Catarina Santos Coelho

Males and females with their backs turned — a study of sexual segregation in red deer

Junho 2018

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UNIVERSIDADE DE COIMBRA

Agradecimentos

Quero começar por agradecer ao Professor Doutor José Paulo Sousa pela oportunidade de realizar este projeto.

O meu especial agradecimento vai para a minha orientadora Doutora Joana Silva Alves, por todo o apoio, paciência e conhecimento que me transmitiu ao longo desta jornada. Muito obrigada por acreditares e me fazeres acreditar nas minhas capacidades, exigindo sempre um pouco mais do que aquilo que eu estava a dar. Não podia deixar de agradecer também ao Mestre António Silva por toda a sua disponibilidade e colaboração em todo o processo. Obrigada pelo tempo que abdicaste do teu trabalho para ajudar a resolver todos os problemas técnicos do meu.

Um agradecimento aos colegas de laboratório por tornarem os dias de trabalho mais animados e por estarem sempre disponíveis para ajudar mesmo quando as minhas amostras empestavam a sala dias a fio. Um especial obrigada à Fernanda por toda a ajuda e conselhos que foi dando ao longo deste trabalho.

Um grande obrigada ao Grupo de Cordas, à Estudantina Feminina de Coimbra da SF/AAC, e aos amigos que lá fiz, pelas horas semanais em que me fizeram esquecer que tinha uma tese para escrever. Obrigada por manterem a minha (in)sanidade.

À Bia e à Sónia, pela amizade e apoio, mesmo estando a quilómetros de distância.

Ao Tiago, pelos desabafos ouvidos e por não me deixar desacreditar.

À minha madrinha, pelo exemplo que representa e por acreditar sempre em mim.

À minha família, em especial aos meus avós, pelo incentivo e força que me dão, mesmo não percebendo muito bem o que é que eu andei para aqui a fazer.

Por último, mas não menos importante, um sincero obrigada à minha mãe, ao meu pai e ao meu irmão, por toda a confiança que depositam em mim e pelo apoio incondicional que me transmitiram ao longo desta etapa. Obrigada por não me deixarem desistir.

Resumo

Apesar de se tratar de um comportamento amplamente descrito e estudado no reino animal, especialmente em ungulados, a segregação sexual carece de uma definição consensual entre autores. Ao longo dos anos, várias hipóteses foram sugeridas e estudadas com o objetivo de explicar quer a componente social quer a componente de habitat deste complexo comportamento. No entanto, nenhuma foi capaz de fornecer uma justificação universal para este fenómeno, o que sugere a contribuição simultânea de vários fatores para a origem do mesmo.

Segundo a hipótese da seletividade alimentar, machos e fêmeas possuem diferentes eficiências digestivas e necessidades energéticas, decorrentes do seu dimorfismo sexual em termos de tamanho, pelo que selecionam habitats distintos em detrimento da qualidade de alimento. Este estudo tem, então, como objetivo avaliar o pressuposto que serve de base à hipótese da seletividade alimentar. Através da análise de excrementos de veado, recolhidos por observação direta e em animais abatidos em montarias na Serra da Lousã, comparou-se o tamanho dos fragmentos de plantas excretados por machos e fêmeas de forma a avaliar diferenças na eficiência digestiva entre sexos. Nos excrementos recolhidos na época reprodutiva foi possível encontrar diferenças significativas no tamanho dos fragmentos, o que levou a concluir que os machos possuem maior eficiência digestiva do que as fêmeas quando a qualidade da dieta e os padrões de atividade são idênticos entre sexos, o que só acontece na época reprodutiva. Deste modo, os nossos resultados apoiam a hipótese da seletividade alimentar, não sendo as diferenças obtidas suficientes para explicar na totalidade o padrão de segregação sexual nesta população de veados.

Sendo também um pressuposto da hipótese da sincronização de atividades, as diferentes eficiências digestivas de machos e fêmeas poderão ser o ponto de partida para encontrar uma explicação para a segregação sexual, relacionando as componentes de habitat e social.

Palavras chave: *Cervus elaphus*; segregação sexual, dimorfismo sexual, tamanho corporal, eficiência digestiva

Abstract

Despite being a behaviour widely described and studied in the animal kingdom, especially in ungulates, the sexual segregation lacks a consensual definition between authors. Over the years, many hypotheses have been suggested and studied with the objective of explaining either the social or the habitat component of this complex behaviour. However, none was able to provide a universal explanation for this phenomenon, which suggests a simultaneous contribution of various factors to be in its origin.

According to the forage selection hypothesis, males and females present different digestive efficiencies and energetic requirements, due to their sexual body size dimorphism, which results in the selection of distinct habitats as consequence of the forage quality. Therefore, this study aims to evaluate the assumption in which the forage selection hypothesis is based. Through the analysis of red deer faeces, collected by direct observation and in hunted animals in the Lousã Mountain, the size of the plant fragments excreted by males and females was compared to evaluate differences between the digestive efficiencies of both sexes. It was possible to identify significative differences in the size of the fragments present in the faeces collected during the rut season, which leads to the conclusion that males have a higher digestive efficiency than females when the food quality and the activity patterns are similar between sexes, which only happens during the rut season. As so, our results provide support to the forage selection hypothesis, but the differences obtained do not seem enough to explain the full patterns of sexual segregation of this red deer population.

The fact that the differential digestive efficiency of males and females is also an assumption of the activity budget hypothesis might suit as a basis to find an explanation for sexual segregation, relating the habitat and social components.

Key words: *Cervus elaphus*, sexual segregation, sexual dimorphism, body size, digestive efficiency

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Chapter 1
General introduction

1.1. Sexual Segregation

1.1.1. Definition

In the social organization of the red deer, males and females live apart most of the year, behaviour designated as sexual segregation. This phenomenon is described as the differential use of space by sexes (Bowyer 1984; Bowyer et al 1996; Bleich et al 1997; Kie & Bowyer 1999; Barboza & Bowyer 2001) or as the segregation into different social groups (Conradt 1998; Ruckstuhl & Neuhaus 2005) and it has been documented in birds, fishes and mammals (Bleich et al 1997; Ruckstuhl & Neuhaus 2000). Sexual segregation is especially evident in species with a notable sexual dimorphism in terms of body size, such as ungulates (Miquelle et al 1992; Main et al 1996; Mysterud 2000; Ruckstuhl & Neuhaus 2002).

Despite the definition here presented, not all authors describe this behaviour the same way, with some emphasising a social approach and others highlighting the habitat component. Therefore, is important to consider two components within the sexual segregation, the social segregation and the habitat segregation, which might have an independent or collective contribution to such phenomenon (Conradt 1998).

The social segregation reflects the organization of males and females in different social groups (Conradt 1998; Ruckstuhl & Neuhaus 2005) while the habitat segregation refers to the use of different types of habitat by sexes (Newsome 1980; Clutton-Brock et al 1987; Ruckstuhl & Neuhaus 2005).

1.1.2. Main hypotheses

Throughout the years many hypotheses have been proposed to explain the sexual segregation, either by explaining the social segregation or the habitat segregation.

Three main hypotheses have been suggested to explain social segregation: the social factor hypothesis, the social affinity hypothesis and the activity budget hypothesis. Regarding the social factor hypothesis, individuals should aggregate in same sex groups by the necessity to develop significant life skills, like fighting experience in males and search of food resources and maternal skills in the case of females (Main et al 1996). Secondly, the social affinity hypothesis postulates that the cause of sexual segregation is the dissimilar social motivations of individuals which should lead to the aggregation in same sex and age groups (Geist 1971; Bon & Campan 1996). The activity budget hypothesis suggests that distinct foraging and resting patterns between sexes, resulting

from their different digestive efficiency, may be on the origin of the sexual segregation. Since the cost of activity synchronization is very high, especially between individuals of different size, sex and age class, it should result in the aggregation of individuals with similar activity patterns (Conradt 1998; Ruckstuhl 1998, 1999; Ruckstuhl & Neuhaus 2002).

Concerning the habitat segregation, the reproductive strategy and the forage selection hypotheses seem to be more suitable to explain it (Ruckstuhl & Neuhaus 2002; Alves et al 2013). The reproductive strategy or predation risk hypothesis suggest that sexual segregation rely on the fact that males and females present different reproductive approaches. While females watch over for the survival of their offspring, having preference for protected habitats especially during calving and lactation, males tend to choose areas with high quality food to enhance their physical condition in time for the rut season (Main & Coblentz 1990, 1996; Main et al 1996). Lastly, the forage selection, also denominated gastrocentric hypothesis, proposes that sexual segregation is as outcome of the distinct energetic requirements and digestive efficiency that results from the body size dimorphism between sexes. Thereby females should prefer areas with high quality food while males would choose areas with higher food quantity, even if it implies lower quality (Bowyer 1984; Demment & Soest 1985; Beier 1987; Barboza & Bowyer 2000, 2001).

1.2. Digestion and body size relations

1.2.1. The gastrocentric model

Barboza and Bowyer (2000) proposed a model for the forage selection hypothesis, also known as gastrocentric hypothesis, by projecting that body size dimorphism between sexes is responsible for distinctive energetic requirements and digestive efficiency of males, non-reproductive females and reproductive females, which should be reflected in their diets and choice of habitat.

Therefore, they postulated that males, being notably bigger than females, have a higher gut capacity and longer retention, as well as higher energetic requirements. Consequently, they should be able to ingest higher amounts of food, fulfilling their energetic requirements in shorter periods of time and based on lower food quality (high fibber content).

However, gestation and lactation incur in nutritional demands that exceed the energetic requirements of males despite the gut capacity being similar between

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reproductive and non-reproductive females (Barboza & Bowyer 2000). Subsequently, reproductive females may increase the length of the small intestine, like documented in several species (Fell 1972; Barry 1977; Jenks et al 1994), to enhance the retention time and the consequent ability of nutrient uptake.

1.2.2. Digestive efficiency

The digestive capabilities are directly related with the size of the food particles since smaller particles allow a greater surface-volume ratio facilitating the mechanical and microbial actions of the process (Ulyatt et al 1986; Bjorndal et al 1990). Therefore, several factors that may shape the digestive efficiency must be mentioned. From the anatomic perspective, mouth and teeth sizes, rumen volume and gut capacity are important factors influencing the digestive capabilities. In more detail, mouth and tooth sizes determine the amount of ingested food per bite and the chewing efficiency by affecting the contact area between the food and the teeth surface (Gross et al 1995; Pérez-Barbería & Gordon 1998). Rumen volume and gut capacity are related with the amount of food that can be accommodated and the contact area between the food particles and the mucosal surface, regulating the retention capacity (Demment & Soest 1985). Nevertheless, it's also important to refer that behavioural parameters, like chewing frequency and feeding and rumination times, might be shaped in response to these anatomical factors and to environmental aspects, like the quality of the available food resources (Pérez-Barbería & Gordon 1998; Ruckstuhl 1998; Pipia et al 2008), which, ultimately influence the digestive efficiency. Additionally, physiological parameters, like age and body size, play an indirect role in the digestive capabilities once they are related with the anatomical factors. Since age will be considered in this study, relationships between body size and other physiological and anatomical parameters need to be highlighted.

Starting with the daily energetic requirements, in mammals, they scale with body size as $BM^{0,75}$, being BM the body mass (Geist 1974; Demment & Soest 1985). The teeth size and incisor arcade breadth, which can be related with bite size and chewing efficiency, also present an allometric scaling with body size (Clutton-Brock & Harvey 1983; Mysterud 2000). However, the same does not apply to the rumen volume and gut capacity that scale proportionally to body mass (Demment & Soest 1985; Illius & Gordon 1987; Fritz et al 2009). Consequently, it's expected an increase in the chewing efficiency and retention of bigger animals, reflecting a higher digestive capacity with the increase of body mass (Demment & Soest 1985). Another important concept is the Jarman-Bell principle (Bell 1970; Jarman 1974) which relates the increase in body size with a

tendency to feed on the most accessible resources and be less selective. In consequence, larger animals should present a poorer diet, with less amounts of protein and higher fibre contents (Geist 1974).

1.3. Ecology and behaviour of the red deer

1.3.1. Morphology and sexual dimorphism

Cervus elaphus is a mammal, commonly known as red deer, included in the Ungulata superorder and the Cervidae family. This species presents a very robust body as well as a large head with an elongated muzzle and a great pair of ears (Baskin & Danell 2003). They have a thick coat which varies in colour according to the season, ranging from a reddish brown in summer to a darker brown in winter (Oliver 1999). The tail is short and brownish in contrast with the perianal area which has a whiter coloration (Baskin & Danell 2003; Wilson & Mittermeier 2011).

The sexual dimorphism is very noticeable either by the body size or by the presence of secondary sexual characters. Males measure between 175 and 250cm and weight around 130 to 180kg while females are 160 to 210cm long and weight 80 to 120kg. These differences express a sexual dimorphism of 37±3% in terms of body size for the studied population (Alves et al 2013). Besides that, the presence of antlers is the most visible sign to discriminate the sexes since it's an exclusive character of males developed for the first time around the second semester of their lives (Peixoto 2014).

1.3.2. Distribution and habitat

The current geographic range of the red deer includes North America, Europe, North Africa and Middle East (Gill 1990; Clutton-Brock & Albon 1992; Koubek & Zima 1999; Wilson & Ruff 1999, Wilson & Mittermeier 2011). In Europe it is largely widespread apart from Scandinavia, Finland and Iceland (Zachos & Hartl 2011).

In Portugal, the red deer was almost extinct but with the efforts of reintroduction programmes the species is now present throughout the country, with stable populations. The biggest populations in the wild can be found in the International Tagus Natural Park, Lousã Mountain, Montesinho Natural Park, and Alentejo. The population present at the Lousã Mountain results from a reintroduction program that took place between 1995 and 1999, with the release of 96 animals (Salazar 2009; Alves 2013).

Cervus elaphus is capable of inhabit a vast diversity of areas, from woodlands to shrublands and meadows, which reflect their need for protection and food, respectively (Clutton-Brock et al 1982; Bobek et al 1984; Soriguer et al 1994; Manly et al 2002; Borkowski 2004; Alves et al 2014).

1.3.3. Feeding ecology

Being an herbivore and ruminant, red deer presents a very specialized digestive system with the stomach compartmentalized in four chambers (rumen, reticulum, omasum and abomasum, successively) to allow the breakdown of cellulose (Hofmann 1989). This compartmentalization guarantees the efficiency of the rumination and digestive process, so that bigger particles do not pass to the lower tract, maximizing the nutrient absorption (Spalinger et al 1986).

According to Hofmann (1989), *Cervus elaphus* is classified as an intermediate feeder since it alternates between grazing and browsing according to the seasonality and availability of food resources (Bugalho et al 2001; Dumont et al 2005). In Mediterranean ecosystems, including the Lousã Mountain, red deer is predominantly browser, feeding manly of shrubs, especially in summer when grasses become senescent (Milne et al 1978; Bugalho et al 1998; Bugalho et al 2001; Alves 2013). Besides the alternation between species, they can also eat different plant structures like stems which are richer in fibber or leaves with a higher protein value (Bell 1970; Garcia 2016; Garcia et al *(In prep)*).

1.3.4. Social organization and reproduction

The red deer presents a markedly sexual segregation, living associated in unisex groups most of the year, except in the rut season. With a typical matriarchal society, females usually form social groups with their mature daughters or close relatives and their respective offspring. Sub-adult males may remain in the matriarchal groups until reaching 2 or 3 years old (Alves et al 2013), which is approximately the age at which they disperse from the familiar group and join other males of similar age (Mitchell et al 1977; Wilson & Mittermeier 2011). With the increase of age, mature daughters can also abandon the familiar group to form their own group or to join another, typically within the same home range (Clutton-Brock et al 1982).

As the rut season gets closer, the social organization of the red deer changes, with males dispersing from each other and joining groups of females, forming a personal

harem or mixed sex group. This period is marked by the loss of the velvet in the antlers, increasing the levels of testosterone, mane and neck muscles growth and displays like roaring and rubbing the antlers against the vegetation to mark the territory (Lincoln 1971; Clutton-Brock et al 1982; Pépin et al 2001; Alves 2013). The rut season in the studied population occurs from mid-September till the end of October, after which males disperse and the social organization of the species returns to a normal sexual segregation (Alves et al 2013). The gestation lasts approximately 34 weeks with the calving, of a single calf or not often two, occurring between May and June (Clutton-Brock et al 1982).

1.4. Study area

1.4.1. Location, topography and climate

The Lousã Mountain is located in the Central Region of Portugal (40º3'N, 8º15'W), and extends for about 170km^2, integrating seven municipalities from Coimbra (Miranda do Corvo, Penela, Lousã and Góis) and Leiria (Figueiró dos Vinhos, Pedrógão Grande and Castanheira de Pêra) districts, and in which are included twelve hunting areas (Alves 2013).

This area presents an accentuated variation of altitude that goes from 100m to 1205m above the water level, with deep valleys and accentuated slopes to the hilltops (Alves 2013).

The climate is typically Mediterranean, being characterized by hot and dry summers, and rainy and windy winters with an occasional occurrence of snow (Archibold 1995; Alves et al 2013). The area is drained by a complex hydrological network inserted in the Mondego and Tejo Rivers basins (Alves 2013).

1.4.2. Flora and Fauna

The land cover of the Lousã Mountain is mainly composed by a mixture of coniferous and broadleaf trees combined with shrublands. Concerning the coniferous species, they are essentially *Pinus pinaster*, *Pinus sylvestris*, *Pinus nigra*, *Pseudotsuga menziesii* and *Cupressus lusitanica*. The broadleaf trees consist of *Quercus* sp., *Castanea sativa*, *Prunus lusitanica* and *Ilex aquifolium*, and are less common than the coniferous trees, with which form the mixed forests. Exotic species of the genus *Eucalyptus* and *Acacia* are becoming more frequent in the Lousã Mountain vegetation, especially in areas of lower elevation and near water courses, respectively. In terms of

shrublands they are dominated by *Erica* spp., *Calluna vulgaris*, *Ulex* spp. and *Pterospartum tridentatum*. In the surrounding areas is common to find agricultural fields with fruit trees and vegetable plantations (Alves 2013).

The faunal composition of the area includes a wide variety of avifauna, amphibians, reptiles and mammals. With focus in the ungulates, besides the red deer, two other species are present, being them the roe deer *Capreolus* and the wild boar *Sus scrofa*.

1.5. Aims

The main aim of this study is to evaluate if the differential digestive efficiency between sexes, resulting from body size sexual dimorphism, is the crucial factor to explain the behaviour of sexual segregation. Therefore, we hypothesise that differences in the digestive capabilities of males and females would be shown by differential reduction in size of the ingested food fragments. By comparing the size of the plant fragments present in the faeces of hunted animals from the Lousã Mountain, we predict that if males have higher digestive efficiency, despite ingesting bigger fragments, they would excrete fragments of equal size or even smaller than females. Nonetheless, is important to adjust the predictions considering the variations in diet quality and activity patterns of each sampled season, since the interaction between these factors may change the outcome. So, when diet quality is similar between sexes, and assuming synchronized activity patterns that may not benefit both sexes – rut season – we predict that males exhibit smaller fragments than females. On the other hand, when diet quality is similar between sexes, but they differ in the activity patterns, and assuming each sex shapes the activities in their benefit – autumn and winter – we predict that males present equal sized fragments than females. When diet quality differs between sexes, being higher in males, and assuming the activity patterns benefit each sex – spring – we predict that males present equal sized fragment than females.

Chapter 2

Linking digestive efficiency and sexual segregation:
can sexual differences in digestibility explain the
sexual segregation patterns in red deer?

2.1. Introduction

Red deer males and females have been proved to live apart all year, with exception of the rut season, phenomenon known as sexual segregation (Alves et al 2013). This behaviour has been broadly described in the animal kingdom, but whether or not it consists in the differential use of habitat (Clutton-Brock et al 1987; Kie & Bowyer 1999) or purely in their separation into distinct social groups (Conradt 1998; Ruckstuhl & Neuhaus 2005) is yet to be clarified. In fact, despite the variety of hypotheses proposed throughout the years, none was found to provide a universal explanation to this behaviour, leading to believe that a multiplicity of behavioural and environmental aspects play a combined role to promote sexual segregation (Main et al 1996; Bonenfant et al 2004; Pérez-Barbería et al 2005; Alves et al 2013). Therefore, it's important to keep in mind that hypotheses proposed for habitat and social segregation are not mutually exclusive and might complement each other.

Authors defending the forage selection hypothesis suggest that body size dimorphism produces distinct energetic requirements, as well as different digestive efficiencies in both sexes, leading to differences in the diet choices, and consequently on the habitat used. A model, known as gastrocentric model, was proposed to better explain the implications of body size in the digestive capabilities of males, non-reproductive females and reproductive females (Barboza & Bowyer 2000). They predicted that males should be able to better digest forage of lower quality than non-reproductive females, since they have higher gut capacity and longer retention times due to their higher body mass. Thereby, male's capacity to ingest higher quantities of forage in shorter periods of time and to better digest lower food quality would compensate for their superior energetic requirements. Nonetheless, non-reproductive and reproductive females, despite having similar gut capacities, differ in the energetic requirements, since gestation and lactation imply higher demands of nutrients and energy. Therefore, they proposed that reproductive females would select higher food quality and increase the food intake, as well as increase the post-ruminal segments of their digestive tract to allow a higher absorption ability, compensating their higher nutritional demands.

Since the size of the particles have a great influence in the digestive process, because smaller particles are better exposed to the digestive agents due to a higher surface-volume ratio (Ulyatt et al 1986; Bjorndal et al 1990), this might be a good measure of the digestive efficiency. Nevertheless, to understand how body size can influence the digestive efficiency is important to know how it affects the parameters involved in the process. Chewing efficiency depends on the teeth and bite sizes, since they determine the contact area between the food and the teeth surface and the size of

the ingested food fragments, and both scale allometrically with body size (Clutton-Brock & Harvey 1983; Pérez-Barbería & Gordon 1998; Mysterud 2000). On the other hand, rumen volume and gut capacity increase proportionally to body size and are responsible for controlling the amount of food that can be accommodated, and also the retention capacity (Demment & Soest 1985; Illius & Gordon 1987; Fritz et al 2009). However, constraints resulting from anatomic and environmental characteristics, for example diet quality, might generate behavioural responses in terms of the time spent feeding and ruminating and in the chewing frequency, which would also have impact in the digestive efficiency (Pérez-Barbería & Gordon 1998; Ruckstuhl 1998; Pipia et al 2008). Therefore, even if the digestive capabilities differ between males and females, as expected from their distinct anatomical features, changes on the behavioural aspects involved in the digestive process might occur as a compensation mechanism to overcome the anatomical disadvantages (Dryden et al 1995; Gross et al 1995). Baring this in mind, besides causing differences in the digestive efficiency, body size dimorphism may also be responsible for differential activity patterns in males and females (Ruckstuhl 1998), which would relate the forage selection and the activity budget hypotheses. Such relation raises, once again, the question if habitat and social components of sexual segregation can be dissociated from each other, or may play a combined role in such a complex behaviour.

Previous studies approached the forage selection hypothesis in a comparative perspective, focusing on the feeding behaviour of males and females, and postulating predictions related with food quality. According to this hypothesis, males would ingest and select habitats of poorer quality food than females, since they are expected to have a higher digestive efficiency resultant from their higher body size. Several of the studies analysed this prediction in multiple ruminant species using direct observations or faecal content analysis. While some of them corroborated the prediction of males selecting lower quality habitats (i.e. Beier 1987; Pérez-Barbería et al 1997; Mysterud 2000; Neuhaus & Ruckstuhl 2002), the majority didn't provide support for the forage selection hypothesis (i.e. Miquelle et al 1992; Main & Coblentz 1996; Ruckstuhl 1998; Pérez-Barbería & Gordon 1999; Mysterud 2000; Ruckstuhl & Neuhaus 2002; Bonenfant et al 2004), including in *Cervus elaphus* (Clutton-Brock et al 1982).

However, in this study we followed a different approach to the same hypothesis by focusing on the assumption that body size dimorphism causes differential digestive efficiency in both sexes. By taking in account the model proposed by Barboza and Bowyer (2000) and other physiological relations with body size, the target of the study is to evaluate the size of the plant fragments present in the faecal material of males and females. The aim is to understand if the body size dimorphism reflects a distinct digestive

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efficiency between sexes, in terms of reduction of the food particles along the digestive process, that might explain the sexual segregation patterns. If males have higher digestive efficiency than females, it's expected that male's faeces present equal or smaller sized fragments than female excrements, even though males ingest larger food fragments. However, specific predictions can be made for each sampled season concerning the diet quality and activity patterns of each sex. In the rut season, we predict that males exhibit smaller fragments than females, since they feed on similar quality forage and assuming that the activity patterns presented during aggregation do not benefit one of the sexes. Regarding autumn and winter, males are expected to excrete equal sized fragments than females, once the diet quality is similar between them but the activity patterns may vary to benefit each sex. In spring, we predict equal sized fragments since the diet quality is different between sexes and activity patterns should also be shaped in benefit of each sex.

2.2. Methods

2.2.1. Study area

This study took place in the Lousã Mountain, characterized by a typical Mediterranean climate and a high range of altitude among the deep valleys and round hilltops (Archibold 1995; Alves 2013). Since the reintroduction of red deer, the species has expanded within 435km^2 of the mountainous region and the surrounding areas, becoming an important game species (Alves 2013). The hunting network in the Lousã Mountain comprehends twelve hunting areas, subdivided in national hunting areas (ZCN), municipal hunting areas (ZCM), associative hunting areas (ZCA) and area without game management (TNO) (Alves 2013). Every year, between October and February, organized hunting events, called "montarias", take place in several of these areas, usually once or twice per hunting area per year.

2.2.2. Sample collection

In this study, only samples from adult individuals were used to guarantee a highly sexual dimorphism that might justify the differences in the digestive efficiency of males and females. The samples were collected in four periods – rut, autumn, winter and spring – in several sampling points of the Lousã Mountain (Fig.1).
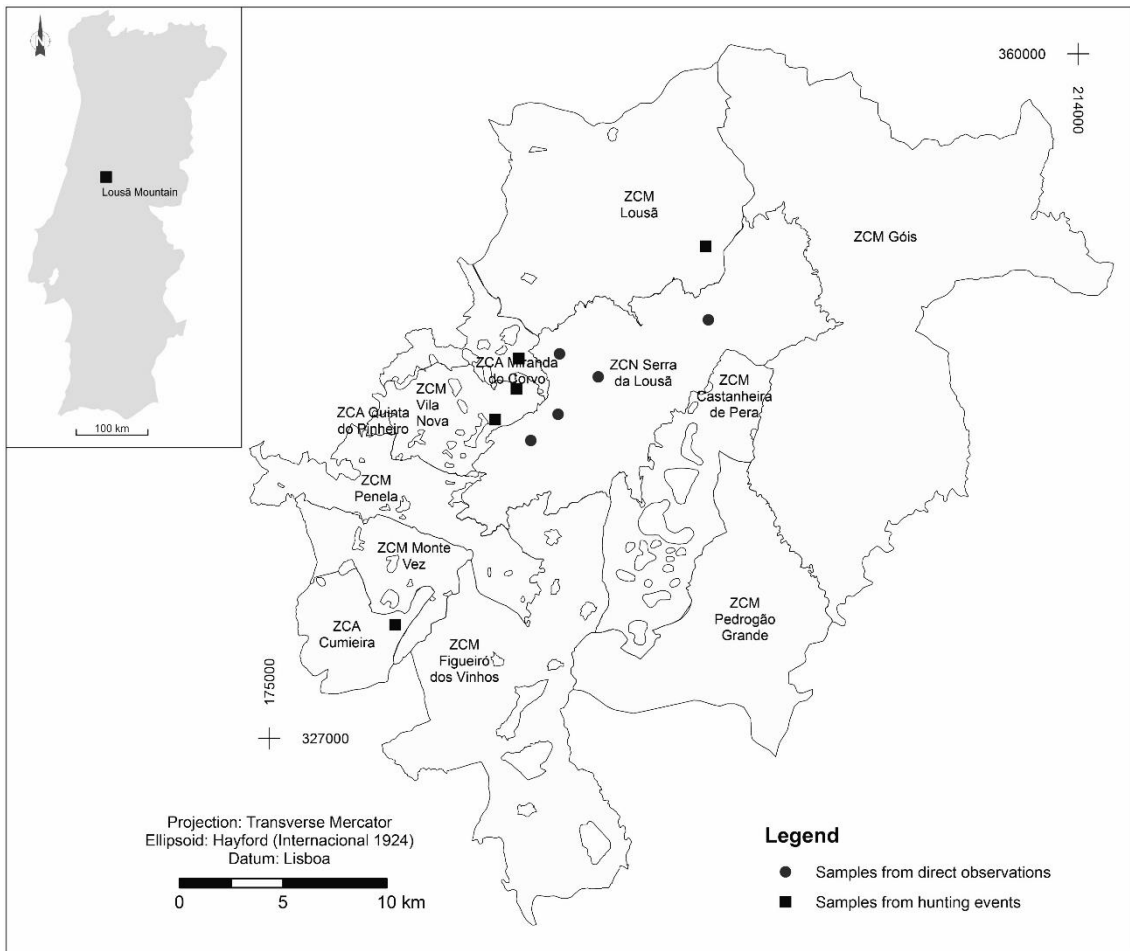
Fig. 1 – Map of the study area, hunting areas and sampling points.

Faecal samples were collected directly from animals hunted in "montarias", between 2013 and 2017, allowing an easy identification of the age and sex of each individual. These parameters, as well as several body size measurements, were taken, and the samples were placed in plastic flasks and labelled with a unique identification for each animal sampled. A total of 58 samples were collect during the rut season (9 females and 11 males), autumn (11 females and 11 males) and winter (10 females and 6 males).

Faecal samples were also collected during April to June of 2013 through direct observation, allowing to have samples from spring. The direct observations were made in defined points of the Lousã Mountain with binoculars and telescope, and whenever an animal was observed defecating, the age, sex and geographic parameters were registered to allow the collection of the respective faeces. A total of 15 samples were collected during spring, being 8 from females and 7 from males.

All the samples were stored in a freezer at -20ºC until further analysis.

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2.2.3. Sieve analysis

After being defrosted, 5 pellets of each sample were separated, weighted and soaked in water with detergent for about one week to allow the sieving without damaging the fragments. Posteriorly, the samples were washed, with current water, through a sequence of sieves (1mm, 0.5mm, 0.106mm and 0.075mm) and the portions retained in each sieve were transferred to identified petri dishes. The portions retained in the 0.5mm sieve were stained with methylene blue and preparations were made by placing a couple of drops of each in clean petri dishes and covered with a coverslip to eliminate the excess of water. Since each preparation contained about 5 to 10 fragments, 20 preparations were made, for each sample, and photographed in the magnifier with the ampliation of 0.7x, to allow the measuring of 100 fragments. All the samples were dried at 60ºC for 48h and then weighted (± 0.001g).

Posteriorly, the fragments were analysed in the software Fiji – Image J. The scale was set by using a photograph of a metallic ruler for the ampliation used, and the photographs were converted into black and white images to allow the software to automatically measure the fragments (the procedure is presented in more detail in Appendix 1). A total of 100 fragments were measured in each subsample and the measurements taken were the area (mm^2) and perimeter (mm).

2.2.4. Data analysis

Since the sieve of 0.5mm was the first in which a considerable amount of matter was retained and the sieves below showed fragments of standardized sizes, to analyse the differences in the fragments size only the 0.5mm sieve was considered. So, to analyse the size of fragments of males and females, two generalized linear models (GLM) were performed for the area and the perimeter of the fragments retained in the 0.5mm sieve. The mean area and perimeter were calculated for each individual and these measures were used in separated GLM's as the dependent variable and fitted with a gamma with log link distribution, since the values were strictly positive. The sex of the individuals and the season were used as factors, with the interaction between them being also evaluated, and pairwise comparisons performed using Bonferroni correction.

To analyse the weight results, the mean particle size was calculated for each individual, using two indexes – modulus of fineness (MOF) and discrete mean (dMEAN). First, the sieves where numbered from 1 to 4, from smaller to bigger, and the weights where expressed as the proportion of particle mass retained in each sieve relatively to

the total mass of particles retained for each sample. The MOF (Poppi et al 1980; Fritz et al 2012) was then calculated as:

$$MOF = \sum_{i=1}^{n} p(i) \times i$$

being *i* the number of the sieve and *p(i)* the proportion of particle mass retained in the respective sieve. The dMEAN (Fritz et al 2012; Clauss et al 2015) was calculated as:

$$dMEAN = \sum_{i=1}^{n} p(i) \times \frac{S(i+1) + S(i)}{2}$$

being *S(i)* the pore of the sieve. For the mean particle size of the particles retained in the maximum sieve used, the maximum particle size *S(n+1)* was defined as 2mm. The indexes were then used as dependent variable in two separated GLMM's in the same way as described above for the fragment size analyses.

The statistical analyses were made using the IBM SPSS Statistics 23 software. The results are presented as estimated means ± standard error, and 95% confidence intervals. *P* values < 0,05 were considered significant.

2.3. Results

When analysing the area of the fragments (Fig. 2), significant differences were found for the interaction between sex and season ($F_{(3.65)} = 2.967$; $p = 0.038$). The pairwise comparisons allowed to detect that significant differences were found between sexes within the rut season ($t_{(65)} = 2.737$; $p = 0.008$), revealing that the fragments excreted by males had a lower mean area (0.487 ± 0.023 mm^2) than those excreted by females (0.583 ± 0.026 mm^2), in that season.
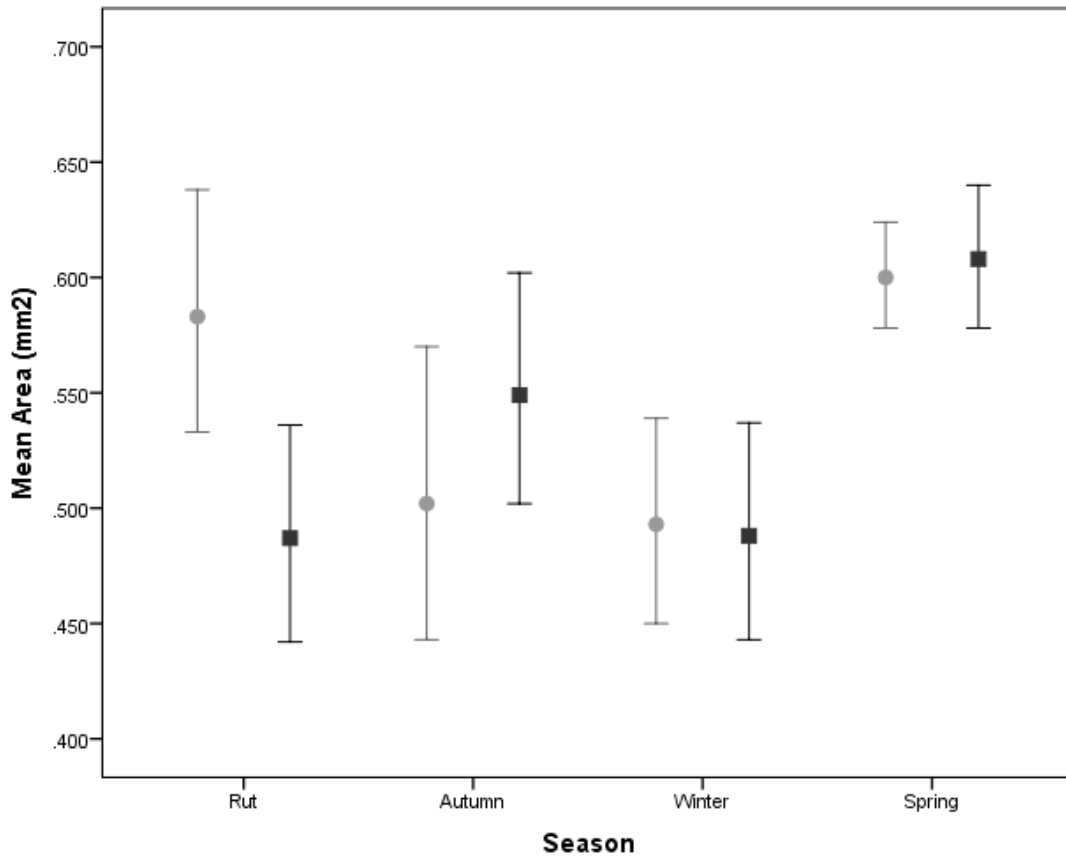
Fig. 2 – Mean area of the fragments (mm^2) by season for females (grey circles; $N = 38$) and males (black squares; $N = 35$).

In terms of perimeter of the fragments (Fig. 3), similarly to what was assessed previously with the mean area, significant differences were found for the interaction between sex and season ($F_{(3,65)} = 5.057$; $p = 0.003$). Through the pairwise comparisons it was verified, once again, that significant differences were found for sexes within the rut season ($t_{(65)} = 3.101$; $p = 0.003$), with males excreting fragments of lower perimeter (3.415 ± 0.108 mm) than females (3.851 ± 0.090 mm), and for sex within the spring ($t_{(65)} = 2.334$; $p = 0.023$), revealing males with larger fragments (4.290 ± 0.104 mm) than females (4.016 ± 0.55 mm).
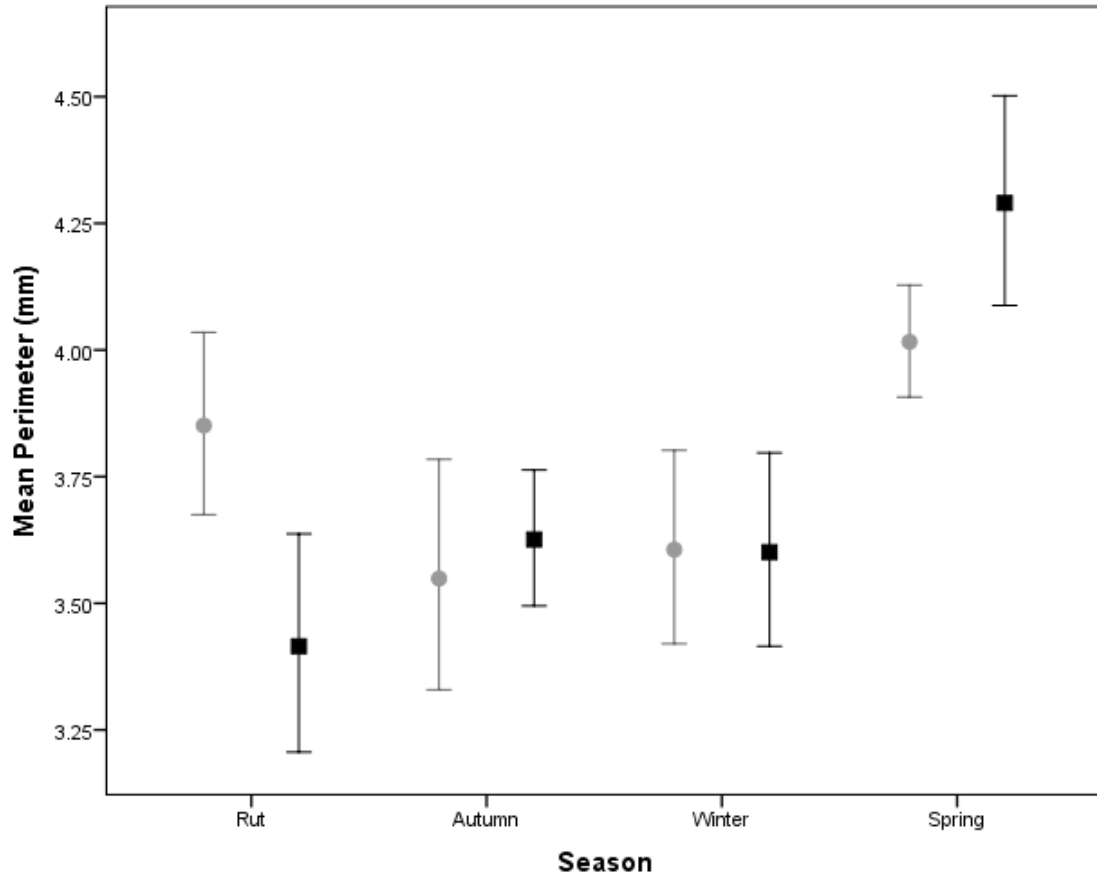
Fig. 3 – Mean perimeter of the fragments (mm) by season for females (grey circles; $N = 38$) and males (black squares; $N = 35$).

Regarding the two indexes calculated, since the results showed the same patterns, and according to Fritz et al (2012) the dMEAN is a better descriptor of the mean particle size than MOF for sieve analysis, only the result of dMEAN is presented (Fig. 4). Significant interaction between sex and season ($F_{(3,65)} = 3.368$; $p = 0.024$) was found. The pairwise comparisons revealed significantly different values of dMEAN for each sex within the winter ($t_{(65)} = 3.340$; $p = 0.001$), with males showing higher dMEAN values (0.499 ± 0.016) than females (0.430 ± 0.013).
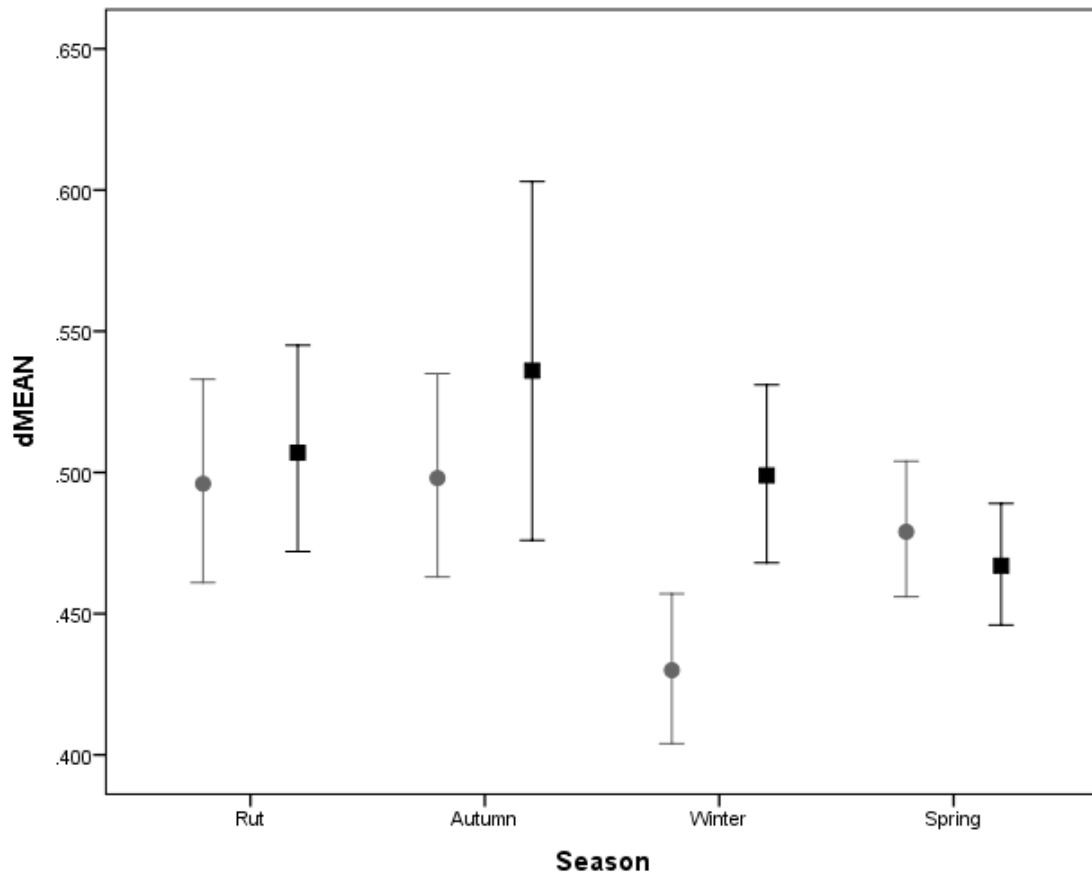
Fig. 4 – Discrete mean of the fragments by season for females (grey circles; $N = 38$) and males (black squares; $N = 35$).

2.4. Discussion

The results obtained for the area and perimeter of the fragments excreted reveal that significant differences between males and females occur in the rut season, and in spring in the case of the perimeter. Before takin any further conclusions about the meaning of this results, it's important to remember that digestibility depends on various factors, particularly the anatomic features, the feeding and rumination times and the quality of the diet (Demment & Soest 1985; Gross et al 1995; Pérez-Barbería & Gordon 1998, Barboza & Bowyer 2000). Consequently, a certain caution it's needed when interpreting the results. Since the bite size increases with body size (Clutton-Brock & Harvey 1983; Pérez-Barbería & Gordon 1998; Mysterud 2000), males ingest bigger fragments than females, but if their digestive efficiency is higher, as suggested in the forage selection hypothesis (Demment & Soest 1985; Barboza & Bowyer 2000), the fragments excreted by males are expected to be equal or smaller than the ones excreted by females. This assumption should be true, at least, when the anatomic characteristics

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are the only variable between sexes, that is, when the other parameters affecting digestive efficiency are identical for both sexes.

According to a previous study, the red deer population from the Lousã Mountain only aggregates in the rut season (Alves et al 2013), which implies certain changes on the grazing, rumination and resting times of males and females so they can synchronize their activity patterns, allowing the aggregation (Conradt 1997, 1998; Ruckstuhl 1998; Ruckstuhl 1999). The diet of this same population was also evaluated in another study, revealing no significant differences between the diet quality ingested by males and females in the rut, autumn and winter (Garcia et al (*In prep)*). Taking this into account, the rut is the only season that allows a comparison of the digestive efficiency based exclusively in the anatomic characteristics of each sex. In fact, our results show significant differences between sexes in this season, both in terms of area and perimeter, with males presenting smaller fragments than females. Since males ingest bigger plant fragments, the differences reflect a greater particle size reduction through the digestive process of this sex, therefore revealing a superior digestive efficiency relatively to females, when both feed on similar quality diets (Garcia et al (*In prep)*) and are expected to have synchronized activity patterns (Conradt 1997, 1998; Ruckstuhl 1998; Ruckstuhl 1999). These results confirm our prediction for this season, supporting the main assumption of the forage selection hypothesis (Demment & Soest 1985; Barboza & Bowyer 2000). Despite the expected synchrony of activities, the possibility that the activity patterns performed in this season might be favouring one of the sexes cannot be excluded, but since these patterns are not known, no further conclusion can be made about this subject.

Regarding autumn and winter, despite sexes being segregated (Alves et al 2013), thus being able to change their activity patterns in their benefit and independent of the other sex, the quality of the diet is still similar (Garcia et al (*In prep)*). Thereby, the digestive efficiency in these seasons might be due, not only to the anatomy of each sex but also to their specific behavioural response that might be shaped accordingly to their body size constraints (Ruckstuhl 1998; Pipia et al 2008). The fact that no significant differences were found in terms of area and perimeter, doesn't mean that males don't have higher digestive capabilities than females, because they ingest bigger and excrete equal sized particles, nevertheless presenting a greater reduction of the particles than females. These results go in accordance to our prediction, therefore supporting the forage selection hypothesis, only this time, the distinct digestive efficiency between sexes should be the result of an interaction between their anatomic characteristics and activity patterns. Studies comparing the activity patterns of males and females in several ungulates, including red deer, showed that females spent more time feeding than males

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(Duncan 1975; Leuthold & Leuthold 1978; Clutton-Brock et al 1982; Ruckstuhl 1998; Neuhaus & Ruckstuhl 2002; Pipia et al 2008). This also makes sense from the theoretically point of view, once males have bigger bite sizes (Clutton-Brock & Harvey 1983; Mysteryd 2000) and rumen volume (Demment & Van Soest 1985), they are able to ingest and accommodate larger amounts of food in shorter periods of time (Barboza & Bowyer 2000). Therefore, they would be able to spend more time ruminating, which would contribute to the reduction of the ingested plant fragments, accentuating their higher digestive efficiency. The fact that the differences in the digestive efficiency in these seasons are less notable than in the rut might be because the diet in the rut has poorer quality (Garcia et al (*In prep)*), which may cause the digestive capabilities of males to stand out, despite the activity patterns. It's also noteworthy that in autumn and winter the fragments were smaller in terms of mean area and perimeter, which goes in accordance to the fact that these are the seasons where the diet quality is higher, and so both sexes should be able to equally digest it (Demment & Soest 1985).

Relatively to the spring, our results showed that females excreted smaller fragments, in terms of perimeter, than males, which would suggest that females had a higher digestive efficiency in this season, contrarily to our predictions. Nevertheless, unlike in the previous analysed seasons, besides being segregated (Alves et al 2013), the sexes ingest different quality forage, so any hasty conclusions need to be analysed in more detail. In terms of diet quality, the differences found in spring reflected an ingestion of higher quality by males (Garcia et al (*In prep)*), contrarily to one of the predictions of the forage selection hypothesis. This occurrence doesn't invalidate the fact that males are able to digest poorer quality forage due to higher a digestive efficiency, only enhances the idea that this ability doesn't necessarily force them to choose this type of food when superior quality food is available (Illius & Gordon 1987). And a likely explanation for why males ingest higher quality food than females in spring is that this season corresponds to the period of final gestation and births for females, so, it's possible that females seek for more protected habitats and reduce their home range in this particular season even if it compromises the quality of the food available, as proposed by the reproductive strategy hypothesis (Main & Coblentz 1990, 1996; Alves et al 2013). Besides females already having a smaller bite size (Clutton-Brock & Harvey 1983; Mysteryd 2000), they might be shaping their activity patterns to compensate for feeding on less quality forage (Pérez-Barbería & Gordon 1998; Pipia et al 2008) and potentially having increased energetic demands if gestating or lactating (Barboza & Bowyer 2000), which may be reflecting in the smaller size of the excreted fragments in comparison to males. Additionally, the fact that males are ingesting higher quality forage may also reflect a need to reduce less the fragments to extract the maximum energy and

nutrients. Contrarily to the perimeter, no differences were found relatively to the area of the fragments in the same season which suggests a bigger digestive efficiency of males. The fact that the results from the spring are contradictory and that a multiplicity of factors, other than the anatomic features of each sex, may be affecting the digestive process makes it difficult to compare the digestive efficiency of males and females. The spring is also the season in which the ingested diet present poorer quality (Garcia et al (*In prep)*), in general, reflecting the largest fragments in comparison to the remaining seasons.

In regard to the dMEAN, is important to mention that it represents an estimated value for the mean particle size based on the proportion of particles retained in each of the used sieves, considering for the calculation all the sieves together (Fritz et al 2012; Clauss et al 2015). Thereby, these results give an overall idea of the fragment size without focusing only in the 0.5mm sieve.

In the rut, no differences were found between sexes, contrarily to what happened relatively to the mean area and perimeter and to our prediction. Although, once again, the fact that males excreted equal sized particles also reveals a superior reduction of the particles during the digestion when compared to females. Therefore, the mean particle size corroborates the higher digestive capabilities of males, when both sexes eat similar quality forage (Garcia et al (*In prep)*) and are expected to have synchronized activity patterns (Conradt 1997, 1998; Ruckstuhl 1998; Ruckstuhl 1999), which supports the assumption of the forage selection hypothesis.

Concerning autumn and winter, these are seasons in which the sexes are segregated but present a diet of similar quality (Alves et al 2013; Garcia et al (*In prep)*). In autumn, no differences were found in the mean particle size similarly to the area and perimeter, as predicted Thus indicating that in this season males present a higher digestive capability than females which might result not only from their anatomy but also from their activity patterns. Contrarily to our predictions, in winter the mean particle size varied between sexes, with females presenting smaller fragments than males, suggesting a higher digestive efficiency of females. If we assume the previous results of the area and perimeter, this might be simply the result of their activity patterns compensating the anatomical disadvantages imposed by their body size (Ruckstuhl 1998). Besides, winter is the second season with the higher diet quality (Garcia et al (*In prep)*), in general, which could also be contributing to accentuate the differences, once males might extract the maximum energy without the need to further reduce the particles.

Relatively to spring, no differences were found in terms of mean particle size, as expected, which would suggest a higher digestive capability of males and support the main assumption of the forage selection hypothesis, but, as previously mentioned, it's

especially difficult to make any comparisons in this season, since the quality of the diet differs between sexes.

Few studies were made concerning the faecal particle size and the one's made focused on relating the mean particle sizes with body size across ruminant feeding types (Clauss et al 2002) and ruminant species (Clauss et al 2015), rather than doing intraspecific comparisons and using actual measures of the fragments. Instead they used indexes to estimate the mean particle size, like the dMEAN which was also used in this study. The comparison of the dMEAN within ruminant species, fed on a grass hay diet, revealed that the mean particle size is approximately constant independent of the body size (Clauss et al 2015). Assuming the increasing bite size with the body size (Clutton-Brock & Harvey 1983; Mysteryd 2000), these results support that bigger ruminants have higher digestive efficiency in terms of particle size reduction when fed on the same diet, which is also supported by our results. Nonetheless, that study only refers one type of diet and does not consider the possible differences in the activity patterns of each species.

Comparing the results from the area and perimeter with the mean particle size, some slight differences were observed. The fact that the dMEAN represents a global measure of the faecal particles can cause the significant differences found in the particles retained in the upper sieve to be mitigated by the largest amounts of particles retained in the remaining sieves. In the other hand, the differences found in the mean particle size that were not found in the mean area and perimeter might be a result of major portions of particles being retained in different sieves, other than the upper one, for each sex. Nevertheless, in general, both the results allow to conclude that males present higher digestive efficiency than females, when they feed on identical food quality (Garcia et al (*In prep)*) and are expected to have similar activity patterns (Conradt 1997, 1998; Ruckstuhl 1998; Ruckstuhl 1999), which supports the forage selection hypothesis but does not provide a complete explanation to the annual patterns of segregation of this red deer population. Further studies comparing the size of the fragments in different stomach compartments may help to clarify if these differences result from the mechanical action of rumination or from the action of digestive enzymes. Besides, it's possible that the higher digestive efficiency of males results from higher rumination times, which would also support the activity budget hypothesis (Ruckstuhl 1998). As so, our results suggest that sexual segregation may be the outcome of a variety of factors, and that feeding behaviour, digestibility and activity patterns are some of the factors that may help to clarify the sexual segregation patterns verified in this population.

Chapter 3
General conclusions

In terms of methodology, the mean particle size provides a global overview of the size of the fragments based on the proportion of matter retained in the sequence of sieves (Fritz et al 2012; Clauss et al 2015). Therefore, it's possible that this measure might mitigate the differences found in the upper sieve, besides being an estimated value. Using actual measures of fragments retained in the upper sieve was an innovative aspect of this study and allowed to have a better perception of the differences between the size of the fragments without them being masked by the standardised particles that are retained on the sequentially inferior sieves. However, this last method might also mislead the overall size of the faecal particles, besides being a much more time-consuming method. So, it seemed cautious to use both methods and compare the results. Concerning the sampling effort, 100 fragments was proven to discriminate 85% of the species richness in the red deer diet (Garcia 2016), being, therefore, chosen as the sampling effort for this study, since it represents a good commitment between cost and reliability.

This study allowed to confirm the assumption that males have higher digestive efficiency than females, supporting the forage selection hypothesis in the studied population, although not being able to explain in a complete extent their patterns of segregation. In addition, some of the predictions of the forage selection hypothesis have not been supported in other studies (i.e. Clutton-Brock et al 1982; Miquelle et al 1992; Main & Coblentz 1996; Ruckstuhl 1998; Pérez-Barbería & Gordon 1999; Mysterud 2000; Ruckstuhl & Neuhaus 2002; Bonenfant et al 2004), including in the same red deer population (Garcia et al (*In prep*)), regarding the diet quality of each sex. In fact, all the previous studies were consistent in one thing, none of them could provide a universal explanation for the occurrence of sexual segregation. This reinforces that idea that no single factor is responsible for this behaviour, but instead they complement each other and might, simultaneously, play a role in sexual segregation. However, body size dimorphism and the consequent different digestive capabilities of males and females seem to be an important starting point in future studies of sexual segregation, since it allows to relate several concepts of the habitat and the social components of this behaviour. So, the attempt to simplify a complex behaviour like sexual segregation is probably the wrong approach and more robust studies involving multiple factors, including feeding behaviour, digestibility and activity patterns, may help to better understand this phenomenon.

References

Alves, J. A. D. S. (2013). Ecological assessment of the red deer population in the Lousã Mountain (PhD thesis, Universidade de Aveiro).

Alves, J., da Silva, A. A., Soares, A. M., & Fonseca, C. (2013). Sexual segregation in red deer: is social behaviour more important than habitat preferences? Animal behaviour, 85(2), 501-509.

Alves, J., da Silva, A. A., Soares, A. M., & Fonseca, C. (2014). Spatial and temporal habitat use and selection by red deer: The use of direct and indirect methods. Mammalian Biology-Zeitschrift für Säugetierkunde, *79*(5), 338-348.

Archibold, O. W. (1995). Mediterranean ecosystems. In Ecology of world vegetation (pp. 131-164). Springer, Dordrecht.

Barboza, P. S., & Bowyer, R. T. (2000). Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. Journal of Mammalogy, 81(2), 473-489.

Barboza, P. S., & Bowyer, R. T. (2001). Seasonality of sexual segregation in dimorphic deer: extending the gastrocentric model. Alces, 37(2), 275-292.

Barry Jr, R. E. (1977). Length and absorptive surface area apportionment of segments of the hindgut for eight species of small mammals. Journal of Mammalogy, 58(3), 419-420.

Baskin, L., & Danell, K. (2003). Ecology of ungulates: a handbook of species in Eastern Europe and Northern and Central Asia. Springer Science & Business Media.

Beier, P. (1987). Sex differences in quality of white-tailed deer diets. Journal of Mammalogy, 68(2), 323-329.

Bell, R. (1970). The use of herb layer by grazing ungulates in the Serengeti. Pages lll–123 in A. Watson, ed. Animal populations in relation to their food resources. In Symp. Br. Ecol. Soc (Vol. 10).

Bjorndal, K. A., Bolten, A. B., & Moore, J. E. (1990). Digestive fermentation in herbivores: effect of food particle size. Physiological Zoology, 63(4), 710-721.

Bleich, V. C., Bowyer, R. T., & Wehausen, J. D. (1997). Sexual segregation in mountain sheep: resources or predation?. Wildlife Monographs, 3-50.

Bobek, B., Boyce, M. S., & Kosobucka, M. (1984). Factors affecting red deer (*Cervus elaphus*) population density in southeastern Poland. Journal of Applied Ecology, 881-890.

Bon, R., & Campan, R. (1996). Unexplained sexual segregation in polygamous ungulates: a defense of an ontogenetic approach. Behavioural Processes, 38(2), 131-154.

Bonenfant, C., Loe, L. E., Mysterud, A., Langvatn, R., Stenseth, N. C., Gaillard, J. M., & Klein, F. (2004). Multiple causes of sexual segregation in European red deer: enlightenments from varying breeding phenology at high and low latitude. Proceedings of the Royal Society B: Biological Sciences, 271(1542), 883.

Borkowski, J. (2004). Distribution and habitat use by red and roe deer following a large forest fire in South-western Poland. Forest ecology and management, 201(2), 287-293.

Bowyer, R. T. (1984). Sexual segregation in southern mule deer. Journal of Mammalogy, 65(3), 410-417.

Bowyer, R. T., Kie, J. G., & Van Ballenberghe, V. (1996). Sexual segregation in black-tailed deer: effects of scale. The Journal of Wildlife Management, 10-17.

Bugalho, M. N., Milne, J. A., & Mayes, R. W. (1998). The diet of red deer in southern Portugal in relation to food availability. In Advances in Deer Biology. Proceedings of the IV International Conference on Deer Biology, Kaposvar. Pannon University of Agriculture, Pannon (pp. 216-218).

Bugalho, M. N., Milne, J. A., & Racey, P. A. (2001). The foraging ecology of red deer (*Cervus elaphus*) in a Mediterranean environment: is a larger body size advantageous? Journal of Zoology, 255(3), 285-289.

Clutton-Brock, T. H., & Albon, S. D. (1992). Trial and error in the Highlands. Nature, 358(6381), 11-12.

Clutton-Brock, T. H., & Harvey, P. H. (1983). The functional significance of variation in body size in mammals. Mammal behaviour and ecology, 632-663.

Clutton-Brock, T. H., Guinness, F. E., & Albon, S. D. (1982). Red deer: behavior and ecology of two sexes. University of Chicago press.

Clutton-Brock, T. H., Iason, G. R., & Guinness, F. E. (1987). Sexual segregation and density-related changes in habitat use in male and female Red deer (*Cervus elaphus*). Journal of Zoology, 211(2), 275-289.

Conradt, L. (1997). Causes of sex differences in habitat use in red deer *(Cervus elaphus* L.*)* (Doctoral dissertation, University of Cambridge).

Conradt, L. (1998). Could asynchrony in activity between the sexes cause intersexual social segregation in ruminants? Proceedings of the Royal Society of London B: Biological Sciences, 265(1403), 1359-1368.

Daan, S., & Aschoff, J. (1982). Circadian contributions to survival. In Vertebrate circadian systems (pp. 305-321). Springer, Berlin, Heidelberg.

Demment, M. W., & Van Soest, P. J. (1985). A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. The American Naturalist, 125(5), 641-672.

Dryden, G. M., Stafford, K. J., Waghorn, G. C., & Barry, T. N. (1995). Comminution of roughages by red deer (Cervus elaphus) during the prehension of feed. The Journal of Agricultural Science, 125(3), 407-414.

Dumont, B., Renaud, P. C., Morellet, N., Mallet, C., Anglard, F., & Verheyden-Tixier, H. (2005). Seasonal variations of red deer selectivity on a mixed forest edge. Animal Research, 54(5), 369-381.

Duncan, P. (1975). Topi and their food supply (Doctoral dissertation, University of Nairobi).

Fell, B. F. (1972). Adaptations of the digestive tract during reproduction in the mammal. In World review of nutrition and dietetics (Vol. 14, pp. 180-256). Karger Publishers.

Fritz, J., Hummel, J., Kienzle, E., Arnold, C., Nunn, C., & Clauss, M. (2009). Comparative chewing efficiency in mammalian herbivores. Oikos, 118(11), 1623-1632.

Fritz, J., Streich, W. J., Schwarm, A., & Clauss, M. (2012). Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. Journal of animal physiology and animal nutrition, 96(5), 783-797.

Garcia, F. F. (2016). Sexual segregation in red deer: a question of food? (Master's thesis, Universidade de Coimbra).

Garcia, F., Alves da Silva, A., Ruckstuhl. K.E., Sousa, J.P. & Alves, J. (In prep.) Can feeding preferences of males and females be the reason behind sexual segregation in red deer?

Geist, V. (1971). Mountain sheep. A study in behavior and evolution. University of Chicago Press.

Geist, V. (1974). On the relationship of social evolution and ecology in ungulates. American zoologist, 14(1), 205-220.

Gill, R. (1990). Monitoring the status of European and North American cervids (Global Environment Monitoring System information series no. 8). United Nations Environment programme, Nairobi.

Gross, J. E., Demment, M. W., Alkon, P. U., & Kotzman, M. (1995). Feeding and chewing behaviours of Nubian ibex: compensation for sex-related differences in body size. Functional Ecology, 385-393.

Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. Oecologia, 78(4), 443-457.

Illius, A. W., & Gordon, I. J. (1987). The allometry of food intake in grazing ruminants. The Journal of Animal Ecology, 989-999.

Jarman, P. (1974). The social organisation of antelope in relation to their ecology. Behaviour, 48(1), 215-267.

Jenks, J. A., Leslie Jr, D. M., Lochmiller, R. L., & Melchiors, M. A. (1994). Variation in gastrointestinal characteristics of male and female white-tailed deer: implications for resource partitioning. Journal of Mammalogy, 75(4), 1045-1053.

Kie, J. G., & Bowyer, R. T. (1999). Sexual segregation in white-tailed deer: density-dependent changes in use of space, habitat selection, and dietary niche.

Koubek, P., & Zima, J. (1999). *Cervus elaphus.* The atlas of European mammals, 388-389.

Leuthold, B. M., & Leuthold, W. (1978). Daytime activity patterns of gerenuk and giraffe in Tsavo National Park, Kenya. African Journal of Ecology, 16(4), 231-243.

Lincoln, G. A. (1971). The seasonal reproductive changes in the red deer stag (*Cervus elaphus*). Journal of Zoology, 163(1), 105-123.

Main, M. B., & Coblentz, B. E. (1990). Sexual segregation among ungulates: a critique. Wildlife Society Bulletin (1973-2006), 18(2), 204-210.

Main, M. B., & Coblentz, B. E. (1996). Sexual segregation in Rocky Mountain mule deer. The Journal of Wildlife Management, 497-507.

Main, M. B., Weckerly, F. W., & Bleich, V. C. (1996). Sexual segregation in ungulates: new directions for research. Journal of Mammalogy, 77(2), 449-461.

Manly, B. F. L., McDonald, L., Thomas, D. L., McDonald, T. L., & Erickson, W. P. (2002). Resource selection by animals: statistical design and analysis for field studies. Springer Science & Business Media.

Milne, J. A., Macrae, J. C., Spence, A. M., & Wilson, S. (1978). A comparison of the voluntary intake and digestion of a range of forages at different times of the year by the sheep and the red deer (*Cervus elaphus*). British Journal of Nutrition, 40(2), 347-357.

Miquelle, D. G., Peek, J. M., & Van Ballenberghe, V. (1992). Sexual segregation in Alaskan moose. Wildlife Monographs, 3-57.

Mitchell, B., Staines, B. W., & Welch, D. (1977). Ecology of red deer: a research review relevant to their management in Scotland. Institute of Terrestrial Ecology.

Mysterud, A. (2000). The relationship between ecological segregation and sexual body size dimorphism in large herbivores. Oecologia, 124(1), 40-54.

Neuhaus, P., & Ruckstuhl, K. E. (2002). Foraging behaviour in Alpine ibex (*Capra ibex*): consequences of reproductive status, body size, age and sex. Ethology Ecology & Evolution, *14*(4), 373-381.

Newsome, A. E. (1980). Differences in the diets of male and female red kangaroos in central Australia. African Journal of Ecology, 18(1), 27-31.

Oliver, M., & Oliver, J. M. (1999). El ciervo y el monte: manejo y conservación:(*Cervus elaphus* L.).

Peixoto, R. D. S. (2014). Biologia populacional de um repovoamento de veado (*Cervus elaphus* L.) em ambiente mediterrânico: padrões de uso do espaço, expansão geográfica e dinâmica de uma população fundadora (PhD thesis, Universidade de Évora).

Pépin, D., Cargnelutti, B., Gonzalez, G., Joachim, J., & Reby, D. (2001). Diurnal and seasonal variations of roaring activity of farmed red deer stags. Applied animal Behaviour science, 74(3), 233-239.

Pérez-Barbería, F. J., & Gordon, I. J. (1998). Factors affecting food comminution during mastication in herbivorous mammals: a review. Biol J Linn Soc, 63, 233-256.

Pérez-Barbería, F. J., & Gordon, I. J. (1999). Body size dimorphism and sexual segregation in polygynous ungulates: an experimental test with Soay sheep. Oecologia, *120*(2), 258-267.

Peréz-Barberia, F. J., Oliván, M., Osoro, K., & Nores, C. (1997). Sex, seasonal and spatial differences in the diet of Cantabrian chamois *Rupicapra pyrenaica parva*. Acta Theriologica, *42*(1), 37-46.

Pérez-Barbería, F. J., Robertson, E., & Gordon, I. J. (2005). Are social factors sufficient to explain sexual segregation in ungulates?. Animal Behaviour, 69(4), 827-834.

Pipia, A., Ciuti, S., Grignolio, S., Luchetti, S., Madau, R., & Apollonio, M. (2008). Influence of sex, season, temperature and reproductive status on daily activity patterns in Sardinian mouflon (*Ovis orientalis musimon*). Behaviour, 145(12), 1723-1745.

Poppi, D. P., Norton, B. W., Minson, D. J., & Hendricksen, R. E. (1980). The validity of the critical size theory for particles leaving the rumen. The Journal of Agricultural Science, 94(2), 275-280.

Ruckstuhl, K. E. (1998). Foraging behaviour and sexual segregation in bighorn sheep. Animal Behaviour, 56(1), 99-106.

Ruckstuhl, K. E. (1999). To synchronise or not to synchronise: a dilemma for young bighorn males? Behaviour, 136(6), 805-818.

Ruckstuhl, K. E., & Neuhaus, P. (2000). Sexual segregation in ungulates: a new approach. Behaviour, 137(3), 361-377.

Ruckstuhl, K. E., & Neuhaus, P. (2002). Sexual segregation in ungulates: a comparative test of three hypotheses. Biological Reviews, 77(1), 77-96.

Ruckstuhl, K. E., & Neuhaus, P. (2005). Sexual segregation in vertebrates: ecology of the two sexes. 1st edn. Cambridge: Cambridge University Press.

Salazar, D. C. (2009). Distribuição e estatuto do veado e corço em Portugal (Master's thesis, Universidade de Aveiro).

Soriguer, R. C. (1994). El ciervo en Andalucía. Junta de Andalucía.

Spalinger, D. E., Robbins, C. T., & Hanley, T. A. (1986). The assessment of handling time in ruminants: the effect of plant chemical and physical structure on the rate of breakdown of plant particles in the rumen of mule deer and elk. Canadian Journal of Zoology, 64(2), 312-321.

Uliyatt, M. J., Dellow, D. W. J., John, A., Reid, C. S. W., & Waghorn, G. C. (1986). Contribution of chewing during eating and rumination to the clearence of digesta from the reticulum-rumen. Control of digestion and metabolism in ruminants. Englewod Cliffs. New Jersey, 498-515.

Wilson, D. E. & Mittermeier, R. A. (2011). Handbook of the Mammals of the World. Vol. 2. Hoofed Mammals. Barcelona: Lynx Edicions.

Wilson, E. B. D. E., & Ruff, S. (1999). North American I Mammals. Smithsonian Institute, Washington, DC.

Zachos, F. E., & Hartl, G. B. (2011). Phylogeography, population genetics and conservation of the European red deer *Cervus elaphus*. Mammal Review, 41(2), 138-150.
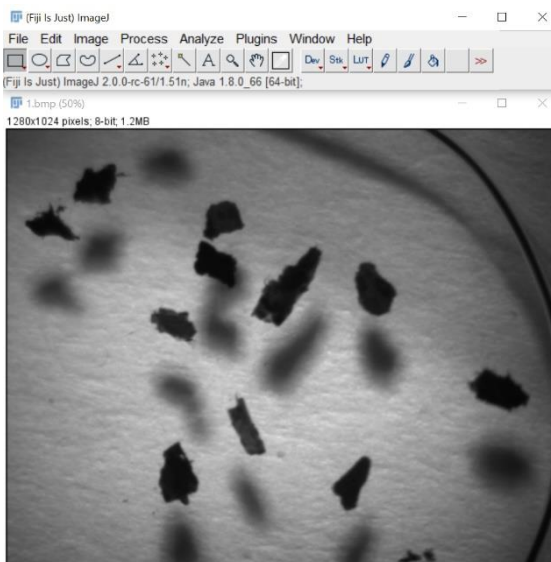
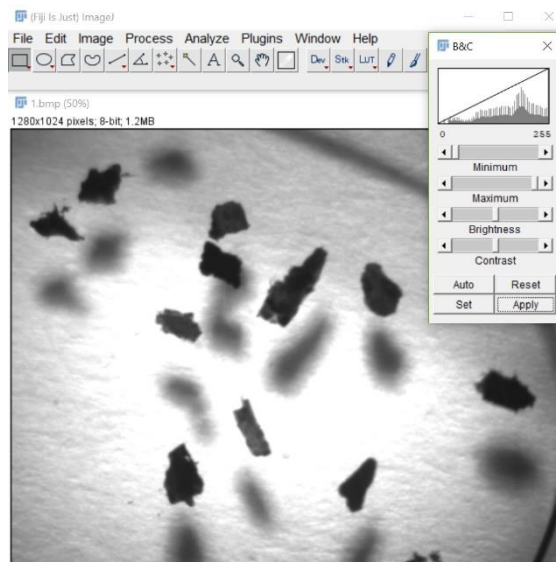# Appendix 1

How to measure fragments using Fiji (Image J)

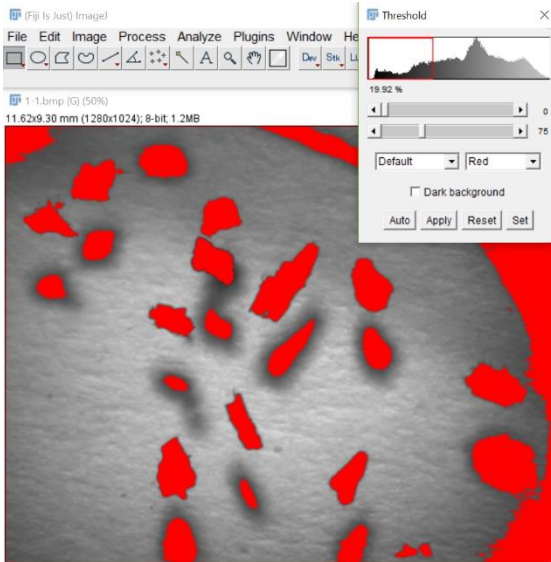**Step 1:** Open the image with scale > Trace the pretended scale measure > Analyze > Set scale
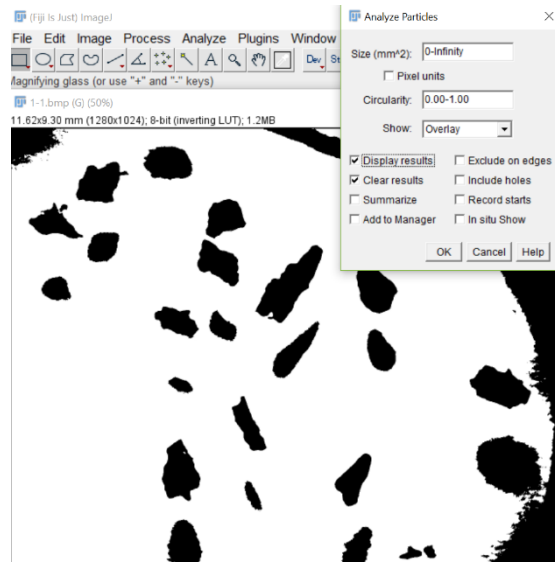

Step 2: Open the image of the fragments
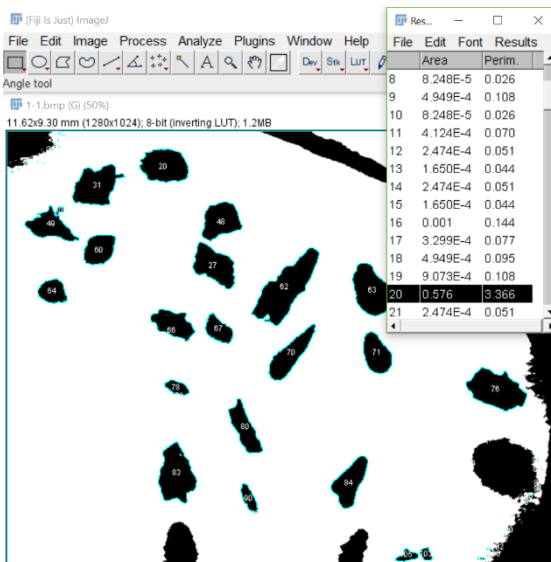

Step 3: Image > Type > 8-bit

Step 4: Image > Adjust > Contrast

Step 5: Image > Adjust > Threshold

Step 6: Analyze > Analyze particles

Step 7: From the analysed particles output choose the ones that correspond to fragments, being aware of false fragments that result from excessive shadows in the original image).