Diversity of fecal microbiota of endangered Western capercaillie (Tetrao urogallus)

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THE UNIVERSITY OF ZAGREB

THE FACULTY OF VETERINARY MEDICINE

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Diversity of fecal microbiota of endangered Western capercaillie (*Tetrao urogallus*)

Master's Thesis

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LIST OF SUPPLEMENTS

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1. INTRODUCTION

Ecosystems worldwide are currently facing increasing climate and anthropogenic interferences. Such adjustments have highlighted the importance of mountain habitats in the global preservation of biodiversity (FOLEY et al., 2005; STRITIH et al., 2021). Therefore, it is essential to monitor the condition of mountain habitats and in particular, their key species, making use of their presence as factors in the assessment of environmental health. Capercaillie (*Tetrao urogallus*), due to their specific habitat requirements, are listed as one of the key species in mountain environments. As such, they are highly dependent on optimal forest conditions and management. From a broader point of view, capercaillie populations are still of sufficient size in parts of northern boreal forests, however, populations in central and western Europe are increasingly endangered and are facing a constant decline and even disappearance of local populations. This is especially visible at the margins of their geographical distribution (including Croatia). Therefore proving the necessity to understand the biology and physiology of capercaillie to minimise and/or prevent the impact of potentially harmful factors on their population status.

Besides capercaillie, other members of the so-called 'forest grouse' group such as hazel grouse (*Bonasa bonasia*), rock ptarmigan (*Lagopus muta*), and black grouse (*Lyrurus tetrix*) are also considered key species. Their interesting appearance, mating requirements, cultural importance, economic consideration, and very distinct habitat requirements have contributed immensely to their public profile and highlighted a need for their conservation. Currently, the only species from this group that are still present in Croatia are hazel grouse and capercaillie. According to the EU Birds Directive 79/409/EEC, member states are obligated to maintain the population of the capercaillie at an appropriate level or to adapt the population of the species to that defined in cf Article 2. One important factor to consider is the composition and diversity of capercaillie faecal microbiota. Capercaillie's individual feeding habits and the perspective of forest management can be linked in relation to the local disappearance of spruce (*Picea abies*) forests which constitute an important part of the winter feed source for capercaillie.

Along with habitat preservation and modelling, one of the potential measures in population recovery is artificial breeding and reintroduction of captive-bred animals. It must be noted that nutrition in captive conditions can differ from that in free-living animals and result in the development of microbiota, which is not ideal for the digestion of naturally available feed. This can reduce the survival rate of released animals. Therefore, this research aims to

identify the diversity of faecal microbiota in endangered Western capercaillie (captive and free-living from different habitats) to better facilitate a diet that appropriately promotes a healthy intestinal microbiota to ensure maximal survival rates of reintroduced animals.

2. OVERVIEW OF CURRENT KNOWLEDGE

2.1. SPECIES DESCRIPTION

Based on morphology and behaviour, Western capercaillie (*Tetrao urogallus*) were previously divided into 12 subspecies (POTAPOV and FLINT, 1989) that were later reduced to 8 (MADGE and MCGOWAN, 2002). The development of sophisticated molecular tools has led to further studies about the validity of such taxonomy (SEGELBACHER et al., 2003; SEGELBACHER and PIERTNEY, 2007). Finally, according to the genetic analysis, two main western capercaillie lineages have been defined, the southern one known as *T. u. aquitanus* and the northern one or *T. u. urogallus* (DURIEZ et al., 2007; RODRÍGUEZ-MUÑOZ et al., 2007). It was proposed that their expansion in Europe was separate, in a way that the southern lineage expanded throughout Europe (reaching Romania and Bulgaria), while the boreal one expanded in Asia and North-Eastern Europe (DURIEZ et al., 2007). Contrary, the study performed by

Fig. 1. Capercaillie males and females in the aviary (photo: D. Konjević).

RODRÍGUEZ-MUÑOZ et al. (2007) states that the boreal lineage evolved from the southern one in the late Pleistocene. A study on Dinaric populations of capercaillie showed both lineages to be present in this area, with the majority of analysed samples (96%) belonging to *T. u.*

urogallus (BAJC et al., 2011). According to the same authors, the subspecies *T. urogallus major* is present in Croatia.

Western capercaillie males are distinctly black and much larger than females who have

Fig. 2. Capercaillie female (photo: I..Križ).

a mixture of brown, cream, and grey coloured feathers, which makes a good camouflage during the nesting period. Males usually weigh between 4 to 5 kg, however, during his studies at Mala Kapela mountain, TURKALJ (1934) reported males weighing up to 7 kg. Females are smaller and usually reach around 2 kg.

2.2 DIET, BEHAVIOUR AND SEASONAL DISTRIBUTION OF CAPERCAILLIE

Capercaillie inhabit areas at altitudes above 900 metres above sea level (m.a.s.l.), which consists predominantly of coniferous forests however capercaillie have also been seen in mainly deciduous forests (BLANCO-FONTAO et al., 2010). Similar differences were also observed in Dinaric Alps in the case of the so-called 'Dinaric inversion'. These variations are understandable when considering that ecological conditions can vary over the distribution range resulting in variability in the demographic and behavioural characteristics of the local

populations (CHASE and LEIBOLD, 2003). Moreover, in the case of peripheral populations (populations at the margins of species distribution area), habitat characteristics and habitat

Fig. 3. View on the capercaillie habitat, mountain areas above 1000 m.a.s.l. (photo: D. Konjević).

utilisation can vary so much meaning that these populations can be less tolerant and more vulnerable to habitat changes. Besides altitude and type of forest associations, when choosing their habitat, capercaillie relies on several factors such as the age of the tree stands, an abundance of Bilberry plants, habitat fragmentation, social organisation and human activities.

Forest association is one of the most important factors for habitat selection by capercaillie. Coniferous forests are especially important during the winter period as they provide efficient shelter, better microclimate (higher average daily temperature, lower exposure to wind), shoots, and needles, which are their main winter food. It was also observed that while coniferous forests are preferred during the winter periods, predominantly spruce forests with bilberry are chosen during the autumn (UNANDER, 1980; ROLSTAD, 1984). As previously said, mainly deciduous forests are used as a habitat in the case of certain populations, like the Cantabrian one (BLANCO-FONTAO et al., 2010). Beech (*Fagus sylvatica*) forests are

preferred during the mating season as they provide a better view of the surrounding terrain (especially when males are sitting on their branches).

Several studies have also highlighted the relationship between the age of the forest and the presence of capercaillie. In the winter and early spring, older pine forests provide bigger branches, broad crowns and an open canopy where birds can fly between and feed on pine needles, especially when snow is present (GJERDE et. al., 1985; KLAUS et al., 1985; ROLSTAD and WEGGE, 1988, 1996). During the summer period when plant material is more abundant, capercaillie are less dependent on old forests (SEMENOV-TJAN-SANSKIJ, 1960; MARCSTROM et al., 1982; ROLSTAD et al., 1988). Furthermore, these preferences may also vary in accordance with the age and sex of the birds. As such, males are usually found in old tree stands all year round, while during the vegetation period, females can also be found on forest clearings (STORCH, 1993). This is probably related to the increased food demands of growing chicks. On the other hand, areas that are more open are utilised as lek sites for the bird's specific courtship ritual and as areas that provide different food during spring, summer, and autumn periods. ROLSTAD (1988) showed that during autumn, both males and females have ''equally strong'' affinities for old forests. He also noted that males preferred more dry vegetation than females, and attributed that to the use of shrub cover. In addition to that, ROLSTAD and TOVERUD (1984) proved the affinity of males to the ''edges'' and ''clearcut'' areas of older forests whereas females avoided clearer areas but could be found with a more even distribution throughout the forest, unrelated to its age. Such preferences towards areas with more cover could be due to the smaller size of females, which enables them to move more easily in denser areas compared to males, but also to a better shelter that is provided by such forests.

Regarding the seasonality, during the winter time capercaillie can be found in older spruce-beech-fir forests, while predominantly common spruce forests (*Picea abies*) are preferred during the summer period, probably due to the presence of blueberry (*Vaccinium myrtillus*) which constitute an important food source (SANIGA, 2004). After the ceasing of the mating period in June, both males and females move from their lek sites to summer habitats. During this period, it was reported that males are non-territorial and that in June and July, they tend to use successional stages of the forest, whereas in August they preferred older, natural forest (ROLSTAD et al., 1988). Females were shown to have smaller home ranges (103 compared to 170 ha) to prefer denser habitats.

Once a male has elected his lek site, he will continue to use it throughout his life, forming temporary harems each mating season. Broods of 6-8 eggs are laid from mid-April
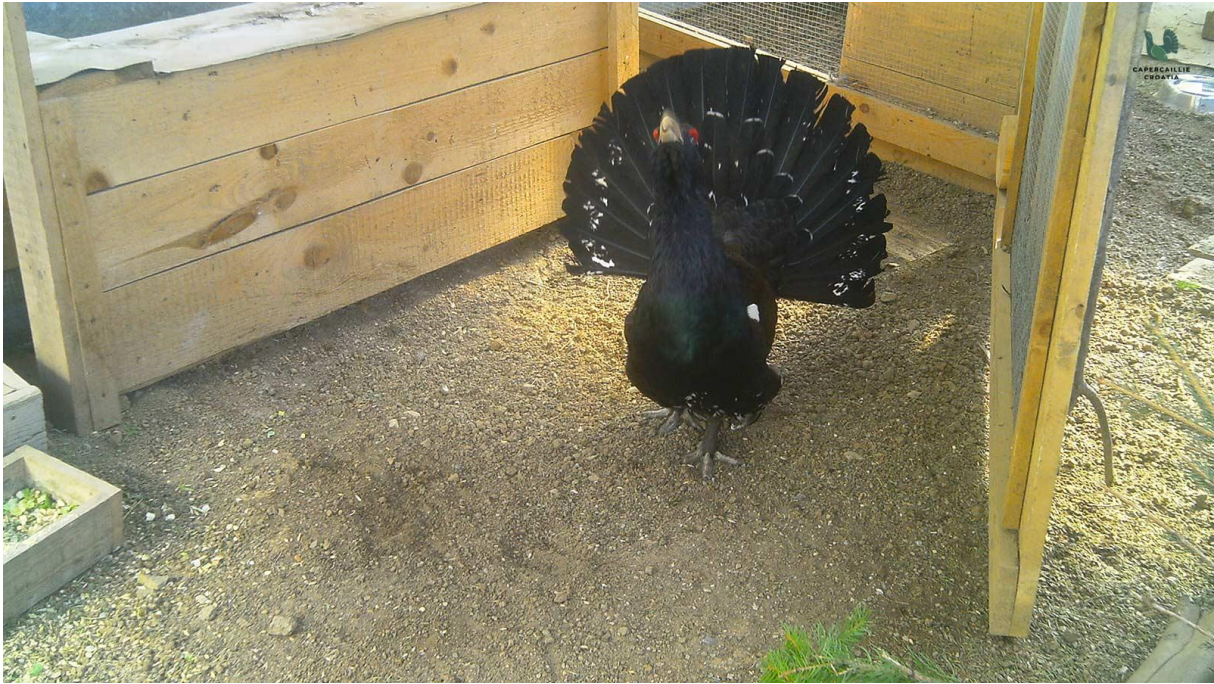
Fig. 4. Male capercaillie displaying in the aviary (photo: I. Križ).

until early May depending on the altitude, which causes the process to arise later in areas where it is increased. Similarly, climate and latitude also influence the time hatching after 26 days between May and June. While males are solitary during the majority of the year, females are occasionally seen in groups of 2 to 4 individuals.

Until 2010, the majority of studies described the diet of capercaillie in singular parts of the year (SUMMERS et a., 2004). SUMMERS et al. (2004) were among the first to describe ''seasonal changes'' in the diet of chicks and birds. Their study describes variations of Scots Pine needles in the diet of adult birds throughout the year. In April, 13.5% of droppings consisted of Scots Pine needles, which increased to over 95% from November to April. They also proved that in May, June, and July pollen and cones are capercaillie's main food in Scotland. Other food sources to note are blueberry leaves and sedges (*Carex* spp.) seeds during the summer until September. Chicks are also known to utilise insects as a protein-rich food supply enabling them to grow feathers and muscles faster, which gives them the means to escape from predators. During June chicks of ages between 2 and 13 days, eat predominantly larvae of *Coleoptera*, *Lepidoptera* and *Formicidae* (SUMMERS et al., 2004). As they age, they begin to eat more 'plant material, particularly blueberry fruit, but also sedge seeds, ericaceous leaves and moss' (SUMMERS et al., 2004). Past studies indicate that the abundance of food

sources plays a massive role in habitat selection by capercaillie. According to COPPES et al. (2021), capercaillie broods in the Black Forest preferred open forest stands with canopy gaps. Such habitats provided chicks with longer exposure to the sun and secured an intermediate cover of blueberries. Adult birds are mainly folivores, and the type and low energy content of their food make them spend a large amount of time feeding (BLANCO-FONTAO et al., 2010). CHUA et al. (2021) applied molecular techniques to differentiate dietary preferences in capercaillie using faecal samples. According to their study, the most frequently occurring group of plants in the capercaillie diet are trees, dwarf shrubs and forbs. Of trees, trees from the pine family (*Pinaceae*) and heath plants (*Ericaceae*) were the most frequently found (CHUA et al., 2021). This has confirmed previous findings (SEISKARI, 1962; LIESER, 1966; PICOZZI, 1996; SUMMERS et al., 2004). CHUA et al. (2021) also found that heath plants constitute a more significant part of the diet in the autumn, while pine trees are more important in the winter and spring period, before the start of the vegetation period. The ratio of pine needles is strongly correlated with the snow depths and its longer presence, extending deeply into the spring period. The diet is also related to the shifts in habitat use, showing more considerable use of dwarf shrubs, especially blueberry. According to this study capercaillie diet contains only several species at each time of year, the most frequently detected species being the shoots and needles of coniferous trees, followed by blueberries (CHUA et al., 2021).

2.3 CAPERCAILLIE POPULATION STATUS IN EUROPE

According to the IUCN red list, western capercaillie are listed as a ' 'Least Concern'' (LC) species (https://www.iucnredlist.org/species/22679487/85942729). However, this could be considered misleading. Due to its extremely large range, capercaillie simply do not meet the threshold to be declared as a 'Vulnerable' species. These criteria include Extent of Occurrence <20,000 km2 combined with a declining or fluctuating range size, habitat extent/quality, or population size, and a small number of locations or severe fragmentation. In total, the population size is due to the area of Scandinavia and Russia is still rather large. The boreal area is a non-fragmented habitat with an estimated capercaillie population of about 1000000 birds. In contrast to the boreal, the alpine population is estimated at only 30,000 birds that live in a highly fragmented area, frequently lacking corridors that should connect different populations. The Alpine population is divided into small fragmented local populations (subpopulations) ranging from 100 to 1000 birds which are protected to ensure their survivability. This division is a result of habitat loss and uneven distribution patterns of conifer forests. Consequently, a

sharp population decline in capercaillies' populations was observed in the majority of European countries, especially in fragmented habitats of central Europe, or the more peripheral range of southern countries (STORCH, 2000; DURIEZ et al., 2007; DROZDOWSKI et al., 2021). Another example of dramatic decline has been seen in German capercaillie populations in the Black Forest (COPPES et al., 2019). There were 570 males during monitoring in 1971, while only 167 males were observed in 2018. The most pronounced decline was observed in the eastern (a drop from 48 males in 1993 to 8 males in 2018) and southern parts (a drop from 160 males in 1993 to 26 males in 2018) (COPPES et al., 2019). In Croatia, monitoring data have shown that in the middle of the 20th-century capercaillie was regularly present in the five main mountain areas (Risnjak, Velika Kapela, Mala Kapela, Velebit, Ličko sredogorje and Lička Plješivica). The largest population was present on Risnjak Mountain (Gorski Kotar) with 112 leks, while the smallest population was present on Ličko sredogorje mountain with only one lek (FRANIĆ, 1910; RUCNER, 1954; TURKALJ, 1956; CAR, 1970; LUKAČ, 2004; FRKOVIĆ, 2012). More recent monitoring, performed mainly by foresters or National Parks indicate a 77% decrease in lek numbers over the last 60 years. Interestingly, the highest decline was observed on Risnjak Mountain, with a fall from 112 to only 5 leks. According to data from local forestry management units, lek numbers on Velebit mountain have dropped from 50 in 1957 (ANDRAŠIĆ, 1957) to the present 0.

In a modern, constantly changing landscape capercaillie have one important requirement that limits its preservation in highly developed countries, as well as on the limits of their dispersal range. This requirement is a need for large areas of specific habitat (SCHROTH, 1992; STORCH, 1995; SJÖBERG, 1996). Forest fragmentation, directly linked to this requirement, is one of the most important factors for their preservation (ROLSTAD and WEGGE, 1989). All mentioned before, and the fact that capercaillie are an umbrella species for some endangered birds (SUTER et al., 2002) makes them a highly important keystone species that indicate the conservation status of biodiversity in mountain habitats. This is further emphasised by the fact that capercaillie are associated with historical and ethical values in many countries (ULICZKA et al., 2004). Some studies have highlighted the importance of certain ''more individual'' factors on the preservation of capercaillie. For example, KLAUS et al. (1997) demonstrated that the interlinked effects of emissions on ground vegetation and predation of nests and chicks due to the forestry industry contribute to the 'disappearance of capercaillie'. During their study, the density of capercaillie decreased from 24 birds per 10,000 ha to 1 from 1970 to 1990. According to their study, this decrease was a result of eutrophication

from increased emissions. The disruption in the natural pH of the soil after contamination with factory emission led to a decreased pH favouring an increase in the variety of plant species of plant and hence decrease in the availability of blueberries, which is a favoured source of cover for chicks and feed for adults (KLAUS et al., 1997). STORCH (1994) also noted that ''home range size was inversely related to the availability of Bilberry''. Additionally, the territorial and orientation behaviour of capercaillie have been described in reference to infrasound (LIESER et al., 2006). They described how ''non-vocal sounds'' of capercaillie are ''likely to play an important role in territorial behaviour''. Therefore, it could also be concluded that anthropogenic activity such as wind turbine use, could be destructive to wild capercaillie populations. Other factors, presented as highly important, are non-routine human activities as seen in the example of ski and winter tourism in general (THIEL et al., 2008, 2011). These stresses provoke high-energy demands in capercaillie and are therefore especially dangerous during the wintertime (flight response) when it is of vital importance to conserve energy.

Yet, even though these studies have shown the relation of certain negative factors with the decline of capercaillie populations, it is more likely that this negative trend is multifactorial. Presently, wildlife management is oriented largely on principles and measures for species protection, and not to a combination of these measures and practical land management (SUCHANT, 1999). As such, a non-integrated approach leads to conflicts between human activities and wildlife management (SUCHANT, 1999). For example, several studies have lately reported that lek sites are not only related to the age of forest stands but are also highly dependent on landscape scale habitat quality (STORCH et al., 2002; ROLSTAD et al., 2007; SIRKIÄ et al., 2011; ZIZAS et al., 2017). Therefore, habitat analysis and habitat preferences are an extremely important part of capercaillie's conservation programs, and in particular one of the main preconditions for successful reintroduction (MOSS et al., 2010; MERTA et al., 2015). In Croatia, habitat preference analysis for capercaillie was performed for the area of Velebit and Lička Plješivica Mountain (KOLETIĆ, 2018; KRAPINEC et al., 2022). Lička Plješivica Mountain was selected since it is the only area in Croatia where the capercaillie population is stable. As seen so far, an integrative approach including habitat modelling and management and the creation of corridors to connect isolated populations coupled with more successful reintroduction programs is already a necessity for capercaillie survival in many European countries.

2.4 PREVIOUS STUDIES ON CAPERCAILLIE GUT MICROBIOTA

The gut microbiome is a term used to describe a complex community of microorganisms that inhabits the intestines of both humans and animals (JARRET et al., 2021). BERG et al. (2020) expanded this definition and stated that besides microorganisms, the term microbiome also refers to their activity and formation of an interactive micro-ecosystem that is crucial for the normal functioning and health of the organism. E. Metchnikoff of the L. Pasteur Institute (METCHNIKOFF, 1910) recognized this connection between the gut microbiome and the health status of an individual already in 1900. He hypothesised that the longevity of Bulgarians was related to the consumption of fermented milk products. In other words, he concluded that the introduction of 'good' bacteria could be beneficial in fighting off the 'bad' ones. This doesn't mean that organisms are free of 'good' bacteria and that they have to be introduced. Each species has its normal flora. The hologenome theory says that the evolution of organisms occurred together with their microbiome, which, if normal, can increase survival, fitness, reproduction success and coping with environmental stress (ZILBER-ROSENBERG and ROSENBERG, 2008). The importance of the gut microbiome in maintaining functional metabolism, ensuring energetic balance, healthy intestinal mucosa, helping the immune system, etc., was further emphasised in later studies (CHO and BLASER, 2012; SHREINER et al., 2015). The gut microbiome contains a large number of different species and its variety depends on several factors that can potentially have an impact on the health of an animal. For example, the human microbiome contains trillions of microorganisms (CRESCI and BAWDEN, 2015). Exactly the species is the most important factor that dictates the diversity of the gut microbiome. That is why birds with over 10000 species and adaptation to so many ecological niches are extremely interesting to study. Another important factor, besides animal species, that determines the composition of the gut microbiome is diet (DAVID et al., 2013; CLAYTON et al., 2018). Several studies proved that altering the gut microbiome was negative for nestling growth and fecundity (see GROND et al., 2018). Another field of interest is the potential of wild birds to carry various pathogens, of which some might have zoonotic potential (TSIODRAS et al., 2008). This is of particular importance when the migration potential of certain birds is considered (AHMED et al., 2015) or the fact that an increasing number of birds is present in urban environments (Phillips et al., 2018). However, even more important is to analyse the gut microbiome and its relation to avian health, especially in the case of declining species (VICKERY et al., 2014; DODD and GRUEBER, 2021). Capercaillie is of special importance due to its declining populations in central and Western Europe, but also as a part of

the reintroduction program. So far, the reintroduction of captive-raised birds has not met the expectations. According to LIUKKONEN-ANTTILA et al. (2000) and SIANO et al. (2006), the success of reintroduction is low mainly due to the high predation rates, explained by morphological, physiological and behavioural deficits of released birds. From these studies, it is clear that habitat management is one of the most important factors in the pre-release procedure. Other factors include good development of flight musculature, recognition of danger, knowing how to find food, and having a well-developed digestive system (including microbiome) to be able to survive in wild conditions. SCHALES et al. (1993) analysed 173 faecal samples from captive and 92 samples from wild capercaillie and found that almost all samples contained Escherichia coli and Bacillus spp. Beside them frequently isolated were *Streptococcus* spp., other *Enterobacteriaceae*, and *Clostridium perfringens*. WIENEMANN et al. (2011) analysed bacteria from the ceca of captive and wild capercaillie using the 16S rRNA gene. They found differences in the bacterial communities between wild and captive birds but have related them to disturbances in microbiota rather than to differences in the diet. Interestingly they found an absence of *Clostridium* spp. in captive birds, even though it is known that *C. perfringens* causes necrotizing lesions in the digestive tract of captive *T. urogallus* (STUVE et al., 1992). Anyhow, WIENEMANN et al. (2011) have concluded that such differences may lead to decreased survival of released birds. Intestinal flora can modify after the release of captive birds (ZHANG et al., 2022), in the case that they survive long enough. That is why it is important to achieve a functional microbiome in the intestines of captive birds before reintroduction.

3. HYPOTHESIS AND AIMS OF RESEARCH

This research hypothesises that the breeding of capercaillie in the conditions of a large aviary located in a natural habitat will ensure the development of a gut microbiota similar to that of wild birds.

Aims:

1)   To analyse the composition and diversity of western capercaillie faecal microbiota during the winter and early spring.

2)   To compare findings with previous studies.

3)   To evaluate the potential of adapting natural feed resources in the case of reintroduction.

4. MATERIALS AND METHODS

4.1. SAMPLING LOCATION AND SAMPLES

In this study capercaillie faecal samples were collected from wild populations originating from two different habitats (NP Sjeverni Velebit and Lička Plješivica Mountain) and a captive population kept in the aviary of the Middle-European Capercaillie Breeding Centre, Farjev Laz, Gorski Kotar (Risnjak massif).

Velebit Mountain

Velebit Mountain ranges from Vratnik (near the city of Senj) some 145 km to the southeast. It can be divided into the Northern, Middle, Southern and Southern-Eastern parts. Since Velebit creates a barrier between the Adriatic coast and the continental part of Croatia, it can be divided into seaside, sub-mountain and continental areas. Two National Parks were

Fig. 5. Beech-Fir forest association (photo: D. Konjević).

formed on Velebit Mountain, NP Paklenica in 1949 and NP Northern Velebit in 1999. The whole area was proclaimed a Nature Park in 1981. NP Northern Velebit (sampling location) covers an area of 11 157.29 ha (TOMLJANOVIĆ, 2019). The vegetation is mainly composed

of coniferous trees (Common spruce, Silver fir (*Abies alba*), Mountain pine (*Pinus mugo*) and Black pine (*Pinus nigra*)), with beech forests (*Fagus sylvatica*) and partly downy oak (*Quercus pubescens*) and Oriental hornbeam (*Carpinus orientalis*). Consequently, the following forest associations are established: *Querco pubescenti – Carpinetum orientalis* Horvatić 1939, (*Seslerio autumnalis-Fagetum sylvaticae* Horvat M. Wraber ex Borhidi, *Omphalodo-Fagetum* Tregubov 1957 corr. Puncer 1980, *Polysticho lonchitis-Fagetum* Horvat 1938, *Lamio orvalae-*

Fig. 6. Premontane beech forest (photo: C. Stiles).

Fagetum sylvaticae, Horvat 1938, *Aremonio-Piceetum*, Horvat 1938, *Listero-Piceetum abietis, Calamagrostio-Abietetum* Horvat, 1950, *Adenostylo alliariae-Piceetum*, *Lonicera borbasianae-Pinetum mugi*, *Cotoneaster tomentosi-Pinetum nigrae* Horvat 1938. Along with forest associations, grass communities characteristic of meadows and grazing areas are present, but are slowly replaced by forest vegetation due to the depopulation and consequent decline of livestock populations. According to W. Köppen's classification climate is categorised as Cfsbx or moderate warm rainy climate with dry periods during the summer. The quality of Velebit as a habitat for capercaillie was analysed by KOLETIĆ (2018). According to this study, capercaillie prefers colder areas above 1000 m.a.s.l. with above average precipitation, with

longer duration of snow cover and a higher share of forests. KOLETIĆ (2018) concluded that the most suitable area for capercaillie in the NP Northern Velebit in total covers approximately 2 052 ha.

Lička Plješivica

Lička Plješivica Mountain is the second largest mountain in Croatia, partly located in neighbouring Bosnia and Herzegovina. The area above 1000 m.a.s.l. covers a surface of 42697 m2. Dominant forest association is premontane (upper montane belt) beech forest in association with large white buttercup (*Ranunculo platanifolii-Fagetum* Marinček et al., 1993).

Fig. 7. Blueberries (photo: D. Konjević).

KRAPINEC et al. (2022) evaluated the habitat quality on an uninterrupted part of the area above 1000 m.a.s.l. in a total surface of 24205 m2. Similarly to the populations on Velebit Mountain, capercaillie at Lička Plješivica prefers higher altitudes, higher slopes, longer duration of snow cover, and a higher number of cold days. Capercaillie also prefers more forested areas and older forest stands (KRAPINEC et al., 2022). According to the same authors, only 6% of the analysed area is declared as unsuitable for capercaillie, while the most suitable area covers 15%.

Risnjak massif

Farjev Laz (aviary) is located in the hunting ground VIII/110 ''CRNA GORA'', which is part of the Risnjak massif. The characteristics of the Risnjak massif are briefly discussed here. The Risnjak massif represents a connection between the Alps and the Dinaric Mountains. Due to the vicinity of the Adriatic coast, Risnjak is partly under the influence of the Mediterranean climate, which in combination with its geology leads to a high floristic diversity (TOMLJANOVIĆ, 2020). Forest vegetation is mainly composed of coniferous (Common spruce, Silver fir, Mountain Pine) and beech associations. Following forest associations are present: *Lamio orvalae-Fagetum sylvaticae* Horvat 1938, *Omphalodo-Fagetum* Tregubov 1957 corr. Puncer 1980, *Polysticho lonchitis-Fagetum* Horvat 1938, *Ostryo-Fagetum*, *Blechno-Fagetum*, *Blechno-Abietetum*, *Calamagrostio-Abietetum* Horvat 1950, *Clematido alpinae-Piceetum, Aremonio-Piceetum* Horvat 1938, *Listero-Piceetum abietis*, *Lonicero borbasianae-Pinetum mugi* (KONJEVIĆ, 2017, TOMLJANOVIĆ, 2020). According to W. Köppen's classification and data from the meteorological station Parg, the climate is Cfsbx.

Capercaillie are kept in the large aviary (72 m x 72 m) in the Middle European Capercaillie Breeding Centre ''Prezid''. The aviary is located at an average altitude of 900 m.a.s.l., and contains plant species typical for the Gorski Kotar region. Both the plant species present and the size of the aviary, mean that it perfectly mimics the wild conditions for capercaillie in Gorski Kotar.

Faecal samples

Fresh faecal samples were collected during the spring, summer and autumn periods of 2022 from captive male and female capercaillie, while samples from free-living populations were collected during the winter period from individuals of unknown sex. Due to the dense vegetation cover and small population size, faecal samples from free-living animals are only available during the presence of snow cover, which makes them visible. In total, 29 faecal samples were collected (Table 1). There was no antibiotic treatment for any of the captive birds at least 6 months before the sampling. Birds were classified as captive ones (more coniferous-based diet), birds from Lička Plješivica (diet based on more deciduous species) and Northern Velebit (more coniferous-based diet). Both captive and wild animals had access to food of animal origin (larvae and ants) and forest fruits, seeds and leaves. Samples were collected using gloves, placed in plastic bags, marked and transported to the refrigerator. Upon arrival at the Veterinary Faculty samples were stored -80oC until further preparation. The samples from

Northern Velebit were also stored in ethanol, which could influence the results of sequencing later on.

Table 1. Collected faecal samples.

Location	Captive	Lička Plješivica	Northern Velebit
No of samples	14	7	8
Samples sent to sequencing	11	6	7

4.2. DNA ISOLATION

The DNA of the microbiota was eluted from the samples using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich, USA) using a slightly adjusted manufacturer's protocol as described below.

The samples were frozen at a temperature of -80°C until the preparation day. Collected frozen faecal samples were thawed at room temperature and a sample of 25 mg was taken from each and placed into 1.5 mL microcentrifuge tubes. 180 µl of lysis solution T and 20 µl of Proteinase K solution was added to each sample and mixed by vortexing. The samples were then incubated at 55oC for 4.5 hours and centrifuged for 30 seconds at 8,000 rpm until the tissues were completely homogenous. 200 µl of Lysis Solution C was added to 100 µl of supernatant from each sample and vortexed for 15 seconds to ensure further homogeneity and then incubated at 70oC for 10 minutes. A volume of 500 µl of Column preparation solution was added to each pre-assembled binding column and centrifuged at 13,000 rpm for 1 minute. The flow through liquid was then discarded and the samples were prepared for binding by adding 200 µl of 95% ethanol to the lysate. The sample was then mixed thoroughly by vortexing to ensure homogeneity. The lysate from the preparatory step described above was put into the DNA binding column using a wide-bore pipette tip. It was then centrifuged at 13,000 rpm for 1 minute. The collection tube containing flow-through liquid and the binding column was placed into a new 2 mL collection tube. The first wash was performed by adding 500 µl of prepared wash solution concentrate to the binding column and centrifuging at 13,000 rpm for 1 minute. The collection tube was then discarded and the contents were placed within the binding column in a new 2 mL collection tube. The second wash was performed by adding 500 µl of

wash solution to the binding column and centrifuging for 3 minutes at 13,000 rpm speed to enable the binding column to dry. It was ensured the binding column was free of ethanol before the DNA was eluted. The collection tube containing the flow through liquid and the binding column was placed in a new 2 mL collection tube. 50 μl of elution solution was pipetted into the centre of the binding column incubated for 10 minutes and room temperature and centrifuged for 1 minute at a 13,000 rpm.

4.3. QUALITY CONTROL OF DNA SAMPLES BEFORE SENDING THEM TO THE EXTERNAL LABORATORY

The concentration and purity of the obtained genomic DNA were assessed using BioDrop Ltd. (Cambridge, UK) spectrophotometric analysis. On confirmation of the samples meeting the stated appropriate limits of ≥ 200 ng amount of DNA, ≥ 20 μL volume of each sample and A260/280 = 1.8-2.0 purity they were sent to the external laboratory.

4.4. SEQUENCING

The samples were sent for metagenomic analysis by an external laboratory (Novogene Co., Ltd., Cambridge, UK). The 16S rRNA bacterial genes of distinct regions (16SV4/16SV3/16SV3-V4) were amplified using specific primers. Phusion® High-Fidelity PCR Master Mix (New England Biolabs) was used for PCR reactions. The obtained PCR products were mixed with a loading buffer and analysed using electrophoresis. Only samples with clear main strips between 400-450 bp were sent to further procedure and purified using Qiagen Gel Extraction Kit (Qiagen, Germany). NEBNext Ultra DNA Library Pre® Kit for Illumina was used for library preparation while its quality was assessed by the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated. The samples were analysed as a combination of captive and Lička Plješivica samples and captive and Velebit samples. Paired-end reads were assigned to samples and merged using FLASH (MAGOČ and SALZBERG, 2011). Quality filtering of raw tags was performed as previously described (CAPORASO et al., 2010; BOKULICH et al., 2013). Effective tags were obtained following chimaera sequences removal using the UCHIME algorithm (EDGAR et al., 2011; HAAS et al., 2011). The obtained sequences were classified into operational taxonomic units (OTU) using

the UPARSE algorithm and a 97% threshold of similarity (EDGAR, 2013). OTUs abundance information was normalised using a standard sequence number corresponding to the sample with the least sequences. The phylogenetic relationship was studied using MUSCLE software (EDGAR, 2004). Beta diversity analysis, to evaluate differences of samples in species, were calculated using QIIME software (Version 1.7.0). Principal component analysis (PCA) was applied to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). Principal Coordinate Analysis (PCoA) was displayed by the WGCNA package, stat packages and ggplot2 package in R software. A hierarchical clustering method conducted by QIIME software was used to interpret the distance matrix.

5. RESULTS

5.1. PART I - FAECAL MICROBIOME OF CAPTIVE AND WILD CAPERCAILLIE FROM LIČKA PLJEŠIVICA MOUNTAIN

The metagenomic analysis of the V3-V4 region of 16S rRNA genes was performed for each faecal sample. The samples were grouped into 4 groups – cecal faecal samples (C), male faecal samples (M), female faecal samples (F), all originating from captive animals, and wild capercaillie faecal samples marked as LP (Lička Plješivica).

Metagenomic analysis was used to study and compare the composition of microbial communities in each sample. Four phyla were represented across all samples in the highest abundance: Actinobacteriota, Proteobacteria, Bacteroidota and Firmicutes (Figure 1).
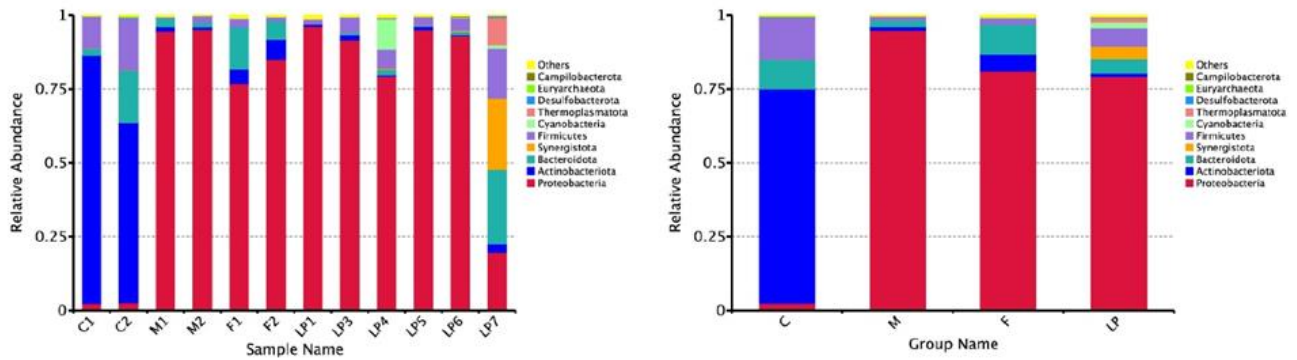
Fig. 8. Taxa relative abundance in phyla for each studied sample.

The analysis showed a significant distinction in the composition of the cecal faecal samples when compared to other groups. Cecal faecal samples had the highest abundance of Actinobacteriota, while other groups showed an abundance of Proteobacteria. The samples collected from the wild capercaillies showed higher overall heterogeneity of the microbial community, which was probably a result of a different diet. Interestingly, a distinction was also detected in samples from males and females (Figure 2).
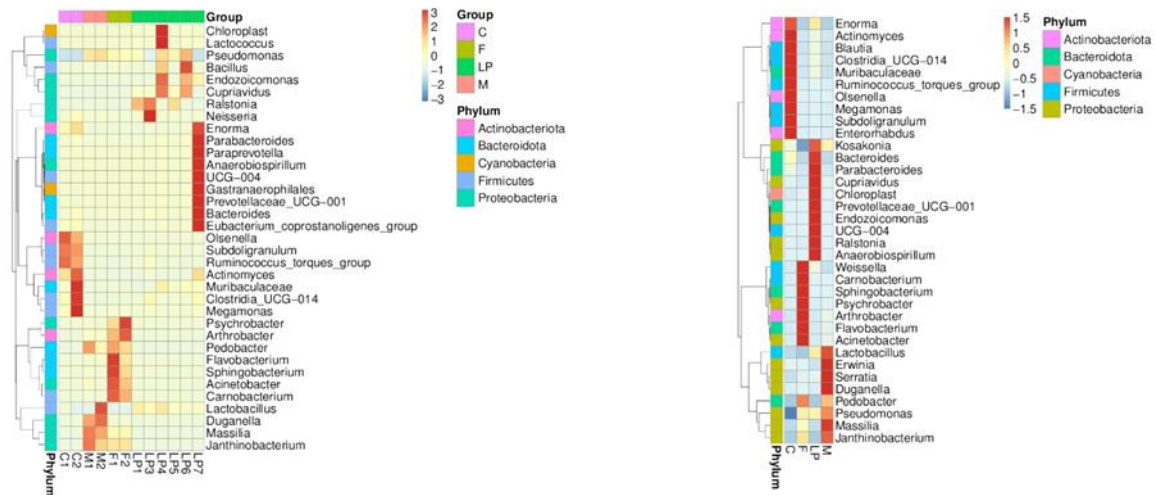
Fig. 9. Taxonomic abundance cluster heatmap for individual samples and groups.

Alpha diversity analysis was used to analyse the diversity within samples and represent the richness of microbial communities in each sample. The richness and diversity were highest in the samples from wild capercaillies, as expected (Figure 3). The similarity of all studied samples was presented in the UPGMA cluster tree (Figure 4), showing two main clusters – one with the samples from males and females in the aviary, and the other with the cecal and wild capercaillie samples.
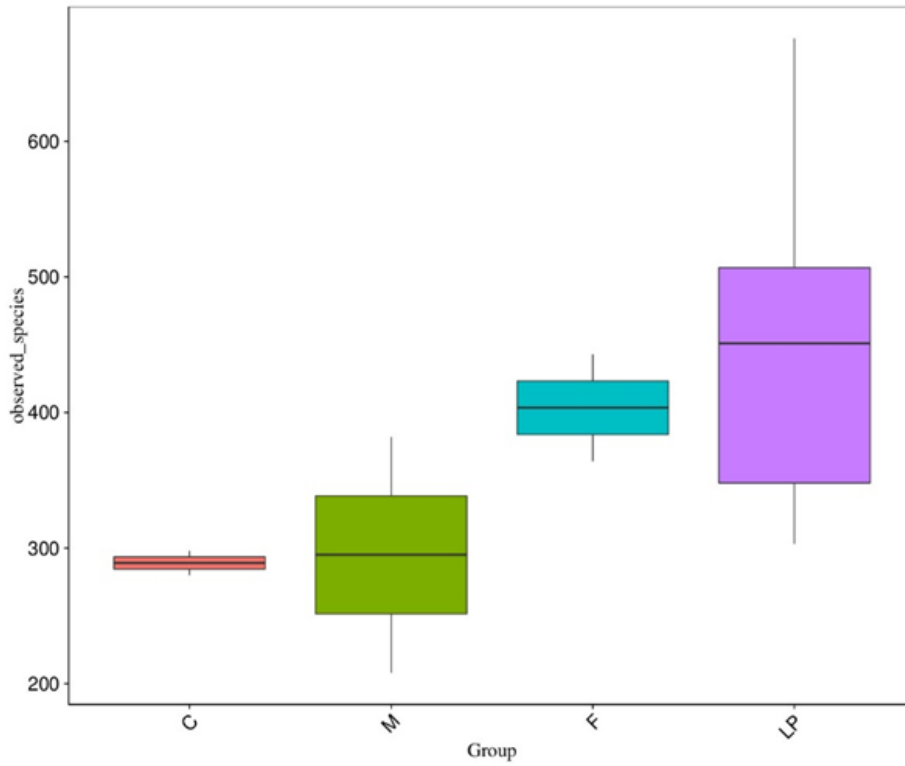
Fig. 10. Box plot of the differences of observed species in each studied group.
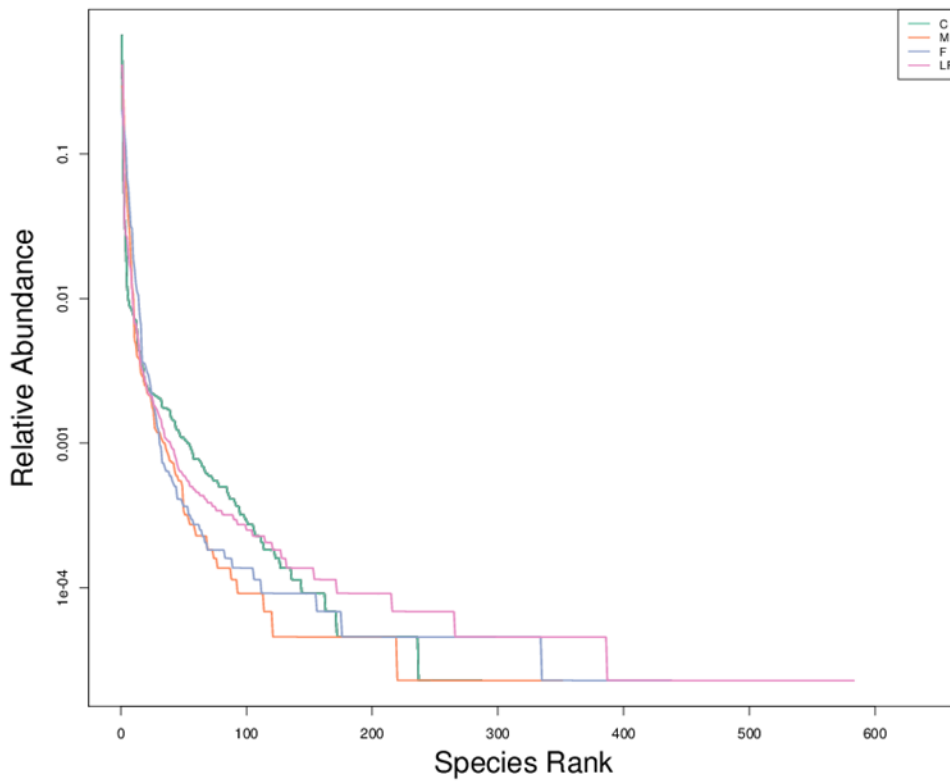
Fig. 11. Relative abundance of species in different samples.
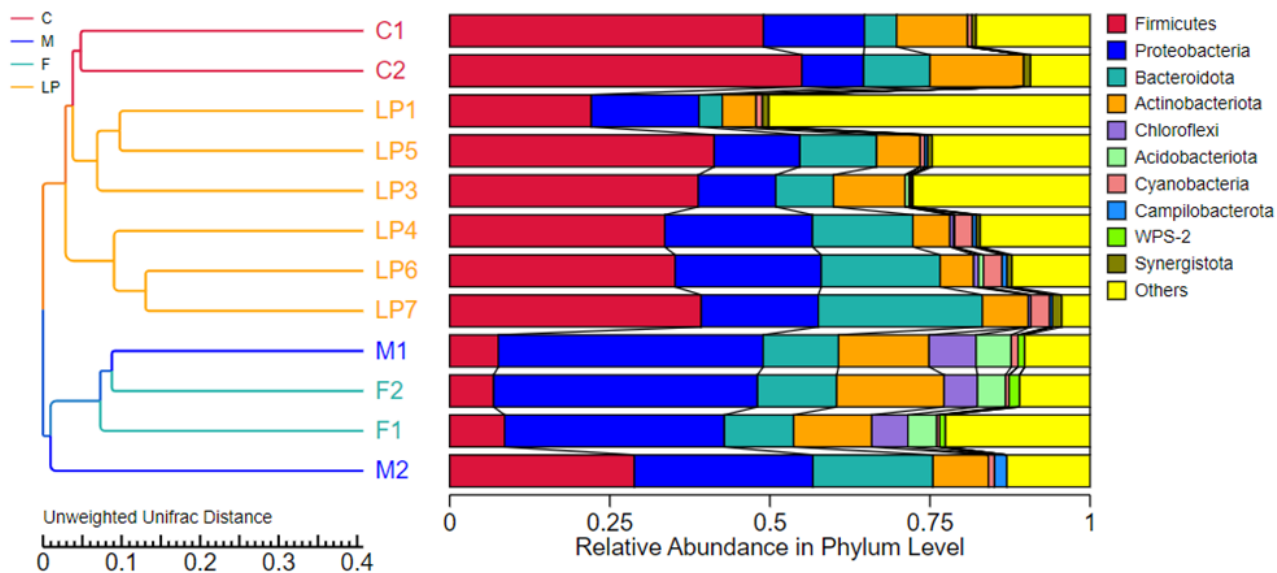
Fig. 12. UPGMA cluster tree showing the similarity among different samples.
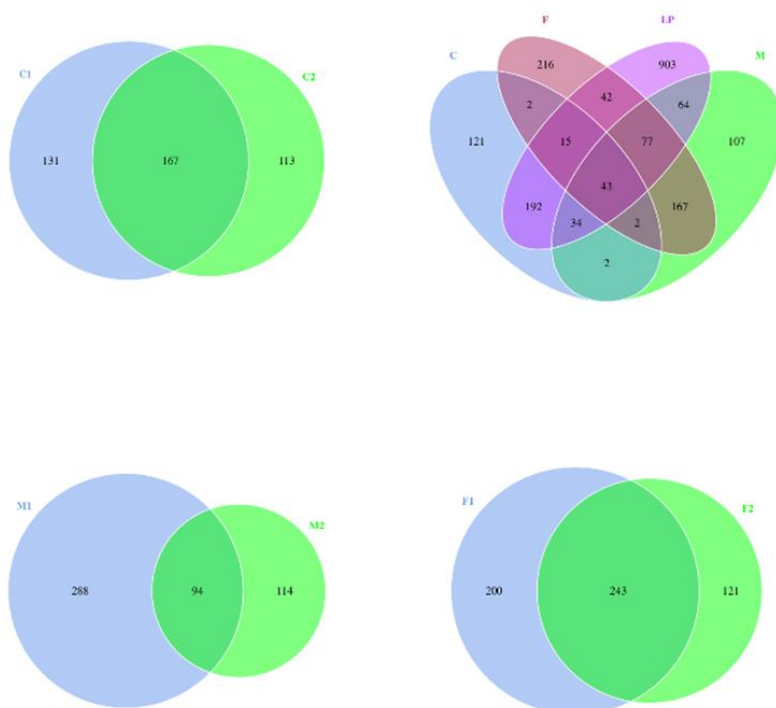
Fig. 13. Venn's diagram showing overlapping in bacterial communities between samples collected from males (M), females (F), caecum (C) and Lička Plješivica (LP). Interestingly more overlapping was observed in females than in males. Similarly, samples from females showed higher overlapping with samples from wild capercaillie.
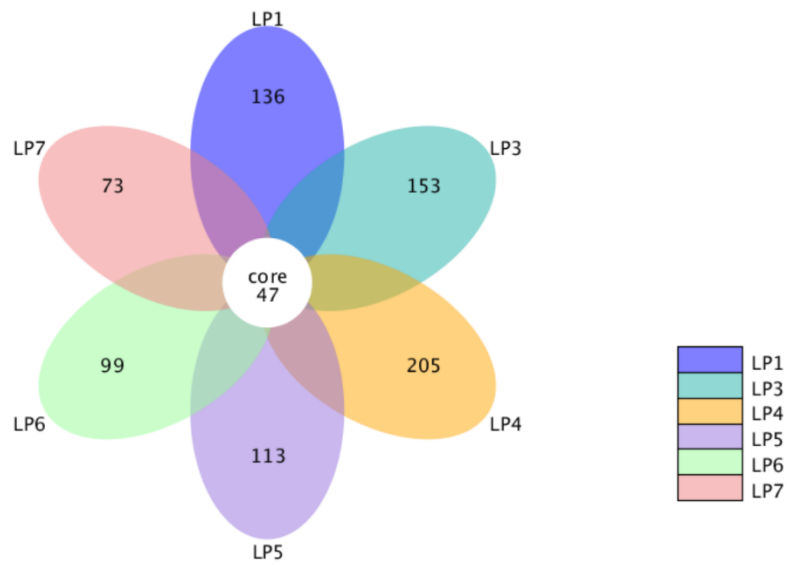
Fig. 14. Flower plot showing number of species shared between all samples from Lička Plješivica, as well as each sample separately. Core area, species common for each sample, include 47 species.
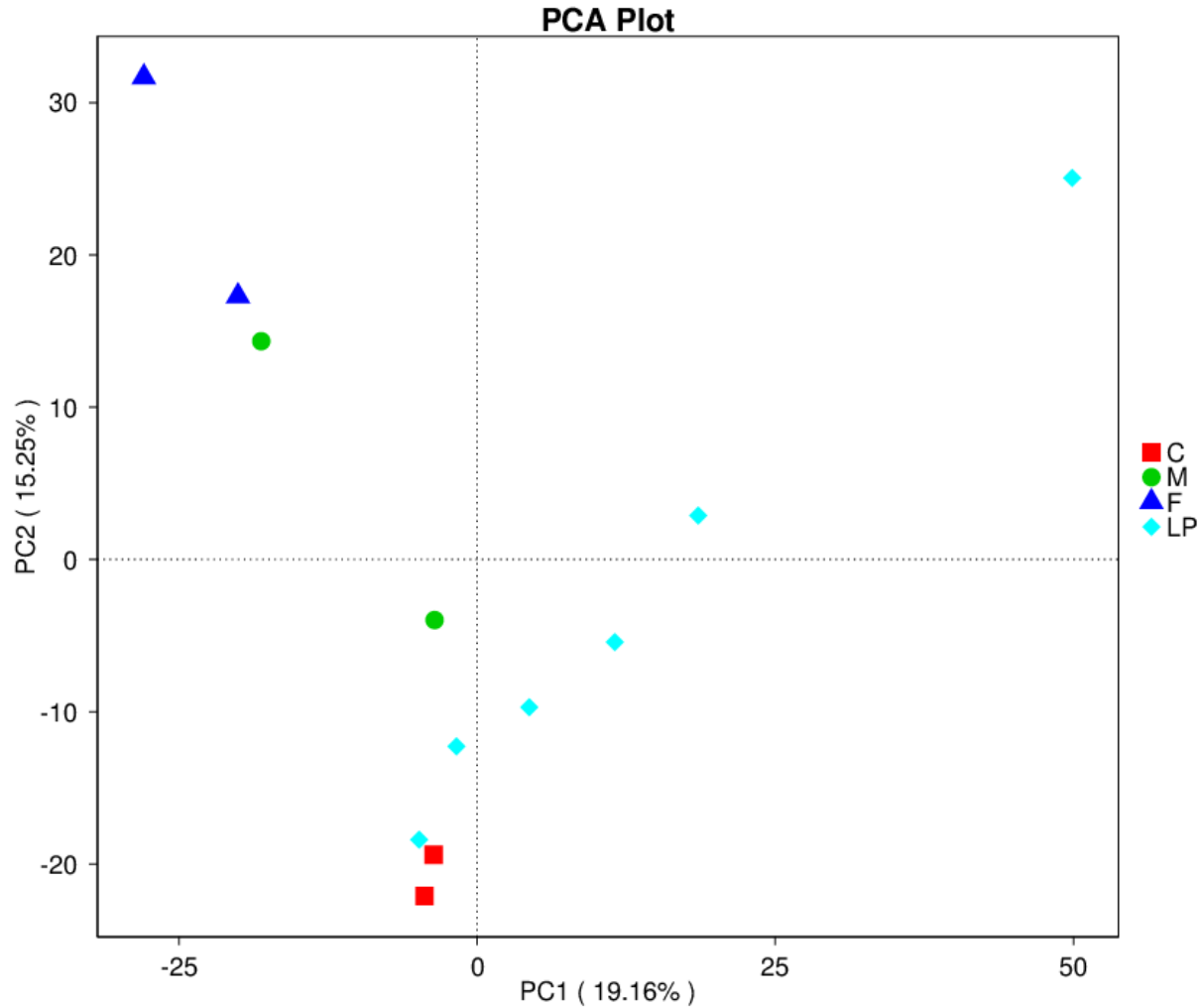
Fig. 15. PCA plot showing similarities between aviary samples and a separate cluster of samples from Lička Plješivica Mountain.

5.2. PART II: FAECAL MICROBIOME OF CAPTIVE AND WILD CAPERCAILLIE FROM NORTHERN VELEBIT MOUNTAIN

As in previous group of samples, the metagenomic analysis of V3-V4 region of 16S rRNA was done for each faecal sample. There were three groups of samples – adult captive animals (O), juvenile captive animals (P) and wild adults from Northern Velebit (R).

Regarding abundance of different phyla represented across samples highest was for: Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria and Bacteroidota, similar as in previous sampling but in different ratios (Fig. 5). Results showed clear distinction between juvenile and adult captive samples, where in faecal samples from adult birds Proteobacteria were found in highest abundance, while in samples from juveniles Firmicutes were most

common. On the other hand, samples of wild capercaillie from Northern Velebit, as in previous sampling, had higher heterogeneity, with highest abundance of Proteobacteria, followed with high presence of Firmicutes. Abundance of microbiota in adult samples is more similar to the wild one (Fig. 6).
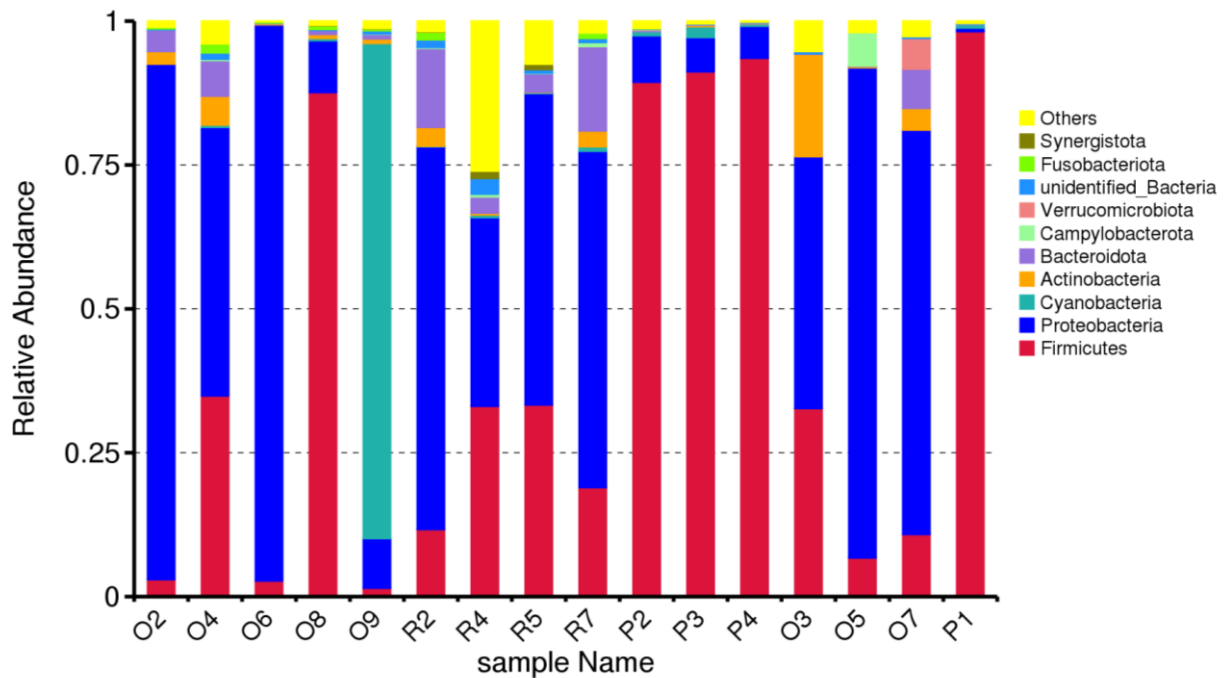
Fig. 16. Taxa relative abundance in phyla for each studied sample in second sampling.
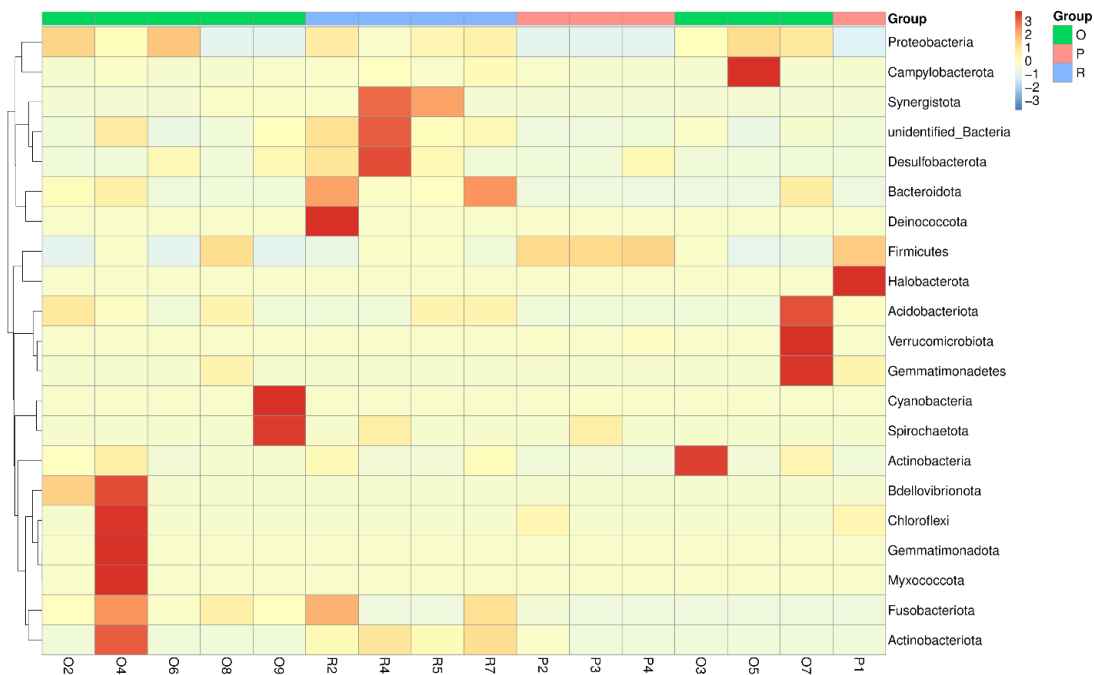
Fig. 17. Taxonomic abundance cluster heatmap for individual samples in second sampling.

Regarding alpha diversity results, it again showed higher richness and diversity in the samples from wild capercaillies (Figure 7), with also higher richness and diversity in adult samples, compared to juvenile captive samples, representing probable developmental phase of microbiota. When UPGMA cluster tree is analysed, it is clearly showing two clusters. One in which samples from adult captive animals are mixing and show highest similarity with samples from wild animals from Northern Velebit Mountain (Figure 8). Second representing captive juvenile samples in the phase of development.
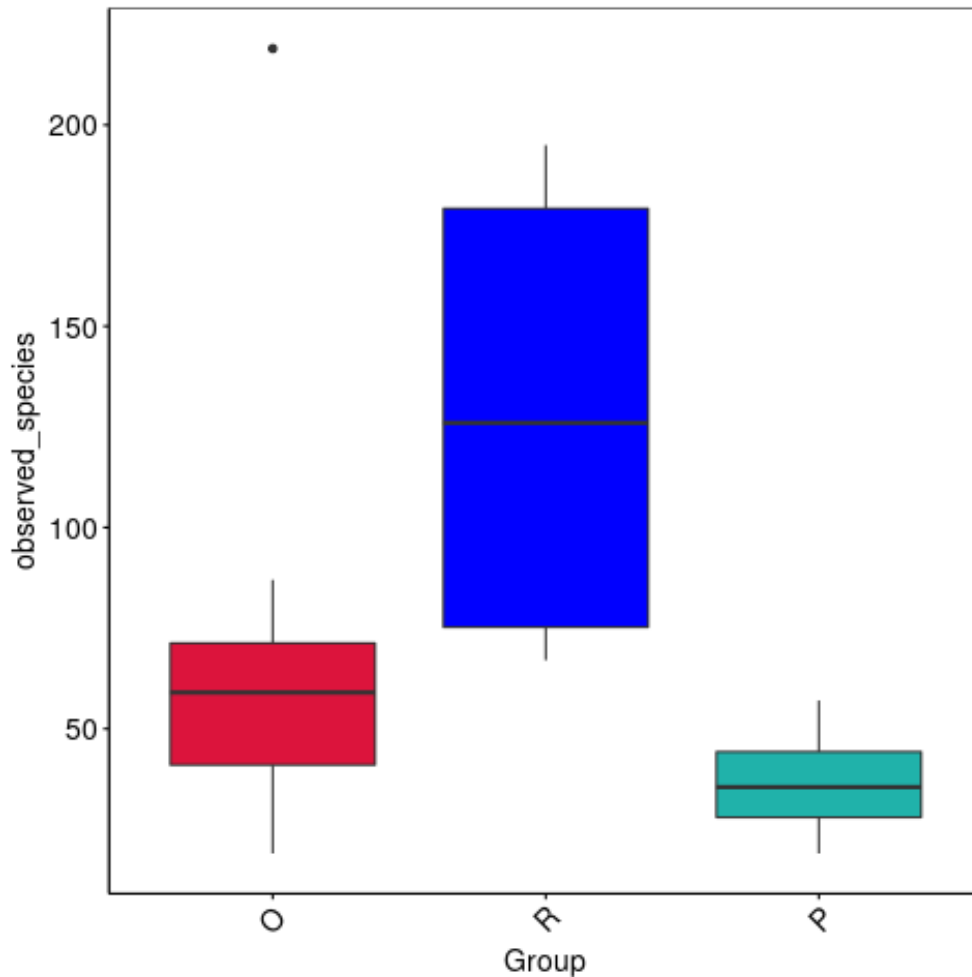
Fig. 18. Box plot of difference of observed species in each studied group in second sampling.
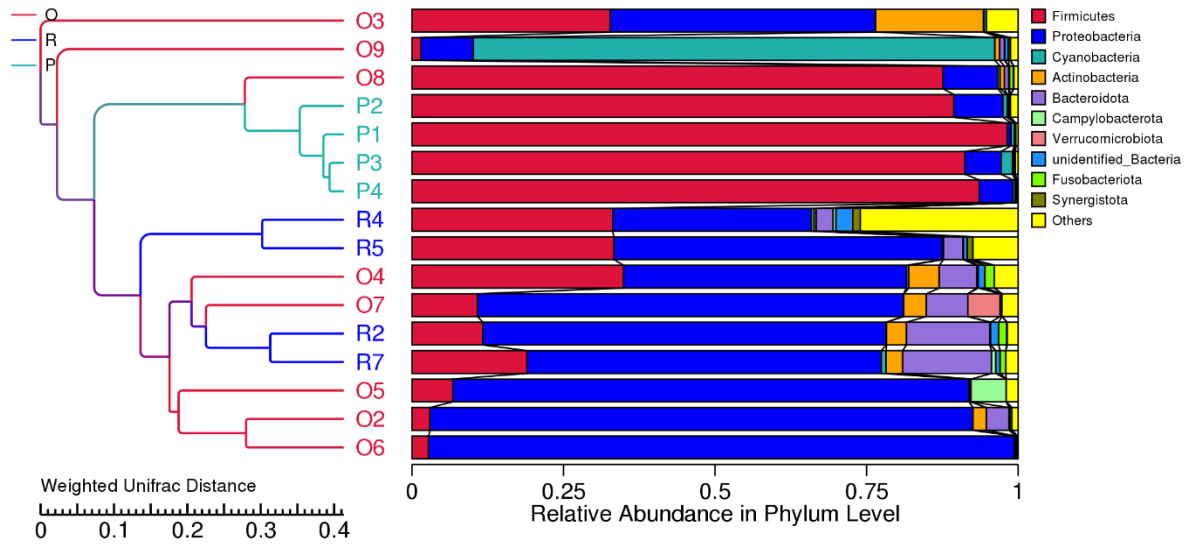
Fig. 19. UPGMA cluster tree showing the similarity among different samples in second sampling.

6. DISCUSSION

In general, many scientists and conservation biologists agree that the world is facing another mass extinction (CEBALLOS et al., 2015). Such an event can be caused by a combination of factors and are also partly caused, and definitely accelerated by human activities. To counter these events, numerous actions must be taken to help conserve the affected species and habitats. One of the common measures is the reintroduction, introduction, and supplementation of captively bred species, which has lately become a central tool in wildlife conservation (CONDE et al., 2011; CRATES et al., 2023). The significance of captive breeding for releasing purposes is raised further by IUCN, which recommends it as the most significant conservation tool for more than 2000 endangered species (IUCN conservation planning specialist group, 2020). Still, despite its emphasised significance, it should also be noted that captive breeding requires a lot of resources and labour investment. Such investment includes the building and maintenance of facilities, establishing a maternal group of adults, and costs associated with feeding, veterinary care and husbandry. This means that potential investors often look for successful post-release survival of animals within effective conservation programs (GERBER, 2016; CRATES et al., 2023). Older studies indicate that the post-release survival of captive breed animals is far lower than wild ones (KLEIMAN, 1989; SNYDER et al., 1996). In the majority of cases, this remains accurate and means that there are still many issues to be addressed in order to improve the survival rate post-release. Another potential problem is that the reintroduction of captive animals can have negative effects on the resident population, primarily through the introduction of various pathogens (PETERS et al., 2014). Therefore, the process of captive breeding must ensure that birds are healthy, of good genetic characteristics (representing the same subspecies) and well prepared for survival in the wild pre-release.

While genetics and potential health problems are beyond the scope of this study, our research will focus primarily on the phenotypic characteristics that are necessary for the survival of released animals. CRATES et al. (2023) describe captive phenotypic characteristics within three groups, behaviour, morphology, and health, which are observed in captive animals and differ from those in free-living animals. The morphology and partly health status groups of characteristics will be further described in this study. Morphological characteristics include the size of certain body parts and the development of skeletal muscles and organs. Among other observed modifications (from reptiles to mammals), not only shorter legs and wings were observed in captive birds (CAR and ZANN, 1986; DOMÍNGUEZ et al., 2010), but also flight

feathers (STOJANOVIC et al. 2021). Furthermore, among many other factors (i.e. cranium and teeth characteristics, bite force, etc) modifications of a gastrointestinal system such as intestinal length or villi characteristics were also observed (MOORE and BATTLEY, 2006). Even though the impact of these modifications on post-release survival is still unclear and debated, it is appropriate to assume that they may reduce the survival and migratory characteristics of released animals (DAVIS et al., 2020).

The health status group of characteristics is divided into several areas such as stress, diseases, parasites, the condition of the gastrointestinal system and physical ability (CRATES et al., 2023). MUELLER and SACHS (2015) emphasise gut microbiome as an important part of gastrointestinal characteristics. It can also be said that since gut microbiome largely depends on diet, animals with a diet that is similar to the one available in the wild are better prepared, and are more likely to adapt to free-living conditions (YANG et al., 2020). This was previously confirmed by WHITESIDE et al. (2015) who improved the post-release survival of pheasant chicks through feeding a more natural diet prior to release. One of the characteristics (physical abilities) that is classified under the health status group would, according to our opinion, fit more into the morphology group. An example of this includes less-developed flight muscles that preventing released animals from fleeing from predators or flying over long distances.

All previously mentioned implies that gut microbiome is largely influenced by diet and has an impact on the efficiency of the digestive system and overall fitness of the animal. This is of special interest in highly specialised species such as capercaillie. In this study, a comparison between the faecal microbiome of captive capercaillie (with further division of adult males, adult females, and juveniles) and wild capercaillie from two dietary different habitats (Lička Plješivica Mountain characterized by premontane beech forests, and Northern Velebit characterized by more coniferous forest associations) was performed. In total 10 microbial phyla were detected in each part of the study. In first part, four phyla were represented across all samples in the highest abundance: Actinobacteriota, Proteobacteria, Bacteroidota and Firmicutes. It was observed that the microbial community was more diverse in the case of wild capercaillie from Lička Plješivica, and that most abundant were Firmicutes, followed by Proteobacteria. In the case of captive animals, more abundant were Proteobacteria, followed by Firmicutes. Other more represented phyla included Cyanobacteria and Actinobacteriota. As expected, in the second part of the study the samples from captive animals revealed a similar pattern as in the first study, while samples from wild animals from Northern Velebit Mountain contained mostly Proteobacteria, followed by Firmicutes and Actinobacteriota. The most

dominant phyla in juvenile birds was Firmicutes. Again, community richness was higher in the case of the samples from wild birds. Similarly, RASMUSSEN and CHUA (2023) collected and analysed samples from captive (1) and wild capercaillie (7) from Norway and found greater richness of microbial communities in the samples from wild capercaillie. At the same time, the most abundant were Proteobacteria, followed by Firmicutes, Bacterioidota, etc. In total they recovered 11 phyla, and a group characterised as others (RASMUSSEN and CHUA, 2023). GAO et al. (2023) made a comparison between the faecal microbiome of five wild black-billed capercaillie (*Tetrao parvirostris*) flocks and found that at the phylum level the most abundant were Camplyobacterota, Bacillota, Cyanobacteria, Actinomycetota, and Bacteroidota.

Firmicutes (syn. Bacillota) is a phylum that is commonly found in birds and includes mainly gram-positive bacteria, among which are classes like *Bacilli*, *Clostridia* and *Mollicutes* (GROND et al., 2018). Even though each of the detected phyla are quite diverse and contain several classes, meaning that it is not easy to describe and understand its function in wild birds, Firmicutes are generally linked with digestion and absorption of proteins and are relatively abundant in insectivorous and omnivorous birds (BERRY, 2016; YAN et al., 2022). In the studies on human gastrointestinal microbiome, TOMOVA et al. (2019) found that Firmicutes are more present in the case of diets with lots of protein and fat rich food. This is in accordance with our finding that Firmicutes are predominant phylum in juvenile birds, as it is known that young animals feed predominantly on food of animal origin, in particular insects and their larvae. Additionally, a higher abundance of Firmicutes in one captive adult bird may indicate its frequent presence on ant nests provided in the aviary. Some members of this phyla also play a key role in the degradation of fibre into fatty acids (FLINT et al., 2008), which explains their relatively high abundance in adult birds during the winter period. Along with its positive aspects, Firmicutes also contain some pathogenic bacteria (BENSKIN et al., 2009), among which *Clostridium botulinum* and *C. perfringens* should be emphasized since the latter is associated with necrotizing enteritis in captive capercaillie (STUVE et al., 1992).

Unlike mammals, birds have higher relative abundance of Proteobacteria in their guts. Differently to Firmicutes, Proteobacteria are mainly composed of gram-negative bacteria and contain five large classes, which gives them a large spectrum of potential functions.

Actinobacteria are also one of the most abundant phyla in birds. They play a role in the stability of the GI tract (BINDA et al., 2018) and have been used as probiotics in livestock and humans (KAILASAPATHY and CHIN, 2000). Another important function is their role in the digestion of cellulose and chitin.

Bacteroidetes are important for their role in breaking down cellulose and in the digestion of carbohydrates and polysaccharides, which are otherwise indigestible by the host (THOMAS et al., 2011). It is also known that Bacteroidetes inhibit the colonization of pathogens, and that their metabolites interact with the immune system and contribute to the inactivation of pathogens (NKOSI et al., 2022). Contrary to Firmicutes, TOMOVA et al. (2019) found that Bacteroidetes are more frequent in the GI system of individuals feeding predominantly on more plant-based food.

As previously mentioned, the roles of these bacteria in wild birds remain understudied. This research shows that Firmicutes play a major role in the intestinal flora of juvenile birds. This is understandable since in this age category the diet consists of 70% of animal proteins, and 30% of plants. As the birds age their diet will change to incorporate a larger percentage of material of plant origin. Accordingly, adult animals have a higher ratio of Proteobacteria in their intestinal flora. The results of our study show a greater richness in the microbial community of wild birds, which is the result of the variety of available food. This is in accordance with SMITH (2019) who previously reported a lower microbial diversity in captive animals. Additionally, the detected phyla were more similar between captive and Northern Velebit capercaillie, due to the similarities in their diets.

Overall, despite the fact that post-release survival rates are influenced by a range of factors, it is clear that the microbiome plays a very important role in the fitness and adaptation of released birds. This means that designing a method to adjust gut microbiota according to a specific habitat should have a positive impact on survival of released animals (SMITH, 2019).

7. CONCLUSIONS

- ➢ Ten different microbial phyla were detected in capercaillie faeces.
- ➢ The most abundant phyla were Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes.
- ➢ The richness and diversity of microbial species was higher in the samples from wild capercaillie.
- ➢ Juvenile birds had higher proportions of Firmicutes, indicating more animal based nutrition, whilst adults had more Proteobacteria and Bacteroidetes, indicating a more plant-oriented diet.
- ➢ The results from the captive capercaillie samples were more similar to the wild capercaillie samples from Northern Velebit, indicating a similar nutritional potential of both mentioned habitats.
- ➢ Similar findings to our study were obtained by RASMUSSEN and CHUA (2023) who found 11 microbial phyla, in which Proteobacteria were the most prominent.
- ➢ The composition of the gut microbial community is one of the factors that can influence a better adaptation to a new habitat and therefore potentially increase the post-release survival rate of animals.

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9. SUMMARY

Diversity of fecal microbiota of endangered Western capercaillie (*Tetrao urogallus*)

Charlotte Francesca Stiles

Western capercaillie (*Tetrao urogallus*) are a large galliform species that inhabits mostly coniferous forests at altitudes above 1000 m.a.s.l. Over the last 60 years, a sharp decline in capercaillie populations was observed in many of central European habitats, especially at the peripheral range of the species distribution. Accordingly, recent monitoring data in Croatia indicate a 77% decrease in lek numbers. Measures aimed at recovery and restoration of the capercaillie population are complex and include a variety of factors such as habitat modelling, pest control, and the potential reintroduction or supplementation of captive bred animals. The breeding of animals in captivity may result in behavioural, morphological and health differences that may reduce post-release survival rates. The aim of this study was to analyse the faecal microbiome of captive birds from the aviary in the Gorski Kotar region (which has a more coniferous forest) with that from wild birds in Lička Plješivica (premontane beech forests) and Northern Velebit Mountain (more coniferous forests). Samples were collected from known animals in the aviary and opportunistically from wild animals, kept cold using ice packs and transported to the Veterinary Faculty. The DNA of the microbiota was isolated using a commercial kit, purified and then sent for sequencing and bioinformatic analysis to an external service. In total, 11 microbial phyla were detected with Firmicutes, Proteobacteria, Actynobacteria and Bacteroidetes being the most abundant. Juvenile animals had higher proportions of Firmicutes indicating a higher ratio of animal protein in the diet. With increasing age, proportions of Proteobacteria and Bacteroidetes increased, because of the shift to a more plant oriented diet. The richness and diversity of the microbiome was higher in samples from wild animals. The gut microbiome of captive animals was more similar to that of wild birds from Northern Velebit, indicating similar dietary conditions. By adjusting the gut microbiota according to a specific habitat, post-release survival rates and the efficiency of conservation programs should improve.

Key words: capercaillie, captive birds, wild birds, gut microbiome, habitat conditions

10. Sažetak

Raznolikost mikrobiote izmeta ugroženog tetrijeba gluhana (*Tetrao urogallus*)

Charlotte Francesca Stiles

Tetrijeb gluhan (*Tetrao urogallus*) je velika ptica iz porodice kokoški koja nastanjuje pretežito crnogorične šume na nadmorskim visinama iznad 1000 m. Značajan pad brojnosti populacije tetrijeba zabilježen je tijekom proteklih 60 godina u većini staništa središnje Europe, a posebice na rubnim područjima rasprostiranja ove vrste. Slično, i noviji podatci praćenja ove vrste u Hrvatskoj ukazuju na 77%-tni pad brojnosti pjevališta. Mjere oporavka i obnove populacije tetrijeba su složene i uključuju brojne aktivnosti poput modeliranja staništa, kontrolu vrsta s negativnim utjecajem na tetrijeba te potencijalnu reintrodukciju/popunu populacije pticama iz umjetnog uzgoja. Uzgoj u zatočeništvu može mijenjati biheviorističe, morfološke i zdravstvene značajke jedinki te dovesti do njihovog smanjenog preživljenja po ispuštanju. Cilj ovoga istraživanja bio je analizirati mikrobiom izmeta ptica iz volijere na području Gorskog kotara (pretežito crnogorične sastojine) s mikrobiomom divljih jedinki s područja Ličke Plješivice (pretplaninska šuma bukve) i Sjevernog Velebita (crnogorične šume). Uzorci su prikupljeni od poznatih jedinki u volijeri i oportunistički od divljih jedinki u prirodi, zamrznuti na -20oC i prevezeni na Veterinarski fakultet. DNK mikrobiote izolirana je komercijalnim kitom, pročišćena i poslana na sekvenciranje i bioinformatičku analizu u komercijalni servis. Utvrđeno je ukupno 11 skupina, pri čemu su najzastupljenije bile Firmicutes, Proteobacteria, Actynobacteria i Bacteroidetes. Mlade ptice imale su veći udio skupine Firmicutes što ukazuje na pretežitu prehranu hranom životinjskog podrijetla. Odrastanjem jedinki povećavaju se i udjeli skupina Proteobacteria i Bacteroidetes. Broj vrsta i raznolikost mikrobioma bila je veća u uzorcima od divljih tetrijeba. Mikrobiom crijeva tetrijeba iz uzgoja usporedljiviji je s divljim jedinkama sa sjevernog Velebita što ukazuje na slične prehrambene uvjete u tim staništima. Prilagodba crijevne mikrobiote specifičnom staništu trebala bi povećati preživljavanje jedinki nakon ispuštanja, kao i učinkovitost programa očuvanja.

Ključne riječi: tetrijeb gluhan, ptice iz umjetnog uzgoja, divlje ptice, mikrobiom crijeva, uvjeti staništa

11. BIOGRAPHY

I, Charlotte Francesca Stiles, was born on the 14th of May 1998 in Warwickshire, England. I attended the village primary school when I was five and then when I was eleven, moved to the village high school. When I was sixteen, on completion of my GCSEs, I moved schools to complete my A-levels at Stratford Girls' Grammar School for 2 years.

After I finished my A-levels, I worked for 6 months then completed a FGASA-accredited field guide course for 6 months in South Africa before beginning my studies in Zagreb.

In 2019, alongside my studies I took part in the Plavi Project and produced a poster for coastal tourist agencies to promote 'dolphin friendly behaviour'. During my studies I also volunteered in the University's Equine Team, in the Clinic for Obstetrics and Reproduction, and within Wildrescue Vef.

During the summer of 2021 I completed an erasmus externship at the equine hospital of the university of Liege for 2 months, where I admitted patients, administered therapies and followed cases of internal medicine, surgery and infectious diseases.

During the Day of Student Science 2021 I gave a presentation entitled 'Involvement in wildlife science as a student'.

I have been a member of the editorial board of the scientific journal 'Veterinar' for the past 4 years and as a Handling Editor am responsible for the checking of articles written in English.

During the past couple of years I have taken part in and contributed academically to wildlife research activities within the faculty within the Department of Epidemiology and Economics. I have assisted in the preparation of anti-parasitic medication for deer and performed necropsies to assess for its effectiveness.

I published the case report 'Severe pulmonary fascioloidosis in a wild Mouflon (Ovis musimon)' in 2021, in the journal Helminthologia. I presented an abstract of my case report 'Severe case of contagious ecthyma in balkan chamois (Rupicapra rupicapra balkanica) at the III Internal Rupicapra Symposium in 2021.

I attended the Eastern European Regional Veterinary Conference in Ljubljana in May 2022.

In June 2022 I completed a CEEPUS scholarship placementat the VetMedUni Vienna within the Research Institute for Wildlife Ecology. My placement lasted one month during which I assisted in necropsies of wild animals and animals from the zoo, attended lectures and seminars relating to projects, completed a wildlife immobilisation course, wrote an academic paper about wild boar anaesthesia and assisted with general husbandry activities of the animals housed there.

I then completed another externship at WildCom Research Group (Autonomous University of Barcelona) for 6 weeks where I took part in necropsies, fieldwork, lab work, seminar presentations, sample collecting, working at a government wildlife rehabilitation centre, writing up of research and participating in discussions relating to projects working with wildlife such as birds, amphibians, reptiles and mammals.

I was a member of the organising committee for the 10th International Deer Biology Congress (September 2022) where I was responsible for proof-reading and editing the book of abstracts and assisted with the organisation of the congress.

In November 2022 I won the Dean's Award.

In 2023 I published an article in the journal VetRecordCareers about my experiences working with wildlife as a student entitled 'Lure of the wild leads student to combine conservation with veterinary science'.

From March 2022 to March 2023 I am taking part in the Wildlife Disease Association/European Association of Zoo and Wildlife Veterinarian and Mentoring Programme 2022/2023 under the mentorship of Professor Ignacio Sanches (Wildcom Research Group, Autonomous University of Barcelona).

From April to June 2023 I completed an internship at a small animal clinic in Germany undertaking tasks such as assisting in surgery, monitoring anaesthesia during surgery, preparing animals for surgery, administering therapies, assisting with diagnostic imaging, general husbandry tasks, admitting patients, performing clinical examinations and helping clinicians on duty as needed.