

Breeding biology of ring ouzel (*Turdus torquatus*) in the West Carpathians, Slovakia

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Abstract. Reproduction is an important life history trait, affecting both parental fitness and population persistence. To contribute to knowledge of breeding ecology of birds living in high mountain ecosystems we monitored reproductive periods of ring ouzel (*Turdus torquatus*) in northern Slovakia. Between May 1985 and July 1989 essential characteristics of breeding biology of this species were recorded. Field data on 40 nests from the West Carpathians were obtained. In 38 complete clutches we observed clutch sizes of three (n = 3), four (n = 24), five (n = 10) and six (n = 1) eggs. The total success rate was recalculated using 34 nests. Out of 146 eggs laid 116 young hatched (79.5 %) and 52 young fledged (35.6 %). We assume that our research caused some increased predation, so if we eliminate (statistically) this impact then the revised numbers are: 115 eggs laid, 90 young hatched (78.3 %) and 63 young fledged (54.8 %). Length of incubation was between 13 and 15 days (n = 14 nests), with an average of 14 days. The main incubation period ran from May 10th-19th. Breeding care for young in the nest lasted between 11 and 15 days, with an average of 13 days (n = 13). Shorter length of breeding care in the nest (11 days) was a result of external disturbances, mainly by raptors. Fledging largely occurred between May 21st and June 9th.

Key words: ring ouzel, *Turdus torquatus*, breeding biology, the West Carpathians

Introduction

Mountain and high mountain ecosystems are facing increasing threat. Contrary to the lowlands and forest avifauna, sufficient knowledge on the basic ecology of alpine and sub-alpine bird species is still lacking, in both Slovakia as well as the balance of Europe (Lehikonen *et al.* 2019). This is especially true for species that live particularly remote lives outside of

human interest. The supramontaneous species, ring ouzel, has been shot in the past for its distinctive black and white coloration (Čaputa *et al.* 1982). This species is generally overlooked by European Union (EU) member states because is not listed on Annex I of the Birds Directive. However, as a migratory species it should be subject to the same conservation measures as Annex I species, under Article 4.2 of the Birds Directive. The EU holds around 60 % of the global population of ring ouzel, and the Carpathians are the highest-density region in the world for this species. After the retreat of the last glaciers, habitat for this species has been reduced to northern Europe and subalpine mountain regions of Europe. We rank the species among typical glacial relics. Subspecies *T. torquatus alpestris* breeds in montane areas from Spain east to Romania, with high densities in the Pyrenees, Massif Central, Alps, Balkan, Greece, Asia Minor and the Carpathians (del Hoyo *et al.* 2005). In Eastern Europe, birds of this species largely winter at lower elevations south of their breeding range (Hagemeijer and Blair 1997). Migration to overwintering grounds in southern Spain and North Africa occurs mostly in September, October and November (White 1907; Glutz von Blotzheim and Bauer 1988; Busche 1993). The ring ouzel is also a good example of a subalpine species for which information on small-scale ecological requirements is still largely lacking across its breeding range (Bocheński 1968; Burfield 2002; D'Amicis 2002; Holupírek 2004; Beale *et al.* 2006; Ciach and Mrowiec 2013; Bacht *et al.* 2013; Sim *et al.* 2013a, 2015). Breeding areas for British birds are typically comprised of steep slopes with crags, gullies, scree or boulders, as well as more gently sloping or flat areas, and are often dominated by heather, pasture or bracken (Poxton 1986, 1987; Cramp 1988). In Fennoscandia, *T. torquatus torquatus* breeds in similar open areas on fells above pine (*Pinus*) forests, but also nests on the edge of birch (*Betula*) or spruce (*Picea*) stands (Bannerman 1954). In contrast, *T. torquatus alpestris* habitually breeds in open conifer woods close to moist, grassy areas at altitudes of 600-2000 m a.s.l., favouring spruce, fir (*Abies*) and occasionally beech (*Fagus*) forests. The main range of the ring ouzel in the Alps or Caucasus is similar to the upper limits of the coniferous forest and the mountain pine and *Rhododendron* sp. zone (Demetiev and Gladkov 1954; Glutz von Blotzheim 1964). In the northern Carpathians this species is found between 500 and 1700 m a.s.l. (Bocheński 1960; Głowaciński and Profus 1992; Janiga 1992; Janiga and Poxton 1997; Tomiałojć and Stawarczyk 2003).

The species is usually single-brooded in the Carpathian area, and we assume that this is the most common reproductive strategy in its other central and eastern European mountain habitats (Walter 1995). Nests are built in trees, mostly in larches close to the trunk, at a height from 1.5 to 20 m (Janiga and Višňovská 2004). Females take care of nest building and brooding, with very few exceptions of male assistance in these tasks. Most birds leave the study area immediately after reproduction, (i.e., before the end of June), spending the post-breeding period at higher elevations (Glutz von Blotzheim and Bauer 1988). Because the Carpathians are host to the most plentiful populations of ouzels, information on the central European population is essential for preservation of this species.

Moreover, due to climate change, a better understanding of the ecology of ring ouzel is a priority. For example, British nest records show that the species currently lays its first clutch approximately eight days earlier than in 1968 (Baillie *et al.* 2005), suggesting that climate change has already affected breeding life of ouzels. During breeding season, adults feed nestlings almost exclusively on invertebrates, particularly earthworms (*Lumbricidae*) (Burfield 2002). *Lumbricidae* availability is largely determined by rainfall and temperature (Askew and Yalden 1985), and thus temperature and rainfall during breeding are determinants of nest survival. In Britain and Iceland, the decline of the species has continued, with perhaps as much as a 58 % decline occurring between 1988 and 1999 (Wotton *et al.* 2002; Buchanan *et al.* 2003; Burfield and Brooke 2005; Sim *et al.* 2010; Mee 2018). In parts of the Scotland the local population remains stable (Rebecca 2001). In Switzerland, the species decreased by 36 % between 1990 – 2018 (Barras *et al.* 2020). Climatic factors have also an indirect impact on the occurrence of the ring ouzel (Beale *et al.* 2006; von dem Bussche *et al.* 2008). Withdrawal of the ring ouzel into higher habitats under the influence of changing rainfall and temperature is predicted in many regions (Gubitz and Spath 2002; von dem Bussche *et al.* 2008). Changes are also probable in terms of population size in the Carpathians region, where an altitudinal shift may occur in the subalpine zone. The aim of this study is to contribute to knowledge on the breeding biology of the ring ouzel, and this historical data also helps to establish a baseline against which current changes should be monitored to determine population trends of the species.

Material and Methods

The area around Veľký Brankov, in the Low Tatra National Park, Slovakia (May 1985 to July 1989)

was selected for this study. We recorded essential characteristics of breeding biology of the species, including: nest locations, nest structure, number of eggs laid, number of young hatched, success and length of incubation, number of nestlings and fledglings, and success rate of breeding. Eggs and nestlings were marked with a felt-tipped pen and older nestlings were ringed with a combination of colored plastic rings, so that each individual was identifiable. Field data on 35 nests from Brankov was collected. Additionally, information from one nest in Choč Hills (1989) and four nests from the West Tatra mountains (1998-1999) was also used (Table 1). The survey of the nests took place in 3 - 4 day intervals. After finding the nests, they were regularly visited during the incubation period in 2 - 3 day intervals.

The real numbers of nests (or number of cases) in the final evaluation of individual statistical characteristics (in the chapter – Results) can differ, because not all nests were equally accessible for gathering all of the characteristics. Following the initial discovery of the nests, basic measurements were carried out. For additional details see Janiga and Višňovská (2004) or Schirutschke (2005). Incubation was regularly watched from a prescribed distance from the nest, (i.e., whether the female was actively brooding or not). The date and order of hatching were determined (hatching scheme) either during hatching afterward based on developmental characteristics of individual birds (Janiga 1992). The total number of eggs in a clutch, the number of chicks hatched from eggs that were laid, and the number of young that flew out of the nest were all recorded. Losses of eggs, losses of chicks and total losses during nesting, as well as the causes of these losses, were determined. During this research, a researcher inadvertently negatively affected the nesting process in several nests, and this problem is discussed under Results. For chicks that successfully left the nest, this date was recorded, as well as the time period of time chicks remained in the nest from hatching to leaving the nest.

During recording of nest care, the activity of parents in defence of the nest, (i.e., the aggressiveness of the nesting pair), was also ascertained. We distinguished four categories of this behaviour:

- 1 – Very aggressive individual; attacks a researcher while defending the nest.
- 2 – Aggressive sounds when in close proximity to researcher, but does not attack.
- 3 – Louds sounds from a distance and/or “whistle” warnings.
- 4 – Flies away and observes the nest from afar, or is not present.

Using this scale, the degree of aggression of parents during the incubation period and during the care of the chicks was determined, as well as the ratio of

	1985	1986	1987	1988	1989	1998	1999	Total
Brankov – Low Tatra mountains	2	6	7	8	12	0	0	35
West Tatra mountains	0	0	0	0	0	1	3	4
Choč Hills	0	0	0	0	1	0	0	1
Number of nests	2	6	7	8	13	1	3	40

Table 1. Summary of the number of examined nests of ring ouzel.

the aggressiveness of the female and the male. After fledglings left the nest, some of the nests were taken for analysis of nesting material and some for analysis of parasites that live in nests. The parasites were extracted in photoelectors at the Zoological Institute of the Faculty of Science, Comenius University. Larvae and images of bird fleas were determined (Dr. Cyprich, Dr. Krumpál).

Results

161 eggs were found in 38 nests. Ring ouzels laid three to six eggs per clutch, but clutches of four were the most frequent (Fig. 1). The average number of eggs in a complete clutch was 4.3. ($s = \pm 0.6$).

In this study, the data is evaluated in two ways. In the first case, the real breeding success of ouzels is calculated, and in the second case, the increased breeding success is calculated that likely would have occurred without the influence of this research. In twelve nests, the birth rate was likely negatively affected in several ways by our presence near the nests. In seven cases, predators were inadvertently attracted to the nest as the breeding pair were defending the nest, or predators were attracted to the nest as a result of human presence (i.e., pine marten followed our footprints in the snow). Three nests were directly affected by humans, where parents abandoned their eggs (once), or abandoned hatchlings (twice), which subsequently died. In one case, a female was trapped in the mist net, and did not return to the nest the following day, resulting in the death of two young. The male stayed with the young near the nest, fed them, defended them and managed to preserve them. The remaining young flew out of the nest at 11 - 12 days after hatching, earlier than is commonly observed. This example shows how dangerous this collection process (netting, ringing and weighing) can be to a breeding pair during this sensitive nesting period.

While human-induced losses were minimal during incubation, when nest visits occurred with similar frequency, nests appear to be more vulnerable during this juvenile stage. Data on breeding biology is presented in Table 2.

Incubation success in ouzels was very high (Fig. 2); in the majority of nests chicks hatched from all laid eggs. 30 nests with nestlings were monitored until the young fledged (Fig. 3). The effect of field research on the mortality of nestlings was highly significant, the difference was approximately 20 % in the young production.

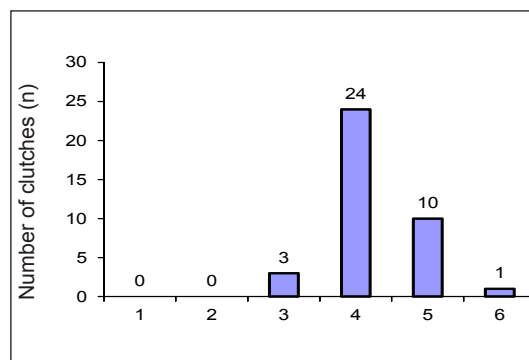


Fig. 1. Number of eggs in a complete clutch.

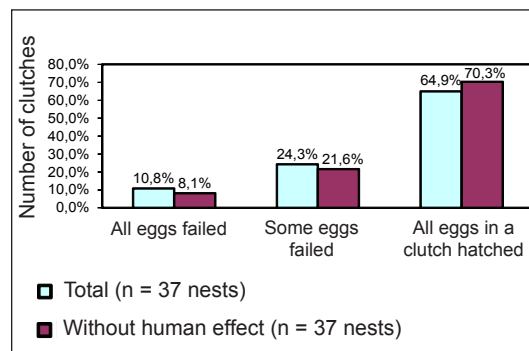


Fig. 2. Success rate of hatching in ring ouzels.

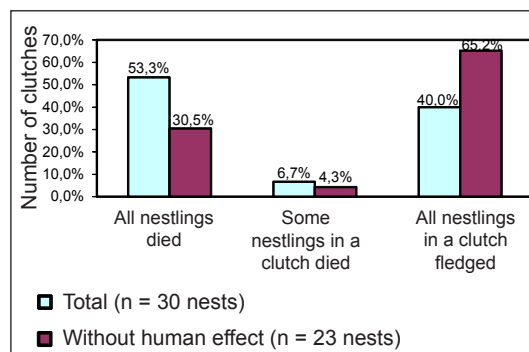


Fig. 3. Success rate of fledging in ring ouzel.

Total success rate – number of young fledged from number of eggs laid was recalculated from 34 nests (Fig. 4). Out of 146 eggs laid, 116 young hatched (79.5 %), and 52 young fledged (35.6 %). If we eliminate the impact of our research then the adjusted numbers are: 115 eggs laid, 90 young hatched (78.3

Variable	Variant	x (n)	S	min – max
Number of eggs in complete clutch	Total	4.2 (38)	0.62	3 – 6
	Without human effect	4.2 (38)	0.62	3 – 6
Number of hatchlings per clutch	Total	3.5 (37)	1.37	0 – 5
	Without human effect	3.6 (37)	1.24	0 – 5
Number of fledglings per clutch*	Total	1.7 (30)	1.93	0 – 5
	Without human effect	2.7 (23)	1.94	0 – 5

* at least one young successfully hatched

Table 2. Breeding biology characteristics of ring ouzel (x – mean; n – number of nests; S – standard deviation).

%), and 63 young fledged (54.8 %). In general, females must lay two eggs to rear one fledgling.

Losses caused by field research were described earlier. Other causes of failed eggs were mainly strong storms, snowstorms, deceased parents or abandonment (22 eggs or 68.7 % of failed eggs). Three eggs (9.4 % of failed eggs) were destroyed by a squirrel or bird of prey. Seven sterile eggs (21.9 % of failed eggs) were found in seven nests; four in four-egg nests, two in five-egg nests and one in a six-egg clutch. Mortality of nestlings was higher than mortality of eggs. Cold weather or abandonment of nestlings caused the death of 13 young in a nest (20.3 % of all dead nestlings). 51 chicks were killed by predators; nine nests were impacted by a bird of prey, two by a mustelid, and two by a squirrel.

The dates of the laying of the first egg are summarized in ten day intervals in Fig. 5. The average days to lay the first eggs, hatch the first nestlings and fledging of the first young are summarized in Table 3. It was found that 92 per cent of eggs were laid between April 22nd and May 10th.

The incubation period lasted between 13 and 15 days ($n = 14$ nests), and the average was 14 days. More detailed account of the hatching scheme of chicks is presented in Fig. 6. The primary period was between 10th and 19th May. The pattern of hatching in a given nest was very variable and often depended on the number of eggs in a clutch (Table 4). The chicks in the nest usually did not hatch at once, but at intervals of half a day to one day. Hatching asynchrony of two days was observed only twice (7.1 % of 28 exactly observed nests).

Breeding care for young in the nest lasted between 11 and 15 days, (13 days on average) in the 13 nests observed. We can assume that shorter length of breeding care (11 days) was a result of external disturbance – generally predation by raptors. The date of fledging was commonly between May 21st and June 9th, though in one nest the fledging of young occurred on May 7th (Table 3).

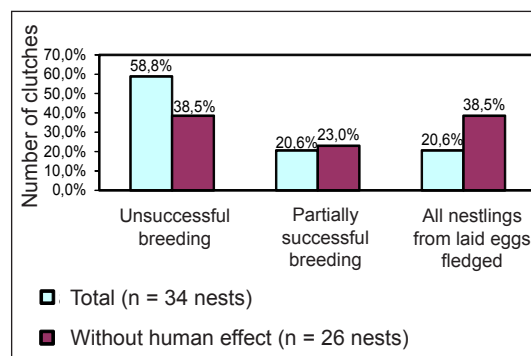


Fig. 4. Overall success rate of breeding of ring ouzel.

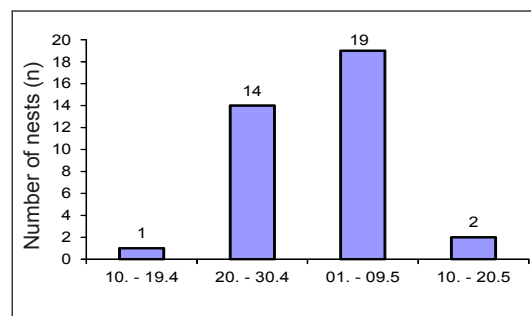


Fig. 5. The breeding season of the ring ouzel as shown by the number of first-egg laying dates in „third-month“ periods.

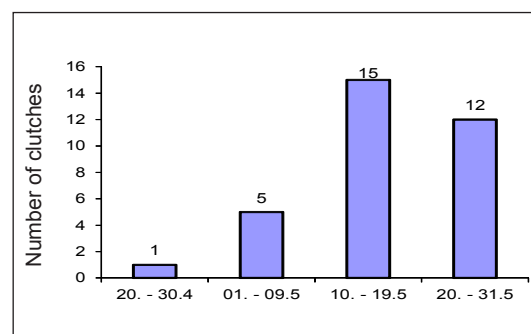


Fig. 6. Dates of chick hatching divided into decades of days in April and May.

Variable	x (n)	min - max	S
Date of the laying of the first egg	1 st – 2 nd May (36)	10 th April– 17 th May	7.31
Date of the first chick hatched	16 th May (33)	24 th April – 30 th May	7.13
Date of the first young fledged	28 th – 29 th May (13)	7 th May – 9 th June	8.21

Table 3. The date of laying, hatching and fledging of young (n – number of nests measured; S – standard deviation).

Nests with three young (5)		Nests with four young (18)		Nests with five young (5)	
Hatching asynchrony pattern	Number of cases	Hatching asynchrony pattern	Number of cases	Hatching asynchrony pattern	Number of cases
3 + 0	2	4 + 0	2	5 + 0	0
2 + 1	2	3 + 1	13	4 + 1	2
1 + 2	1	2 + 2	3	3 + 2	3

Table 4. Pattern of hatching asynchrony in ring ouzel nests. The eggs did not hatch all at once, in a given day, the first group of nestlings usually hatched (for example, three chicks in the pattern 3 + 1), followed by the second group of eggs, which hatched from 0.5 to 1 day after the first hatch (for example when one chick hatched later, the scheme is 3 + 1).

Despite being more itimid and elusive than other European thrushes, ring ouzels are highly territorial during breeding season, and often very actively defend the nesting area against both intra – and interspecific intruders. During incubation (23 nests observed), in 19 cases (82.6 %) the degree of aggression was the same in both sexes (whether aggressive or non-aggressive); a slightly higher to definitely higher rate of aggression was observed in females in three cases (13 %), and in one case (4.4 %), the male was more aggressive than the female in defence of the clutch. In nests with young ($n = 28$), the parent pair participated in the defense of the nest to the same extent in 20 cases (71.4 %). The female was more aggressive than male in three nests (10.7 %), and the male was more active in defense than female in five nests. In females ($n = 15$), in eight cases (53.3 %) the degree of aggression was the same during the incubation period and during the rearing of the young in the nest, (i.e., they behaved more or less the same during the entirety of the nesting period). Only one female (6.7 %) was more aggressive during incubation than during the care period for nestlings, but up to six females (40 %) showed increased aggression in nests with young. Males ($n = 15$) behaved in the same way in seven cases (46.7 %), throughout the nesting period. Similarly to females, only one male (6.7 %) showed an increased degree of aggression during incubation, but up to seven males (46.7 %) were more aggressive when there were young present in the nest.

In 1986, six nests were collected: three immediately after successfully fledging, two after predation of nestlings, and one in which the eggs failed. The presence of different groups of invertebrates was also examined. There were larvae and adults of Diptera, Coleoptera, Hymenoptera, Acarina, Heteroptera, Psocoptera, Colembola, Araneae, Chilopoda and Siphonaptera. Extreme numbers of flea larvae were found in three nests, in two from which young fledged (1200 and 28) and in one where the nestlings were killed by a predator (500 larvae and 2 imagos).

Discussion

Ecotones or environmental discontinuities with a median cover of forest, with open canopy cover consisting of spruce, stone-pine and larch, characterise the optimal habitat of ring ouzel in the Alps or Carpathian mountains. The altitudinal extent of the ring ouzel in the Carpathians is lower than that in the Alps. An increase in abundance of ouzels occurs between 900–1500 m a.s.l., in the subalpine vegetation zone. Nutrient-poor grasslands, forbs and copice of the high-elevation sites are additional characteristics of preferred habitats. The proximity of clearcuts and young tree stands may also create a landscape, temporarily suitable for the occurrence of ouzels, especially in lower mountain locations (Marisova and Vladyshevskii 1961; Oberwalder *et al.* 2002; Karaska 2002; Janiga and Višňovská 2004; Bashta 2005; Kajtoch 2011). However, nutrient-rich valley grassland and farmlands may have a negative effect on ring ouzel existence, as do large amounts of debris and settlements. Climate change has also

proven to be an important indicator of ring ouzel occurrence (Beale *et al.* 2006; von dem Bussche *et al.* 2008), predicting the most suitable ring ouzel habitats in the subalpine temperature range. Snowpack plays a crucial role for the occurrence of birds in spring, when its melt provides much of the water supply in subalpine and alpine ecosystems (Resano – Mayor *et al.* 2019). The water supply resulting from snow highly influences soil moisture and penetrability during breeding season, as well as increases ouzels' access to their primary foodsource – earthworms (Marisova and Vlayshevskii 1961; Glutz von Blotzheim and Bauer 1988; Tyller and Green 1994; Burfield 2002). Earthworms are more active in the upper ground layers and hence more accessible when soil is humid and soft, (i.e., in the mountains during or shortly after rainfall or during the snowmelt period). Since they are considered climate-sensitive invertebrates, ouzels that rely on them as a food source are significantly vulnerable in the face of climate change (von dem Bussche *et al.* 2008). The positive effect that wet and penetrable soils have on the availability of earthworms is thus key for ouzels and some other species of birds in alpine ecosystems (Resano-Mayor *et al.* 2019, Barras *et al.* 2020). When ouzels arrive at the alpine breeding grounds in April, their habitats are still characterized by a dense snowpack. Birds perform daily elevational movements, and overnight in breeding sites, but during the rest of the day, they visit snow-free meadows at lower elevations to forage. An earlier spring snowmelt can accentuate the risk of a phenological mismatch for migratory ouzels, with negative consequences for their population dynamics (Barras *et al.* 2021a, b). Beale *et al.* (2006), suggest that once the trend in climatic variables is taken into account, the ring ouzel territory occupancy will decline by an average of 3 - 6 % per year, as a direct consequence of the recent trend towards warmer summers. In the mid and western European mountains, Alpine ring ouzels have shown a negative population trend since 1990, with a local retreat to higher altitudes (Kronshage 2003). Withdrawal of the ring ouzel to higher ground under the influence of climate change is predicted in the Alps (von dem Bussche *et al.* 2008) and changes are also probable in the distribution of this species in the Carpathians, where an altitudinal shifting of the extent of the lower subalpine zone may occur (Ciach and Mrowiec 2013).

Laying dates for ouzels from the Carpathians or Alps are highly dependent on snowcover and snowmelt. Birds begin breeding at one year of age, and all the evidence points toward a monogamous mating system. Within our research area, 92 per cent of eggs were laid between April 22nd and May 10th. Thus, ouzels reproduce particularly early compared to other sympatric mountain bird species (Barras *et al.* 2020, 2021a). In the Carpathians, females began breeding earlier in the season than in Fennoscandia (Pulliainen *et al.* 1981, Hudec *et al.* 1983). Although there is evidence for the occurrence of second broods in the mid and western European mountains (Walter 1995; Gubitz and Spath 2002), ouzels are generally single-brooded. In the Alps, Carpathians or Fennoscandia, breeding conditions are only suitable for a short time (Barras *et al.* 2020). In some cases, second clutches are in fact substitute lay-

ings. For example, at our locality, one female laid the first egg and then was disturbed by a squirrel. The bird was subsequently located in a nearby vacant Song Thrush nest, and laid her next eggs in this new nest. An additional substitute clutch was found in mid-May. However, if breeding conditions remain suitable for longer periods, it is possible that the species may become multi-brooded. In Great Britain, for example, 62 % of females made second breeding attempts. These females usually often laid their first eggs in April, earlier than the mean laying date for all ouzels. Triple-brooded females likely begin breeding the earliest in the season, and have shorter inter-brood intervals than other females (Sim *et al.* 2012, 2013a). The extent of double-brooding is unclear, but likely varies with latitude throughout Britain (Cramp 1988). Tyller and Green (1994) summarized the data from nest records for Wales, Scotland and England, where the laying peak occurred during the first 10 days of May; slightly later than described by Flegg and Glue (1975), and continued until early July. In England and Scotland there was no clear relationship between altitude and laying date, while in Wales, laying was delayed by four days on average for every 100 m in altitude gain. Circumstantial evidence that the extent of double-brooding in ouzels is associated with rainfall, was provided by Appleyard (1994). The number of second clutches was highly influenced by rainfall in June. In the Alps and Carpathians, ouzel are mainly single brooders, and we assume that effective natality is compensated by larger clutch size than in the British Isles (Table 5).

The female alone builds an open-cup nest, lays one egg per day, and performs most, if not all, incu-

bation and brooding (Marisova and Vladyshevskii 1961; Korodi Gál 1970; Durman 1977). However, there is rare evidence of male participation in incubation (Flegg and Glue 1975), usually at night (Gubitz and Spath 2002). The average complete clutch size from the Carpathians and Alps is between 4.24 to 4.6 eggs, and is larger than in Britain (3.7 to 4.2 eggs) (Table 5). Ring ouzel clutches in Welsh and Scottish/English samples are generally similar, and clutches of four predominate (Tyller and Green 1994; Burfield 2002). Durman (1977) and Poxton (1986) recorded more clutches of five eggs in Great Britain during wet years, due to an abundant supply of earthworms for adults during the laying period. Many such clutches were also found in Northumberland (Galloway and Meek 1984). Occasionally, clutches of six were recorded (Flegg and Glue 1975).

During incubation, adult ouzels often fly long distances from the nest to feed. However following the hatch at 12-14 days of incubation, it is advantageous for parents to have a good food source nearby, as both parents provision their nestlings until they fledge at 11-15 days of age (Marisova and Vladyshevskii 1961). During nesting, ouzels largely forage in very short grass swards, with 90 % of the selected foraging sites offering ground vegetation shorter than 10 cm. In the mid-European mountains, grass usually exceeds this height in the first half of June. Because ring ouzel use the brief window between snowmelt and the growth of ground vegetation for their breeding period, they elect to forage at sites with predominantly short grass (Barras *et al.* 2020). This strong selection preference for short grass indicates that prey accessibility may be the

Sub-sp.	Country	n	Clutch size (%)					x	References
			2 eggs	3 eggs	4 eggs	5 eggs	6 eggs		
<i>torq.</i>	England	79		10 (12.7 %)	56 (70.9 %)	12 (15.2 %)	1 (1.3 %)	4.05	Flegg and Glue (1975)
<i>torq.</i>	Scotland	38		1 (2.6 %)	30 (78.9 %)	7 (18.4 %)	0	4.16	Poxton (1986)
<i>torq.</i>	Scotland	99		(7-15 %)	(76-88 %)	(4-11 %)	0	3.75- 4.17	Burfield (2002)
<i>torq.</i>	Scotland	75		7 (9.3 %)	60 (80 %)	8 (10.7 %)	0	4.01	Arthur and White (2001)
<i>torq.</i>	Wales	89	2 (2.2 %)	13 (14.6 %)	63 (70.8 %)	11 (12.4 %)		3.93	Tyller and Green (1994)
<i>torq.</i>	Scotland, England	301	3 (0.9 %)	32 (10.6 %)	228 (75.7 %)	38 (12.6 %)		4.00	Tyler and Green (1994)
<i>alp.</i>	Swiss	92		8 (8.7 %)	38 (41.3 %)	43 (46.7 %)	3 (3.3 %)	4.45	Glutz von Blotzheim (1964)
<i>alp.</i>	Czecho-Slovakia	24		3 (12.5 %)	11 (45.8 %)	10 (41.7 %)	0	4.29	Hudec <i>et al.</i> (1983)
<i>alp.</i>	Slovakia	38		3 (7.9 %)	24 (63.2 %)	10 (26.3 %)	1 (2.6 %)	4.24	This study
<i>alp.</i>	Ukraine	17		0	mostly	mostly	rarely	?	Marisova and Vladyshevskii (1961)
<i>alp.</i>	Romania	39		0	12 (30.7 %)	27 (69.3 %)	0	4.6	Korodi Gál (1970)

Table 5. Clutch size of ring ouzels from different regions of Europe (n = number of nests examined, x average clutch size).

main cause drawing ouzels toward higher ground, as a result of the influence of climate change (as predicted in the Alps and Carpathians) (Beale *et al.* 2006; von dem Bussche *et al.* 2008). The transition to more dense vegetation at lower altitude sites formerly inhabited by ring ouzels, is followed by the shift of ring ouzel to sites at higher elevation, and the movement of blackbirds and fieldfares into the newly-suitable lower elevation habitats (Kronshage 2003; Janiga's personal observations).

Eggs may be at considerable risk during incubation. While losses during incubation are usually lower than losses among nestlings (Korodi Gál 1970; Durman 1977; this study), other studies suggest that more losses may occur during incubation (Poxton 1986). The main causes of loss are infertile or failed eggs, cold weather, egg predation (in our case by squirrels), and nest and parent predation by raptors and mammals (Poxton 1986; this study). Several studies have reported nest predation by fox, mustelids and corvids, in which case ouzels may disappear from the breeding area (Thompson *et al.* 1997). Losses of nestlings are usually as a result of an unspecified cause (Barras *et al.* 2021a), although wet weather (Flegg and Glue 1975), limited food (Barras *et al.* 2021a) or predation (this study) are the main causes of brood reduction. Our study showed that while ouzels are not common prey for some raptors, the conspicuous behaviour of ring ouzels may make them vulnerable to predation, and even limited predation may adversely affect their numbers. Repeated visitation of nests by humans may attract the attention of raptors during aggressive defence of nests by ouzels, and may reduce breeding success. We found that ring ouzels are highly territorial, and interactions between parents and intruders were often recorded (60 % of nests). From this point of view, potential disturbances must be minimized during ornithological research. The average number of chicks fledged per nest is usually between three and four (Tyller and Green 1994; Burfield 2002; Sim *et al.* 2010, 2012; Barras *et al.* 2021a), but in our case, only two young per nest successfully fledged. Poor reproductive success, (1.6 fledglings/nest) was also reported by Walter (1995) in the Alps. Thus, an important element in the protection of this single brooding species in the mid-European mountains is to reduce disturbance of breeding birds. Fledglings usually remain dependent on parents for a further 2-3 weeks (Cramp 1988). If the females re-nest, the male often provides most post-fledging care (Appleyard 1994). However, in the Alps and Carpathians, parents leave the breeding grounds to move towards higher elevations as soon as the brood have fledged, most probably to track suitable feeding grounds. At many study areas, food sources procured for fledglings included earthworms, tipulids, green caterpillars, coleopterans, spiders, dipterans, geometrid larvae, millipedes, and lizzards (Marisova and Vladyshevskii 1961; Korodi Gál 1970; Tyller and Green 1994). Blueberries may also be an important food source for the survival of juveniles, as the birds change their diet from invertebrates, (potentially highly invaded by helminths) (Sitko and Okulewicz 2010), to fruits. Blueberries and other fruits may flush and reduce the amount of helminths in the gastrointestinal tract of hosts (Sitko in verb). In the late summer,

when worms may be inaccessible, ring ouzels feed predominantly on fruit; notably blueberries, rowan, and yew berries (Marisova and Vladyshevskii 1961). This period is crucial for ouzels because most estimated first-year mortality occurs in this species in the first five weeks post-fledging (Sim *et al.* 2011). An ouzel juvenile's survival may depend on its mobility within its habitat in order to locate suitable habitat for foraging (Sim and Rebbeca 2003; Sim *et al.* 2007), remain concealed from predators (Sim *et al.* 2013b; this study), and avoid endo and ectoparasites (e.g., Sitko *et al.* 2006; Sitko and Okuliewicz 2010; Sitko 2011; Bush *et al.* 2018).

This research provides baseline data on breeding ecology of ring ouzel in Slovakian mountains. It would be interesting to compare this historic data to recent samples from the same locality to assess the impact of afforestation, climate change and the increase in tourism on breeding behaviors within this species. An important element in the protection of ouzels may be the maintenance of extensive forms of land-use which are conducive to the short, grassy vegetation responsible for generating a sufficient food source in mountain areas. Important context may also be offered through the comparison of potential differences between mountain and lowland populations, and the different land-use pressures experienced by both habitats.

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The influence of the environment on growth parameters of great tits (*Parus major*)

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Abstract. The data from previous studies on the ontogeny of *Parus major* were used in this study. Allometry of growth characters was compared at three independent sites and at different times. A total of 186 young were measured in three habitats: Low Tatras - an ecotone between a mountain meadow and forest community; High Tatras - forest habitat near the road; Šúr - a habitat of a wetland alder forest. In terms of allometric characteristics such as tarsometatarsus growth, wing skeleton (ulna/radius), and weight, it can be concluded that the best environment for development is the border of forest and meadow habitats (Low Tatras). The forest with a network of transport roads represents a lower quality environment. In waterlogged alder forests, the trajectories of juvenile development were different than in the mountain environment of spruce forests. Rather, they indicated more difficult development conditions for the nestlings, which suggests that the wetland alder forests are not the optimal habitat for the breeding of *P. major*.

Key words: *Parus major*, environment, trajectories of juvenile development

Introduction

The study of avian life history holds an important position in various areas of ecological research (Ye *et al.* 2021). The life history theory explains the general features of the life cycle, (i.e., lifespan, growth rate, number of offspring, and reproductive attempts), which are connected with constraining relationships among each other and specific environmental requirements (Wawrzyniak *et al.* 2020). Environmental conditions during early development stages can have long-term effects on an individual's life history (Lindström 1999; Monaghan 2008). In altricial birds, the nestling period is an especially sensitive time within the life cycle that strongly influences their further survival and general reproductive success (Langham 1972; Bryant 1978; Amiot *et al.* 2014). The studies of nestling develop-

ment and growth present the diversity of growth patterns among birds associated with their morphology, ecology, and behavior (Ricklefs 1968; Nilsson and Svensson 1996; Bize *et al.* 2006), parental effort (Gilby *et al.* 2011), as well as other life-history traits (Remeš and Martin 2002; Mainwaring *et al.* 2010). The body condition of birds is related to their survival, reproductive success, behavior, and evolutionary ecology, but also provides the knowledge necessary in wildlife management and conservation biology (e.g., Bustnes *et al.* 2002; Blums *et al.* 2002, 2005; Bachman and Widemo 1999). In studies with regard to body condition, morphological and physiological measurements replace direct measurements (Labocha and Hayes 2012). Quantitative measures of nestling growth and development are important for understanding avian breeding biology and reproductive strategies (Fernaz *et al.* 2012). Determining the exact age of nestlings is an important prerequisite for determining the nesting strategy, and helps identify the impact of environmental variables on body condition, growth, and reproductive success (Shaffer 2004; Jongsomjit *et al.* 2007).

The great tit (*Parus major*, L. 1758) is a widespread and common species across most of Europe, the Middle East, Central Asia, and east across the Palearctic to the Amur River. Their population in Europe accounts for less than half of its global range. Its European breeding population is extremely large (> 46,000,000 pairs), and was stable between 1970–1990. Although there were declines in a handful of countries during 1990–2000, populations were stable across the vast majority of Europe (BirdLife International 2021). The most common occurrence of the species is in open deciduous and mixed forests and edges and clearings in dense forests, including conifer forests and boreal taiga. It is also found more widely in plantations, hedgerows, orchards, parks, gardens, the edges of cultivation, and almost any group of trees or bushes (Gosler *et al.* 2013; Hinsley *et al.* 2008). It feeds on a wide variety of insects, especially caterpillars and larvae as well as spiders, seeds and fruit (Perrins 1991; Snow and Perrins 1998; Seki and Takato 1998).

The egg-laying period differs mainly among latitudes. In Europe, laying begins in March and April in southern lowlands, and in May in the north (Snow and Perrins 1998). The great tit is a typical cavity nester, breeding in a hole that is usually inside a tree, (although occasionally in a wall or rock face) and it frequently uses nestboxes for nesting (Gosler *et al.* 2013). They are monogamous breeders, and establish breeding territories (Krebs 1971).

Though clutch size can vary, it is most often 6 to 12 eggs. The incubation period is between 12 and 15 days. As altricial birds, they hatch unfeathered and blind, and chicks are fed by both parents. The nestling period is between 16 and 22 days, with chicks being independent of the parents eight days after fledging (Gosler *et al.* 2013). Great tits are seasonal breeders. The exact timing of breeding varies based on a number of factors, including location, sunlight, and daytime temperatures (Van Noordwijk *et al.* 1995; Gosler *et al.* 2013). The start of nesting may also be influenced by individual factors such as the age of the female, as younger females tend to start laying later than older females (Jarvine 1991). The timing of hatching is synchronized with peak availability of prey, but can be manipulated when environmental conditions change after the laying of the first egg by delaying the beginning of incubation, laying more eggs, or pausing during incubation (Cresswell and McCleery 2003).

Postnatal development is an important period in the life history of birds. Birds with high postnatal growth rates (altricial species) are characterized by the rapid early development of “supply” organs, such as digestive organs (Blom and Lilja 2005). The difference in growth rate, (an increase of mass and size), mainly at fledging, depends on external environmental factors (season, nest location, food availability) as well as on the individual predispositions of the individual (e.g., might be sex-specific, Tilgar and Mänd 2006). The rates of biological processes measured in extrinsic time or on an astronomic time scale may fluctuate within growing birds according to environmental conditions (Janiga 1986). Several studies have confirmed that in the great tit population, the growth rate of nestlings between broods is related to some reproductive parameters, such as hatching date or brood size (e.g., Ricklefs and Peters 1979; Murphy 1983; Orell 1983; Skagen 1987; Singer and Yom-Tov 1988), as well as to external variables, such as ambient temperature or food supply (e.g., Quinney *et al.* 1986; Blancher and Robertson 1987; Skagen 1987).

Body mass (weight) is a variable that can be easily and reliably measured and is a good indicator of condition index (Labocha and Hayes 2012). Weight of nestlings is the criterion generally used as a measure of body size, and is dependent on brood size, season, and habitat (Orell 1983). The study of nestling development presents the diversity of growth patterns among birds associated with their morphology, ecology, and behavior (Ricklefs 1968). The growth rates of birds are species dependent and can vary within a certain range (Ricklefs 1968, 1973, 1976). In great tits, the growth rate is most obvious in the first days of life, as the absolute growth rate (g/day) increases during the first days of life and is highest at 3-7 days (Orell 1983). Eight day old nestlings weigh about ten times their hatching weight. After 7 days, the daily weight increase becomes smaller, and between the 10th and 14th days the weight curve begins to level off as nestlings approach adult weight (Gibb 1950). Slight weight recession may occur before flying (Gibb 1950; Orell 1983).

Wing length. During development in nestlings of great tits, weight increase is more rapid than increase in wing length. The growth rate is low during the first several days (0-4), increasing from 1.0 to 2.0 mm per day, and became linear at a much

higher rate, (4.5-5.0 mm per day) at 5-10 days. Toward the end of the nestling period growth slows down, but the wings are still growing during fledging, at a rate of about 2.5 mm per day (Orell 1983).

The tarsus length is one of the basic input parameters for calculating fitness index (i.e., physical condition, Veľký and Kaňuch 2008). In the great tit, the tarsus stops growing at the age of 12 days and has even been found to shorten in many chickens just before flying out (Orell 1983). This is because the ankle joints are not as swollen at this age as in younger chickens. General growth curves of *P. major* tarsus indicate that a size of about 12 mm was found in 6-day-olds from different environments (Gosler 1993).

In this study, we tried to evaluate the impact of environmental conditions on the growth curves of great tit nestlings in three different habitats. Two of them represent a forest zone in a submontane area. One of these localities is congested by traffic (nests near roads), which represents a burden due to the environmental impact of lead from motor fuel mixtures (Kočvara *et al.* 2021). The third habitat is at a lower altitude, in a waterlogged alder forest. We assumed that the trajectories of development and the success of reproduction would be different in all localities.

Material and Methods

Study sites and data collection

Research was conducted at three different locations in Slovakia. Data were collected in different years as part of previous research on the nesting biology of birds (see Paliesková *et al.* 1990; Kočvara *et al.* 2021).

The first locality was in the submontane area of the High Tatras (at 850 to 1350 m a.s.l.). The nest boxes were placed at a height of 2.5-3.0 m above ground near the road leading to the cities of Štrbské Pleso, Starý Smokovec, and Tatranská Lomnica. Nest boxes were placed 100 meters apart over 1.5 km. The habitat presents a typical forest community with a dominant representation of spruce (*Picea abies*). The research was carried out from May to July during the years 1995 and 1996. The second locality was in the Low Tatras, in the Brankov nature reserve (about 900 – 1100 m a.s.l.). This study area is characterized by various types of forest, from spruce monocultures to mixed fir (*Abies alba*), beech old wood (*Fagus sylvatica*) and meadow communities. The location of the nest boxes was similar to the most frequent observations of *P. major*, as reported in the study by Janiga and Korec (2019). The research was carried out from May to July during the years 1985 and 1987. The third locality was in the Šúr National nature reserve (130 m a.s.l.), located in the northwestern tip of the Danube plain between Svätý Jur, Bratislava-Vajnory and the Chorvátsky Grob at the foot of the Little Carpathians. The area is represented by original communities of peat alder (*Alnus glutinosa*) forest with characteristic barrel roots. The nest boxes were placed on trees similar to the previous sites. The research was carried out from May to July during the years 1985 and 1986.

At eachy site, boxes were monitored regularly during the nesting period, and every 2-3 days af-

ter hatching. During monitoring, the following parameters of hatchlings were consistently measured: length of tarsometatarsus, length of wings skeleton (ulna/radius), and weight of nestling.

Age of the nestlings

During postnatal development, there is a disproportionate growth of internal organs, bones, muscles, and weight gain. In altricial birds, this disproportion in size and weight gain is evident during the first days of life and continues until the nestling leaves the nest. The concept of heterochrony in the study of growth trajectories deals with changes in growth rate and timing of developmental processes, and therefore includes time as an essential component. (McKinney and McNamara 1991). Allometry only refers to time implicitly, with respect to the rate at which growing organisms move through the space of the morphological characters (Teather and Weatherhead 1994; Badyev and Martin 2000). Because heterochrony deals with changes in the rates and timing of growth processes, the most useful way to study it is to compare the actual curves depicting measures of size or shape as a function of physical time (Alberch *et al.* 1979). The age scale is calibrated with a measure of physical time (measured usually in days). In altricial and semi-altricial birds, the age (physical time) does not increase linearly with many growing variables, and the scale of age may be very wide to detect important proportional changes in the growth of organs of birds (Paliesková *et al.* 1990; Janiga *et al.* 1993). As an alternative, the concept of physiological age (intrinsic time) may be used (Lebeau *et al.* 1986). Strauss (1987) suggests that overall body size is preferable as an estimate of biological age because it is more directly tied to growth than chronological time. In this sense, physical time (age in days) is replaced by physiological age (body size) in this study.

Statistical analysis

Statistical analysis of the obtained data was performed in the software Statistica Ver. 12. To determine the growth curves at specific localities, linear graphs were made, taking into account the dependence of growth factors on tarsometatarsus length, wing bones (ulna/radius), and weight.

Results

In total, 186 great tit nestlings were measured (Low Tatras: $n = 51$; High Tatras: $n = 88$, Šúr: $n = 47$). Growth curves depending on tarsometatarsus size and weight (Figs. 1 and 3) confirm that the nestlings in the Low Tatras locality achieved the best development due to their earlier departure from the nest. The higher weight due to tarsometatarsus rates in the second half of nest care suggests better feeding by adults and a more suitable habitat (fir-beech forests). A comparison of the growth curves of wing length and weight (Fig. 2) confirms that nestlings developed best in the Low Tatras. In the second half of nest care, the nestling wings in the High Tatras developed slightly better than in Šúr, unlike tarsometatarsus measurements.

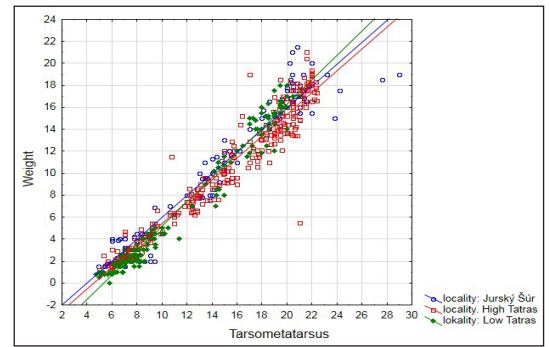


Fig. 1. Comparison of tarsometatarsus growth depending on weight in *P. major* nestlings in three different localities.

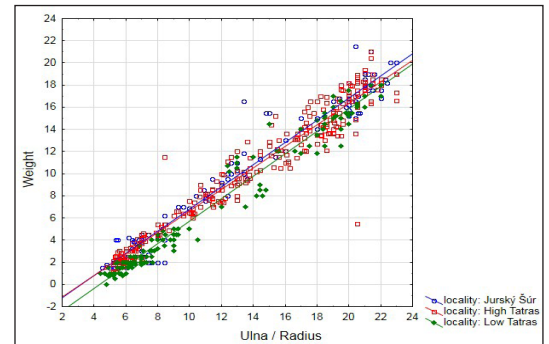


Fig. 2. Comparison of wing bone (ulna, radius) growth dependent on weight in *P. major* nestlings in three different localities.

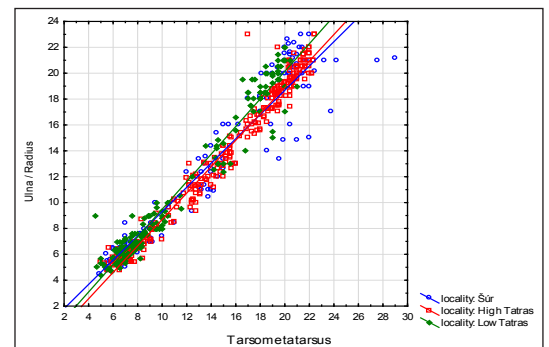


Fig. 3. Comparison of tarsometatarsus growth depending on wing bone (ulna, radius) growth in *P. major* nestlings in three different localities.

Discussion

Birds are generally considered to be suitable indicators for detecting the effects of many environmental factors acting simultaneously since they may respond to subtle changes in the environment which would otherwise remain undetected (Koskimies 1989; Furness *et al.* 1993). In this study, we tried to compare the growth curves of nestlings of great tits from different habitats in order to determine the impact of the habitat on the quality of development. Our results confirm that the growth curves in the three different tit populations are very similar, but show slight differences. However, additional studies have confirmed differences in growth rates among populations of great tits from different geographical areas or altitudes (Barba *et al.* 1993; Bor-

djan 2013). Changes in growth rates will show up as differences in slopes of the growth curves, either upward (acceleration) or downward (retardation) McKinney and McNamara (1991). The development period in a bird species is a comparison between various selective pressures favoring slow or rapid growth, the main factors at work here being food supply and chick mortality (Lack 1968). Birds developing at a slower rate of growth have less frequent feeding, which allows parents to raise more juveniles. On the contrary, rapid growth is balanced by predation pressure (Orell 1983). Growth rates are optimized among species that occupy different environmental conditions (Remeš and Martin 2002) and can vary considerably between different populations (Barba *et al.* 1993). The difference in growth rate might be limited by physiological limitations and subjective predispositions of the individual, such as sex (Ricklefs 1969; West *et al.* 2001), and/or is year-dependent (Tilgar and Mänd 2006), relating to external environmental variations (season, nest location, food supply) (Remeš and Martin 2002). Although the nestlings from warmer and lower altitude areas (Šúr) hatch with a larger tarsometatarsus, in the second half of nest development, the growth rate slows down. Juveniles from the High Tatras lag behind the most in development (Fig. 1). As we compared the growth of tarsus with weight gain, we can say that nestlings from the Šúr and the High Tatras had lower quality or less food, or worse environmental conditions. Differences in populations caused by the environment are also documented in the studies by Gil-Delgado *et al.* (2005); Biard *et al.* (2017); and Ye *et al.* 2021.

Overall body size is preferable as an estimate of biological age because it is more directly tied to growth than chronological time (Strauss 1987). Rates of biological processes measured extrinsic time or using an astronomic time scale may fluctuate within growing birds according to environmental conditions (Janiga 1986). Chronological time is usually replaced by size as a measure of intrinsic time (Strauss 1987). In studies evaluating the influence of several factors on the development of the organism, it is, therefore, more appropriate to use the physiological age of the young (expressed by changes in size) than the chronological age. It is, however, appropriate to use more than one morphometric measurement to estimate age (Brown *et al.* 2011). Because individual birds have a remarkable capacity to vary their mass and volume depending on their nutritional status (Emlen *et al.* 1991), it is biologically most meaningful to define size from skeletal measurements, and therefore, independent of nutrient reserves (Piersma and Davidson 1991). Morphometric data have been used to estimate the nestling age of various species (see Wails *et al.* 2014).

To compare growth rates from different populations of great tits, we chose only three basic measures. Tarsometatars length and wing bone length are endogenous factors that are individual for each individual. Weight gain is an exogenous factor that correlates most with the availability of food resources and thus with the benefits of the habitat. The primary growth pattern can be hypothetically defined as rapid tarsus growth with weight gain in the first days of development (tarsus growth stabilizes around day 10, see in Orell 1983). In the sec-

ond half of nesting development, weight gain and tarsal growth are both slower, while the growth of the wing is accelerated. The high energy costs of development are offset by a sharp increase in weight within the first 10 days (Barba 1993). Early lower limb development is important for nest mobility, which may be related to eating behavior or hygiene. The development of the wing is related to the ability to fly earlier and leave the nest. The parts of the body which are essential for mobility, the wing and tarsus, are much more developed at the fledging stage than is the tail, which a bird can manage without for short periods (Orell 1983).

Wing feathers of juveniles grew faster relative to tarsus bones in localities with the best environmental conditions (Kočvara *et al.* 2021). If juveniles in the Low Tatras have the fastest wing development relative to tarsus, this could indicate development and fledging. Thus we can infer that conditions for leaving the nest quickly are the most satisfactory in the Low Tatras locality and the least satisfactory in the Šúr locality (Fig. 3). Acceleration of growth is important for the success of the growth strategy, but is also crucial to maturation, with respect to the degree of functional maturity Bjorklund (1996). Relatively heavier nestlings with longer wings (in our study in the Šúr locality, Fig. 2) should, according to Orell (1983), be more developed due to their more advanced maturity. The prolonged nesting period in hole nesters is due to the long interval between the day of attaining maximum weight and the day of fledging (Haartman 1954, 1957).

Individual growth rate and fledgling mass is determined by food intake (Gill 1994; Keller and Van Noordwijk 1994), and can be reflected in the difference between two environments (Richner 1989). Thus, the growth curve may be used for comparing differences in quality between breeding environments within the range of species (Janssens *et al.* 2003; Eeva *et al.* 2009). Great tits are altricial nesters and all food consumed by juvenile is provided by the parents (Tanner *et al.* 2007).

The quality and quantity of food resources is determined by food availability in the breeding territory (Gibb and Betts 1963), and can be further influenced by unfavorable conditions such as weather or competition (Minot 1981; Keller and Van Noordwijk 1994). There may be variation in feeding capacity due to differences in foraging efficiency between the parent birds, and the “quality” of the territory (Askenmo 1973; Högstedt 1980), as both quantity and quality of food has been shown to impact growth rate (Berthold 1976).

Variation in growth rate of the great tit can be high in years when there was marked nestling starvation, or when some pairs experience difficulty in finding food for their nestlings, resulting in delayed brood growth. It was found that chicks hatched in years that were poor in food have a shorter tarsus than those that hatched in years when food was sufficient (Horak 1994). The difference between the two environments can be reflected in fledgling mass (Richner 1989), thus growth curve may be used for comparing the quality difference between breeding environments within the range of species (Janssens *et al.* 2003; Eeva *et al.* 2009). Differences in the growth curves in our monitored habitats can be explained by the different nesting environments of tits.

In Šur, vegetation is comprised of high barrel alder forests, often flooded with water, which is perhaps a more suitable habitat for such hole-nesting species as *Ficedula albicollis* (see Paliesková *et al.* 1990). This species catches insects out of the air, whereas tits feed on larvae and small insect caterpillars that are better represented in spruce and mixed forests. Habitats poor in food sources or offering more uniform food equate to higher daily energy expenditures of feeding parents (Veľký and Kaňuch 2008).

Altitude is an important factor in reproduction success. The effect of altitude on breeding phenology and clutch size of tits in Europe has been confirmed (Gil-Delgado *et al.* 1992; Krementz and Handford 1984; Sanz 1998). Individuals breeding at the limit of species distribution show markedly different breeding parameters than their counterparts in more optimal environments (Orell and Ojanen 1980, 1983; Veistola *et al.* 1994). Bordjan (2013) confirmed that at lower altitudes, weight curves are approximately the same, and differ only at higher altitudes (approximately 1000 m a.s.l.). Our results confirm that the growth curves within areas at the same altitude are similar. It can be assumed that the nestlings in the High Tatras localities, which were at approximately the same altitude as in the Low Tatras locality, were impacted in their development due to proximity of the road and the associated pollutant loads (e.g., lead; Kočvara *et al.* 2021).

Another negative environmental factor in the Šur area is higher temperatures and waterlogged and flooded areas. This factor is crucial for the development of insects that hatch in water (e.g., mosquitoes), but is also a vector of various parasites - particularly haemosporidian. It is known that the type of nesting affects the occurrence of blood parasites specifically as well as parasites in general. Open nest birds are prone to more haemosporidian parasites and longer developing chicks have a higher prevalence (longer nest time results in more parasites) (Dunn *et al.* 2017). An extremely high number of vectors can have the same effect on hole-nesting birds. Although the prevalence of haemosporidian has not been studied, it can be assumed that the increased presence of vectors encountered in the Šur locality during research may have increased its occurrence, which could negatively affect the condition and growth of nestlings.

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The effect of altitude on accumulation of heavy metals in *Vaccinium myrtillus* organs in the Low Tatra mountains (from the Veľká Chochuľa mountain to the Prašivá mountain)

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Abstract. Bilberry samples were collected in the Low Tatras (the Carpathian mountain range) for analysis of heavy metals in roots, stems and leaves. We focused on heavy metal concentrations, which indicate pollution in alpine environments. Collection of samples was performed in two transects to compare samples from different altitudes. Each transect was divided into two parts based on the collection area. We have hypothesized that pollution in bilberries has contributed to the decline in marmot population in the area. Therefore, we decided to verify the presence of pollution in bilberries, which could also be related to escalating precipitation at higher altitudes. We found significant differences between accumulation of some elements in different plant organs as well as between the eastern and western parts of the mountain ridge. Elevation had a significant effect on the accumulation of several elements in bilberries.

Key words: bilberry, heavy metals, accumulation, bioindicator

Introduction

Bilberries are dwarf shrubs that constitute one of the major drivers in ecosystem dynamics of boreal forests. They provide food, regulate species, and affect seedling regeneration and soil nutrient and carbon cycles. Bilberry grows primarily in acidic soils and is not very demanding with respect to nutrients or water; but long-term nitrogen deposition often leads to a decline in bilberry and replacement by ferns (Höcke *et al.* 2016). Bilberry is capable of growing on sites contaminated by heavy metals, and therefore, is useful as a bioindicator (Mikkonen and Huttunen 1981; Uhlig *et al.* 2001) as well as for the efficient colonization of contaminated sites (Białońska *et al.* 2007; Kandziora-Ciupa *et al.* 2013; Kukla and Kuklová 2008; Reimann *et al.* 2001; Sailemaa *et al.* 2004; Sheppard 1991; Taulavuori *et*

al. 2013). Experimental germination indicates that copper is more toxic to bilberry seeds than nickel. It has been found to delay germination and suppress seedling growth by inhibiting cell expansion and division (Lyanguzova 1999).

Heavy metals (HM), when present in higher than optimal concentrations, affect different cellular components, thereby interfering with the normal metabolic functions of plant cells (Tkalec *et al.* 2014). As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly, and some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Assche and Clijsters 1990; Jadia and Fulekar 1999). The negative influence of heavy metal on the growth and activities of soil microorganisms also indirectly affect the growth of plants. Reduction in the number of beneficial soil microorganisms due to high metal concentration may lead to a decrease in organic matter decomposition leading reduced soil fertility. Enzyme activities are useful for plant metabolism, but can be hampered due to interference of HM with activities of soil microorganisms. These toxic effects (both direct and indirect) lead to a decrease in plant growth, ultimately resulting in the death of plant (Schaller and Diez 1991). With an excessive amount of heavy metals, the surface of the plant is disturbed, and manifestations vary (e.g., leaf necrosis begins to reduce plant biomass. Metals that do not play any beneficial role in plant growth, such as Pb, Cd, Hg, and As, can cause adverse effects at very low concentrations in the growth medium (Kibra 2008). Plants on land tend to absorb HM from the soil and retain the majority in their roots. There is some evidence that plant foliage may also take up HM (and it is possible that this lead is moved to other parts of the plant). Pb is one of the ubiquitously distributed but most abundant toxic elements in soil. Uptake of lead by roots of the plant may be reduced with the application of calcium and phosphorus to the soil. Lead has an adverse effect on morphology, growth and photosynthetic processes of plants (Morzck and Funicelli 1982), and also induces proliferative effects on the vascular plant repair process (Kaji *et al.* 1995). Conversely, iron toxicity is not common. Iron as an essential element for all plants, and has many important bio-

logical roles in processes such as photosynthesis, chloroplast development, and chlorophyll biosynthesis. Although most mineral soils are rich in iron, the expression of iron toxicity symptoms in leaf tissues occurs only under flooded conditions, which involves the microbial reduction of insoluble Fe^{3+} and insoluble Fe^{2+} (Becker and Asch 2005). High chromium concentration can induce lipid peroxidation in plants and disturb the chloroplast ultrastructure, thereby disturbing the photosynthetic process (Peralta *et al.* 2001).

Generally, elevated levels of heavy metals are also associated with increased oxidative stress by increasing ROS production in plants (Reddy *et al.* 2005; Emamverdian *et al.* 2015), interfering with various macromolecules and disrupting normal cellular functions and metabolism (e.g., resulting in lipid peroxidation, inactivation or damage of proteins and chlorophyll, DNA injury) (Anjum *et al.* 2016). Once formed, ROS must be detoxified as efficiently as possible to minimize damage. Antioxidant systems in plants are complex and involve an array of non-enzymatic and enzymatic mechanisms capable of preventing the cascades of uncontrolled oxidation (Gratao *et al.* 2005, 2008; Kandziora-Ciupa *et al.* 2013).

Bilberries have a large capability to accumulate Mn. A positive correlation was found between the level of non-protein thiols and Cd and Zn concentrations, as well as between proline and these metals. Increased Mn accumulation caused a decrease in antioxidant response (Kandziora-Ciupa *et al.* 2017). Many other authors have mentioned the large capability of bilberry to accumulate Mn “as a manganese hyperaccumulator”. As a hyperaccumulator of manganese, bilberry has detoxification mechanisms and shows no symptoms of phytotoxicity (Korcak 1989; Reeves 2006). Manganese occurs unevenly in the soil environment (Fiala *et al.* 2013), and its presence is associated with its relative content in the parent rock or with emissions. Manganese is a microelement needed by plants for their normal growth and development. Dominant forms accessible to the plants are Mn^{2+} compounds taken up by means of epidermal cells of plant root parts (Marschner 2006; Pittman 2005). Manganese is a heavy metal needed for plant growth, and if it is not present in a given amount for a given plant, the plant will become stunted and overgrown by others, resulting in Mn deficient plants being pushed out, unless in the presence companion plants (i.e., *Vaccinium vitis-idaea*) with different nutrient or soil requirements. Plants can suffer from lack and or surplus of Mn. Excessive uptake occurs in more acidic soils with a $\text{pH} < 5.5$ (Bergmann 1988; Kabata-Pendias 2011; Mengel and Kirkby 2001; Xue *et al.* 2004). The general hypothesis points to the differentiated change of manganese content in the assimilatory apparatus of *Vaccinium myrtillus* over the years, as well as the effect of manganese concentrations in soil during the growing season, as influenced by precipitation levels. In the second half of the vegetation period, Mn content in leaves is generally higher than in branches. Although manganese content in branches and leaves was above toxic limits, evidence of leaf damage from toxicity was not detected (Reeves 2006). Bilberry,

as a medium accumulator of manganese, has suitable mechanisms for Mn elimination. Antioxidant systems in plants may be used as early indicators of environmental stress on target organisms preceding morphological or ultrastructural damage, and as warning indicators for the ecosystem (Białońska *et al.* 2007; Kandziora-Ciupa *et al.* 2016).

Our paper is a part of wider study focused on decline in marmot populations in the Low Tatra Mountains. The main aim of this study was analysis of heavy metals in roots, stems and leaves of *Vaccinium myrtillus* close to abandoned marmot burrows in selected parts of a mountain ridge in the Low Tatra Mountains. This research is intended to answer two basic questions:

1. Are bilberries contaminated in alpine meadows above the tree line in the Low Tatras?
2. How does altitude affect pollution?

Material and Methods

Sample collection

This study was conducted during summer seasons between 2018 and 2019 in a mountain ridge of the Low Tatra Mountains (between Prašivá Mountain and Veľká Chochuľa Mountain). Samples of bilberry were collected along two horizontal transects using 1×1 - metre plots. The first transect was located at the upper line of the occurrence of abandoned marmot (*Marmota marmota latirostris*) burrows, and in parallel with the upper line of dwarf shrubs (1545 – 1743 m a.s.l.). Based on our previous research (unpublished), it is likely that marmots have abandoned this location due to cessation of livestock grazing and subsequent spreading of the dwarf shrub species *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Empetrum nigrum*. The second horizontal transect was located 100 altitudinal meters below the first transect. Quadrat plots were situated each 100 m along both horizontal transects. For each 1×1 -metre plot, environmental variables (altitude, aspect) and data on the coverage of dominant plant species were recorded. Localization, altitude, and aspect of each vegetation plot was measured using Garmin Oregon 300 equipment. The tallest plant of bilberry was collected from each plot. Plants were cut at ground level, labelled, and transported to the laboratory in zip-sealed plastic bags.

Laboratory work

The first transect was not specifically divided. Samples consist of whole stems with leaves. The second transect was divided into three groups: leaves marked as A; stems as B; and roots as C. These samples were dried in a laboratory incubator (IF 160 Plus) (Mettler, Germany). Temperature was set to 80°C with the air circulation fan set at 30 %. Samples were dried for 8 hours. Dried samples were crushed in a cryomill (Retsch, Germany). Crushing time depended on sturdiness of samples, particularly roots, but took approximately one minute for leaves and stems with a frequency of 30 Hz. Roots were cut into small pieces for better results.

Dried and crushed samples were analysed using DELTA Environmental Handheld XRF Analyzer (Olympus, Innov-x Systems, USA). Measurements ran in a closed Delta XRF portable workstation. The spectrometer was calibrated using a certificated reference material of INCT-PVTL-6 Virginia tobacco leaves (ICHTI, Poland). Samples were analysed in plastic cuvettes with plastic foil on the bottom for 240 seconds in three equal measurements. From these the arithmetic average was calculated.

Statistical analyses

Principal component analysis (PCA) was used to determine main components, the component weights, and the percentage of variation. We examined a total of 399 samples. From these we considered eleven factors, of which six constituted more than 5% of the total variability. One-way ANOVA of principal component scores and linear regression was used to compare the effects of environmental variables (elevation, locality, combination of locality and month) or different plant organs (stems, leaves, roots).

Results

Effect of altitude on concentrations of elements in stems of bilberries from the first transect

Principal component analysis (PCA) shows correlations between elements in bilberry samples us-

ing five factors (Table 1). The first factor indicates the negative correlation between S, Cl, K, Ca, Cr and Ba. The second factor shows the positive correlation with Pb. The third factor represents the negative correlation with Rb (Fig. 1). The fourth factor showed a decline in Mn. The fifth factor indicates a negative correlation with Zn. For factor 3 the correlation of Rb increases with altitude (Fig. 1). Linear regression for factors 1, 2, 4, 5 is shown in Table 2. Differences in Pb accumulation (factor 2) by mountain are show in Fig. 2. A complete overview of Pb accumulation from both transects is shown in Fig. 3.

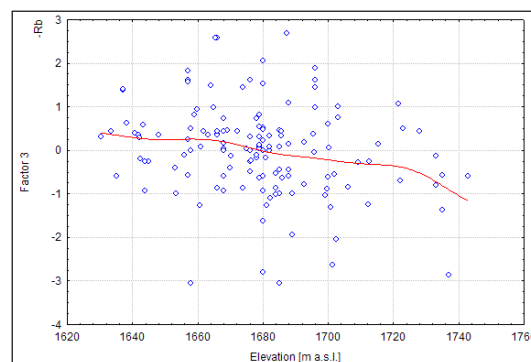


Fig. 1. Concentration of Rb increases with elevation ($y = 17.892 - 0.0107 \cdot x$, $r = 0.24$, $n = 142$, $F(1,140) = 8.3711$, $p < 0.0044$) in stems (with leaves) of *V. myrtillus* in the first transect from October. The line is calculated as distance weighted least squares.

Element	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
S	-0.970	-0.076	0.013	0.064	0.023
Cl	-0.878	-0.308	-0.017	0.192	-0.041
K	-0.894	-0.339	-0.153	0.011	-0.001
Ca	-0.938	-0.144	0.111	0.093	-0.101
Cr	-0.956	0.023	0.071	0.164	0.068
Mn	-0.512	-0.492	0.389	-0.572	0.120
Fe	-0.791	0.519	0.089	-0.048	0.120
Zn	-0.490	0.693	-0.143	-0.276	-0.419
Rb	-0.246	-0.190	-0.920	-0.186	0.135
Ba	-0.944	0.048	-0.015	0.038	-0.042
Pb	-0.302	0.873	0.033	-0.030	0.330
Eigenvalue	6.5	2.0	1.1	0.5	0.4
Total variance [%]	58.9	18.4	9.7	4.7	3.2

Table 1. Eigenvectors with the percentage of variance in principal component analysis of elements in the *Vaccinium myrtillus* from the first transect. The highest correlations are in bold.

Factor	Regression formula	R	Result
Factor 1	$y = 25.3298 - 0.0151 \cdot x$	0.14	$F(1,140) = 2.6597$, $p < 0.10517$
Factor 2	$y = -9.0674 + 0.0054 \cdot x$	0.08	$F(1,140) = 0.86857$, $p < 0.35296$
Factor 4	$y = -3.6617 + 0.0022 \cdot x$	0.07	$F(1,140) = 0.68353$, $p < 0.40978$
Factor 5	$y = 2.27 - 0.0014 \cdot x$	0.05	$F(1,140) = 0.38918$, $p < 0.53374$

Table 2. Results of linear regression analyses of factors from PCA with elevation ($n = 142$).

Analyses of elements in Vaccinium myrtillus from the 2nd transect from June

PCA shows correlations between elements in bilberry using six factors (Table 3). The first factor indicates a positive correlation between S, Cl, K and Ca. The second factor shows a positive correlation between Cr, Fe and Ba. The third factor

shows the antagonistic relationship between Fe and Zn. While Fe levels increase, Zn levels decrease and vice versa. The fourth factor shows a decline in Rb. The fifth factor indicates a positive increase in Mn. The sixth factor captured the positive accumulation of Pb. Factor 1 for bilberry leaves showed a positive correlation with S, Cl, K and Ca, as they are mostly accumulated in leaves

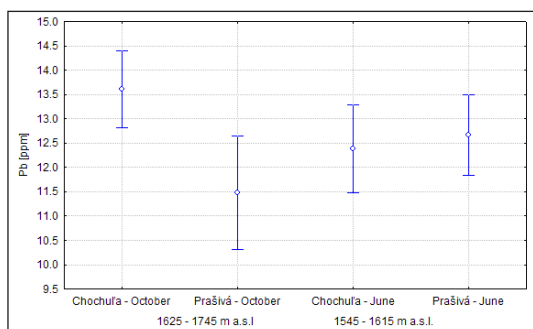


Fig. 2. Concentrations of Pb are higher in stem samples of *V. myrtillus* (with leaves) from the area of Velká Chochuľa and Malá Chochuľa than from the area of Prašivá from an elevation of 1625 – 1745 m a.s.l. from the first transect from October (One-way ANOVA, $n = 142$, $F(1, 140) = 16.280$, $p = 0.00009$).

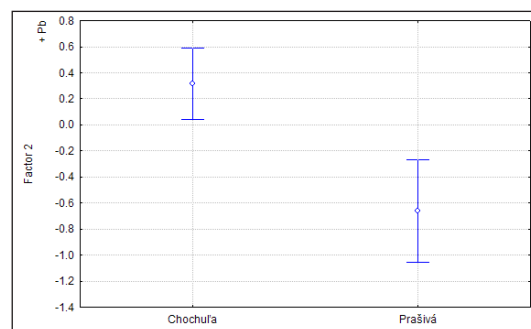


Fig. 3. Differences in Pb distribution of stem and leaf samples of *V. myrtillus* between ridge areas of two transects from autumn and early summer months (One-way ANOVA, $n = 314$, $F(3, 310) = 3.3105$, $p = 0.02042$).

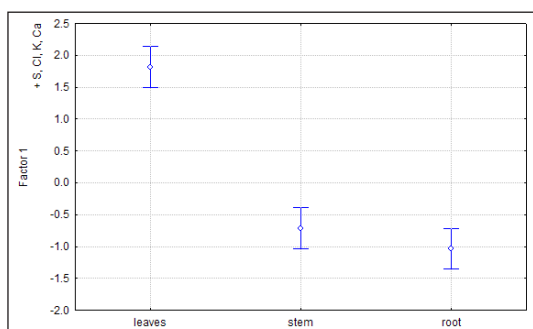


Fig. 4. Comparison of differences in effects of factor 1 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 90.965$, $p = 0.0000$). LS means with 0.95 confidence limits of scores of factor 1. Vertical bars denote $\pm$ standard errors.

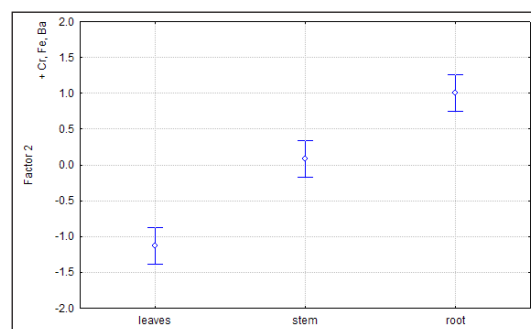


Fig. 5. Comparison of differences in effects of factor 2 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 67.511$, $p = 0.0000$). LS means with 0.95 confidence limits of scores of factor 2. Vertical bars denote $\pm$ standard errors.

Element	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
S	0.822	-0.182	-0.067	0.315	-0.242	0.134
Cl	0.860	-0.397	0.019	-0.087	0.075	-0.020
K	0.891	-0.385	0.056	-0.075	0.001	-0.009
Ca	0.839	-0.034	-0.420	0.043	-0.097	-0.142
Cr	0.494	0.629	0.493	0.211	0.096	-0.192
Mn	0.274	0.264	-0.473	0.079	0.689	0.388
Fe	0.356	0.631	0.626	-0.018	0.207	-0.090
Zn	-0.167	0.432	-0.636	-0.433	-0.013	-0.298
Rb	0.417	-0.218	0.246	-0.788	0.169	-0.072
Ba	0.469	0.615	-0.470	0.142	-0.148	-0.168
Pb	0.222	0.584	0.067	-0.374	-0.440	0.514
Eigenvalue	3.8	2.2	1.7	1.1	0.8	0.6
Total variance [%]	34.8	19.6	15.8	10.3	7.5	5.6

Table 3. Eigenvectors with the percentage of variance in principal component analysis of elements in the *Vaccinium myrtillus* from the second transect. The highest correlations are in bold.

while stems have lower amounts and roots exhibit the lowest (both with negative cumulation) (Fig. 4). Leaves do not accumulate Cr, Fe and Ba, but bilberry root accumulates these elements the most (Fig. 5). In factor 3, roots and leaves show a positive correlation between Zn, Fe, while stems show a negative correlation (Fig. 6). In June, Rb accumulates the most in stems of bilberries, whereas lower levels of Rb are found in leaves and roots (Fig. 7). We did not find any significant effect in bilberries organs from Mn accumulation

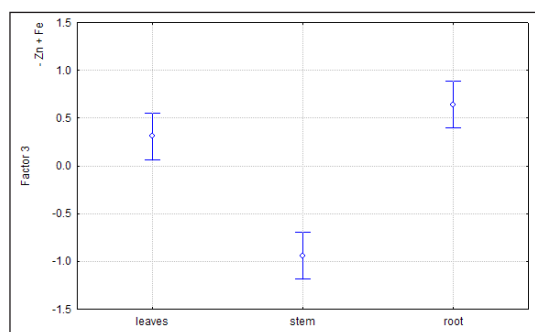


Fig. 6. Comparison of differences in effects of factor 3 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 46.168$, $p = 0.0000$). LS means with 0.95 confidence limits of scores of factor 3. Vertical bars denote $\pm$ standard errors.

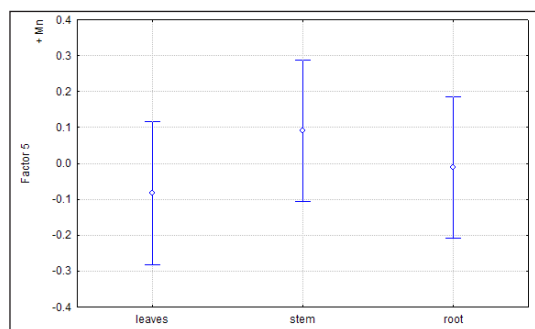


Fig. 8. Comparison of differences in effects of factor 5 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 0.74197$, $p = 0.47723$). LS means with 0.95 confidence limits of scores of factor 5. Vertical bars denote $\pm$ standard errors.

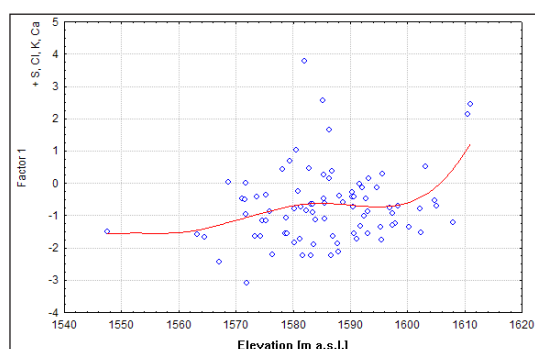


Fig. 10. Concentrations of S, Cl, K, and Ca increase in stems of *V. myrtillus* with elevation ($y = -38.5643 + 0.0239 \cdot x$, $r = 0.23$, $n = 85$, $F(1,83) = 4.8185$ $p < 0.03095$) in the second transect from June. The line is calculated as distance weighted least squares.

(Fig. 8). Factor 6 shows Pb concentration, with which leaves and roots correlate almost equally; they show a slightly positive correlation, while stems show a negative correlation (Fig. 9).

Effect of altitude on concentrations of elements in stems of bilberries from the second transect

For the first factor from the second transect we have recorded a positive accumulation of the following elements: S, Cl, K, Ca. The accumula-

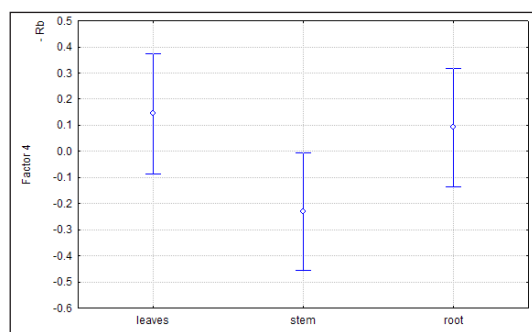


Fig. 7. Comparison of differences in effects of factor 4 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 3.1195$, $p = 0.04591$). LS means with 0.95 confidence limits of scores of factor 4. Vertical bars denote $\pm$ standard errors.

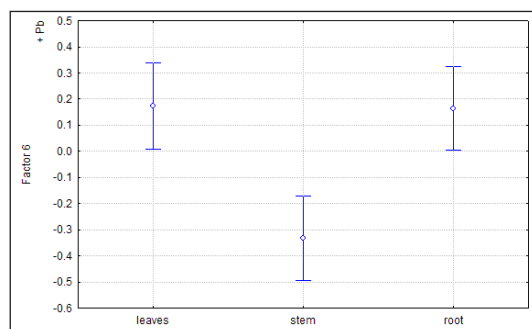


Fig. 9. Comparison of differences in effects of factor 6 in the different parts of *V. myrtillus* from June (One-way ANOVA, $F(2, 249) = 12.488$, $p = 0.00001$). LS means with 0.95 confidence limits of scores of factor 6. Vertical bars denote $\pm$ standard errors.

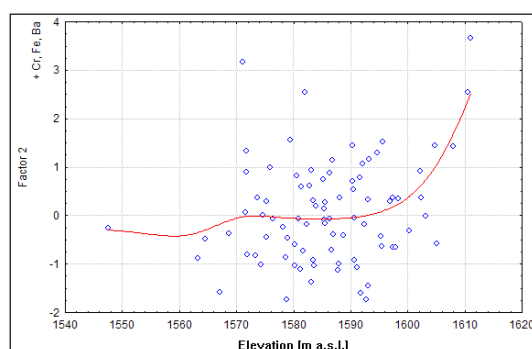


Fig. 11. Concentrations of Cr, Fe and Ba increase in stems of *V. myrtillus* with elevation ($y = -37.9224 + 0.024 \cdot x$, $r = 0.25$, $n = 85$, $F(1,83) = 5.7164$ $p < 0.01907$) in the second transect from June. The line is calculated as distance weighted least squares.

tion of elements increases with higher elevation (Fig. 10). The second factor shows accumulation of Cr, Fe, Ba. Element concentrations increase with higher altitude (Fig. 11). In factor 3, the inverse effect of (-Zn and +Fe) is not related to elevation (Fig. 12). In factor 4, Rb values appear to increase with altitude. The latter decreasing may be due to fewer samples and so cannot be taken as indicative (Fig. 13). In factor 5, concentration of Mn significantly decreases with elevation (Fig. 14). In factor 6, concentration of Pb

decreases with elevation. Lower-lying samples are the most affected by Pb contamination (Fig. 15). In comparison of groups 3 and 4, group 3 represents Chochuľa mountain, and Group 4 represents Prašivá mountain. Significant differences in the second transect were observed only for factors 4 and 6. The concentrations of Rb were higher in the area of Velká Chochuľa and Malá Chochuľa than from the area of Prašivá (Fig. 16). In factor 6 the amount of Pb was higher on Prašivá (Fig. 17).

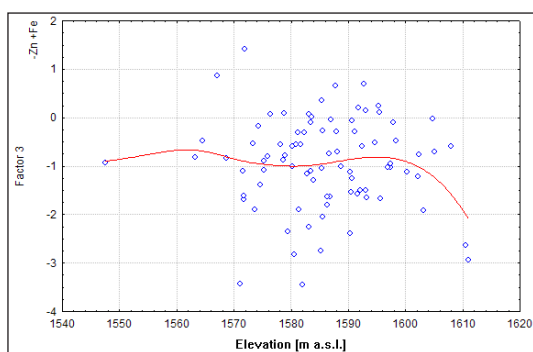


Fig. 12. Inverse effect of Zn and Fe concentrations in stems of *V. myrtillus* is not related to elevation ($y = 7.8784 - 0.0056 \cdot x$, $r = 0.07$, $n = 85$, $F(1,83) = 0.36568$, $p < 0.54702$) in the second transect from June. The line is calculated as distance weighted least squares.

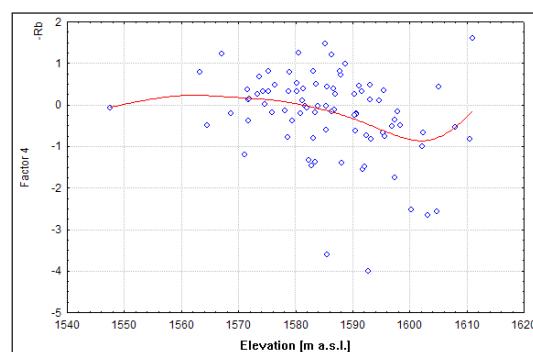


Fig. 13. Concentration of Rb increases with elevation ($y = 41.5011 - 0.0263 \cdot x$, $r = 0.29$, $n = 85$, $F(1,83) = 7.6663$, $p < 0.00694$) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

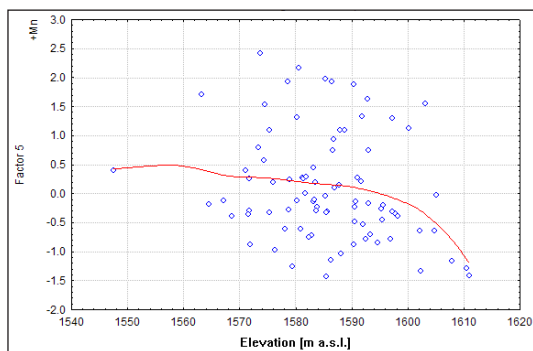


Fig. 14. Concentration of Mn decreases with elevation ($y = 30.9601 - 0.0195 \cdot x$, $r = 0.24$, $n = 85$, $F(1,83) = 4.9245$, $p < 0.02921$) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

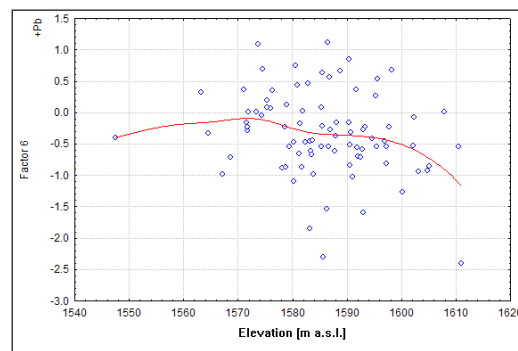


Fig. 15. Concentration of Pb decreases with elevation ($y = 21.7749 - 0.0139 \cdot x$, $r = 0.24$, $n = 85$, $F(1,83) = 4.9245$, $p < 0.02921$) in stems of *V. myrtillus* in the second transect from June. The line is calculated as distance weighted least squares.

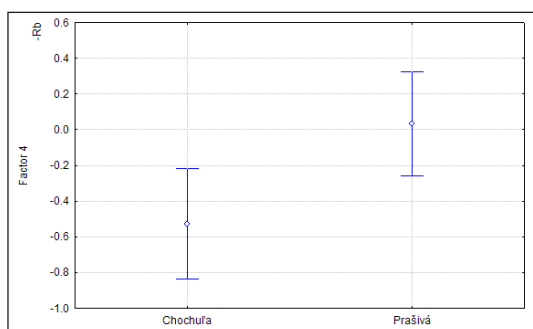


Fig. 16. Concentrations of Rb are higher in stem samples of *V. myrtillus* from the area of Velká Chochuľa and Malá Chochuľa than from the area of Prašivá in the elevation 1545 – 1615 m a.s.l from the second transect from June (One-way ANOVA, $n = 85$, $F(1, 83) = 6.8$, $p = 0.01060$).

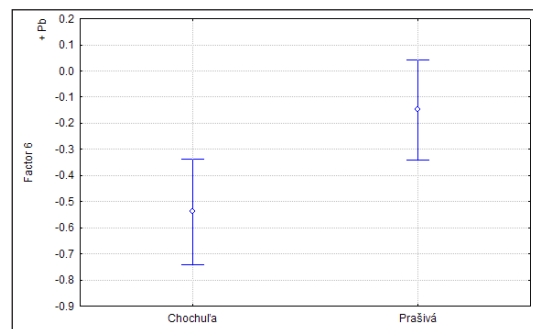


Fig. 17. Concentrations of Pb are higher in stem samples of *V. myrtillus* from the area of Prašivá than from the area of Velká Chochuľa and Malá Chochuľa in the elevation 1545 – 1615 m a.s.l from the second transect from June (One-way ANOVA, $n = 85$, $F(1, 83) = 7$, $p = 0.00648$).

Discussion

Pollution

Pollution in alpine zones is caused mainly by rain. Therefore, it is necessary to define where precipitation comes from, and it can be classified into several groups. The first of these is local precipitation affected by domestic production and transportation. There are four larger cities in the vicinity of the Low Tatras, as well as 4 main road connections. Each city has its own production. In Ružomberok, industry mainly produces pulp and paper; Banská Bystrica is known for its metallurgical and mining industry; Brezno is predominantly known for its engineering industry; and Liptovský Mikuláš manufactures leather goods. These cities, together with their roadways, are the largest sources of local pollution. Consequently, precipitation also affects global pollution, as evidenced by the presence of sand from the Sahara Desert in locations far removed. Like sand, various emissions are transmitted. Air flow has a huge effect on this transmission, as well as high and low pressure affecting wind direction.

Elevation

In terms of elevation, the samples collected below were visibly older than samples collected on the mountain ridge. This is because bilberry colonies spread from lower altitudes and gradually replaced other vegetation on the mountain ridge, which affected the natural conditions of the alpine ecosystem. One of the biggest consequences of this shift was the decline in marmot populations. The precondition for their disappearance is lack of food, as bilberries do not provide a sufficient nutrient source during spring. It was assumed that samples collected from lower elevations would be more affected by pollution, precisely because of their older age. We found that Mn and Pb accumulate in lower altitudes to a greater extent than in higher altitude environments. In terms of Mn, this is natural, as bilberry is a hyperaccumulator of manganese (Korcak 1989; Reeves 2006). Therefore, older samples from lower altitudes were able to store more Mn over their lifespan. Our greatest concern was Pb accumulation at lower altitudes, as the accumulation of heavy metals may affect the food chain (Marques *et al.* 2009). On the other hand, samples from the mountain ridge could also be exposed to more precipitation, causing an increase in elements such as S, Cl, K, Ca, Cr, Fe, and Ba. Some of these are used by bilberries as nutrients and may simply represent a possible difference in soil nutrients, as the younger colony found at higher altitudes is not yet fully developed. For each 100 m in altitude gain, precipitation sum increases. This depends on several factors, and different locations may show variation based on climate, rainfall, or precipitation conditions. According to Shparyk and Parpan (2004), snowfall pollutes the environment more than rainfall. They also identified more chemical elements in snow samples than in soil or moss (e.g., *Pleurozium schreberi*) in the Ukrainian Carpathians.

Distinctive accumulation patterns of selected elements in different parts of bilberry

Many elements found in the environment are essential. Leaves are seasonal parts of the plant that perform photosynthesis and are important for nutrition during the growing season. To some extent, they eliminate heavy metals as they fall off, because pollution is mainly absorbed through soil. Usually, lead is deposited in the roots of plants, but there are cases where it can move into other parts as well. There is some evidence that plant foliage may also absorb lead (and it is possible that this lead has moved between parts of the plant) (Morzck and Funicelli 1982). Our research has clearly shown us that bilberry leaves store Pb in almost the same amount as roots during the month of June. As there is no significant shift in accumulation between June and October, it is likely that some Pb is being eliminated as the bilberry sheds its foliage in the fall. However, the lead from this foliage is ultimately deposited into the soil and may be reabsorbed by the plant or consumed by animals, though distributed over a larger area.

Changes in bilberry

Bilberries exhibited no observable changes in leaves or on the plant, though potential changes would likely only be visible under microscope. Despite lead pollution, bilberry plants have not experienced stunted growth despite the fact that metals such as Pb, Cd, Hg, and As do not play an advantageous role in growth of the plant, and could act adversely even at low concentrations (Kibra 2008). A certain level of resistance to lead is due to the presence of phosphorus and calcium in the soil, which directly influences the absorption of lead into the root of the plant by reducing the amount of lead (Morzck and Funicelli 1982).

The effect of altitude and organ on accumulation of heavy metals in Vaccinium myrtillus in the Low Tatra mountains

The most positive aspect of this research lies in the number of samples studied. As there were a multitude of samples, we were able to eliminate many biases and inaccuracies that would have occurred with a smaller number of samples. However, not all sections of the graphs were free of outliers. This was most evident with Rb, where the smaller number of the highest-altitude bilberry began to influence the results of the others. As a result, we assert that Rb decreases with altitude, as this correlation is only shared by 8 samples. For the other factors, the number of samples is significantly higher. Due to the nature of deposition of this element, we found its values to be quite variable, and thus, we must take into account that variation in these sections that differs from the others. Nevertheless, it can help us in gaining a perspective with regard to the conditions and deposition of heavy metals in bilberries.

Sample season

The period during which samples were collected played a crucial role, due to variation in recorded

values. For Chochuľa, the difference was shifted by a quarter. In October the highest amount of Pb was recorded, while in June Pb concentrations were lower. On the other hand, we can see the opposite phenomenon with regard to Pb accumulation on Prašivá, where the amount of Pb in October was less than what was recorded in June. For more consistent results, we recommend collecting samples during the same time period, (even when the variable studied is altitude), as values change during the year due to both weather conditions, and growth and development of the bilberry. In some cases the position of the slope or the location of the snowbank could also play a crucial role in accumulation (Shparyk and Parpan 2004). Due to changing conditions, the potential for additional research increases, and further studies could be conducted with regard to variation in season on heavy metal accumulation in bilberry, or heavy metal accumulation in soil or as a result of bilberry detritus, that would provide additional information and value to this field of study.

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Patterns of select element accumulation in tissues and feces of *Rupicapra rupicapra tatrica*

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Abstract. We analyzed 352 samples of chamois tissue and feces and found lead in most of the samples, confirming the high frequency of lead occurrence in the alpine zone. In juvenile bones, lower Sr and P values were found than in adult individuals, which may be related to faster metabolism in younger chamois. In female bone samples, the elements P, Ca, Rb, Ba, Mn, Sr were found in higher concentrations than in male bones. In tongue tissue, Rb and Ca content was higher in adult individuals than juveniles and were also found in higher concentrations in females than males. Liver tissue had higher Cu concentrations and lower Zn concentrations in males.

Key words: *Rupicapra rupicapra tatrica*, heavy metal and other element accumulation, Tatra Mountains

Introduction

The Tatra chamois as a glacial relict and endemic subspecies, is a significant element of Tatra fauna. Chamois remain active throughout the year in the Tatra Mountains - even in the coldest winter conditions. These animals live in the alpine and subalpine vegetation zone year-round.

During the vegetative season, chamois prefer herbs, particularly grasses and juicy plants, and they like to graze on plant buds. They do not tear the plants, but catch them gently with their lips and consume only the upper juicier parts of plants. During the growing season, chamois graze up to 117 plant species. The vegetation of the alpine and subalpine zones is rich in many juicy and fragrant plants, which are popular with these animals. Chamois living in the mountains are often subject to human influences and air pollution.

Many heavy metals accumulate on the ridges of mountains where chamois live as a result of atmospheric deposition, as confirmed by many studies. Gábriš (1998) writes that several factors influence metal toxicity, including: the quantity, form, properties, and reactivity of the metals; environmental conditions (pH, temperature and others); and the individual ability of each living organism to accumulate the metal (element) in underground and above-ground organs, plant seeds, or more precisely, in the body of an animal. Some of this content may be excreted in urine, excrement, and the like. In terms of toxicity of chemical elements in eatables, Brtková (1991) ranks cadmium, lead and mercury among the most toxic heavy metals. As the concentration of heavy metals increases in nature, animal toxicity increases as well. Heavy metals such as Cd, Pb, Hg and As are most toxic to animals, humans and the environment. Excess of these elements can cause serious problems. Heavy metals result in a lack of minerals in the body. Decreasing Ca concentration can lead to osteoporosis, tooth decay, periodontal disease, heart disease, muscle spasms and colic. Al relocates Ca so it is not available for the formation of teeth, bones and muscles (including heart muscle), and these body structures weaken. Heavy metals can enter the body through the skin, respiratory tract, or through intestinal absorption. Following absorption, heavy metals can be distributed to various organs, including glands and CNS. Some of the elements may be deposited in the skeletal system or in teeth (Athar and Vohora 1995). The availability and mobility of heavy metals in the terrestrial environment is influenced by soil pH, soil type, oxidation-reduction potential and cation exchange. Metals first enter the food chain through absorption by plants, which are subsequently ingested by both humans and herbivores, (Hrudey *et al.* 1995). While some of these grazed plants are poisonous to humans, as well as to animals, (e.g., white hellebore (*Veratrum*)), chamois often consume young shoots of this poisonous plant without being harmed by the poisonous alkaloids contained in the whole plant (Teren 1987). Heavy metals accumulate in most of these plants, which can affect chamois life. The plant can absorb substances and metals through the root system or leaf surfaces. In order for metals to penetrate leaves, they must either pass through the epidermis of the leaf, which is covered with a waxy cuticle, or through the vents. The vents are equipped

with a mechanism that regulates the penetration of substances into the leaves by changing the diameter of the vent hole. This mechanism determines the entry of substances into the leaf based on molecular weight (Kvesitadze *et al.* 2006). Plant root activity has a major impact on soil properties, including water dynamics, as well as solutes and gases in the soil (Hinsinger *et al.* 2009). The plant root system takes on metals through two phases; the initial rapid phase, when metals penetrate from the surrounding rhizosphere into the root system by diffusion, and the subsequent slower phase of metal accumulation in plant tissues (Puckett *et al.* 2010). Metals received by root cells are accumulated in the root or are translocated through xylem to tissues and organs in the aerial parts of the plant (Prasad 2004; Jabeen *et al.* 2009).

Because metal ions enter root system cells accompanied by water, the speed of their uptake by the root is also dependent on current soil moisture; and thus impacted by the capacity of soil to hold water. Soil moisture plays an important role in its use, and in decision-making within land and soil resources related to the bioavailability of metals in soil. Significant sorption (binding) of metals takes place in soils rich in organic matter (e.g., humic and fulvic acids) (Kvesitadze *et al.* 2006; Marschner 2011).

The goal of this paper is to determine element concentrations in the tissue and feces of chamois.

Material and Methods

Sample collection

Chamois specimens used for this analysis were naturally deceased, due to avalanche conditions or predation. Often, chamois are startled by human interactions (e.g., rescue helicopters, or tourists), resulting in a slip and fall, followed by predation by the wolf population. Samples were labeled with the date, location, and the name of the researcher they were found by.

Most chamois were from the TANAP state forests. Samples were moved to the Institute of High Mountain Biology (IHMB) in Tatranská Javorina. Samples were taken of organs including: liver, heart, lungs, hair, bone, muscle, spleen, kidney, tongue and feces. We moved the samples to Petri dishes and dried them in the dryer for 10-13 hours. Subsequently, samples were x-rayed using spectrophotometers. We examined 352 samples of Tatra Chamois.

X-ray fluorescence spectrometry

Organ samples from the Tatras were analyzed by the hand-held XRF spectrometer DELTA CLASSIC (USA), using the DELTA portable workstation. Samples of organs were mechanically processed. Every sample was transferred to a small plastic cuvette with a clear bottom and analyzed by the spectrometer. The device was calibrated using the certificated reference of 'Beef liver standard NCS ZC 71001' (Haizhou 2015). When a sample was large or unformed we had to fit its shape it was cut down to fit. Each sample was analyzed for 240 seconds in three

80 second measurements, and the results were averaged. The data was processed using the EveryWAN Remote Support (Personal Edition) program.

Statistical analyses

We used Principal component analysis (PCA); a mathematical statistical method that uses orthogonal transformation to convert elements of a set of observations that are potentially correlated to elements of a set of values that are linearly uncorrelated. Amounts of elements were statistically compared using one way ANOVA and Unequal N HSD test at the 95% confidence level ($p < 0.05$). One-way ANOVA of principal component scores was used to test the differences in mutual concentration of more elements in the tissues and feces of chamois. Another statistical method that was used was the Mann-Whitney U Test, which was suitable for evaluating an insufficient number of samples in individual tissues and feces. We also used Fisher's exact test on 2 x 2 contingency tables with data of positive and negative measurements of elements in samples divided by the research area in where the sample was taken. This last method was used on elements found at all localities but not in all samples.

Results

The following elements were detected in our samples: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Zr, Mo, Cd, Sn, Sb, Ba, and Pb. Other elements that were measured but not found in any of our samples were Ag, and Hg. Elements belonging to heavy metals like As, Cd, and Se were measured, but their content was less than the detection limit in most of our samples. We found evidence of Pb, Ba, Sr and Rb, which are classified as toxic heavy metals. Out of the heavy metals (Ti, Ni, Zr, Cd, Sn, Sb and Pb) that were not measured in significant amounts in our samples, only Pb was found in enough samples for statistical evaluation. Fig. 1 shows higher accumulation of Pb in feces when compared to all other studied tissues of Tatra chamois. Contingency tables (Table 3 and 4.) show differences in lead accumulation between individual localities. The highest lead accumulation was in High Tatras, which contributed the most samples of chamois. Table 1 shows a comparison of the average values of elements in sex and generation. Table 2 shows a comparison of the average values of elements in individual tissues and feces.

Bones

PCA of bones revealed eight factors. The first six of these show a mutual relationship of elements in the bone with 81 % variance (Appendix 1). Factor 1 is in strong positive correlation with Ca, P and Rb. These elements have increased values in bone tissue of female Tatra chamois (Fig. 2). At factor 2, a strong mutual relationship among elements (Zn, Sr, Cu, Pb and Fe) was found. Factor 3 expresses a strong negative correlation of the biogenic elements S, Cl, and K that rises or decreases simultaneously. Factor 4 is a bipolar vector, which

	Female	Male	Adult	Juvenile
P	170188.998	74757.284	99867.531	51127.922
S	12792.547	9698.365	17352.623	11937.831
Cl	8701.386	6691.526	7064.692	6202.369
K	13792.870	12922.762	9918.507	12152.855
Ca	69209.373	56135.628	78554.451	47346.490
Ti	10.904	22.296	17.543	30.775
Cr	56.545	45.047	54.871	42.028
Mn	109.861	102.640	90.169	99.914
Fe	7066.479	5364.395	4399.107	4211.290
Co	0.614	0.001	0.209	0.001
Ni	40.219	22.106	23.925	15.989
Cu	38.692	47.734	31.985	37.768
Zn	193.850	199.742	188.855	174.239
As	0.001	0.001	0.001	0.001
Se	0.801	1.142	0.659	0.821
Rb	42.396	33.771	31.751	34.904
Sr	16.344	16.610	39.894	17.990
Zr	0.148	0.598	0.306	0.822
Mo	6.154	6.223	5.131	5.917
Ag	0.001	0.001	0.001	0.001
Cd	0.657	0.775	0.693	0.319
Sn	0.001	0.768	0.764	0.001
Sb	2.227	3.444	2.831	3.154
Ba	32.758	25.466	38.763	27.198
Hg	0.001	0.001	0.001	0.001
Pb	5.818	8.935	5.358	8.135

Table 1. Average values of elements between sex and generation.

states that when Fe and Cu increase, toxic elements Pb and Ba decrease and vice versa. Factors 2, 3 and 4 did not manifest between age groups and sexes. Factor 5 is in negative correlation with Mo. Mo decreased with a simultaneous increase of Ba in the bones of females, whereas in males, Ba decreases when Mo increases (Fig. 3). Factor 6 shows us the difference between the accumulation of Mn in male and female samples. While higher concentrations are found in males (Fig. 4), a negative correlation is observed in Mn at factor 7 with simultaneously increasing Cr in males and the opposite in females (Fig. 5). Factor 8 is in negative correlation with P and Sr (Table 5). These elements increased in female bones when compared to male bones (Fig. 6). Juveniles showed lower contents of Sr and P than adults, in whom these elements increased (Fig. 7).

Kidneys

PCA revealed that the first three factors show a mutual relation of elements in the kidney tissues of Tatra chamois with variability of 67.2 %. First factor showed variability, of which the highest instance was a unipolar vector, in which elements

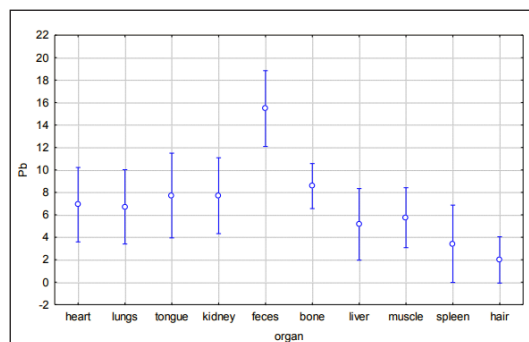


Fig. 1. Concentrations of Pb (ppm) in the feces and tissues of Tatra chamois (One-way ANOVA, $F(9, 342) = 6.1057$, $p = 0.00000$). Feces significantly differ from hair ($p = 0.000014$) and spleen ($p = 0.000061$), Unequal N HSD test, $MS = 0.78723$, $df = 342.00$.

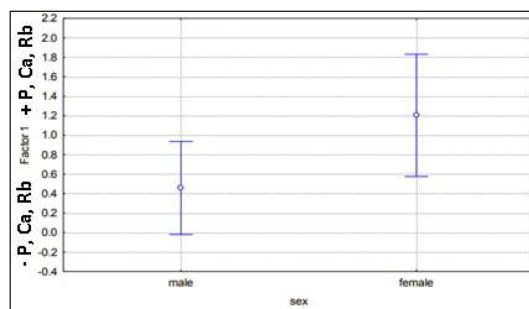


Fig. 2. Differences between sexes in concentration of elements (P, Ca, Rb) in bones of Tatra chamois, based on PCA values (One-way ANOVA, $F(1, 28) = 3.7534$, $p = 0.06285$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

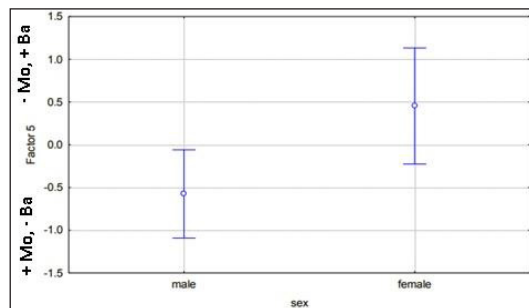


Fig. 3. Differences between sexes in elements (Mo, Ba) accumulation in bones of Tatra chamois, based on PCA values (One-way ANOVA, $F(1, 28) = 6.1162$, $p = 0.01973$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

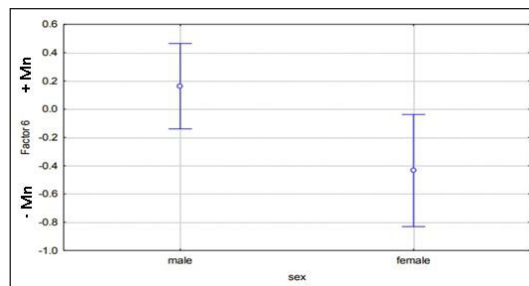


Fig. 4. Comparison of the amounts of Mn between male and female bones of Tatra chamois based on PCA values (One-way ANOVA, $F(1, 28) = 6.0207$, $p = 0.02062$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

	Bone (71) mean	Kidney (25) mean	Tongue (20) mean	Feces (25) mean	Liver (28) mean	Spleen (24) mean	Muscle (40) mean	Heart (26) mean	Lungs (26) mean	Hair (68) mean
P	383471.41	31771.91	8586.88	10694.24	21974.50	4798.14	14055.62	4066.97	17370.75	23192.73
S	889.25	6489.30	6302.30	2218.73	4358.33	4996.77	5075.42	5807.76	6229.46	69951.91
Cl	1743.53	9470.57	6780.53	2144.53	6534.32	7637.41	5650.59	7103.51	15544.75	11176.52
K	2264.58	18198.78	13719.92	10682.35	11323.47	15231.36	11490.08	13203.24	19578.21	9964.77
Ca	329184.88	3075.39	2872.97	15359.53	3095.63	1135.64	10415.86	1200.08	2337.00	16093.65
Ti	3.85	N/D	5.25	211.56	N/D	N/D	1.51	0.79	N/D	26.02
Cr	16.66	29.26	33.60	20.22	18.59	16.09	24.89	24.60	43.75	180.29
Mn	66.99	90.27	37.31	587.40	63.11	42.18	32.43	36.76	51.78	53.21
Fe	211.18	2420.83	914.83	2297.56	2843.52	50753.45	688.36	1713.61	3752.58	955.16
Co	0.80	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Ni	16.11	9.22	4.35	4.29	4.41	N/D	0.92	8.17	14.75	87.29
Cu	0.59	31.97	4.69	21.42	335.17	N/D	7.23	26.58	15.35	3.74
Zn	197.83	263.70	172.87	281.15	295.21	160.27	262.40	114.46	117.75	109.07
As	0.20	N/D	N/D	0.42	N/D	N/D	N/D	N/D	N/D	N/D
Se	0.09	9.75	N/D	N/D	0.20	N/D	N/D	N/D	N/D	N/D
Rb	6.93	57.47	41.91	48.01	43.91	39.70	43.99	44.27	46.70	17.58
Sr	151.96	0.70	N/D	47.67	0.53	2.63	3.82	N/D	N/D	2.72
Zr	0.29	N/D	N/D	5.53	N/D	N/D	N/D	N/D	N/D	N/D
Mo	3.48	6.50	5.90	5.02	6.51	5.07	5.05	5.76	6.22	6.61
Ag	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Cd	0.51	5.04	N/D	0.61	0.48	N/D	N/D	N/D	N/D	0.25
Sn	N/D	N/D	N/D	N/D	0.52	N/D	2.35	N/D	N/D	1.42
Sb	1.37	2.48	4.25	2.90	2.96	3.27	4.54	4.50	2.00	2.74
Ba	114.65	8.07	1.81	50.53	2.45	N/D	5.37	6.34	7.21	36.95
Hg	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pb	8.21	7.83	7.27	15.75	5.12	3.04	5.60	6.92	6.71	1.30

Table 2. Average values of elements. The number in brackets shows the number of examined samples.

Sample type	HT	WT	Total
Frequencies of positive samples	136	2	138
Percent of total	43.871 %	0.645 %	44.516 %
Frequencies of negative samples	159	13	172
Percent of total	51.290 %	4.194 %	55.484 %
Column totals	295	15	310
Percent of total	95.161 %	4.839 %	

Table 3. Contingency table of Pb measurements with statistical values between localities: HT - High Tatras and WT - West Tatras (Fisher's exact test, $p = 0.0102$).

Sample type	HT	BT	Total
Frequencies of positive samples	136	4	140
Percent of total	42.633 %	1.254 %	43.887 %
Frequencies of negative samples	159	20	179
Percent of total	49.843 %	6.270 %	56.113 %
Column totals	295	24	319
Percent of total	92.476 %	7.524 %	

Table 4. Contingency table of Pb measurements with statistical values between localities: HT - High Tatras and BT - Belianske Tatras (Fisher's exact test, $p = 0.0037$).

(P, S, Cl, K, Ca, Mn, Cu, Zn, and Mo) simultaneously increased or decreased. The effect of factor 1 was not significantly different in adults than juveniles (Fig. 8) nor between generations (One-way ANOVA, $F(1, 23) = 0.44832$, $p = 0.50980$). Concentrations of P and Cl at factor 2 increased with a simultaneous decrease in Mo and Pb. We also noted an increasing concentration of Cd and Se in kidneys with a simultaneous decrease in Ca, Mn and Ba (Appendix 2, factor 3).

Tongue

There was no significant difference between generations and sexes among factors 1, 2, 3 and 4 (Mann-Whitney U Test, $p < 0.05000$). Appendix 3 shows that factor 5 showed the most significant relationship between the content of elements in a generation ($p = 0.02$) and sex ($p = 0.01$). Higher negative correlation occurred in juvenile individuals. Greater prevalence of positive correlation was seen in adult individuals

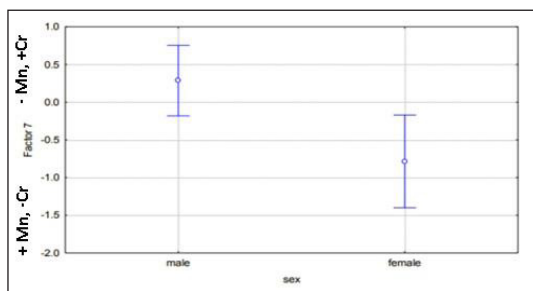


Fig. 5. Concentrations of Cr, Mn in male and female bones of Tatra chamois, based on PCA values (One-way ANOVA $F(1, 28) = 8.0707$, $p = 0.00829$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

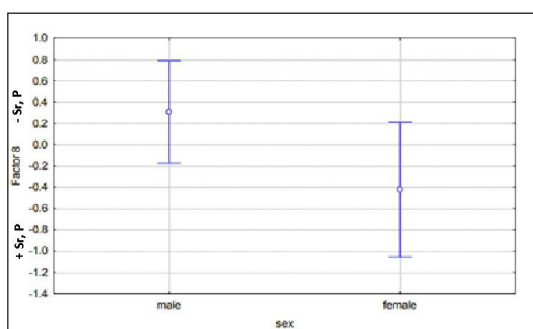


Fig. 6. Comparisons of concentrations of elements (Sr, P) between male and female sex in bones of Tatra chamois, based on PCA values (One-way ANOVA $F(1, 69) = 4.0112$, $p = 0.04913$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

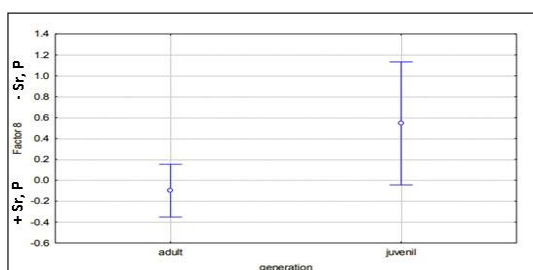


Fig. 7. Difference in concentration of element (Sr, P) between adult and juvenile generation in bones of Tatra chamois, based on PCA values (One-way ANOVA $F(1, 69) = 4.0112$, $p = 0.04913$). The means of the principal components scores (PC1) with their 95 % confidence limits are compared.

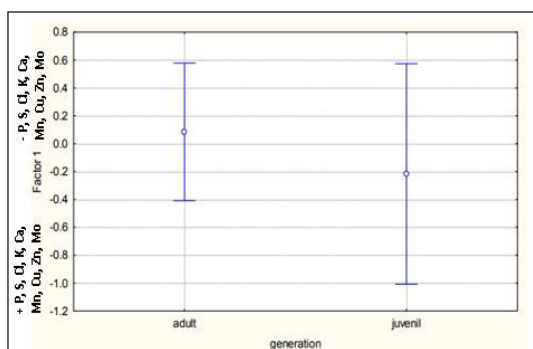


Fig. 8. Mutually decreasing or increasing concentrations of elements (P, S, Cl, K, Ca, Mn, Cu, Zn, Mo) in kidneys of Tatra chamois based on principal component coordinates of PCA analysis (One-way ANOVA, $F(1, 23) = 0.44832$, $p = 0.50980$).

(Fig. 10). Expression of factor 5 was also significant within individual sexes ($p = 0.01$). Male values showed a negative correlation in more than 75 % of the investigated samples (Fig. 9). Approximately 75 % of female values had a positive correlation. Some elements, including Ca, Rb and Ba were higher in females than in males.

Feces

In the analysis of feces samples, the only significant result was the effect of factor 2 between sexes (Mann-Whitney U Test, $p = 0.03$). There was no significant relationship between the generations. Females had higher amounts of Ca, Zn and Sr and lower concentrations of Cr than males, who exhibited the opposite correlation (Fig. 11). Factor 2 had a variance of 17.98 %. A negative correlation was seen in Cr and a positive correlation was observed in Ca, Zn, and Sr (Appendix 4).

Liver

The effect of factor 6 was the most notable. The variance of the samples was 4.58 %. This factor was characterized by the negative correlation of Zn and the positive correlation of Cu (Appendix 5). Factor 6 did not show up significantly between generations, nor between sexes, but it does have an important effect. In male tissues, the greater content of Cu is reflected in Fig. 12. These results indicate that copper content is higher in males than in females, while the content of Zinc is higher in females than in males.

We did not find differences between sexes or age groups in synergic contamination of elements when we examined spleen, muscle, heart, lung or hair tissues.

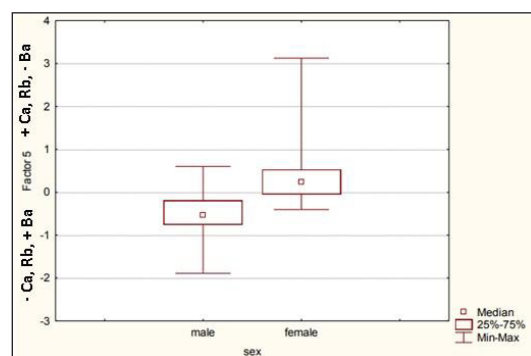


Fig. 9. The box plot graph compares median factor coordinates. Rb, Ca and Ba were higher in females compared to males.

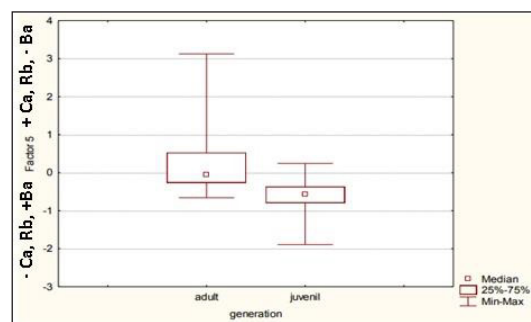


Fig. 10. The box plot graph compares median factor coordinates. Ca and Rb were higher in adult individuals.

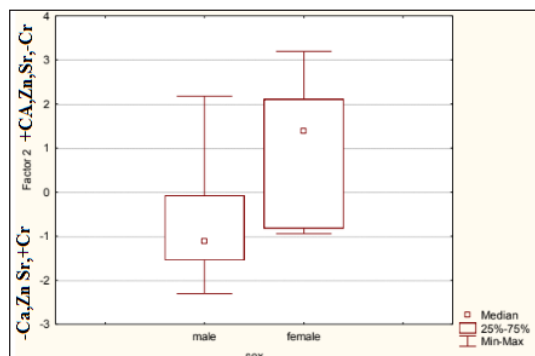


Fig. 11. The box plot graph compares median factor coordinates. Ca, Zn and Sr was higher in females.

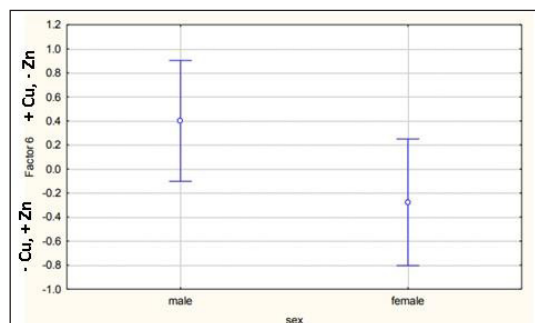


Fig. 12. LS Means with 0.95 confidence limits of factor coordinates. Male liver contained proportionally more Cu and Zn than female liver tissue (One-way ANOVA, $F(1, 19) = 3.7851$, $p = 0.06666$).

Discussion

Concentration of elements in hard tissues (bones)

We found significant increases of P, Ca and Rb in the bones of chamois. The metabolism of calcium and phosphorus is linked, so these two elements should not be considered separately. Both are very important in tooth and bone metabolism. Calcium is an essential nutrient, necessary for growth and development. It builds the skeleton and helps to prevent many skeletal disorders during adolescence and childhood. Phosphorus, along with other elements such as Zn, Pb, Sr, Ca, are accumulated in higher concentration in skull bones than in teeth (Matkovic 1991, 1992; Matkovic *et al.* 1990). The increased amount of Rb can be a result of a higher concentration in the Earth's crust at a given location, where it contributes to the soil and is ingested by animals. The lowest Rb content of the flora was determined on keeper weathering soils. The Rb content of plants is subject to remarkable variation based on the geological origin of the soil when compared to other trace elements (Nyholm and Tyler 2000). In factor 2, strontium follows the physiological pathway of calcium, with 99 % of its content present in bone, it is thus considered essential and associated with the occurrence of calcium (Wadhwa and Care 2000). The animal body absorbs strontium as if it was calcium. The metabolism of strontium is similar to calcium, but in general, animals utilize and retain strontium less effectively than calcium (Comar 1967; Comar and Wasserman

1964). Strontium is thought to play a critical role in bone health. It tends to migrate to sites where active remodeling is taking place and promotes mineralization of bones and teeth. In fact, because of its similarities, strontium is capable of replacing a small proportion of calcium in calcified crystals of bone and teeth. As it appears, strontium adds strength to these tissues, making them more resistant to breakdown. Strontium also appears to draw extra calcium into the bone. The presence of these elements (Ca, P and Sr) is linked (Alina 2013). Another interesting element we measured in bones was Rb. Elements which are absorbed in the body through digestion don't only have a plant origin. Elements from the soil, including lead, are absorbed through the intestines of organisms.

Mn and Cr were also detected in the bones of chamois. For plants, manganese availability in soil increases with decreasing soil pH and decreases with increasing organic content (Schulte and Kelling 1999). The primary source of manganese to chamois was plants. Schroeder *et al.* (1966) confirm in their study that all human tissues contain manganese in concentrations remarkably constant throughout most of life. Chromium is an element that occurs naturally in both animals and humans, and is necessary for life. Chromium is responsible for preserving bone material by reducing the loss of calcium in urine, promoting collagen production, increasing adrenal DHEA levels and improving insulin regulation. It helps the skeleton regulate energy metabolism. This complex process includes osteocalcin (a hormone secreted by the bone-building osteoblast cells) acting on the pancreas to enhance insulin production and in peripheral tissues to increase glucose (Anderson 1987). In female chamois, when Mn concentration in bones increases, chromium decreases and males present the opposite correlation. Because these elements are essential for bodily function, interactions between them may represent complex metabolic and physiologic processes.

Heavy metals in bones

The main factors affecting the accumulation of potentially-toxic metals by grazing animals are the presence of the metal, its concentration at the soil surface or in herbage, and the duration of exposure to the contaminated pasture and soil. With regard to heavy metals, we only found Cd in two bone samples and As in one bone sample out of 352 total chamois samples. Hg was not found in any samples. Lead was present in most samples. Lead accumulation in plants and animals in the alpine zone is a highly discussed topic in many studies. Lead accumulates in vascular plants primarily through the leaves and to a lesser degree in the roots (Kabata-Pendias and Pendias 1984). Janiga (1998) describes the role of lead as a pollutant that is transported several thousand kilometers depending on meteorological conditions. Wind speed and prevailing direction play a very important role in heavy metal transport in the mountains. In high altitude habitats, where chamois occur, north-western and western winds mainly prevail in the West and High Tatras (Konček *et al.* 1973). Janiga *et al.* (1998) confirmed that lead is deposited

in the Tatras, which represents a barrier to pollutants from the northern part of central and east Europe. About 50 % of lead is physiologically accumulated in vascular plants through their tissues and the other 50 % is washed away (Bednářová and Bednář 1978). Additionally, most heavy metals stored in soil are not natural but have an anthropogenic origin. Heavy metals (cadmium and lead) are found more frequently in the upper levels of soil than in the lower levels, due to emissions (Ewers and Schlipkoter 1991). Lead as the only heavy metal present in multiple samples, and in general is considered to be a major contaminants that enters the food chain. It has a negative impact on the environment, so its use has been banned in most countries (Greenwood 1993). We can also confirm high lead deposition in the West Carpathians in ruminant *Rupicapra rupicapra tatrica* populations. In the contingency tables (Table 3 and 4) we processed the frequency of lead accumulation according to localities. The highest lead accumulation was recorded in samples from the High Tatras. Pb levels in Tatra lake sediments increased in the samples from the 20th century, and were likely deposited as a result of air pollution due to the development of road transportation (Szarlowicz 2013). Pb, as a pollutant, can be transported from Zn-Pb smelters due to north-western winds in the Tatra mountains (Steblez 2005). Lead has not been mined recently in surrounding areas (including the Czech Republic), and mining and smelting of lead were recently reduced in Poland.

Concentration of elements in soft tissues and feces.

The majority of kidney samples came from juvenile individuals. We surmise that stress hormones could be affecting young individuals to a greater degree in their interactions with tourists, skiers or rescue helicopters and further impact could also have an environmental burden. However, such an impact would depend on an increased metabolic rate in young individuals, and is as yet unconfirmed in Blagojević *et al.* (2012), where the authors state that at early age stages, due to increased metabolic rate, heavy metals are more rapidly accumulated. Bioaccumulation may also be affected by the general fitness of young animals. Therefore, it is not surprising that bioaccumulation is more effective at an unpolluted locality, due to the better condition of the animals (on average). At that age (neonates and juveniles) bioaccumulation is mainly affected by the high rate of metabolism, no matter how high the concentration of heavy metals is in the environment. Moreover, the higher metabolic rate of juveniles, implying a high uptake of food, may explain the increased amounts of xenobiotics, such as Cd and Pb, from polluted areas (Blagojević *et al.* 2012). With regard to cadmium, plant absorption has been shown to be significantly easier than absorption of lead, which may also contribute to greater food-borne cadmium toxicity (Ye *et al.* 2015). In tongue tissue, we found males had higher Ba content and lower Ca and Rb content, while females had higher Ca and Rb content in the tongue and lower Ba content. Prasad (2004) claim significant variation in arsenic intake occurs between sexes in bank voles,

where they found stomach arsenic concentrations to be higher in females than in males. Interestingly, this gender difference couldn't be explained on the basis of dietary preference, as both sexes of bank vole ingest similar diets. The impact of sex on trace element concentrations in sample species cannot be fully attributed to behavioural characteristics and thus requires further investigation. In feces, the accumulation of Ca and Cr. Ca and Cr was found, which are essential elements that the body regularly excludes. We cannot infer why the content of these elements correlated with sex, and to do so a further study would be required.

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Appendix 1

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
P	0.809	0.045	0.117	-0.195	0.319	-0.049	-0.157	-0.329
S	-0.367	0.001	-0.787	-0.115	0.065	0.124	-0.033	-0.263
Cl	-0.194	-0.116	-0.796	-0.063	0.313	-0.098	0.049	0.257
K	0.570	0.012	-0.645	-0.054	-0.031	-0.065	-0.313	-0.090
Ca	0.870	-0.178	0.204	0.087	0.229	-0.011	-0.121	-0.184
Cr	0.639	-0.240	-0.067	-0.273	0.397	0.255	0.396	0.094
Mn	-0.303	-0.370	0.184	-0.063	0.225	0.669	-0.447	0.159
Fe	0.330	-0.474	-0.005	-0.703	-0.143	0.105	0.240	0.084
Cu	-0.202	-0.595	0.198	-0.522	-0.185	-0.300	-0.230	0.067
Zn	-0.196	-0.885	-0.117	0.042	-0.184	-0.091	-0.051	-0.003
Rb	0.783	-0.198	-0.166	0.268	-0.171	-0.182	-0.168	0.266
Sr	-0.462	-0.693	0.085	0.043	0.178	-0.214	0.062	-0.328
Mo	0.657	-0.326	-0.071	0.311	-0.432	0.088	0.068	0.098
Ba	-0.102	-0.442	0.136	0.482	0.634	-0.230	0.027	0.195
Pb	0.006	-0.525	-0.142	0.532	-0.246	0.322	0.189	-0.222
TV %	25.81	18.04	12.65	10.67	8.45	5.99	4.57	4.06
CV %	25.80	43.85	56.50	67.17	75.62	81.61	86.18	90.24

Appendix 1. Factor coordinates of the variables, based on correlations of elements in bones of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold.

Appendix 2

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
P	-0.688	0.606	0.134	-0.127	-0.168	0.191	-0.044	0.004	-0.108
S	-0.767	0.314	0.169	0.268	0.059	-0.007	0.213	0.285	-0.096
Cl	-0.584	0.695	-0.049	0.016	-0.282	0.168	-0.126	0.019	0.038
K	-0.876	0.286	-0.055	-0.147	-0.305	0.111	-0.013	0.019	-0.036
Ca	-0.601	-0.290	-0.605	0.185	0.190	0.175	0.119	0.039	-0.217
Cr	-0.589	0.142	0.134	0.531	0.013	-0.293	-0.241	-0.411	-0.047
Mn	-0.718	-0.379	-0.523	0.047	0.132	-0.001	0.087	-0.066	-0.073
Fe	-0.408	0.224	0.063	-0.688	0.184	-0.011	0.366	-0.357	0.029
Cu	-0.765	-0.312	0.288	-0.174	-0.013	-0.157	-0.121	0.222	0.154
Zn	-0.783	-0.309	0.364	-0.169	0.139	0.074	-0.127	0.014	0.112
Se	-0.412	-0.009	0.664	0.308	0.178	-0.267	0.381	0.043	-0.046
Rb	-0.432	0.106	0.112	-0.160	0.782	0.155	-0.327	0.043	-0.039
Mo	-0.730	-0.523	0.157	0.061	-0.284	0.011	-0.121	-0.149	-0.024
Cd	0.295	-0.177	0.442	0.387	0.054	0.690	0.124	-0.142	0.078
Ba	-0.546	0.271	-0.565	0.329	0.154	-0.016	0.140	-0.007	0.380
Pb	-0.574	-0.735	-0.025	-0.134	-0.279	0.109	0.063	0.025	0.036
TV %	39.83	15.35	12.04	8.53	7.15	5.06	3.85	3.01	1.71
CV %	39.83	55.18	67.22	75.75	82.90	87.96	91.81	94.82	96.53

Appendix 2. Principal component weights of the first nine factors of PCA of elements in kidneys of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 1 express 39.83 % of total variance.

Appendix 3

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
S	-0.800	0.355	0.047	0.161	-0.164	-0.138	-0.209	0.207	-0.249
Cl	-0.426	0.792	-0.204	-0.070	-0.056	0.043	0.303	0.029	0.182
K	-0.764	0.401	-0.290	0.153	-0.105	0.132	0.324	0.058	0.009
Ca	-0.014	-0.122	0.654	0.058	0.629	-0.082	0.303	0.239	-0.013
Cr	-0.251	0.657	0.467	-0.291	-0.034	-0.190	0.103	-0.340	-0.187
Mn	-0.933	-0.060	0.057	0.184	-0.065	-0.047	-0.119	0.199	-0.011
Fe	-0.530	0.532	0.282	-0.237	0.226	0.060	-0.416	0.002	0.241
Cu	-0.339	-0.449	-0.122	-0.547	-0.191	-0.543	0.131	0.092	0.089
Zn	-0.794	-0.494	-0.077	-0.126	0.144	0.003	-0.141	-0.088	0.185
Rb	0.013	0.228	-0.507	0.474	0.480	-0.449	-0.073	-0.164	0.022
Mo	-0.814	-0.417	0.140	0.061	0.155	0.195	0.060	-0.246	0.006
Ba	0.051	-0.106	0.601	0.561	-0.468	-0.223	0.019	-0.079	0.172
Pb	-0.781	-0.558	-0.060	0.104	-0.021	0.073	0.142	-0.120	0.004
TV %	35.75	20.35	11.71	8.50	7.90	5.21	4.61	2.96	1.76
CV %	35.75	56.10	67.81	76.31	84.21	89.42	94.03	96.99	98.75

Appendix 3. Factor coordinates of the variables, based on correlations of elements in tongue of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 5 express 7.90 % of total variance.

Appendix 4

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
S	-0.331	-0.113	0.691	-0.535	-0.063	0.211	-0.005	0.058	-0.146
Cl	-0.703	0.219	-0.311	-0.293	0.112	-0.218	0.314	0.312	0.037
K	-0.800	0.134	0.241	-0.344	0.022	-0.311	0.120	-0.042	0.054
Ca	-0.146	0.862	0.008	-0.079	0.261	0.020	-0.266	0.006	-0.249
Cr	-0.474	-0.501	0.375	-0.201	0.255	0.458	-0.034	-0.082	0.137
Mn	-0.724	0.448	-0.298	-0.034	-0.035	-0.054	-0.075	-0.359	0.127
Fe	-0.797	-0.377	0.144	0.272	-0.140	0.017	-0.231	0.103	-0.037
Cu	-0.060	0.346	-0.217	-0.324	-0.796	0.277	-0.049	0.037	0.021
Zn	-0.314	0.584	0.151	0.337	0.081	0.443	0.435	-0.084	-0.069
Rb	0.024	0.201	0.821	0.055	-0.189	-0.450	0.064	-0.135	0.020
Sr	0.187	0.560	0.536	0.495	-0.084	0.068	-0.096	0.221	0.180
Mo	-0.821	-0.282	-0.089	0.363	-0.135	0.001	0.190	0.008	-0.031
Ba	-0.822	0.313	-0.108	-0.034	0.174	0.074	-0.311	0.102	0.136
Pb	-0.800	-0.320	-0.040	0.389	-0.159	-0.095	-0.088	-0.023	-0.179
TV %	34.18	17.98	13.85	9.77	6.64	6.43	4.23	2.40	1.53
CV %	34.18	52.16	66.01	75.78	82.42	88.85	93.08	95.48	97.00

Appendix 4. Factor coordinates of the variables, based on correlations of elements in feces of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 2 express 17.98 % of total variance.

Appendix 5

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
P	0.886	-0.189	0.080	-0.208	0.155	-0.060	0.059	-0.254	0.003
S	0.727	0.132	-0.235	-0.368	-0.304	-0.081	0.344	-0.058	-0.156
Cl	0.880	-0.076	-0.087	-0.165	0.203	0.158	-0.268	0.047	-0.042
K	0.925	0.061	0.213	0.062	0.099	0.094	-0.132	-0.128	-0.117
Ca	0.106	-0.122	0.696	-0.498	0.407	-0.114	0.140	0.057	0.181
Cr	0.751	-0.426	-0.172	0.110	-0.321	0.002	0.158	0.133	0.196
Mn	0.876	-0.206	0.068	0.355	0.014	-0.086	-0.051	0.100	-0.005
Fe	0.626	0.478	-0.099	-0.502	-0.091	0.100	-0.107	0.256	-0.021
Cu	0.243	0.771	-0.140	0.202	0.102	0.459	0.133	-0.096	0.185
Zn	0.253	0.759	-0.104	0.014	-0.155	-0.517	-0.167	-0.081	0.139
Rb	0.027	0.134	-0.676	0.119	0.658	-0.182	0.160	0.093	-0.062
Mo	0.769	-0.138	0.118	0.578	0.066	-0.085	0.043	0.036	0.046
Pb	0.034	0.563	0.701	0.336	-0.002	-0.038	0.153	0.109	-0.167
TV %	41.55	15.85	12.61	10.41	7.07	4.58	2.83	1.70	1.54
CV %	41.55	57.39	70.00	80.41	87.48	92.06	94.89	96.59	98.13

Appendix 5. Factor coordinates of the variables, based on correlations of elements in liver of Tatra chamois (TV % - total variance %; CV % - cumulative variance %). The most important correlations are in bold. Factor 6 express 4.58 % of total variance.

Biology of alpine accentor (*Prunella collaris*) VI. Interspecific relationships among alpine accentors and other species of birds during winter

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Abstract. Observations were carried out on winter aggregations of alpine accentors. This species of bird was more likely to feed at dawn and dusk. During winter, regular feeding groups of different species of birds congregating at the same time at feeding sites, were consistent in composition and space. Dominant hierarchies formed that were weight related. The following ranking orders of dominance were observed among different species of birds (> - indicates a higher position in the dominance ranking, = indicates an equal position): *Pica pica* > ***Prunella collaris*** > *Dendrocopos major* > *Chloris chloris* => *Fringilla coelebs* = *Emberiza citrinella* => *Parus major* > *Poecile montanus*, ***Prunella collaris*** > *Picus canus*, *Garrulus glandarius* > ***Prunella collaris***, *Corvus corax* > ***Prunella collaris***, *Nucifraga caryocatactes* > ***Prunella collaris***, *Turdus pilaris* > ***Prunella collaris***, *Turdus merula* > ***Prunella collaris*** => < *Parus major* > *Periparus ater* > *Cyanistes caeruleus* > *Lophophanes cristatus*, ***Prunella collaris*** > *Sitta europaea* > *Periparus ater*, *Erithacus rubecula* > *Chloris chloris* => *Emberiza citrinella*, *Coccythraustes coccythraustes* > *Emberiza citrinella*. Additionally, the possible transmission of diseases between different bird species, within different ecosystems, and in the food chain between accentors and birds of prey during autumn is discussed.

Key words: alpine accentor, *Prunella collaris*, dominance and hierarchy among bird species, winter flocks, supplementary feeding

Introduction

Food availability is one of the most influential factors affecting bird subsistence and as a result many authors assume that supplementary feeding enhances survival and fitness in birds. However, the short-term effect of supplementary feeding may also correlate negatively with natural selection. Supplementary feeding can alter body condition, reproduction, survival, diseases prevalence, and migration in bird species (Plummer *et al.* 2015; Galbraith *et al.* 2017a). In

addition, the practice of garden bird feeding often activates interspecific interactions between bird species that would normally not interact in the absence of contact at these supplementary bird feeders. Thus, the sites where birds are fed are not only the locale of their atypical concentration, but also the location for increased transmission of infectious diseases (Timko and Kmeť 2003; Novotný *et al.* 2007). Based on natural selection, such a locality may also impose a potential threat from unknown predators. The ecological effects of a dense concentration of birds at feeders remains poorly understood (Robb *et al.* 2008), though supplementary feeding has increased rapidly since the second half of the 20th century, and current estimates suggest that in some countries more than 50 % of households repeatedly feed wild birds (Cowie and Hinsley 1988).

The alpine accentor is a passerine species living in alpine regions. It is a trans-migratory and partially migratory species, making significant local migrations throughout winter. Adult males are commonly observed throughout the year near breeding sites, forming changing aggregations of one to three individuals. At lower elevations, several hundred kilometers from breeding sites, aggregations often involve more individuals. Birds forage mainly in rocky areas of the mountains, with larger groups also foraging in lower altitude pastures. Feeding on artificial resources within human proximity - at bird feeders or near settlements and winter resorts - usually represent only a small fraction of foraging time. At bird feeders, alpine accentors encounter various other species of birds and mammals, and these encounters are only possible during winter. This study presents a list of many species that accentors encountered over many years of research. In some cases, interactions are described in more detail, particularly in terms of dominance or daily behavioural cycles. Competitive interactions amongst many passerine and other bird species were recorded in order to verify the dominance hierarchy between alpine accentors and other bird species. The importance of body size as a determinant of rank in the hierarchy was also examined.

Material and Methods

This study describes how alpine accentors and other bird species compete for food during winter. Field work was conducted at two sites. At the first site, accentors were studied between 1984 and 2005 in the Great Fatra Mountains National Park, at the

Malinô Brdo ski resort. Birds were monitored from the beginning of November to the end of April. Details on the number of birds seen in respective years and the number of visits per year are presented in Janiga (2020). In the majority of years, the presence of birds was dependent on heavy snow and deep snow cover in the mountains, but in some years, birds had a continued presence at the resort on sunny days when snow cover disappeared in many areas. Accentors foraged near hotels, pensions and restaurants. Daily visits lasted a minimum of five hours, but many visits lasted from dawn till dusk. To study feeding preferences (Janiga and Novotná 2006), a feeder with several sections containing different dietary sources was positioned near the hotel where the birds also roosted, preened or rested. Supplementary food was provided ad libitum. Sections of different food in the feeder ensured that foraging behaviour was not influenced by the location of high and low value foods. 27 accentors were captured with a cheese/millet-baited falling trap. Each bird was individually marked with colour rings. The majority of birds were individually distinguished and re-sighted over the next years. Binoculars of various powers were of assistance for observation. Notes on behaviour were simply written or dictated onto tape. Competitive interactions and visits of accentors and other bird species were video recorded, and the recordings were later analysed in the laboratory.

At the second site, two wintering accentors were systematically monitored at the Spišská Magura, near the village of Ždiar (Antošovský hill). The presence of accentors was initially detected from footprints; the size and shape of which usually differed from other bird species. (Fig. 1).



Fig. 1. The footprints of alpine accentors are specific and different from those of many other bird species.



Fig. 2. Experiments utilizing free feeding of accentors without the presence of other bird species. Trophic Cameras (left) and supplementary feeders (right) were used.

Birds were also attracted to feeders by a large winter flock of yellowhammers (*Emberiza citrinella*). The period of observation lasted from the beginning of December to the end of March in winters 2018/2019 and 2019/2020. Four Bushnell Trophy Cameras were used to take pictures and continual video recordings (Fig. 2). Maintenance activities (feeding, resting, preening, interactions, etc.) of birds were recorded 24 hours per day over 80 days. Data collection took place each day (weather permitting). The two general forms of competition among different species of birds are interference competition and exploitation. Interference competition occurs when competing species confront directly (usually at bird feeders). Exploitation competition occurs when species use the same resources but do not inhibit each other. Both exploitation competition (e.g., Gustafsson 1987) and interference competition (Slagsvold 1975) seem to be common between different passerine bird species. Interference competition of accentors was recorded at the bird feeder. Both exploitation competition and more complex behaviours were recorded in an enclosed yard where accentors were provided with their favorite diet and were not disturbed by other bird species (Fig. 2). Aggressive interactions between birds can take several forms, but this study focussed on competitive interactions. This included subtle interaction, when one bird waited for another to finish feeding as well as displacement, when one bird suddenly arrived and frightened off other birds, or displaced or chased another from a perch at or near a bird feeder.

Results

Winter flocks are characteristically formed by the association of birds that nest separately, and thus often consist of several species. The results of this study, based on direct or recorded observations of interactions at bird feeders, demonstrate that body size differences amongst different species of birds significantly influence patterns of feeding behaviour and the ability to monopolise food resources. Accentors are a good example to illustrate this phenomenon.

Coal tit (Periparus ater) and crested tit (Lophophanes cristatus)

Although coal tit sometimes successfully managed to usurp food, successful takeovers were rare and were often associated with waiting or desertion of feeding opportunities. Accentors always gained the



Fig. 3. Coal tit and alpine accentor at feeder with different kinds of food (left). Coal tit had precedence over crested tit (right).

best position when feeding. Fifteen interactions between accentors and coal tits were recorded. In eleven cases, the tits avoided accentors, while in three others they moved close to the accentors with a threat display but did not feed (Fig. 3). In one case the tit was driven away.

When a number of species join forces, there is usually a pecking order amongst them. This can be easily observed at bird feeders where the competition is high. Alpine accentor always maintained precedence over coal tit, while coal tit had precedence over crested tit. Coal tit avoided Eurasian nuthatch, while crested tit avoided alpine accentor.

Blue tit (Cyanictes caeruleus) and crested tit (Lophophanes cristatus)

Alpine accentor had precedence over blue tit at both sites (Malinô Brdo and Ždiar) in multiple

years (Fig. 4). But the type dominance varied from day to day. Out of 18 recorded interactions, the tit flew away five times and waited 10 times at a short distance. In three cases, when the tit distinguished no danger from the accentor, it gradually began to feed alongside this bigger species. In general, dominance rank is determined by body mass across species, and heavier species monopolise access to food. However, in contrast to this general theory, some individuals of lighter species may be seen feeding with larger birds (Fig. 5). The strategy of mutual feeding between two bird species of different size is based on experience. These findings suggest that a species' position within the bird hierarchy can influence its experience, and thus this factor must be taken into account in studies on social hierarchy in birds. It was also observed that the accentor learned to respond to the alarm call of the blue tit.



Fig. 4. Blue tit usually waited at the distance 10 – 20 cm until the accentor was fed.



Fig. 5. The blue tit fed only when the alpine accentor was no longer interested in the food source. After tits experienced non-antagonistic accentor behaviour, both species fed together.

Blue tit always exhibited precedence over crested tit. Crested tit avoided more aggressive blue tit or waited some distance from the feeder until the blue tit fed (Fig. 6). When closely related species have similarities in foraging preferences, it is highly probable that dominance hierarchy between these species is a result of evolutionary history. Blue tit always avoided great tit.

Willow tit (Poecile montanus)

The Willow tit is a relatively aggressive species and can actively chase smaller species of birds. The tit avoided accentors, but usually only from a short distance (Fig. 7). At the feeder, however, the Willow tits always avoided great tits, and avoided but sometimes also defended against yellowhammers or European greenfinches.

Great tit (Parus major)

At both field sites, a total of 68 interspecific interactions between great tits and accentors were recorded at supplementary feeders. In general, it was confirmed that dominance is determined by body mass between these two species. Tits tended to avoid accentors, particularly at the second locality (Ždiar), where they flew away or waited several metres away (35 observed interactions, Fig. 8). At the first site (Malinô Brdo), heavier accentors also frequently monopolised access to the food source (7 interactions, Fig. 8 – right). However, on occasion, lighter great tits were able to defend their food (Fig. 9), particularly when subordinate accentor specimens (in the hierarchy of a flock of accentors) chose to feed on a food source preferred by tit. Although body size is an important factor in food availability, this may not be true if the



Fig. 6. The crested tit avoided blue tit and waited until there was free access to the feeder.



Fig. 7. Examination of interactions around species' ranks suggests that Willow tit are considerably more dominant than expected given their body mass. In the figure on the left, the tit attacks the yellowhammer, while accentors continually maintained precedence over tits (right).



Fig. 8. Interactions between alpine accentors often depend on individual birds. At the second experimental field site, both male and female tits avoided accentors. At the first experimental site, female tits usually waited near the feeder for the accentor to feed (right).



Fig. 9. Interactions between different species of birds is dependant on individual birds. Through agonistic threat displays (above – right and left) smaller species or some individuals of smaller species may gain control of limited resources (right bottom). Sometimes two accentors recognized this position and accepted it (left bottom).

food source is limited. In this case, some individuals - even from smaller species - are able to defend their food source against a larger species. This study suggests that these differences in body mass often result in the creation of an interspecies dominance hierarchy, granting the heaviest species the greatest control of feeding sites. However, in select cases, smaller species have been shown to gain control of limited resources through agonistic interactions, including threat displays (Fig. 9) or physical combat. Six interactions were recorded when great tits exhibited precedence over alpine accentors. In 20 cases, tits and accentors behaved neutrally, feeding without evidence of dominance between individual birds.

In this relationship between accentors and great tits, the tits tend to find new food sources earlier and faster than accentors. During early morning, tits arrived earlier and often scattered food around the feeder, while accentors usually arrived later in the day and first collected scattered millet before utilizing the feeder. While

scavenging, they mirrored the feeding pattern of tits.

Eurasian nuthatch (Sitta europaea), *Eurasian blackbird (Turdus merula)* and *fieldfare (Turdus pilaris)*

Nuthatch spent less time feeding at the supplementary feeder during each visit than accentors, as they had to spend more time waiting in close proximity to feeding sites until feeding opportunities arose. Dominance in interactions often depended on individual birds. 13 interactions were recorded. The accentors actively pushed the nuthatch twice. When less food was available, stress was higher and direct aggressive contact between these two species was more likely to occur. The nuthatch avoided the accentor six times, while accentors ran and flew away from nuthatches twice, and three other interactions were neutral (Fig. 10). However, accentors consistently avoided blackbirds (three interactions observed, Fig. 11) and fieldfares (two interactions observed).



Fig. 10. Nuthatch may benefit from feeding quickly; reducing the amount of time spent on feeders to avoid costly interactions with dominant accentors.



Fig. 11. Consistent with expectations, the dominant blackbirds were more likely to be observed utilising supplementary diets than less dominant accentors.

Yellowhammer (Emberiza citrinella)

The bird feeder in Ždiar is used every winter by a flock of more than one hundred yellowhammers. Thousands of photo-trap records were obtained. Some specimens were found to feed very close to accentors, but accentors can usually reach the food source first due to their greater size and more dominant behaviour (Fig. 12).

The usual pattern for wintering birds is for submissive individuals to stand just out of range of a feeding neighbour's attack, as a method of avoidance behaviour. For individual yellowhammers, this minimum closure distance tends to be more consistent, resulting in the yellowhammers encircling the accentors, whereby movement of the circle is dependent on movement of the accentors (Fig. 13).

European greenfinch (Chloris chloris) and common chaffinch (Fringilla coelebs)

There was considerably less inter-species competition directly below the feeder, where food was scattered by both birds and the elements. This allowed subordinate species, like greenfinch to gain greater access to the food source during winter, and subordinate species like chaffinch to gain greater access to food during the early spring (Fig. 14). Both species avoided accentors. At the feeders, greenfinch had a slight precedence over yellowhammer and great tits. However, this relationship was more dependent on individuals than on species. In such

a feeding aggregation, each individual has its own place in the flock and accepts that it must give way to more dominant species both within and outside of its position at the feeding site. In many cases, greenfinches, tits or yellowhammers fed together. (Fig. 15). Further more, a bird's dominance over another might alter from day to day.

Crows and woodpeckers

A species' position within the hierarchy of a flock can influence food choice and foraging tactics. At the feeders found at both experimental sites, common raven (*Corvus corax*), spotted nutcracker (*Nucifraga caryocatactes*), Eurasian jay (*Garrulus glandarius*) and magpie (*Pica pica*) were significantly more likely to use the feeders or feeding grounds, compared to less dominant accentors. Encounters were rare (ravens – two times, nutcrackers – five times, jays – two times, and magpies – six times), and accentors always avoided the larger species. Crows can always reach the food source first due to their large body size and domineering behaviour, which can consequently deter other bird species (Fig. 16). This is the way pecking orders or dominance hierarchies are developed, and following the initial jostling for power, these relationships become fairly stable. However, while this tendency may reduce the risk of aggression between individuals, it can also be detrimental to lower-ranking species. Dominance rank was not found to be significantly positively associated with body mass be-



Fig. 12. Dominant, heavier accentors exert greater control over natural foraging sites than subordinate, lighter yellowhammers (left). However, the relationship between some individuals may be more neutral, allowing some yellowhammers to feed very near to accentors (right).



Fig. 13. Yellowhammers create circles around heavier and dominant accentors.



Fig. 14. The relationship of greenfinches (left, right) or chaffinches (right) to accentors was most commonly a consequence of body size. Both species avoided accentors.



Fig. 15. Joint feeding of greenfinches and great tits (left) and yellowhammers and great tits (right).

tween woodpeckers and accentors. Great spotted woodpecker (*Dendrocopos major*) avoided accentors and other birds in large interspecific flocks (yellowhammers, greenfinches), and a grey-headed woodpecker (*Picus canus*) was chased away by an accentor defending access to the feeder with an upraised wing.

Other songbirds

The interaction of accentors with other songbirds that occurred in the experimental plot were never observed or recorded by cameras, because at this time, accentors were no longer present in the plot. The European robin



Fig. 16. Magpies (left) and great spotted woodpeckers (right) frequented the same feeding site as accentors.

(*Erithacus rubecula*), hawfinch (*Coccothraustes coccothraustes*), Eurasian tree sparrow (*Passer montanus*) and bullfinch (*Pyrrhula pyrrhula*) that hold territory in early spring may join up with the flocks of yellowhammers as they pass through. Aggregations of different species at the same feeding site in winter or spring can have serious epidemiological implications. Birds originate or migrate from very different ecological conditions and feeding sites may serve as a source of potential viral and bacterial disease transmission. At the plots, droppings are often mixed with seeds from supplementary food. Robins had precedence over yellowhammers and greenfinches, and yellowhammers avoided hawfinch (Fig. 17). Dominance rank was not found between tree sparrows and yellowhammers or bullfinches and yellowhammers (Fig. 18).

Birds of prey and mammals

Flocks may reduce the chance of attack by predators, and in many groups of birds there seems to be inter-specific recognition of alarm calls; at least between blue tits, coal tits and accentors. On the other hand, flocks draw attention to themselves, and it is often easy for a predator to target one atypical bird from of a flock of yellowhammers. Moreover, accentors seem to be less adapted to more unknown predators in forest environments. While yellowhammers or tits quickly fly up into the trees at the alarm call, the response of accentors was often delayed. On occasion, an accentor remained standing alone in the snow. Birds are not primarily adapted to domestic cats, dogs, or fox. Cats in particular can effectively hunt songbirds under feeders. In February 1992, a cat killed one accentor specimen this way. The birds reacted most effectively



Fig. 17. Yellowhammers and greenfinches created distant circles around robins; robins had precedence over them (left); yellowhammers also avoided hawfinch (right).



Fig. 18. Eurasian tree sparrows (left) and bullfinches were also rare visitors to the feeding site (right).

to attacks by common kestrel (*Falco tinnunculus*) and managed to fly away. Conversely, if they saw the silhouette of a Northern goshawk (*Accipiter gentilis*), they remained motionless. The sites below the feeders were also sporadically frequented by European pine marten (*Martes martes*), least weasel (*Mustela nivalis*), and short-tailed weasel (*Mustela erminea*), but there is no evidence of accentors being caught by these predators during winter.

Information transfer and learning

Feeding advantages of group foraging lie in the number of trained eyes employed in seeking food. Some species are more skilled at foraging than others, and those less skilled often take advantage of what the former have found. In some cases, when a new food source became available at a feeding site, alpine accentors responded, and other present bird species (great tits, woodpeckers) learnt of the new feeding prospect from their behaviour. Conversely, the opposite occurred, wherein flocks of yellowhammers helped accentors find food (Fig. 19).

Factors of weather in winter

Alpine accentors were significantly more likely to seek out food immediately after sunrise and before sunset. Because it requires a relatively short handling time, supplementary diets enable birds to gain a greater energetic benefit at a lower foraging cost. This is the reason accentors place particular emphasis on foraging for valuable foods in the early morning or just before dark-

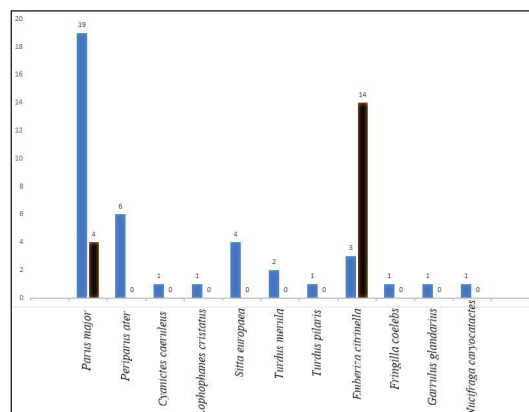


Fig. 19. Recorded number of observations of transmission of information on feeding sites. The left histograms (numbers for each species) show that when a food source becomes available at a feeding site, accentors respond, and some other bird species may learn of the feeding prospect from the behaviour of accentors. Usually these were species exhibiting solitary behaviour during winter. The early morning feeding area likely represented the primary center for directed information transfer. On the contrary, wintering yellowhammers undertake foraging, flight and roosting in aggregations of flocks. It likely that a yellowhammer in a flock not only “feels” safer but actually is safer from most forms of predation in winter. When yellowhammers (*E. citrinella*) located food and started eating, they were quickly joined by accentors (right histograms).

ness. During winter, birds are likely to experience higher thermoregulatory costs overnight due to decreased ambient temperatures. In addition to



Fig. 20. Snowing and windy. During snowstorms (left), the accentors would arrive at the feeding sites early in the morning and rest or feed there all day (right).



Fig. 21. Snow cover. Accentors can accurately locate snow-covered food sources (left). At that time they usually arrive at early dawn (right). It is evident that this adaptation helps them to survive in high-altitude alpine ecosystems.

low temperatures, accentor flights from higher altitudes are caused by snowfall, strong winds and thickness of snow cover (Figs. 20, 21, 22 and 23).

Figs. 24 and 25 show that when these factors act together, the probability of accentors arriving at feeders is very high.



Fig. 22. Snow cover and drinking. Accentors use snow as a water source in winter. Feeding on snow allows them to save energy in freezing periods, as they do not have to search for water sources.



Fig. 23. Temperature. During frosts, accentors can ruffle up their feathers very quickly. It takes them about ten seconds to increase the volume and achieve a suitable "spherical" shape to provide sufficient insulation.

Discussion

Rank orders in winter. When a number of species commonly join forces and hunt together in mixed flocks, there may be a pecking order between them (Popp *et al.* 1990). This can be easily observed in bird tables where competition for available food may be high (Woodrey 1990; Galbraith *et al.* 2017b). In many bird species, a highly significant positive association between dominance rank and body mass across species suggests that the hierarchy is largely driven by variation in body mass or size (Alatalo and Moreno 1987; Cowie and Hinsley 1988; Galbraith *et al.* 2017b). Inter-species interactions at supplemental feeding sites in this study confirmed that birds with greater body mass were more dominant than smaller species. The alpine accentor is the largest species in the family Prunellidae, and also lives at extremely high elevations in the mountains. This alone guarantees non-aggressive accentors a dominant position at feeding sites in winter, when they encounter smaller songbird species. Experimental video-recordings suggest that differences in body mass may lead to the formation of dominance hierarchies between species, leaving the heaviest species in the greatest control of higher value foods at supplemental feeding stations. Lower-ranking species move

quickly to avoid dominant birds, and their foraging tactics may allow them to avoid engaging in costly interactions with dominant birds (Birkhead 2008; Zeng and Lu 2009; Miller *et al.* 2017). However, in other situations, these foraging strategies may impact fitness, as smaller species may be at greater risk of predation than larger more dominant species. The following dominance rank orders were observed among different species of birds (> - indicates a higher position in the dominance ranking, = indicates an equal position): *Pica pica* > *Prunella collaris* > *Dendrocopus major* > *Chloris chloris* => *Fringilla coelebs* = *Emberiza citrinella* => *Parus major* > *Poecile montanus*, *Prunella collaris* > *Picus canus*, *Garrulus glandarius* > *Prunella collaris*, *Corvus corax* > *Prunella collaris*, *Nucifraga caryocatactes* > *Prunella collaris*, *Turdus pilaris* > *Prunella collaris*, *Turdus merula* > *Prunella collaris* => *Parus major* > *Periparus ater* > *Cyanistes caeruleus* > *Lophophanes cristatus*, *Prunella collaris* > *Sitta europaea* > *Periparus ater*, *Erithacus rubecula* > *Chloris chloris* => *Emberiza citrinella*, *Coccothraustes coccothraustes* > *Emberiza citrinella*. Competitive interactions, where one individual is consistently successful, can lead to the formation of a system in which each animal is either dominant or subordinate to another. Accentors required a relatively short handling time to gain a

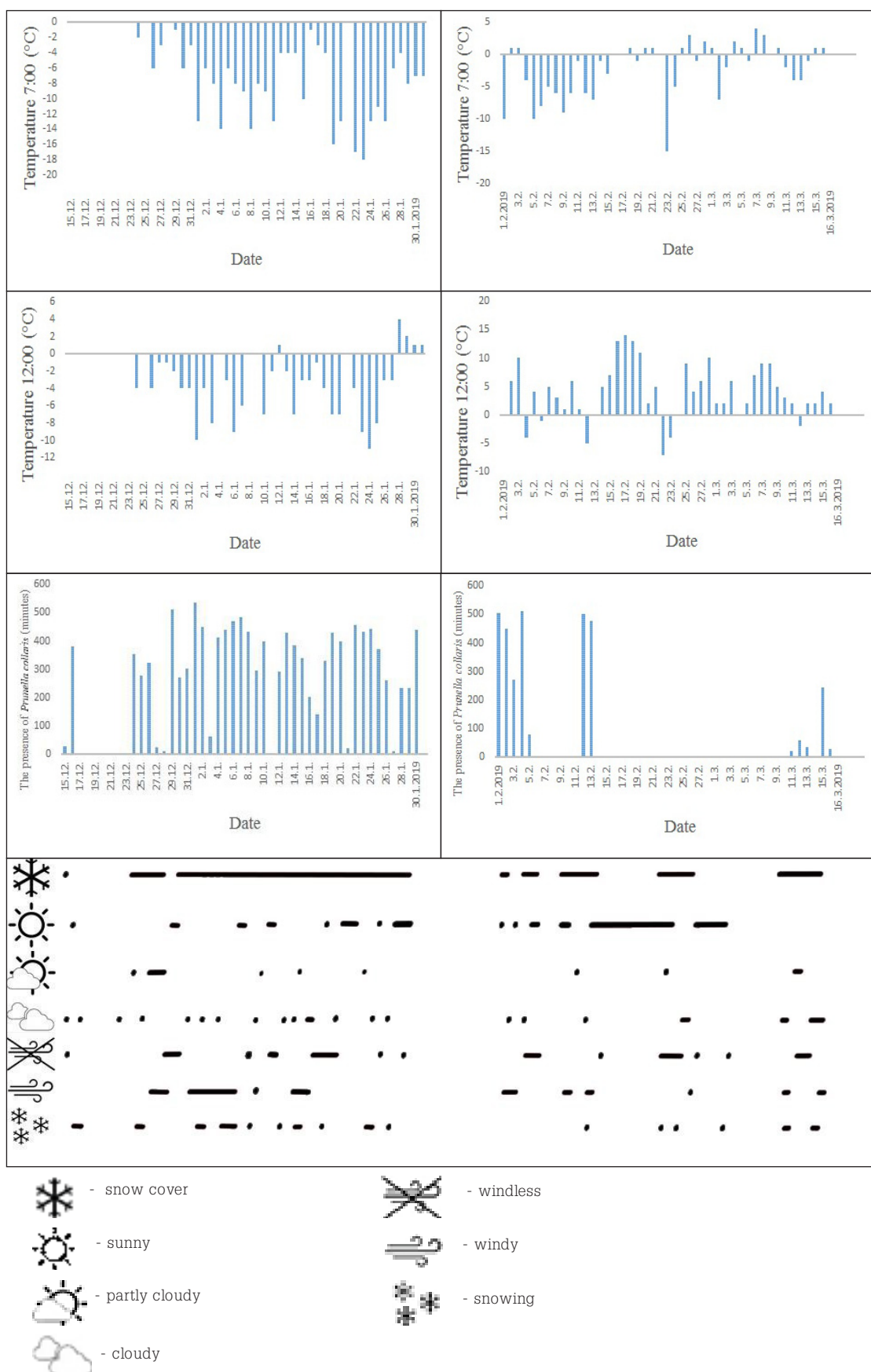


Fig. 24. Occurrence of alpine accentors at the feeding site depending on the most important factors of weather in winter 2018/2019 (Spišská Magura).

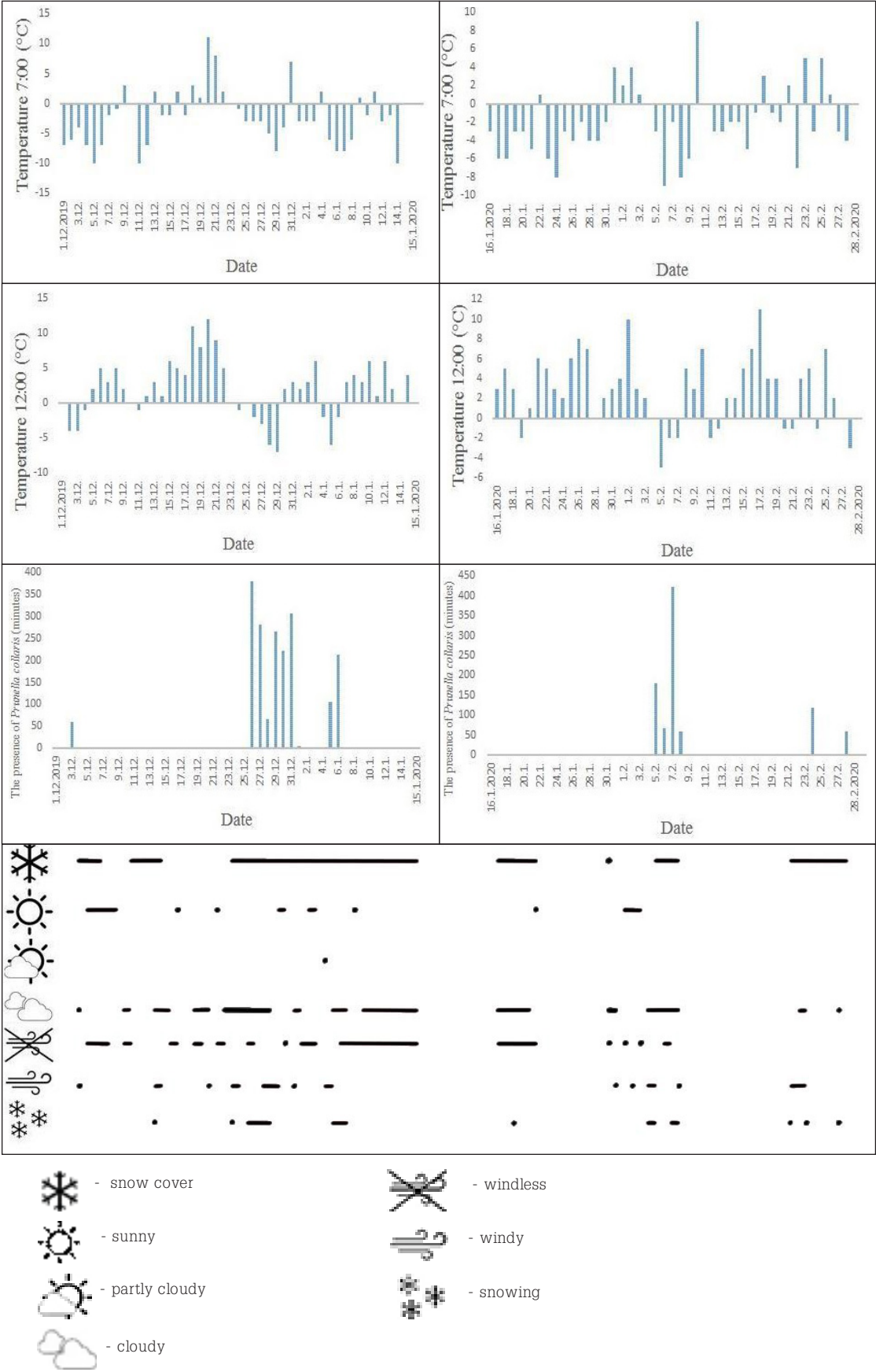


Fig. 25. Occurrence of alpine accentors at the feeding site depending on the most important factors of weather in winter 2019/2020 (Spišská Magura).

greater energetic benefit at a lower foraging cost. During winter, birds are likely to experience higher thermoregulatory costs overnight or during snow flurries during the day, due to decreased ambient temperatures (Olsson *et al.* 2000). Therefore, they place particular emphasis on foraging for high value foods in the hours near dawn or just before darkness. This allows accentors to establish themselves effectively in a hierarchy of multi-species bird aggregation. Hierarchy can decrease the number of aggressive interactions. The structure of such inter and intraspecific hierarchies can be determined by many factors, including; sex, age, previous experience, aggressive nature, and body mass. Species with greater body mass had increased competitive success and often displaced smaller species from food sources. However, the relative aggressiveness of some smaller species (e.g., the great tit) allowed them to defend food sources from bigger species; most notably accentors. The same behaviour was observed among dunnocks (*Prunella modularis*) in the High Tatras, where one individual dunnock was able to drive more than ten accentors away from the food source during an April snowfall.

Diseases, hygiene and conservation. Individual birds and species that make frequent use of feeders are more likely to experience both the benefits and detrimental impacts of provided food. For example, supplementary feeding can influence disease transmission rates and disease dynamics in birds (Wilcoxon *et al.* 2015; Galbraith *et al.* 2017a), while winter flocks comprised of different species may draw the attention of additional predators (Orros and Fellowes 2015), and feeding in gardens may drive evolutionary change in bird migrants (Plummer *et al.* 2015). The positive effects of supplementary feeding in some species include: a reduction in individual stress; an increase in immune defense; faster

feather growth; and improved body condition. On the other hand, some individuals exhibit symptoms of conjunctivitis, pox, dermal disease or cloacal disease (Wilcoxon *et al.* 2015). The risk of infections increases in late winter and early spring (Kocianová *et al.* 1985; Janiga 1991; Janiga *et al.* 2007; Kisková *et al.* 2011) when migratory and wintering birds meet at feeding sites. The most important genera of bacteria identified in faecal, cloacal, and pharyngeal samples of alpine accentors during this period are *Klebsiella*, *Yersinia* and *Staphylococcus* (Janiga *et al.* 2007). Intermixing of droppings and seeds from feeders (Fig. 26) can be a significant source of disease transmission between birds during different seasons and between differing ecosystems, as well as throughout the food chain between predator and prey. Autumn is the second-most important period for the occurrence of these different genera of bacteria in birds (Janiga 1991; Janiga *et al.* 2007). At this time of year, accentors are most frequently caught by birds of prey and other predators.

Interspecific interactions - from late spring to autumn, birds of prey, songbirds, other vertebrates

The alpine accentor is the subject of a long-term study by the Institute of High Mountain Biology. The first data on this bird species was systematically collected in 1984, and a multitude of research has been collected in the interim. In addition to this systemic data collection, many other phenomena in the life of this species were observed. The findings of this study are some of the first to shed light on how alpine accentors compete for access to food in winter. In recent decades, bird feeding has become an increasingly popular activity throughout the world, but its ecological impacts are still poorly understood (Robb *et al.* 2008). Although there are



Fig. 26. A mixture of scattered seeds and droppings (top left) under the feeder (top right) can be a source of disease transmission in birds. An accentor found a seed lying near the droppings (bottom left) and then ate it (bottom right).

many experimental supplementary feeding studies in both captive and natural settings, few studies discuss the wider impacts of supplementary winter feeding on bird life throughout the seasons. Accentors are a food source for some birds of prey and mammalian predators, particularly in autumn. Although some species of falcon are known as predators of accentors, the Northern goshawk (*Accipiter gentilis*) and the Eurasian sparrowhawk (*Accipiter nisus*) are likely the most common Eurasian bird predators of alpine accentors. In the Alps, accentors may compose a large percentage of the diet of some hawks in winter as well as in summer. In samples of the prey of a pair of sparrowhawks nesting in the Western Alps on the upper treeline, 156 samples of different animals were found over three years, 72 of which were alpine accentors (Glutz von Blotzheim and Bauer 1988). During research conducted through this study in the West Carpathians, nine direct interactions between hawks and accentors were recorded. One of these occurred in May, but the others took place during September and October, when hawks fly into the alpine zone to hunt migratory birds. Accentors, upon observing hawks, always flew sharply into the mountain cauldrons, and often did not return. On two occasions, remnants of accentor feathers were found and identified as caught by hawks. Falcons, such as the Eurasian hobby (*Falco subbuteo*) use a similar strategy to hunt migratory songbirds travelling over high mountains in autumn. In September, such an attack on accentors was recorded. In this case the accentor spotted the hobby, and quickly flew down into the valley. Another species of falcons, the kestrel (*Falco tinnunculus*), is dangerous to fledglings of accentors in summer (Dyrce 1976; Heer 1998). Adult male and female alpine accentors utilize loud alarm calls when these predators appear, then systematically attack and drive it away. Several such interactions were observed in July.

Songbirds. Adult accentors were also found to attack some species of songbirds, such as the Northern wheatear (*Oenanthe oenanthe*). In the West Carpathians, accentors generally avoid common ravens (*Corvus corax*), though some interactions were observed near summits during the month of September. During this same timeframe, accentors were observed to avoid alpine clough (*Pyrrhocorax graculus*) in the Tian Shan. During autumn in the Tian Shan, alpine accentors responded to alarm calls of cloughs and plain mountain finches (*Leucosticte nemoricola*). Accentors were recorded foraging with the mountain finches, but the finches had precedence over the accentors. In Kyrgyzstan, interactions between accentors and wallcreeper (*Tichodroma muraria*) were also observed. Between these two species, the stronger and more aggressive appears to be the wallcreeper. During five observed attacks, a wallcreeper demonstrated precedence over an accentor four times, with the accentor claiming victory once. Between spring and autumn, accentors often come into contact with water pipits (*Anthus spinoletta*). Interactions between these species in the West Carpathians mainly occurs between July and September. In most cases, accentors chase pipits away from food, with only the occasional instance of the inverse.

Inexperienced juvenile accentors sometimes form small flocks with juvenile pipits in summer (seen in July), but in other circumstances a 19-day-old accentor was observed to attack a water pipit (Glutz von Blotzheim and Bauer 1988). In a cage, an accentor can chase away a red crossbill (*Loxia curvirostra*) (Glutz von Blotzheim and Bauer 1988).

The Eurasian ermine (*Mustela erminea*) is a very dangerous mammalian predator of accentor nestlings during summer. The presence of ermine during this period always results in strong anti-predation behaviour by adult birds, whereby accentors will cooperatively attack ermines and aggressively defend nests. Sometimes higher vigilance to predation pressure can also be achieved through breeding aggregation with other species (Heer 1998). In autumn, when an accentor sees an ermine, it stands and does not move. In one case, a chamois (*Rupicapra rupicapra*) was observed to walk half a metre alongside an accentor that did not react at all. The common European viper (*Vipera berus*) can also be very dangerous for accentors during nesting. In the Pyrenees, 18 percent of broods were preyed on after hatching, and predators included both mustelids and the common viper (del Hoyo *et al.* 2005).

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