



Research Institute of
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University of Natural Resources
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Body condition and effects of coccidian infections in European Ground Squirrels (*Spermophilus citellus*) in a large population in eastern Austria

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Declaration in lieu of oath

I herewith declare in lieu of oath that this thesis has been composed by myself without any inadmissible help and without the use of sources other than those given due reference in the text and listed in the list of references. I further declare that all persons and institutions that have directly or indirectly helped me with the preparation of the thesis have been acknowledged and that this thesis has not been submitted, wholly or substantially, as an examination document at any other institution.

Date

Signature

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1 Abstract

The European ground squirrel (*Spermophilus citellus* Linnaeus, 1766) is considered an endangered species by the IUCN red list and its populations in Austria continuously decreased over the recent decades. European ground squirrels (EGS) are hibernators and utilize fat reserves as predominant energy source during hibernation. As such, these fat reserves constitute an important element in the survival of EGS. Depending on sex, age and season, the individuals in a population differ regarding the utilization of these energy reserves. EGS are known to harbor intestinal *Eimeria* parasites and as parasites generally intervene in the energetic system of their host, 63 roadkill EGS were examined to determine the influence the *Eimeria* abundance had on their body condition. The retroperitoneal fat index (RFI) was used as an indicator for body condition and the reliability of the RFI to predict body fat percentage was verified using reference fat percentages obtained from Soxhlet fat extraction of 20 animals. Parasite loads of the individuals were quantified using a modified McMaster counting method. In an information-theoretic approach, utilizing model averaging, the effects of *Eimeria* abundance, along with sex and age of the host individuals and month of collection on the RFI were investigated between May to August. *Eimeria* abundance had a negligible, but positive influence on body condition. Juvenile individuals generally had a lower body condition than adults and body condition generally increased until August. Males generally had a higher body condition than females, whereas females caught up with males around mid-June and reached higher RFI levels than males before the hibernation period. Due to the small sample size and high model uncertainty, the present study should be understood as explorative to identify important factors of body fat condition in EGS.

2 Introduction

2.1 The European Ground Squirrel (EGS)

The European ground squirrel (*Spermophilus citellus* Linnaeus, 1766) is a medium sized, hibernating rodent belonging to the family of Sciuridae (Helgen et al. 2009). EGS are living in colonies, inhabiting the steppe and grassland regions from central to south-eastern Europe. Their distribution range is separated by the Carpathian Mountains into a north-western and a south-eastern range. The south-eastern distribution extends from European Turkey and Greece, up north to Romania and Moldova; the north-western distribution ranges from Hungary, over Slovakia and Austria, to the Czech Republic (Coroiu et al. 2008; Ruzic 1978). EGS populations in Austria are restricted to the north-eastern states of Lower Austria, Vienna and Burgenland, where they are present in the Pannonian low- and highlands in the planar to sub mountainous zone. EGS are adapted to grove free grasslands with low vegetation heights and, due to their ground-dwelling nature, require deep soil types with low subsoil water levels (Spitzenberger and Bauer 2001). Because of declining population trends, *Spermophilus citellus* is classified as 'Vulnerable' (VU) on the IUCN Red List and is also listed in the Appendix II of the Bern Convention and Annexes II and IV of the EU Habitats and Species Directive (Coroiu et al. 2008). In Austria, EGS is classified as 'Endangered' on the national Red List (Wallner et al. 2005-2010) and its protective status is further acknowledged by protection laws on national and state levels (Enzinger and Gross 2013; Herzig-Straschil 2007; Janák et al. 2013). Loss of suitable habitat, mostly due to habitat fragmentation, has led to a decline in EGS populations in Austria since the 1970's. Continued reduction into small isolated populations and loss of most of the large and densely populated colonies marked this ongoing trend (Spitzenberger and Bauer 2001). Lower Austrian EGS populations experienced a negative trend development of 48% from 2009 to 2012 and extinction of 27 of formerly 168 populations (Enzinger and Gross 2013). In Burgenland, nearly all populations decreased in size or density between 2004 and 2007. Habitat degradation is assumed to have led to this development, but even habitats with low degradation had declining EGS populations (Herzig-Straschil 2007). Around 45% of the Austrian populations are located in Natura 2000 areas, but despite the species' protection status, potentially suitable EGS habitats are mostly not covered by the Natura 2000 network (Janák et al. 2013).

The major threats to EGS are influences of agricultural management, fragmentation by urbanization, residential, commercial and traffic development (Janák et al. 2013). Habitat fragmentation and isolation can lead to low genetic variability and high inbreeding coefficients were already reported in a study on the genetic variation in EGS populations in the Czech Republic (ŘÍČANOVÁ et al. 2011). Degradation and shrinkage of habitats can also impact

population dynamics by influencing parasite-host relationships (Sinclair et al. 2006), as transmission rates increase with higher host presence (Scott 1988). Parasites and their effects on EGS are scarcely investigated and research is needed in this field to provide knowledge for conservation management (Janák et al. 2013; MATĚJŮ 2010).

2.1.1 Biology and ecology of EGS

A distinct male biased sexual dimorphism in EGS is reflected in significant differences in larger mean cranial length, body size and body weight (Klenovšek and Kryštufek 2013; Ruzic 1978; Spitzenberger and Bauer 2001). Body size ranges for adult females are 174-217 mm, for adult males 176-228 mm. Adult male body weights range from 125g-380 g, and 131-353 g in adult females (Ramos-Lara et al. 2014).

Each burrow in a colony is occupied by a single individual and only females share their burrow with their offspring until juvenile dispersal (Millesi et al. 1998; Váczi et al. 2006). The burrow serves as a shelter from extreme environmental conditions, cover from predators and after emergence, as hibernacula. EGS are active during the day and retreat into their burrows in the evening (Everts et al. 2009). Home ranges of single individuals in a colony often overlap and are on average 0.1 to 0.4 ha. The extents of surface exploitation range from 40m up to 338m and depend on habitat structure, season, population density, sex, and age (MATĚJŮ 2008; Turrini et al. 2008).

Significant knowledge about EGS ecology is derived from a long studied Austrian population near Langenzerndorf (48°31 N, 16°36 E) north of Vienna (Hoffmann et al. 2004; Huber et al. 1999; Huber et al. 2001; Huber et al. 2002; Millesi et al. 1998; Millesi et al. 1999a; Millesi et al. 1999b; Millesi et al. 2000; Millesi and Hoffmann 2008). I decided to compare my results mainly to the studies on the Langenzerndorf population and additionally to studies on North American ground squirrel species, as far as a comparison is appropriate (Buck and Barnes 1999; Jameson and Mead 1964; Neal 1965).

2.1.2 Differences in energy allocation

The length of the active season determines an individual's ability to accumulate energy reserves needed for hibernation. Depending on population density, differences in energy allocation during the season of above ground activity can vary distinctly between sexes and age classes (Millesi et al. 2004; Millesi and Hoffmann 2008).

Emergence from hibernation starts in Austria at the beginning of March (or even around late February, pers. comm. F. Suchentrunk) and lasts to the end of April. Males are the first to emerge, followed by male yearlings and lastly females (Millesi et al. 1999a; Millesi and Hoffmann 2008). The active season is 168 days for adult and yearling males, and 135 days for adult and yearling females. The active season for juvenile males is around 102 days and around 95 days for juveniles females, according to averaged values based on data from Millesi and Hoffmann (2008).

Males

Around 3 weeks after female emergence, the mating period starts (Millesi et al. 2000) and involves male courtship behavior towards females (Huber et al. 2002; Millesi et al. 1998). Males exhibit intensive levels of aggression during the mating phase, mainly directed towards competitors, often resulting in injuries (Millesi et al. 1998; Millesi et al. 2004; Strauss et al. 2007). Shortly after the mating period, males begin to dig their individual burrows. Reproductive males at high population densities have been observed to spend time and energy digging litter burrows for their mates. This energy allocation in paternal effort benefits their offspring in terms of higher body weight at first emergence (Huber et al. 2002). Reproductive males have the highest energy expenditures during the mating period, indicated by the highest weight loss over the whole season (Millesi et al. 1999b). Male yearlings generally have a higher overwinter body weight loss than adult males (Millesi et al. 1999b). The ability of male yearlings to reach maturation can be highly variable depending on the population density. Low population densities, for example, lead to a higher proportion of reproductive yearling males (Hoffmann et al. 2003; Millesi and Hoffmann 2008). To reach sexual maturity, yearling males need to reach a certain body weight threshold before hibernation. Male yearlings in low density populations have a higher body weight at emergence than in high density populations, presumably, due to higher food availability. Having a higher body mass and therefore being reproductively active, also results in an earlier emergence compared to non-reproductive individuals (Millesi et al. 1998; Millesi and Hoffmann 2008). From around mid-June onwards, fat accumulation starts for reproductive males and their body mass increases rapidly until immergence. At this point, adult males reach higher body weights than females of the same age (Millesi et al. 1999b). Male yearlings that do not participate in reproduction, reach their lowest body weight after hibernation (Hoffmann et al. 2008; Millesi et al. 1998) and can accumulate fat storages from then on to reach the same body weight levels as adult males shortly before hibernation. In contrast, reproductive yearlings do not reach this level at the end of the season (Millesi et al. 1999b; Millesi et al. 2004; Strauss et al. 2007). Non-reproductive yearlings also continue growth after their second emergence (Millesi et al.

1999b), whereas growth in reproductive active yearlings stagnates after first hibernation (Millesi et al. 2004).

Females

Females generally start reproduction when reaching the yearling class, after their first hibernation (Millesi et al. 1998; Millesi et al. 1999b). They have one litter per year (Kryštufek and Vohralík 2005; Ruzic 1978), with around two to ten juveniles per litter (Huber et al. 2001; Millesi et al. 1999a; Ruzic 1978), and an average gestation period of 29 days (Aschauer et al. 2006; Millesi et al. 1999a). The reproduction rates are often high with 90-100% (Millesi et al. 1998; Millesi et al. 1999a; Millesi et al. 1999b). Females have their lowest body weight right after emergence and female yearlings have higher body weight loss over the winter than adult females. Female body weight increases from emergence until parturition and is then relatively constant over the weaning phase, but drops in the last two weeks of weaning. After the weaning phase, body mass increases until immergence, at which point female adults and yearlings reach the same weight levels (Millesi et al. 1999b).

Older females start reproduction earlier and their litters tend to include more males and are mostly larger compared to litters of female yearlings. Their litters are also nursed longer (Huber et al. 2001; Huber et al. 2002). Heavier female yearlings have the tendency to produce more litters with lighter offspring, whereas heavier older females tend to produce heavier offspring, but not more offspring per litter (Huber et al. 1999). As a consequence of the prolonged nursing period for older females, their fat deposition starts later in the season and their relative mass after the following hibernation is lower than for females with shorter gestation periods (Huber et al. 2001; Millesi et al. 1999a). Population densities also affect female yearlings, having lower body weights and emerging earlier at high density levels than at low density levels (Millesi and Hoffmann 2008).

Juveniles

Juveniles are born in the beginning of May, after a four weeks gestation period (Millesi et al. 2000) and emerge around the beginning of June (Millesi et al. 1999a; Millesi et al. 1999b; Millesi and Hoffmann 2008). They disperse at around 9 weeks of age from their natal burrows and male juveniles tend to cover longer distances in a shorter time than females. This exposes them to greater risks, when searching for a new home range according to Hoffmann et al. (2004). Overwinter survival of juveniles is strongly influenced by their body mass before hibernation, which in turn is influenced by maternal effort (Millesi et al. 1999a).

Immergence

Immergence timing in EGS from Austria varies between regions and years, but generally starts in August for both sexes, but males and yearlings typically immerge later than female adults. Juvenile immergence can last until the beginning of October (Millesi et al. 1999a; Millesi and Hoffmann 2008; Strauss et al. 2007). The median durations of hibernation are 185 days for males, 228 days for females and 192 days for juveniles (Millesi et al. 1999b).

2.1.3 Fat storages and their role during hibernation in EGS

The accumulated fat reserves at the end of the season play an important role during hibernation in EGS. A few weeks before immergence, the animals experience low food availability and enter a fasting state, which in turn induces a shift from carbohydrate to lipid catabolism. This metabolic change, combined with other environmental cues, e.g. lower ambient temperatures and shorter day length, is an important element in the induction of torpor (Melvin and Andrews 2009). During hibernation, the metabolic rate is greatly reduced and the body temperature is lowered to the surrounding ambient temperature. The animals stay in this deep torpor state for several days. This is then followed by an energy demanding arousal phase, where body temperature and metabolic rate return to *normal* levels. Deep torpor and arousal alternate over the course of the hibernation period (Heldmaier et al. 2004; Nedergaard et al. 1990). Hut et al. (2002) reported body temperatures in EGS during deep torpor as low as 0°C. During the arousal phases, the temperatures returned to around 36°C.

Deep torpor and torpor arousal are predominantly fueled by fat storages (Dark 2005; Melvin and Andrews 2009) and their depletion can contribute up to 84% of the weight loss during hibernation, as demonstrated by Dark et al. (1989) for golden-mantled ground squirrels. In experiments with Syrian hamsters, body mass loss during torpor was directly correlated to the loss of fat depots in the retroperitoneal and periepididymal cavities (Weitten et al. 2013).

2.2 Body condition

A condition index (CI) generally serves as an indicator for the condition and the health of the studied animals, and in a broader sense, for the energy reserves that are contained in their bodies (Krebs and Singleton 1993; Peig and Green 2009; Schulte-Hostedde et al. 2001). Because *health* is a relative term and difficult or even impossible to measure, it is especially important to precisely define the indicator used to express it. The indicator, i.e. the CI, should therefore reflect important determinants about the studied species in context of their ecological

and physiological needs (Peig and Green 2009). Several CIs are used by biologist and ecologists, ranging from the 'Fulton's index', developed for the condition assessment of fish, to more sophisticated indices, as the 'Scaled mass index', re-developed by Peig and Green (2009). CIs are most often expressed as various ratios or residuals of body mass relative to body frame size. The underlying assumption for these CIs is that the relationship between the two measurements is directly related to energy reserves contained in the animal's body and that these energy reserves themselves are an indicator for the individual's health, nutritional status or fitness (Wilder et al. 2016). Concerning the nutritional status of an animal, its fat content is most often the presumed target variable to be indicated by the CIs. But body condition indices based on body weight and size are often, not always, poor indicators for the body fat content (Krebs and Singleton 1993; Labocha et al. 2014; Schulte-Hostedde et al. 2001; Schulte-Hostedde et al. 2005).

In case the study design permits the destruction of some study animals or dead animals can be acquired, energy reserves can be directly assessed by utilizing destructive methods. These commonly use solvent agents to extract lipids from the whole animal carcass or tissue samples. With the aid of direct measurements, the quality of weight-length based CIs can be validated, so they can reliably be used to predict the study animal's body condition. The most commonly used fat extraction method is the Soxhlet technique (Reynolds and Kunz 2001).

Body Condition Index for EGS

Using the importance of fat reserves as a basis for a CI, the CI should effectively reflect fat/energy reserves. In the available literature on EGS, body weight was commonly used as an indicator for the individual condition or simply as a mean to compare individuals between or within classes. In contrast, many studies on various North-American ground squirrel species utilized fat contents, in addition to body weight, to express body condition, respectively the nutritional status (Buck and Barnes 1999; Jameson and Mead 1964; Neal 1965). Because roadkill animals were available in the present study, it was possible to employ a direct measurement as CI, namely the retroperitoneal fat deposits situated below the diaphragm, mainly around the kidneys and the neighboring regions. The selection of retroperitoneal fat as a CI was further based on the use of RFI by Riney (1955) for red deer (*Cervus elaphus*) and by Suchentrunk (1984), in a study on the feeding ecology and body condition of European red foxes (*Vulpes vulpes*). The RFI was also used by Hackländer et al. (2011) in a study on the effects of continental differences on body condition and reproductive output in female European hares (*Lepus europaeus*). Skryja and Clark (1970) used retroperitoneal fat weights as CI in a study on seasonal changes in golden-mantled ground squirrels (*Callospermophilus lateralis*).

2.3 *Eimeria* parasites

The species of the genus *Eimeria* belong to the phylum Apicomplexa, subclass of Coccidia and are intracellular host specific parasites of the gastrointestinal system of many vertebrates and invertebrates. They have an apicomplexan, monoxenous life cycle, that can be divided into three developmental stages: merogony and gamogony, that take place inside the host, and sporogony, which takes place outside of the host (Duszynski and Upton 2001; Lucius and Loos-Frank 2008).

Infection occurs by ingestion of the oocysts, the reproductive stages of *Eimeria*. Every oocyst contains four sporocysts, each containing two sporozoites (Fig. 1, no. 1). When the oocysts are ingested, the contained sporozoites hatch in the intestine and invade epithelial and subepithelial cells, where they encapsulate and start feeding (Fig. 1, no. 2). Inside the cells they progress to schizonts/meronts by cytotgenesis and finally develop into the first generation of merozoites (Fig. 1, no. 3). The merozoites rupture the host cell and invade new epithelial cells (Fig. 1, no. 4), where they undergo the schizont to merozoite development again (Fig. 1, no. 5). The number of these merogonous generations depends on the *Eimeria* species. The last generation of merozoites (Fig. 1, no. 6) develops into microgamonts (Fig. 1, no. 8) or macrogamonts (Fig. 1, no. 7). The microgamonts leave their cell and fertilize mature macrogamonts in their respective host cell (Fig. 1, no. 9). After fertilization, an oocyst wall is formed around the zygote and the unsporulated oocyst is released via the feces (Fig. 1, no. 10). Sporogony takes place outside of the host (Fig. 1, no. 11) under aerobic conditions and enables the oocyst to survive under outside conditions (Fig. 1, no. 1). Further, only sporulated oocysts can infect a new susceptible host (Duszynski and Upton 2001; Mehlhorn 2012).

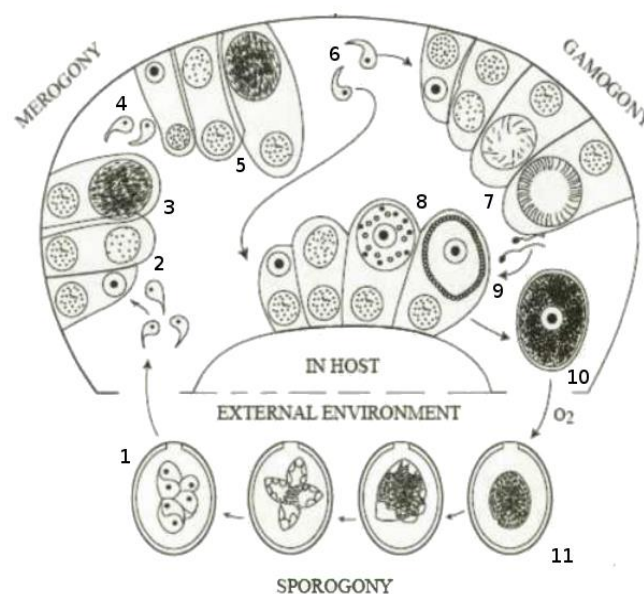


Figure 1: Line drawing of *Eimeria* life cycle from Duszynski and Upton (2001), modified by the author.

2.3.1 *Eimeria* in wildlife

For understanding coccidia infections, the distinction between *infection* and *coccidiosis* is important. Infections by coccidia are common, whereas the term coccidiosis characterizes a coccidian infection as a pathological condition. Not every animal infected by *Eimeria* develops coccidiosis (Duszynski and Upton 2001).

In livestock and other captive held animals, severe infections and high transmission rates are facilitated by crowded housing conditions, as in factory farming, where avoidance of infected individuals is limited. Infections in young individuals can be lethal, which is of high concern in cattle (Lucius and Loos-Frank 2008) and rabbits (Duszynski et al. 2013).

Eimeria infections in wildlife are also common, but are mostly of relatively low grade, due to a presumed immunization after first infection. Severe coccidian infections in wildlife mainly occur in high density populations or where habitat degradations, resulting in spatial stress, lead to increased contact with infected individuals or infectious material. In contrast to livestock, clinical signs of infection are seldom expressed in wild animals, even though high levels of *Eimeria* oocysts can often be found in their feces. Experimental infections with high loads of coccidia led to weakness, anorexia, dehydration, emaciation, fever, weight loss, diarrhea and nervous distress (Duszynski and Upton 2001). The damage to the epithelial walls can lead to malabsorption of nutrients, as well as to neurological dysfunctions as a result of a fluid to electrolyte imbalance. In juvenile rabbits for example, an acute weight loss of over 20%, due to a coccidian infection, can lead to death on the same day (Duszynski et al. 2013). *Eimeria* can also have impacts on the body condition of their hosts. In Finnish bank vole populations, for example, *Eimeria* Infections were negatively related to body mass of the individuals. Because the mother's post-partum body mass was positively related to offspring mass, the negative effects of *Eimeria*, indirectly led to lowered offspring body mass (Hakkarainen et al. 2007).

2.3.2 *Eimeria* infections in EGS

The newest revision of literature concerning *Eimeria* spp. for the Marmotini tribe by Wilber et al. (1998) concluded 26 *Eimeria* species occurring in Marmotini, of which three species are proven to occur in *Spermophilus citellus*:

- i. *E. citelli* Kartchner & Becker, 1930
- ii. *E. callospermophili* Henry, 1932
- iii. *E. cynomysis* Andrews, 1928



Figure 2: i. *E.callospermophili*, ii. *E.citelli*, iii. *E. cynomysis*, photographs made by the author

These *Eimeria* species are well known and described in the literature, but to the author's knowledge, no connections between their abundance and the body condition of EGS were made before. Moreover, there is no known account that reported the presence of *E. callospermophili* and *E. cynomysis* in Austrian EGS populations.

In a study on *Eimeria* in EGS in Bulgaria by Golemansky and Koshev (2009), 88.05% of 109 animals were positive for *Eimeria*. Prevalence was reported for *E. citelli* (92.7%), *E. callospermophili* (66.6%) and *E. cynomysis* (14.6%). Categorized infection intensities were evaluated for 96 individuals on a semi-quantitative basis. No differences between sexes were found, but juveniles had higher infection intensities than yearlings and adults. For EGS in Greece, Diakou et al. (2014) reported *Eimeria* oocysts in 94.4% of 125 samples, which consisted of *E. callospermophili* (73.6%), *E. citelli* (60.8%), *E. cynomysis* (32.8%) and unknown *Eimeria* spp. (17.6%). Mixed infections of these three species seem to be common in EGS.

Little information on the pathogenicity of these three species in EGS are available. Pellérdy (1974) reported catarrhal enteritis in EGS with severe *E. citelli* infections. Besides the occurrence in EGS, the three species can also be found in several *Spermophilus* species and, despite the generally presumed host specificity of *Eimeria*, in other members of the Marmotini tribe, as in the genera *Cynomys* and *Marmota* (Seville and Stanton 1993; Stanton et al. 1992; Wilber et al. 1994; Wilber et al. 1998). Todd and Hammond (1968a) inoculated non-native hosts with *E. callospermophili*, which resulted in bloody mucoid diarrhea, inactivity and food refusal in severely infected *Urocyon v. richardsonii*. Repeated infections did not lead to a development of resistance and oocyst output remained roughly the same during the trials. Infected *Urocyon v. armatus* shed around 9 million oocysts per gram feces, but exhibited no behavioral signs of coccidiosis, despite having severely altered epithelia. Infected *Callospermophilus lateralis* and *Otospermophilus variegatus* were also behaviorally

inconspicuous. In *Urocitellus elegans*, repeated infections with *E. callospermophili* led to increased prepatent and decreased patent periods, suggesting an immune response by the host. These varying disease progressions were also observed for other *Eimeria* species in various ground squirrels (Todd et al. 1968; Todd and Hammond 1968b). The removal of endo- and ectoparasites in *Urocitellus columbianus*, by which *Eimeria* were also removed, indicated varying effects on their host's body condition (Neuhaus 2003; Raveh et al. 2011; Raveh et al. 2015). The effects of *Eimeria* on the body condition of ground squirrels are rather inconclusive and especially in EGS, so far unknown.

2.4 Study aims

The main goal of this study is to investigate the influence the *Eimeria* abundance has on the body condition of EGS. As the body condition is likely to be influenced by sex, age and season of sample collection, these variables are also incorporated into the analysis. Because fat reserves play an important role during hibernation, the RFI will be used as a direct indicator for the individual body condition. The ability of the RFI to predict body fat in EGS will be validated using reference fat percentages of total EGS corpses via Soxhlet fat extraction. The *Eimeria* abundances will be determined using the coproscopic McMaster technique. This study will employ an information-theoretic approach, utilizing model averaging of linear models, to identify probable predictors of RFI.

The main working hypothesis for the influence of *Eimeria* abundance on RFI is:

- i) Due to the generally destructive nature of *Eimeria* on the epithelial cells of the intestine and the possibility of clinical infections, the abundance is expected to have a negative influence on the individual's body condition, thus animals with high *Eimeria* load should have a lower RFI.

The working hypotheses for the influences of age, sex and season on RFI are:

- ii) Male individuals will likely decrease in fat until and during mating in April and will start to accumulate fat afterwards.
- iii) Female adults will likely decrease in fat during mating and gestation and will commence fattening after lactation.
- iv) Because juveniles have a shortened time window to accumulate fat before hibernation and they also must invest energy into growth, they are expected to have overall lower RFI levels than adults before immergence.

(The hypotheses ii) and iii) can only be analyzed partially because of sample size restrictions, which are further explained in 3.1)

3 Material and Methods

3.1 Sample area and sample collection

Altogether 149 roadkill EGS were collected between March 3rd and August 8th, 2016. The collection took place within an approximately 2400 ha large area (red area in Figure 3) north-east of 'Krems an der Donau' (+48° 26' N, +15° 39' E), situated in the largest connected EGS population in the whole of central Europe, with an estimate of 50,000 – 100,000 individuals. Despite its large size, the population has a low genetic variability (Suchentrunk unpublished, see also Jilge (2017)).

The area has an altitude of around 240 m above sea level and is characterized by a Pannonian-Atlantic climate, with a mean annual temperature of 9.2°C and a mean annual precipitation of 480 mm. Land use is dominated by vineyards (58%) and farmland (40%), only 2% is used as grassland. Main soil type is the chernozem, a typical soil of continental steppe climates, consisting of loess, lime sands, marl and other fine sand types (Jaburová 2009).

The animals were collected in the course of vehicle driven counts of active ground squirrels by Franz Suchentrunk along a circa 25 km long road transect (black line in Figure 3), around and through the area. Because of the protected status of EGS, the animal collection was performed under official permission 'Sammelbewilligung für das Aufsammeln von totgefahrenen Ziesel im Bundesland Niederösterreich', 'Kennzeichen: RU5-BE-364/003-2013', 'Bezug: BD2-N-200/114-2013', 'Naturschutzabteilung der NÖ Landesregierung', by Franz Suchentrunk, Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna.

Necropsy was performed on the animals by wildlife pathologists at the Research Institute of Wildlife Ecology, Vienna, within three days after collection. The examination included assessment of signs of disease, evaluation of various fat reserves and tissue sampling for further histological examinations. Measurements taken relevant to this study included body weight (to 0.5 grams) and body length (to 0.1 cm). Retroperitoneal fat was extracted and weighted to 0.01 grams. Feces were collected from the rectum and, if necessary, from the colon, and stored in a sample tube at 4°C for later examination for coccidian parasites. The animals were classified by sex and age class, as far as status of degradation of the animals permitted. The yearling and adult classes were pooled, because missing data on tooth erosion analysis that would otherwise enable distinction of several age classes (Ruzic 1978), could not be performed at this time.

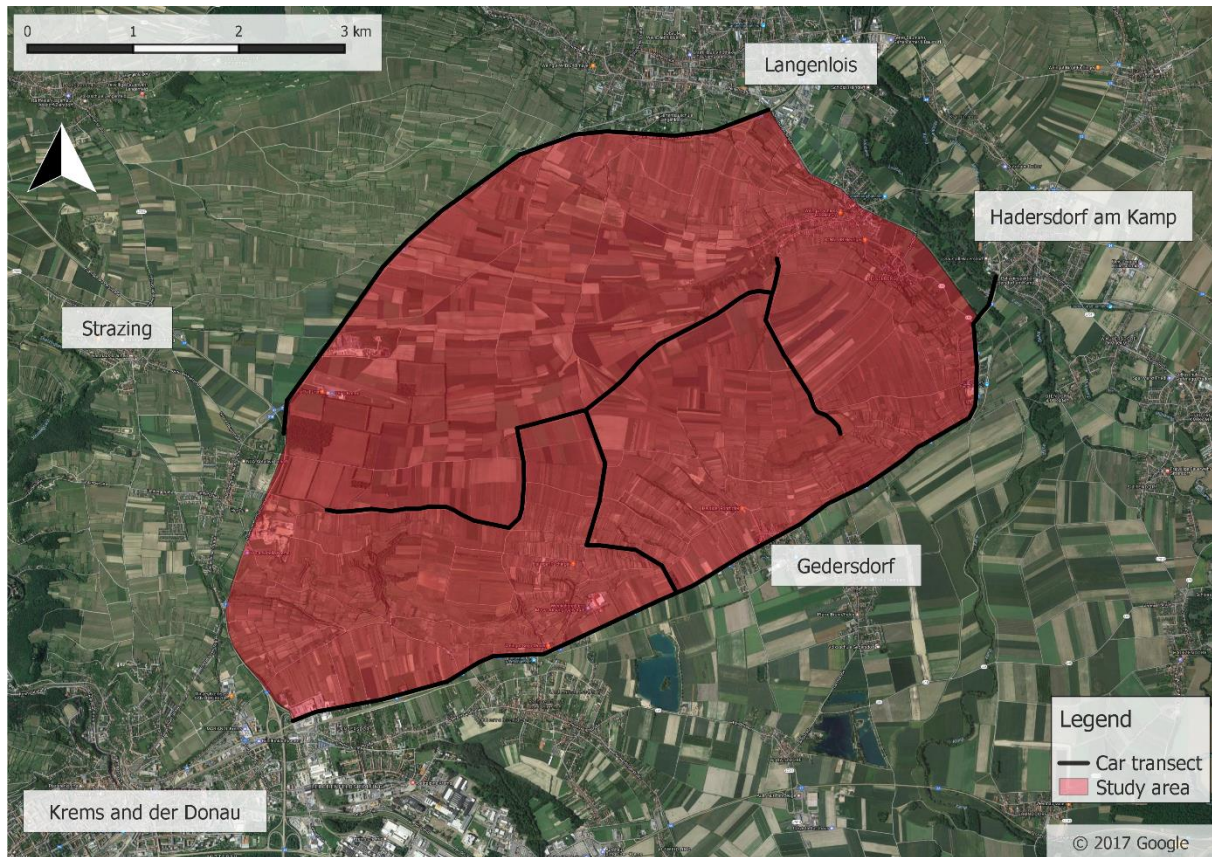


Figure 3: Map of the study area (red area) and road transects (black lines)

Sample size restrictions

Originally, it was intended to identify the different *Eimeria* species and use their prevalence as an additional parameter in the model. However, due to technical inadequacy, when using incubated samples for *Eimeria* determination, the April and May samples could not be used for analysis. Additionally, only animals that had a corresponding body weight, retroperitoneal fat weight, determined sex, age class and *Eimeria* count could be included in the analysis. From 80 individuals with available oocyst counts, only 63 animals collected between May 14th and August 28th, 2016 could be used.

3.2 Soxhlet method

To validate the relationship between RFI and body fat content, 20 animals with various amounts of retroperitoneal fat were selected for Soxhlet fat extraction, to obtain an approximately homogenous sample distribution. Petroleum ether was used as fat solvent agent. In short, every individual was separately minced with a meat grinder and insufficiently homogenized pelt tissue was cut into small pieces and mixed with the meat mass. Two samples were prepared for every individual. For each sample, a weighted amount of meat mass (around 30 g) was mixed with the same proportion of purified sea sand. The samples were then dried in an oven for 24 hours at 100°C. The samples were reweighted after drying to obtain the weight of lean dry mass and were then homogenized in a plastic bag with a hammer. The pulverized matter was filled into paper shells and placed into the Soxhlet apparatus. Weighted flasks were filled with petroleum ether, placed in the apparatus and heated to boiling point (at around 60°C). After 6 hours and multiple extraction cycles, resulting in a solvent-fat solution in the flasks, these were then placed into an oven for at least 3 hours at 100°C to allow the ether to evaporate. The extracted fat masses remained as residuals in the flasks and the fat weights were determined by comparing the weights of the flasks, from before and after the procedure. The resulting fat weights were divided by the respective lean dry masses of the samples. The mean of both samples was calculated to get the relative body fat content for each individual (Fig. 4).

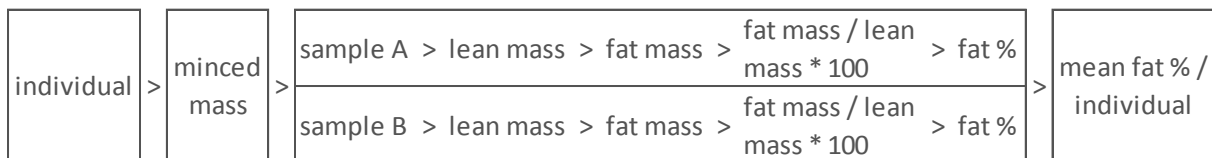


Figure 4: Schematic of the Soxhlet procedure principle.

Statistical model for the validation of RFI

A linear regression model was used to predict relative body fat content based on relative retroperitoneal fat. The retroperitoneal fat index (RFI) is expressed in per thousand:

$$\text{RFI} = \text{retroperitoneal fat in (g)} / \text{body weight in (g)} * 1000$$

The corresponding whole body fat from Soxhlet extraction (SOX) is expressed in percent:

$$\text{SOX} = \text{extracted fat in (g)} / \text{sample weight (g)} * 100$$

A linear model was constructed using SOX as response variable and RFI as predictor:

$$lm(\text{SOX} \sim \text{RFI})$$

An additive model was also tested and AICc was slightly higher than AICc for the linear model.

3.3 *Eimeria* abundance

Because it is not possible to assess the abundance of endogenous stages of *Eimeria* in the intestines per se by any known method, the oocysts contained in the host's feces are counted instead, by using a coproscopic technique. For this study, *Eimeria* oocysts counts were determined via a modified McMaster method (Schmäschke 2014). The principle is to dissolve a known weight of feces in a known volume of a flotation solution, extract a sample and count the oocysts contained in the sample under a microscope. The eggs per gram (EPG) can then be easily calculated.

The flotation solution was prepared beforehand, by mixing sodium chloride (NaCl) with Sucrose in a 2:1 volume/weight ratio. The specific gravity of the resulting solution was 1.27. A two-chamber McMaster glass slide was used for examination under the microscope.

Either one, two, or three grams of feces, depending on the available amount, were extracted from the sample tube and placed into a glass beaker. Per gram feces, 15 ml of flotation solution was added and mixed with a magnetic stirrer. A small amount of the homogenized feces solution was then extracted with a syringe and transferred into the two McMaster chambers. The slide was then placed onto the microscope table and left to rest for 10 minutes. Each McMaster chambers was then examined along their grid lines for *Eimeria* oocysts at a 200x magnification and oocysts were counted accordingly. In case the difference in counts between the two chambers was higher than 50%, the chambers were emptied, a third chamber filled and examined. Samples that contained no oocysts were double checked using a combined sedimentation-flotation technique (Schmäschke 2014).

The number of oocysts per gram was calculated by dividing the obtained oocyst count by the feces weight and multiplying the result by the amount of flotation solution divided by the volume of the McMaster chambers. The resulting EPG values per individual were used in the statistical analyses.

Due to a prolonged time between the collection of the samples and the examination in the beginning of the study, some samples started to develop mould on the surface. Because effects of mould on *Eimeria* oocysts are unknown to the author, 'mould' was included as a variable in the statistical analysis to account for a possible effect on *Eimeria* abundance in the samples.

3.4 Statistical analysis

Linear regression models were used for the analysis of the dataset, using R-Studio version 1.0.44, R version 3.3.1 (R Core Team 2016). The MuMIn package version 1.15.6 (Barton 2016) was utilized for model selection and model averaging functions. Graphs were created using the ggplot2 package version 2.2.0 (Wickham 2009), ggforce package version 0.1.0 (Pedersen 2016) and sjPlot package version 2.1.2 (Lüdtke 2016).

3.4.1 Information theoretic approach

To analyze the effects of *Eimeria* abundance on RFI, an information-theoretic approach was used (Burnham and Anderson 2002).

Common hypothesis testing and model selection approaches generally try to find a “best” model that is the best approximation of the information in the data set and then inferences about the system are made based on this single model. The philosophy of an information-theoretic model selection approach is to acknowledge that there is a loss of information inherent in the process of model selection and that there are often many valid models to make inferences from. The goal is to select one or more models that minimize the information loss. This can be accomplished by comparing all plausible models (Burnham and Anderson 2002). The information theoretic approach starts with the construction of a working hypothesis, based on a priori knowledge and information about the test subject, stemming from research and rationale. This is a common procedure for other analysis approaches as well. Based on these hypotheses, a ‘global model’ is constructed. This model includes all plausible variables and their interactions, formulated in the hypothesis. From the global model, other plausible candidate models can be created in a hierarchical way (not containing other additional variables, but potentially less), to further address relevant questions about the information contained in the data set. The models are then ranked by use of Information criteria, such as the Akaike information criterion (AIC). These information criteria serve as indicators for the quality of the model with regards to the information loss. Inference should then be made on the best model or models. In case no single model in the model set is clearly superior over the others, a model averaging attempt can be made. Model averaging calculates averaged estimates for each predictor, weighted by the probability that a given model is the best model in the given set of candidate models. Instead of basing inferences on one single model, inferences can then be made on the entire set of models (Burnham and Anderson 2002).

Because of the different nature of the information theoretic approach, I will refrain from using the terms *(not) significant* and *(not) rejected* when discussing results, because these

expressions are heavily linked to null-hypothesis testing. The *p values* in the global model output table 2 do not reflect significance values as they are used in the context of null-hypothesis testing. In this case, they reflect the probability that the true, but unknown parameter value is equal 0 or smaller (higher) at a positive (negative) estimate.

AICc was used as information criterion for small sample sizes. DeltaAICc is the AICc difference to the best model with lowest AICc. LogLik is used by the averaging function to calculate AICc. The Akaike weight ω_i is the probability that a given model is the best model, given a set of candidate models. The relative variable importance (RVI) is the Sum of Akaike weights over all models that include this variable. It is the probability that a given variable is included in the best model and an expression for the importance of the variable. The higher the value, the higher the relative importance of this variable in the model set. As a rule of thumb, RVI values over 0.7 are considered meaningful.

3.4.2 Construction of a global model

A global model was created with RFI as response variable and EPG as predictor. As additional influences on RFI, the variables sex and age class were added for compensation. Because EPG, as well as sex and age class may have seasonal dependent effects, the month of the collection date was added as an additional variable. To control for combined effects, two-way interactions (2) were chosen for these four variables. A separate interaction term of EPG and mould was added for unknown interaction effects of mould on *Eimeria* oocysts, as explained before. A linear model was calculated from the global model to predict influences of EPG, month, sex and age class on RFI.

$$lm(RFI \sim (month + sex + agecl + EPG)^2 + EPG:mould)$$

The RFI (+1) was log transformed after the first model computation to compensate for skewness of the residual-distribution. The variables RFI and EPG are metric. For the variable sex, females were coded as 1 and males as 2. For age class, adults were coded as 1 and juveniles as 2. For month, the corresponding numerical month of the collection date was coded as integer from 5 to 8 and used as a metric variable. The variable mould was coded as 0 for absence of mould and 1 for presence of mould.

To find probable variables, all possible candidate models were computed from the global model via the dredge function from the MuMIn package and ranked by AICc. Model averaging was performed on all models, which resulted in an output with averaged estimates and relative variable importance values.

4 Results

4.1 Validation of RFI

The body weights of the 20 individuals chosen for Soxhlet extraction ranged from 109 – 352.5 g (mean = 262.4 ± 34.72 g). Retroperitoneal fat weights ranged from 0 – 6.1 g (mean = 2.27 ± 1.97 g). This resulted in a mean RFI of $8.14 \pm 6.46\%$ (range = 0 – 21.58%). SOX values of lean mass ranged from 0.44 – 47.46%, with a mean of $24.21 \pm 15.00\%$. SOX could be predicted from RFI by the equation $y = 6.98 + 2.12 * x$. The adjusted R^2 for this model was 0.845. Table 1 shows the linear model output and Fig. 5 illustrates the linear regression of the retroperitoneal fat index against body fat content from Soxhlet extraction. The results validate the assumption about RFI being a useful predictor of relative body fat content. The RFI was further used for the construction of the global model.

Table 1: Summary of linear model estimation of RFI on whole body fat content from Soxhlet fat extraction n=20.

Variables	Estimate	Std. Error	t value	p value	
Intercept	6.983	2.154	3.241	.004	**
RFI	2.118	0.207	10.225	< .0001	***
Adjusted R^2 : 0.845					
Significance codes: 0 '***' 0.001 '**'					

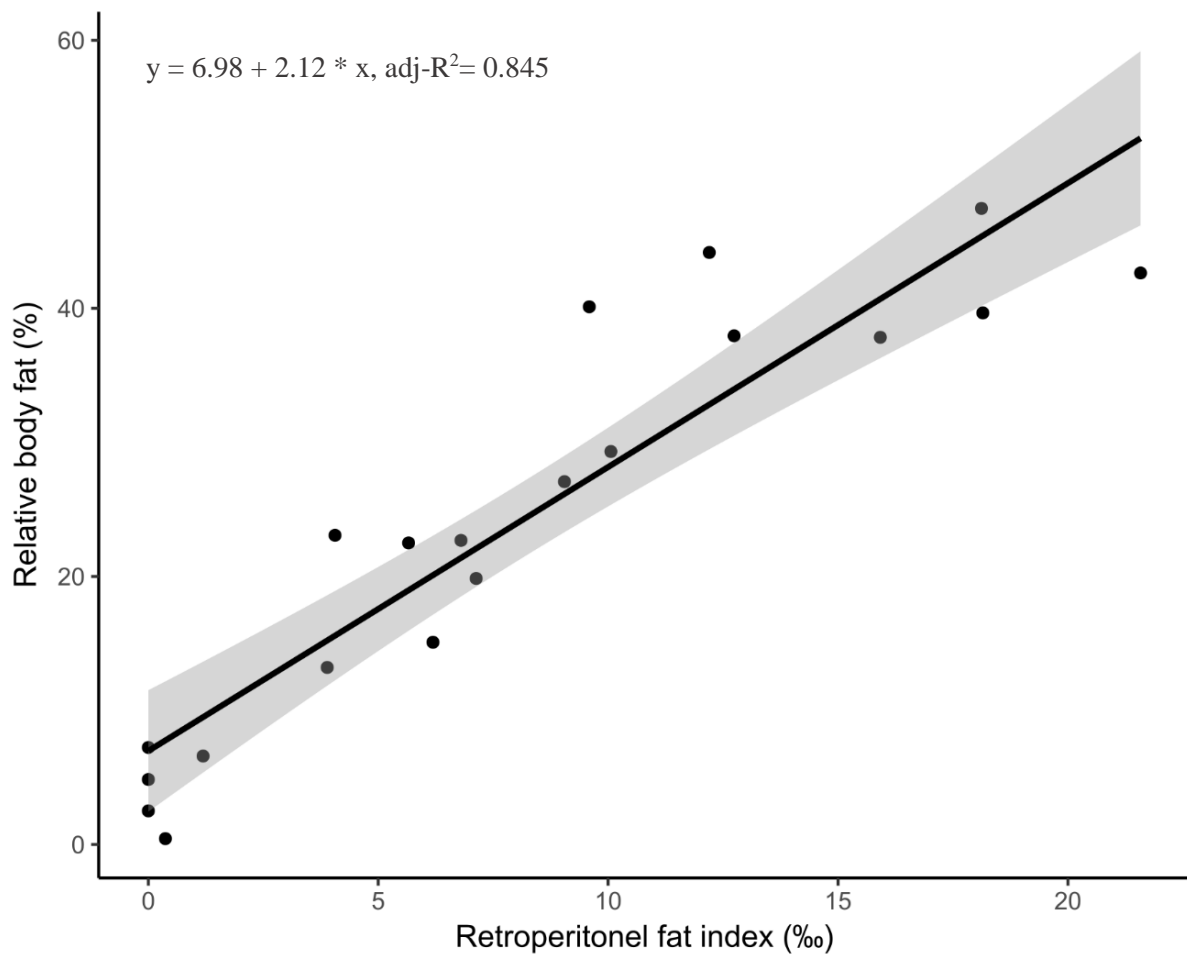


Figure 5: Linear regression plot of retroperitoneal fat index (RFI) to relative body fat content of lean mass (SOX), 95% confidence band for prediction in gray, n=20.

4.2 Body weights and RFI

63 animals were used for the construction of the global model. 43 individuals were categorized as adults of which 14 were females and 29 were males. 20 animals were categorized as juveniles, of which 10 were females and 10 were males. The first individual contained in this data set was a female found on the 14th of May, the last individual, a male juvenile, was found on the 28th of August. The last female, a juvenile individual, was found on the 4th of August. There was a gap between mid-June to mid-July, where no females were found. Male adults were found consistently during the study.

Body weights

Mean body weight over all individuals was 229 ± 55.7 g. The individual with the lowest body weight was a juvenile male with 120 g, the heaviest individual a male adult with 341 g.

Adult males found later in the season had increasingly lower body weights than in May, whereas adult female found later in the season tended to have slightly higher body weights (Fig. 6). Juveniles were found from the beginning of June and their body weights were increasingly higher until the end of the study.

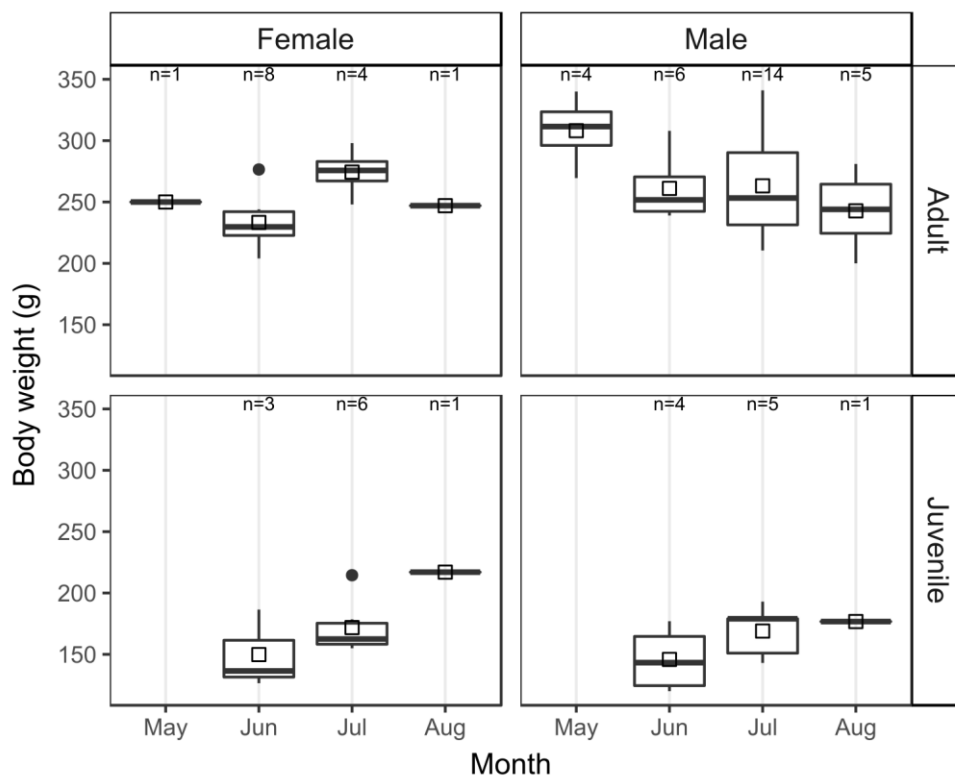


Figure 6: Boxplots of body weight by month, separately for sex and age class, squares show mean body weights, n=63.

RFI

Retroperitoneal fat weights ranged from 0 – 6.28 g (mean = 1.23 ± 1.67 g). Several adult females in May and June had no retroperitoneal fat reserves, whereas every male during this time had at least low RFI levels (Fig. 7). Adult females had the highest mean RFI ($6.8 \pm 8.4\%$), but their lower median ($1.69 \pm 7.84\%$) reflected the individuals with absent RFI (Fig. 8). An adult male individual had the highest RFI value (23.7%). Juveniles had an overall shorter RFI range and a lower mean RFI than adults. One juvenile female in August had a considerable higher RFI than the others, but body weight and size indicated her as juvenile.

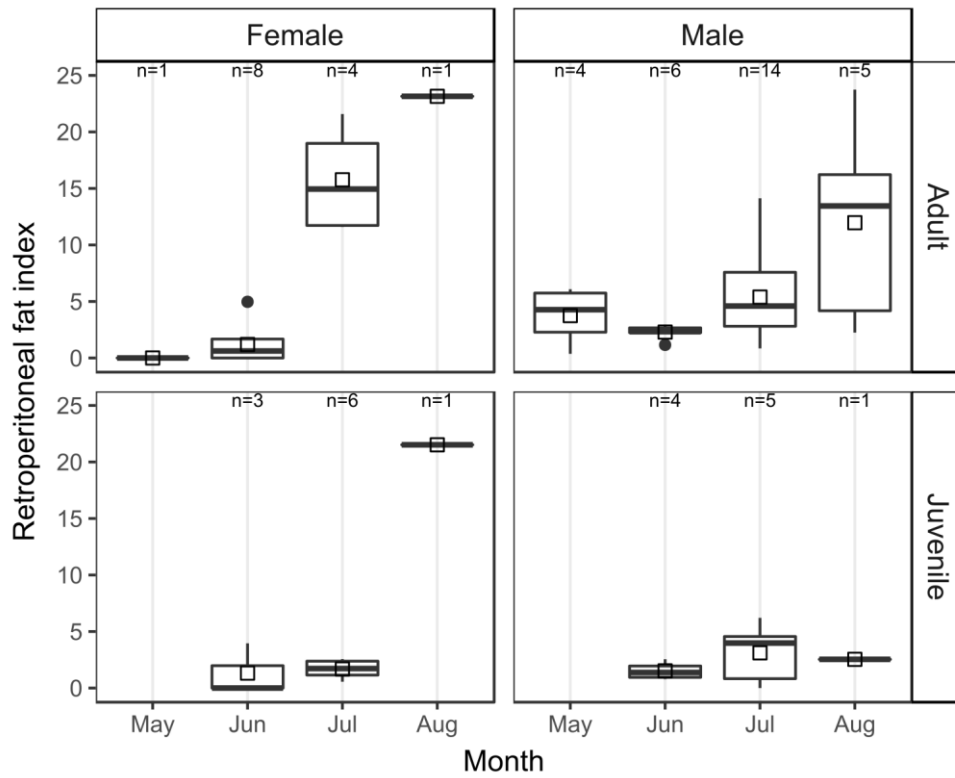


Figure 7: Boxplots of RFI by month, separately for sex and age class, squares show mean body weight, n=63.

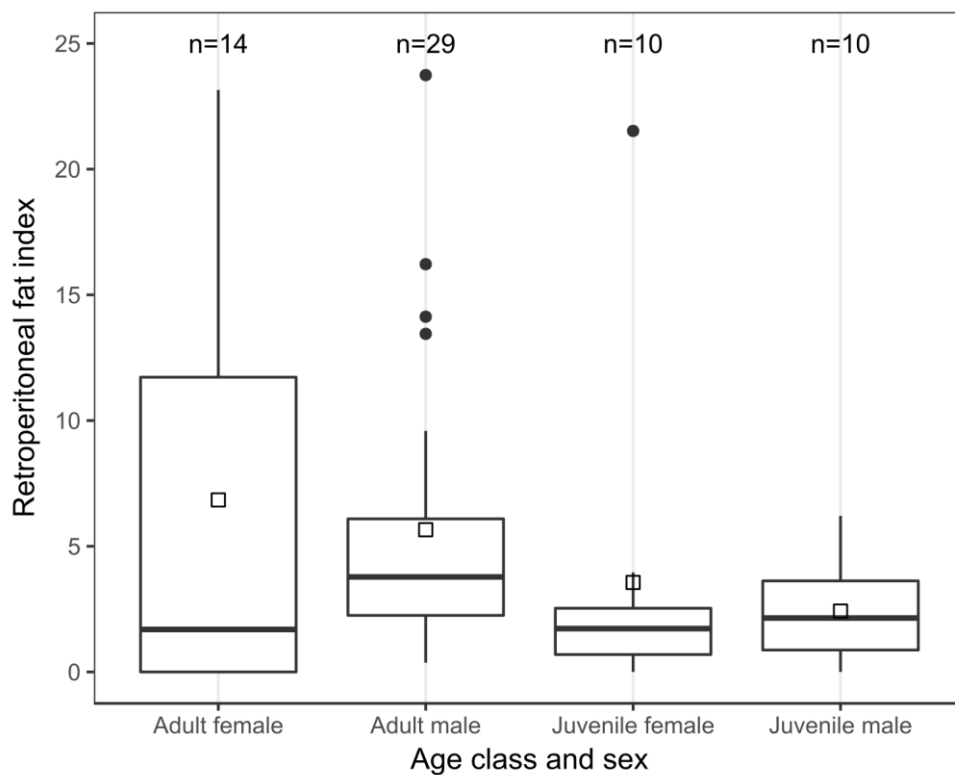


Figure 8: Boxplots of RFI values by age class and sex, squares show mean RFI, n=63.

4.3 *Eimeria* abundance

Altogether 80 samples were examined for oocysts, but only the results of the samples corresponding to the 63 individuals used in the global model analysis are reported here. *Eimeria* oocysts were found in 61 of the 63 samples (96.8%). Positive EPG counts ranged from an adult female with 70 EPG to 770,300 EPG in a juvenile female. Mean EPG was 39000 (median = 13700 EPG) over all individuals. The individual with the maximum EPG count had over 550,000 EPG more than the next highest EPG count (Figure 9). Most individuals had relatively low *Eimeria* abundances, when compared to the three highest counts.

Individuals with lower than average RFI tended to have higher EPG abundances. Considering the median RFI and EPG, individuals with higher RFI had seemingly higher EPG values (Fig. 10).

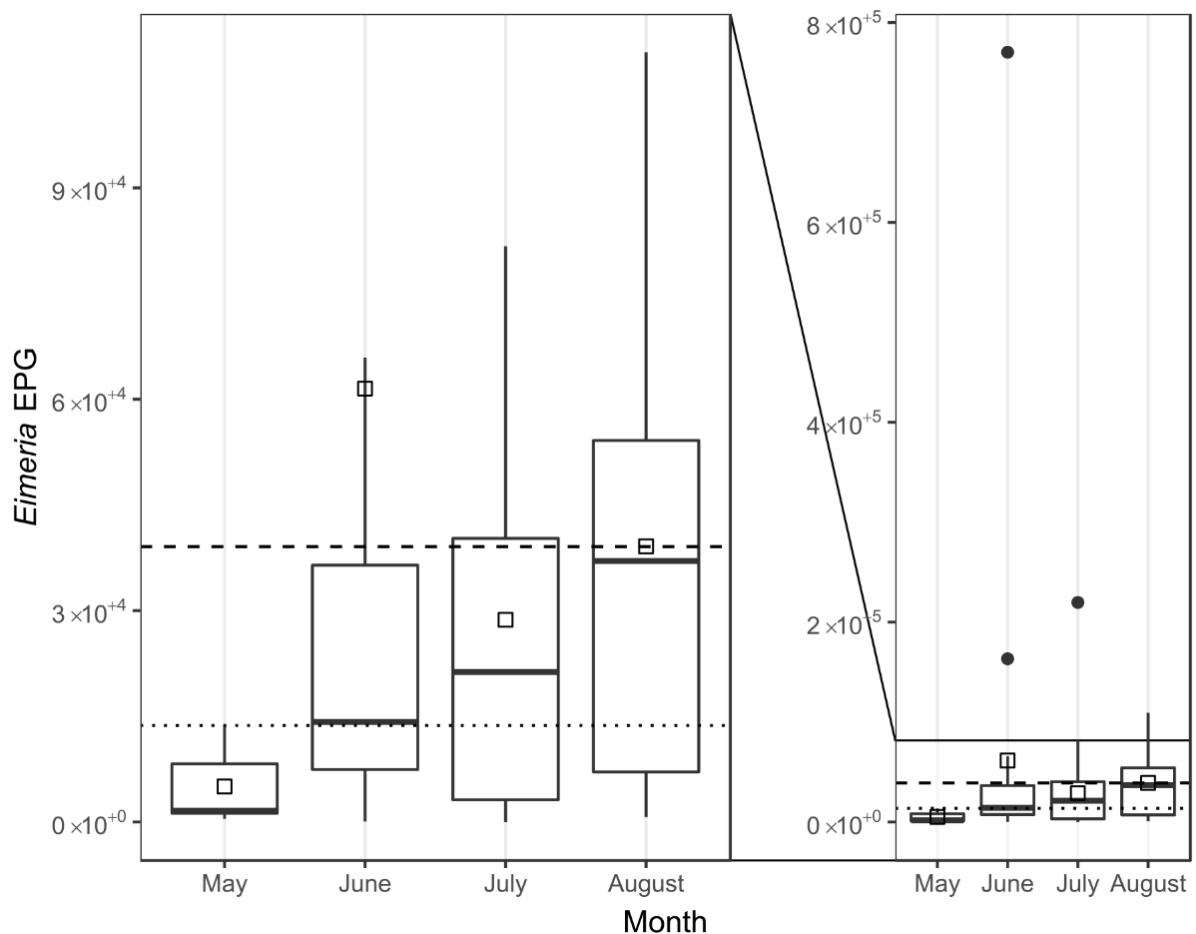


Figure 9: Boxplot of EPG by month, the right plot shows the entire plot, the left plot shows a cutout, squares show mean EPG per month, the dashed line shows overall mean EPG, the dotted line shows overall median EPG, n=63 (n in May = 5, June = 21, July = 29, August = 8)

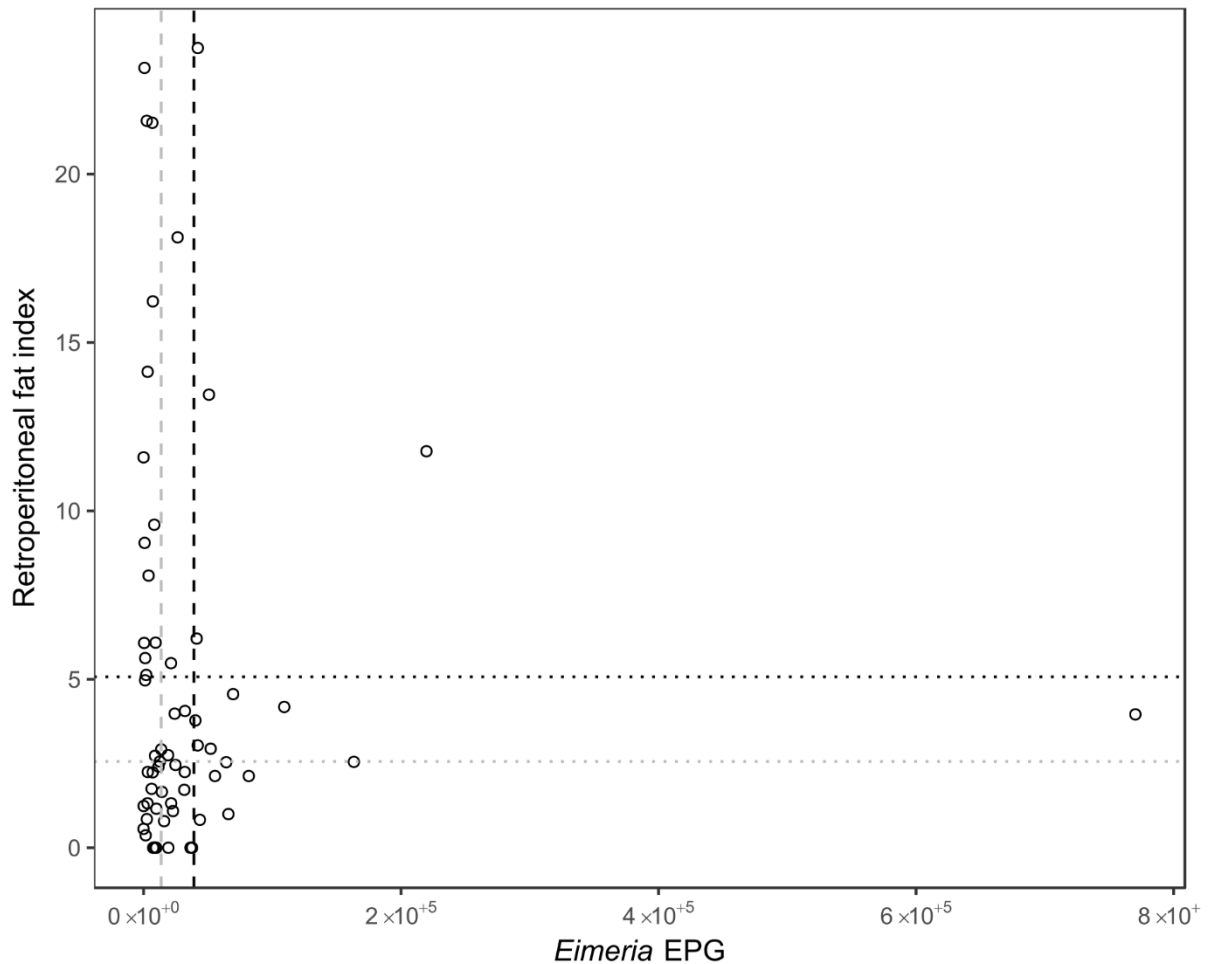


Figure 10: Scatterplot of RFI to EPG, black dashed line shows mean EPG, black dotted line shows mean RFI, dashed and dotted lines in gray show respective median EPG and RFI, n=63

4.4 Model analysis

Global model

A linear model was calculated from the global model to predict influences of EPG, month, sex and age class on RFI. All possible models were computed and ranked by AICc. The model selection table for all computed models with Akaike weights > 0.01 can be found in the Appendix I. Because Akaike weights were small, and no single model was superior to the others, model averaging was performed. The most probable variables by RVI after model averaging were month (1.00), sex (1.00), age class (1.00), the interaction effect of month with sex (1.0) and *Eimeria* abundance (0.97). The presence of mould had a slightly lower estimate on EPG abundance than absence, but this difference was not found to be a mayor influence on body condition. No discernable differences in the influence of month, sex or age class in

combination with *Eimeria* abundance on the body condition were found, as these interaction effects were not considered important variables for the final model due to their low RVI.

Table 2: Summary of the model averaging output for the global model: Variables and interaction terms, regression coefficients, adjusted standard error, z values and p values. RVI values of the most probable variables in bold.

Variables	Estimate	Adjusted SE	z value	p value	RVI
intercept	-13.980	2.899	4.839	< .0001	...
age class	-1.110	1.105	1.005	.315	1.00
EPG	7.480×10^{-7}	1.346×10^{-5}	0.056	.956	0.97
month	2.544	0.459	5.543	< .0001	1.00
sex	7.072	1.514	4.672	< .0001	1.00
mont : sex	-1.102	0.235	4.701	< .0001	1.00
age class : sex	0.512	0.373	1.372	.170	0.44
age class : EPG	3.463×10^{-6}	3.688×10^{-6}	0.939	.348	0.31
EPG : month	-2.730×10^{-7}	3.398×10^{-6}	0.080	.936	0.23
age class : month	-0.080	0.285	0.281	.779	0.21
EPG : sex	-9.663×10^{-7}	3.563×10^{-6}	0.271	.786	0.21
EPG : mould	2.608×10^{-7}	1.281×10^{-5}	0.020	.984	0.20

Final model

A final model was estimated with the probable variables ($RVI > 0.7$) from the model averaging results (Tab. 3 and Figure 11). *Eimeria* abundance had a positive influence on RFI ($\beta = 2.17 \times 10^{-6}$, $SE = 8.13 \times 10^{-7}$). RFI was positively associated with consecutive months ($\beta = 2.442$, $SE = 0.387$). Males generally had higher body condition than females ($\beta = 7.145$, $SE = 1.446$), although females only had lower RFI values in May, but passed males at around July and reached higher RFI levels than males before hibernation in August ($\beta = -1.068$, $SE = 0.219$, Fig. 13). Juvenile individuals had a generally lower body condition than adults ($\beta = -0.836$, $SE = 0.178$).

Table 3: Summary output of the final model: fixed variables, regression coefficients, standard error, t value and p values.

Variable	Estimate	Std. Error	t value	p value
intercept	-13.85	2.515	-5.507	< .0001
EPG	2.107×10^{-6}	8.128×10^{-7}	2.669	< .001
month	2.442	0.387	6.305	< .0001
sex	7.145	1.446	4.941	< .0001
month : sex	-1.068	0.219	-4.887	< .0001
age class	-0.836	0.178	-4.691	< .0001
Adjusted R-squared: 0.5341				
p-value: < .0001				

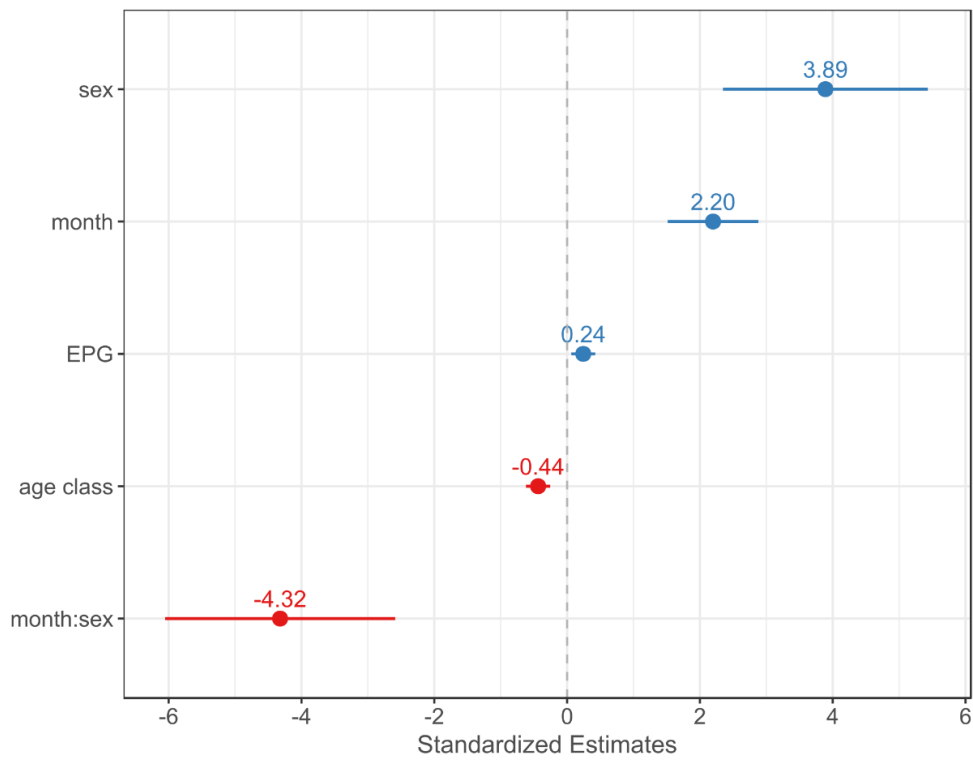


Figure 11: Plot of standardized beta coefficients, with their respective confidence intervals

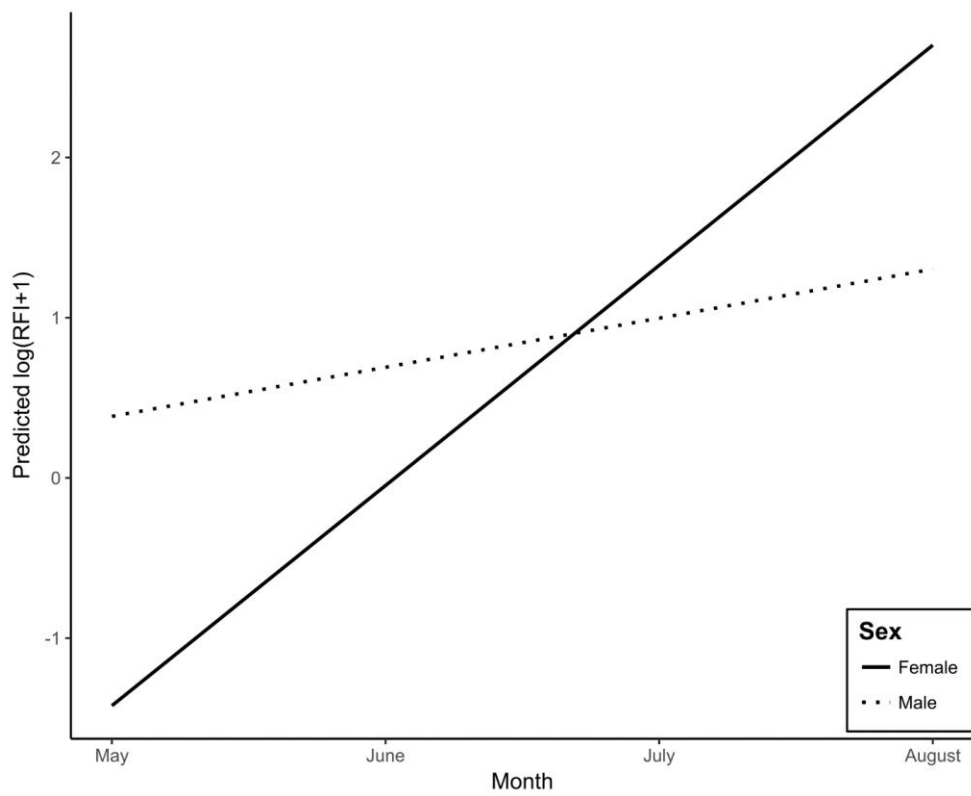


Figure 12: Interaction plot of month to predicted RFI, solid line for females, dotted line for males

5 Discussion

5.1 RFI as body condition index

In the present literature on European ground squirrels (EGS), body weight changes were commonly used as an indicator for the individual condition or simply as a mean to compare individuals within categories. Using these changes as an indicator for condition is practicable in capture-mark-recapture studies or long-term studies with high sample sizes, as it was done in the LP by Millesi et al. (1999b). However, Dark (2005) emphasized that body mass changes in ground squirrels do not always represent changes in body fat content, neither for mass loss nor for mass gain. Therefore, it seemed appropriate to directly utilize the fat reserves as an indicator for condition, as the fat content was of interest during this study.

Initial comparison of individual body weights and their corresponding RFI values showed that the overall increase in RFI values over the season was not reflected by body weights. This was especially distinct in adult males, where body weights decreased over the season, the RFI, however, increased. For juveniles, body weights reflected retroperitoneal fat more accurately. But even here, the strength of the RFI was shown in the before mentioned juvenile outlier in August (Fig. 7). This individual had a considerable higher condition, compared to the other juvenile individuals, and matched the RFI of adults. Its body weight alone did not represent this female's superior condition.

The linear regression model confirmed a strong relationship between retroperitoneal fat reserves and whole body fat contents. As there is a biological limit to what degree body fat can be stored in an animal, a larger sample size might suggest a non-linear relationship between retroperitoneal and whole body fat. This was tested, but the additive model was slightly inferior to the linear model, probably due to the small sample size. Nevertheless, the RFI can be seen as a useful indicator for the animal's condition in terms of its recent energy budget and its preparation status for hibernation.

The RFI has of course its limitations, as it can only be applied to dead animals. Because fat reserves play such a vital role during hibernation in EGS, further investigations into the predictability of fat reserves utilizing body mass and several structural dimensions are planned. The retroperitoneal fat index may provide a useful basis to verify a condition index, that can reflect the individual fat condition in live individuals. This would be a valuable tool for the conservation management of EGS. In addition, several indices of body condition as described in the literature (see Peig and Green (2010)) will be calculated and compared to the presently used RFI, utilizing a bigger sample from the currently studied population encompassing the entire period from emergence from hibernation to immergence.

5.2 Limitations of inference for model analysis

By the $n/\text{parameters}$ sample size rule, only one predictor per 10 samples should be added to a model (Burnham and Anderson 2002). For such a small sample size ($n=63$), the global model was stressed with eleven estimated parameters + intercept to incorporate plausible interactions between the variables. This was reasoned by the lack of knowledge about the relationship between body condition and *Eimeria* abundance in the existing literature on EGS and should be understood as an explorative approach. Model uncertainty was high, with small Akaike weights over all computed models. All estimated effect sizes were comparatively low, with *Eimeria* abundance having the lowest effect on RFI, followed by age class, month, sex and the interaction effect of month and sex, which had the strongest effect. As the individuals from March/April were missing for analysis, statements can only be made regarding the progression of the body fat condition from May until the end of the above-ground season.

The animals collected in this study were killed in accidents or perished near the road. As such, they were considered being a random sample of the population. However, it cannot be fully excluded that those samples were not truly random and influenced by unknown parameters. There seems to have been a bias towards adult males being victims of accidents more frequently, as they were found twice as often as adult females. Reasons could be that males have larger home ranges (MATĚJŮ 2008) or they may be more active than females during certain times (Everts et al. 2009) and thus, must cross roads more frequently. Dispersal behavior of yearlings and juvenile could also be of importance in this regard (Hoffmann et al. 2004).

5.3 Effect of *Eimeria* abundance on RFI

The main aim of this study was to determine the influence of *Eimeria* abundance on the body condition of EGS. The assumption that high *Eimeria* abundances would negatively affect body condition could not be confirmed. The results suggest rather the opposite, as *Eimeria* abundance was positively associated with RFI. However, an experimental jackknife removal of every single individual revealed that the individual with the highest EPG count had an inordinate influence on the model outcome. The removal of this individual led to the exclusion of EPG as a probable variable for the final model. Removal of every other individual did not change the model outcome or added additional interaction effects as probable predictors for RFI. However, there was no reasonable biological justification to remove this individual from the data set that was ultimately used for analysis. The presented results provide low strength of evidence for an effect of *Eimeria* parasites on the body fat condition of EGS in the context of this data set, i.e., without including the early period after emergence from hibernation, when

squirrels may face particularly high infestation loads and may possibly not have yet achieved enough immunocompetence after hibernation. Additionally, shortly after hibernation, individual energy expenditures for the reproduction period may lead to a trade-off in energy allocation to reproductive or immunocompetence, particularly in males.

Studies reporting positive associations between *Eimeria* abundance and their host's body condition do not exist to the author's knowledge. Nevertheless, positive relationships between parasite burden and body condition are known to occur for other parasite species. Winternitz et al. (2012) found no effects of *Eimeria* and nematode intensities on the body condition of *Microtus montanus*, but they found a positive correlation between cestode intensity and body condition in adult individuals. Mixed infections with nematodes and cestodes were also observed during the present study and have been reported in EGS before, summarized by Ramos-Lara et al. (2014). The nematode, cestode and trichurid eggs were counted accordingly to the McMaster protocol, but were ultimately not included in the analysis, due to the already stressed model and the unknown validity of the McMaster method to reliably detect these heavier eggs. These parasites may have also influenced the body condition and/or had an interspecific influence on *Eimeria* abundance. Vaclav and Blazekova (2014) found that helminth infections facilitated reproductive rates of *Eimeria* in Alpine marmots (*Marmota marmota latirostris*) presumably by suppressing the inflammatory response of their host. Tapeworm infections on the other hand, had a decreasing effect on *Eimeria* oocyst shedding.

Individuals with a higher body condition could also have been more active and therefore had more contact with other animals or contaminated material, i.e. with feces. Halvorsen (1986) found higher nematode intensities in heavier juvenile *Rangifer tarandus* than in individuals with lower body weights. In this case, juvenile weights were positively related to the social status of their mothers. The dominant juveniles had better access to food, which ultimately led to a higher ingestion rate of nematode larvae contained in the feed. Thus, a high *Eimeria* abundance could be related to higher food intake for animals with a higher body condition and the associated higher probability to ingest parasite oocysts.

The overall high prevalence of *Eimeria* was in accordance with the findings of Golemansky and Koshev (2009) and Diakou et al. (2014). The relatively low, but consistent EPG levels suggest a generally stable occurrence of *Eimeria* in the population. As mentioned before, the knowledge about the pathogenicity of *Eimeria* species in EGS is scarce. The severity of symptoms in ground squirrels in general, seems to depend on the specific *Eimeria* species and varies between the different host species (Todd et al. 1968; Todd and Hammond 1968a). Further, Seville et al. (1992) found no effect of *Eimeria* infections on the nutritional assimilation

in *Uroditellus elegans*, even though some animals shed over 3×10^6 EPG. They hypothesized that host and parasites had formed a *commensalism*, due to their co-evolution. Considering the consistent levels of infections in nearly all individuals in this study, this may also be the case for the *Eimeria* species in EGS. However, *Eimeria* still infest the epithelial cells of the intestine, destroying these during the merogony cycles. During this study, severe alterations of the intestinal mucosa were observed in several animals (per. comm. A. Posautz, Vet.Med.Univ. Vienna). Species-specific differences in pathogenicity of the three *Eimeria* species found presently in EGS may exist and/or infestation levels might need to reach a certain threshold to cause (clinically relevant) coccidiosis. This was investigated, for example, in broiler chickens with subclinical *Eimeria* infections, but with inconclusive results (Haug et al. 2008; Hodgson 1970). Stanton et al. (1992) reported that *U. elegans* with multiple-species infections shed more oocysts than individuals with single-species infections. For the present study, a preliminary analysis of the prevalence of the different *Eimeria* species indicated high levels of mixed infections, as was also shown by Golemansky and Koshev (2009) and Diakou et al. (2014). Additionally, the EPG counts found in this study were relatively low when compared to *Eimeria* abundances found in North-American ground squirrels. Thomas et al. (1995) reported over 10^8 EPG in several infected *U. elegans*, which have similar body weight and size as EGS. Individuals with higher *Eimeria* infestations and (presumed) progressed clinical symptoms, could have been inhibited in their activity and died in or near their burrows; thus, were not found during this study.

Eimeria, as other parasites, undergo a prepatent and patent period until oocysts are shed by their host. Therefore, an infestation of the intestine can be present, but oocysts are not yet developed and shed by the host (Duszynski and Upton 2001). This could have influenced the abundance of oocysts, as the longer the infestation occurs, the more oocysts could be produced and excreted. The time and progression of the infection in the examined individuals was of course unknown and therefore oocyst counting methods might be inadequate to measure the effects of infections. Irvine (2006) proposed, that the best study design to investigate effects of gastro-intestinal parasites on their hosts are experimental field studies involving parasite removal. Studies on the effects of endo- and ectoparasite removal in different ground squirrel species showed, that parasites can have negative effects on the animal's energy balance, but parasite free individuals had rather low short term benefits regarding body condition. The removal of endoparasites in *Uroditellus columbianus* resulted in only slight differences in body mass gain between treated and non-treated male individuals during the active season and during hibernation (Raveh et al. 2011). However, Neuhaus (2003) reported, that endoparasite removal had a positive effect on body weight gains in reproductive female *U. columbianus* during the weaning phase. Parasite-removal before the mating phase resulted in no differences in body weight gains from mating until parturition.

To explore these possible connections, further investigations on other parasites occurring in EGS, as well as on the prevalence (and abundance) of the different *Eimeria* species in context of the observed histopathological alterations are needed.

5.4 Effects of age class, sex and month on RFI

Unfortunately, the condition status after emergence could not be analyzed with this data set. Accordingly, the second and third working hypotheses could not be addressed. However, the model estimates for sex and age class still provide interesting tendencies for the progression of individual body fat condition over the time from May to August. The overall higher RFI for males, in combination with females reaching higher RFI levels than males before hibernation, was the most intriguing tendency. It indicates, that females could gain enough fat reserves for hibernation faster than males, even though they start later with its accumulation. Some of the females still carry during May and the other females must already invest energy into lactation. In the 'Langenzerndorf population' (LP), body weights increased during the gestation period until mid-May and decreased at the end of lactation (Millesi et al. 1999b). However, despite the body weight increase in females during gestation, fat depletion during this time generally continues (Dark 2005). The low retroperitoneal fat reserves during May and June indicated this, as these females seemingly had no excess energy to store as fat reserves. Curiously, no adult females were found between the middle of June and mid-July, even though their aboveground activity should be heightened during the lactation period, as they spend more time aboveground, foraging (Everts et al. 2009). The females which were then found after mid-July, had substantially higher RFI values than the individuals found in June, indicating a rapid fat accumulation after lactation, as was expected. In the previous years, fewer females were also found during this time window. It is currently unknown, if this represents an annual pattern or reflects a possible male biased sex ratio. The gap coincides with the above mentioned decrease in female body weights at the end of the lactation period, observed in the LP by Millesi et al. (1999b). It may well be, that adult females decrease their aboveground activity during this time or do not move far from their burrow, as they need to recover from lactation.

At the onset of hibernation, females had higher body fat reserves than males. As females hibernate for a longer time, they also need larger energy reserves than males. This was also observed in *Urocitellus parryii*, where adult females had a 11.5 % higher mean lean body fat content than male individuals prior to hibernation and immersed earlier into hibernation than adult males. But contrarily to male EGS, male *U. parryii* are known to cache food for hibernation and thus can bridge low fat reserves (Buck and Barnes 1999). In the LP, female body weights before hibernation did not indicate higher fat contents for females in comparison to males

(Millesi et al. 1999b). In the present study, the RFI was able to identify this difference in body fat condition and provides with it a possible explanation for the earlier immergence into hibernation of female EGS.

The male individuals collected during May were large adults with high body weights and were expected to have a relatively low RFI, due to commonly high energy expenditures during the mating period. Even though mating takes place in early April, the decrease in body mass until the beginning of May is high and should reflect overall body fat loss, as suggested by Millesi et al. (1999b). The relatively high RFI values in May suggest that these males were already able to accumulate some fat or had remaining fat reserves, even though the mating period just ended. This is of course based on the assumption, that the timing of reproductive activity is not overly different between the LP and the study population.

In June, however, some adult males had as low RFI values as juveniles with body weights half that of adult males. This is remarkable, as it indicates that the depletion of fat reserves in adult males might last until end of June, contrary to Millesi et al. (1999b), who reported that male body weights already started to increase in the beginning of May, which could be understood as a first sign of an increase in fat reserves.

The tendency for higher RFI values for males than for females during May and June could have been influenced by yearlings that were pooled in the adult class, as indicated by low body weights of several males in June. Non-reproductive male yearlings can theoretically use the whole season for fat accumulation and thus, could have influenced RFI for males. Coincidentally, Hoffmann et al. (2008) found the highest population densities and largest proportion of immature yearlings in vineyards compared to other habitat types and suggested lower predation pressure in vineyards, due to better cover from predators. This could also fit the present study population, as vineyards make up half of the land use in this area (Jaburová 2009). However, the proportions of reproductive and non-reproductive yearling males in the studied population were unknown at the time of this study. Further analysis of tooth erosion, testes sizes and the population density will help to identify possible influences on male body fat condition.

Juveniles had considerable lower relative retroperitoneal fat reserves than adults. This matches the current understanding of the weight and fat gain pattern in juvenile EGS and confirms the fourth working hypothesis. Juveniles have a substantially shorter season for fattening and must also invest a large proportion of their available energy into growth, hence they enter hibernation later than adults (Millesi et al. 1999b; Millesi and Hoffmann 2008). Similar observations, regarding lower relative fat content in juveniles, were made for

Xerospermophilus tereticaudus (Neal 1965), *C. lateralis* (Jameson and Mead 1964) and *U. parryii* (Buck and Barnes 1999).

Surprisingly, the interaction effect of month and sex suggests the same fat accumulation pattern for juveniles as for yearlings and adults. Millesi et al. (1999b) reported that juvenile males in the LP were larger in body size and mass at immergence than juvenile females. In the present study, juvenile females had a higher body condition at the end of the season than juvenile males. One could conclude from these results, that, even though both sexes terminate structural growth for the ongoing season after around two month of age and use the rest of the season to accumulate fat (Millesi et al. 1999b), juvenile males seem to trade off fat accumulation with growth. In male ground squirrels, insufficient fat reserves before hibernation suppress testosterone production and hence, suspend sexual maturation (Dark 2005). In case a juvenile EGS male cannot mature over winter, the high mortality rates for yearlings over the active season (Millesi et al. 1999b) could diminish the chances of this individual to pass on its genes. However, early participation in mating can also negatively affect yearlings, as only a few of them can acquire a female in spring (Millesi et al. 1999b) and they are additionally inhibited in growth and in body weight gain over the season (Millesi et al. 2004; Strauss et al. 2007).

6 Conclusion

The positive association between *Eimeria* abundance and body condition was contrary to prior expectations and probably due to a strong influence of a single individual in the data set. As no comparable cases in present literature exist that report a positive relationship between *Eimeria* and their host's body condition, this association seems unlikely when considering exclusively *Eimeria* parasites as a cause. Interspecific influences of other parasites could provide proximate explanations and need to be further investigated with a larger sample size and additional data on the other parasites occurring in EGS. Equally, potential (negative) effects of *Eimeria* infections during the early period of activity after emergence from hibernation, may be particularly evident in reproductively active males. This period has currently not been covered by the analysis and will be further addressed in future research.

As females have a considerable shorter time window for fattening and hibernate considerably longer, they need larger fat reserves than males. This was distinct in higher RFI values for females at the end of the active season. This difference in fat accumulation between sexes also held true for juveniles, as male juveniles might trade-off fat accumulation with growth. Even though these patterns in energy allocation were largely expected from a biological point of view, it was intriguing how well the RFI picked up this pattern and provided a possible explanation for the early immergence of female EGS.

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APPENDIX I: Model selection table

Appendix table 1: All models with Akaike weight > 0.01, variable abbreviations: age = age class, mon = month, mld = mould, models sorted by AICc, with logLik, deltaAICc and Akaike weights (ω_i)

Models	logLik	AICc	deltaAICc	ω_i
age + epg + mon + sex + mon:sex	-55.48	127	0	0.14
age + epg + mon + sex + age:sex + mon:sex	-54.48	127.62	0.62	0.10
age + epg + mon + sex + age:epg + mon:sex	-54.93	128.53	1.53	0.06
age + epg + mon + sex + age:epg + age:sex + mon:sex	-53.69	128.77	1.77	0.06
age + epg + mon + sex + epg:mon + mon:sex	-55.32	129.3	2.30	0.04
age + epg + mon + sex + age:mon + mon:sex	-55.45	129.56	2.56	0.04
age + epg + mon + sex + epg:sex + mon:sex	-55.46	129.58	2.58	0.04
age + epg + mon + sex + epg:mld + mon:sex	-55.46	129.58	2.59	0.04
age + epg + mon + sex + age:sex + epg:mon + mon:sex	-54.15	129.69	2.69	0.04
age + epg + mon + sex + age:sex + epg:sex + mon:sex	-54.24	129.88	2.88	0.03
age + epg + mon + sex + age:mon + age:sex + mon:sex	-54.44	130.29	3.29	0.03
age + epg + mon + sex + age:sex + epg:mld + mon:sex	-54.45	130.29	3.29	0.03
age + epg + mon + sex + age:epg + epg:mon + mon:sex	-54.76	130.93	3.93	0.02
age + epg + mon + sex + age:epg + age:mon + mon:sex	-54.88	131.16	4.16	0.02
age + epg + mon + sex + age:epg + epg:sex + mon:sex	-54.92	131.24	4.25	0.02
age + epg + mon + sex + age:epg + epg:mld + mon:sex	-54.93	131.25	4.25	0.02
age + epg + mon + sex + age:epg + age:sex + epg:mon + mon:sex	-53.59	131.41	4.41	0.02
age + epg + mon + sex + age:epg + age:sex + epg:mld + mon:sex	-53.59	131.41	4.41	0.02
age + epg + mon + sex + age:epg + age:sex + epg:sex + mon:sex	-53.64	131.5	4.50	0.01
age + epg + mon + sex + age:epg + age:mon + age:sex + mon:sex	-53.64	131.52	4.52	0.01
age + mon + sex + mon:sex	-59.19	131.88	4.88	0.01
age + epg + mon + sex + epg:mon + epg:mld + mon:sex	-55.3	131.99	4.99	0.01
age + epg + mon + sex + age:mon + epg:mon + mon:sex	-55.3	132	5.00	0.01
age + epg + mon + sex + epg:mon + epg:sex + mon:sex	-55.32	132.03	5.03	0.01
age + epg + mon + sex + age:mon + epg:mld + mon:sex	-55.39	132.17	5.17	0.01
age + epg + mon + sex + age:mon + epg:sex + mon:sex	-55.41	132.23	5.23	0.01
age + epg + mon + sex + epg:mld + epg:sex + mon:sex	-55.43	132.26	5.26	0.01
age + epg + mon + sex + age:sex + epg:mon + epg:sex + mon:sex	-54.06	132.35	5.35	0.01
age + epg + mon + sex + age:sex + epg:mon + epg:mld + mon:sex	-54.1	132.43	5.43	0.01
age + epg + mon + sex + age:mon + age:sex + epg:mon + mon:sex	-54.14	132.51	5.51	0.01
age + epg + mon + sex + age:mon + age:sex + epg:sex + mon:sex	-54.19	132.61	5.61	0.01
age + epg + mon + sex + age:sex + epg:mld + epg:sex + mon:sex	-54.21	132.65	5.65	0.01
age + epg + mon + sex + age:mon + age:sex + epg:mld + mon:sex	-54.43	133.1	6.10	0.01
age + mon + sex + age:mon + mon:sex	-58.66	133.35	6.35	0.01
age + epg + mon + sex + age:epg + age:mon + epg:mon + mon:sex	-54.6	133.42	6.42	0.01
age + mon + sex + age:sex + mon:sex	-58.78	133.6	6.60	0.01