab

Figure 6.14: Tabs of the page with the information about each animal

The Temperature tab (Figure 6.14a) shows a chart with the animal temperature, where the user can choose the range of the data being shown, facilitating the data interpretation. The chart background colour changes according to the light levels at the time of each measurement (grey during the dark phase and white in the light phase). If a treatment is applied to an animal, a vertical red line appears in the chart in the date and time of the treatment application.

The Activity tab (Figure 6.14b) is similar to the temperature tab, but instead of showing the temperature of the animal, it calculates the distance walked by the animal in each time bin, which duration is defined by the user. This activity in each bin is obtained by summing the distance between the centre of the antennas where the animal was detected in each consecutive measurements, in each time bin.

The Preference tab (Figure 6.14c) shows a bar chart with the time that the animal spent in each position of the cage. This preference is calculated by counting the time that it took for the animal to be detected in a different position. For example, if the animal was detected in position A, the system considers that it stayed in that position until it was detected in a different position.

Finally, the Alarm tab (Figure 6.14d) shows the alarms that are active for that animal. If the user decides to add an alarm, they can choose what parameter they want to control, as well as the condition that must be meet to activate the alarm. The user can also choose to automatically set the same alarm to all the animals in the same cage. When an alarm is triggered, the user receives an email informing which alarm was activated.

6.3.2.2 Cages Menu

The "Cages" page work similarly to the Animals page, showing a table with the information of the cages in the project and a button to add new cages to the system (Figure 6.15).

Figure 6.15: Page where the user can check the list of cages in the project

The page with more detailed information about each cage is also similar to what happens to the animals, including tabs for each of the signals measured and a tab for the alarms. The main difference is that there is an additional tab with a list of all the animals that are housed in that cage. As an example, the tab with the humidity of the cage can be seen in Figure 6.16.

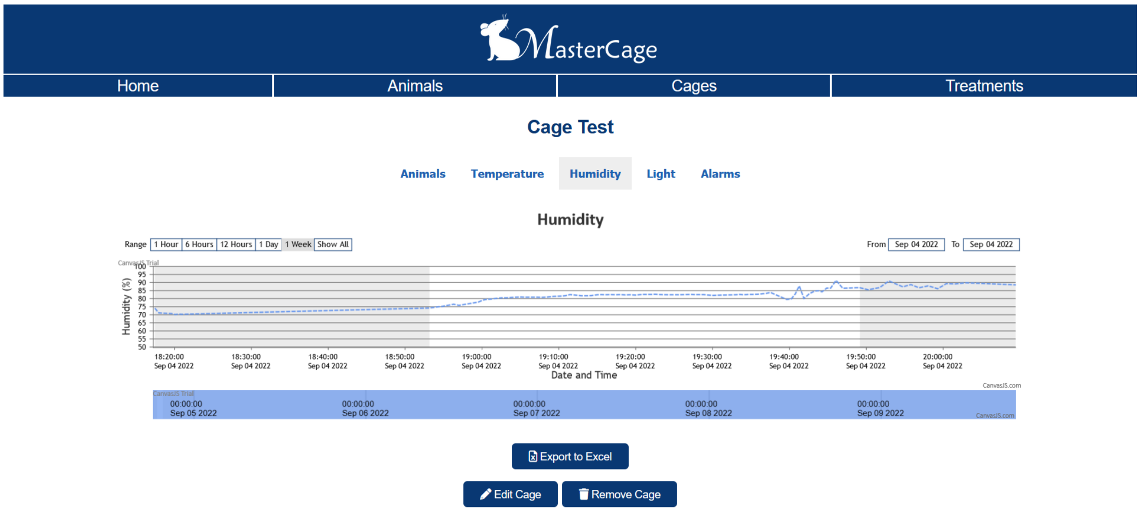


Figure 6.16: Page with the information about each cage. The tab with the humidity is selected in this figure

6.3.2.3 Treatments Menu

The "Treatments" page shows the list of treatments in the database (Figure 6.17). The user can create, edit or delete treatments.

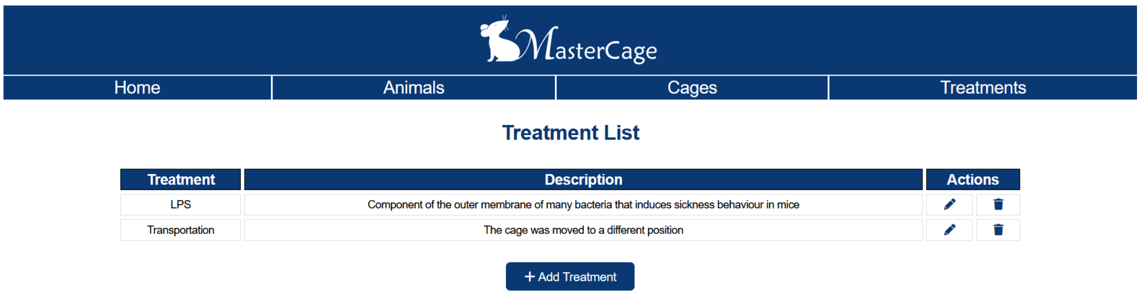


Figure 6.17: Page where the user can check the list of treatments in the database

The user can add to this list any procedure that was applied to the animals that may be relevant, including drugs, treatments or even any procedure done to the cage that may cause physiological changes in the animals (for example, the transportation of the cage).

6.4 Summary

The microcontroller plays a major role in the built system, controlling the activation of the antennas, communicating with the various sensors and even ordering the Serial Wi-Fi Module to update the database with the measured information.

One of its functions is receiving the temperature of each tag. Since this temperature is coded into a hexadecimal number, a conversion algorithm needed to be developed. To do that, several tests were done where the hexadecimal code sent by the tag was compared with the temperature obtained by other temperature sensors.

Another important part of the system is the user interface. In this case, a website where the user can check all the measurements was developed. This website also enables the user to do several other actions, like adding, editing, and deleting animals and cages from the database, setting alarms, exporting the results to Excel and adding treatments in specific time instants.

Chapter 7

System Testing

This chapter presents the test done in mice to validate the system built. This test had a duration of about 2 hours and was carried out at the i3S animal facility.

7.1 Preparation for the pilot test

Before starting the two hour pilot testing of the system at the i3S animal facility, two mice were subcutaneously implanted in the left flank with the Biotherm13 PIT tags under short (< 5 minutes) isoflurane anaesthesia, using a syringe with 12 G needle, with the puncture site sealed by cyanoacrylate-based surgical glue. The tags were implanted in the left flank to reduce the distance between the tags and the antennas.

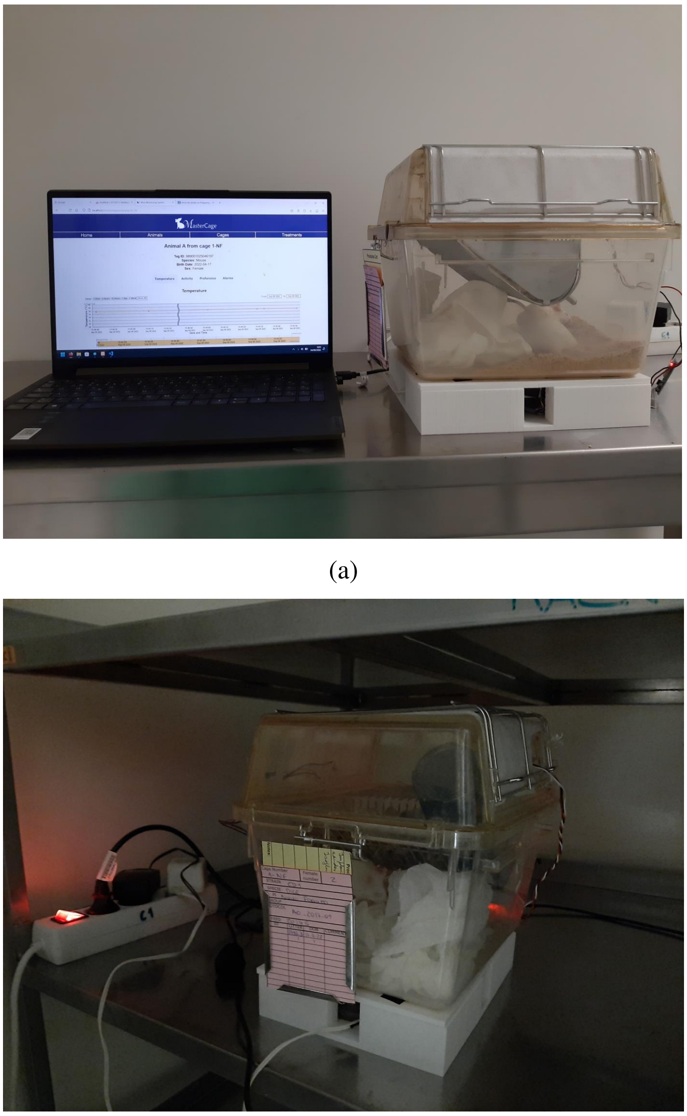
Then, the 3D printed box with the hardware components already assembled was placed below the cage where the two mice were housed, as seen in Figure 7.1. A computer was placed next to the system since it needed to create the local server where the data was being sent, as well as to check whether the system was running correctly.

It was also important to add the cage and the animals where the tests where going to be done to the database, before starting the experiment.

7.2 Pilot test results

During this pilot test, it was possible to see that the antennas did not always detect the mice, especially in the zones of the cage that had more bedding. This can be explained by two main reasons: all the wiring and components of the assembled system reduced the reading range of the antennas, and the time intervals between the activation of each antenna resulted in the mice only being detected if they were next to the antenna that was currently active.

The reading range could be improved by doing a more careful assembly of the system, reducing the interference that may result from the wiring of the antennas to the circuit. Since the wire



(a)

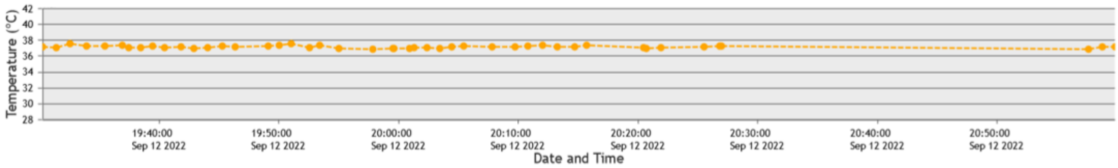
(b)

Figure 7.1: (a) System placed below the cage where the test was conducted, next to a computer showing the measurements being done, and (b) system in the rack where it stayed during the testing. The lights are turned off in (b) because the tests were done during the dark period

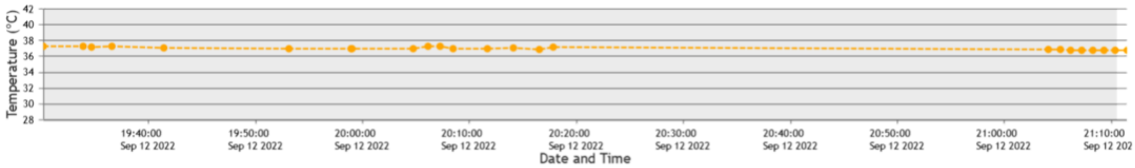
of antennas was very fragile, it sometimes broke during the assembly, which can result in a small change of the resonant frequency of the antenna, and consequently reducing the reading range.

The timing between the activation of each antenna could be reduced by replacing the HE721A0500 relays with other types of switches that opened and closed more quickly. Replacing the DHT22 temperature/humidity sensor for other sensor would also help since this sensor had some instabilities when it had short timings to operate, possibly resulting from the fact that it only uses 1 pin to communicate with the Arduino. A sensor that included a pin for a clock signal would be more robust.

Even though the mice were not always detected, they were detected enough times to evaluate their temperature throughout the time. The temperature of the two mice can be seen in Figure 7.2.



(a) Mouse "A"



(b) Mouse "B"

Figure 7.2: Temperature of mice "A" and "B" during the 2 hours of testing

Looking at these graphs, it is possible to see that both mice maintained a constant temperature around 37 ºC during the 2 hours of testing, which was to be expected. These graphs also show that between around 20:30 and 20:50, the antennas did not detect any of the animals. One possible explanation of this is that both mice were huddling together next to the same antenna, which would result in the system not detecting any of the tags. It is also possible to see that mouse "A" was detected more often than mouse "B", which can indicate that the implant site of the tag in mouse "A" was closer to the antennas than the tag in mouse "B", or spatially oriented in a more favourable way (i.e. more vertically).

When it comes to the activity and zone preference, the results would be more conclusive if the tags were always detected by the antennas. Either way, by looking at the activity of mouse "A" in Figure 7.3, the time period where both mice were standing still can be perceived, since the activity between 20:30 and 20:45 was 0 cm. This graph also shows that in the first hour of testing the animal had more activity than in the second, which is expected since mice have patterns of increased and decreased activity throughout the day.

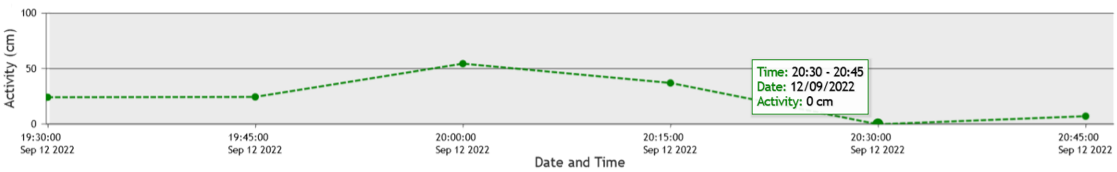


Figure 7.3: Activity of mouse "A" during the 2 hours of testing. The tooltip for the activity between 20:30 and 20:45 is being shown, since the computer cursor is over that point

The chart with the zone preference of mouse "A" can be seen in Figure 7.4. This chart shows an unexpected preference for the spots over antennas "C" and "D". Since these two antennas correspond to the antennas in the middle, one possible explanation is that they detected the mice more often than the other antennas, since the mice have to cross them to move from one side of the cage to the other. If the mice were always detected by every antenna, a more even distribution between the different zones would be expected. Nevertheless, each antenna detected mouse "A" at least once, proving that all antennas were working.

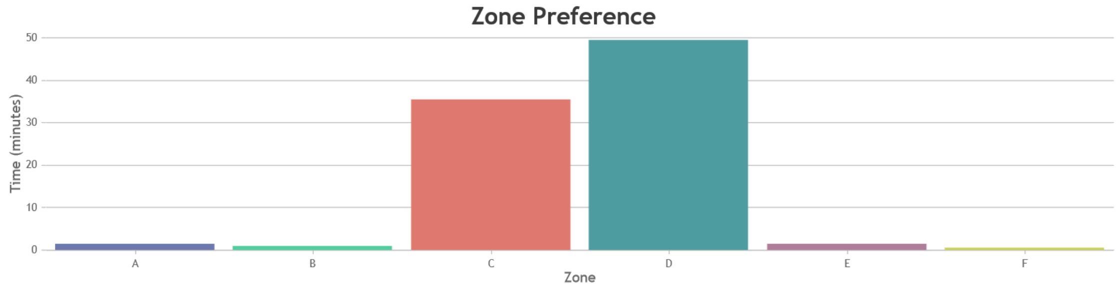
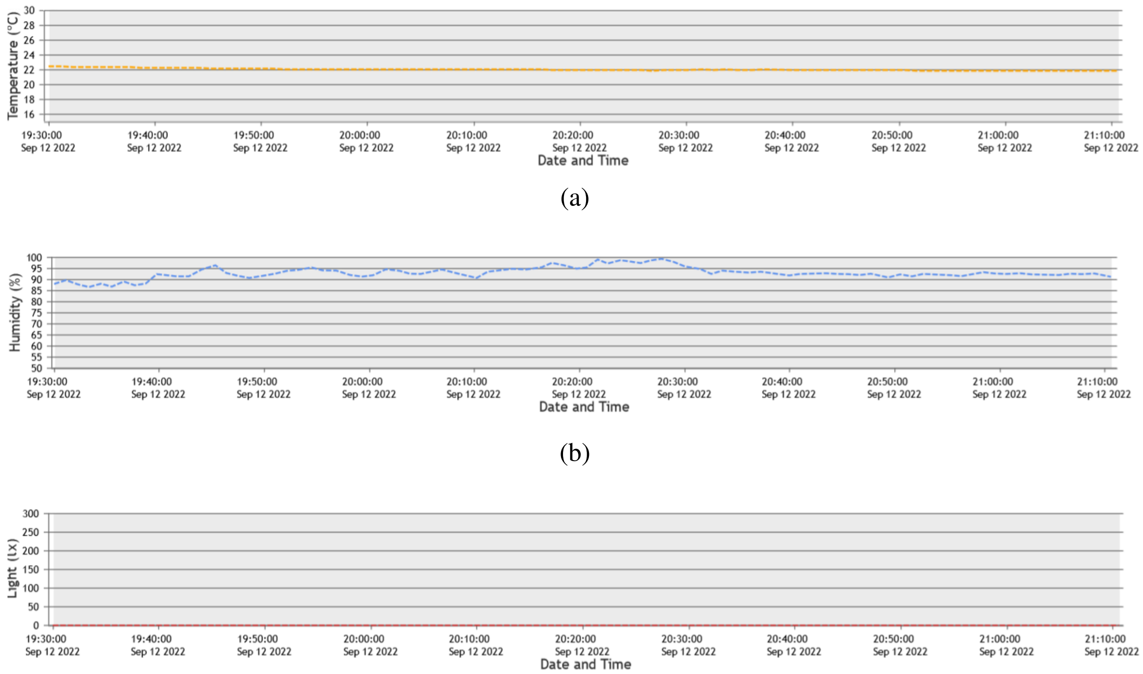


Figure 7.4: Zone preference of mouse "A" during the 2 hours of testing

When it comes to the cage environment, the temperature, humidity, and light intensity of the cage can be seen in Figure 7.5.



(a)

(b)

(c)

Figure 7.5: (a) Temperature, (b) relative humidity, and (c) light intensity in the cage during the 2 hours of testing

As it is possible to see, the cage temperature remained constant around 22 ºC during the 2 hours of testing, which is expected since the room temperature was defined to be around 20 ºC and, as was previously said, the temperature inside the cage tends to be different from the room temperature.

Looking at the relative humidity, it oscillated around 85% and 100%. Although these values may seem high, the relative humidity of the room was defined to be around 100%. The relatively large oscillation may be related to the fact that the DHT22 was operating near the top of its reading range, resulting in a worse accuracy.

Finally, the light intensity had a value of 0 lux throughout all the testing time, since the tests were done during the dark period.

7.3 Summary

The system was validated at the i3S animal facility in a cage with two mice during 2 hours.

Even though the system was not able to always detect the position of the mice, the results for the mice temperature and the cage environment were as expected. On the other hand, the activity and zone preference would benefit from a system that was able to fully detect all the positions where the mice were.

Chapter 8

Conclusions and Future Work

8.1 Conclusions

Animal experimentation, particularly with rodent models, is essential for biomedical research. Since there are many associated ethical challenges, it is important to reduce the suffering caused to the animals, as well as to produce results as reliable as possible, minimising subjective bias and effects caused by the presence of the experimenters themselves. One possible approach for these problems is the use of automatic monitoring systems. These systems should record data 24/7, not needing the direct influence of the experimenter. They can also help detecting clinical signs earlier, so that action can be taken as soon as possible, reducing or avoiding animal suffering.

With all this in mind, an affordable automatic monitoring system for mice or rats based on implantable RFID tags technology was developed. This system not only monitors each individual animal in a cage, but also the environment of each cage.

The monitoring of each animal is done by implanting each animal with a thermosensitive PIT tag and then placing six RFID reader antennas below the cage, so that by knowing which antenna detects each tag at each time-point, the position of each animal can be estimated, alongside its body temperature. Using this information, it is also possible to estimate if each animal has a preference for a certain zone of the cage, and whether it is more or less active. An RFID reader that follows the same communication protocol as the PIT tags was used. Since this reader could only be connected to one antenna at a time, each antenna was connected to a reed relay, so that each antenna was only connected to the RFID reader every time the respective reed relay had its switch closed. By quickly switching between the relay that was closed it was possible to read tags in all the antennas sequentially. The system was controlled using an Arduino Leonardo and the measured information was sent to a database using a Wi-Fi Module. Finally, the hardware components were assembled in a box that was designed and 3D printed.

Since the temperature information of the tag was a hexadecimal number, it was necessary to develop an algorithm to convert that information into the temperature. To do that, the hexadecimal temperature number sent by the tag was compared with the temperature obtained by other sensors in different temperatures. It was possible to conclude that the temperature could be obtained by converting the three last digits of the tag temperature data from hexadecimal to decimal (while forcing the third to last digit of the hexadecimal number to be 1), dividing by 10 and then subtracting the obtained value by 1 ºC.

A hand-made antenna was built in the lab and tested with the RFID reader. This antenna was then compared with other two commercially available antennas to see which one had the longer reading range. The antenna with the longer reading range was then chosen, since it was expected that the reading range would decrease due to the interference of the other components.

Some environmental parameters of the cage are also monitored. The temperature and humidity is measured using a temperature and humidity sensor and the light is measured with a LDR. These sensors were placed inside the cage, above the cage grid.

A website was created to show the data that was sent to the database. The website also has several other additional features, such as adding, editing, and deleting animals and cages from the database, setting alarms, exporting the results to Excel, adding treatments in specific time instants, and also checking the results in a detailed way.

Finally, the system was tested at the i3S animal facility using two mice during 2 hours. This final test showed that the system was capable of continuously monitoring the mice temperature, as well as the cage environment temperature, humidity, and light. However, the tags were not always detected by the antennas, making the activity and zone preference measurements less reliable. This has two main explanations: the assembly of the system significantly reduced the reading range of the antennas and the system took too much time to switch between each antenna, which led to the mice not being detected most of the times.

Either way, this work showed that it is possible to develop an affordable automatic monitoring system for mice and rats, contributing to more reliable results and less suffering caused to animals.

8.2 Future Work

There are several improvements that can be done to the system.

Firstly, more antenna shapes and sizes should be tested to better understand how each of the properties of the antenna affect the reading range. With that information, antennas with specific reading ranges could be obtained.

Although a close approximation for the temperature that is measured by the Biotherm13 PIT tags was obtained, further studies where more values of temperatures are tested and the tags do not need to be emerged from the water before the measurement should be carried.

Looking at the built system, the main improvements would be improving the reading range and reducing the time that it takes to switch between the different antennas.

The reading range could be improved by choosing other antenna models and by trying to do a better assembly of the system, where the interferences to the antenna operation are reduced.

The switching time could be improved by using a switch that is able to open and close more quickly than the reed relays used. Some possible components are multiplexers with good isolation between the different independent outputs (so that there is not interference between the different antennas) or transistors. The DHT22 sensor would also need to be replaced by other temperature and humidity sensor, since it has some difficulties sending the data to the Arduino when the system is operating very quickly.

Using an Arduino with integrated Wi-Fi communication would also make the system more robust.

Since internet connection may not always be available at the animal facility, adding a SD card module to the system would be interesting. The system could record the measurements to the SD card if no Wi-Fi signal was detected, so that the user could then export the data that was stored in the card into the website.

Finally, the monitoring system should be tested for a longer time period, including both the dark and light phases. This would be important to test if the system is capable of detecting the expected decrease of activity during the light phase.

Appendix A

Summary of the State of the art

This appendix contains a table with a summary of the products that were mentioned in this work. Apart from the product name (or paper author), description and parameters measured, this table also presents if each product can distinguish between multiple animals in the same cage and if it is invasive for the animal.

Table A.1: Summary of several products that are available in the market or described in literature

Product Name or Paper Authors	**Small Description**	**Distinguishes measurements of animals on the same cage?**	**Invasive?**	**Animal parameters measured**	**Reference**
STARR Life Sciences - In-Cage Running Wheels	Running Wheel added to the cage	No	No	Distance traveled and time spent on the wheel	[84]
Panlab - Grip Strength Meter	Grid that measures the maximum force shown by the animal	Yes, by individual handling by the experimenter	Minimally, as it requires handling by the experimenter	Gripping Strength	[85]
González-Sánchez et al.	Electrodes placed on the cage as well as environmental sensors	No	No	Heart Rate and Breathing Rate	[4]
STARR Life Sciences - Home Cage Infrared Motion Detector	Infrared light motion detector	No	No	Activity	[86]
Panlab - SMART Video Tracking System	Video analysis system	Yes, with the Social Interaction module	No	Activity and certain behaviours	[87]

Table A.1: Summary of several products that are available in the market or described in literature

Product Name or Paper Authors	**Small Description**	**Distinguishes measurements of animals on the same cage?**	**Invasive?**	**Animal parameters measured**	**Reference**
N. H. Franco et al	Automatic thermal image analysis system	No	No	Body surface temperature	[46]
UID - Mouse Matrix	Reading units placed on several places of the cage	Yes, with RFID	Minimally Invasive	Activity, preference and temperature	[91]
Actual Analytics - Home Cage Analyser	Reading units placed on several places of the cage and an intrared video camera on the side	Yes, with RFID	Minimally Invasive	Activity, preference, interactions, temperature and some behaviours	[91]
Ahloy-Dallaire et al.	Reading units next to a food dispenser and a body weight sensor	Yes, with RFID	Minimally Invasive	Food eaten and body weight	[12]
STARR Life Sciences - E-Mitter Telemetry Implants	Small devices implanted on the animal	Yes, with a technology similar to RFID	Yes, since surgery is needed, and are larger than PIT tags	Temperature, activity, and heart rate	[92]

Table A.1: Summary of several products that are available in the market or described in literature

Product Name or Paper Authors	**Small Description**	**Distinguishes measurements of animals on the same cage?**	**Invasive?**	**Animal parameters measured**	**Reference**
STARR Life Sciences - MouseOx Plus	Device attached to the animal that measures physiological signals	Yes	Yes	Heart rate, breathing rate, arterial saturation, temperature, activity, and others	[93]
TSE Systems - Neurologger	Device implanted in the head of the animal that measures physiological signals	Yes	Yes	EEG and EMG	[93]
TSE Systems - IntelliCage	Multiple water bottles with nose poke holes that can be personalised	Yes, with RFID	Minimally Invasive	Behaviour and water consumed	[102]
TSE Systems - PhenoWorld	Multiple modules that share the same interface	Yes, with RFID	Minimally Invasive	Depends on the modules	[25]

Appendix B

Pinout of the components

This appendix contains the pinout of the components used in this work. A description of the pins is also presented.

B.1 RFIDRW-E-TTL

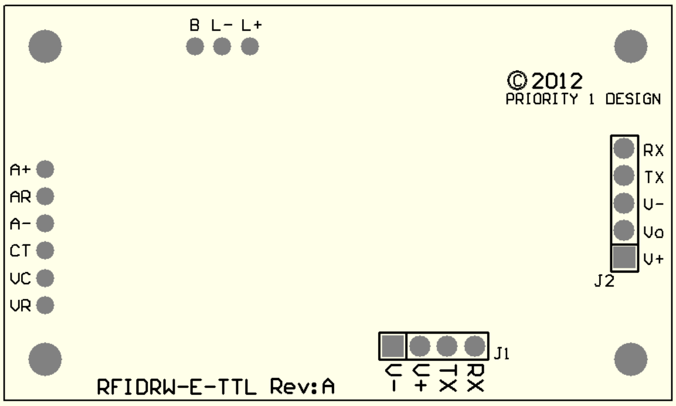


Figure B.1: Pinout of the RFIDRW-E-TTL

Table B.1: Description of the RFIDRW-E-TTL pins

Pin	Description
V+	Supply voltage
V-	Ground
RX*	UART RX input (commands to the reader are sent on this line)
TX*	UART TX output (RFID data and responses to the commands are sent on this line)
Vo	3.3 V power supply
L+	Positive polarity for dual colour LED
L-	Negative polarity for dual colour LED
B	Output for buzzer
A+	Pin for one side of the antenna, that is connected to a 22 Ω series resistor
AR	Similar to A+ but without the 22 Ω series resistor
A-	Pin for the other side of the antenna
CT	Capacitor can be placed between CT and VC to tune the operating frequency
VC	Capacitor can be placed between VC and VR to adjust the signal strength at VR
VR	Signal sense point

* The RX and TX pins operate with 0 - 5 V in the J1 connector and 0 - 3.3 V in the J2 connector

B.2 HCF4051

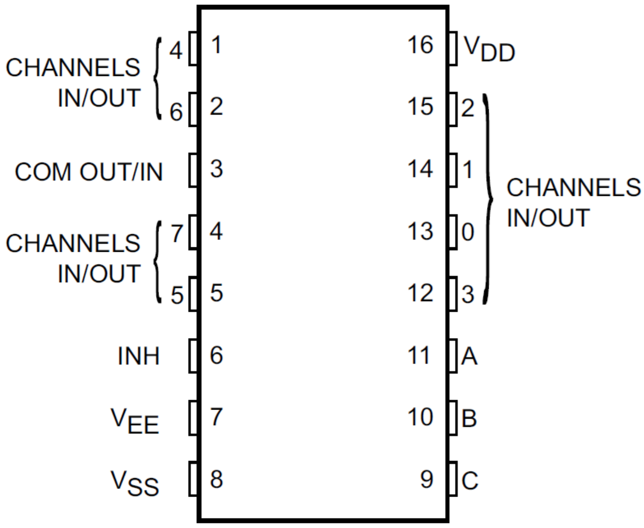


Figure B.2: Pinout of the HCF4051

Table B.2: Description of the HCF4051 pins

Pin(s)	**Description**
A, B, C	Binary control inputs
INH	Inhibit input (disables all channels)
CHANNELS IN/OUT	Independent inputs/outputs
COM OUT/IN	Common output/input
V_{DD}	Positive supply voltage
V_{SS}	Ground
V_{EE}	Negative supply voltage

B.3 HE721A0500

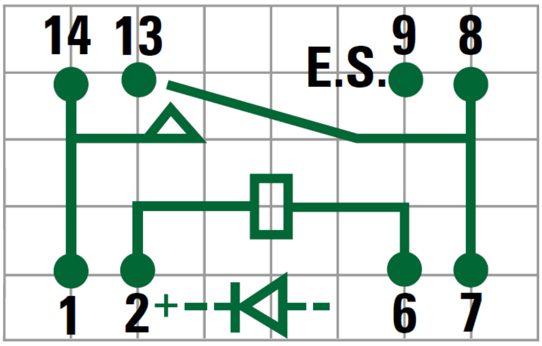


Figure B.3: Pinout of the HE721A0500

Table B.3: Description of the HE721A0500 pins

Pin(s)	**Description**
1, 14	Normally open contact
13	Not connected
7, 8	Common contact
9	External Magnetic Shield
2	Supply Voltage to close the switch
6	Ground

B.4 DHT22

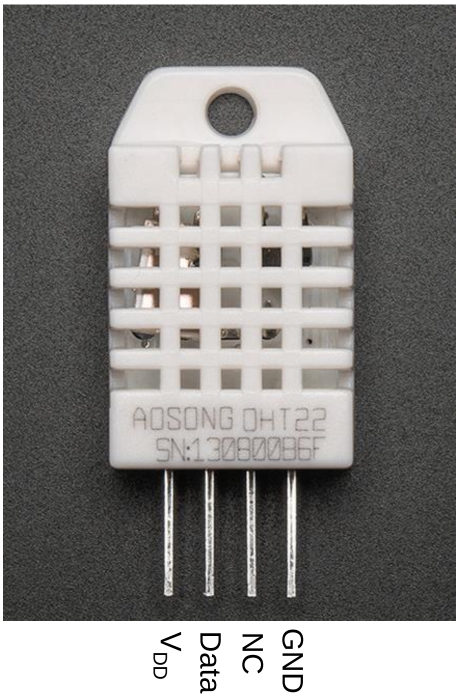


Figure B.4: Pinout of the DHT22

Table B.4: Description of the DHT22 pins

Pin	**Description**
V_{DD}	Supply Voltage
Data	Serial Data, bidirectional port
NC	Not connected
GND	Ground

B.5 ESP-07 Serial Wi-Fi Module

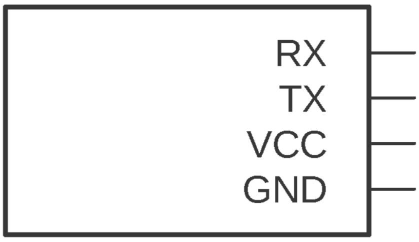


Figure B.5: Pinout of the ESP-07 Serial Wi-Fi Module

Table B.5: Description of the ESP-07 Serial Wi-Fi Module pins

Pin	**Description**
RX	UART RX input (commands to the Wi-Fi module are sent on this line)
TX	UART TX output (responses to the AT commands are sent on this line)
V_{CC}	Supply Voltage
GND	Ground

B.6 Arduino Leonardo

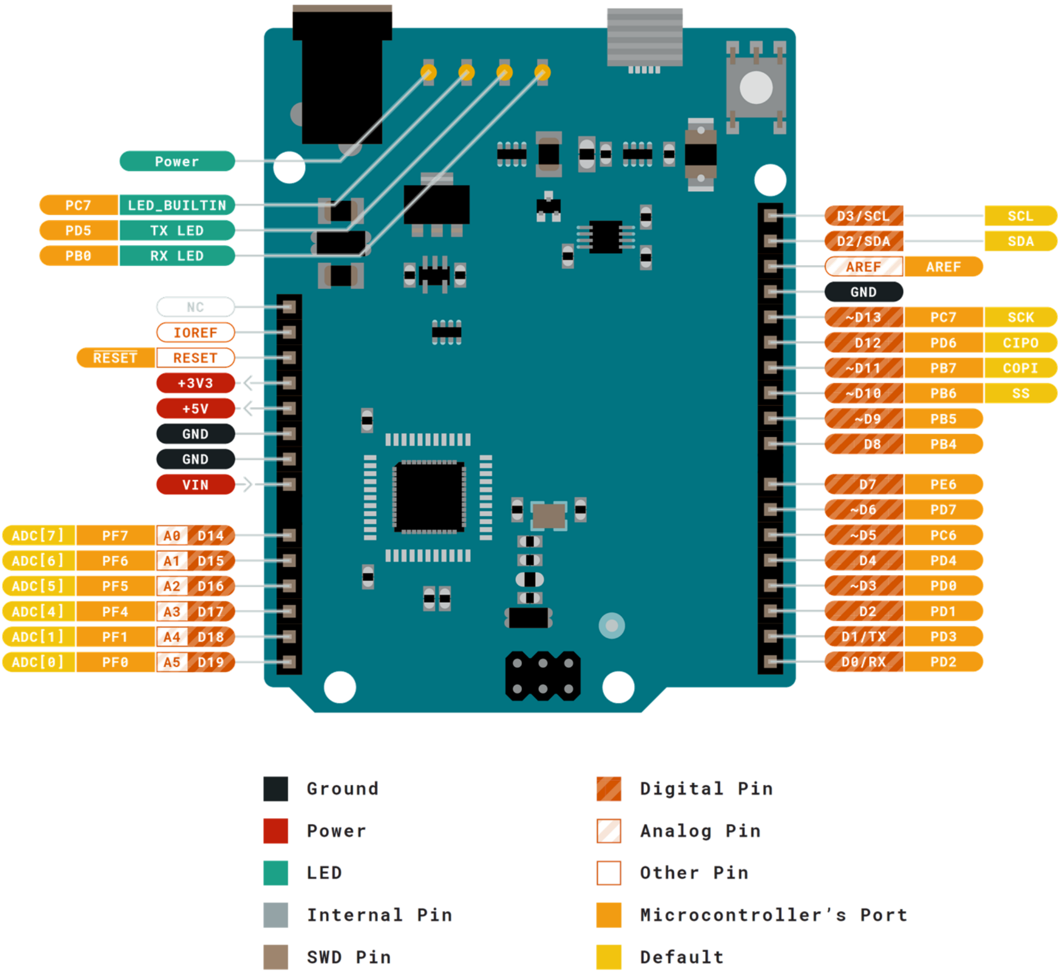


Figure B.6: Pinout of the Arduino Leonardo

Appendix C

RFIDRW-E-TTL Capacitors and Resistors Calculation

This appendix contains the formulas used to determine the capacitors and resistors that should be used in order for the RFIDRW-E-TTL reader to operate with different types of antennas.

C.1 Formulas used

The internal and external resistors and capacitors connected to the RFIDRW-E-TTL reader that are important for these calculations can be seen in Figure C.1.

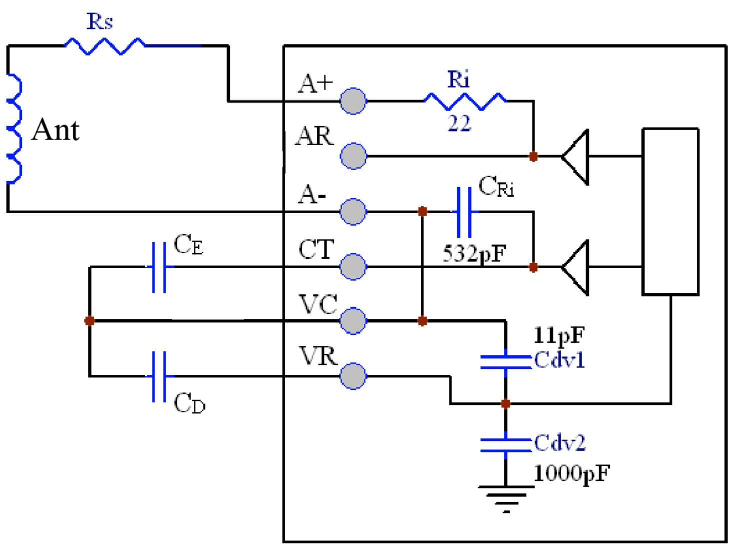


Figure C.1: Internal and external capacitors and resistors connected to the RFIDRW-E-TTL

Tuning the reader for the right resonant frequency

The resonant frequency (f_0) of the RFIDRW-E-TTL reader can be calculated using:

$$f_0 = \frac{1}{2\pi\sqrt{L_R C_R}} \tag{C.1}$$

where L_R corresponds to the inductance of the used antenna and $C_R = C_E + C_{R_i}$.

Knowing that, the value of C_E that tunes the reader for the frequency f_0 is given by:

$$C_E = \frac{1}{(2\pi f_0)^2 \cdot L_R} - C_{R_i} \tag{C.2}$$

Maximum voltage and current across the coil

The peak to peak voltage across the coil can be calculated using:

$$V_{Ant,\ pp} = \frac{2}{f_0 \cdot C_R \cdot (R_T + 6)} \tag{C.3}$$

where $R_T = R_{Ant} + R_i + R_S$.

The peak current in the coil can be determined using:

$$I_{Ant,\ peak} = \frac{6.37}{R_T + 6} \tag{C.4}$$

The maximum value of current should not exceed 200 mA. This can controlled by adding the R_S resistor to the circuit, if needed.

Maximum voltage in the sensing point VR

The voltage in the sensing point V_R can be calculated using the formula:

$$V_R = V_{Ant,\ pp} \cdot \frac{C_{dv1} + C_D}{(C_{dv1} + C_D) + C_{dv2}} \tag{C.5}$$

The maximum voltage allowed in V_R is 4.0 V, so the C_D capacitor should have the capacitance that produces that value, so that the signal in V_R is large enough to be sensed in the presence of the much higher voltage across the coil. So, the formula used to obtain the value of C_D is:

$$C_D = \frac{V_R \cdot C_{dv2}}{V_{Ant,\ pp} - V_R} - C_{dv1} = \frac{4 \cdot C_{dv2}}{V_{Ant,\ pp} - 4} - C_{dv1} \tag{C.6}$$

C.2 Calculation example for the D-Think Inc. coil

The coil by D-Think Inc. has the following properties:

- $L_R = 333.8\ uH$
- $R_{Ant} = 10.5\ \Omega$

Since the RFID tags used operate with a f_0 of 134.2 kHz, we have:

$$C_E = \frac{1}{(2\pi f_0)^2 \cdot L_R} - C_{R_i} = \frac{1}{(2\pi \cdot 134.2 \times 10^3)^2 \cdot 333.8 \times 10^{-6}} - 532 \times 10^{-12} = 3.68\ nF \quad \text{(C.7)}$$

When it comes to the voltage and current in the coil, if we consider $R_S = 0\ \Omega$, we have:

$$V_{Ant,\ pp} = \frac{2}{f_0 \cdot C_R \cdot (R_T + 6)} = \frac{2}{134.2 \times 10^3 \cdot 4.212 \times 10^{-9} \cdot (10.5 + 22 + 0 + 6)} = 91.9\ V \quad \text{(C.8)}$$

$$I_{Ant,\ peak} = \frac{6.37}{R_t + 6} = \frac{6.37}{10.5 + 22 + 0 + 6} = 165.5\ mA \quad \text{(C.9)}$$

Since $I_{Ant,\ peak}$ is below 200 mA, R_S is not needed.
Finally, the obtained value for C_D is:

$$C_D = \frac{4 \cdot C_{dv2}}{V_{Ant,\ pp} - 4} - C_{dv1} = \frac{4 \cdot 1 \times 10^{-9}}{91.9 - 4} - 11 \times 10^{-12} = 34.5\ pF \quad \text{(C.10)}$$

Appendix D

Components box dimensions

This appendix contains 2D drawings of the bottom part and top part of the box that was 3D printed. Figure D.1 corresponds to the bottom part of the box where most of the components will be placed and Figure D.2 is the lid of the box, containing the grooves for the antennas.

All the dimensions in the drawings are expressed in mm.

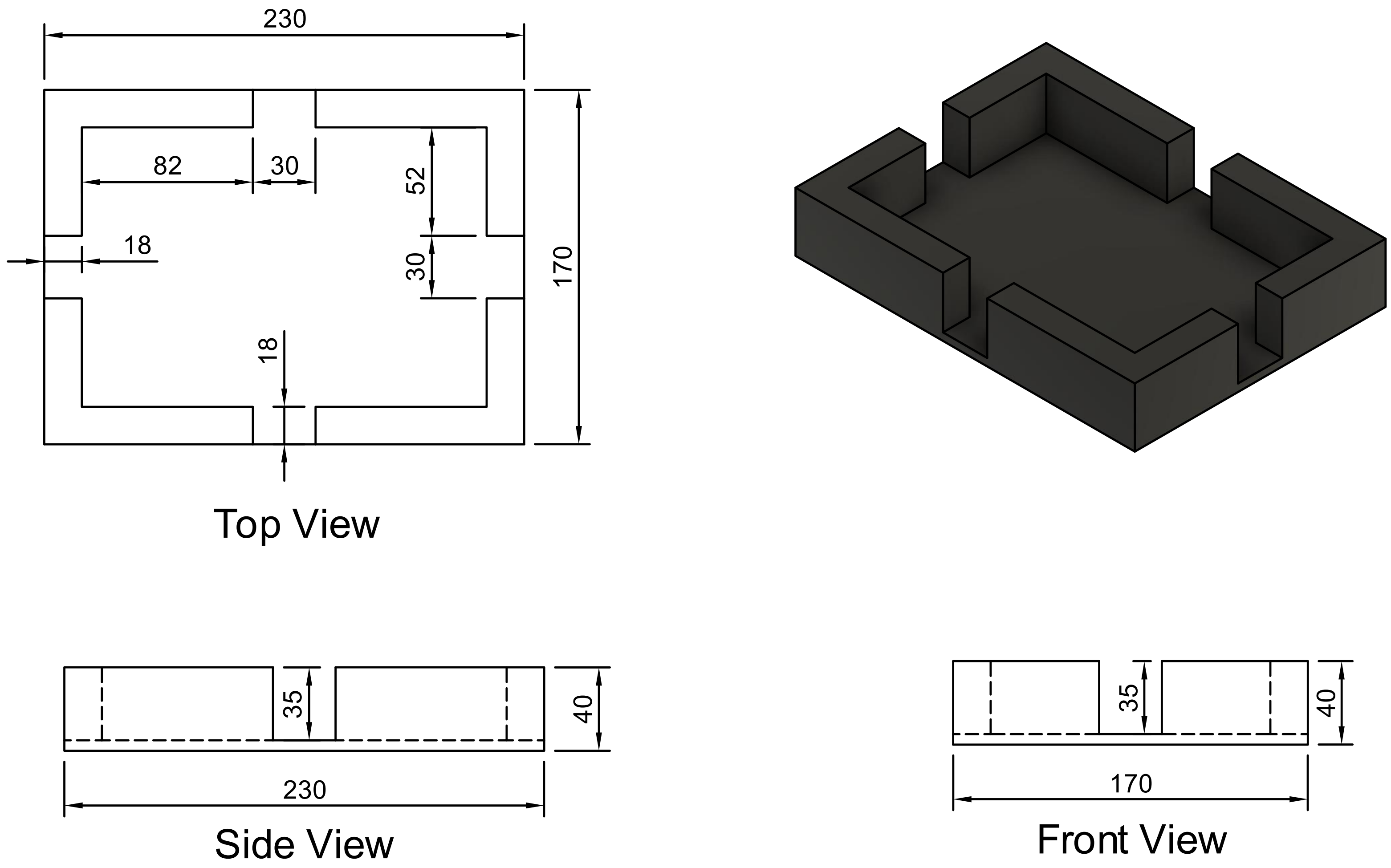


Figure D.1: 2D drawing of the bottom part of the box. All dimensions are in mm

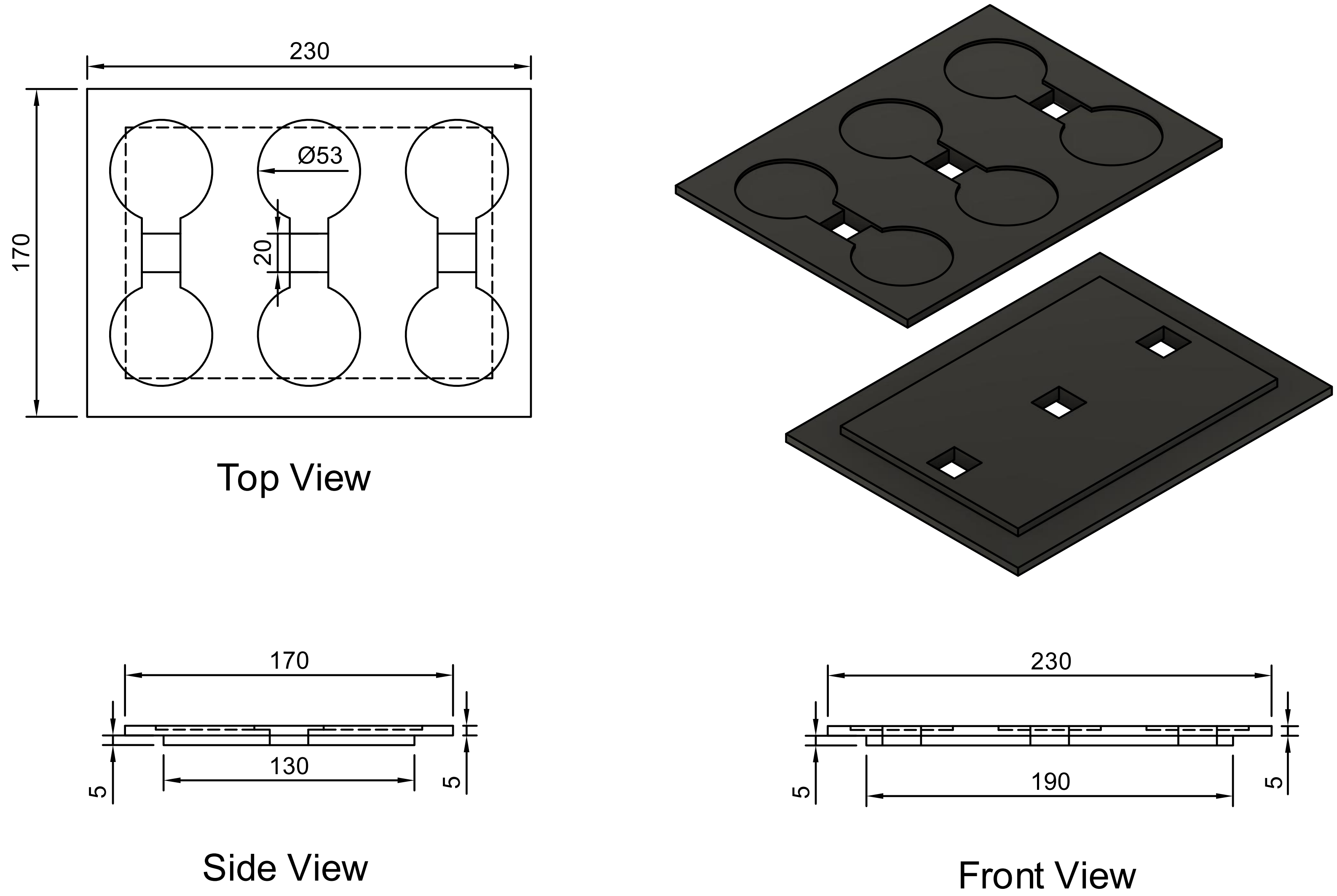


Figure D.2: 2D drawing of the top part of the box. All dimensions are in mm

Appendix E

Arduino Code

This appendix contains the entire code that was uploaded to the Arduino board.

```
/* Libraries */
#include <WiFiEspAT.h>
#include <DHT.h>
#include <SoftwareSerial.h>
#include <RFIDTag.h> // Library that was created

/* Define Constants */
#define rxPin 8 // RX Pin for the RFID Reader (On Arduino Leonardo
    only 8, 9, 10, 11, 14, 15 and 16 can be used)
#define txPin 9 // TX Pin for the RFID Reader

#define channel_A 4 // Pin for the A control of the multiplexer
#define channel_B 3 // Pin for the B control of the multiplexer
#define channel_C 2 // Pin for the C control of the multiplexer
#define maxChannel 5 // The multiplexer will switch between the
    channels 0 to maxChannel

#define DHTPIN 13      // Pin for the DHT 22 Sensor
#define DHTTYPE DHT22   // DHT 22 (AM2302)

#define LDR_pin A0 // Pin used to measure the LDR resistance
#define R1 10000 // Resistor used in the LDR voltage divider

#define tagStringSize 16 // Size of the C-Strings with the tag ID
#define floatStringSize 10 // Size of the C-Strings with the sensor
    measurements
```

```
/* Cage Environment Variables */
float env_hum;  // Stores humidity value
float env_temp; // Stores temperature value
char env_hum_string[floatStringSize] = ""; // C-String with the humidity value
char env_temp_string[floatStringSize] = ""; // C-String with the temperature value
float vLDR, R_LDR; // Stores voltage and resistance of the LDR
int env_lux; // Stores light values


/* Timing Variables */
unsigned long lastEnvironmentTime = 0; // Last time the environmental data was measured (in milliseconds)
unsigned long lastTagTime = 0; // Last time the tag data was measured (in milliseconds)
unsigned long lastSwitchTime = 0; // Last time the active antenna switched (in milliseconds)
const unsigned long environmentInterval = 60000L; // Milliseconds between sending the environmental data to the server
const unsigned long switchInterval = 400L; // Milliseconds each antenna is active
const unsigned long offInterval = 600L;  // Milliseconds between deactivating a antenna and activating another one (needs to be at least about 600 ms)


/* RFID Variables */
char c;
String code = "";
bool onlyID = true; // True if only the first 15 digits of the code (ID) are being read
float animal_temp;
char animal_temp_string[floatStringSize] = "";
char previousTagID[tagStringSize] = "";
Tag currentTag;
```

```
int i = 0; // independent channel of the multiplexer that is
   activated
bool channelOn = true;


/* Network SSID and Password */
char ssid[] = "arduino";  // your network SSID (name)
char pass[] = "arduino1234";      // your network password


/* Server and port to connect to */
char serverAddress[] = "10.227.144.42"; // Address of the server (IP
    of the computer if a local server is being used)
int port = 80;


/* Serial Wifi Variables */
char apiKeyValue[] = "tPmAT5Ab3j7F9"; // Key that should be equal to
    the one in the website code
char cage[] = "1"; // ID of the cage that is being monitored
int status = WL_IDLE_STATUS;     // Wifi radio's status
char postData[90];
char PostHeader[270];
char table[25] = "";


/* Initialization of other important objects */
DHT dht(DHTPIN, DHTTYPE); // DHT sensor for normal 16mhz Arduino
WiFiClient client; // Client that will send the HTTP Request
SoftwareSerial RFID_Serial(rxPin, txPin); // Software Serial for the
    RFID Reader


void setup()
{
  // Prepare the pins that control the multiplexer
  pinMode(channel_A, OUTPUT);
  pinMode(channel_B, OUTPUT);
  pinMode(channel_C, OUTPUT);
  selectInputChannel(i);
```

```

  // Initialize ESP8266 module
  Serial.begin(9600);
  Serial1.begin(115200);
  WiFi.init(&Serial1); // use hardware serial for the ESP8266

  // Initialize serial for RFID Reader
  RFID_Serial.begin(9600);

  // Check for the presence of the shield
  if (WiFi.status() == WL_NO_SHIELD) {
    Serial.println("WiFi shield not present");
    while (true); // don't continue
  }

  // Attempt to connect to WiFi network
  while ( status != WL_CONNECTED) {
    status = WiFi.begin(ssid, pass); // connect to WPA/WPA2 network
    Serial.println("Connecting to the network...");
  }
}


void loop()
{
  // Interval between activating each antenna (to not cause interference between multiple antennas)
  if (millis() - lastSwitchTime > switchInterval and channelOn) {
    Serial.println("Off Time");
    selectInputChannel(7); // channel without any antenna (alternatively, the multiplexer inhibit channel can be used)
    channelOn = false;

    // If certain time has passed since last posting the environmental data
    if (millis() - lastEnvironmentTime > environmentInterval) {
      Serial.println("Temperature/humidity reading");
      env_temp = dht.readTemperature(); // read environment temperature
      env_hum  = dht.readHumidity(); // read environment humidity
```

```
      vLDR = analogRead(LDR_pin) * 5.0 / 1024; // read LDR voltage
         in the divider

      // If the environmental conditions were successfully read
      if (!isnan(env_temp) and !isnan(env_hum) and vLDR != 5.0) {
        lastEnvironmentTime = millis();

        R_LDR = (R1 * vLDR) / (5 - vLDR);
        env_lux = round(23852124.3 * pow(R_LDR,-1.23623));

        dtostrf(env_temp, 0, 2, env_temp_string);
        dtostrf(env_hum, 0, 2, env_hum_string);

        strcpy(table, "EnvironmentMeasurement");
        httpRequest(table);
      }
    }

    // If a tag was detected
    if (strlen(previousTagID) != 0) {
      Serial.println("Tag Reading");
      lastTagTime = millis();
      strcpy(table, "TagDetection");
      httpRequest(table);
    }

    strcpy(previousTagID, "");

  }

  // Switch the antenna that is active (and send the tag
     measurements to the database when the last antenna is reached)
  if (millis() - lastSwitchTime > switchInterval + offInterval) {
    Serial.println("Switch Antenna");
    lastSwitchTime = millis();
    i++;

    // If the the cycle is starting all over
    if (i > maxChannel) {
      Serial.println("Cycle restarting");
```

```
      i = 0; // restart the antenna cycle
    }
    selectInputChannel(i);
    channelOn = true;
  }

  // If the RFID Reader detected a tag
  if (RFID_Serial.available()) {
    Serial.print(".");
    c = RFID_Serial.read();

    // Last characters were reached
    if (c == '\r') {

      // The 15 digits of ID were read
      if (onlyID) {
        RFID_Serial.write("RAT\r"); // Ask for the RFID Reader to send the complete code
        code = "";
      }
      // The full code was read
      else {

        // If the code was correctly detected
        if (code.length() == 32) {
          currentTag = Tag(code);
          code = "";

          currentTag.getID().toCharArray(previousTagID, tagStringSize);
          dtostrf(currentTag.getTemperature(), 0, 2, animal_temp_string) ;
          currentTag.print();
        }
      }
      onlyID = !onlyID;
    }

    // Add the new character to the code
    else {
```

```
      code = code + c;
    }
  }
}

// Function to make a HTTP connection to the server
void httpRequest(char table[])
{
  Serial.println("Starting Request");

  if (strcmp(table, "EnvironmentMeasurement") == 0) {
    sprintf (postData, "api_key=%s&table=%s&cage=%s&t=%s&h=%s&l=%i",
        apiKeyValue, table, cage, env_temp_string, env_hum_string,
       env_lux);
  }

  else if (strcmp(table,"TagDetection") == 0) {
    sprintf (postData, "api_key=%s&table=%s&ID=%s&t=%s&p=%c",
       apiKeyValue, table, previousTagID, animal_temp_string, char(i
       +65));
  }

  // If there is a successful connection
  if (client.connect(serverAddress, port)) {
    // Send the HTTP POST request
    sprintf (PostHeader, "POST http://%s:%i/monitoring/post-esp-data
       .php HTTP/1.1\r\nConnection: keep-alive\r\nContent-Type:
       application/x-www-form-urlencoded\r\nHost:%s:%i\r\nContent-
       Length:%u\r\n\r\n", serverAddress, port, serverAddress, port,
        strlen (postData));
    strcat (PostHeader, postData);

    client.println(PostHeader);
    Serial.println(PostHeader);
  }
  else {
    // If the connection failed
    Serial.println("Connection failed");
  }
}
```

```
// Function to select the active independent channel of the
   multiplexer
void selectInputChannel(int channel) {

  int A = bitRead(channel, 0);
  int B = bitRead(channel, 1);
  int C = bitRead(channel, 2);

  digitalWrite(channel_A, A);
  digitalWrite(channel_B, B);
  digitalWrite(channel_C, C);

  Serial.println("\n\nChannel " + String(channel));
}
```

Appendix F

RFIDTag Code

This appendix contains the full code of the RFIDTag library (RFIDTag.cpp file).

```
/*

  RFIDTag.cpp - Library for decoding and showing the codes read by
     the RFIDRW-E-TTL.

  Created by Diogo Espinheira Moutinho, 2022.

*/

#include "RFIDTag.h"

Tag::Tag() {
    countryCode = nationalCode = checksum = "0";
    temperature = 0.0;
    extraData = animalApplication = false;
}

Tag::Tag(String code)
{
    int len = code.length();

    countryCode = code.substring(0, 3);
    nationalCode = code.substring(4, 16);
    animalApplication = stringToBool(code.substring(17, 18));
    extraData = stringToBool(code.substring(19, 20));
    checksum = code.substring(21, 25);
```

```

    String tempString = code.substring(29);
    tempString[0] = '1'; // change the third to last digit into 1

    temperature = ((float)strtol(tempString.c_str(), NULL, 16)) / 10.0 - 1.0; // convert to hexadecimal, divide by 10 and subtract by 1
}

void Tag::print()
{
    Serial.println("\nCountry Code: " + countryCode);
    Serial.println("National Code: " + nationalCode);
    Serial.println("Extra Data: " + String(extraData));
    Serial.println("Animal Application: " + String(animalApplication));
    Serial.println("Checksum: " + checksum);
    Serial.println("Temperature: " + String(temperature) + " °C");
}

String Tag::getID()
{
    return countryCode + nationalCode;
}

float Tag::getTemperature()
{
    return temperature;
}

bool Tag::stringToBool(String c)
{
    return c != "0"; // if c=="0", returns false; if c=="1", returns true;
}
```

Appendix G

Full water cooling experiment

This appendix contains the full data of the water cooling experiment, presenting the measurements that were made by the tag and the pt100 every 30 seconds during the 1 hour of experiment.

Table G.1: Comparison between the temperature given by the pt100 and the code sent by the tag during the entire water cooling experiment

Time since beginning (hour:min:sec)	**Temperature pt100 (ºC)**	**Extended Data**	**Processed Extended Data**	**Temperature PIT (ºC)**
00:00:00	46.4	0001CC	1CC	46.0
00:00:30	46.1	0001C9	1C9	45.7
00:01:00	45.7	0001C6	1C6	45.4
00:01:30	45.4	0001C3	1C3	45.1
00:02:00	45.1	0001C0	1C0	44.8
00:02:30	44.7	0001BD	1BD	44.5
00:03:00	44.4	0000BA	1BA	44.2
00:03:30	44.1	0001B8	1B8	44.0
00:04:00	43.8	0000B5	1B5	43.7
00:04:30	43.5	0001B2	1B2	43.4
00:05:00	43.2	0001AF	1AF	43.1
00:05:30	43.0	0000AD	1AD	42.9
00:06:00	42.7	0001AA	1AA	42.6
00:06:30	42.4	0000A8	1A8	42.4
00:07:00	42.2	0001A5	1A5	42.1
00:07:30	41.9	0001A3	1A3	41.9
00:08:00	41.7	0000A1	1A1	41.7

Table G.1: Comparison between the temperature given by the pt100 and the code sent by the tag during the entire water cooling experiment

Time since beginning (hour:min:sec)	Temperature pt100 (ºC)	Extended Data	Processed Extended Data	Temperature PIT (ºC)
00:08:30	41.4	00009D	19D	41.3
00:09:00	41.1	00009B	19B	41.1
00:09:30	40.9	000199	199	40.9
00:10:00	40.6	000097	197	40.7
00:10:30	40.4	000195	195	40.5
00:11:00	40.2	000193	193	40.3
00:11:30	40.0	000091	191	40.1
00:12:00	39.8	00008F	18F	39.9
00:12:30	39.6	00018D	18D	39.7
00:13:00	39.3	00018B	18B	39.5
00:13:30	39.1	000089	189	39.3
00:14:00	38.9	000187	187	39.1
00:14:30	38.7	000085	185	38.9
00:15:00	38.5	000184	184	38.8
00:15:30	38.3	000182	182	38.6
00:16:00	38.1	000181	181	38.5
00:16:30	37.9	00007F	17F	38.3
00:17:00	37.7	00017E	17E	38.2
00:17:30	37.6	00007C	17C	38.0
00:18:00	37.4	00007A	17A	37.8
00:18:30	37.3	000079	179	37.7
00:19:00	37.1	000177	177	37.5
00:19:30	36.9	000075	175	37.3
00:20:00	36.7	000174	174	37.2
00:20:30	36.6	000172	172	37.0
00:21:00	36.4	000171	171	36.9
00:21:30	36.2	000070	170	36.8
00:22:00	36.1	00006E	16E	36.6

Table G.1: Comparison between the temperature given by the pt100 and the code sent by the tag during the entire water cooling experiment

Time since beginning (hour:min:sec)	**Temperature pt100 (ºC)**	**Extended Data**	**Processed Extended Data**	**Temperature PIT (ºC)**
00:22:30	35.9	00016C	16C	36.4
00:23:00	35.8	00016A	16A	36.2
00:23:30	35.6	000169	169	36.1
00:24:00	35.5	000068	168	36.0
00:24:30	35.3	000067	167	35.9
00:25:00	35.2	000166	166	35.8
00:25:30	35.1	000064	164	35.6
00:26:00	34.9	000163	163	35.5
00:26:30	34.7	000061	161	35.3
00:27:00	34.6	00015F	15F	35.1
00:27:30	34.4	00015F	15F	35.1
00:28:00	34.3	00005E	15E	35.0
00:28:30	34.2	00015C	15C	34.8
00:29:00	34.1	00005B	15B	34.7
00:29:30	33.9	00015A	15A	34.6
00:30:00	33.8	000058	158	34.4
00:30:30	33.7	000057	157	34.3
00:31:00	33.6	000156	156	34.2
00:31:30	33.5	000155	155	34.1
00:32:00	33.4	000054	154	34.0
00:32:30	33.2	000153	153	33.9
00:33:00	33.1	000051	151	33.7
00:33:30	33.0	000051	151	33.7
00:34:00	32.9	00004F	14F	33.5
00:34:30	32.7	00014E	14E	33.4
00:35:00	32.6	00014D	14D	33.3
00:35:30	32.5	00014D	14D	33.3
00:36:00	32.4	00014B	14B	33.1

Table G.1: Comparison between the temperature given by the pt100 and the code sent by the tag during the entire water cooling experiment

Time since beginning (hour:min:sec)	**Temperature pt100 (ºC)**	**Extended Data**	**Processed Extended Data**	**Temperature PIT (ºC)**
00:36:30	32.3	00004A	14A	33.0
00:37:00	32.2	000049	149	32.9
00:37:30	32.1	000148	148	32.8
00:38:00	32.0	000147	147	32.7
00:38:30	31.9	000046	146	32.6
00:39:00	31.8	000046	146	32.6
00:39:30	31.7	000045	145	32.5
00:40:00	31.6	000144	144	32.4
00:40:30	31.5	000142	142	32.2
00:41:00	31.4	000142	142	32.2
00:41:30	31.3	000141	141	32.1
00:42:00	31.2	000141	141	32.1
00:42:30	31.2	000040	140	32.0
00:43:00	31.1	00013F	13F	31.9
00:43:30	31.0	00003E	13E	31.8
00:44:00	30.9	00003D	13D	31.7
00:44:30	30.8	00003D	13D	31.7
00:45:00	30.8	00013C	13C	31.6
00:45:30	30.7	00003B	13B	31.5
00:46:00	30.6	00013A	13A	31.4
00:46:30	30.5	000139	139	31.3
00:47:00	30.4	000038	138	31.2
00:47:30	30.4	000038	138	31.2
00:48:00	30.3	000037	137	31.1
00:48:30	30.2	000037	137	31.1
00:49:00	30.1	000136	136	31.0
00:49:30	30.1	000135	135	30.9
00:50:00	30.0	000034	134	30.8

Table G.1: Comparison between the temperature given by the pt100 and the code sent by the tag during the entire water cooling experiment

Time since beginning (hour:min:sec)	**Temperature pt100 (ºC)**	**Extended Data**	**Processed Extended Data**	**Temperature PIT (ºC)**
00:50:30	29.9	000034	134	30.8
00:51:00	29.8	000133	133	30.7
00:51:30	29.7	000032	132	30.6
00:52:00	29.7	000032	132	30.6
00:52:30	29.6	000031	131	30.5
00:53:00	29.5	000130	130	30.4
00:53:30	29.4	000130	130	30.4
00:54:00	29.4	000130	130	30.4
00:54:30	29.3	000130	130	30.4
00:55:00	29.2	00012E	12E	30.2
00:55:30	29.1	00012E	12E	30.2
00:56:00	29.1	00012D	12D	30.1
00:56:30	29.0	00012D	12D	30.1
00:57:00	29.0	00002C	12C	30.0
00:57:30	28.9	00002C	12C	30.0
00:58:00	28.9	00012B	12B	29.9
00:58:30	28.8	00012B	12B	29.9
00:59:00	28.7	00002A	12A	29.8
00:59:30	28.7	000029	129	29.7
01:00:00	28.6	000029	129	29.7
01:00:30	28.5	000128	128	29.6
01:01:00	28.5	000127	127	29.5
01:01:30	28.4	000127	127	29.5
01:02:00	28.4	000026	126	29.4
01:02:30	28.3	000026	126	29.4
01:03:00	28.3	000025	125	29.3
01:03:30	28.2	000025	125	29.3

Appendix H

MySQL Code

This appendix contains the MySQL code that was used to create the user interface database.

```sql
CREATE TABLE User (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  name TEXT NOT NULL,
  email TEXT NOT NULL
);


CREATE TABLE Project (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  name TEXT NOT NULL,
  user INTEGER NOT NULL,
  FOREIGN KEY (user) REFERENCES User(id)
);


CREATE TABLE Cage (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  name VARCHAR(5) NOT NULL,
  type TEXT,
  project INTEGER NOT NULL,
  FOREIGN KEY (project) REFERENCES Project(id)
);


CREATE TABLE Animal (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  code TEXT,
  tagID VARCHAR(15) UNIQUE,
  species TEXT NOT NULL,
  birthDate DATE NOT NULL,
  sex TEXT,
  cage INTEGER,
```

```
  project INTEGER NOT NULL,
  FOREIGN KEY (cage) REFERENCES Cage(id),
  FOREIGN KEY (project) REFERENCES Project(id)
  );


CREATE TABLE Treatment (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  name TEXT NOT NULL,
  description TEXT
);


CREATE TABLE AnimalMeasurement (
    id INTEGER PRIMARY KEY AUTO_INCREMENT,
    animal INTEGER NOT NULL,
    dateTimeMeasured DATETIME NOT NULL,
    temperature DECIMAL(4,2),
    position VARCHAR(1),
    FOREIGN KEY (animal) REFERENCES Animal(id)
);


CREATE TABLE CageMeasurement (
    id INTEGER PRIMARY KEY AUTO_INCREMENT,
    cage INTEGER NOT NULL,
    dateTimeMeasured DATETIME NOT NULL,
    temperature DECIMAL(4,2) NOT NULL,
    humidity DECIMAL(4,2) NOT NULL,
    light INTEGER(4) NOT NULL,
    FOREIGN KEY (cage) REFERENCES Cage(id)
);


CREATE TABLE AnimalTreatment (
    animal INTEGER,
    treatment INTEGER,
    dateTimeTreated DATETIME NOT NULL,
    PRIMARY KEY (animal, treatment),
    FOREIGN KEY (animal) REFERENCES Animal(id),
    FOREIGN KEY (treatment)  REFERENCES Treatment(id)
);


CREATE TABLE AlarmAnimal (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  animal INTEGER NOT NULL,
  parameter TEXT NOT NULL,
  value DECIMAL(5,1) NOT NULL,
```

```
  valueCondition TEXT NOT NULL,
  numActivations INTEGER(2) NOT NULL,
  FOREIGN KEY (animal) REFERENCES Animal(id)
);


CREATE TABLE AlarmCage (
  id INTEGER PRIMARY KEY AUTO_INCREMENT,
  cage INTEGER NOT NULL,
  parameter TEXT NOT NULL,
  value DECIMAL(5,1) NOT NULL,
  valueCondition TEXT NOT NULL,
  numActivations INTEGER(2) NOT NULL,
  FOREIGN KEY (cage) REFERENCES Cage(id)
);
```

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