BioDiNet

MREŽA ZA VAROVANJE
BIOTSKE RAZNOVRSTNOSTI
IN KULTURNE KRAJINE

RETE PER LA CONSERVAZIONE
DELLA BIODIVERSITÀ E DEL
PAESAGGIO CULTURALE

BIODIVERSITY AND CONSERVATION OF KARST ECOSYSTEMS

EDITED BY ELENA V. BUZAN
AND ALBERTO PALLAVICINI

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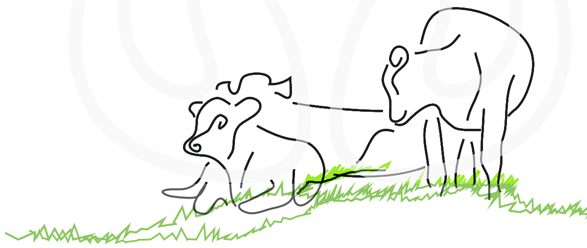

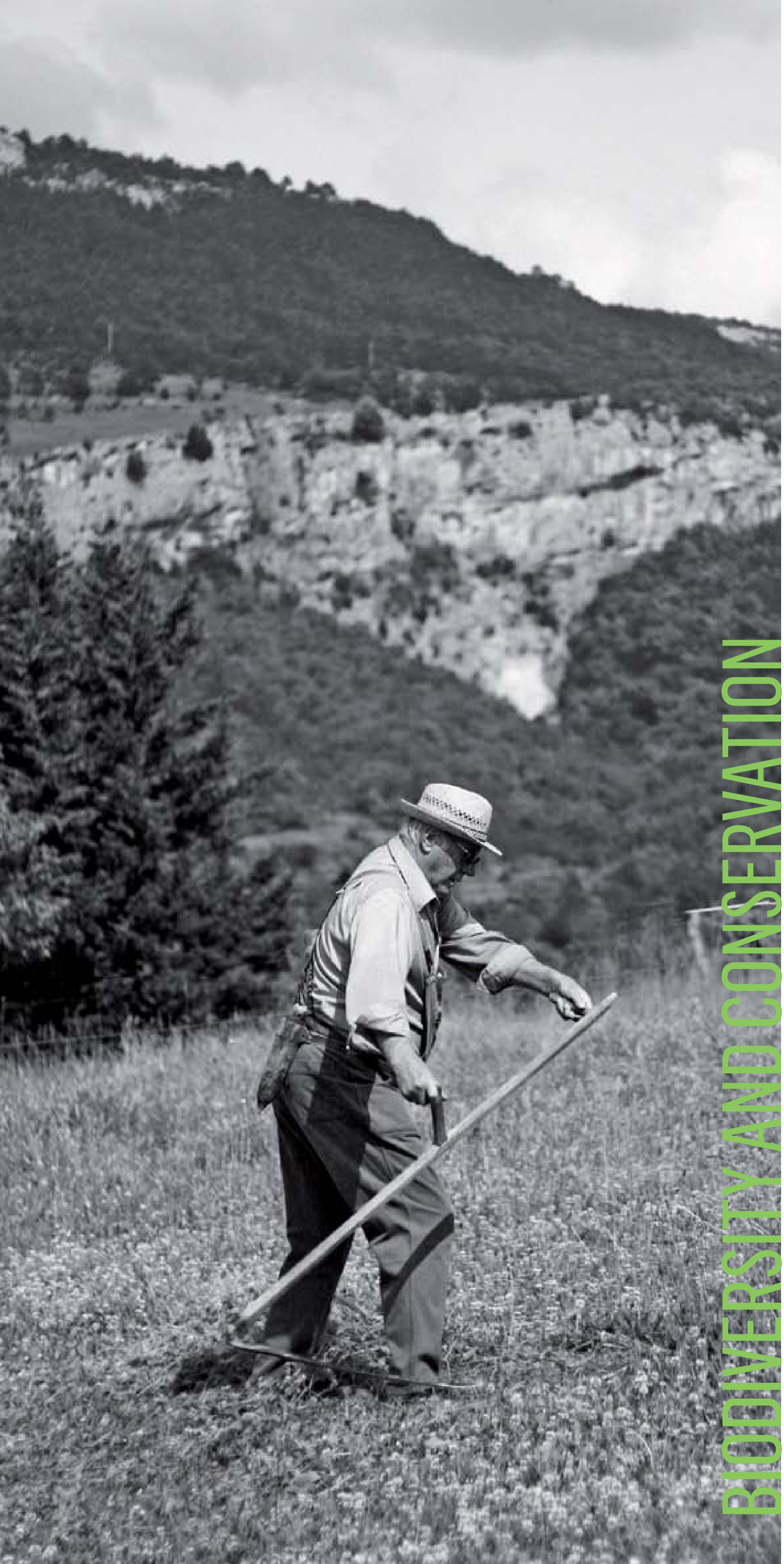

BIODIVERSITY AND CONSERVATION OF KARST ECOSYSTEMS

BIODIVERZITETA IN
VARSTVO KRAŠKIH
EKOSISTEMOV

BIODIVERSITÀ E
CONSERVAZIONE DEGLI
ECOSISTEMI CARSICI

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EDITORS:

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NETWORK FOR THE PROTECTION OF BIODIVERSITY AND LANDSCAPE

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PREFACE
UVOD
INTRODUZIONE

Biodiversity is an important factor in maintaining existing karst landscapes and nowadays provides the important indicator of degraded karst ecosystems.

The Biodiversity and conservation of karst ecosytems is a synthesis of knowledge about geological and geomorphological and biological aspects, which clarifies the required conditions for maintaining biodiversity on the karst and Karst edge, a broad region located along the border of Italy and Slovenia.

In this book we provide brief scientific summaries of the research work conducted in BioDiNet project – a network for biodiversity and cultural landscape conservation. Funding was provided by the European program of cross-border collaboration (Slovenia-Italy 2007-2013).

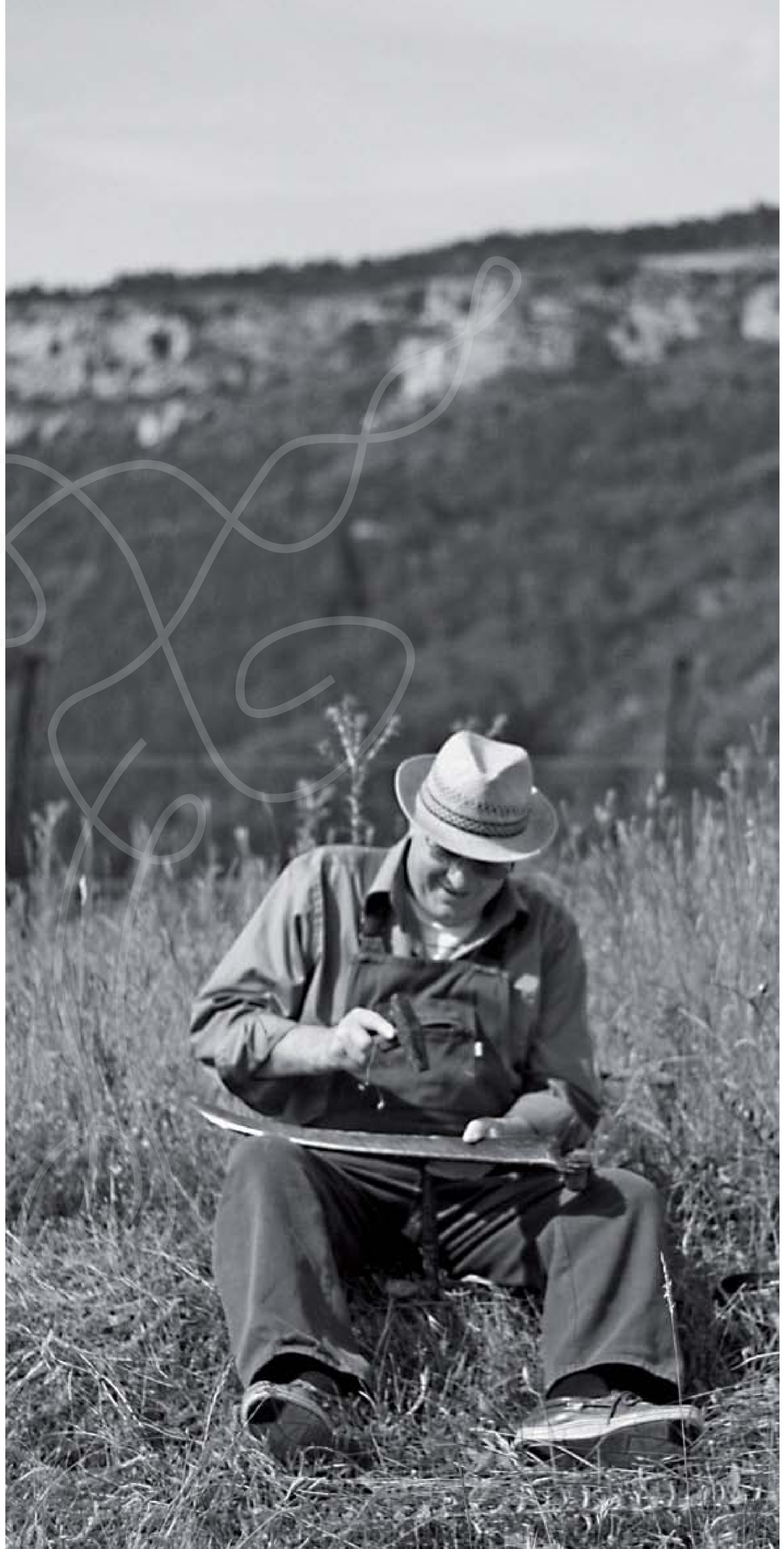
Four major areas were identified as key to the management of this landscape and require further investigation.

- Research related to genetic variation, species and ecosystems needs to be integrated and strengthened in order to provide better conservation management of endemic species, ecotypes and habitats.
- There is a need to nominate and research bio-indicator species involved in habitat maintenance to serve as the basis of landscape scale monitoring programs.
- A more directed effort is required to estimate the impact of different pollutants upon cave ecosystems.
- Design and implementation of an effective conservation monitoring system based on standardize surveys to aid with the prediction of future landscape changes and biodiversity patterns.

The book provides an annex named *Guidelines for the biodiversity conservation of karst ecosystems.* These guidelines are designed for stakeholders and local public administration and are written in Slovenian and Italian.

We express our sincere gratitude to the students and volunteers who have helped researchers during numerous field trips and with laboratory analyses. We acknowledge and praise the dedication and expertise of our co-authors, without their input and insights this book would not have been possible.

Elena V. Buzan
Alberto Pallavicini



PART 1
BIOINDICATORS AND THREATS

BIOINDIKATORJI
IN GROŽNJE

BIOINDICATORI E
MINACCE

(EDITOR ELENA V. BUZAN)

BioDiNet

MREŽA ZA VAROVANJE BIOTSKE RAZNOVRSTNOSTI IN KULTURNE KRAJINE

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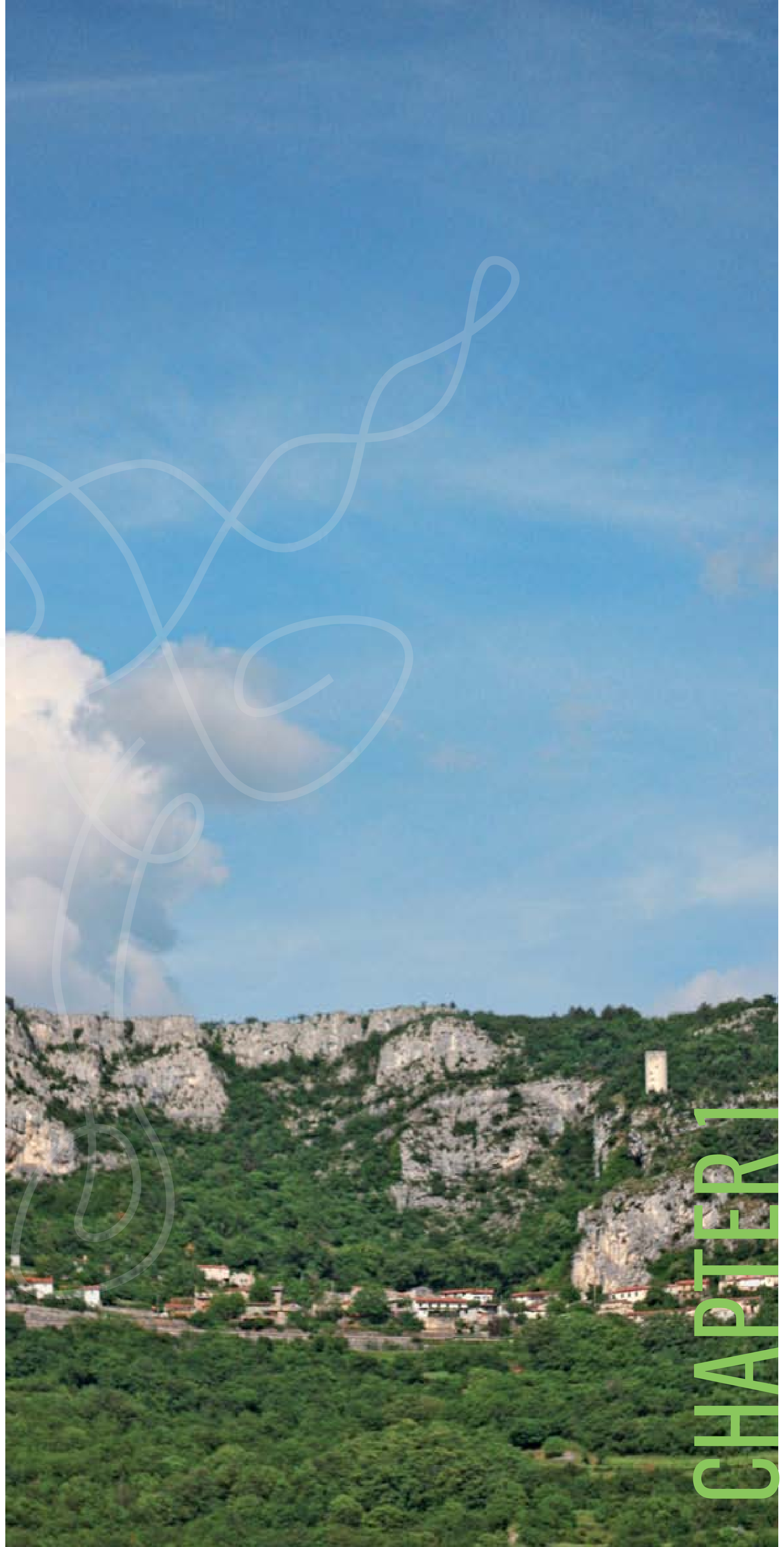
NETWORK FOR THE PROTECTION OF BIODIVERSITY AND LANDSCAPE



CHAPTER 1

BIODIVERSITY AND CONSERVATION OF KARST ECOSYSTEMS IN THE TRANSBOUNDARY AREA

BIODIVERZITETA IN VAROVANJE KRAŠKIH EKOSISTEMOV V ČEZMEJNEM OBMOČJU

BIODIVERSITÀ E SALVAGUARDIA DEGLI ECOSISTEMI CARSICI NELLA ZONA TRANSFRONTALIERA

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Karst environments, as we see them today, are the result of geological, hydrological, biological and sociological processes that over millennia contributed to the creation of a unique and highly sensitive landscape. Karst ecosystems are one of the most fragile in the world. On the surface thin layer of soil makes them highly susceptible to erosion, while below, rapid infiltration of precipitation water can quickly spread pollution over large distances (Zwahlen, 2004).

The characteristic archetype of the karst landscape in southwestern Slovenia and the neighboring Friuli Venezia Giulia region of Italy is a product of economic, social and cultural influences that have taken place in the last few decades. The region is a mosaic of diverse habitats, such as dry rocky grasslands, forest fragments, karst ponds and hedges. Many of these habitats are the result of traditional agricultural practices that persisted for centuries. However, in modern times there has been a marked reduction of agrarian practices in this region; in some localities farming has been completely abandoned. As agriculture is removed from the landscape grasslands undergo a series of succession processes, firstly with the emergence of scrublands and over time ending in the restoration of the forest cover (Kaligarič *et al.*, 2005).

The Karst edge is the border between the internal limestone Karst and the coastal flysch that extends from the Italian Karst through Slovenia and into Croatia in the eastern part of Istria. This margin is part of the "Karst" *Natura 2000* site (Figure 1.1). Historically, the area of the Karst edge was isolated from the economic development of major population centers; as a consequence elements of agrarian landscape were preserved well into the twentieth century. Semi-natural dry grasslands cover over 20% of the region with more than 200 karst ponds present. These ponds provide unique freshwater habitats for many species of plants and animals. Dry karst meadows and pastures of the class *Festuco-Brometea* represent one of most diverse habitats in Europe (Kaligarič *et al.*, 2005). Karst pastures are characterised by dry, warm conditions where the substrate is stony and the soil layer shallow, while karst meadows are mostly found in more moist conditions with deeper and more humified soils (Pipenbaher *et al.*, 2011). Calcareous grasslands and karst ponds are a focal issue in nature conservation throughout Europe since they support high number of species and represent an important component of both cultural and natural heritage. Some parts of these habitats are included in protected areas (Figure 1.1).

Today, this area is sparsely populated with a prevailing migration of young people away from the region's small villages as they seek out opportunities in larger population centres. There is a strong trend toward the abandonment of traditional land use practices and as a consequence grasslands are in the process of becoming overgrown or being adapted to intensive agricultural practices. Part of this process is an accelerated rate of decline and loss also of karst ponds and hedges.

When historical elements of the karst and Karst edge are left intact they provide valuable ecosystem services and resources. Unfortunately, conservation management in this region has generally lacked substantial scientific data, which is essential for evidence-based decision making and planning (Margules & Pressey, 2000). Ideally, factors affecting the richness and distribution of karst biodiversity should be incorporated into quantitative guidelines for karst conservation and *Natura 2000* sites.

To address this need, some of the conservation issues in the region were examined to develop sustainable guidelines for *Natura 2000* sites as part of the cross-border collaboration project BioDiNet, which carried out studies on the karst and Karst edge. The first aim of the project was biodiversity characterization and monitoring using genetic and ecological tools in order to achieve baseline information for the long-term maintenance of biodiversity and protection of karst ecosystems. In addition, BioDiNet reviewed the geological and hydrological features connected with the known and potential pollution of underground ecosystems.

The results of the project provide an important scientific knowledge base, for the production of conservation guidelines in format that is useful for stakeholders and local public administration.

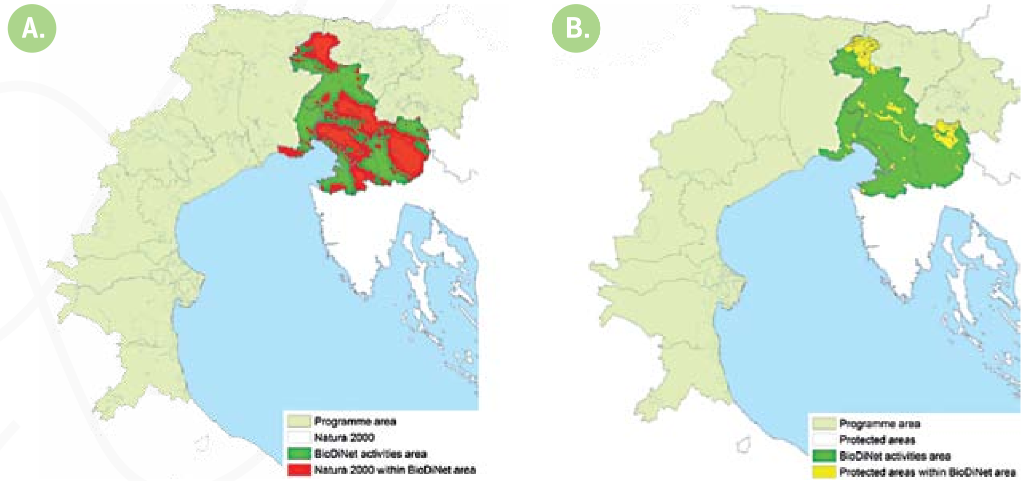

Figure 1.1: Map A. Illustration of the overlap between EU *Natura 2000* network of protected areas (red) and BioDiNet study region (green and red).
Map B. Illustration of the extent of the program area for cross border collaboration between Italy and Slovenia 2007-2013 and BioDiNet activities (shown in dark green and yellow). Areas protected by national laws are shown in yellow.

The BioDiNet contribution can be divided into project themes: Karst grasslands, Grassland biodiversity as an indicator of land use intensity, Biodiversity and pollution of fresh water ecosystems and Conservation of endemic species.

1.1 KARST GRASSLANDS

The grasslands of the North Adriatic Karst are among the most species rich habitats in Europe, they maintain very high plant species densities when compared to other terrestrial habitats (Kaligarič *et al.*, 2006). These grasslands are of semi-natural origin, meaning that they have emerged through centuries or millennia marked by low-intensity human land use, and are believed to have replaced naturally occurring forests.

Different moisture and soil conditions determine distinct vegetation types, such as meadows composed of mesophyllous plants in areas with mesic conditions or semi-natural pastures composed of sclerophyllous plants in oligotrophic areas (Pipenbaher *et al.*, 2013). In this region grasslands are mainly used for grazing (pastures) or hay-making (meadows), but in some areas they have been abandoned and have entered succession. Currently, spontaneous reforestation is a strong driving force for abandoned fields in this region (Kaligarič *et al.*, 2006). The meadows and the pastures are important habitats that serve to maximize local biodiversity because they contain different species when compared to the surrounding forests (Andrič & Willis, 2003).

Karst grasslands have been recognized by the EC Habitats of Community Interest with the code 62A0 (Eastern sub-mediterranean dry grasslands) (*Scorzoneratalia villosae = Scorzonero-Chrysopogonetalia*). The first goal of the research was to analyze the floristic and vegetation structure of the various types of grasslands (meadows and pastures under different models of management in the Italian (above Gorizia, Alture di Polazzo - Gorizia) and Slovenian karst (Hrastovlje, the Podpeč uplands and Rakitovec) to help estimate their conservation value.

The research also estimated spatial and seasonal variation of both the yield and quality for karst pastures, the production of karst meadows were also characterized and evaluated.

Researchers created digital 3D models, produced by photogrammetric reconstruction, to estimate the height of vegetation within the research area. With a clear separation of different classes of land cover they were able to estimate the density of vegetative regrowth (scrubbing-over), which aided in the identification sites where investments in grassland restoration would give the greatest returns.

In order to assess the impact of grazing on abandoned pastures researchers carried out floristic/vegetational surveys on an experimental area of the Italian Karst at Bazovizza within the Special Area of Conservation (SAC) "Karst of Trieste and Gorizia".

Agriculture is the predominant land use in the area, with primary production a key economic sector, which impacts biodiversity in both positive and negative ways. Due to their climatic and geomorphological characteristics the meadows in the karst and Karst edge fall into the productive category of dry grasslands. Sustainable management of grazing areas must consider the way in which vegetation responds to climatic change in relation to the geomorphology of the area. Knowledge of vegetation responses to climate permits better management of plant communities in pastures by reducing production losses through modifying grazing practices. Grazing livestock greatly effects the composition of pasture plant communities. With proper grazing management, animals always cause a pasture to be a more complex mixture of plants than in the absence of grazing. The vegetation compositions of grasslands are closely linked to the availability of nitrogen. Fertilizers in the karst region should only be used on meadows that are traditionally managed for hay production, as they have an adverse effect upon biodiversity and pollute aquifers. There is an urgent need for scientific information upon the effects of grazing management including all aspects of biological diversity, vegetation processes, soil properties and dynamics.

Researchers from University of Padua provide estimates of how environmental factors impact upon the productivity of karst meadows. They also deliver guidelines for cut anticipating, which can provide better forage quality and palatability, especially from more productive meadows, due to higher crude protein content.

1.2 GRASSLAND BIODIVERSITY AS AN INDICATOR OF LAND USE INTENSITY

Bioindicators are taxa or functional groups which reflect the state of the environment, either acting as early warning indicators of environmental change (environmental indicator), used to monitor a specific ecosystem stress (ecological indicator) or to indicate the levels of taxonomic diversity at a site (biodiversity indicator) (McGeogh, 1998).

Butterflies are often considered good bioindicators. Their fairly specific ecological requirements manifest as rapid responses to environmental changes (Čelik, 2007; Stefanescu *et al.,* 2004). Their ecology is generally well understood, taxonomic identification is relatively easy and they are often species-specific towards their host plants. They also play an important role as pollinators, prey for other species and hosts for parasites (Čelik, 2007).

In this research theme scientists tried to assess the influence of different management practices through surveying butterfly diversity (Jugovic *et al.,* 2013). The studies of butterfly communities from different habitat types (dry karst meadows, pastures and overgrown areas), and monitoring of a target species, the black-veined white *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) that, like many other butterfly species has shown a steep decline in recent decades, illustrate the importance of mosaic landscapes and traditional activities such as rotational grazing. Researchers also demonstrate the influence of different habitat types (dry karst meadows, pastures and scrubbed-over areas) upon butterfly assemblages.

In pastures, dung beetles can contribute significantly to the ecological processes of nutrient cycling, bioturbation, pollination and seed dispersal (Nichols *et al.,* 2008). In the Karst edge grazing has been the main activity that has maintained open pastures within a mosaic of scrublands and forests. In this context, researchers from University of Primorska, investigated the utility of dung beetles as a bioindicator for habitat changes connected with grassland management such as decreases and increases in grazing.

Birds have been used as bioindicators for many reasons, their ecology is well known, the links among bird communities, vegetation associations, and territory has been clearly demonstrated (Petty & Avery, 1990), they cover different ecological level (Burrough, 1986) and they are easily detected. The loss of karst grasslands is mirrored by changes in bird populations. Research from DOPPS - BirdLife Slovenia and their collaborator tried to monitor changes in bird populations in three different karst areas.

1.3 BIODIVERSITY AND POLLUTION OF FRESH WATER ECOSYSTEMS

Due to a porous limestone substrate surface water in the karst region is scarce or absent. Man-made karst ponds are the only surface water bodies and support variable biotic communities, depending on ecological parameters and human activities (Zelnik *et al.,* 2011). These habitats represent a network of suitable stepping stone habitats for a large numbers of species. Studies of different animal groups give us more complete knowledge of the biodiversity, function and conservation status of threatened karst ponds ecosystems. Conserving aquatic biodiversity in the area requires well-planed conservation management.

The main aim of the research in this theme was to estimate amphibian species composition, newt abundance and genetic diversity in karst ponds. Additionally, a preliminary analysis of zooplankton composition was also carried out. The influence of ornamental fish upon amphibian community structure was evaluated. In collaboration with Australian researchers from the University of Adelaide and James Cook University we are carrying out a survey of zooplankton biodiversity in Karst ponds.

The biodiversity of the Dinaric hypogean habitats is known to be the highest in the world and the richness of endemics is very high (Sket, 2012). The caves are fragile ecosystems and constantly threatened by human activities on the surface (Sket, 2012). In our research we tried to identify the appropriate tools for making reliable estimates of cave biodiversity. Within the project we analysed the composition of cave biota from seven caves.

There is an important need to establish a better groundwater monitoring program in karst and Karst edge. This system is important for maintaining biodiversity but it is also an important reservoir for local human populations. Unfortunately, many important karst aquifers and springs are improperly protected. In this theme researchers make contributions to identifying the impact of different pollutants upon cave environments and biota.

1.4 CONSERVATION OF ENDEMIC SPECIES

By employing techniques in conservation genetics it is possible to investigate and characterize many aspects of genetic variability. This type of analysis provides additional data for conservation programmes (Frankham, 1995).

Endemic species are often important objects in nature conservation planning due to their local distribution and vulnerability. Researchers focused on the genetic structure of *Moehringia tommasinii* populations, a stenoendemic plant to northern Istria. They obtained spatial analyses of genetic variation in order to estimate the genetic makeup and gene flow between populations. Guidelines for preserving this fragile, yet remarkable species are given.

Genetic analyses were conducted on bee populations by researchers from University of Trieste. They estimated hybridisation rates in two bee ecotypes, the yellow Italian bee (*Apis mellifera ligustica*) and the grey Slovene bee (*A. m. carnica*). The hybridisation is a consequence of repeated imports of non-native bees for commercial purposes. They investigated if the genetic relic, in the Kast area, of *A. mellifera carnica* is adapted to coastal environments and if the Istrian-Dalmatian ecotype deserves special attention to ensure its preservation.

The present book identified the need for further studies to provide a link between ecology, taxonomy and genetics of karst biodiversity in transboundary area. It is also advisable that future research should study the relationships between different taxa and ecosystems management considering the variations in biophysical condition in light of future climate changes. However, the results should be useful to regional decision-makers in understanding the potential value of karst ecosystem and to planning its sustainable management.

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CHAPTER 2

MOEHRINGIA TOMMASINII,
AN ENDEMIC CHASMOPHYTE FROM THE KARST EDGE

MOEHRINGIA TOMMASINII,
ENDEMIČNI HAZMOFIT KRAŠKEGA ROBA

MOEHRINGIA TOMMASINII,
UNA SPECIE ENDEMICA CASMOFITA DEL CIGLIONE CARSICO

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ABSTRACT

Endemic species play an important role in understanding biogeographical patterns and are often used in planning nature conservation strategies. *Moehringia tommasinii* is stenoendemic to Northern Istria (Croatia, Italy and Slovenia). It inhabits overhanging rocks on calcareous cliffs where water occasionally oozes and provides nutrients. Due to its rarity and vulnerability, it is considered in all the main national and international conservation acts. According to the IUCN, recreational activities such as rock climbing represent the main threat to *M. tommasinii*. A genetic analysis using AFLP markers, conducted in this study, showed genetic structuring into three clusters: the three Slovenian populations belong to one genetic cluster, while the Italian and the Croatian populations are assigned to two different clusters. This could be partly explained through isolation by distance, which is concordant with the possible limited dispersal of this species. However, future studies on the biology of the species, especially pollination biology and reproduction strategies are required to properly understand the isolation mechanisms detected by this study.

Key words: endemic species, Karst edge, *Natura 2000*, AFLP, rock climbing

IZVLEČEK

Endemične vrste igrajo pomembno vlogo pri razumevanju biogeografskih procesov in jih pogosto uporabljamo pri načrtovanju varstvenih strategij. Tommasinijeva popkoresa je stenoendemit severnega dela Istre (Hrvaška, Italija in Slovenija). Najdemo jo v previsnih stenah apnenčastih klifov, kjer voda občasno mezi iz skalnih razpok in prinaša hranila. Zaradi redkosti in ogroženosti je vrsta vključena v vse pomembnejše nacionalne in mednarodne varstvene akte. IUCN opredeljuje rekreativne dejavnosti, kot je športno plezanje, za eno izmed glavnih groženj vrsti. V okviru projekta smo s pomočjo AFLP markerjev izvedli genetsko raziskavo, ki je pokazala genetsko strukturiranost vrste v tri klastre: prvi klaster združuje vse tri slovenske populacije, medtem ko italijanska in hrvaška populacija predstavljata vsaka svoj klaster. Rezultate lahko deloma razložimo z izolacijo zaradi razdalje, kar je v skladu z omejeno disperzijo vrste. Za razumevanje izolacijskih mehanizmov, zaznanih v raziskavi, bi bilo potrebno dodatno poznavanje biologije vrste, posebno njenega opraševanja in reprodukcijskih strategij.

Ključne besede: endemična vrsta, Kraški rob, *Natura 2000*, AFLP, plezanje

RIASSUNTO

Le specie endemiche hanno un ruolo importante nella comprensione dei modelli biogeografici e sono spesso utilizzate nella pianificazione di strategie di conservazione della natura. La *Moehringia tommasinii* è stenoendemica dell'Istria settentrionale (Croazia, Italia e Slovenia). Abita le nicchie rocciose delle pareti calcaree, dove l'acqua filtra occasionalmente e fornisce sostanze nutritive. A causa della sua rarità e vulnerabilità è considerata in tutti i principali atti nazionali ed internazionali di conservazione della natura. Secondo la IUCN attività ricreative come l'arrampicata rappresentano la principale minaccia per *M. tommasinii*. L'analisi genetica mediante markers AFLP condotte in questo studio, ha evidenziato una strutturazione genetica in tre gruppi: le tre popolazioni slovene appartengono a un cluster genetico, mentre la popolazione in Italia e la popolazione in Croazia sono assegnate a due gruppi diversi. Ciò potrebbe essere in parte dovuto dall'isolamento a distanza, che è in concordanza con la dispersione limitata di questa specie. Tuttavia sono necessari studi futuri sulla biologia della specie, in particolare la biologia d'impollinazione e le strategie di riproduzione, per capire bene i meccanismi d'isolamento rilevati da questo studio.

Parole chiave: specie endemiche, Ciglione carsico, *Natura 2000*, AFLP, arrampicata

2.1 INTRODUCTION

Endemic species play an important role in understanding biogeographical patterns and are often used in planning nature conservation strategies. The Mediterranean is considered a hotspot of European, as well as global biodiversity, harbouring a large number of endemic plants (Blondel & Aronson, 1999). The long-term persistence of biota, even during the glacial stages of the Pleistocene, is among the reasons for today's high biodiversity rates around the Mediterranean. Recently, Medail & Diadema (2009) proposed a series of fine scale glacial refugia according to known phylogeographical patterns in plants, considering Northern Istria to be one of them.

Northern Istria is characterised by two geological substrates, moist and consequently cold flysh and dry and warm limestone, The Karst edge, a long line of vertical cliffs extending from the northwestern to the southeastern part of the Istrian peninsula, represents the contact zone between the two bedrocks and represents an excellent habitat for many thermophytic representatives of the Mediterranean flora (Kaligarič, 1992). The vertical limestone cliffs represent a habitat for many chasmophytes, the most emblematic representative being *Moehringia tommasinii* (Caryophyllaceae; Fig. 2.1).

Figure 2.1: *Moehringia tommasinii* (top) and the limestone cliffs of Osp, one of the species' localities (down).

2.2 *MOEHRINGIA TOMMASINII*, AN EMBLEMATIC ENDEMIC PLANT FROM THE KARST EDGE

Moehringia tommasinii is stenoendemic to Northern Istria. It inhabits overhanging rocks of calcareous cliffs where water occasionally oozes and provides nutrients (Fig. 2.1). Although this habitat seems dry at first glance, the overhanging rocks provide higher moisture due to shading (Martini, 1990).

This plant is however not the only narrow endemic species from the genus. A recent taxonomical revision of sandworts (genus *Moehringia*) (Fior & al., 2006, Fior & Karis, 2007), based on molecular data, identified two phylogenetic groups within *Moehringia* s.s., namely *Moehrigia* sect. *Latifoliae*, with some widely distributed taxa, and *Moehringia* sect. *Moehringia*, restricted to the wider Alpine region with many narrow endemics distributed south of the Alpine arc and confined to limestone cliff habitats (Marchesetti, 1879; Merxmüller & Guterman, 1957; Sauer, 1959; Mayer, 1960; Sauer, 1965; Merxmüller, 1967). *Moehringia tommasinii* represents one of the many narrow endemics from this section (Fior & Karis, 2007).

The plant has been recorded in six localities (Fig. 2.2): one in Italy (cliffs in the Glinščica Valley - Val Rosandra), three in Slovenia (cliffs at Osp, Črni Kal, and Podpeč) and two in Croatia (both in the proximity of the locality Istarske toplice) (Kaligarič, 2004; Brana, 2005; Kaligarič & Surina, 2005). A seventh locality was reported by the Austro-Hugarian botanist Eduard Pospichal (1897-1899) from Nugla (north of the town Buzet, Croatia), but this finding was not confirmed in recent times.

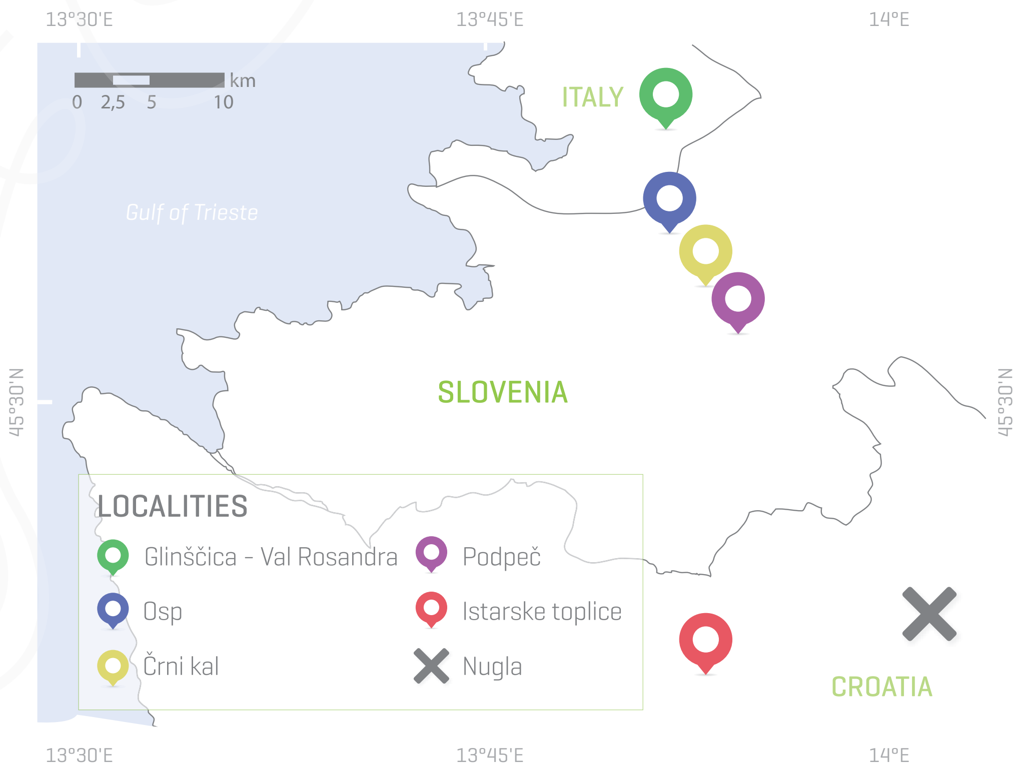

Figure 2.2: Distribution map of *Moehringia tommasinii* (according to Pospichal, 1897-1899; Martini, 1990; and Kaligarič, 2004).

Osp, with its imposing vertical limestone wall (Figure 2.1), is considered to be the *locus classicus* of *M. tommasinii* (Wraber, 1992; Fig. 1). The taxonomical history of this plant somehow denotes the regional botanical activity during the second half of the 19th century. The species was described by the leading botanist from the nearby Italian city Trieste, Carlo Marchesetti in 1879 on the provision of plant material from Osp. In the name he honoured his botanical teacher, the botanist Muzio de Tommasini, who had first found this plant in the same location in 1843.

2.3 CONSERVATION STATUS

Due to its rarity and vulnerability, *M. tommasinii* is considered in all main national and international conservation acts. At national level it is protected in all three countries in which it occurs (Uradni list RS 46/2004; Narodne novine 70/2005; Repertorio della flora italiana protetta, 2013). It is also listed as Rare (R) in the national Red Lists of threatened species in Slovenia (Uradni list RS 82/2002; Kaligarič, 2004), Endangered (EN) in Croatia (Nikolić & Topić, 2005) and Nearly threatened (NT) in Italy (Rossi *et al.*, 2013).

Even higher concern is given to the plant at international level. The IUCN treats the species as endangered (EN) (Montagnani *et al.*, 2011). The species is included in the Appendix I (Strictly Protected Flora Species) of the Bern Convention (Convention on the Conservation of European Wildlife) and also in the Appendix II of the Habitat Directive as one of the qualifying species for the establishment of *Natura 2000* sites. It occurs in no less than four *Natura 2000* sites in all three countries: Aree Carsiche della Venezia Giulia (IT3341002) and Carso Triestino e Goriziano (IT3340006) in Italy, Kras (SI3000276) in Slovenia, and Istarske Toplice (HR2001011) in Croatia. However, even though the area of the Karst edge was declared a *Natura 2000* site, no current management exists for the disturbing activities on rocky cliffs, apart from the closure of some climbing paths in order to preserve the nesting sites of some bird species.

2.4 THREATENED DUE TO UNCONTROLLED HUMAN ACTIVITIES?

Rocky cliffs have been relatively unaffected by humans over centuries due to their inaccessibility. However, in the last few decades they have become an increasingly popular spot for outdoor recreational activities. The IUCN recognises recreational activities such as mountaineering, rock climbing, speleology, the taking or removal of flora and vandalism as main threats to *M. tommasinii* as well as to some other chasmophytic species. Probably the most direct threat to the species is represented by the very popular sport of rock climbing. This activity has been increasing since the 1970s and today the Karst edge (with climbing sites in Osp, Mišja peč and Črni Kal) represents one of the most visited venues for this outdoor sport in the entire North Adriatic region. The problems regarding climbing on the Karst edge have already been emphasized by Wraber (1992) and Škornik (1992) and the negative effects of rock climbing on chasmophytes have been documented by several other studies from different parts of the world (eg. Kelly & Larson, 1997; Vogler & Reisch, 2011).

Climbing is however not the only cause for concern. Degradation of the environment caused by intense use and permanent human presence on these sites is evident also by the occurrence of a large number of invasive species that can lead to the loss of adequate habitat for chasmophytes. Particularly in Osp, we observed a worrying presence of a range of invasive species such as Tree-of-heaven *Ailanthus altissima*, Virginia creeper *Parthenocissus quinquefolia* and silverlace vine *Fallopia baldschuanica*, which have already started penetrating into rocky habitats.

2.5 FIRST ATTEMPTS OF POPULATION STATUS CENSUS

Within the project BioDiNet we aimed to assess the status of the species by measuring the population size and analysing the genetic variation and structure of *M. tommasinii* populations.

The population size was estimated in all three Slovenian localities by observing the habitat from distance using binoculars and a telescope. The largest population was observed in Osp with estimated population size between approx. 570 and 650 individuals. Free climbing is most intense in this area. The cliffs of Osp are one of the most visited climbing sites in the region with 201 climbing routes of a length of 7040 m (website Plezanje.net). The highest climbing intensity is on the western side (the Babna, Banje and Srnjak cliff sections) of the wall where the smallest numbers of plants were observed (see Table 2.1). Plants remnants, observed on several occasions at the base of the wall, point to the negative effects of climbing on this plant. Parts of the routes are closed to restrictions regarding conservation of protected bird species (Golden eagle *Aquila chrysaetos*, Peregrine falcon *Falco peregrinus*, Eagle owl *Bubo bubo* and Blue rock thrush *Monticola solitarius*; Uradni list RS, 5/2006). On the eastern part of the cliff we observed the largest number of plants, especially on the patches without climbing routes.

Table 2.1: Abundance estimation of *Moehringia tommasinii* on Osp cliffs in relation to climbing intensity. Climbing intensity is considered as 0 - no disturbance, 1 - low intensity, 2 - medium intensity and 3 - high intensity (according to website Plezanje.net).

Cliff section		Number of climbing routes	Total length of climbing routes	Rock climbing intensity	Estimate of species abundance (individuals)
B I-III	Babna	114	3145	3	75 - 90
A I-II	Banje	48	1127	2	1
C I-III	Srnjak	20	1008	1	55 - 75
C / IV	Stena	17	1600 m	1	100 - 110
C / V - 1		2	160 m	1	140 - 150
C / V - 2		-	-	0 (closed)	200 - 220
Total					571-646

54 climbing routes of a length of 1454 m represent the climbing site of Črni Kal. Here we recorded between 320 and 340 individuals. The majority of individuals occur in very small cushions, while larger plants were observed only in the most inaccessible sites. The only Slovenian site with *M. tommasinii*, where climbing is not allowed, is the cliff in Podpeč (Plezanje.net). Here we recorded between 250 and 260 individuals, growing on two distinct parts of the wall.

2.6 POPULATION GENETIC STUDY OF *MOEHRINGIA TOMMASINII*

In order to conduct a genetic study, we collected between 6 and 12 leaf samples from each of the five localities across the whole species range: Črni Kal, Osp, Podpeč (all three in Slovenia), Val Rosandra - Glinščica Valley (Italy) and Istarske Toplice (Croatia). Samples were collected between 2012 and 2014 and silica-dried. DNA was extracted using a modified CTAB protocol (Doyle & Doyle, 1990). We used Amplified Fragment Length

Polymorphism (AFLP) to determine the population structure.

AFLP is a PCR-based technique, which uses the selective amplification of digested DNA fragments to generate and compare unique fingerprints for genomes of interest. The digestion of DNA, that is to say the cutting of DNA molecules into smaller pieces, is accomplished using special enzymes called Restriction Endonucleases. Amplification of fragments is performed in a similar fashion to regular PCR using randomly chosen primer pairs. The result is a large number of length polymorphisms, which are caused by mutations that create or abolish restriction endonuclease recognition sites. As this method does not require prior information regarding the targeted genome, it is widely used in population studies of many species, especially plants and bacteria, but also fungi and animals. Moreover, being highly reproducible and sensitive in detecting polymorphisms it is widely used for assessments of genetic diversity within species or among closely related species, to infer population-level phylogenies and biogeographic patterns and other elements of interest.

Altogether, 45 samples were used and 74 polymorphic markers were obtained using two Mse-Pst primer combinations: PstI-AGA + MseI-CAG and PstI-ACA + MseI-CAG. Data analysis was performed using GeneMarker 2.6.2 software (SoftGenetics, LLC, State College, PA, USA).

2.7 POPULATION GENETIC STRUCTURE

Genetic relationships among all genotyped individuals are displayed using factorial correspondence analysis (FCA) using GENETIX4.05.2 (Belkhir *et al*, 1996-2004). The FCA plot clearly supports distinction of individuals into the three main regions according to their rough geographical origin (Figure 2.3). The first axis (explaining 49 % of variation, P = 0.016) separates the Croatian population from three Slovenian and Italian populations. The second axis (explaining 33 % of variation, P = 0.011) mainly separated the Italian and Slovenian individuals.

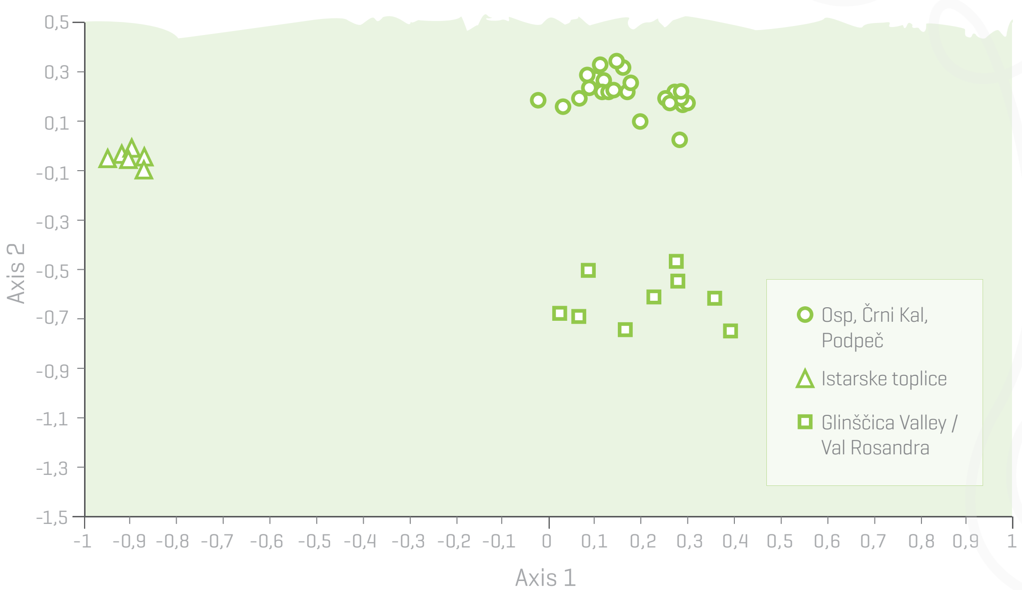

Figure 2.3: Distribution of individuals based on FCA. The first axis explains 49% of the variance, and the second axis explains additional 33% of the variance.

We further analysed the data in STRUCTURE v.2.3.2 (Pritchard *et al.*, 2000, Hubisz *et al.*, 2009), which implements a model-based clustering method for inferring population structure. The programme classifies individuals into the set number of clusters so that the Hardy-Weinberg Equilibrium in these clusters, i.e. populations, is achieved. We tested different number of clusters (K), using the admixture ancestry model and correlated allele frequency model (with $\lambda = 1$). The results obtained in STRUCTURE analysis are congruent with FCA analysis (Figure 2.3). The best model for K = 3 (using ΔK according to Evanno *et al.*, 2005) clearly separates populations from the three regions. The three Slovenian populations belong to a single genetic cluster whilst the Italian and Croatian populations are assigned to two separate clusters.

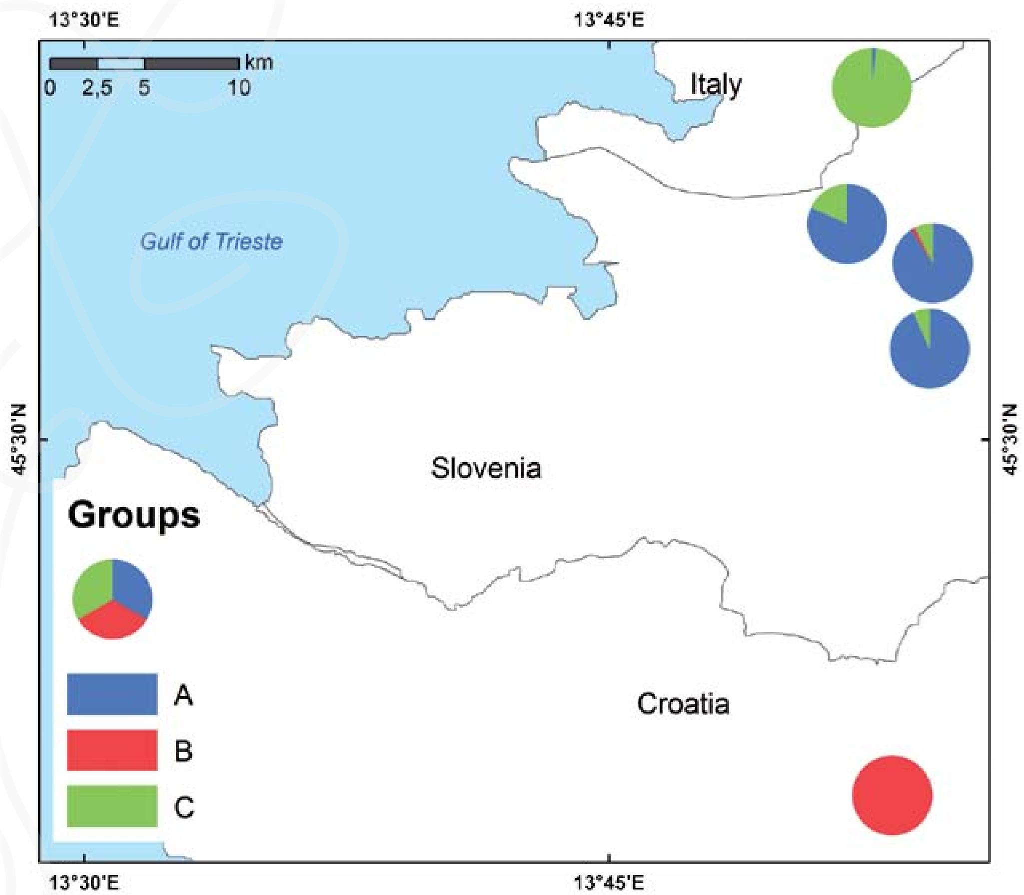

Figure 2.4: Genetic structure of *Moehringia tommasinii* from five sampled locations in Slovenia, Italy and Croatia. The graph is based on the program STRUCTURE. The colour segments in pie charts correspond to the membership in particular clusters (K=3).

CONCLUSION

The biology of *M. tommasinii* is practically unknown. Literature data on pollination biology of the genus *Moehringia* is scarce. Hind (1988) reported field observations of Coleoptera (f. Curculionidae, Dermestidae, Elateridae), Diptera (f. Empidae, Syrphidae, Chloropidae) and Hymenoptera (f. Formicidae) as visitors to the flowers of some other species of *Moehringia*. Honeybees were observed only as visitors to flowers on cultivated sandworts. He suggests that the absence of large hymenopteran visitors on wild *Moehringia* populations, capable of relatively long-distance pollen transfer, may be indicative of low nectar production and may consequently lead to genetically highly structured populations even on a small spatial scale.

The genus *Moehringia* is characterized by the presence of lipid-rich structures called elaiosomes on their seeds, a feature connected with myrmecochory – dispersion of diaspores by ants. In a study of dispersal mechanisms in *Moehringia* Casazza et al. (2008) pointed out that myrmecochory is particularly important in species growing in rocky habitats and on cliffs. Ants are regarded as having limited seed dispersal capabilities. It was estimated that seeds can be transported by ants to a distance ranging from 0.01 to 77 meters (mean global distance 0.97 m) (Gomez & Espadaler, 1998). Nevertheless, such dispersion enables the plants to be maintained in less competitive habitats (*Casazza et al.*, 2008).

Even though all localities lie in the radius of 25 kilometres, the genetic study showed clear structuring of the (sub)populations into three clusters. The three Slovenian localities and the Italian one are separated into two clusters but share some genotypes (Figure 2.4), whereas plants from Croatia belong to a distinct cluster. This could be partly explained by geographical isolation, which is concordant with the biological features within the genus. Plants from the three Slovenian localities lie along the Karst edge and the distances between sampling sites are approximately 3 km, enabling the gene flow between (sub)populations. The results indicate that the samples from three Slovenian localities could be treated as one population. In addition the Italian locality lies close by, less than 5 km from the closest locality site in Osp, but is more isolated compared to the other Slovenian localities, as it is confined within a valley. The Croatian locality is the most isolated, located 15 km from the closest Slovenian population in Podpeč. Apparently, this is enough to prevent gene flow between populations. However, future studies on the biology of the species are required to properly understand the isolation mechanisms detected by this study. Pollination biology and reproduction strategies in particular should be examined for a comprehensive understanding of the issue.

In order to preserve this fragile, yet remarkable species, more restrictive management rules should be applied in all localities. This is important not only for conservation of *M. tommasinii*, but also for other highly specialised species that are adapted to this unique environment.

SUGGESTED READINGS

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CHAPTER 3

BUTTERFLY DIVERSITY ON KARST EDGE: INTEGRATIVE APPROACH FOR THE MONITORING OF SELECTED SPECIES

DIVERZITETA METULJEV NA KRAŠKEM ROBU: INTEGRATIVNI PRISTOP K SPREMLJANJU IZBRANIH VRST

LA DIVERSITÀ DELLE FARFALLE SUL CIGLIONE CARSICO: UN APPROCCIO INTEGRATO AL MONITORAGGIO DELLE SPECIE SELEZIONATE

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ABSTRACT

Karst meadows of the class *Festuco-Brometea* are species-rich habitats of national and European importance. Although these habitats are semi-natural, given that they developed with traditional farming activities, they host high numbers of vertebrate and invertebrate taxa. They are especially important for their butterfly fauna as these vulnerable animals quickly disappear with the intensification of land use. The studies of butterfly communities from different habitat types (dry karst meadows, pastures and overgrown areas), and monitoring of a target species, the black-veined white *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) that, like many other butterfly species has shown a steep decline in recent decades, illustrate the importance of mosaic landscapes and traditional activities such as rotational grazing. An integrative approach provides a detailed insight how these areas should be managed in order to maintain high biodiversity of butterflies in future.

Key words: grazing, monitoring, bioindication, *Aporia crataegi*

IZVLEČEK

Kraški travniki razreda *Festuco-Brometea* so vrstno bogati habitati nacionalnega in evropskega pomena. Čeprav so polnaravni, saj so njihov razvoj omogočile tradicionalne kmetijske dejavnosti, so pomembni za številne vretenčarske in nevretenčarske vrste. Še posebej so pomemben habitat za metulje, saj te občutljive živali ob intenzifikaciji rabe območij s teh hitro izginevajo. Pričujoča študija združb metuljev, še posebej pa glogove belinke *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) v različnih habitatih (na suhih kraških travnikih, pašnikih in zaraščajočih površinah), katere številčnost tako kot pri mnogih drugih vrstah metuljev v zadnjih desetletjih upada, prikazuje pomembnost mozaične pokrajine in tradicionalnih kmetijskih dejavnosti, na primer rotacijske paše. Integrativni pristop k študiji nam je omogočil tudi vpogled v to, na kakšen način bo potrebno upravljati s travišči na Kraškem robu v prihodnje, če želimo obdržati visoko vrstno pestrost metuljev.

Ključne besede: paša, monitoring, bioindikacija, *Aporia crataegi*

RIASSUNTO

Le praterie carsiche della classe *Festuco-Brometea* sono habitat ricchi di specie d'importanza nazionale ed europea. Nonostante le origini semi-naturali, siccome l'origine è collegata alle attività agricole tradizionali, ospitano un numero elevato di specie di vertebrati e invertebrati. Questi habitat sono particolarmente importanti per la fauna dei lepidotteri poiché questi animali vulnerabili scompaiono rapidamente con l'intensificazione dell'uso del suolo. Gli studi effettuati su diverse comunità di farfalle provenienti da diversi tipi di habitat (prati carsici aridi, pascoli e aree incolte) e il monitoraggio di specie target - pieride del biancospino (*Aporia crataegi* Linnaeus, 1758) (Lepidoptera: Pieridae) che come molte altre specie ha evidenziato un forte calo negli ultimi decenni - illustrano l'importanza del paesaggio a mosaico e delle attività tradizionali come il pascolo a rotazionale. Un approccio integrativo fornisce una visione dettagliata di come queste aree dovrebbero essere gestite al fine di mantenere l'elevata biodiversità di lepidotteri in futuro.

Parole chiave: pascolo, monitoraggio, bioindicatori, *Aporia crataegi*

3.1 MANAGEMENT OF KARST MEADOWS AND PASTURES AND IMPORTANCE OF BUTTERFLY TAXA FOR BIOINDICATION

Biological indicators are taxa that can be used to monitor the health of an environment. As they respond to changes in their environment, they are also suitable for the assessment of the influence of different kinds of management upon species composition and communities (Stefanescu *et al.*, 2004). Butterflies are often considered as good bioindicators for their specific ecological requirements and rapid response to environmental changes, including human activities (e.g. Čelik, 2007; Stefanescu *et al.*, 2004). They are relatively easy to recognize to a species rank, their ecology is generally well understood and they are often species-specific towards their host plants. Butterflies also play an important role as pollinators, as prey for other species and as hosts for different parasites (Čelik, 2007).

In the Mediterranean, agricultural activities, urbanisation and other human impacts often lead to impoverishment of biodiversity (Stefanescu *et al.*, 2004), as habitat becomes unsuitable or fragmented. There are important differences between management practices, such as mowing, grazing and abandonment; the latter leading to areas becoming overgrown. In mowed areas there is an intensive and constant loss of nutrients from the ground, whereas with grazing, nutrients are mainly restored through the excrements of grazers (Kooijman & Smith, 2001). Extensive grazing may have both positive and negative impacts upon the environment. Grazers can defoliate competitive plant species which can increase the plant diversity in an area (Grime, 2001; Bakker, 1989; Bullock *et al.*, 2001). Moreover, grazing or traditional mowing conducted once or twice per year may prevent or slow down vegetation succession processes on dry and semi-dry karst meadows (Baba, 2003) providing the space for species of open habitats. Areas however should not be overgrazed, as this may change the composition of plant species; edible plants are often more affected and as a consequence less edible species expand (Hartley & Mitchell, 2005; Jewell *et al.*, 2005, Krahulec *et al.*, 2001; Louault *et al.*, 2005). In some plants, reproductive success may decrease as the grazers selectively feed on the most nutritious parts of the plants, that being often seeds and flowers (Peterlin & Gorkič, 1998).

Dry karst meadows and pastures of the class *Festuco-Brometea* in southwestern Slovenia represent one of most diverse habitats in Europe (Kaligarič, 2005). Karst pastures are characterised by dry, warm conditions where the soil is stony and shallow, while karst meadows are mostly found in more moist conditions with deeper and more humified soils (Pipenbaher *et al.*, 2011). These habitats are the result of traditional agricultural practices and other human activities in the past and therefore are fragile and threatened.

Due to their reduction of these practices karst pastures and meadows are extremely rare and in some areas completely abandoned. The abandonment usually leads to succession and the development of scrublands and, eventually, pioneer forests (Kaligarič, 2005; Stergaršek, 2009).

The Karst edge is a part of the "Karst" *Natura 2000* site. The climate is submediterranean and for its butterfly fauna it is known to represent one of the hotspots within the country as well as the wider area. In the submediterranean region of Slovenia, 152 or 85 % of all confirmed butterfly species for Slovenia were recorded (Verovnik *et al.*, 2012). Some of these species are registered on red lists of endangered species in Slovenia and the wider area (Verovnik *et al.*, 2012). Five species of Lepidoptera are also qualifying species for the "Karst" *Natura 2000* site (marsh fritillary *Euphydryas aurinia* Rottemburg 1775, false ringlet *Coenonympha oedippus* Fabricius 1787, Eastern eggar *Eriogaster catax* (Linnaeus, 1758), Jersey tiger moth *Callimorpha quadripunctaria* (Poda, 1761) and the geometer *Erannis ankeraria* (Staudinger, 1861)).

On the Karst edge, different management practices were assessed through inspection of butterfly diversity (see Jugovic *et al.*, 2013) and the ecological requirements of a target species, the black-veined white, *Aporia crataegi* (Pieridae) (Jugovic *et al.*, in prep.; Figure 3.1a, b, this chapter). Three sampling plots of roughly the same sizes and approximately 1.5–3 km apart were chosen in the area (Table 3.1); a pasture with a scrubbed-over area nearby, and two dry karst meadows. From 15th May until 29th June 2012 mark release-recapture (MRR) was applied to black-veined whites in the three plots. The method was repeated each second or third day, weather permitting. Additionally, also diversity and abundances of other butterfly species (or species complexes) were recorded for each (sub)plot. The scale used was: 0 = unnoted, 1 = single individual, 2 = 2–5 individuals, 3 = 6–10 individuals, 4 = 11–20 individuals, 5 = 20–50 individuals, and 6 = >50 individuals. For the mark-release recapture of black-veined white, an open population approach with an imposed superpopulation (a modified Jolly-Seber model) was applied. Moreover, ecological requirements of black-veined white's larvae (Figure 3.1c, d) were recorded by means of microhabitat selection (the inventarisation of host plants and different environmental parameters) in April 2013 (Jugovic *et al.*, in prep.).

Figure 3.1: (a) Adult male of black-veined white, *Aporia crataegi* (Pieridae) feeding on meadow clary, *Salvia pratensis* (Lamiaceae). (b) Marked male resting on smoketree, *Cotinus coggygria* (Anacardiaceae). (c) Larva of A. crataegi feeding on common hawthorn, *Crataegus monogyna* (Rosaceae). (d) Larva of *A. crataegi* sun bathing on the tip of a branch of common hawthorn. Photographs: Jure Jugovic.

Table 3.1: Summary data on sampling plots (adapted from Jugovic *et al.*, 2013).

Village	Sampling plot	Geographic position	Habitat type (short description)	Area [ha]	Altitude [m a. s. l.]	Distance R1-R2-Z [m]
Rakitovec	R1	N 45°28'19.37" E 13° 57' 29.32"	extensive dry grasslands (partially mowed and partially in an early stage of overgrowing)	5.32	520–540	0-1430-3160
	R2	N 45° 28' 52.80" E 13° 56' 35.03"	extensive dry grasslands (mainly mowed)	5.18	500–520	1430-0-1770
Zazid	Z	N 45° 29' 43.76" E 13° 55' 56.10"	extensive dry grasslands (Z_A: fenced pasture & Z_B: shrubs and overgrowing area)	5.76	600–620	3160-1770-0
Total				16.26	500–620	

3.2 BUTTERFLY DIVERSITY IN CORRELATION WITH THE MANAGEMENT OF HABITATS

In three plots from the Karst edge, 63 species or species complexes (Table 3.2; see also Jugovic *et al.*, 2013, Table 2) were recorded over 16 sampling occasions from the middle May until the end of June in 2012 (Jugovic *et al.*, 2013). Species diversity and composition were highly connected to the management types; nearly all species being recorded on dry karst meadows (60 species, 95 %), while in pasture only 34 species (54 %) were recorded. The diversity on the scrubbed-over area close to the pasture was similarly low (28 species, 44 %). As the species accumulation curves approach a plateau or show a similar decline in the discovery of new species (see Figure 1 in Jugovic *et al.*, 2013), differences in species diversity can be attributed to the habitat differences. Except for one sampling day, the number of species from the two karst meadows exceeded the numbers of species from pasture and the scrubbed-over area. A decrease in the number of species after a peak in middle June partly coincided with mowing of the meadows (see Figure 2 in Jugovic *et al.*, 2013).

Table 3.2: Numbers of butterfly species recorded at plots and subplots on Karst edge.

Village	Plot (subplot)	Habitat	No. of species/plot	No. of species/habitat
Rakitovec	R1	Dry karst meadow	47	60
	R2	Dry karst meadow	55	
Zazid	Z (Z_A)	Pasture	34	34
	Z (Z_B)	Overgrowing area near the pasture	28	28

Two distinct groups with only a few mismatches were recovered from a cluster analysis of samplings recorded in the study plots and subplots (Figure 3.2). Species compositions and abundances from 64 samplings reflected habitat types and localities (meadows near Rakitovec *vs.* pasture and scrubbed-over area near Zazid). These two clusters exhibit a high rate of dissimilarity, exceeding 55 %. Only eight samples from dry meadows clustered together with samples from the pasture and the neighbouring scrubbed-over area, and three samples from the pasture clustered together with majority of samples from the two dry meadows. All except one of samples from the meadows that clustered together with samples from pasture and scrubbed-over area correspond to the time when parts of meadows have already been mown, and all three mentioned samples from the pastures are from the period when most plant species were flowering (see Jugovic *et al.*, 2013).

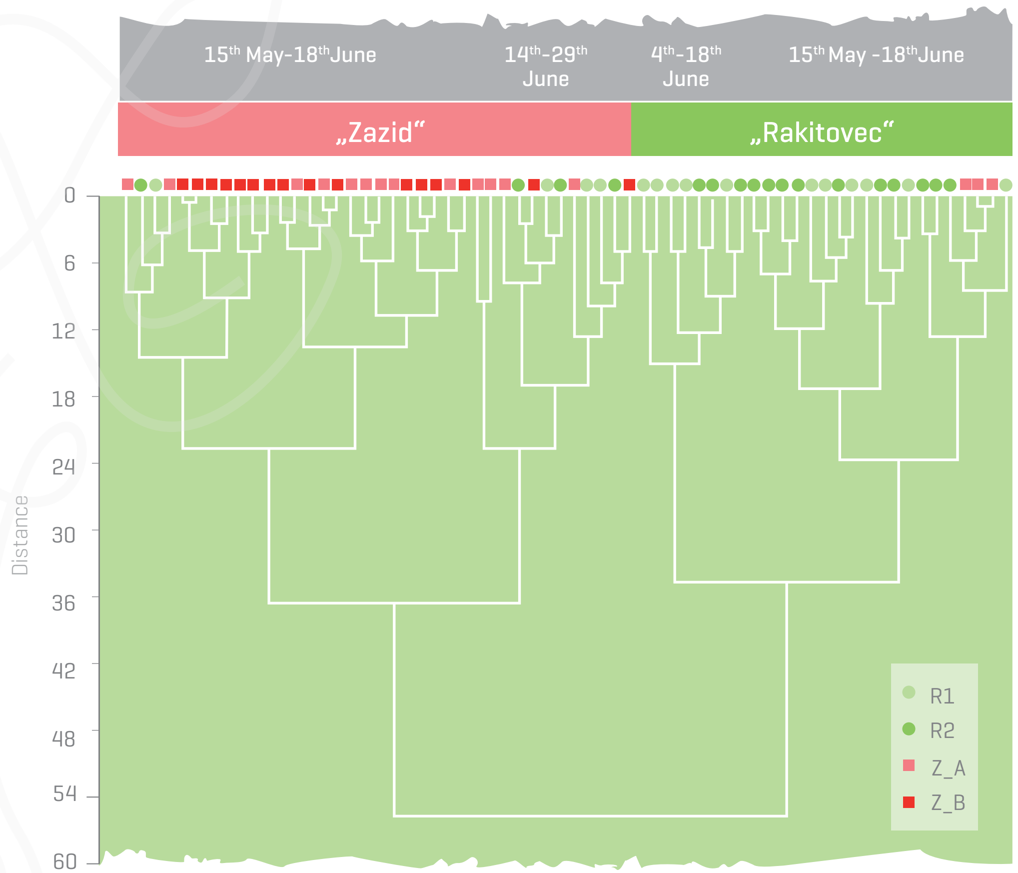

Figure 3.2: Cluster analysis (Euclidean distance, Ward's method) of 64 samplings of butterfly species recorded at three sampling plots from the Karst edge (15th of May–29th of June, 2012). Abbreviations: Z – pasture near Zazid (Z_A – fenced and grazed part; Z_B – neighbouring overgrowing area); R1, R2: dry meadows near Rakitovec (R1: partly overgrown). Adapted and redrawn from Jugovic *et al.*, 2013.

3.3 INTEGRATIVE APPROACH TO MONITORING OF BLACK-VEINED WHITE, *APORIA CRATAEGI*

Altogether, 1711 butterflies were marked during 16 days of fieldwork (1184 males, 527 females, for a photo of a marked individual, see Figure 3.1b). Most of butterflies were marked in one of two dry meadows near Rakitovec with rather fewer in the partially overgrown pasture in Zazid. In the latter plot accounting for 35 % of the entire sampling area, only 14 % of all butterflies were recorded. Migrations of butterflies were recorded between all three sampling plots.

We estimated total population size derived from all three plots together using a modified Jolly-Seber open population model with a superpopulation approach. The module POPAN in programme Mark was used with the overdispersion parameter, c-hat = 1.30. Estimations were derived from model averaging of 58 models in total. Four best-fit models that contributed most to the population size estimates (delta QAICc < 7) are presented in Table 3.3.

Table 3.3: Four best-fit models (delta QAICc < 7) describing seasonal population demography of black-veined white (*Aporia crataegi*) from three plots on Karst edge.

Model	QAICc	Delta QAICc	AICc weights	Model likelihood	Num. par.
Phi(.) p(t) pent(g*t) N(g)	2131.608	0	0.51382	1	37
Phi(.) p(g) pent(g*t) N(g)	2133.019	1.4109	0.25377	0.4939	27
Phi(g) p(t) pent(g*t) N(g)	2133.566	1.9578	0.19306	0.3757	38
Phi(t) p(g) pent(g*t) N(g)	2137.482	5.8738	0.02725	0.0530	37

Population estimates (± standard error) for males and females were 2775 ± 189 (95 % confidence interval (CI): 2403–3146) and 1596 ± 232 (CI: 1141–2052) animals, respectively. From these data, densities were calculated for males and females, amounting to 170 (CI: 148–193) and 98 (CI: 70–126) animals per hectare, respectively.

During 11 days in spring 2013 (April 24th–30th and May 2nd–5th), all three plots were surveyed for presence of potential host plants (shrubs) for black-veined white. Four species of potential host plants were recorded on the Karst edge (Figure 3.3). Of these four, hawthorn (*Crataegus monogyna*) and blackthorn (*Prunus spinosa*) were selected preferably, whereas two other potential host shrubs, rosehip (*Rosa* sp.) and rock cherry (*Prunus mahaleb*) were not preferentially selected (Figure 3.3). There was an emphatic preference towards blackthorn, which represented 29.1 % of the plants investigated, but more than half of all plants occupied by larvae (Jugovic *et al.*, in prep.).

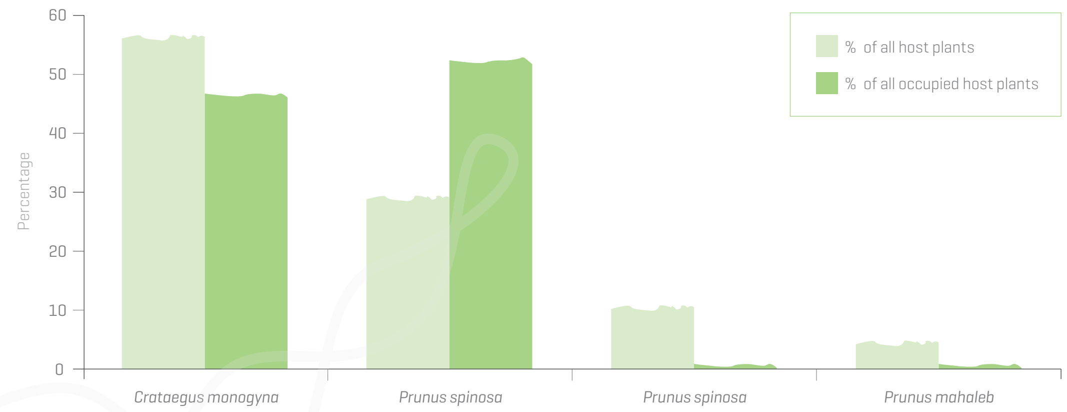

Figure 3.3: Black-veined white larval occupancy of four host plant species with their relative abundances recorded in three plots from Karst edge.

Most of plants with larvae were smaller and more exposed to the sun than unoccupied ones. Moreover, ground cover below the occupied blackthorns consisted mostly of grass or grass and leaves (> 80 % of cases), whereas the percentage of these two categories was much lower under occupied hawthorns (53 %).

In most cases, only a single larva or nest was found on a plant. In more than 80 % of cases no more than three larvae or two nests were recorded per plant. This may simply be a consequence of the abundance of host plants or a strategy for spreading (see Garcia-Barros and Fartmann 2009) when egg-laying females seek out a range of different places in order to ensure development for at least some of the offspring (Jugovic *et al.*, in prep.).

The two preferred host plants, common hawthorn and blackthorn are either mesophilic or thermophilic and so do not grow on very windy sites or in shaded areas. This is especially the case for blackthorn that is a shrub from the first phases of succession but disappears later as the succession progresses (field work observations). As this species seems to better match the ecological requirements of larvae on the Karst edge than common hawthorn does, it comes as no surprise that in later successional stages where hawthorn may still persist, almost no larvae were recorded. Such example was evident in the area subject to scrubbing-over (Jugovic *et al.*, in prep.).

3.4 WHAT DOES THE INTEGRATIVE APPROACH TELL US?

Different studies on diversity and ecological requirements of selected species all show that human activities have the potential to influence the species composition of the flowering plant community (Poldini, 2009). As a consequence this disturbs the nectar feeding of adult butterflies. This said, the effects of grazing are minimal when compared to those of consistent scrubbing-over. The latter negatively affects species of open habitats, leading to a decrease in biodiversity (Kaligarič & Čarni, 1991). Grazing can slow down this scrubbing-over process, thus having a positive impact by leaving some space for the communities found in earlier stages of succession (Kaligarič & Čarni, 1991). Different habitat types were shown to host different butterfly assemblages, with most species being encountered in warm dry karst meadows, following by pastures, and smallest numbers being found in scrubbed-over areas. This is borne out by the numbers and densities of the adults of *Aporia crataegi* encountered and marked from each of these areas. Nevertheless, it should be noted that in some species, larval habitats differ significantly from the ones required by the adult butterflies. In *Aporia crataegi*, adult animals need open spaces with nectar-bearing plants, while their larvae are most abundant in the early stages of succession where their host plants occur at high densities. However, the succession should not proceed too far as nearby flowering plants are also needed by the adults. Hedges that separate different plots are thus important not only as a part of an area's cultural heritage but also as larval habitats for many invertebrate and other species.

Mowing also diminishes butterfly diversity in dry karst meadows, especially when huge areas are mowed early in the season, thus not allowing juvenile stages of butterflies to develop. It has been recorded that species diversity quickly decreased after some areas of the study plots were mowed (see Jugovic *et al.*, 2013). It should not go unnoticed, however, that the end of flight period of some species could also be the reason for observed decline in species diversity. Traditional grazing can therefore maintain species-rich habitats such as those encountered in dry karst meadows (see Fahrig *et al.*, 2011) that are known to host high biodiversity. Nevertheless, grazing should be traditional and rotational, so constant pressure is not applied to a given area. Furthermore, this allows the presence of different stages of succession that can host many more species than only a single stage can. The advantage of a mosaic of successional stages that support high diversity has already been stressed as best option for high butterfly diversity (Balmer & Erhardt, 2000).

CONCLUSION

Although all three activities and processes, mowing, grazing and scrubbing-over, result in a decrease in species richness, we believe that grazing can also play an important role in increasing the diversity of dry karst meadows. It slows down or halts the scrubbing-over process and leaves the habitats more open (Kaligarič & Čarni, 1991). The presence of species of conservation concern at all survey sites further supports the importance of traditional activities that create and maintain the mosaic structure of the area. Such a structure is vital for the feeding, resting, reproduction, growth and development of butterflies (see Jugovic *et al.*, 2013).

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CHAPTER 4

DUNG BEETLE (COLEOPTERA: SCARABAEOIDEA) *COMMUNITIES* IN KARST PASTURES

ZDRUŽBE PLOJKAŠEV (COLEOPTERA: SCARABAEOIDEA) NA KRAŠKIH PAŠNIKIH

COMMUNITÀ DI SCARABEI STERCORARI (COLEOPTERA: SCARABAEOIDEA) SULLE PRATERIE CARSICHE

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ABSTRACT

We assessed biodiversity of dung beetles from two pastures near Zazid and Hrastovlje on the Karst edge, where four pitfall traps were set at each of the three sampling plots (D1: open pasture, D2: pasture with overgrowth, D3: meadow at the forest edge outside the fenced pasture) of the pasture and neighbouring area from spring to autumn 2012. Cattle excrements that were used as bait were added to two pitfall traps at each sampling plot and another two traps were used as a control. We analysed differences in species composition and abundances with regard the study site (the pasture in the surroundings of village Zazid *versus* the pasture in the surroundings of village Hrastovlje), the use of bait (absent *versus* present), degradation stage (D1, D2, D3) and stage of the bait (fresh *versus* old). As an identification tool, external morphology was used. However in cases of closely related taxa, genitalia structure and the DNA barcoding method were employed. A clear preference of dung beetles towards traps with fresh bait was shown. Most species and individuals of dung beetles were recorded from the overgrown part of the pasture (D2). Other ground living invertebrate groups, however, were most abundant outside the pasture (D3), hence the mosaic structure of the landscape is favoured to maintain highest biodiversity possible.

Key words: pitfall traps, biodiversity, degradation, grazing

IZVLEČEK

Ocenjevali smo diverziteto hroščev koprofagnih plojkašev na dveh pašnikih pri Zazidu in Hrastovljah na Kraškem robu. Na vsakem od obeh pašnikov smo izbrali po tri vzorčne ploskve (D1: pašnik, D2: pašnik v zaraščanju, D3: travnik ob gozdnem robu izven ograjenega dela pašnika) in na vsaki vzorčili s po štirimi talnimi pastmi od pomladi do jeseni 2012. V dve pasti na vsaki vzorčni ploskvi smo dodali za vabo goveji iztrebek, preostali dve pasti sta bili kontrolni. Analizirali smo razlike v vrstni sestavi in abundancah vrst med obema pašnikoma (Hrastovlje, Zazid), med tremi vzorčnimi ploskvami (D1, D2, D3), v pasteh z in brez vabe, ter glede na starost vabe. Taksone smo določali na podlagi zunanjih znakov. Vendar pa v primeru ozko sorodnih taksonov ti znaki pri določanju pogosto niso jasni, zato smo v teh primerih taksone določali na podlagi zgradbe genitalnih struktur ali uporabili črtne kode DNA. Plojkaši so izkazali jasno preferenco za sveže iztrebke, večino plojkašev (tako živali kot vrst) pa smo zabeležili na zaraščajočem delu pašnika (D2). Drugi talni nevretenčarji, ki so se ulovili v pasti, so bili v največjem številu zastopani na travniku izven pašnika (D3). Zaključujemo, da je za vzdrževanje visoke biotske raznovrsnosti treba vzdrževati mozaičnost pokrajine.

Ključne besede: talne pasti, biodiverziteta, degradacija, paša

RIASSUNTO

In questo studio abbiamo valutato la biodiversità degli scarabei stercorari su due pascoli nei pressi di Zazid e Hrastovlje nell'area del Ciglione carsico, dove quattro trappole a caduta sono state collocate in ciascuno dei tre siti di campionamento (D1: pascolo aperto, D2: pascolo incolto, D3: prato ai margini della foresta al di fuori del pascolo recintato) del pascolo e nell'area prossima al pascolo nel periodo dalla primavera all'autunno del 2012. Escrementi di bestiame sono stati utilizzati come esca è aggiunti in due trappole a caduta per ogni sito di campionamento e le altre due trappole sono state utilizzate come controllo. Abbiamo analizzato le differenze nella composizione delle specie e l'abbondanza di esemplari in relazione all'area di studio (il pascolo nei dintorni del villaggio di Zazid rispetto al pascolo nei dintorni del villaggio di Hrastovlje), all'uso di esche (assente contro presente), allo stadio di degradazione (D1, D2, D3) e allo stadio dell'esca (fresco rispetto a vecchio). Come strumento di identificazione abbiamo utilizzato la morfologia esterna. Tuttavia, in casi di taxa strettamente correlati, abbiamo utilizzato come metodo d'identificazione la struttura degli organi genitali e il DNA barcoding. È stata dimostrata una chiara preferenza degli scarabei stercorari verso trappole con esca fresca. La maggior parte delle specie e individui di scarabei stercorari sono stati registrati dalla parte incolta del pascolo (D2). Altri gruppi d'invertebrati del suolo erano più abbondanti al di fuori del pascolo (D3), quindi la struttura a mosaico del paesaggio è evidentemente favorevole per la manutenzione di un alto livello di biodiversità.

Parole chiave: trappole a caduta, biodiversità, degradazione, pascolo

4.1 DUNG BEETLES AS THE INDICATORS OF THE HEALTH OF AN AGRO-ECOSYSTEM

Indicators of environmental conservation in agro-ecosystems have received a great deal of recent attention, which remains desirable as a basis for managing agro-ecosystems. Unlike the majority of beetles, scarabaeoids are well known to most people due to their relatively large size, often bright colouration, significant economic importance and the association of the genus *Scarabaeus* Linneaus, 1758 with the sacred symbols of ancient Egypt (Grebennikov & Scholtz 2004). Dung beetles are members of the diverse and numerous superfamily Scarabaeoidea, and include their coprophagous representatives, usually those of the families Geotrupidae, Scarabaeidae and Aphodiidae (Halffter & Matthews, 1966). They represent a relatively small group of insects, with some 5000 species known worldwide (Scholtz *et al.*, 2009). Most species are associated with herbivore dung, but some species also use other non-dung food sources, like carrion or detritus. Adults and larvae split the resource further by feeding on different fractions of the food. What is astonishing in this group of beetles is the well-developed brood care, which permits a smaller energetic investment in reproduction than would otherwise be the case, and, ultimately, results in low juvenile mortality. Dung beetles fall into three basic nest building categories, tunnelers (paracoprids), dwellers (endocoprids), or rollers (telecoprids). Dung beetles known as rollers, roll dung into round balls, which are used as a food source or brooding chambers. Other dung beetles, known as tunnelers, bury the dung wherever they find it. A third group, the dwellers neither roll nor burrow, but stay and live in the dung (Bertone *et al.*, 2012).

Dung beetles fulfil vitally important functions such as nutrient cycling, bioturbation, pollination, seed dispersal, parasite suppression, and nutrient cycling in many different ecosystems (Andresen, 2001, 2003; Losey & Vaughan, 2006; Nichols *et al.*, 2008; Hanski & Cambefort, 1991).

Ecosystem services benefit human well-being and are heavily depended on viable natural systems to provide ecosystem functions that directly or indirectly serve and support human life in countless ways. This also includes services provided in agro-ecosystems; especially so since humanity developed a pastoral lifestyle (Wallace, 2007). As dung beetles consume large amounts of dung as adults and larvae, their actions have been credited in reducing pasture fouling, adding nutrients to soil, aerating the soil and competing for nesting habitat and food resources with flies. They have the potential to improve pastures through the incorporation of manure into pasture soils. There is clear evidence that tunnelling dung beetles in the pasture ecosystem are not only responsible for the removal of dung, but also play a critical role in the nutrient cycle of cattle pastures, facilitating the return of nitrogen to the soil resulting in improved plant growth (Bertone *et al.*, 2006).

Dung beetles are excellent indicators for landscape biodiversity studies as they are ecologically sensitive and can compositionally respond to slight changes in the local environment (Nichols *et al.*, 2008). They are useful indicators both of high biodiversity in untransformed pastoral regions and of healthy pastures in untransformed or intensively farmed areas. At a local scale, characteristic differences in species assemblage structure between natural and transformed habitats are useful both for conservation issues and for certification and marketing of high quality products from natural pastures. This permits the testing of reserves for relative naturalness and the certification of farms with a high proportion of natural vegetation, and high dung beetle diversity or endemicity, as 'natural' for the marketing of natural products such as meat (Davis *et al.*, 2004).

4.2 OBJECTIVES OF THE STUDY

In this chapter we review the case of the use of dung beetle assemblages in different habitats (Figures 4.1, 4.2) at local scale on the Karst edge. In period from March to October 2012, we set pitfall traps (with propylene glycol) in two karst pastures on Karst edge, the region being characterized by higher altitudes (78 % territory is higher than 600 m a. s. l.) and a low population density (Gams *et al.*, 1998). The region is a mosaic structure of forests, grasslands and anthropogenic areas. Two pastures near the villages of Hrastovlje and Zazid were chosen for study sites. In each of the pastures, traps were set in three different habitats (for the details, see Table 4.1; see also Figure 4.2). Abundance of individuals and species was analysed to understand the influence of different types of human-modified ecosystems upon dung beetles. For the detailed statistical analysis, samples from March to June were included. The species list, however is prepared for the whole sampling period.

As far as their ability to live in different habitats the dung beetles were categorized into three categories: "eurytopic" – species able to tolerate a wide range of environments, "oligotopic" – species capable of living in somewhat different environments and "stenotopic" – able to tolerate only a narrow range of environmental changes. Taxonomy follows Alonso-Zarazaga (2004), while the chorotype and ecology were determined according to Brelih *et al.* (2010).

Table 4.1: Summary data on two study sites and six sampling plots (habitats). At each plot, two pitfall traps with and two without a bait (cattle excrement) were checked after four and fourteen days. During each visit, traps were emptied, and the dung was changed each fourteen days.

Village	Altitude	Geographic position	Habitat 1 (D1)	Habltat 2 (D2)	Habitat 3 (D3)
Zazid	387 m	45°29´33.58´´S, 13°56´13.29´´E	open pasture	pasture with overgrowth	meadow at forest edge
Hrastovlje	94 m	45°31´20.18´´N, 13°56´15.63´´E	open pasture	pasture with overgrowth	meadow with hedgerow

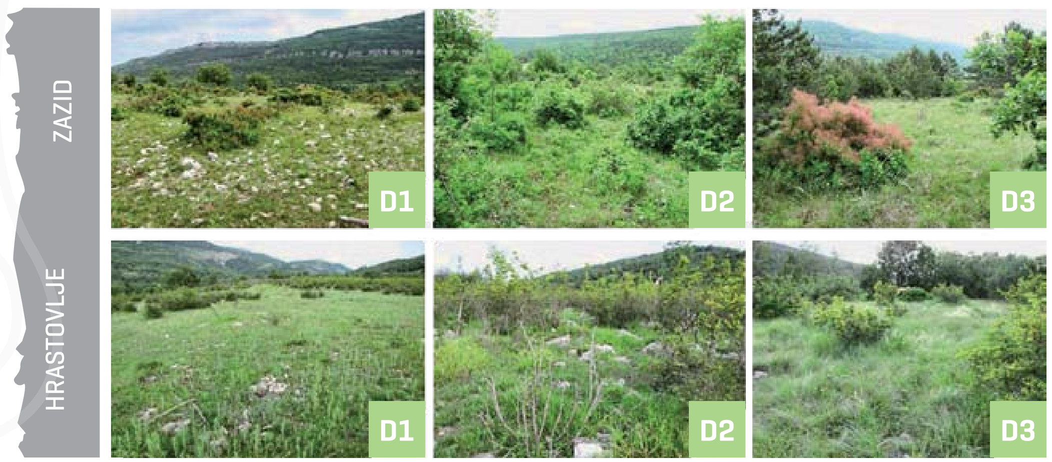

Figure 4.1: Three habitats from pastures near villages Zazid and Hrastovlje with denoted degradation levels (D1 – most degraded, D3 – least degraded; see also Table 4.1). Photograph: Nataša Koprivnikar.

While developing time-efficient and cost-effective ecological research, taxonomic challenges in hyper-diverse invertebrate groups, such as dung beetles in our case, represent a major barrier (Samways, 2002). The taxonomical relationship between European dung beetles has only been superficially studied, and mostly involved species from the Iberian peninsula (Villalba *et al.*, 2002). In this study we used classical morphometry for taxa identification but also DNA barcoding as a tool for the correct identification of problematic dung beetle taxa, which cannot be easily or reliably identified merely on the basis of external morphology (Fišer Pečnikar & Bužan, 2014).

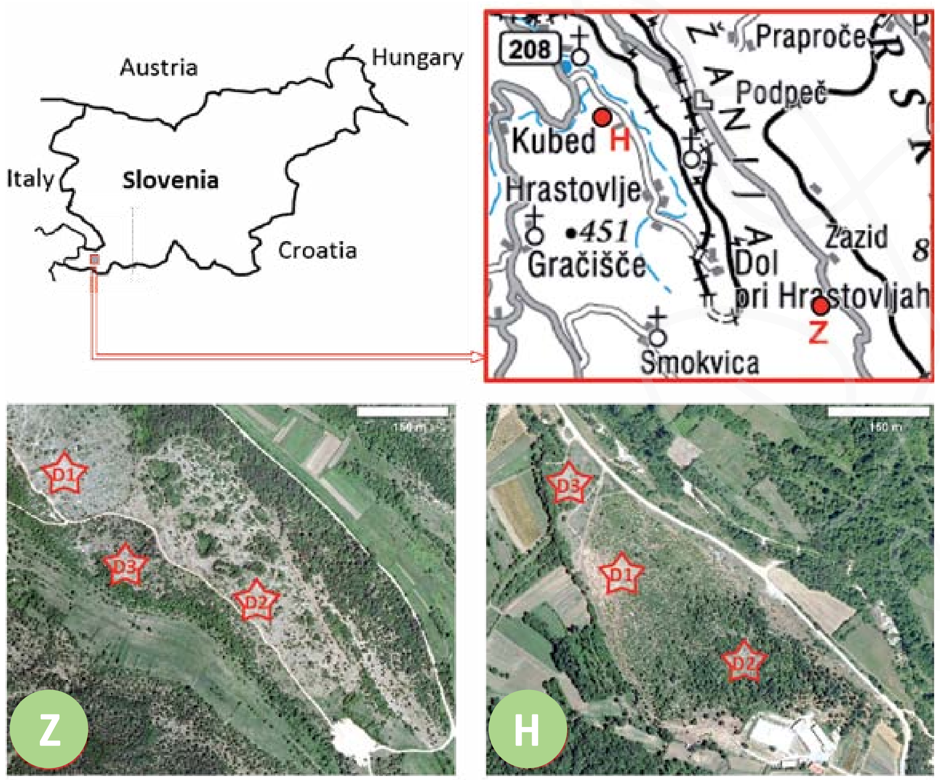

Figure 4.2: Position of Slovenia and two selected karst pastures (study sites) located near Zazid (Z) and Hrastovlje (H). Red stars represent the localities on which traps were set. D1 – open pasture; D2 – pasture with overgrowth (both within the fenced pasture); D3 – meadow at the forest edge outside the fenced pasture. Cartography: Peter Glasnović.

4.3 SPECIES DIVERSITY AND ABUNDANCE

In total we sampled 29 dung beetle species, belonging to three families, Geotrupidae (2 species), Scarabaeidae (15) and Aphodiidae (12) (Table 4.2). The karst pasture at Zazid proved to be more favourable for dung beetle communities, both in terms of species diversity and the number of recorded specimens, with 24 species and 2745 specimens recorded. On the other hand, at the study site near Hrastovlje, 20 species and 1027 specimens were recorded. Fifteen species were recorded at both study sites, while nine and four species were recorded only at Zazid and Hrastovlje, respectively (Table 4.2).

Table 4.2: Systematic list of recorded dung beetle species, number of specimens, chorotype and ecology. When a species was recorded exclusively in one study site, numbers are denoted in bold type.

	Species	No. of specimens		Chorotype*	Ecology
		Hrastovlje	Zazid		
	Scarabeidae Latreille, 1802				
1.	*Sisyphus schaefferi* (Linnaeus, 1758)	547	1831	Turanic-European-Mediterranean	Stenotopic
2.	*Copris lunaris* (Linnaeus, 1758)	**2**	0	Asian-European	Stenotopic
3.	*Euoniticellus fulvus* (Goeze, 1777)	4	2	Palearctic	Stenotopic
4.	*Caccobius schreberi* (Linnaeus, 1767)	**22**	0	Turanic-European-Mediterranean	Stenotopic
5.	*Onthophagus coenobite* (Herbst, 1783)	2	12	Turanic-European	Eurytopic
6.	*Onthophagus fracticornis* (Preyssler, 1790)	4	25	Siberian-Turanic-European	Eurytopic
7.	*Onthophagus grossepunctatus* (Reitter, 1905)	229	278	Southern-middle European	Stenotopic
8.	*Onthophagus illyricus* (Scopoli, 1763)	3	5	Turanic-European-Mediterranean	Stenotopic
9.	*Onthophagus lemur* (Fabricius, 1781)	0	**11**	Turanic-European	Stenotopic
10.	*Onthophagus medius* (Kugelann, 1792)	1	44	Turanic-European-Mediterranean	Stenotopic
11.	*Onthophagus ovatus* (Linnaeus, 1767)	10	20	Turanic-European	Eurytopic
12.	*Onthophagus ruficapillus* (Brullé, 1832)	7	1	Turanic-European	Stenotopic
13.	*Onthophagus taurus* (Schreber, 1959)	14	5	Palearctic	Stenotopic
14.	*Onthophagus verticicornis* (Laicharting, 1781)	9	99	Turanic-European	Eurytopic
15.	*Ontophagus joannae* (Goljan, 1953)	13	29	European	Eurytopic
	Aphodiidae Leach, 1815				
16.	*Colobopterus erraticus* (Linnaeus, 1758)	0	**2**	Asian-European-Mediterranean	Oligotopic
17.	*Esymus pusillus* (Herbst, 1789)	0	**1**	Asian-European	Eurytopic
18.	*Acrossus luridus* (Fabricius, 1775)	0	**2**	Palearctic	Oligotopic
19.	*Aphodius fimetarius* (Linnaeus, 1758)	**12**	0	Subcosmopolit	Eurytopic
20.	*Loraphodius suarius* (Faldermann, 1835)	0	1	South-European	Oligotopic
21.	*Euorodalus coenosus* (Panzer 1798)	1	**1**	Turanic-European	Oligotopic
22.	*Melinopterus prodromus* (Brahm, 1790)	**3**	0	Asian-European-Mediterranean	Eurytopic
23.	*Oxyomus sylvestris* (Sopoli, 1763)	5	13	Turanic-European-Mediterranean	Eurytopic
24.	*Phalacronothus biguttatus* (Germar, 1824)	0	**1**	Asian-European-Mediterranean	Oligotopic
25.	*Sigorus porcus* (Fabricius, 1792)	0	**14**	European	Stenotopic
26.	*Subrinus sturmi* (Harold, 1870)	**1**	0	Palearctic	Stenotopic
27.	*Teuchestes fossor* (Linnaeus, 1758)	0	**1**	Palearctic	Oligotopic
	Geotrupidae Latreille, 1796				
28.	*Geotrupes spiniger* Marsham, 1802	0	**5**	Turanic-European	Eurytopic
29.	*Trypocopris vernalis* (Linnaeus, 1758)	318	341	Western-European	Stenotopic
	Individuals (total):	1027	2745		
	Species (total):	20	24		

*according to the biogeographical affilitaion (Brelih *et al.*, 2010)

As far as the habitat preferences were concerned, ten species were characterized as eurytopic, six as oligotopic and thirteen as stenotopic, indicating that the dung beetles are mostly highly specialised. The presence of such a high number of stenotopic species on two pastures is good bioindicator for ecologically diverse microhabitats present in the area. From our samples in early season (March – June), it was clear that dung beetles preferably chose traps with bait over traps without it; this applies for two parameters: number of individuals as well as for number of dung beetle species (Mann-Whitney test, $p<0.001$). Nevertheless, no differences were found in number of individuals or species between the two study sites or the state of the dung (i.e. new (after four days) *versus* old (after 14 days) baits; Mann-Whitney test, range of p values: 0.113–0.972). No differences were obtained in the number of individuals or species from the three degraded stations either (both study sites pooled, Kruskall-Wallis test, $p > 0.17$). When the two study sites were analysed separately, significant differences in the number of individuals and number of species were confirmed for both sites between traps with and without baits (Mann-Whitney test, $p < 0.001$), while in the location near Hrastovlje, we found significant differences in both parameters (Mann-Whitney test, $p < 0.05$) between degraded stations D1 (open pasture) and D2 (part of pasture with overgrowth). In both parameters the values were highest in degraded station D2. This was also the case in location near Zazid, although statistical tests were not significant ($p > 0.05$).

The results prove the importance of pastures for dung beetles. It should be pointed out, however, that dung beetles mostly prefer shaded areas of the pasture (in our case D2) over open parts (D1) or shaded parts of meadows outside (D3). This means that both, presence of grazers with their excrements, and shade are preferred, probably because of higher relative humidity and the plethora of hiding places. In another ongoing study in Croatian Istria only 70 km south of our study sites, the number of species recorded was much lower despite the same sampling methodology (Koren, *in prep.*). Inspected habitats (grasslands, forest edge and forest) from the mentioned study, however were different from those on the Karst edge as no pastures were included in the study. It should also be noted that fifteen species of the subfamily Scarabeinae from only two study sites represent over one third of all the Mediterranean species of this subfamily (40 species in total; Mikšić, 1958). This clearly shows the importance of pastures for dung beetles biodiversity, even more when these areas are maintained over longer periods when obviously, numbers of species increase through years of pastoral activity (Imura *et al.*, 2014).

Records of two species, *Copris lunaris* (Linnaeus, 1758) and *Onthophagus joannae* (Goljan, 1953) are worthy of note (Table 4.2). The first had not been recorded in Slovenia for over fifty years, and it was thought to be probably extinct (Brelih *et al.*, 2010) in the country. Two individuals of *C. lunaris* were found at the location near Hrastovlje, and its presence in Slovenia has been confirmed. *O. joannae* was recorded in Slovenia for the first time, with more than 40 individuals in total from both study sites (see Table 4.2). As this species is morphologically very similar to *Onthophagus ovatus* (Linnaeus, 1767), we have confirmed the correct identification by checking genital structures. Correct identification was further confirmed by DNA barcoding, sequencing the gene for cytochrome oxidase I (1131 base pairs). For the comparison, we used already-published sequences of European dung beetles (Villalba *et al.*, 2002).

The results confirmed our correct identification of *O. joannae*, and for the first time showed that the two species also differ genetically, not only morphologically (Ballerio *et al.*, 2010; Figure 4.3).

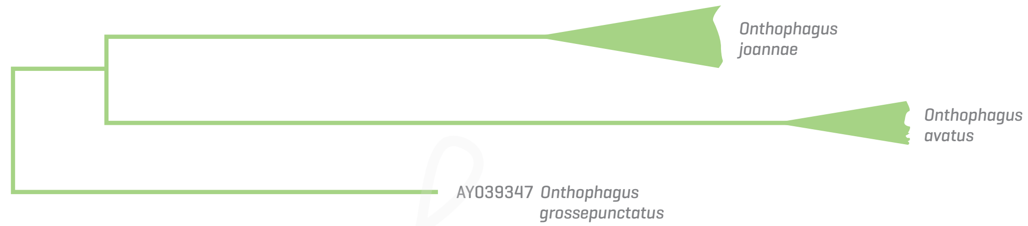

Figure 4.3: Phylogenetic tree (Neighbour joining tree, maximum likelihood) of the genus *Onthophagus*, showing the diferentiation between *O. ovatus and O. joannae. O. grossepunctatus* was used as an outgroup. The analysis was conducted in program MEGA 6.0.

CONCLUSION

Pastures are very important for the biodiversity of dung beetles, especially when different stages of succession (or degradation) are present in the area. Areas with the most overgrowth were preferably selected, indicating that overgrazing is not desirable when seeking to maintain high biodiversity in dung beetles. Other ground-living invertebrates also avoid open pastures, as their biodiversity was always highest at the D3 stations outside the pastures. Therefore rotational grazing that leads to a mosaic landscape is favoured.

SUGGESTED READINGS

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CHAPTER 5

THE BIRDS OF DRY MEADOWS ABOVE THE KARST EDGE

PTICE SUHIH TRAVNIKOV NAD KRAŠKIM ROBOM

GLI UCCELLI DELLE PARATERIE ARIDE SOPRA IL CIGLIONE CARSICO

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ABSTRACT

Below we present the research carried out so far on the birds of dry meadows in the Karst. We also present some results of the BioDiNet project including a survey of the birds of the Podgorski Kras in 2012, with a comparison to the year 2007. The comparison was done especially with regard to the presence of birds in relation to the grazing intensity. We also present a survey of the birds of Golič plateau in 2013 and a comparison with the year 2002 and a survey of the birds of an area of restored grazing close to the village of Basovizza in 2013 and 2014 with some comparisons with earlier years. Grazing Grazing on Podgorski Kras had positive impact on two important species in conservation terms: wood lark *Lullula arborea* and corn bunting *Emberiza calandra* with, on the other hand, a negative impact on skylark *Alauda arvensis*. Hoopoe *Upupa epops* and nightjar *Caprimulgus europaeus* have benefited from deforestation in the Basovizza area but it looks as if the deforestation was not radical or extensive enough for other meadow species to resettle the area.

Key words: grazing, land abandonment, deforestation, vegetational succession, karst meadows, Karst, black pine plantations, transect count, hoopoe, wood lark, nightjar, tawny pipit, skylark, ortolan bunting

IZVLEČEK

V prispevku predstavljamo dosedanje raziskave ptic na suhih travnikih Krasa. Predstavljamo tudi nekatere rezultate projekta BioDiNet, predvsem popis ptic na območju Podgorskega Krasa v letu 2012, ki ga primerjamo s popisom iz leta 2007; raziskovali smo predvsem prisotnost vrst glede na intenzivnost paše. Predstavljamo tudi popis ptic na planoti Golič iz leta 2013 in ga primerjamo s popisom iz leta 2002 ter popis ptic v okolici vasi Bazovica, kjer je bila paša pred kratkim ponovno vzpostavljena. Ta popis smo naredili v letih 2013 in 2014 in ga primerjamo z rezultati popisov v prejšnjih letih. Paša na Podgorskem Krasu je imela pozitiven vpliv na dve varstveno pomembni vrsti: hribskega škrjanca *Lullula arborea* in velikega strnada *Emberiza calandra*, negativen vpliv pa se je pokazal pri poljskem škrjancu *Alauda arvensis*. Na smrdokavro *Upupa epops* in podhujko *Caprimulgus europaeus* je razgozdovanje v okolici Bazovice vplivalo pozitivno, vendar ni bilo dovolj obsežno, da bi se naselile tudi ostale travniške vrste ptic.

Ključne besede: paša, opuščanje kmetovanja, razgozdovanje, vegetacijska sukcesija, kraški travniki, Kras, plantaže črnega bora, transektni popis, smrdokavra, hribski škrjanec, podhujka, rjava cipa, poljski škrjanec, vrtni strnad

RIASSUNTO

In seguito presentiamo la ricerca eseguita fino ad oggi sull'avifauna delle praterie aride del Carso. Riportiamo anche alcuni risultati del progetto BioDiNet, incluso lo studio su gli uccelli del Carso Podgorski, eseguito nel 2012, e messo a confronto con l'anno 2007, soprattutto per quanto riguarda la presenza degli uccelli in relazione all'intensità del pascolo; lo studio su gli uccelli del Monte Golič nel 2013 rispetto all'anno 2002 insieme allo studio degli uccelli nel 2013 e nel 2014 in un'area di pascolo ripristinata, situata vicino al villaggio di Basovizza, con alcune comparazioni rispetto agli anni precedenti. Il pascolo ha prodotto un impatto positivo su due specie importanti in termini di conservazione: la Tottavilla *Lullula arborea* e lo Strillozzo *Emberiza calandra*, accompagnato, dall'altra parte, da un impatto negativo sull'Allodola *Alauda arvensis*. l'Upupa *Upupa epops* e il Succiacapre *Caprimulgus europaeus* hanno tratto beneficio dalla deforestazione nell'area di Basovizza, sembra però che questa non sia stata abbastanza radicale o estensiva per il ripopolamento di altre specie di prateria.

Parole chiave: pascolo, abbandono dell'agricoltura, disboscamento, successione della vegetazione, prati carsici, Carso, piantagioni di pino nero, censimento su transetto, upupa, tottavilla, succiacapre, calandro, allodola comune, ortolano

5.1 INTRODUCTION

The Karst today looks very different from the Karst of a hundred years ago, due to changes in land management, mostly abandonment. Today, a barren Karst landscape of the past is more of an exception than the rule. It can be mostly found in areas exposed to the wind (the '*bora*' or '*burja*') and where black pine *Pinus nigra* planting did not take place (Perko & Orožen Adamič, 1998). The loss of grasslands on the Karst is mirrored by changes in bird populations. Species such as the short-toed lark *Calandrella brachydactyla* and calandra lark *Melanocorypha calandra* that breed on dry and rocky grasslands disappeared decades ago. From a nature conservation point of view, the loss of common grassland birds for which the Karst does not represent the edge of their breeding range, is of greater concern. One of the most threatened species is the ortolan bunting *Emberiza hortulana*. Its population in Slovenia (and in the Karst) has dramatically declined over the past 10 years, in 2013 an all time low of 29 singing males were recorded, all of them were registered in the Slovene Karst (Figelj & Kmecl, 2013). No territorial males have been recorded in the Italian Karst since 2007.

Abandonment has progressed even further in the Italian Karst where, since the Second World War, animal husbandry has undergone a major decline, leading to the progressive scrubbing-over of the *landa* (*gmajna*) and the re-establishment of forest cover through a series of intermediate stages. At present woodland communities cover more than 60% of the Karst above Trieste and near-natural herbaceous communities make up less than 20% of the land cover.

Very few surveys of the karstic meadows above the Karst edge have been carried out since 2000, besides those presented in this book. In the wider area of the Slovene Karst, the surveys for the new ornithological atlas of Slovenia were done mostly in 2004 (data of DOPPS – BirdLife Slovenia) but are not yet published. Geister (2002) did a survey on the Golič plateau in 2002 but rather late in the year, in the second half of June. For some *Natura 2000* species which require meadows as a habitat, regular monitoring has continued since 2004, namely rock partridge *Alectoris graeca*, scops owl *Otus scops*, nightjar *Caprimulgus europaeus*, wood lark *Lullula arborea* and ortolan bunting *Emberiza hortulana* (Denac *et al.*, 2013). Results of the monitoring show that some grassland species still have relatively strong populations on the Karst compared to other areas in Slovenia.

During our research we were interested in population changes on a larger area of Karst grasslands and in testing appropriate methods for the monitoring of *Natura 2000* species. In this chapter we present case studies in three areas in the Karst: on Podgorski Kras where grazing took place during the research period, on Golič plateau where the karst meadows have been abandoned for decades and in the Basovizza area where a large area was deforested recently. All these environmental changes are discussed in relation to the changes in bird populations.

5.2 CASE STUDIES

5.2.1 INFLUENCE OF GRAZING ON THE AVIFAUNA OF PODGORSKI KRAS

In 2012 we repeated the bird survey of the Podgorski Kras (part of SPA Kras SI5000023) which had already been carried out in 2007 during the project *Natura Primorske* (Interreg IIIA SI-IT 2000-2006). The research area (Figures 5.1, 5.4) altogether covers 29.2 km^2. The method was a transect count with three counting belts on each side of the transect (0-50 m, 50-100 m, above 100 m). We counted 'pairs'; individual birds, pairs and nests were all interpreted as pairs. All pairs were marked on the digital orthophoto map. The survey took part once, from 22 May to 19 June 2012 and was carried out from sunrise till 9 a.m. with a speed of approx. 1 km/h. Altogether 51.682 km were surveyed. Together with birds we also surveyed grazing intensity (Figure 5.2). The population change was estimated with the use of abundance indices between surveys (Tables 5.1 & 5.2).

According to our observations grazing took part in all the years for the period 2007-2012 and it was never very intensive. The average intensity for the year 2007 was 1.6 (on an ordinal scale, for explanation see Figure 5.3) and slightly increased in 2012 with average intensity of 1.7. A grazing intensity of 1 means there is practically no grazing and 5 that the grazing is devastating for the grassland. The majority of grazing took place on the northern, more open part of the research area, where it had an average intensity of approx. 3. Forest areas were mainly without any grazing although in some places traces of grazing (faeces) were also observed in wooded areas. Grazing was very heterogenous as regards the stock species, most numerous animals being horses followed by cattle and donkeys.

The general conclusion is that for the majority of *Natura 2000* species, this type of grazing was beneficial. Some species which have declined at a national level and are becoming increasingly rare, showed increases in population here. These include turtle dove *Streptopelia turtur*, hoopoe *Upupa epops*, wood lark *Lullula arborea*, red-backed shrike *Lanius collurio*, linnet *Carduelis cannabina* and corn bunting *Emberiza calandra*. Even though the area is relatively small, it still holds a substantial percentage of the national population for hoopoe, woodlark and corn bunting and an even more substantial part of the populations inside the SPA Kras (Table 5.3). Tawny pipit *Anthus campestris* is also present here with three pairs. As somewhat expected, scrubland species such as common whitethroat *Sylvia communis*, the population of melodious warbler *Hippolais polyglotta* and yellowhammer *Emberiza citrinella* declined. Grazing intensification negatively impacted ground nesters, most notably skylark *Alauda arvensis* and ortolan bunting *Emberiza hortulana*. Ortolan bunting *Emberiza hortulana* was probably additionally negatively influenced by the clearance of single black pine trees, due to the subsidies which promote this clearance. This species also almost certainly suffered because its foraging grounds have been disappearing in recent decades, especially extensive and traditional agricultural areas. In addition, on a landscape level the factors that positively influence the presence of ortolan bunting on breeding grounds have been disappearing, especially larger meadows and absence of human disturbance, as shown by DeGroot *et al.* (2010). (Table 5.1)

This case study is an example which shows that grazing, if not very intensive, can be beneficial for the birds of karstic meadows. Grazing also hinders further reforestation and fragmentation of their habitats. However, skylark and ortolan bunting show us that appropriate measures for *Natura 2000* species should be included and that the introduction of grazing should be careful and planned. Bird populations respond quickly to inappropriate management.

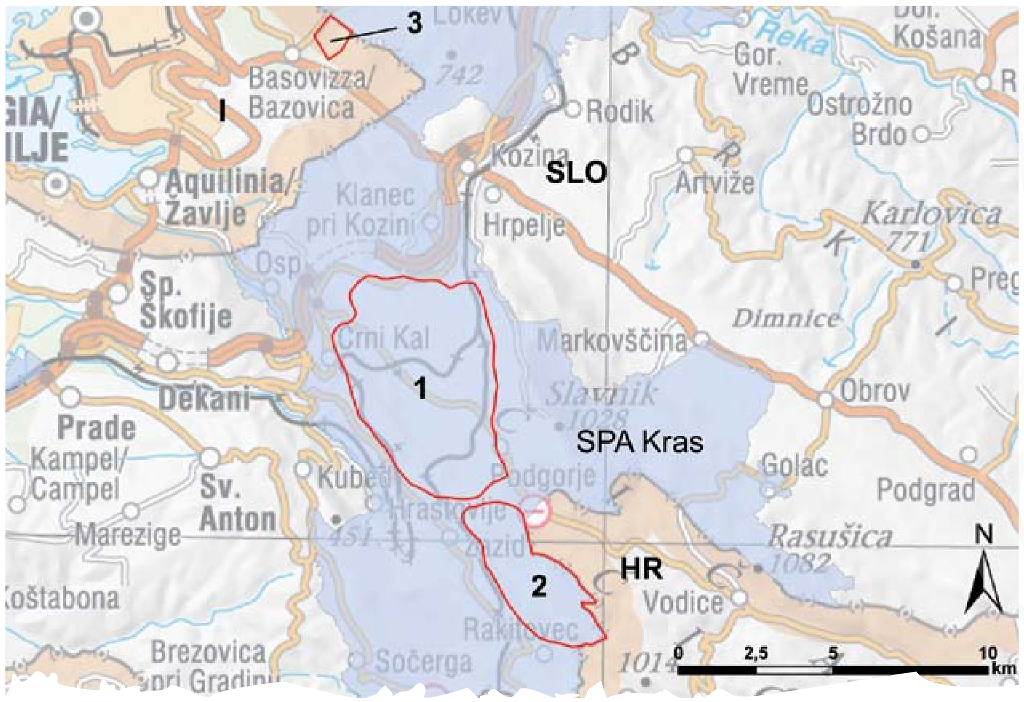

Figure 5.1: The research areas: 1 – Podgorski Kras (SLO), 2 – Golič plateau (SLO), 3 – Basovizza study area (I) map: 1:500.000, GURS – The Surveying and Mapping Authority of the Republic of Slovenia

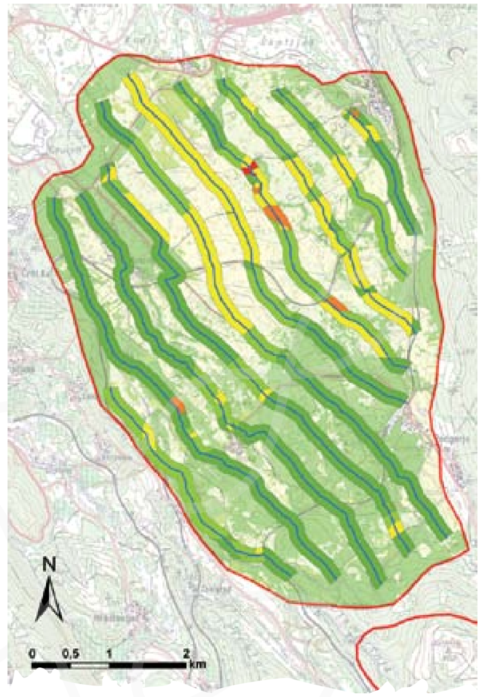

Figure 5.2: Research area 1 (Podgorski Kras); blue lines denote survey transects, areas in the background in dark green are forests; grazing intensity in 2012 in 100 m belt on each side of the transects on the scale 1–5 is also shown:

1 (dark green) – meadow ungrazed, no changes in naturalmeadow seen,
2 (light green) – grazing less intensive, smaller changes of the meadow due to grazing are seen and almost no faeces, 3 (yellow) – grazing moderately intensive,
4 (orange) – grazing is very intensive and meadow substantially changed due to grazing,
5 (red) – meadow devastated due to grazing (e.g. around watering places).

map: 1:50.000 (GURS – The Surveying and Mapping Authority of the Republic of Slovenia); agricultural use: RABA_20120910.shp (MKO - Ministry of Agriculture and the Environment)

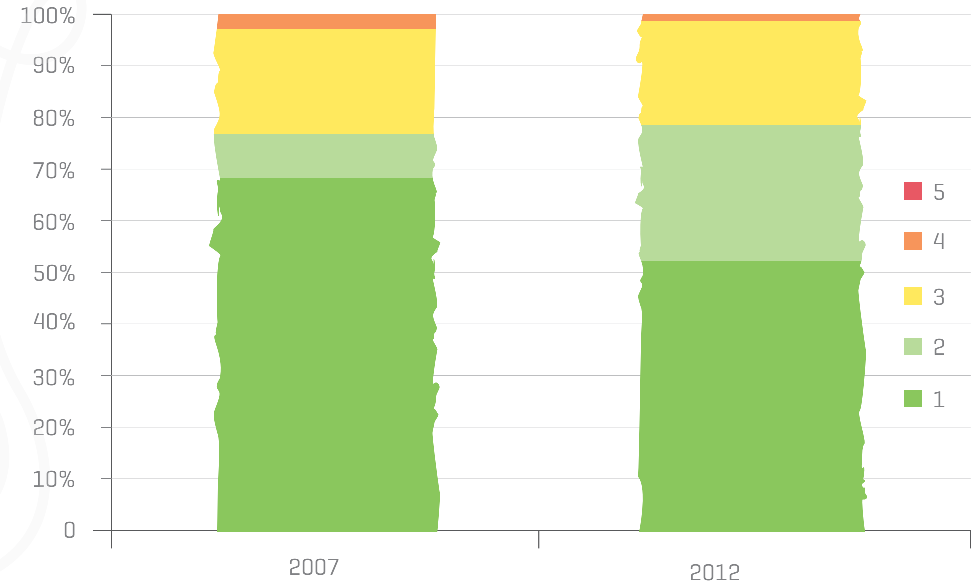

Figure 5.3: Grazing intensity in 100 m belt on both sides of transects: 1 – meadow ungrazed, no changes in natural meadow seen, 2 – grazing less intensive, smaller changes of the meadow due to grazing are seen and almost no faeces, 3 – grazing moderately intensive, 4 – grazing is very intensive and meadow substantially changed due to grazing, 5 – meadow devastated due to grazing (e.g. around watering places).

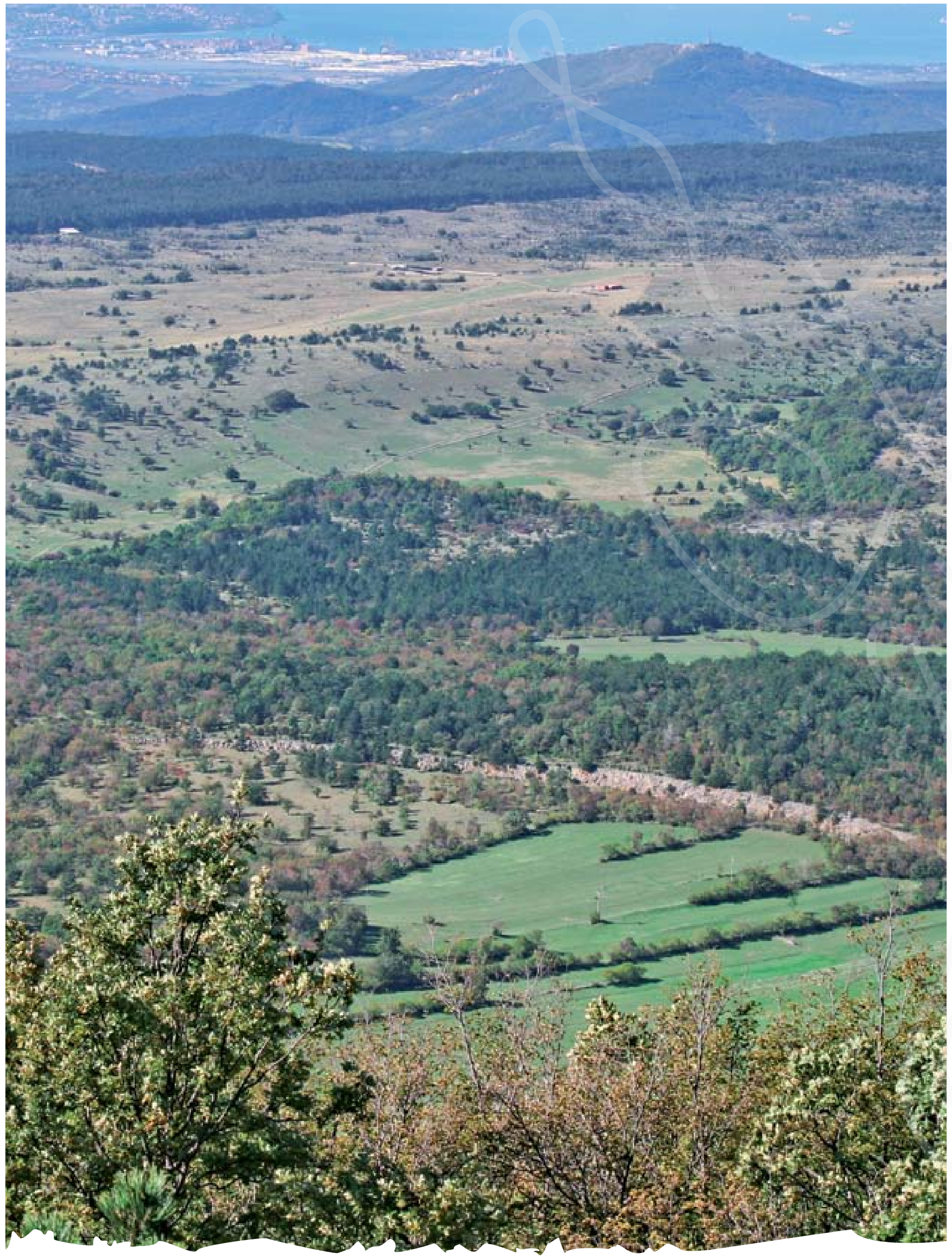

Figure 5.4: Northern (open) part of the research area 1 (Podgorski Kras). Photograph: Tomaž Mihelič.

5.2.2 INFLUENCE OF SUCCESSION ON GOLIČ PLATEAU (CASE 2)

In 2013 we carried out a survey on Golič plateau (part of SPA Kras SI5000023), where we had already undertaken a similar survey in 2002. Golič is a peak standing 890 m a.s.l. on a plateau that lies above the village of Rakitovec in Čičarija, SW Slovenia. Golič plateau is one of the largest remaining karstic grasslands in Slovenia. Decades ago, the majority of Golič plateau was abandoned and left to vegetational succession. The closest comparable grasslands are on Kuk above Movraž and between Prešnica, Črnotiče and Podgorje (Podgorski Kras). These areas differ from Golič in the sense that they have not yet been abandoned and are still managed, mostly by grazing. The aim of our research was to find out how bird populations react to land use abandonment and vegetational succession on karstic grasslands.

The research area (Figures 5.1, 5.6) covers 9.9 km^2 altogether. We used the same transect count method in 2013 as we had in 2002. We counted birds in two belts: an inner belt (0 – 100 m on each side of the transect) and an outer belt (more than 100 m from the transect). We made only one visit each year, at the end of May or at the beginning of June. The total length of the transects was 15.380 km. In 2013 we also carried out a simplified vegetation assessment in which we estimated vegetational succession in the inner belt of the transect. Succession was classified into 5 classes where succession increases from open grasslands as class 1 to forests as class G (Figure 5.5).

Breeding densities were calculated only for species with at least 20 registrations. The majority of transects ran through open land classified into class 1 and class 2 vegetation succession classes which together covered 65.8% of the area 100 m on each side of the transects. Forests covered 17.0 % of this area.

We recorded 45 different species (36 in both years) and altogether we counted 458 breeding pairs in 2002 and 394 breeding pairs in 2013. The population of some grassland species declined substantially during these 11 years. During this period the meadows were totally unmanaged and left to succession. The succession was not very rapid but clearly visible on the satellite images.

Wood lark, ortolan bunting and corn bunting all declined – the population decline of ortolan bunting being substantial with an abundance index of only 17.6 with corn bunting following with an index of 25.5. We counted a slightly higher number of skylarks *Alauda arvensis* (index 110.1). A real surprise was the increase in tree pipit *Anthus trivialis* and it looks as if this early succession stage suits this species. We also noticed a decrease in whitethroat population which is difficult to explain but was probably caused by too late a census date for this species (Table 5.2).

Table 5.1: Table 5.1: Results of the surveys in 2007 and 2012 on Podgorski Kras (research area 1); Index: abundance indices of counted pairs 2012/2007 * 100 (100 = no change); D07, D12: breeding densities in 2007 and 2012, calculated where there were 20 or more counted birds; *Natura 2000* species are shaded.

Species		2007[1] (pairs)	2012[2] (pairs)	Index	D07[3] (pairs/km²)	D12[3] (pairs/km²)
Pernis apivorus	honey buzzard		3			
Circaetus gallicus	short-toed eagle		1			
Accipiter nisus	sparrowhawk	2	1	50.0		
Buteo buteo	common buzzard	7	4	57.1		
Falco tinnunculus	kestrel	5	4	80.0		
Coturnix coturnix	quail	1	2	200.0		
Columba livia (domest.)	feral pigeon		8			
Columba palumbus	wood pigeon	1		0.0		
Streptopelia decaocto	collared dove	1		0.0		
Streptopelia turtur	turtle dove	1	9	900.0		
Cuculus canorus	cuckoo	27	55	203.7		
Caprimulgus europaeus	nightjar	2	1	50.0		
Apus apus	swift	58	11	19.0	17.3	
Tachymarptis melba	alpine swift	3		0.0		
Upupa epops	hoopoe	62	69	111.3	2.4	1.0
Jynx torquilla	wryneck	2	2	100.0		
Picus canus	grey-headed woodpecker	1		0.0		
Picus viridis	green woodpecker	2	5	250.0		
Dryocopus martius	black woodpecker	2	6	300.0		
Dendrocopos major	great spotted woodpecker	20	23	115.0	1.5	1.0
Dendrocopos minor	lesser spotted woodpecker	2	1	50.0		
Lullula arborea	wood lark	89	104	116.9	7.9	7.9
Alauda arvensis	skylark	111	61	55.0	8.6	3.8
Hirundo rustica	barn swallow	27	15	55.6	8.4	
Delichon urbicum	house martin	1	1	100.0		
Anthus campestris	tawny pipit	2	3	150.0		
Motacilla alba	pied wagtail	6	2	33.3		
Erithacus rubecula	robin	55	31	56.4	9.8	3.9
Luscinia megarhynchos	nightingale	26	26	100.0	2.7	1.7
Phoenicurus ochruros	black redstart	1	1	100.0		
Phoenicurus phoenicurus	redstart		1			
Saxicola torquatus	stonechat	5	5	100.0		
Monticola solitarius	blue rock thrush		1			
Turdus merula	blackbird	348	361	103.7	37.3	33.2
Turdus philomelos	song thrush	18	36	200.0		1.6
Turdus viscivorus	mistle thrush	25	41	164.0	2.4	4.2
Hippolais polyglotta	melodious warbler	16	13	81.3		
Sylvia cantillans	subalpine warbler	1	3	300.0		
Sylvia communis	whitethroat	121	86	71.1	16.4	10.0
Sylvia atricapilla	blackcap	198	387	195.5	24.1	45.9
Phylloscopus collybita	chiffchaff	103	82	79.6	10.5	3.9
Muscicapa striata	spotted flycatcher	8	2	25.0		
Aegithalos caudatus	long-tailed tit	10	4	40.0		
Poecile palustris	marsh tit	11	21	190.9		4.6
Lophophanes cristatus	crested tit	16	12	75.0		
Periparus ater	coal tit	7	20	285.7		3.5
Cyanistes caeruleus	blue tit	30	30	100.0	7.4	6.4
Parus major	great tit	65	92	141.5	12.7	12.4
Sitta europaea	nuthatch	5	11	220.0		
Certhia brachydactyla	short-toed treecreeper	10	19	190.0		
Oriolus oriolus	golden oriole	38	54	142.1	1.4	1.8
Lanius collurio	red-backed shrike	32	87	271.9	4.2	9.2
Garrulus glandarius	jay	78	46	59.0	12.1	6.7
Corvus corax	raven	11	6	54.5		
Sturnus vulgaris	starling	6	9	150.0		
Passer domesticus	house sparrow	1	7	700.0		
Passer x italiae	italian sparrow		2			
Fringilla coelebs	chaffinch	353	368	104.2	30.7	36.6
Serinus serinus	serin	1	5	500.0		
Carduelis chloris	greenfinch	14	28	200.0		1.9
Carduelis carduelis	goldfinch	6	14	233.3		
Carduelis cannabina	linnet	14	19	135.7		
Loxia curvirostra	common crossbill	1	11	1100.0		
Coccothraustes coccothraustes	hawfinch	47	49	104.3	9.8	10.8
Emberiza citrinella	yellowhammer	77	37	48.1	4.2	1.6
Emberiza cirlus	cirl bunting	18	35	194.4		3.1
Emberiza cia	rock bunting	35	26	74.3	9.6	7.3
Emberiza hortulana	ortolan bunting	22	7	31.8	1.3	
Emberiza calandra	corn bunting	50	74	148.0	2.9	4.6
	Pairs	**2318**	**2560**	**110.4**		
	Species	**63**	**65**			

[1] project Natura Primorske (Interreg IIIA SI-IT 2000-2006)
[2] this project
[3] breeding densities are calculated according to Bibby et al. (1992); densities are calculated with two belts: 50 m and beyond

Table 5.2: Results of the surveys in 2002 and 2013 on the Golič plateau (research area 2); Index: indices of counted pairs 2013/2002 * 100 (100 = no change); D02, D13: breeding densities in 2002 and 2013, calculated where there were 20 or more counted birds; *Natura 2000* species are shaded.

Species		2002[1] (pairs)	2013[2] (pairs)	Index	D02[3] (pairs/km²)	D13[3] (pairs/km²)
Buteo buteo	common buzzard	1	0	0.0		
Aquila chrysaetos	golden eagle	0	1			
Falco tinnunculus	kestrel	1	1	100.0		
Falco vespertinus	red-footed falcon	0	4			
Alectoris graeca	rock partridge	0	4			
Coturnix coturnix	quail	1	1	100.0		
Streptopelia turtur	turtle dove	1	0	0.0		
Cuculus canorus	cuckoo	19	7	36.8		
Caprimulgus europaeus	nightjar	1	0	0.0		
Apus apus	swift	1	0	0.0		
Dryocopus martius	black woodpecker	2	0	0.0		
Dendrocopos major	great spotted woodpecker	1	0	0.0		
Lullula arborea	wood lark	10	3	30.0		
Alauda arvensis	skylark	109	120	110.1	33.1	24.7
Hirundo rustica	barn swallow	2	0	0.0		
Anthus trivialis	tree pipit	11	24	218.2		4.1
Erithacus rubecula	robin	10	12	120.0		
Luscinia megarhynchos	nightingale	0	1			
Saxicola torquatus	stonechat	7	5	71.4		
Turdus merula	blackbird	32	16	50.0	5.6	
Tudus philomelos	song thrush	5	4	80.0		
Turdus viscivorus	mistle thrush	4	7	175.0		
Sylvia curruca	lesser whitethroat	0	1			
Sylvia communis	whitethroat	27	14	51.9	10	
Sylvia atricapilla	blackcap	38	60	157.9	10.3	14.9
Phylloscopus collybita	chiffchaff	10	9	90.0		
Muscicapa striata	spotted flycatcher	0	1			
Aegithalos caudatus	long-tailed tit	1	0	0.0		
Poecile palustris	marsh tit	0	1			
Lophophanes cristatus	crested tit	4	1	25.0		
Periparus ater	coal tit	6	1	16.7		
Cyanistes caeruleus	blue tit	2	5	250.0		
Parus major	great tit	3	3	100.0		
Sittaaea	Nuthatch	0	1			
Certhia brachydactyla	Short-toed treecreeper	5	4	80.0		
Oriolus oriolus	Golden oriole	1	1	100.0		
Lanius collurio	Red-backed shrike	9	8	88.9		
Garrulus glandarius	Jay	6	1	16.7		
Corvus cornix	Hooded crow	2	0	0.0		
Fringilla coelebs	Chaffinch	49	40	81.6	10.5	8.6
Carduelis cannabina	Linnet	2	6	300.0		
Coccothraustes coccothraustes	Hawfinch	0	2			
Emberiza cia	Rock bunting	11	9	81.8		
Emberiza hortulana	Ortolan bunting	17	3	17.6		
Emberiza calandra	Corn bunting	47	12	25.5	14.5	
	Pairs	458	394	86.0		
	Species	36	36			

[1] A. Figelj, J. Figelj, P. Kmecl *pers. comm.*
[2] this project
[3] densities are calculated according to Bibby et al. (1992); densities are calculated for two belts: 100m and beyond

Table 5.3: Breeding *Natura 2000* species detected in the surveys of research area 1 and research area 2: population in SPA Kras (PKras); population in Slovenia (PSLO); population of the research areas (best expert opinion) (P2012, P2013) and percentage of the population of the research area in SPA Kras population (%Kras); only species for which the methodology of the surveys was appropriate to assess their numbers are shown, these do not include nocturnal species (owls, nightjar), species of the rock cliffs (eagle owl *Bubo bubo*, blue rock thrush *Monticola saxatilis*) and other species which require special methodology (e.g. short-toed eagle *Circaetus gallicus*).

				PODGORSKI KRAS		GOLIČ PLATEAU	
Species		PKras[1]	PSLO[2]	P2012	%Kras	P2013	%Kras
Upupa epops	Hoopoe	300-500	450-750	25-35	7.5	0	0.0
Lullula arborea	Wood lark	1100-1500	3300-4400	200-250	17.3	3-5	0.3
Alauda arvensis	Skylark	2400-3000	10000-18000	100-150	4.6	230-250	8.9
Anthus campestris	Tawny pipit	20-30	30-55	3-5	16.0	0-1	2.0
Lanius collurio	Red-backed shrike	500-1000	20000-30000	250-300	36.7	10-15	1.7
Emberiza hortulana	Ortolan bunting	20-34[3]	20-34	6-8[4]	25.9	3-5[4]	14.8
Emberiza calandra	Corn bunting	1500-2000	4900-10500	130-150	8.0	15-20	1.0

[1] Denac *et al.* (2011)
[2] DOPPS *own data*
[3] estimate for the year 2000 is 90–130 singing males, the population is declining steeply and species doesn't breed outside SPA Kras
[4] singing males

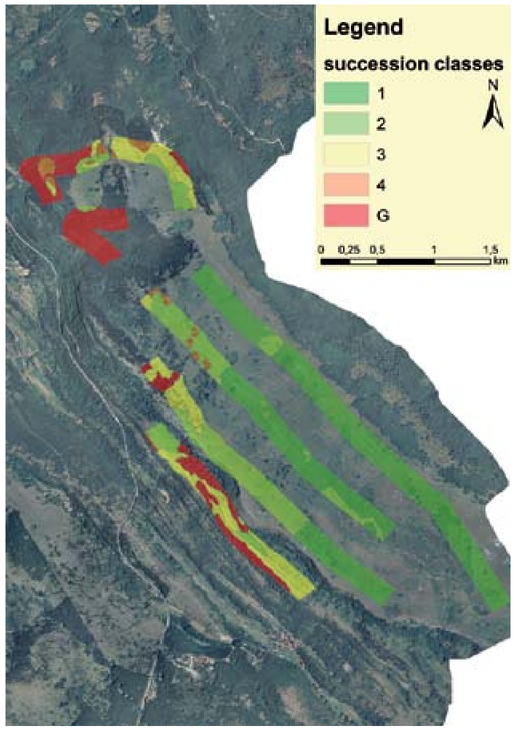

Figure 5.5: Vegetation succession in the research area 1 - Golič; succesion classes:
1 – bare ground, no bushes or trees;
2 – denser grass vegetation, solitary bushes and trees;
3 – dense grass vegetation and bushes;
4 – bushes, scrub, high number of trees present;
G – forest.

Figure 5.6: Research area 2 – dry karstic meadows on Golič plateau above Rakitovec; Koper and Adriatic sea are seen in the background. Photograph: Primož Kmecl.

5.2.3 THE REINTRODUCTION OF GRAZING TO DRY MEADOWS IN THE BASOVIZZA STUDY AREA

The Italian research area is located near the village of Basovizza (Bazovica), in the Municipality of Trieste, NE Italy, next to the Bazovizza-Lipica border crossing, below the southern slopes of Mount Cocusso / Kokoš (674 m a.s.l.) within the SAC SPA - IT3341002 'Karst Areas of Venezia Giulia' / SAC-IT3340006 'Karst of Trieste and Gorizia' (Figure 5.1). It had already been identified as part of a previous project for the restoration and maintenance of the *landa carsica* habitat (62A0 - arid sub-Mediterranean-Eastern grassland (*Scorzoneretalia villosae*), in Annex I of the 92/43/EEC Habitat Directive) through the reintroduction of extensive grazing, carried out by local grazers in the Province of Trieste.

The study area, an area of common grazing land, less than a square kilometre, expropriated in the last century and given over to experimental forestry work, was first surveyed for its biodiversity (flora and fauna) and then partially deforested, fenced and returned to grazing. Bird survey work was carried out in various years between 2006 and 2011 and again under the auspices of the BioDiNet project in the summers of 2013 and 2014. Standardized survey techniques were maintained throughout the survey with the white transect (points 1–8; Figure 5.7) being surveyed throughout both periods 2006 – 2011 and 2013 – 2014 (Figure 5.7).

Figure 5.7: Survey transect in the Basovizza Study Area. Points 1–8 were surveyed throughout both 2006–2011 and 2013–2014). Cartography: authors.

The transect was walked weekly, weather (wind and rain) permitting, from the end of March to the beginning of July (13 visits), beginning not later than 90 minutes after dawn, stopping at the points 1–8 for 5 minutes. All ornithological contacts within 100 metres of the observation point (birds seen, heard and identified) were recorded, including fly-overs. Observations of interest on the walk between the points were also recorded but separately.

The data obtained for 2013 was compared with that obtained using the same techniques in 2009 (when again 13 visits were made between March and July). Two late evening visits were made in fine weather in the months of June and / or July in all the years of the survey (including 2012, when the post-dawn surveys were not carried out) to count nightjar and other nocturnal species. The transect was walked twice, with a torch and nocturnal species were noted.

There were 11 species with at least 20 records in one of the two years. Abundance indices were calculated for 2013 where the data for 2009 provided the baseline for the index (= 100). The data set from the year 2009 was chosen as 'year zero' because this was the first year (and therefore the oldest) for which data was available in which the process of deforestation for the entire site had been completed to the extent that we observe today. The tree removal began, rather hesitantly, in 2006 and 2007 before accelerating in 2008, regular work ceasing and the heavy equipment being withdrawn in 2009 (Table 5.4).

Table 5.4: Results of the surveys in 2009 (13 visits) and 2012 (13 visits) in Basovizza study area (research area 3); abundance indices 13/09 (100 = no change) for the species with at least 20 registrations in at least one year are shown; *Natura 2000* species are shaded.

Species		2009 (sum of individuals)	2013 (sum of individuals)	Index 13/09
Upupa epops	hoopoe	29	24	83
Lullula arborea	wood lark	72	59	82
Motacilla alba	white wagtail	43	19	44
Turdus merula	blackbird	88	96	109
Turdus viscivorus	mistle thrush	25	28	112
Sylvia atricapilla	blackcap	53	66	125
Aegithalos caudatus	long-tailed tit	28	6	21
Parus major	great tit	62	73	118
Fringilla coelebs	chaffinch	110	111	101
Emberizia cirlus	cirl bunting	41	16	39
Emberizia citrinella	yellowhammer	40	19	48
Total (all birds encountered)		949	846	89
Species		62	53	

Five species increased but six declined, some markedly (see '*5.3 Final remarks*'). Overall numbers of birds encountered declined by a little over 10 %. All the other species encountered (a total of 62 in 2009 and 53 in 2013) failed to provide a sufficiently large sample to merit analysis. Many of these were birds seen in transit or on migration or present with just a pair or two on the site or close by.

We present here some species-specific comments for two species surveyed during the night survey and for two *Natura 2000* species.

Scops owl *Otus scops:* Young birds calling from the direction of the Finance Police barracks close to the Basovizza – Lipica border crossing on 13 June 2014 suggest a first breeding for this species recorded since the study began in 2006.

Nightjar *Caprimulgus europaeus:* Evening visits were carried out in fine weather on 16 June and 13 July 2013, (21:15–23:00), for which, respectively 7 and 6 singing males were encountered. Visits were repeated on 6 June and 13 June 2014 when, again, respectively 7 and 6 singing males were encountered. Whilst a far cry from the 22 males found in 2009 immediately following the deforestation work but suggest a species that is quick to respond to changes in the landscape given that no birds were found in 2006 before deforestation work began.

Hoopoe: Present on the site at least since survey work began in 2006, a single pair raising chicks each year (2009–2011) except 2013 when the poor spring may have compromised the breeding effort. 2014 was different in as much as a single territorial pair was present, as usual within the polygon between points 1, 2, 7 and 8 although three separate families totaling 10 young hoopoes were encountered on 6th June, two broods having been brought by their parents to the excellent feeding conditions, perhaps from nearby.

Wood lark: Also present on the site at least since survey work began in 2006, 1 or 2 pairs raise chicks each year. Survey work is made difficult or less accurate by wandering birds singing on the site. This is particularly noticeable in the months of June and July and is a comment that applies equally to bunting species. Climatic issues (*see '5.3 Conclusion'*) as well as disturbance by humans and grazing animals have probably affected this species negatively, offsetting the benefits that have undoubtedly come from the deforestation work.

5.3 CONCLUSION

We can make some quite clear conclusions by comparing the species indices of area 1 (Podgorski Kras) and area 2 (Golič plateau). For instance, the skylark has a negative abundance index on Podgorski Kras while on Golič plateau it has a slightly positive one. We ascribe this to the negative impact of grazing and at the same time it looks as though early succession does not have an impact on this species. The effect of grazing through lessening the nesting success of skylark has already been documented in literature (Pavel, 2004). Grazing has an obviously positive impact on two important species in conservation terms: wood lark and corn bunting which both declined on Golič plateau. The availability of bare ground is important for both of these species since this is where they feed. It looks as if low intensity grazing makes appropriate bare areas (Schaub *et al.*, 2010). One species that declined sharply in both areas is the ortolan bunting. This suggests other reasons for decline beside the inappropriate management of meadows. Its decline in the Karst is well documented for the period since 2005 (Figelj & Kmecl, 2013).

While birds, overall, seem less sensitive than plant communities to poor management practices, adopting a broad acceptance of landscape quality rather than botanical detail, the work at the Basovizza study area has been insufficiently radical in terms of tree removal to bring about a major landscape change and indeed, in parts, appears to be reverting to the pre-existing scrubland, albeit minus the black pine component. Of the birds of open Karst only hoopoe and nightjar have categorically benefitted from the less-than-radical deforestation and other species, such as tawny pipit and red-backed shrike have yet to recolonize. Perhaps the areas are too small and isolated for this to occur. Other species, rare or absent before the deforestation and liking the mixture of short turf and tall trees, such as mistle thrush *Turdus viscivorus*, have increased still further.

It has proved difficult to control all the variables in the Basovizza project and a major (and unmeasured) change has been that of human disturbance. Since the deforestation, with articles about the project in the local press, improved access and the addition of grazing animals, the human presence (dog-walkers, joggers, mountain-bikers, family days-out) has increased markedly, indeed much of the 'figure-of-eight' transect is a route for 'regulars', many of them daily users. The negative influence of dog-walkers has also been observed in England (Langston *et al.*, 2007) as well as the proximity of urban areas (Liley & Clarke, 2003). Trieste (pop. 205,000) is just 10–15 minutes drive from the study area.

Other factors which have undoubtedly affected bird numbers across all three studies, making deductions with regard to habitat changes affecting bird populations difficult, refer to climatic events. The first event, lasting for *c.* 4 weeks from the end of January 2012 involved a period of extremely low temperatures (as low as -15°C in the Italian Karst) and a powerful *bora* gusting at up to 160 km/h for days at a stretch. The second event of note was the extremely cold, wet spring of 2013 in which warm weather did not begin until after 10th June. This undoubtedly affected both the visibility / audibility of the bird communities and the breeding productivity that year for some species.

The days of large karstic meadows are long gone and current landscape is the result of large scale Karst reforestation projects that have been going on since the middle of the 19th century. This process was further enhanced by the depopulation due to post-WWII industrialization and emigration. As one of the locals described it, he still remembers

the period when each house had a cow or two and when they were selling their milk at the town market in Trieste. They fed their cows with the hay they cut on the dry meadows with scythes and which they carried to their homes in large baskets.

If we want to keep the typical species of karstic grasslands, wood lark, corn bunting and so forth, we must imitate the traditional agricultural practices. Recommended management on Golič as well as other areas is the extensive grazing of traditional Karst livestock and the continued maintenance of low-intensity hay meadows. Recreational activities and tourism, especially upland farm and enogastronomic tourism should be encouraged but carefully and firmly regulated to limit disturbance and damage to sites that may come from unrestrained access and certain forms of recreation.

SUGGESTED READINGS

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- Menz, M. H. M. & R. Arlettaz (2011): The precipitous decline of the ortolan bunting *Emberiza hortulana*: time to build on scientific evidence to inform conservation management. Oryx, 46 (1), 122–129.

CHAPTER 6

KARST PONDS:
BIODIVERSITY
AND THREATS

KRAŠKI KALI:
BIODIVERZITETA
IN GROŽNJE

STAGNI CARSICI:
BIODIVERSITÀ
E MINACCE

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ABSTRACT

Over generations, manmade reservoirs, such as ponds, pools and wells, developed into semi-natural wetlands that represent the only aquatic habitat in the area of the Karst edge, forming a network of suitable stepping stone habitats for large numbers of species. Threats to these habitats are: modern farming practices leading to a decline in use of ponds and deterioration; pollution from pesticides and fertilizers; and the establishment of new invasive species such as ornamental fish. In the course of four years 21 ponds were surveyed and amphibian species composition, newt abundance and genetic diversity were studied. Additionally, a preliminary analysis on zooplankton composition was assessed. Nine amphibian taxa were found in the surveyed sites, with smooth newts *Lissotriton vulgaris*, agile frogs *Rana dalmatina*, and common toads *Bufo bufo* being the most prevalent. Abundances of smooth newts were high across all studied ponds and thought to be above the minimal viable population threshold. On the other hand, low numbers of Italian crested newts *Triturus carnifex* did not indicate long-term survival of this species in the area. A genetic study of smooth newt showed three distinct mitochondrial lineages to be in contact in the area, thus indicating a high genetic diversity. The ponds harbour also a high diversity of zooplankton. More research is needed to get a complete species list of zooplankton to link environmental variables to zooplankton community structure so that they can be used as sensitive bioindicators. Studies on selected animal groups add to a better knowledge of the biodiversity, function and conservation status of these endangered habitats. Conserving karst ponds is crucial to maintain aquatic biodiversity as they represent a case where human activity is a driving force of biodiversity rise. These habitats are manmade biodiversity hotspots and require managed conservation approaches to maintain them.

Key words: small waterbodies, Amphibia, *Lissotriton vulgaris*, *Triturus carnifex*, zooplankton

IZVLEČEK

Kraški kali in podobni umetni vodni zbiralniki, so edino površinsko vodno okolje na območju Kraškega roba. Tekom stoletij se preoblikovali v polnaravna mokrišča in zdaj tvorijo povezano mrežo vodnih habitatov številnim vrstam. Grožnjo tem habitatom predstavljajo: sodoben način kmetovanja z opuščanjem rabe kalov in posledično njihovim propadanjem; onesnaževanje s pesticidi in gnojili; ter vnos tujerodnih vrst, kot so zlate ribice. Predstavljamo rezultate raziskav vrstne sestave dvoživk in zooplanktona, številčnosti populacij pupkov in njihove genetske pestrosti v 21 kalih. Na območju smo zabeležili devet vrst dvoživk. Populacije navadnega pupka *Lissotriton vulgaris* so številčne in domnevno dosegajo kriterij minimalne viabilne populacije. Veliki pupek *Triturus carnifex* je manj številčen in tega kriterija domnevno ne dosega, zato je njegovo dolgoročno preživetje na Kraškem robu ogroženo. Filogenetska študija navadnega pupka je pokazala na visoko pestrost vrste na raziskanem območju, saj prihaja do kontakta treh ločenih mitohondrijskih linij. Kali vzdržujejo tudi visoko pestrost zooplanktona. Njegovo vrstno sestavo bi morali z obsežnejšimi raziskavami dopolniti, kar bi omogočilo oceno povezav združbe z okoljskimi spremenljivkami in uporabo te skupine v bioindikaciji. Kraški kali so vroče točke biotske raznovrstnosti vodnih organizmov v kraški pokrajini. Ker zahtevajo stalno vzdrževanje, je človeška dejavnost s stalnim upravljanjem ključna za njihov obstoj.

Ključne besede: majhna vodna telesa, dvoživke, *Lissotriton vulgaris*, *Triturus carnifex*, zooplankton

RIASSUNTO

Gli stagni carsici e simili bacini d'acqua artificiali rappresentano l'unico habitat acquatico superficiale nella zona del Ciglione carsico, formando una rete di habitat idonei per un elevato numero di specie. Le minacce per questi habitat sono rappresentate da: pratiche agricole moderne che causano una diminuzione dell'uso degli stagni e il loro deterioramento; l'inquinamento da pesticidi e fertilizzanti; e la presenza di specie invasive come i pesci rossi. Nel corso di quattro anni abbiamo studiato la composizione delle specie di anfibi e dello zooplancton, l'abbondanza dei tritoni e la loro diversità genetica in 21 stagni carsici. Nove specie di anfibi sono stati censiti nei siti esaminati. L'abbondanza del tritone punteggiato *Lissotriton vulgaris* è risultata alta in tutti i siti esaminati ed è ritenuta sopra il limite della popolazione minima vitale. D'altra parte, il basso numero del tritone crestato italiano *Triturus carnifex* non indica la sopravvivenza a lungo termine di questa specie nella zona. Uno studio genetico del tritone punteggiato ha dimostrato il contatto di tre linee mitocondriali distinte presenti nella zona, indicando così una diversità genetica elevata. Gli stagni supportano anche un'elevata diversità di zooplancton. È necessaria una maggiore intensità di ricerca per ottenere un elenco completo delle specie di zooplancton e di collegare le variabili ambientali alla struttura della comunità di zooplancton in modo che questo gruppo possa essere utilizzato come bioindicatore. La conservazione degli stagni carsici è fondamentale per il mantenimento della biodiversità acquatica in quanto rappresentano un caso in cui l'attività umana è una causa diretta per l'aumento della biodiversità. Questi habitat sono hotspots di biodiversità e richiedono una gestione mediante approcci corretti per la loro conservazione.

Parole chiave: piccoli bacini d'acqua, anfibi, *Lissotriton vulgaris, Triturus carnifex*, zooplankton

6.1 KARST PONDS – THE IMPORTANCE OF MANMADE RESERVOIRS IN A DRY LANDSCAPE

The fissured surface of the limestone Karst plateau in SW Slovenia is characterized by poor water retention and an almost complete absence of natural superficial water bodies. Where natural water bodies do exist they are largely small rock pools that have developed on limestone outcrops with diameters of less than 1.5 meters. For over a thousand years anthropogenic use of the landscape including agriculture, animal husbandry and sedentary occupation has resulted in the construction of manmade reservoirs, such as ponds, pools and wells (Bressi & Stoch, 1999). These traditional farming practices were the driving force behind the establishment of these ponds resulting in an altered pattern of local biodiversity (Denoël & Ficetola, 2007; Knutson *et al.* 2004; Miracle *et al.*, 2010).

These ponds are largely situated near villages and differ in size, structure, location and purpose. The populace of these villages have historically, and in some case continue to maintain these water assets to preserve their water impermeable lining, removed plant material and reconstructed their banks (see Dolce *et al.*, 1991; Ciglič, 2005). Over generations, many of these locations have developed into semi-natural wetlands that represent the only aquatic habitat in the area, forming a network of suitable stepping stone habitats for large numbers of species. In this capacity they are recognised as important conservation sites for freshwater biodiversity (Céréghino *et al.*, 2008). Additionally, these ponds provide ecosystem services such as nutrient interception and hydrological regulation. Due to their small size and stepping stone nature they provide a model system for studies into ecology, evolutionary biology, conservation biology, and metapopulation processes in freshwater systems (see Céréghino *et al.*, 2008 and Della Bella *et al.*, 2008).

Species assemblages at these sites include Cyanobacteria, aquatic and semi-aquatic plants,

hydrozoans, turbellarians, rotifers, nematodes, molluscs (Bivalvia and Gastropoda), annelids (Oligochaeta and Hirudinea), arachnids (Hydracarina), crustaceans (Cladocera, Copepoda and Ostracoda), insects (Plecoptera, Ephemeroptera, Trichoptera, Odonata, Diptera and Coleoptera) and vertebrates (Pisces, Reptilia, Amphibia).

During periods of agrarian subsistence these ponds were essential persistent landscape elements due to regular restoration and maintenance by local famers. In recent times adoption of modern farming practices coupled with a decrease in agrarian subsistence has led to a decline in their use, leading to deterioration of these habitats and breaking their temporal continuity as biodiversity reservoirs. Major factors contributing to this include pollution from pesticides and fertilizers and the establishment of new invasive species such as ornamental fish.

Once a pond is abandoned a series of ecological and geological successions leads to filling of the pond through vegetative overgrowth and infilling, eventually resulting in the desiccation/drainage of the pond. A loss of suitable pond habitats leads to an increased fragmentation and isolation of breeding sites for aquatic organisms, especially amphibians that have limited dispersal abilities (Denoël & Ficetola, 2007). Across Europe and Slovenia there is a growing awareness of pond conservation that is aimed at maintaining and restoring biodiversity (see Céréghino *et al.*, 2008 and Della Bella *et al.*, 2008).

In this chapter we investigate the distribution and biodiversity of amphibians and zooplankton in ponds of the Karst edge (Figure 6.1) that we have been studying since 2006 (Table 6.1). Firstly, we present studies on amphibian populations with species compositions, estimates of smooth (*Lissotriton vulgaris*) and Italian crested newt (*Triturus carnifex*) population sizes, and smooth newt genetic diversity. Additionally, we deliver a snapshot of zooplankton diversity from karst ponds for the first time with the hope that they will be useful for future studies into this poorly understood aspect of biodiversity.

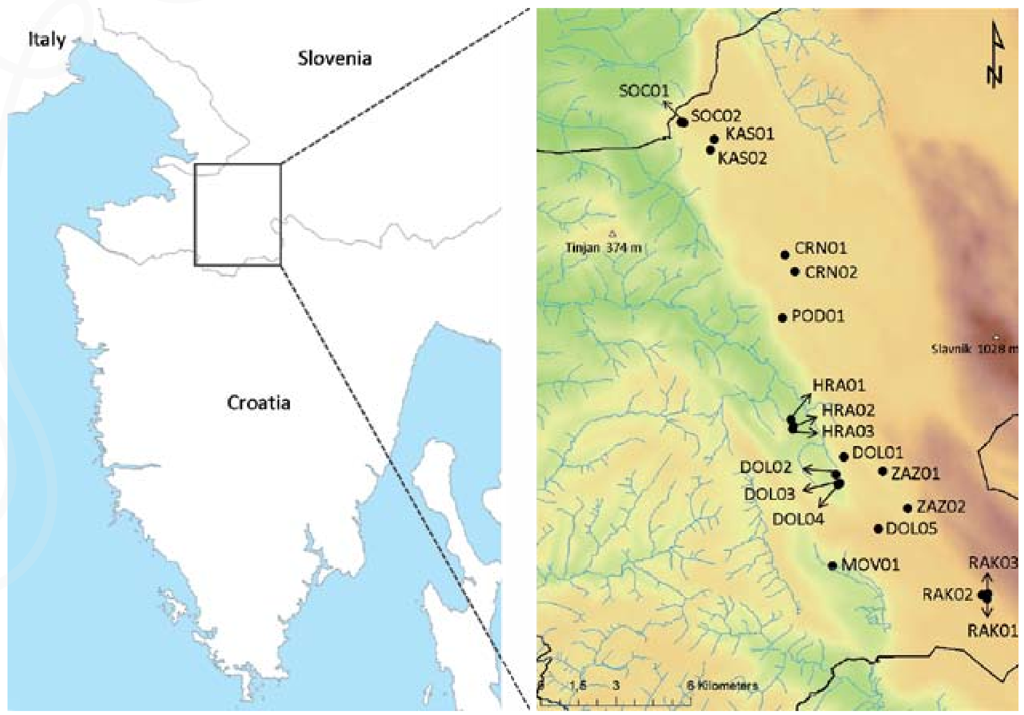


Figure 6.1: Map of Karst edge with sampled ponds. Cartography: Peter Glasnović and Sara Zupan.

Table 6.1: Surveyed ponds in the area of Karst edge, their exact coordinates, years and assessments made. AMPHIB – amphibian species composition; LVUL - ABUN – abundance of *Lissotriton vulgaris*; TCAR - ABUN – abundance of *Triturus carnifex*; LVUL GEN – genetic analysis of *Lissotriton vulgaris*; PHY-CHE – physical and chemical parameters; ZOOPL – zooplankton composition. CP – concrete pond, CT – concrete trench, P – pond, W – well.

									ASSESMENTS					
No.	Site Acronym	Village	Longitude	Latitude	Elevation (m a.s.l.)	Surface area (m²)	Description (Structure)	Years visited	AMPHIB	LVUL - ABUN	TCAR - ABUN	LVUL - GEN	PHY-CHE	ZOOPL
1	SOC01	Socerb	45.5889642	13.8598878	394	41	P	06; 07; 08; 13	+			+	+	
2	SOC02	Socerb	45.5888533	13.8607975	413	69	P	06; 07; 08; 13	+	+	+	+	+	+
3	KAS01	Kastelec	45.5847583	13.8718410	370	38	P	06; 07; 08; 13	+	+	+	+		
4	KAS02	Kastelec	45.5819435	13.8703420	322	36	P	06; 07; 08; 13	+	+		+	+	+
5	CRN01	Črnotiče	45.5554671	13.8972488	424	8	P	06; 07; 13	+			+	+	+
6	CRN	Črnotiče	45.5513601	13.9007162	424	74	P	06; 07; 13	+	+	+	+	+	
7	ZAZ01	Zazid	45.5009911	13.9324357	385	137	P	06; 07; 13	+			+	+	+
8	ZAZ02	Zazid	45.4916333	13.9414654	420	191	P	13	+				+	+
9	RAK01	Rakitovec	45.4688768	13.9699528	516	499	P	06; 07; 13	+			+	+	+
10	RAK02	Rakitovec	45.4697984	13.9679904	510	14	W	06; 13	+	+	+	+	+	+
11	RAK03	Rakitovec	45.4702520	13.9702660	550	37	P	06; 07	+			+		
12	MOV01	Movraž	45.4771792	13.9142503	204	287	P	06; 07; 13	+	+	+	+	+	+
13	DOL01	Dol pri Hrastovljah	45.5819435	13.9144170	240	39	P	07; 13	+			+	+	+
14	DOL02	Dol pri Hrastovljah	45.5001940	13.9154610	225	15	P	07	+					
15	DOL03	Dol pri Hrastovljah	45.4979610	13.9170350	220	14	P	07	+					
16	DOL04	Dol pri Hrastovljah	45.4976650	13.9161500	220	17	W	07	+					
17	DOL05	Dol pri Hrastovljah	45.4865350	13.9308620	490	180	P	07	+			+		
18	POD01	Podpeč	45.5397910	13.8961970	365	7	CP	07	+			+		
19	HRA01	Hrastovlje	45.5140760	13.8993670	144	2	CT	07	+					
20	HRA02	Hrastovlje	45.5123840	13.9001930	150	1	CT	07	+			+		
21	HRA03	Hrastovlje	45.5117490	13.9001560	155	2	CT	07	+					
								TOTAL PONDS	21	6	5	15	11	9

6.2 AMPHIBIAN POPULATIONS IN PONDS OF THE KARST EDGE

Amphibians are considered to be one of the most endangered animal groups globally due to habitat destruction, alteration and fragmentation, introduced species and over-exploitation (see Collins & Storfer, 2003; Marsh & Trenham, 2001). Furthermore, complex and elusive mechanisms including factors such as climate change, increased UV-B radiation, chemical contaminants, emerging infectious diseases (for example chytrid fungus) and their synergistic effects adversely impact upon amphibian populations. All amphibian species in Slovenia are included in the national Red list, with the major threat in the study area being habitat loss due to pond decline both in habitat quality and number.

Karst ponds are highly important habitats for amphibians as they are the only breeding sites in the area, provide habitat for larvae until metamorphosis and for some adult amphibians such as green frogs (*Pelophylax* spp.). Previous research showed eight amphibians taxa present in ponds in the area of Karst edge. The most common urodeles (Lissamphibia, Urodela) are smooth newts *Lissotriton vulgaris* and Italian crested newts *Triturus carnifex*, while fire salamanders *Salamandra salamandra* are only rarely found in them. The most abundant frog species (Lissamphibia, Anura) is the agile frog *Rana dalmatina*, but common toads *Bufo bufo*, yellow bellied toads *Bombina variegata* and European tree frogs *Hyla arborea* are also very frequent. Fewer ponds are inhabited by green frogs (*Pelophylax* spp). (Poboljšaj, 2007; Poboljšaj *et al.*, 2007; France, 2002).

Amphibian species composition in ponds is thought to be dependant on many factors, most importantly by the surrounding terrestrial habitat, water depth and aquatic vegetation cover. Terrestrial habitat requirements vary among species; they have differential demands regarding land-cover (percentage of forest, open or urban areas surrounding the wetlands) and landscape configuration (e.g. proximity to forest). Fish presence has a pronounced negative effect on amphibian communities, extirpating most species except the common toad and to a lesser extent green frogs (Denoël & Ficetola, 2007; France, 2002). Research suggests the necessity of conservation and management of the terrestrial and aquatic habitats in close proximity to ponds, and the control of fish introduction, as an approach to maintaining amphibian diversity.

Table 6.2: Amphibian composition in surveyed karst ponds, with fish presence and number of visits. • – amphibian species present; + – fish present; (+) – fish introduced in the course of the study; SSAL – *Salamandra salamandra*; LVUL – *Lissotriton vulgaris*; TCAR – *Triturus carnifex*; MALP – *Mesotriton alpestris*; BVAR – *Bombina variegata*; BBUF – *Bufo bufo*; HARB – *Hyla arborea*; RDAL – *Rana dalmatina*; PELO – *Pelophylax* spp.

	SITE																					
SPECIES	SOC01	SOC02	KAS01	KAS02	CRN01	CRN02	ZAZ01	ZAZ02	RAK01	RAK02	RAK03	MOV01	DOL01	DOL02	DOL03	DOL04	DOL05	POD01	HRA01	HRA02	HRA03	No. ponds
SSAL		•										•									•	3
LVUL	•	•	•	•	•	•	•		•	•	•	•	•				•	•	•	•		16
TCAR		•	•	•	•	•	•		•	•	•	•										10
MALP									•		•											2
BVAR		•			•	•	•		•		•		•									7
BBUF		•	•	•		•	•				•			•	•	•	•					10
HARB		•	•			•	•						•									5
RDAL	•	•	•	•	•	•	•		•			•	•				•					11
PELO	•	•	•	•																		4
Fish			(+)	+				+		+				+	+	+						7
No. amph. species	3	8	6	5	4	6	6	0	5	2	5	4	4	1	1	1	3	1	1	1	1	Total visits
No. visits	5	7	17	6	2	5	4	1	3	1	2	4	1	1	1	1	1	1	2	1	1	66
No. years	3	3	3	3	2	2	2	1	2	1	2	2	1	1	1	1	1	1	1	1	1	

6.3 AMPHIBIAN DIVERSITY

One of the goals of our study was to assess amphibian composition in these diverse aquatic habitats. The study was carried out in 21 ponds and similar water bodies on the Karst edge during the breeding seasons of 2006, 2007, 2008 and 2013. Ponds were surveyed using standard methods for amphibians: visual search for eggs, larvae and adults and dip-netting for all life stages; additionally, audible clues (male vocalization) were also noted. Visits were infrequent, and some ponds were visited many more times than others (see Table 6.2). The presence of fish species in ponds was noted.

Results were concordant with data from previous studies in this area (see Table 6.2). Altogether nine amphibian taxa were found in 21 studied ponds. The most prevalent were smooth newts, found in 16 ponds. Also frequent were agile frogs (11 ponds), common toads and Italian crested newts (both in 10 ponds). Yellow bellied toads and European tree frogs had a more sparse distribution (7 and 5 ponds, respectively), while marsh frogs were limited to four ponds in the northernmost part of Karst edge. Fire salamander larvae were observed infrequently (3 ponds), presumably due to inappropriate habitat. These terrestrial salamanders usually lay larvae in swift and rather oxygenated streams, but only rarely in ponds. A third species of newt, the Alpine newt *Mesotriton alpestris*, was found in two ponds near the village Rakitovec in the extreme south-western part of Karst edge. The species is occasionally found on the karst plateau, whereas it is not present in lower elevations that characterize Slovenian Istria (Poboljšaj, 2007; Poboljšaj *et al.*, 2007; France, 2002). Due to the transient character of the Karst edge and the higher elevation of these two ponds, the observation was not entirely unexpected.

Amphibian diversity was highest in the large pond situated in the village of Socerb (SOC02, eight species in total), while three ponds held six and three more ponds held five amphibian species. At seven sites only one or two species were present (usually common toad or smooth newt) and no amphibians were recorded from one pond near the village of Zazid (ZAZ02). These sites were either concrete trenches (HRA01-03), concrete ponds (POD01) or were populated by fish (ZAZ02; DOL02-04), and thus less adequate for most amphibians.

In total, we detected seven ponds with fish, predominately goldfish (*Carassius auratus*) but also other unidentified species. Two ponds near the village Kastelec, where fish were observed, had a rather high number of amphibians. One of them (KAS01) was fishless in the beginning of the study, and fish were introduced in 2007. In the other pond (KAS02), common toads were numerous, with only a few specimens of the other amphibian species observed.

Figure 6.2: Amphibians found in ponds of the Karst edge. A. Female smooth newt *Lissotriton vulgaris* and Italian crested newt *Triturus carnifex* larva with prominent gills. B. Yellow bellied toad *Bombina variegata*. C. Common toad *Bufo bufo*. D. Male tree frog *Hyla arborea* calling. Photographs: Peter Glasnović (A and C) and Martina Lužnik (B and D).

6.4 NEWTS ABUNDANCE

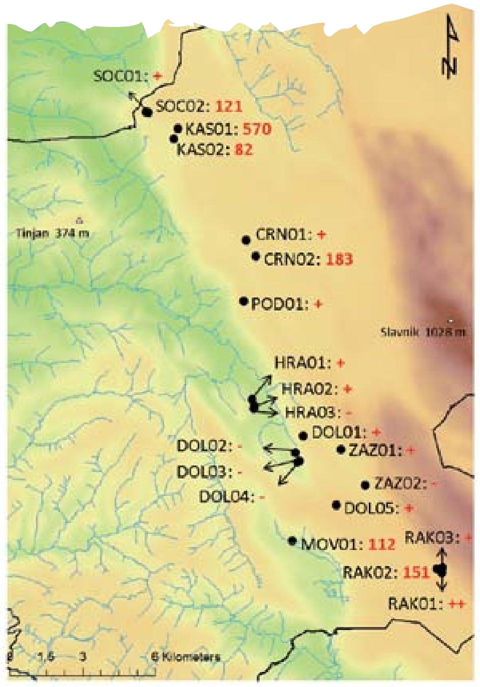
A population study of two syntopic salamandrids, the smooth and Italian crested newts, was conducted in selected ponds on the Karst edge. Both species are denoted as vulnerable in the national red list and the Italian crested newt is also a *Natura 2000* qualifying species.

Newts are prolonged pond breeders that are negatively influenced by fish presence in ponds. Crested newts are highly selective towards breeding habitats, with pond depth being one of the most limiting characteristics. Moreover, the quality and diversity of the terrestrial environment plays a crucial role in newt presence/abundance (Denoël & Ficetola, 2007). Therefore, we propose that newts are good indicators for habitat quality and their populations should to a certain extent reflect the health of aquatic environments.

Abundance of newts was assessed using the MRR (mark-release-recapture) approach. An extensive MRR survey was applied in pond KAS01 near the village Kastelec (Figure 6.3a) during the breeding seasons 2006-2008 and 2013. Population abundance of the smooth newt was estimated using a modified Jolly-Seber open population approach using the module POPAN (assuming a superpopulation) in MARK software (Schwarz & Arnason, 1996; White & Burnham, 1999). For abundance estimation of the Italian crested newt a different approach was used, assuming a closed population within module CLOSED CAPTURES in programme MARK. Abundance in a further five ponds (SOC02, KAS02, CRN02, RAK02 and MOV01) with less intensive field sampling was evaluated using the Petersen Index modified after Chapman for small recapture samples. Other sites in the area were surveyed for newts within the broader study of amphibian diversity, with no evaluation of population size.

Figure 6.3: Examples of Karst ponds. A – intensively studied pond near Kastelec (KAS01); B – visibly eutrophic pond near Črnotiče (CRN02) with high water level; C – pond in Zazid (ZAZ01) with abundant surrounding vegetation; D – pond near Movraž (MOV01) amidst cultivated land. Photographs: Martina Lužnik (A and B) and Scot Mills (C and D).

Population estimates of smooth and Italian crested newts and their presence/absence on the Karst edge are presented on maps (Figure 6.4 and Figure 6.5). For the smooth newt, we were able to calculate abundance in all six investigated ponds, with the highest numbers at Kastelec pond (KAS01). Over the first three years of survey, the abundance of smooth newt in pond KAS01 was quite stable at about 300 males and 300 females (with variable confidence intervals); whereas in 2013 the estimates were around 200 males and females (see Figure 6.6).



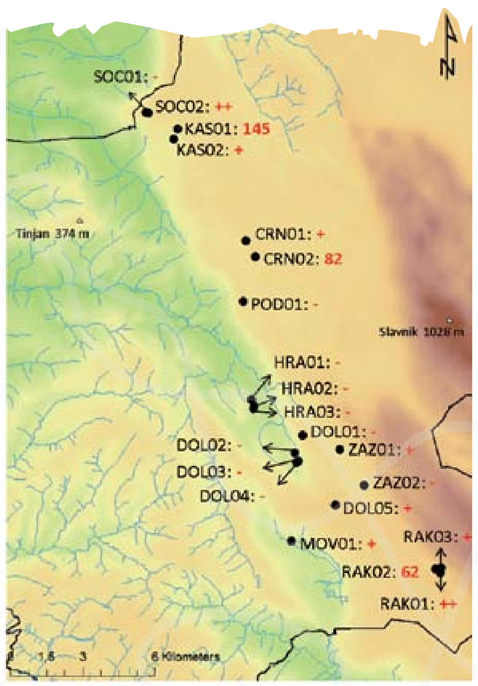


Figure 6.4: Presence and estimated abundance of smooth newt in ponds on the Karst edge. Where abundance was not estimated: (+) less than 50 individuals; (++) more than 50 individuals; (-) newts not present. Cartography: Sara Zupan

Figure 6.5: Presence and estimated abundance of Italian crested newt in ponds on the Karst edge. Where abundance was not estimated: (+) less than 50 individuals; (++) more than 50 individuals; (-) newts not present. Cartography: Sara Zupan

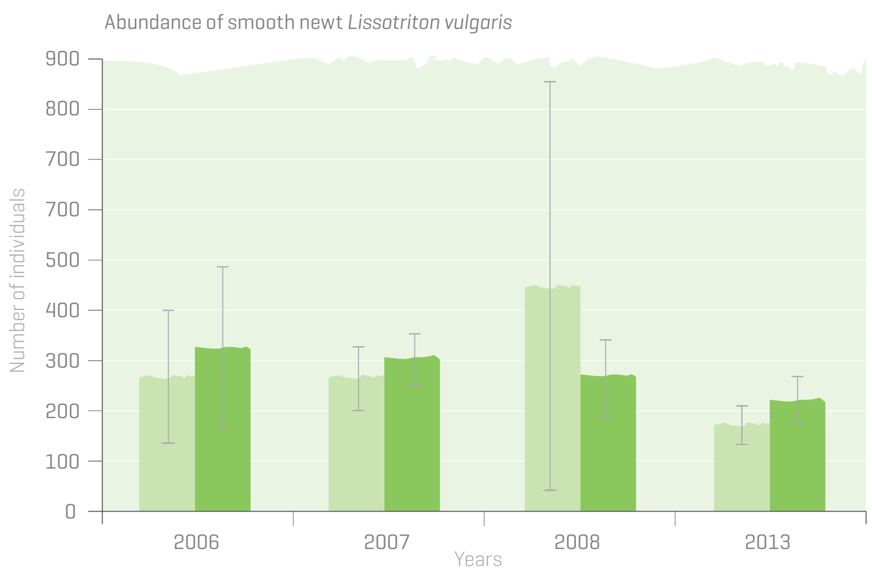


Figure 6.6: Estimated abundance of the smooth newt with 95 % CI in Kastelec pond (KAS01) during four years of study (2006, 2007, 2008 and 2013). Blue: males, purple: females.

Abundances of Italian crested newts were lower compared to those of smooth newts; moreover, this species was less frequent as it inhabited fewer ponds (Figure 6.5). Their abundance at KAS01 was pooled together for males and females and estimated at about 140 individuals (95 % CI: 107 – 204) in 2007 and at 259 individuals (95 % CI: 217 – 325) in 2013 (Figure 6.7). The number of sampled animals in other years of the study was too low for statistically robust population assessments.

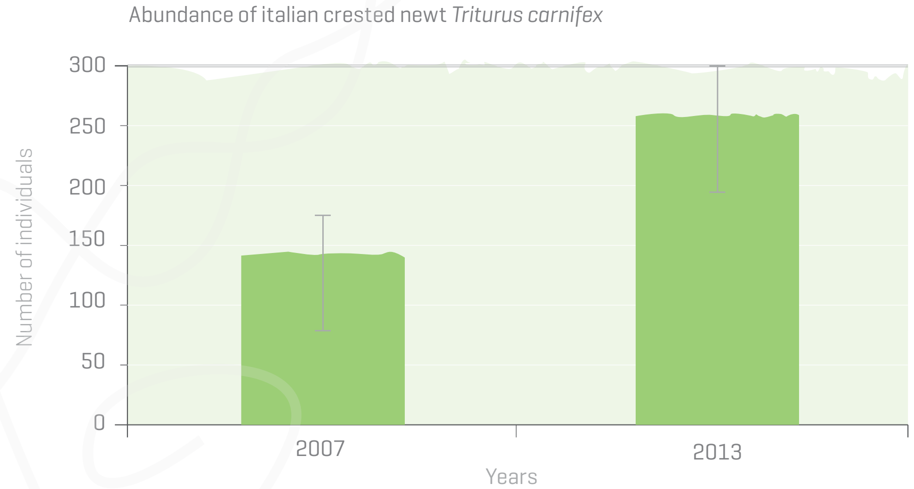


Figure 6.7: Estimated abundance of the Italian crested newt with 95 % CI in Kastelec pond (KAS01) during two years of study (2007 and 2013). Estimates are pooled together for males and females.

Some of the investigated ponds were populated by fish, introduced before and even during the course of the study (e.g. the intensively studied pond in Kastelec). Results suggest the population of smooth newt to be above the minimal viable population threshold on the Karst edge. However, populations of the Italian crested newt in karst ponds allegedly do not meet the minimal viable population threshold, which may indicate a risk to their long-term persistence. In the area both species are further threatened by introductions of fish in certain ponds.

6.5 GENETIC DIVERSITY OF THE SMOOTH NEWT ON THE KARST EDGE

The smooth newt has a wide distribution range across Europe up to western Siberia. The species is polytypic, with seven morphological subspecies described (Raxworthy 1990; Schmidtler & Franzen 2004). Moreover, an analysis conducted by Babik *et al.* (2005) detected a high genetic diversity based on mitochondrial DNA (mtDNA), with 12 lineages present. The oldest three lineages representing separate subspecies are of Pliocene origin (4.5 to 3.0 MA), while other lineages originate in Pleistocene and are not concordant with current taxonomic recognition of subspecies. In Slovenia two subspecies are present; the nominal subspecies *L. v. vulgaris* is confined to the north-eastern part of the country, while the rest is populated by *L. v. meridionalis*. The range borders of these two subspecies are not sharply defined, because of frequent records of males displaying intermediate characters, which concurs with a broad transitional zone with documented gene introgression in the rest of Europe (Križmanić *et al.*, 1997; Raxworthy, 1990; Schmidtler & Franzen, 2004). Until recent, only one mtDNA lineage with a wide distribution in Europe was reported for Slovenia (Babik *et al.*, 2005). However, a detailed phylogeographic study of mtDNA on Slovenian populations of smooth newt showed four distinct lineages present in the country, with one recorded for the first time (Lužnik *et al.*, 2011). Here we present the genetic structure of smooth newt populations of the Karst edge based on two mtDNA genes.

In total, we screened 84 alcohol-preserved tissue samples from 15 localities on the Karst edge (3 to 7 samples per site). Analyses were performed on two mtDNA genes, ND2 and ND4, using a laboratory protocol described in Babik *et al.* (2005). Obtained sequences were evaluated in CodonCode Aligner (v. 1.63; Ewing *et al.*, 1998) and aligned in MEGA 5 (Tamura *et al.*, 2011). To determine haplotypes and their frequencies sequences were analysed using programme DNASp 5.1 (Rozas *et al.*, 2003). A median joining network (programme Network, Bandelt *et al.*, 1999) was used to visualize distribution of haplotypes into lineages.

Results showed three divergent mtDNA lineages (H, L1 and M; names are consistent with Babik *et al.*, 2005) to be present in the investigated area, with a total of 15 haplotypes recorded (Figure 6.8). Lineage H was previously found in Italy and was thought to be associated with *L. v. meridionalis* subspecies (Babik *et al.*, 2005). on the Karst edge this lineage was found in nearly all investigated sites, with 7 haplotypes present. Lineage L1, so far associated with *L. v. vulgaris* subspecies, had a wide distribution throughout Europe from the Balkans to France and Britain (Babik *et al.*, 2005) and also across eastern and central Slovenia (Lužnik *et al.*, 2011). In the investigated area only two haplotypes were present at two sites in the extreme south-eastern part of Karst edge (village Rakitovec). Lineage M was previously not detected in other parts of Europe, where it seemingly is not present. Apparently, this new lineage has a narrow distribution in south-western Slovenia and Istrian peninsula (Lužnik *et al.*, 2011). It is found in nearly all sites on the Karst edge, where it is represented by 6 haplotypes.

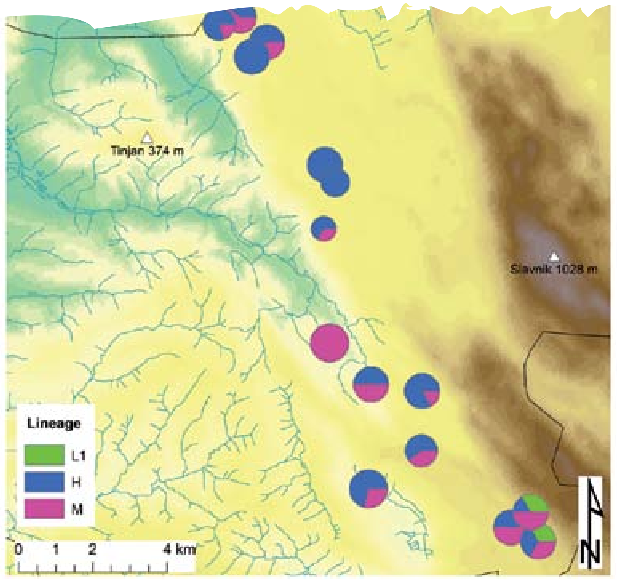


Figure 6.8: Geographical distribution of haplotypes and lineages of smooth newt in sampled ponds of the Karst edge. The area of pie chart is proportional to the number of individuals. Cartography: Peter Glasnović.

Ponds in Rakitovec showed the highest diversity of haplotypes and lineages (three lineages and four to five different haplotypes present per pond). Sympatry of three highly divergent clades On the Karst edge and the wider area could hint at a secondary contact of allopatrically evolved populations (lineages) in small glacial refugia to the south of the Alpine glacier.

6.6 ZOOPLANKTON POPULATIONS IN PONDS OF THE KARST EDGE

The zooplankton assemblages focused upon in this study include species from the phylum Rotifera (the rotifers) and Arthropoda (specifically subclass Copepoda and order Cladocera), see Figure 6.9.

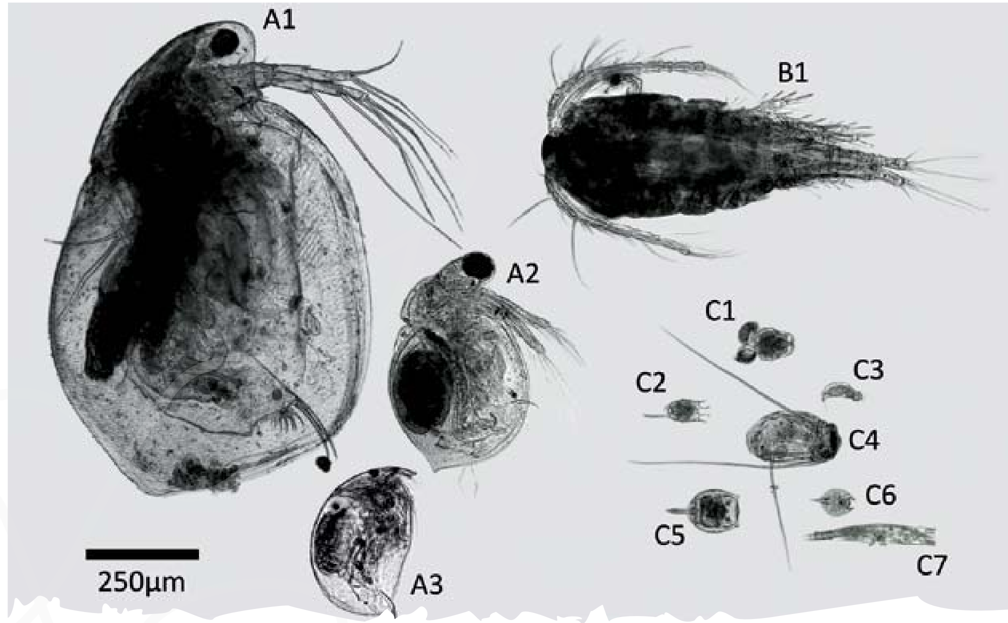


Figure 6.9: Example of zooplankton collected from karst ponds in Slovenia, **Cladocera:** A_1 *Simocephalus* sp., A_2 *Ceriodaphnia* sp., A_3 *Anthalona* sp., **Copepoda:** B_1 Order Cyclopoida, **Rotifera:** C_1 *Brachionus angularis*, C_2 *Keratella valga*, C_3, C_4 *Filinia* sp., C_5, C_6, *Lepadella* sp. C_7 *Rotaria neptunia*. Photograph: Scott Mills.

These organisms are small and extremely vagile with global distributions; Charles Darwin famously noted isolated habitats often contained closely related freshwater taxa:

> *"As lakes and river-systems are separated from each other by barriers of land, it might have been thought that fresh-water productions would not have ranged widely within the same country, and as the sea is apparently a still more formidable barrier, that they would never have extended to distant countries. But the case is exactly the reverse. Not only have many fresh-water species, belonging to different classes, an enormous range, but allied species prevail in a remarkable manner throughout the world."* Darwin (1859: p. 373).

In zooplankton the capacity to disperse is enhanced by the production of diapause states (mostly in the form of diapause eggs) that are capable of withstanding adverse environmental conditions. The evidence for the passive dispersal of small aquatic organisms has, in recent years, leaned toward avian vectors, with both the internal and external transport of propagules (Charalambidou & Santamaría, 2002; Figuerola & Green, 2002; Figuerola *et al.*, 2005). Other vectors such as aeolian transport, flooding events, terrestrial and aquatic animal vectors and anthropogenic transport can not be discounted. The overarching theory is that the potential modes of dissemination in zooplankton are many, and these modes of transport can be expected to facilitate rapid colonization.

Once colonization has taken place established populations deposit a resting egg bank that can endure prolonged anhydrous or adverse conditions that may be maintained for decades or perhaps even centuries until favourable conditions arise (King & Serra, 1998; Kotani *et al.*, 2001). The latter assertion has been demonstrated for the copepod

Diaptomus sanguienus whose diapause eggs have been recovered and hatched from 330 year old sediments collected from Bullhead pond on Rhode Island (USA) (Hairston *et al.*, 1995).

Interestingly, the egg banks in karst ponds have been characterized by a series of depletion events that may have given rise to multiple founding populations. To elaborate, some ponds have had their sediments removed and sold as fertilizer, a process that would have largely removed the resident egg bank.

With zooplankton's capacity for global vagility and stasis it may be hypothesised that species are likely to be widespread and even cosmopolitan, indeed this was the prevailing view until the rise of molecular genetic techniques. Instead, the monopolisation hypothesis of gene flow whereby founding populations adapting to the local conditions, form resident resting egg banks in the sediment and maintain large populations during favourable conditions is now favoured. Under these conditions the resident founding populations effectively buffer against migrant genotypes. It is now thought that geographic speciation as a result of persistent founder effects is indeed a very significant evolutionary driver for microorganisms in a similar fashion to geographic barriers in larger organisms.

Added to this geographic complexity is temporal habitat partitioning, where ponds can go from aquatic to dry and exhibit a multitude of physical and chemical states between these two extremes. As a result pond community structures are in flux, especially in habitats that exhibit strong seasonal influences.

When habitats do dry species emerge from the resident egg bank, each species can exhibit different emergence patterns. Figure 6.10 demonstrates this for three *Cephalodella* sp. rotifers from a lake in Western Australia (unpublished data: Scott Mills), even amongst these closely related species there are clear differences in emergence. The sediments of a small lake or pond can have a very dense egg bank, for example 1683 individual zooplankton belonging to 23 species were hatched from just 60 grams of sediment sourced from a lake in the West Australian outback (unpublished data: Scott Mills).

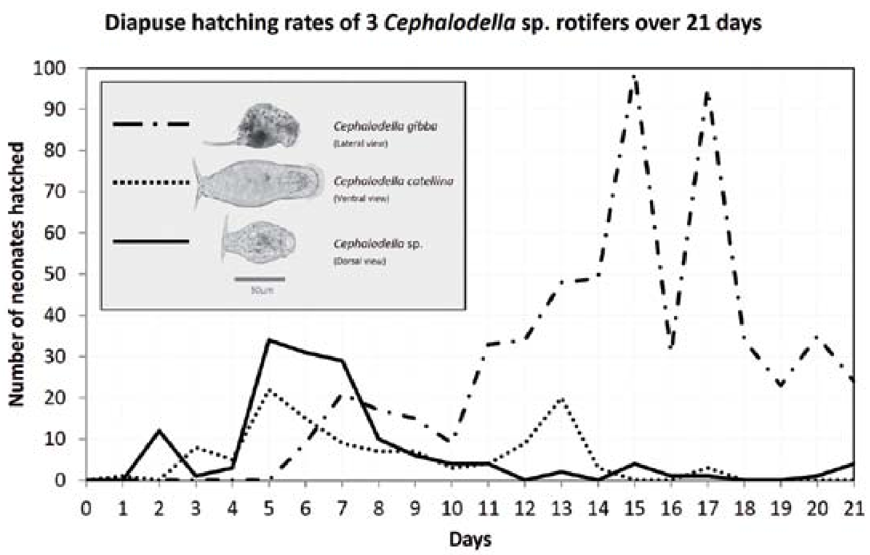


Figure 6.10: Diapause hatching rates of 3 *Cephalodella* sp. rotifers over 21 days. Eggs were recovered from 60 grams of sediments (spread across 12 replicates) using sucrose flotation accelerated with a centrifuge to ≈980 m/s² for 5 minutes in a 50ml Falcon™ tube.

Karst ponds are likely to harbour high levels of diversity, well beyond what can be sampled in single events. Continued sampling across several years or detailed analysis of the resting egg bank would be required to get true estimates of the biodiversity of these habitats. In the next section we present a snapshot of zooplankton diversity from karst ponds.

6.7 BIODIVERSITY SNAPSHOT – ZOOPLANKTON FROM KARST PONDS

Samples were collected from 11 karst ponds in 2013 during two field excursions on May 28 and 29. These habitats were small, with surface areas ranging from 8 to almost 500 square metres. All contained freshwater (93 to 615 μS/cm, <3 to 52 mg/L chlorides), and some indicated carbonate dominance, which is not surprising for water situated on calcium carbonate, see Table 6.3 for some details of the sites water chemistry.

Table 6.3: Ponds sampled and their various physical parameters. Superscripts indicate sample date; 28/May/2013[1] and 29/May/2013[2]. • - indicates analysis was not performed.

Site acronym	Total PO_4^{-3} (mg/L)	Ortho PO_4^{-3} (mg/L)	Nitrates (mg/L)	Chlorides (mg/L)	Conductivity (μS/cm)	pH
SOCO1[1]	0.190	0.089	0.039	28	504.0	6.95
SOCO2[1]	0.280	0.150	0.039	12	535.0	8.94
KASO2[1]	•	•	•	•	615.0	7.99
CRNO1[1]	•	•	•	•	457.0	7.66
CRNO2[1]	0.051	0.012	0.056	<3	157.3	8.06
ZAZO1[2]	0.078	0.056	<0.010	32	423.0	6.97
ZAZO2[2]	0.027	0.008	0.020	<3	93.0	8.32
RAKO1[2]	0.170	0.021	<0.010	52	485.0	7.25
RAKO2[2]	0.170	0.073	0.035	11	148.0	9.35
MOVO1[2]	0.014	<0.006	0.700	9	580.0	6.88
DOLO1[1]	0.026	0.008	0.025	<3	170.8	8.51
Min	0.014	<0.006	<0.010	<3	93.0	6.88
Max	0.280	0.150	0.700	52	615.0	9.35

At least 38 species of zooplankton were present in the 11 ponds; species were dominated by members of the phylum Rotifera with 29 taxa recorded. The greatest number of rotifer species was present in the largest pond located on the outskirts of the village Rakitovec (RAKO1), with a total of 9 taxa present. Of the rotifer taxa identified the most common was *Mytilina mucronata* occurring at a total of five sites located near four separate villages (SOCO1, SOCO2, CRNO1, ZAZO1 and RAKO1). All other taxa were only recorded at 1 (15 species), 2 (7 species), 3 (3 species) or 4 (4 species) sites, see Table 6.4 for more details. This observation may reflect the physiochemical state of the ponds, but also hints at the uniqueness of each of these habitats as rotifer biodiversity reservoirs.

Table 6.4: Rotifers (phylum Rotifera) identified from each sample site, • indicates the species was present.

Class	Order	Family	Species	SOC01	SOC02	KAS02	CRN01	CRN02	ZAZ01	ZAZ02	RAK01	RAK02	MOV01	DOL01
Bdelloidea	Indet.	Indet.	Indet. Bdelloid (S)				•				•		•	
			Rotaria neptunia									•		
			Indet. Bdelloid (L)	•				•			•	•		
Monogononta	*Ploima*	*Brachionidae*	*Brachionus angularis s.l.*	•								•		
			Brachionus calyciflorus								•	•		
			Brachionus quadridentatus							•	•	•		
			Brachionus spp.			•		•		•		•		
			Anuraeopsis sp.			•								
			Keratella valga	•	•		•	•						
		Epiphanidae	*Proalides*						•					
		Dicranophoridae	*Dicranophorus* sp.							•				
		Lecanidae	*Lecane bulla*											•
			Lecane (M.) sp. a								•			
			Lecane (M.) sp. b								•			
			Lecane curvicornis			•								
		Lepadellidae	*Lepadella* spp.				•							•
		Notommatidae	*Cephalodella* sp.										•	
			Eosphora najas		•									
			Notommata sp.					•						
			Notommata copeus						•					
		Synchaetidae	*Polyarthra* sp.		•			•						•
			Synchaeta sp.					•						
		Trichocercidae	*Trichocerca pusilla*								•			
			Trichocerca sp.		•						•			
	Flosculariaceae	*Trichotriidae*	*Mytilina mucronata*	•	•		•		•		•			
		Testudinellidae	cf. Pompholyx			•								
			Testudinella patina		•		•	•		•				
		Filiniidae	*Filinia* sp.				•	•						
		Hexarthridae	*Hexarthra* sp.						•	•				
			Number of rotifer taxa	4	6	4	6	8	4	5	9	6	2	3
			Total number of taxa	29										

Table 6.5: Cladocerans (phylum Athropoda, class Brachiopoda, order Cladocera) sampled from each site, • indicates that the species was present.

Family	Species	SOC01	SOC02	KAS02	CRN01	CRN02	ZAZ01	ZAZ02	RAK01	RAK02	MOV01	DOL01
Chydoridae	*Anthalona* sp.					•		•				
	Alona sp. A							•				•
	Alona sp. B						•	•				
	Chydorus sp.	•	•		•	•	•				•	•
Daphinidae	*Ceriodaphnia sp.*	•			•	•						•
	Daphnia spp.	•			•	•				•		
	Simocephalus sp.	•	•				•				•	•
Number of cladoceran taxa		4	2	0	3	4	3	3	0	1	2	4
Total number of taxa		7										

Table 6.6: Copepods (phylum Athropoda, class Maxillopoda) sampled from each site, • indicates that copepods were present.

Order	Family	Species	SOC01	SOC02	KAS02	CRN01	CRN02	ZAZ01	ZAZ02	RAK01	RAK02	MOV01	DOL01
Cyclopoida	Indet.	Adult	•	•			•	•	•	•		•	
		Copepodites & nauplii	•			•	•		•	•		•	
Calanoida	*Diaptomidae*	cf. *Arctodiaptomus* sp.					•						
		Copepodites & nauplii					•						
Number of copepod taxa			1	1	0	1	2	1	1	1	0	1	0
Total number of taxa			2										

Cladocerans were present at 9 of the sampled ponds, we were able to identify a total of 7 taxa to genus, see Table 6.5. The most common was a *Chydorus* sp. found in 7 ponds (SOC01, SOC02, CRN01, CRN02, ZAZ02, MOV01, DOL01), followed by a *Simocephalus* sp. (see Figure $6.9A_1$) present in 5 ponds (SOC01, SOC02, ZAZ01, MOV1 and DOL01). In ponds where cladocerans were present the number of species ranged from 1 to 4.

Copepods belonging to the Family Cyclopoida were present in 8 ponds while only a single lake had a representative of Calanoida, both adults and juvenile states were recovered. As some observations were of immature stages such as copepodites and nauplii it is uncertain how many taxa this represents, although there were likely only two taxa present. Further investigation will be required to fully identify these copepods.

CONCLUSIONS

Recent scientific research advocates a greater role for ponds as key wetland habitats, especially in dry landscapes (Céréghino *et al.*, 2014). Our study

incorporated four different approaches to assess pond biodiversity and to better define the threats and conservation status of these isolated aquatic habitats in a dry environment.

With nine species present, amphibians showed a rather high species diversity for such a small area. They were found in almost every type of waterbody, from ponds to concrete trenches, especially if devoid of fish. As anticipated, the most frequent amphibian species was smooth newt, while agile frogs were only a little scarcer. On the other hand, alpine newts were found only in two ponds in the highest elevations of the area. Ponds showed a variable range of amphibian species diversity. Amongst the richest were sites in the north western part of Karst edge situated in or near Socerb (SOC02), Kastelec (KAS01), and Zazid (ZAZ01), these sites also exhibited the highest abundance of newts. Populations of smooth newt in the area of Karst edge are abundant enough for their long-term survival, while the Italian crested newt is less abundant. We hypothesise that populations of this *Natura 2000* species do not meet the criteria for a minimal viable population and are therefore particularly at risk. Genetic diversity of smooth newt in the research area is relatively high, comprising three different sympatric lineages. One of these lineages is restricted to south western Slovenia and is dispersed throughout the Karst edge. The present situation is thought to reflect a secondary contact of populations and could point to a glacial micro-refugium for smooth newt in the area.

Most semiaquatic amphibians, such as newts require suitable terrestrial habitats, such as forests, in close proximity to wetlands (Denoël & Ficetola, 2007; Schabetsberger *et al.*, 2006). Newt populations have an increased risk of extinction if they do not live in a suitable habitat and are extremely sensitive to increasing isolation between ponds and terrestrial habitats (Denoël & Ficetola, 2007).

For the first time we have presented data about the zooplankton species composition of Slovenian karst ponds. This data is a starting point for the design of further studies. The data suggests that the ponds harbour a high level of biodiversity that may be even greater if studies were conducted across several years to examine the full extent of the egg bank. Zooplankton can be used in a number of novel ways as biological indicators and in the study of evolutionary mechanisms. It is suggested that a more concentrated study on a selection of ponds be made to get a complete inventory of these habitats' biodiversity. Once this inventory has been made, and linked to physiochemical parameters, zooplankton species compositions may be used as sensitive bio-indicators. Given the young age of these ponds, examination of species level genetic structure would give insights into colonization patterns of recently established zooplankton communities that would shed light on evolutionary processes.

Karst ponds represent a case where human activity is a driving force of biodiversity rise (Denoël & Ficetola, 2007; Knutson *et al.*, 2004; Miracle *et al.*, 2010). Contemporary use of karst ponds has changed with the intensification of agriculture that uses fertilizers, pesticides and other chemicals. These modern farming practices contribute to the degradation of these habitats, and are magnified with predictions of extensive climate change (Corn, 2005). Pollutants accelerate the succession process and thus contribute to a faster loss of suitable habitats. Some karst ponds are even intentionally filled with building materials and refuse (Ciglič, 2005; Dolce *et al.*, 1991).

Another, contradictory, problem with ponds is a lack of human intervention. Natural succession was periodically interrupted by cleaning and other maintenance work. Now this interference is exceedingly rare, which in turn is causing deterioration of these ecosystems and decrease of aquatic organisms.

The introduction of non-native species, such as goldfish, disturbs many organisms living or reproducing in karst ponds. Many researchers have confirmed the negative impact of fish presence on amphibian communities (Denoël *et al.*, 2005; Denoël & Ficetola, 2007; Eby *et al.*, 2006; Ficetola & De Bernardi, 2004; France, 2002; Joly *et al.*, 2001; Kats & Ferrer, 2003; Reshetnikov, 2003]. These effects can be direct, like predation and competition. Indirect effects, such as habitat alteration is mainly seen as a change in the local food web, with cascading effects across all trophic levels.

These habitats are manmade biodiversity hotspots and require managed conservation approaches to maintain them. In order to manage them sound scientific data obtained from long-term monitoring is paramount, in the absence of this information evidence based conservation decisions are not possible. An interdisciplinary approach, taking into consideration both the biotic and abiotic environment, and the way in which it interacts, as well as the cultural landscape is a prerequisite. Furthermore, population genetic studies could reveal the metapopulation structure of selected taxa in the studied system. Monitoring of newt populations in selected ponds is being continued in 2014 and an on-going study using microsatellite loci should shed light on the population genetic structure of these endangered animals. Samples of zooplankton from this study will be processed for DNA barcodes to help facilitate species identification in future research efforts.

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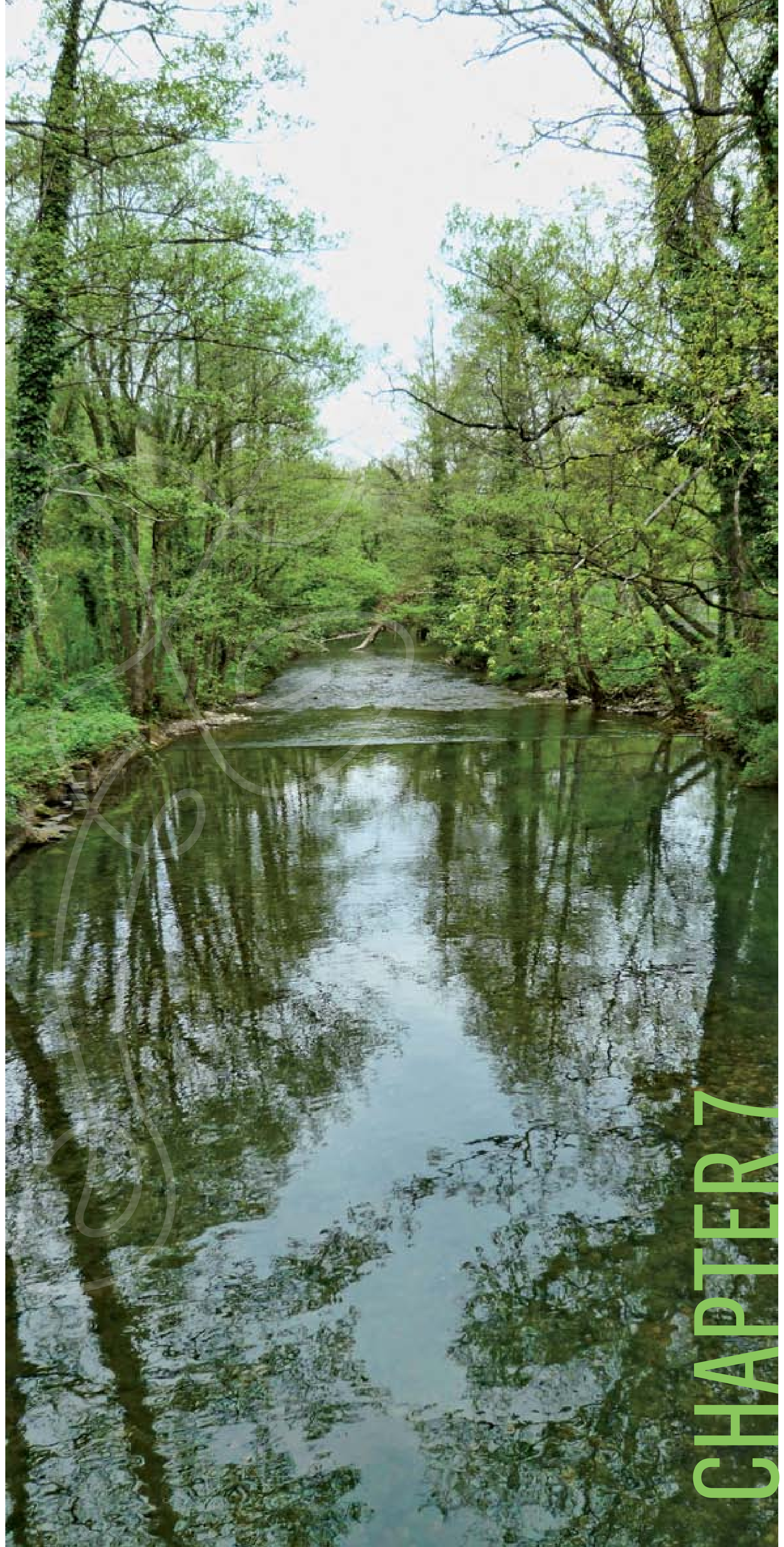

CHAPTER 7

THE IMPORTANCE OF HYDROGEOLOGICAL, GEOLOGICAL AND CLIMATIC FEATURES IN THE KARST LANDSCAPE FOR THE PROTECTION OF WATER RESOURCES AND BIODIVERSITY

POMEN HIDROLOŠKIH, HIDROGEOLOŠKIH, GEOLOŠKIH IN KLIMATSKIH ZNAČILNOSTI KRAŠKE POKRAJINE ZA VARSTVO VODNIH VIROV IN BIODIVERZITETE

L'IMPORTANZA DELLE CARATTERISTICHE IDROGEOLOGICHE, GEOLOGICHE E CLIMATICHE NEL PAESAGGIO CARSICO PER LA PROTEZIONE DELLE RISORSE IDRICHE E DELLA BIODIVERSITÀ

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ABSTRACT

The contribution aims to illustrate the importance of the main climatic, geological and hydrogeological features of the karst landscape, in relation to both floral and faunal biodiversity and to human activities (i.e. grazing management). The geomorphological features of the karst landscape derive from the interaction between climatic factors (e.g. meteorological agents), geological setting, and rock mass properties (mainly solubility). Water represents a key element in this landscape and the absence/scarcity of surface water bodies influences land use and human activities as well as the floral and faunal biodiversity. The high porosity and solubility of the calcareous rocks bring about a rapid and consistent infiltration of water into the ground with the development of cavities within which there is a rapid turnover of groundwater. The high vulnerability of this groundwater and the strategic importance of karst springs (often used for public water supply) demand a sound knowledge of the karst aquifer system in order to protect and preserve water resources. After a regional overview of the geographic, microclimatic, geological and hydrogeological context of the extended area, a test site for the BioDiNet project is presented with particular attention to water resource protection (the Rižana spring catchment, Koper, Slovenia) in relation to grazing activities. Finally the relation between the karst and biodiversity is discussed and an example of the influence of geomorphological and hydrogeological factors on cave fauna biodiversity is provided.

Key words: karst aquifer, aquifer vulnerability, limestone, spring, cave fauna

IZVLEČEK

V poglavju prikazujemo pomen glavnih klimatskih, geoloških in hidrogeoloških značilnosti kraškega sveta v povezavi z diverziteto rastlin, živali in človekovih aktivnosti (npr. paše). Geomorfološke značilnosti kraškega sveta so odvisne od interakcij med klimatskimi dejavniki, geološko podlago in značilnosti kamnin (predvsem topnosti). Voda je ključni element v taki pokrajini in pomanjkanje površinskih vodnih teles močno vpliva na rabo zemljišč in s tem povezanimi človekovimi posegi, nenavsezadnje pa tudi na biodiverziteto. Visoki stopnji poroznosti in topnosti karbonatov pomenita hiter vstop vode v podzemlje; razvijejo se jame z obsežnim sistemom podzemnih voda. Visoka stopnja ranljivosti in strateški pomen kraških izvirov (ki so pogosto v uporabi za javno oskrbo z vodo) zahtevata poznavanje sistema kraških zajetij, kar je pogoj za učinkovito varstvo in ohranitev vodnih virov. Tu predstavljamo pregled regionalnih geografskih, mikroklimatskih, geoloških in hidrogeoloških značilnosti širšega projektnega področja, nato pa predstavljamo ožje, testno območje v okviru projekta BioDiNet (zajetje na reki Rižani, Koper, Slovenija) in stanje ocenjujemo v povezavi s pašnimi aktivnostmi na tem področju. Prikazujemo tudi rezultate krajše študije sestave favne iz sedmih jam s širšega območja Krasa, in razlike v sestavi vrst med posameznimi jamami razlagamo z razlikami v habitatih, predvsem na osnovi razlik v geomorfologiji in hidrogeologiji.

Ključne besede: kraško zajetje, ranljivost zajetja, apnenec, izvir, jamska favna

RIASSUNTO

Il contributo vuole illustrare l'importanza dei principali fattori climatici, geologici e idrogeologici del paesaggio carsico, in relazione sia alla biodiversità della flora e della fauna, che alle attività antropiche (nel caso specifico le attività di pascolo). Le caratteristiche geomorfologiche del paesaggio carsico derivano dall'interazione tra fattori climatici (ad esempio gli agenti meteorologici), struttura geologica e proprietà dell'ammasso roccioso (soprattutto la solubilità). L'acqua rappresenta un fattore chiave in questo paesaggio e l'assenza o scarsità di corpi d'acqua superficiali influenza sia l'uso del territorio, che le attività antropiche, oltre che la biodiversità di flora e fauna. L'elevata porosità e solubilità delle rocce calcaree determina una rapida e significativa infiltrazione d'acqua nel sottosuolo, con lo sviluppo di cavità all'interno delle quali si sviluppa una rapida circolazione delle acque. L'elevata vulnerabilità di queste acque sotterranee e l'importanza strategica delle sorgenti carsiche (utilizzate spesso per uso acquedottistico) richiedono una buona conoscenza del sistema acquifero carsico, al fine di proteggere e preservare le risorse idriche. Dopo un inquadramento a scala regionale delle caratteristiche geografiche, micro-climatiche, geologiche e idrogeologiche dell'area di studio estesa, viene presentato un sito di studio del progetto BioDiNet (il bacino della sorgente Risano, Capodistria, Slovenia), focalizzato sulle protezione delle risorse idriche in relazione alle attività di pascolo. Infine, viene analizzata la relazione tra il Carso e la biodiversità e viene presentato un esempio riguardante l'influenza dei fattori geomorfologici e idrogeologici sulla biodiversità della fauna di grotta.

Parole chiave: acquifero carsico, vulnerabilità dell'acquifero, calcari, sorgente, fauna di grotta

7.1 GEOGRAPHIC AND CLIMATIC FEATURES

The BioDiNet project carries out its studies on the Classic Karst, a broad area located along the border between Italy and Slovenia (Figure 7.1). The area is about 40 km long and 15 km wide, bordered by the Isonzo/Soča River in the NW, by the Vipacco/Vipava River valley towards N and NE, by the Pivka River watershed to the East, by the Adriatic and the Gulf of Trieste towards the W and, along its southern edge, by the Dinaric Karst, the Karst plateau altitudes decreasing irregularly from SE (maximum altitudes of about 650 m a.s.l.) to NW (average altitudes of 200–400 m a.s.l.).

The climate of the Classical Karst is rather complex and very varied in European terms as a result of the climatic transition between different sub-domains: the Mediterranean one, dominant along the coast, and a sub-continental one inland. This rapid transition results in a strong thermal and meteoric contrast between areas very close to one another, in relation to their proximity to the highest mountains and their interaction with the air currents of the Mediterranean basin, which cause intense precipitation events. The average rainfall in the Classical Karst varies from 1000 mm/yr along the coast to about 1800 mm/yr in the hinterland, with an average winter mean temperature of about 3.5°C and an average summer value of about 19.5°C. The mean annual temperature is about 12°C, but this parameter exhibits important variations between coastal districts and areas inland. As a result, a lot of different microclimatic situations are encountered and thus a dense network of weather monitoring stations is required to characterize the climate of the study areas in detail.

For the aims of BioDiNet project, data on precipitation and temperature were collected from all the available weather monitoring stations (Figure 7.1) for the period covered by the project activities, but the entire historical series have been also taken into account. Particular attention was paid to the test sites at Hrastovlje (the Rižana spring catchment, Koper, Slovenia) and the Alture di Polazzo (Gorizia, Italy), where investigations into the grazing lands productivity have been carried out by the University of Padova.

For the Hrastovlje area the climatological analysis was conducted on rainfall datasets for the period 1961–2013, collected from five stations located within a radius of about 20 km: Strunjan/Strugnano and Seča/Seca on the coast together with Movraž/Movrac, Rakitovec and Kozina, moving inland at gradually increasing distances from the coast. Temperature datasets were available only for the stations of Portorož/Portorose Airport (coastal zone) and Sgonico/Zgonik (about 10 km from the sea, on the Karst plateau) and are limited to the period 1990–2013. The annual average temperature is around 13.5°C; those for January and July being, respectively, about 3°C and 24°C. The yearly days with frost (with T_{min}<0°C) number 45 on average, whereas the number of days of ice (with T_{max}<0°C) is very small. The number of tropical days (with T_{max}> 30°C) is 35 on average with the absolute minimum in the time interval being -15°C and the absolute maximum exceeding 37°C.

The recent trend (1990–2013) is characterized by an increase in average temperature of about 0.6°C, slightly higher for the minimum temperatures than the maximum ones. In spite of this, the De Martonne aridity index is over 40 points and the climate is therefore humid without obvious summer water deficits. Finally, according to the Köppen-Geiger climate classification, the climate is temperate sub-littoral, with moderately wet to very hot summers (the "*Cfa*" climate subgroup).

Annual precipitation is quite conspicuously related to the orography and the short distances from the coast, the total average annual just about reaching 1300 mm and distributed over about 94 days. The rainfall regime is sub-littoral, a bimodal type, with a main peak in autumn (October) and a secondary maximum in May. The monthly minimum values (equal to each other and not tantamount to drought) were recorded in February and July.

The half-century precipitation trends show a clear decrease of about 5 mm/yr and thus an overall decrease of 250 mm/yr, approximately corresponding to 19% of current annual rainfall. Winter and autumn are the seasons showing the greatest decreases (about 1.4 mm per season). This signal is partially confirmed by the rainy days trend that shows a minor decline compared with absolute precipitation, but still in the order of 4 rainy days lost over the course of the last 50 years.

In the Alture di Polazzo area, the climate shows similar features. The analyses considered the long time series from Gradisca d'Isonzo (very close to the site) and have been complemented using data from the rain gauge station at Opatje Selo, located nearby but in Slovenia. The average annual temperature is about 13.8°C; those of January and July being about 4°C and 24°C, respectively. The annual temperature range is 20°C, therefore falling between the climatic zones of sub-littoral and sub-continental. The number of days with frost (with T_{min}<0°C) is about 45, with that of tropical days (with T_{max}> 30°C) being 38 on average. The absolute minimum value is -15°C, while the maximum values recorded more than 38°C. The trend shows important rises in temperature of around 1.4°C in the last two decades. The reason for this can be identified in the rapid urbanization that has characterized the area. Nevertheless, the De Martonne aridity index is around 37 points, and therefore the climate is humid without obvious summer water deficits. Finally, according to the Köppen-Geiger climate classification, the climate is defined as humid temperate sub-littoral with a very hot summer (the *"Cfa"* climate sub-group). Average annual rainfall is about 1350 mm, rather abundant in relation to the geographic location of the site, and distributed on about 98 days. Moving towards the "edge" of the Karst plateau, rainfall remains constant, 1374 mm at Opatje Selo, distributed on about 101 days. The rainfall regime in this area pertain also to a sub-littoral type. The rainiest season is autumn, while summer and spring receive quite the same cumulated total rainfall (301 mm vs. 300 mm). The trend for the last 25 years shows a decrease of about 3 mm/yr but looking at the data over the longer term and referring to the last 50 years, it shows a significant increase of around 4 mm/yr. Finally, the recent intensity of daily and hourly maximums show a clear decline.

Figure 7.1: Digital elevation model of the study area and main geographic features of the area with weather stations and study sites. Cartography: Geotema srl.

7.2 GEOLOGICAL SETTING

7.2.1 REGIONAL SETTING AND STRATIGRAPHY

The BioDiNet study area, from a geological point of view, belongs to the Classical Karst with surroundings, the northern sector of the Dinaric Karst that develops towards the SE direction (Figure 7.1). "Classical Karst" is a term used to describe the Italian and Slovenian Karsts as a geological unit, an area belonging to the Adriatic Plate and characterized by the transition from a carbonate platform environment to a terrigenous setting dominated by the Alpine-Dinaric orogenesis. In the Classical Karst the main features are, however, not strictly related to their stratigraphic framework but, remarkably, to the landscape geomorphology.

The Classical Karst defines and represents one of the most historically representative places to observe karst geomorphology and hydrogeology. Its characteristic landscape and its karstic features are controlled by three main factors: lithology, tectonic and weathering. These three factors, acting over a time period spanning from the Albian to the Holocene, control the pedogenesis, the shape of the hill slopes, the subsurface and the surface hydrology. Furthermore, these elements constitute the basis for the floral and faunal biodiversity, the endemic adaption of species and habitat creation and have thus driven the human activities in the area.

The lithologies in the BioDiNet area can be roughly divided into three main groups: the carbonate rocks, here named "Karst Limestones fm", the Flysch sediments, and the Pleistocene-to-Holocene deposits. The carbonate units are the oldest sediments, outcropping in a wide area from the Isonzo/Soča river to the North, straddling the Italian-Slovenian boundary as far south-east as the Val Rosandra/Dolina Glinščice and the northern part of the Koper Municipality. The stratigraphic framework and the lithologic nomenclature have varied many times during the last century and with the pioneering work of Stache (1889) as a basis the Mesozoic to Paleogenic rocks have been separated according to two main criteria, namely their lithology and their age. In order to provide a tentative synthesis of the evolution of geologic nomenclature in the area, we have summarized the main informal stratigraphic schemes proposed over the last 30 years into a diagram (Figure 7.2). Hereafter we will use the Cucchi & Piano *et al.* (2013) lithological classification, referring the reader to the aforementioned diagram to relate the new nomenclature to previous Italian and Slovenian studies.

From the diagram it is possible to observe a generic trend towards an increasing biostratigraphic resolution over the years, leading to a more detailed description of the units. From the bottom of the series, outcropping on the Slovenian-Italian boundary parallel to the gulf of Trieste/Trst the first unit encountered is the "*Calcari di Monte Coste*", a micritic mud-dominated limestone gray to black in colour with an internal stacking pattern characterized by lens of dolomite in the lower part of the formation, then by fossiliferous limestones and finally, in the upper part, by monogenic breccias. The palaeo-environment corresponds to an inner platform and is marked by an angular unconformity at the Aptian-Albian boundary. The unconformity is characterized by a differential subsidence that leaved more accommodation space in the NW sectors, leading the deposition of younger sediments and a tardive emersion of the area. Palaeokarst phenomena are observable, mainly through the bauxite-rich deposits. This unit can be related to the Brje Fm (Jurkovšek *et al.*, 1996) and to the upper part of the "*Calcari del Cellina*" formation (see Tentor *et al.*, 1993).

In this area the Cretaceous sedimentation is frequently interrupted by emersion events, followed by marine transgressions. The Monrupino Fm (Rupingrande mb *sensu* Cucchi *et al.*, 1987), coeval to the Povir Fm, is an example of this regional trend in as much as the base the unit is marked by an emersion horizon, a coarse sand-breccia, usually strongly altered by diagenesis. After the initial erosion the stacking pattern

becomes a general deepening and fining-upward, marking the transition from supratidal/lagoon facies to subtidal ones.

The trend reverses in the Aurisina limestones ("*Calcari di Aurisina*"): from the drowning of the platform at the Cenomanian-Turonian boundary, the facies migrates progressively towards back-reef, reef, lagoon and littoral palaeo-environments. The top of the unit is marked by a large hiatus, usually limited to a part of the Campanian, recognizable by the presence of a breccia horizon.

During the Turonian-Senonian the progradation of the platform was favoured by a tectonic phase that slowed the subsidence to the point of triggering the emplacement of palaeokarsts and palaeosoils enriched in bauxite and outcropping extensively in the Istrian karst. The end of the Cretaceous is marked by short transgressive phases (marked by rudist patch reefs) followed by a new continental phase. Brackish-water facies, rich in porcellanaceous foraminifera, coal deposits and rare karst phenomena characterize the paleogenic Liburnic group, in a complex local heteropy.

The terminal part of the Liburnic group fades more or less abruptly towards subtidal conditions. This led to the establishment of the last carbonate factory in the area, namely a foraminifera-supported reef (mainly nummulites and *Alveolina*). The first appearance of marls at the top of the Liburnic group marks the prelude to the arrival of the silicoclastic turbidites (flysch).

Flysch marls at the base of the turbiditic sequence show low contents of carbonates and a medium textural maturity (quartz 40%; feldspars 16–26%). The fossil content is extremely scarce and diluted. The central part of the series, that may be as thick as 500 meters, is arenaceous to siltitic in size, yellow-to-red in colour when altered and gray on fresh surfaces. From the flysch sediments, pedogenic processes generate a rich and productive soil that may host herbaceous to woody cultivations.

Pliocene to Holocene deposits are dominated by "*Terra Rossa*" (red soil) deposits and by detrital slopes. "*Terra Rossa*" sediments are clayish soils produced by intense weathering of carbonates. The clay is the insoluble, residual sediment that was highly dispersed within Mesozoic limestone. The specific climatic conditions (i.e. Mediterranean to continental temperatures plus intense rainfall) allow for a measurable alteration of the aluminium and iron oxides from bauxite- to limonite- rich deposits. The coalescence of detrital cones at the foot of the limestone walls generates a detrital belt that is partially cemented and usually extremely permeable. The official geological maps of Karst are available in Carulli *et al.* (2006) for the Italian side and in Jurkovšek (2013) for the Slovenian side.

A "unified" lithological map and legend have been produced in the framework of BioDiNet and it is presented in Figure 7.3, as a basemap for the hydrogeological features.

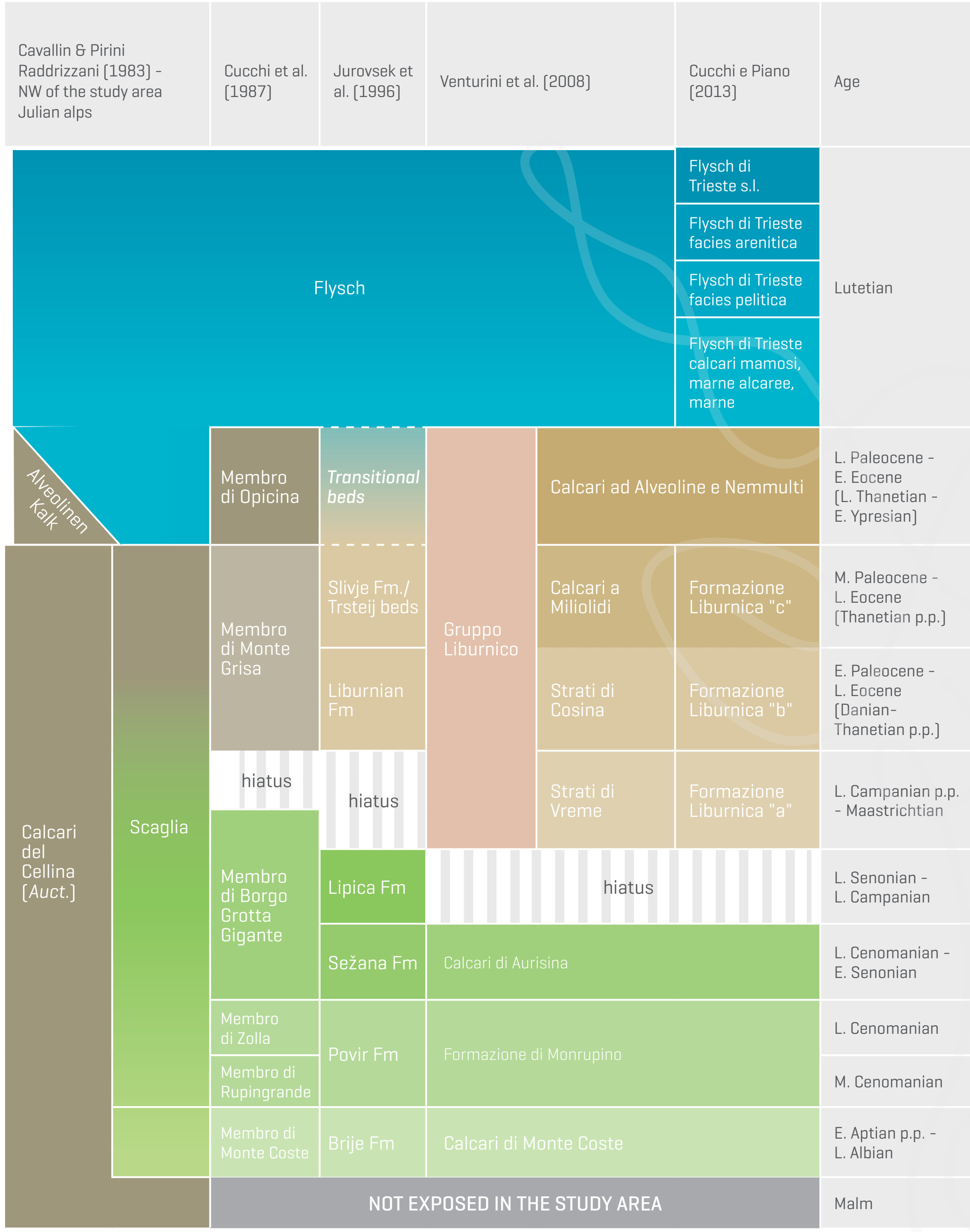

Figure 7.2: Synthesis of the main informal stratigraphic schemes proposed in the last 30 years; formation names in the original languages;the scheme of Cucchi & Piano (2013) is explained in the main text (author: Stefano Furin, Geotema srl).

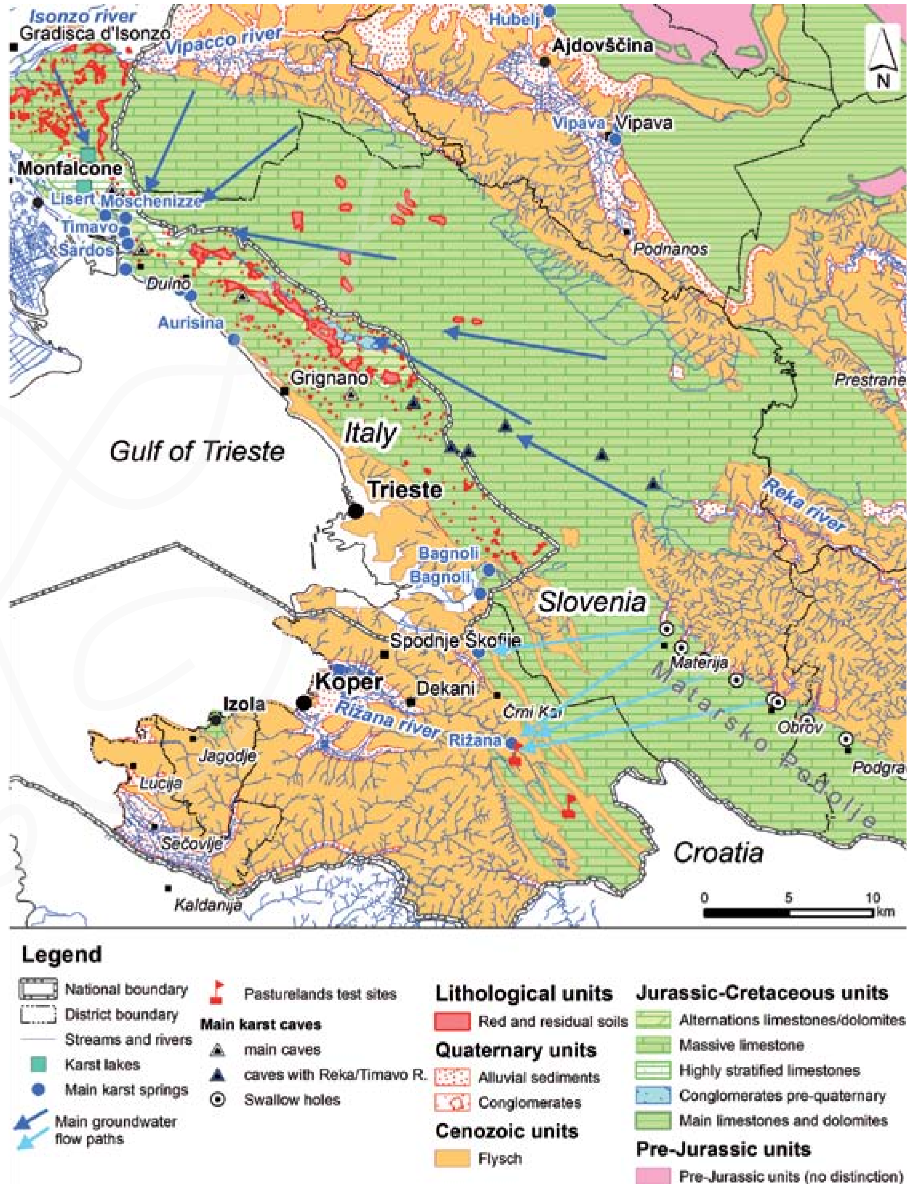

Figure 7.3: Lithological and hydrogeological map of the Classical Karst. Cartography: Geotema srl.

7.2.2 TECTONICS

As stated at the beginning of this chapter, the lithology alone cannot explain the complex morphological features of the area. Since the closure of the Tethyan ocean, tectonics have controlled the accommodation space and the ductile-brittle deformation of the sediments.

The Classical Karst area pertains to a wide structural unit called Adriatic Plate (or Comeno Platform) and is characterized by an asymmetric anticlinalic structure having the axis oriented NW–SE (passing through Doberdò del Lago, Gorjansko, Sežana and M. Straža) with a slight complication provided by secondary folding. The strata are generally dipping 10–30° towards the SW direction, with a local increase in the inclination (reaching vertical orientation) in the Duino–Mt.Grisa area.

The anticlinalic structure is slightly rotated in the Monrupino–Basovizza area by transcurrent faults with minimal horizontal and vertical dislocations. In the Rosandra Valley the structure becomes more complex due to the presence of plicative and disjunctive structures. The entire area is then overlaid by the main compressive structure, the Karst thrust (a Dinaric thrust) and, in the SW area, by minor thrusts repeating part of the flysch series.

More details regarding the tectonics are available in Placer *et al.* (1981, 2010), Bensi *et al.* (2007), Busetti *et al.* (2010) and Cucchi *et al.* (2013). An insight into the tectonics of the Karst edge area in Northern Istria is available in Placer (2005, 2007).

7.2.3 GEOMORPHOLOGICAL FEATURES OF THE KARST LANDSCAPE

A karst landscape is generally characterized by: 1) the absence of a permanent surface flow and by the presence of swallow holes (dolines) and closed depressions; 2) the common occurrence of caves and 3) the existence of large springs frequently located at the base of the carbonate sequence.

Geomorphological features of karst landscapes can be subdivided into exokarst (subaerial), epikarst (subcutaneous) and endokarst (subterranean) features. Another classification distinguishes between dissolutional/destructive forms (due to rock dissolution by water, e.g. dolines, caves, karren fields...) and depositional/constructive forms (due to carbonate precipitation, e.g. the concretions inside caves). Other classifications are based on the concavity of forms (concave/convex), or state of development (initial, mature etc.). General reviews and reference lists regarding karst geomorphology are available in Lewin & Woodward (2009); Ford & Williams (2007), Goldscheider & Andreo (2007) and De Waele *et al.* (2009).

The subterranean aspect of the karst is very important and speleologists explore and study it (Palmer, 2007) but hydrogeologists are also very interested in its characteristics because groundwater flows within these subterranean forms. Exokarst and epikarst features represent a transition zone (for both people and water recharge) via which subterranean features can be reached.

The geological setting of the Classical Karst, specifically its lithological and structural components, control the geomorphology - and therefore the landscape - of the area. As dissolution is the dominant karst process, it is quite obvious that the geomorphological features of a karst area therefore depend substantially upon surface and groundwater flows, and, at the same time, exercise an important influence on water circulation, both on the surface and below ground. The important relationship between karst hydrogeology and geomorphology is analysed in the book by Ford & Williams (2007), while in LaFleur (1998) a review of the geomorphic aspects of groundwater flow is presented.

The GIS analyses of lithological basemap of Figure 7.3, overlayed on a Digital elevation model, shows that lithology influences the behaviour of rocks to dissolution by meteoric water, the *Calcari di Aurisina* and the Opicina mb being the most permeable and thus the most favourable to the development of deep dolines and caves. All the carbonatic units, however, are prone to the development of karstic morphologies. In the Karst area more than 6,000 dolines have been identified, 400 of them being larger than 100 meters in diameter. The distribution of dolines is uneven and can reach more than 100 dolines/km^2 in the area of Monrupino. The depth of the dolines can reach 60–70 meters and at the base they may open into more complex cave systems. Other dissolution phenomena include *Karren* or limestone pavement crossed by fissures and small channel-like structures or, where the strata are almost horizontal and the limestones are isotropous, dissolution tubes as well.

As the Classical Karst is a mature karst system, subterranean karst phenomena are very common:

a review study of its geomorphogenetic evolution is provided in Gams (1998) while Mihevc (2007) reconstructs the evolution and estimates the age of the karst landscape. Subterranean karst may be broadly subdivided into two main categories: (1) caves having a main sub-horizontal development (caves *sensu stricto*); (2) caves having a main sub-vertical development (sinkholes).

It is not uncommon that a major cave is generated from the fall of the floor of a minor one and this usually changes the direction of the water flows greatly, leaving only relict structures (Andriani *et al.*, 2001; Gabrovšek & Stepišnik, 2011).

The main caves and sinkholes of the study area are represented in Figure 7.3, as they are of great importance for the groundwater movement, and can be grouped into the following types: (a) sinkholes are points of intense local recharge to the aquifer from rainfall waters, and they may be dipping points of rivers (*Škocjanske* cave, sinkholes of Materija dry valley) or access points to groundwater flows (Kačna cave, Trebiciano cave/Labodnica, Lazzaro Jerko cave); (b) sub-horizontal caves represent groundwater flow paths, such as the spectacular example of the hypogean Reka/Timavo river between *Škocjanske* cave and Trieste springs; (c) caves with springs are points of groundwater discharge from which originate streams and rivers such as the Timavo springs, Osp/Ospo spring and Rižana spring.

7.3 WATER RESOURCES
7.3.1 PECULIAR FEATURES OF KARST AQUIFERS

Karst aquifers are characterized by the following structures and behaviours (Figure 7.4) (1) in the unsaturated zone there are different types of recharge: widespread across the carbonate platform as well as concentrated at some swallow holes; (2) an epikarst zone is located near the surface zone and may be locally saturated; (3) in the saturated zone there are two main structures: the conduits (sub-horizontal caves) organized in a pattern similar to a surface stream network, where groundwater flows fast and turnover time is short; the storage areas (voids, fractures, matrix pores) where water moves slowly and turnover time is much longer.

Some important features distinguish karst aquifers from other aquifers, such as, for example, the evolution of the system at a human (not geologic) temporal scale, the strong spatial heterogeneity of the medium (i.e. conduits/matrix) and of the hydrodynamic parameters, the duality of recharge (diffuse or concentrated) and the rapid and strong reaction to hydrological events (Goldscheider *et al.*, 2007). Karst aquifers require specific investigation techniques, analysed in detail by Goldscheider & Drew (2007). Apart from the general methods applied in hydrogeology, speleological investigations (Jeannin *et al.*, 2007; Goldscheider *et al.*, 2008) and tracer techniques (Kâss, 1998; Bernishke *et al.*, 2007) have great importance.

Karst aquifers generally provide groundwater of good quality and quantity, but the exploitation and protection of karst water resources must take in account the following main issues: (1) as recharge takes place through enlarged fractures, the filtration, and so the self-purification, of recharge water is little (or null); (2) swallow holes represent potential direct access points to groundwater for pollutants; (3) the very high velocities of groundwater flow (from ten to several hundred meters per hour) can spread pollution over long distances in a short time and (4) changes in water quality may have implications for human health and for ecosystems, including cave communities and surface water ecosystems fed by karst springs.

Karst aquifers are highly vulnerable not only to human activities that result in contamination problems, but also to overexploitation and climate change (Bakalowicz, 2005). So the management and protection of karst aquifers must take into account their high vulnerability, the object of study of various scientific books and monographs (European Commission, 1995a, 1995b; Gunay & Johnson, 1997, Drew & Hötzl, 1999; Zwahlen, 2004).

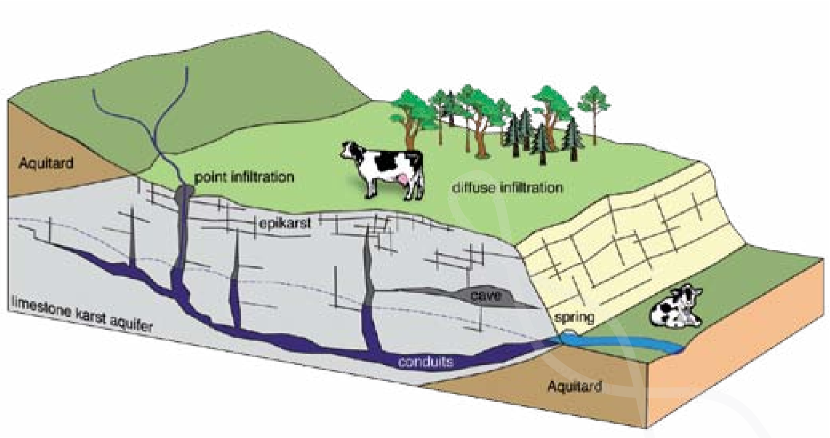

Figure 7.4: Schematic illustration of a heterogeneous karst aquifer system (modified from Goldscheider, 2012; with permission).

7.3.2 REGIONAL HYDROGEOLOGICAL SETTING

The Classical Karst is a wide karst system, with a surface area of about 900 km^2, mostly located in Slovenia (where most of the aquifer recharge takes place), with a small part located within Italy, where a strip, about 10 km wide, faces the sea and the major springs occur. As such it represents a perfect example of a "transboundary aquifer" and requires solid cooperation between the two countries for its management and protection, as discussed in Zini *et al.* (2010).

Referring to its aforementioned geological and geomorphological features, the aquifer develops within the carbonate rocks formed between the Cretaceous and Eocene (mainly within calcareous rocks as the dolomites are less soluble), while flysch formation always acts as an impermeable threshold with respect to the karst aquifer, even though it is a fractured medium that may, locally, bear an aquifer.

As Classical Karst is a mature karst system, within the Comeno unit a complex underground flow network has developed called the hypogean Reka/Timavo river (Cucchi *et al.*, 2001). Its surface pathway starts bearing the name Reka at the foot of M. Dletvo, close to the Croatia/Slovenia boundary and then it flows about 50 kilometers over impermeable flysch, starting its underground course at the Škocjanske caves (a natural monument on the list of UNESCO World Heritage sites). After an underground linear distance of about 33 km, it emerges at the Timavo springs in the Gulf of Trieste, together with some other smaller secondary springs, most of them below the sea level. Based on the data of the Environmental Agency of the Republic of Slovenia for the period 1961–1990, the average discharge of the Reka river is 8.3 m^3/s, with a ratio between low and high flows of 1 to 1,700 with a maximum measured discharge of 305 m^3/s, and a minimum of 0.2 m^3/s (Gabrovšek & Peric, 2006).

The underground pathway of Reka/Timavo River has been investigated since the 19th century, but the first proof of the hydrological connection between *Škocjanske* caves and the springs of Timavo dates back to 1908, when Timeus (1928) carried out the first tracer test. Then a long series of experiments and investigations took place, accurately described in literature (Cucchi *et al.*, 2000; Galli, 1999; Kranjc, 1997; Galli, 2012a; Galli, 2012b), where more information on geology, speleology, hydrogeology and history of exploration and research of the Classical Karst and its aquifer are available. At the moment five caves are known to play an active role in underground flow: the Kačna cave and Trebiciano cave/Labodnica, well known and deeply investigated, and three additional caves where exploration has pushed down to the depths of active Reka/Timavo flow more recently: the Lazzaro Jerko in Italy; the Jama 1 v Kanjaducah and the Brezno v Stršinkini dolini in Slovenia. The river has also been

reached through the Brezno 3G, which turned out to be another entrance of the Kačna cave. The depth of the unsaturated zone reaches down more than 300 m (Gabrovšek & Peric, 2006).

The springs of Timavo have an average discharge of about 35 m^3/s (a minimum of 10 m^3/s and a maximum of 150 m^3/s). In the springs area are located the uptake works for the Slovenian (Klariči station, about 0.25 m^3/s) and Trieste aqueducts (Sardos and Moschenizze Nord springs, also 0.25 m^3). The water quality is good and the size of the reserve is big, but the water resource is highly vulnerable, as highlighted by the monitoring surveys and tracing tests (Gabrovšek & Peric, 2006; Zini *et al.*, 2010).

The Timavo aquifer, in its northern sector, is also fed by the water belonging to the Isonzo/Soča River and the Vipacco/Vipava River, through riverbed losses along a section about a dozen kilometres long, providing a contribution to the karst system of 10 m^3/s at least.

The hypogean Reka/Timavo river flow path is shown in Figure 7.3, where we can observe that it represents the main aquifer system of the Classical Karst, but not the only one: in the southern sector of the Karst plateau, towards the Dinaric Karst, the geological setting is complicated by the subthrusting belt between Trieste (Italy) and Buzet (Croatia) and the flysch hydrogeologic threshold causes the outflow of three springs - from N to S, the Val Rosandra/Glinščica, the Ospo/Osp and the Rižana springs – and gives rise to three rivers.

7.4 PASTURELANDS AND GROUNDWATER PROTECTION: THE CASE STUDY OF RIŽANA SPRING CATCHMENT

Pasturelands and grazing activities represent potential source of contamination for groundwater. As they are located on the surface, the pollutants are driven to the saturated zone by recharge water, and generally are consistently broken down during their flow through the soil and the unsaturated zone, where dispersion and biodegradation processes occur. However, this is often not true for karst aquifers, for example in the case of concentrated point recharge such as swallow holes and dolines, where a rainfall event can rapidly carry the pollutants to groundwater, without any biodegradation.

For example concentrations of animals in small areas (exceeding the contaminant load) close to dolines, poljes on limestone can produce a severe localized contamination with faecal microorganisms and nutrients (Berryhill, 1989). Farm wastes and silage storage directly on the soil are another threat to groundwater and may produce large plumes of contaminated groundwater, as the storage time is much longer (Drew, 1996). If the land is mainly used for pasture and the karst vulnerability is high, karst springs may become contaminated by nitrates, as in the study case of Boyer & Pasquarell (1996), where nitrate concentrations were highest in cave streams draining a dairy farm and in a cave stream draining an area of pasture where cattle congregated for shade and water.

Aside from groundwater quality, impacts can affect also the quantity of karst groundwater, being generally related to important land use changes across wide areas, with consequences for soil erosion, landscape morphology and aquifer recharge and this should be taken into account when undertaking land use conversions from forest to grazing or agriculture and, vice versa, when extensive grazing areas are abandoned and natural reforestation occurs. Many other examples of threats to and impacts on groundwater are available in the interesting reviews involving agriculture and karst systems written by Coxon (1999; 2011).

In the framework of BioDiNet project a detailed study of the Rižana spring catchment has been developed, with the aim to evaluate grazing activities in relation to groundwater protection. The Rižana/Risano spring is the most important water resource for the public water supply of Koper and the entire Slovenian coastal area. The spring is located at the contact between the Upper Cretaceous and Paleocene limestone and Eocene flysch at an altitude of 70 m a.s.l. Its discharge ranges from

0.03 to 91 m^3/s, with a mean discharge for the period 1961–1990 of 4.3 m^3/s (Kolbezen & Pristov, 1998). The spring forms a river flowing from SW to NE direction (see Figure 7.3 and picture Figure 7.5a).

The spring catchment interests a transition zone between the Classical Karst (to the N-NW) and the Dinaric Karst (To the S-SE) and depending on the author it can be associated to either the former or the latter. Following Cucchi *et al.* (2001), we place it together with the Classical Karst in the regional hydrogeological setting (map of Figure 7.3), while other authors associate it to the Dinaric Karst (e.g. Janža, 2010). In any case it should be pointed out that no geological boundary occurs between the two regions.

The area is characterized by repeated alternations of flysch and calcareous units, because it pertains to the underthrusting belt of Kras and Čičarija, where a geomorphological step has formed at sites where limestone are sch rocks, the so called Karst edge (Placer, 2007; see picture of Figure 7.5e), a very peculiar and important feature related to landscape, ecosystems and biodiversity, that forms quite complex hydrogeological conditions.

The entire recharge area is protected by water protection zones for a total area of 244 km^2 (Janža, 2010), which have been delineated on the basis of the geological context and the results from various tracing experiments. Following the first tracer test carried out by G. Timeus in 1910, the tracing tests performed during the years 1985–1987 demonstrated the connections between the spring and the sinking streams of Matarsko podolje/Valsecca di Castelnuovo, on the southern side of the Brkini/Birchini flysch mountains and assessed groundwater velocities at around 700 m/day (Krivic *et al.*, 1987; 1989).

Water from the Rižana River has been used since the early 19th century, since the coastal water supply system was constructed in 1935. Today, during the summer season, the Rižana spring supplies water for more than 120,000 people (Kresič & Stevanović, 2009).

Janža & Prestor (2002) assessed the intrinsic vulnerability of the aquifer supplying the Rižana spring using the SINTACS method in which it is interesting to overlay and compare the vulnerability map with the various potential sources of pollution such as the railway line that passes over the first protection zone in the immediate vicinity of the spring, other local, regional, and main roads and trails passing through the second protection zone as well as agriculture and grazing activities involving about 20% of the catchment (Janža, 2005) and human activities related to the villages.

For the BioDiNet project, after the analysis and review of all the available scientific bibliography, a census of the main springs of the area was organized, aimed at deepening the understanding of the particular hydrogeological setting. The type of spring (in relation to its geology) and the type of human infrastructure for water uptake (well or spring, see examples in Figure 7.5b, c) have been verified in the field for each spring, while the coordinates were taken for GIS mapping and some basic parameters of waters (temperature & electrical conductivity - EC) were measured with a portable instrument (Figure 7.5b).

All the springs are located on the hydrogeological map on Figure 7.6. Apart from Rižana spring, a total of 18 water points were identified (7 wells and 11 springs; Figure 7.7a). Comparing the location with the geological map, as expected, most of the springs/wells are located close to a contact zone between permeable limestone with underlying flysch rocks acting as impermeable threshold (Figure 7.7b). This is the main type of natural occurrence of groundwater in the setting of the Karst edge.

The hydrogeological context, together with the landscape features, have strongly influenced the human settlements in this area since all the old villages sprang up where the groundwater resources were available for most of the year. Most of the old springs are still present (some of them not working today), even if the public water network supplies almost all the villages.

Ponds from which, in the past, cattle could drink represent another interesting water feature typical of this karst landscape. Some of these ponds

only collect rainwater while others are supplied by groundwater from a spring. An example is presented in Figure 7.5d. The main ponds in the area have been also mapped (Figure 7.6) and measured (Figure 7.7). Most of them have been restored in recent years, because they represent an important natural feature in terms of landscape, flora, fauna and biodiversity.

From the data collected in the field some interesting issues arise in as much as the springs located at the highest elevations are representative of local flow paths within the limestone rock strata and the majority are not perennial. There is no clear relation between temperature and water point elevation, due to the complexity of the karst system and microclimatic conditions, while there is a good linear correlation between the electrical conductivity (directly proportional to salinity) of the water and elevation with much lower elevations exhibiting much higher salinities because the underground flow paths are longer and the water-rock exchange is higher. The Rižana spring is an exception because it is located at the lowest elevation (70 m a.s.l.) but exhibits a very low electrical conductivity. Draining the entire karst system, it represents its base level and for this reason is perennial, even if it exhibits very low flow rates during summer. It shows the lowest salinity of all the springs measured and this suggests that it supplies very high quality water. The high value of this water resource should be widely publicized and taken into account in the planning and development of grazing activities for a sustainable land-use management. For example, from the map on Figure 7.6 it is evident that the pasturelands of the Padova University test sites, and so some farms, fall within the groundwater protection zones 1 and 2.

Sustainable management of grazing activities should place great importance on taking into account the hydrogeological features of a karst landscape and any water resource protection zones, because the potential impacts on groundwater quality depend on the location and the type of the activities, as seen in the karst aquifer scheme shown in Figure 7.4 in which the highest pasturelands (located inside the recharge area on limestone outcrops where swallow holes occur) determine a risk of groundwater pollution much more higher than the lowest pasturelands, located on flysch below the spring-line and outside from the recharge area.

Figure 7.5: Some pictures from the Rižana spring catchment: (a) the Rižana stream, downstream to the spring; (b) well in Hrastovlje; (c) old spring at the Zazid station; (d) pond at Rakitovec station; (e) panoramic view of the Karst Edge, seen from Gradišče. Photograph: Valentina Vincenzi

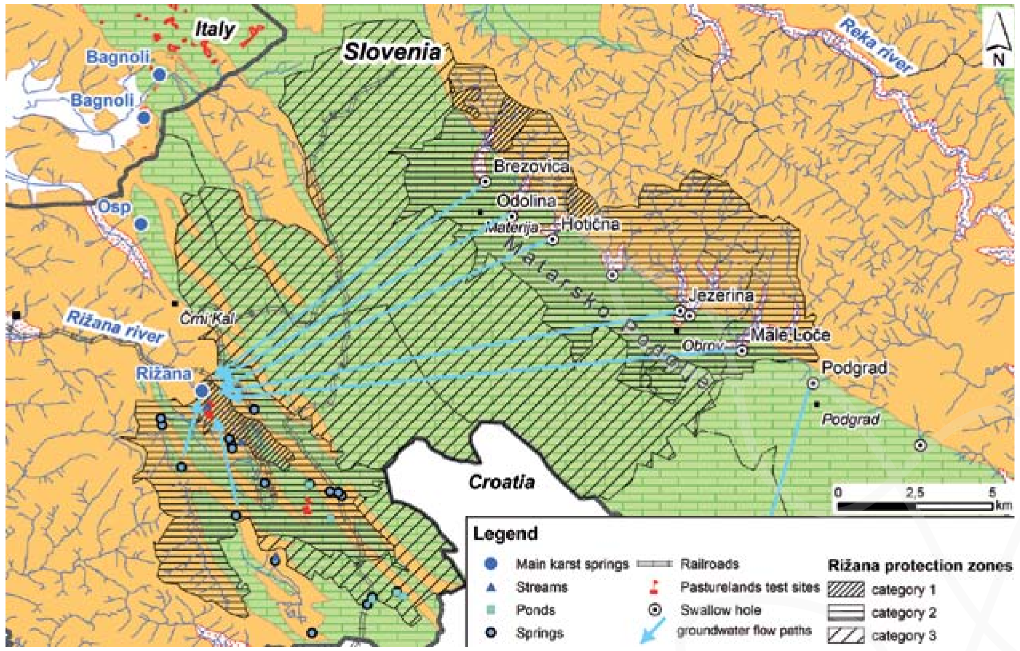

Figure 7.6: Hydrogeological map of the Rižana spring catchment. Lithological legend in Figure 7.3. Cartography: Geotema srl.

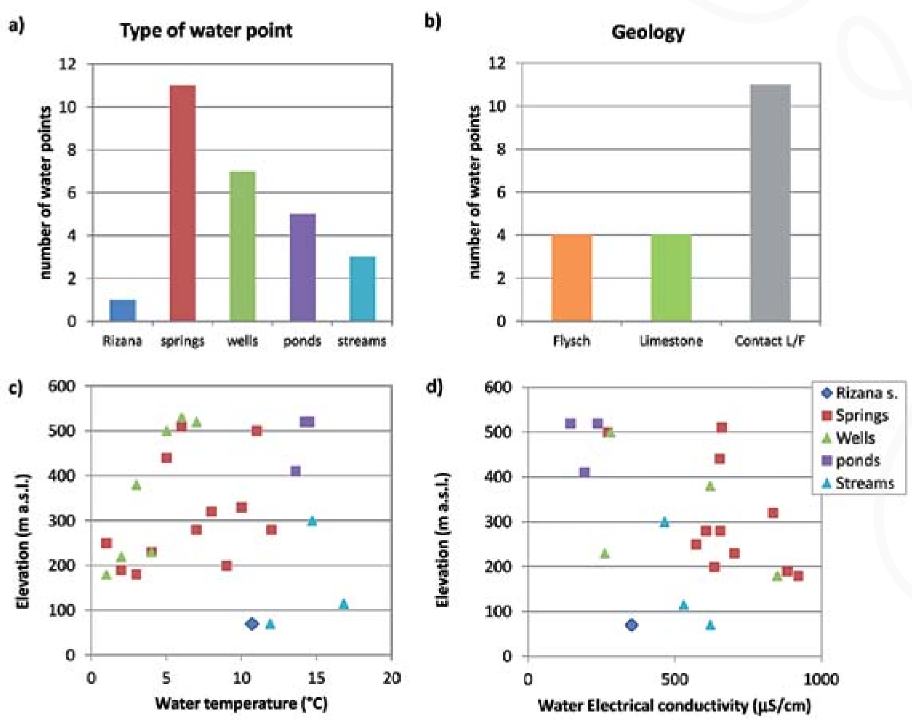

Figure 7.7: Hydrogeological data collected in the Rižana spring catchment.

7.5 KARST LANDSCAPES AND BIODIVERSITY

Karst terrains contain various valuable but vulnerable resources, such as water, soil and vegetation, and they provide a great variety of habitats to many species, both at the surface and underground, including many rare and endemic species. These vulnerable resources require protection and sustainable management. In Goldscheider (2012), following the early publications of Yuan (2001) and Bonacci *et al.* (2009), the complex interconnections between these natural resources are strongly evidenced, putting forward a holistic approach that includes sustainable soil cultivation, landscape and biodiversity preservation and groundwater protection.

Karst landscapes can be divided into two main subgroups: underground landscapes (characterized by the absence of light) and surface karst ecosystems (characterized by a great variety of landscapes and often isolated from their surroundings). The geological, geomorphological and hydrogeological features of a karst landscape have important implications on the ecosystems and their biodiversity. The first evidence for this is that karst aquifers offer a greater diversity of superficial and, particularly, subterranean habitats and larger voids than other subterranean environments and for this reason the karst troglo- and stygofauna exhibits greater biodiversity (Christman & Culver, 2001; Goldscheider, 2012). Apart from morphology, the differences in hydrology, hydrogeology and climate also give rise to a range of different environments, which provide the opportunity for the coexistence of different species, as analysed in Bonacci *et al.* (2009), where the ecohydrological functions of underground karst features (caves, pits, conduits, etc.) is discussed, together with the role of the epikarst and vadose zones, and the importance of the flood factor. Similarly, the research of Sket (1999) on groundwater biodiversity in Europe shows that the particularly high stygobiotic diversity in the wider Dinaric region (S Europe), and in Slovenia in particular, is a consequence of the large karst areas as well as of its particularly turbulent geological past, which enhanced the opportunities for multiple immigrations and speciations. Another example is available in the study of Moldovan *et al.* (2007), where the biodiversity and ecology of the fauna (mainly copepods) in percolating water from Slovenian and Romanian caves was studied. The results indicate that the epikarst (where this fauna originates) is a heterogenous habitat and that a relationship between faunal richness and the physical characteristics of the water was established, with the correlation between surface geomorphology and fauna in percolating water being statistically significant in the Postojna cave system.

In Slovenia, limestone is the dominant soluble rock occurring in the Dinaric Karst (e.g. Zupan Hajna in White & Culver, 2012). Flysch regions, however, occur near the Slovenian coast, where soluble layers can provide suitable places for formation of crevices and caves, so that cave-dwelling fauna can be found in both types of rocks (e.g. Polak *et al.*, 2012). As the biodiversity of the Dinaric caves is known to be the highest in the world and the endemic richness is very high (Sket in White & Culver, 2012), it is important to identify appropriate tools for biodiversity estimation not only generally, but also at the regional scale, also taking into account habitat types. It is also crucial to estimate the impact of different pollutants upon cave environments and biota.

Land-use and human activities represent another important factor affecting biodiversity while, on the other hand, being subject to control by geomorphological and hydrological features of landscape. In Karst these are mainly represented by agricultural and grazing activities and they lead to deforestation. Deforestation occurred in the past created a mosaic of various habitats, thus increasing the surface biodiversity. On the contrary, the agricultural/farming abandonment that has taken place in recent decades has allowed vegetational succession on pasturelands and progressive natural reforestation, bringing about a decrease in biodiversity.

Various examples are available in literature. Alexandrowicz & Alexandrowicz (2003) investigated the Kraków Upland, a karstland influenced through time, firstly by natural and later by anthropogenic factors. The rich network of nature/landscape parks and nature reserves/monuments as well as documentary sites preserve most of the valuable areas and objects. The geological history and geodiversity fit together with rich habitats and the biodiversity in a harmonious fashion. The research of Polak (2005) provides an overview of the current knowledge of the fauna of the land habitats around the Pivka lakes (Slovenia). As a result of the abandonment of land use by humans in the area around the Pivka lakes, rapid vegetational succession on pasturelands and a decrease in biodiversity have been observed. Therefore, in addition to legal protection, the area requires active management and conservation as well as the preservation and encouragement of the formerly extensive farming practices. In a different geological setting, the karstic turloughs of Ireland, similar conclusions are drawn by Sheehy Skeffington & Gormally (2007) with the single greatest threat to turlough biodiversity in the future perhaps being the cessation of traditional farming within their basins. Turloughs are an integral part of the Irish cultural landscape and so it is important to develop a strategy for turlough conservation that involves the land-owners and takes into account local socio-economic factors as well as the conservation of their biodiversity.

All the case studies here presented, together with the contributions contained in this monography, lead to the important conclusion that the sustainable management of the karst landscape must take into account all the factors affecting biodiversity (abiotic, biotic and anthropogenic) together with the related processes and their interconnections.

7.5.1 CAVE FAUNA AND THREATS FROM ABOVE

Organic pollutants are known to alter the oligotrophic status of ground ecosystems, and waste can have major impact on terrestrial as well as aquatic life in caves. As a result, community composition and diversity may change (e.g. see Wood *et al.*, 2008). Pollutants originating from human activities at the surface (e.g. intensive agriculture, waste waters, waste dumping sites, tourism, quarries, and domestic animal cadavers; e. g. see Polak *et al.*, 2012) can increase the food base and attract surface dwelling species, which may invade these eutrophied habitats and successfully compete with subterranean biota (Brown *et al.*, 1994). The most important impact upon aquatic cave fauna is wrought by pollution of underground waters draining a wider area (Polak *et al.*, 2012) and underground ecosystems are therefore under strong pressure (Sket, 1999).

Although the impacts of human activities upon cave fauna are little known and even less understood, it is quite clear these may cause damage to entire underground ecosystems (Bocelli *et al.*, 1992; Miko *et al.*, 2003). Studies of communities and potential bio-indicative taxa are therefore very important as a first step toward the successful protection of underground habitats.

During the present project, two activities were carried out in seven caves from south-western Slovenia.

1.) In two caves in the flysch region (Poljanska buža and Kubik) and five caves in limestone settings (Vipavska jama, Jama *Čebina*, Kraljičevka, Jama v Kavčičih and Pečina v Radotah, Figure 7.8) an inventory of the cave biota in each of the caves was carried out. As samples were collected qualitatively and were incomplete (i.e. we did not use traps), it was necessary to collect samples from each of the caves with the same effort (i.e. two sampling visits per cave, three researchers searching for animals in different microhabitats). The fieldwork was conducted in the period 2012–2013.

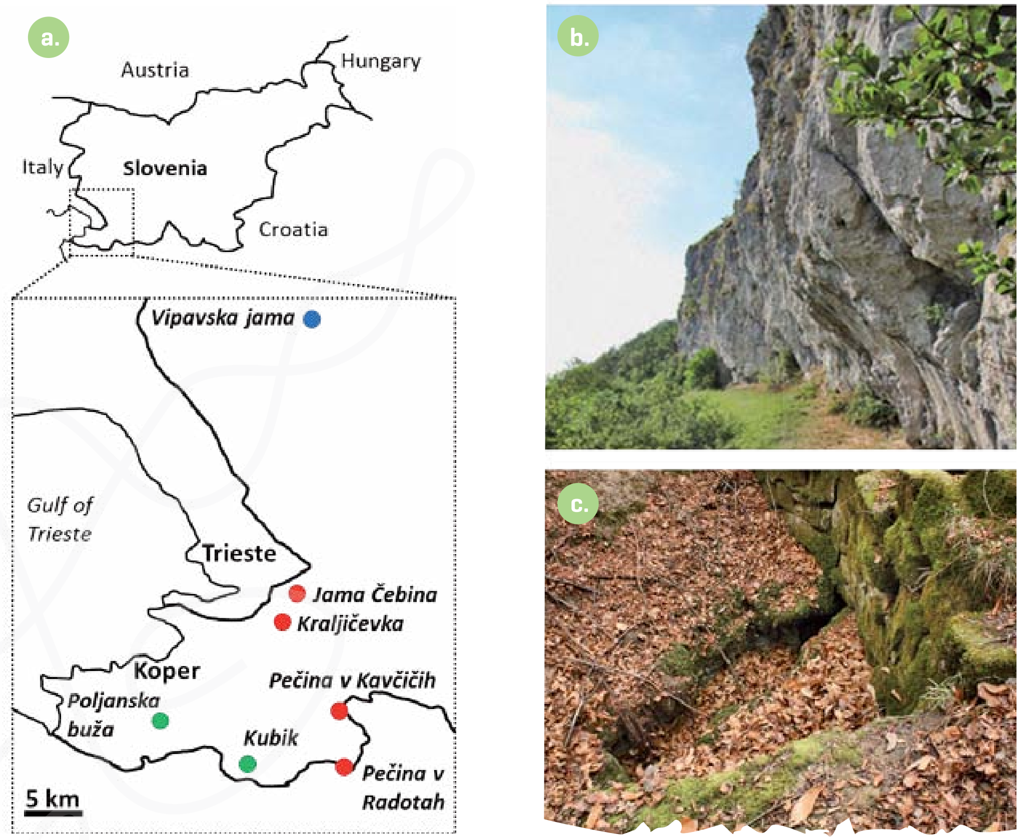

Figure 7.8: (a) Geographic position of seven caves used for study of community assemblages in SW Slovenia. In Vipavska jama, an estimation of population size in Troglocaris aggr. anophthalmus was also carried out; (b) Karst edge, limestone region; (c) flysch region, entrance to the Poljanska buža. Photography and cartography: Jure Jugovic.

Altogether, 90 taxa of animals were recorded, at least 20 % of them were recognised as troglobitic (i.e. animals restricted to cave environments; see Table 7.1). The correlation between number of taxa and length of a cave is positive (Pearson's correlation coefficient = 0.44). The difference in community assemblages (Figures 7.9 and 7.10) are best explained by two factors, (1) bedrock (i.e. flysch *vs.* limestone) and (2) water presence (i.e. Vipavska jama with permanent spring is most distant from the other caves set in limestone). Although a similarity index (Jaccard's index) yielded results that are well correlated to the geographic distances between the caves (compare Figures 7.9a and 7.9b, see also Figure 7.10), the bedrock seems to be most important factor influencing their diversity.

Table 7.1: List of seven caves from SW Slovenia with basic data and numbers of taxa and troglobionts.

Cave	Rock type	Water habitat [a]	Length/depth [m]	No. of taxa	No. of troglobionts (share) [b]
Poljanska buža	flysch	+	876/91	33	7 (21 %)
Kubik	flysch	+	292/10	35	6 (17 %)
Vipavska jama	limestone	++	1392/64	26	7 (27 %)
Jama Čebina	limestone	-	31/12	16	2 (13 %)
Kraljičevka	limestone	-	46/7	10	2 (20 %)
Pečina v Kavčičah	limestone	-	42/7	20	5 (25 %)
Pečina v Radotah	limestone	(+)	402/168	20	5 (25 %)
TOTAL				90	18 (20 %)

[a] -: terrestrial habitats only; +: small stream present, occasional spring; ++: water habitat prevailing, permanent spring (no sampling was done in water part of Pečina v Radotah)
[b] only taxa unambiguously recognised as troglobitic were counted

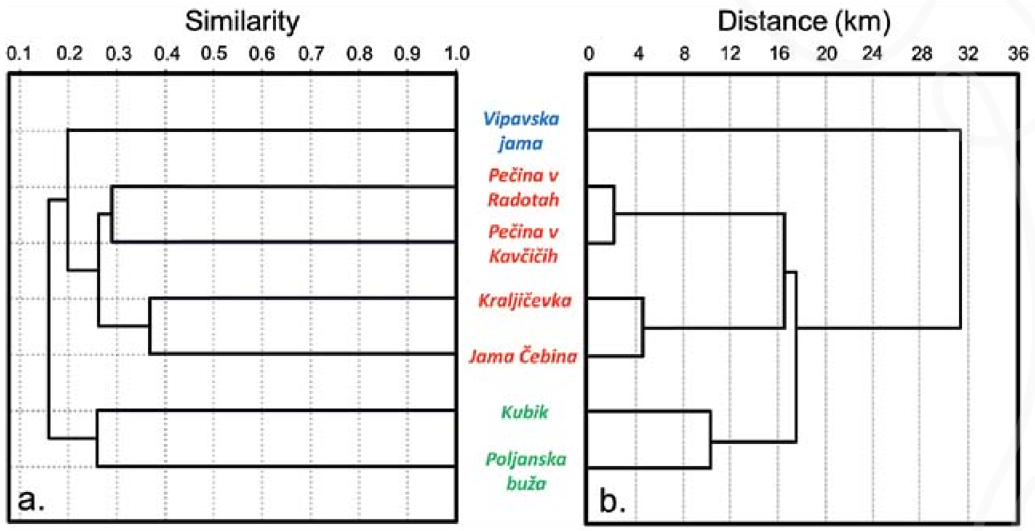

Figure 7.9: (a) Cluster analysis. UPGMA dendrogram (Jaccard's index, presence/absence data) of similarity in faunal assemblages among seven caves from SW Slovenia; (b) geographic distances among caves.

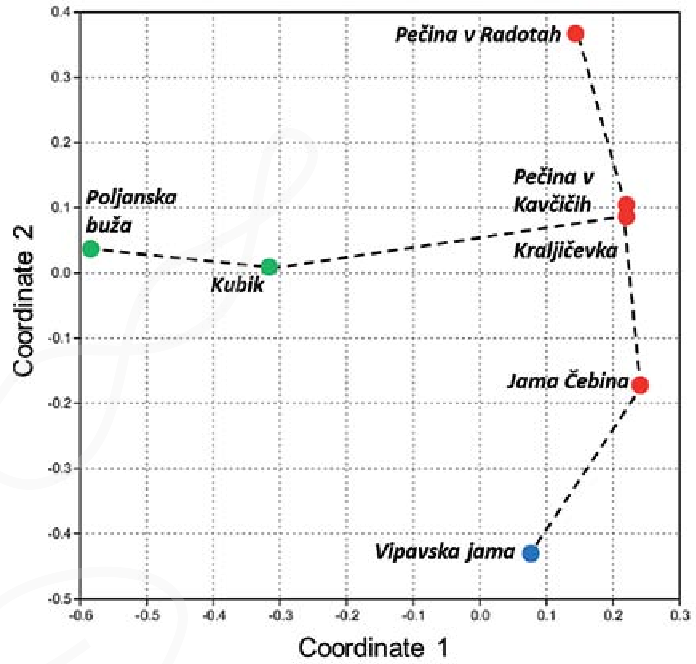

Figure 7.10: Projection of seven caves from SW Slovenia along first two axes in non-metric Multidimensional scaling analysis. Dashed line: Minimum spanning tree. Data on presence of 90 taxa is used.

2.) In Veliko jezero in Vipavska jama (Figure 7.8), the population structure of atyid (Decapoda: Atyidae) cave shrimp *Troglocaris anophthalmus sontica* (i.e. part of *Troglocaris* aggr. *anophthalmus*; Figure 7.11) was studied during the late summer (September 2012) and winter (February 2013) periods, applying mark-release recapture techniques (Jugovic *et al.*, in prep.). As the samplings were conducted during short periods (less than one week) in each of the two periods, the Schnabel method for closed populations was applied. Ovigerous females and juveniles were present in samples during both sampling periods. No differences between the two seasons could be derived from the population size estimates but their wide confidence intervals (CI) did not allow precise conclusions of the population size. Nevertheless, the small sampling area, the low number of recaptures (only 5 %) and fairly high estimates indicated that the population is in good shape.

Figure 7.11: Cave shrimps *Troglocaris* aggr. *anophthalmus* (Decapoda: Atyidae). Photograph: Jure Jugovic.

For the biodiversity assessments and its conservation, faunal lists and studies of species composition have fundamental aspects. Ecological analyses of the composition of cave biota from seven caves showed that different types of cave habitats host different faunal assemblages, regardless of the distance and rock type. As a result, the community assemblage of each cave is very unique (similarity rates being always below 40 %, Figure 7.9a), illustrating the high biodiversity of the Dinaric caves and supporting the need for the conservation of caves from regional to global scale. As caves are fragile ecosystems and constantly threatened by human activities on the surface (e.g. see Sket in White & Culver, 2012) and underground systems are also an important reservoir of (potable) water, these ecosystems should be handled with care.

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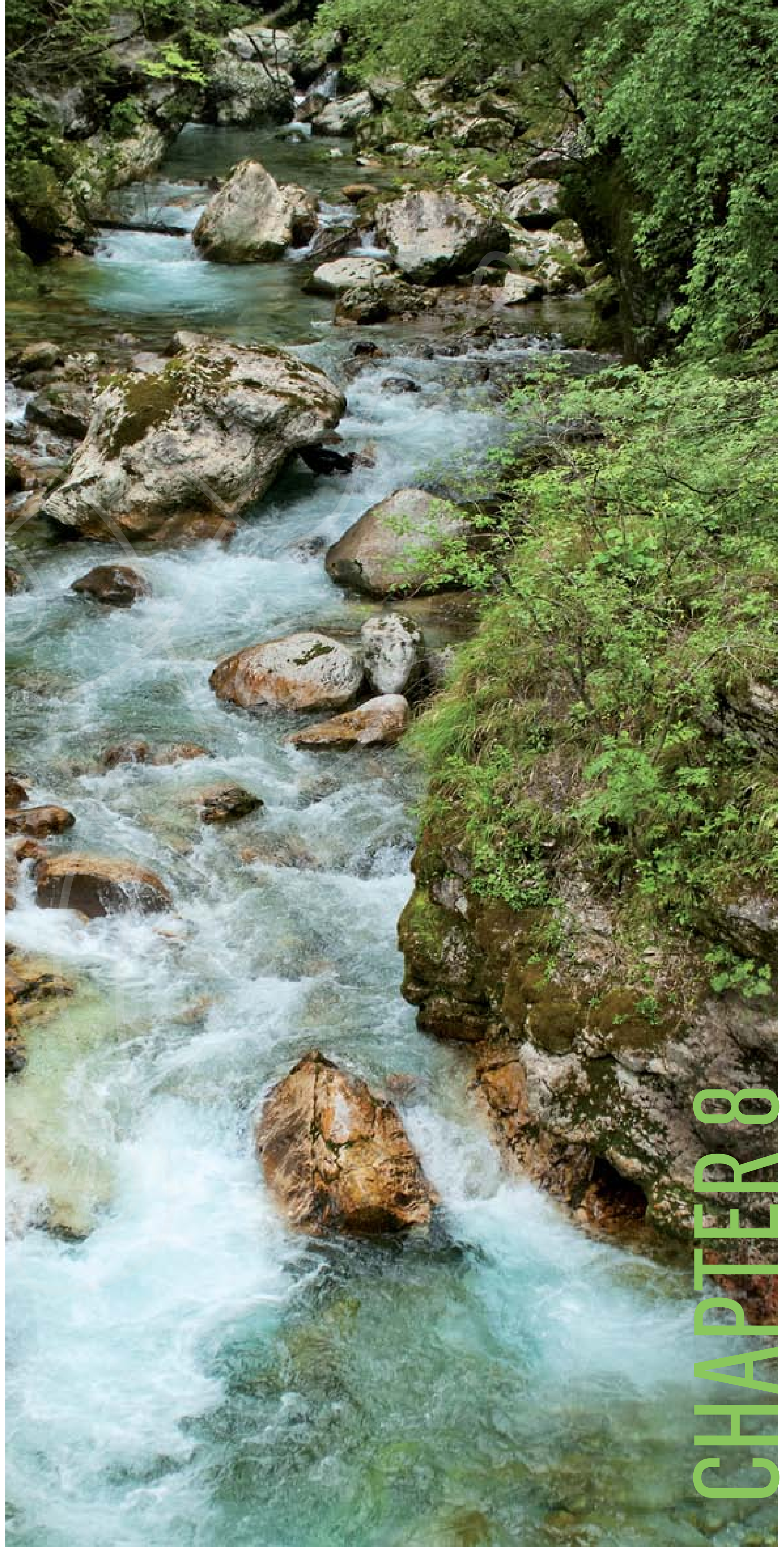

CHAPTER 8

REVIEW OF WATER POLLUTION AND PROTECTION IN KARST REGION

PREGLED ONESNAŽENOSTI IN VARSTVA VODA NA KRASU

REVISIONE DELL'INQUINAMENTO E PROTEZIONE DELLE ACQUE NELL' AREA CARSICA

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ABSTRACT

Due to the specific characteristic of the Karst region and surroundings,, groundwater is extremely vulnerable to pollution. The karst regions extend across 43 % of Slovenia, differing in its regional range, hydrological characteristics and types of karst. Therefore, the Slovenian karst regions are not equally sensitive to groundwater pollution. The aim of this contribution is to review the potential and actual pollution in the Classical Karst, located in a wide area along the border between Italy and Slovenia. In this respect, the contribution aims to review the protection policy that is crucial to preserve the qualitative properties of the Karst's groundwater for exploitation in the future. Based on the data in the literature, the most important source of pollution in the selected area comes from agriculture, sewage from households, polluted water from traffic, polluted industrial wastewaters and from illegal dumping. More studies are needed to identify the influence of the use of fertilizers in agriculture, the impact of the traffic and illegal dumping on the quality of Karst groundwater. So far the quality of Karst groundwater has been satisfactory. However, some signs of contamination have already been recorded in several Karst springs. Therefore, there is an important need to establish a better groundwater monitoring system in the region. In addition, many important Karst aquifers and springs are poorly protected. This may be due to confusing water protection policies or inadequate knowledge with regard to sustainable water management.

Key words: Karst region, pollution, environmental monitoring, environmental policy, groundwater

IZVLEČEK

Zaradi specifičnih značilnosti kraške pokrajine je podtalnica zelo občutljiva na onesnaževanje. Kraške pokrajine obsegajo okoli 43% Slovenije in se razlikujejo po svojih regionalnih in hidroloških značilnosti ter tipu krasa. Podtalnice slovenskih kraških pokrajin niso enako občutljive na onesnaževanje. Namen prispevka je pregledati potencialno in dejansko onesnaževanje na Krasu, ki se nahaja v širokem območju ob meji med Italijo in Slovenijo. Ravno tako smo v poglavju povzeli z zakonodajo predpisane postopke varovanja voda, ki so ključnega pomena za ohranitev kakovosti kraške podtalnice v prihodnosti. Po podatkih iz literature so v izbranem območju najbolj pomembni viri onesnaževanja kmetijstvo, kanalizacijska ureditev, onesnažena voda zaradi prometa, industrijska odpadna voda in divja odlagališča odpadkov. Da bi lahko opredelili vpliv uporabe gnojil v kmetijstvu, vpliv prometa in nezakonitega odlaganja odpadkov na tem področju, bi bilo potrebno narediti več študij. Doslej je bila kakovost kraške podtalnice zadovoljiva. Vendar pa so bili že zabeleženi znaki onesnaževanja v različnih kraških izvirih. Zato je pomembno, da se vzpostavi boljši sistem monitoringa podzemne vode. Poleg tega je veliko pomembnih kraških vodonosnikov in izvirov slabo zaščitenih. To je lahko posledica nejasne politike varstva voda ali neustreznega znanja na področju trajnostnega gospodarjenja z vodami.

Ključne besede: Kras, onesnaževanje, okoljski monitoring, okoljska politika, podtalnica

RIASSUNTO

Date le caratteristiche specifiche del Carso, le acque sotterranee risultano essere estremamente vulnerabili all'inquinamento. La regione carsica si estende sul 43% della superficie della Slovenia e differisce su gamma regionale, in caratteristiche idrologiche e tipi di carsismo. Come risultato le regioni carsiche slovene non sono ugualmente sensibili all'inquinamento delle acque sotterranee. Lo scopo di questo contributo è di rivedere l'inquinamento potenziale e reale sul Carso classico, situato nell'area del confine Italo-Sloveno. A questo proposito il contributo si propone nel rivedere le politiche di protezione che è fondamentale per la preservazione delle proprietà qualitative di acque sotterranee del Carso per l'uso futuro. In conformità a dati bibliografici le fonti principali d'inquinamento nella regione provengono dall'agricoltura, da inquinanti d'uso domestico, inquinanti dovuti al traffico, acque reflue industriali inquinate e da discariche abusive. Successivi studi sono necessari per identificare l'influenza dell'uso di fertilizzanti in agricoltura, l'impatto del traffico e lo scarico illegale di rifiuti sulla qualità delle acque sotterranee carsiche. Finora la qualità delle acque di falda carsica è stata soddisfacente. Tuttavia alcuni segni di contaminazione sono già stati registrati in diverse sorgenti carsiche. Pertanto vi è una necessità urgente per stabilire un sistema di monitoraggio delle acque sotterranee migliore. Inoltre molti corpi acquiferi carsici e sorgenti risultano essere scarsamente protetti. Ciò è dovuto soprattutto a politiche di tutela delle acque poco chiare e ad un' inadeguata conoscenza in materia di gestione sostenibile delle acque.

Parole chiave: area Carsica, inquinamento, monitoraggio ambientale, politiche ambientali, acque superficiali

8.1 INTRODUCTION

This chapter addresses the issues surrounding the pollution and protection of the Classical Karst, a wide area (40 km long and 15 km wide) bordered by Soča River, Vipava River, Pivka, the Adriatic sea the Gulf of Trieste and by the Dinaric Karst.

The area has a characteristic landscape that has been influenced by lithology, tectonics and weathering, effecting the subsurface and the surface during the Abian Age through to the Holocene epoch. The Karst plateau reaches the heights from 200 to 600 m above sea level (Krajnc *et al.*, 1999) and is composed primarily of the carbonate rock, mostly limestone and dolomite from the Cretaceous period and Paleocene epoch, flysch sediments and Pleistocene-to-Holocene deposits. The Karst plateau has more than 600 registered caves with average cave length of 83 m, but only few of them are longer than 500 m (Jamarska zveza Slovenije & Inštitut za raziskovanje krasa, 2003; Kovačič & Ravbar, 2005). Most of the caves are not easily accessible. Therefore, the information regarding the circulation of underground water is very limited (Kovačič & Ravbar, 2005). For an investigation of the Karst aquifers specific investigation methods are required such as tracer techniques and speleological investigation (Goldscheider *et al.*, 2008; Jeannin *et al.*, 2007). In addition to a high density of caves, the absence of a permanent surface flow and springs located at the base of the carbonate depositional sequence are typical for the Karst landscape. The aquifers of the studied area are fed by sinking rivers at its borders, precipitation water and by underground infiltration from the aquifer of the Vipava and Soča rivers (Krivic *et al.*, 1989; Habič, 1984). The Karst aquifers are not only specific because of the different types of recharged, but also due to their rapid reaction to hydrological events, characteristic hydrodynamic parameters and the spatial heterogeneity of the conduits (Goldscheider *et al.*, 2008).

Karst areas are more susceptible to environment problems compared to other areas, due to highly developed subterranean networks and their fragile ecosystems. Moreover, the Karst landscape is suitable for dumping of solid or liquid waste, because it disappears underground and "out of sight is out of mind" (Ford & Williams, 1989). The aquifer of the Karst plateau is an important source of drinking water in southwestern Slovenia. It should be pointed out that, due to specific characteristic of the Karst region, the groundwater is extremely vulnerable to pollution. Therefore, the aim of this chapter is to review the pollution and protection in Karst region with particular regard to groundwater.

8.2 THE POTENTIAL AND ACTUAL POLLUTION IN KARST REGION

In relation to groundwater pollution in the Karst region, an existing and potential source of contamination results from human activities that are taking place mostly on the land surface (Zwahlen, 2004). The most important sources of pollution come from agriculture and farming, sewage from households, polluted waters from traffic, polluted industrial wastewaters and from illegal dumping (Kovačič & Ravbar, 2005). These sources are discussed in the following subchapters.

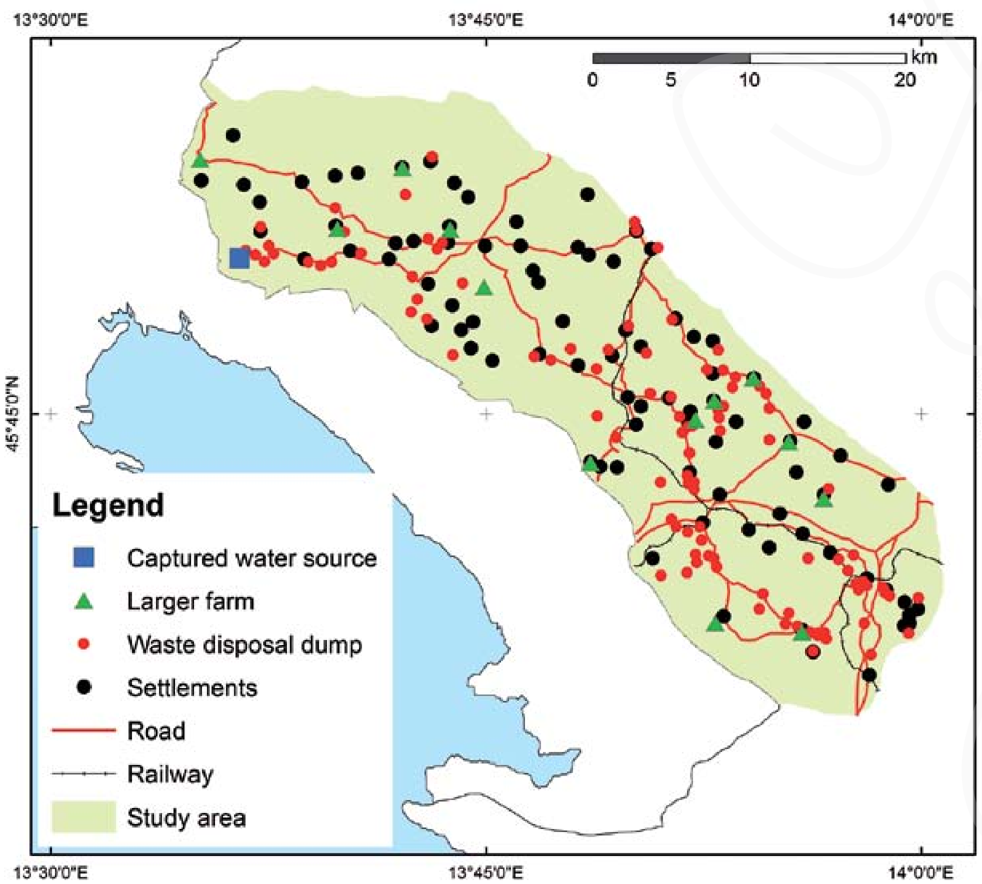

Figure 8.1: Simplified hazard map of the Karst plateau (modified from Kovačič & Ravbar, 2005). Cartography: Peter Glasnović.

8.2.1 AGRICULTURE AND FARMING

The Karst plateau has a population density of about 4 inhabitants per km^2. Therefore, the study area is relatively sparsely inhabited. In this area the agriculture was important in the past. Nowadays, it is reduced to small fields for personal use. However, viticulture is becoming more and more important. In order to achieve higher yields, winegrowers probably use a greater amount of phyto-pharmaceutical products (Rejec-Brancelj, 2001). Uncontrolled fertilizing of grapevines or other plants at the wrong time of the year can also represent a problem in this area. In addition, improperly stored dung heaps and stocks of stable manure or poorly-built cesspits may be the potential point sources for underground water pollution. Overall, uncontrolled use of fertilizers and pesticides in agriculture can lead to the pollution of drinking water supplies. Furthermore, the overuse of such substance has a negative impact on the subterranean fauna (Polak & Pipan, 2011).

8.2.2 SEWAGE FROM HOUSEHOLDS

In the study area are dozens of little villages with up to 200 inhabitants. Only five settlements have more than 500 inhabitants. The largest settlement has 4800 inhabitants. Although the situation has improved in recent years, the small villages have limited regulated sewage and sewage treatment. A source of pollution may come from wastewater cesspits with permeable floors (Kovačič & Ravbar, 2005).

8.2.3 POLLUTED WATERS FROM TRAFFIC

The study area is situated on the crossroads of important routes. According to DARS (2014) the average daily traffic crossing the plateau was about 63,000 vehicles in 2012. Firstly, the rainwater run-off from the road may be quite polluted. Not all the roads have regulated roadside channels and the roadway dirt may be washed directly underground. Consequently, the transport of dangerous material may represent an important threat to underground waters. In the case of spillages of hazardous substances, railway and road accidents, or channeled drainage from motorways, pollution is a point source pollution (Kogovšek, 2011).

In these area "natural" disasters such as spills of hazardous substances, excessive amounts of manure or overturned oil tankers have already taken place (Kranjc, 1999), although it has not yet happened that a pumping station has been shut down or the supply of water has been interrupted. In these cases the contaminants were diluted below detection limits or at least below the permissible limits. According to Kranjc (2010), this is not a long term solution. Opportunities and pollution threats are becoming more common and more likely, because of increasing population density, the growing number of industrial and other production and non-production plants, ever-increasing traffic and the construction of new roads, railways or pipelines. If this development is not followed by the development of water treatment plants, sewage systems, water-tight asphalt surfaces and various other actions to prevent pollution of the Karst, the contamination will increase and mere dilution will no longer be sufficient (Kranjc, 2010). Depending on the size of the catchment area from where the aquifer is powered, this should be applied not only in Karst, but also in the villages above the Ilirska Bistrica, for Košanska valley and its poultry production and processing industry, and for the river Vipava in which rain flushes contaminants from intensive agriculture. After the summer drought, the residues of fuels and oils and also toxic metals, especially cadmium and lead are washed from the roads to the sediments. Although the toxic metals are deposited in sediments, the water can moved them closer to the captured water source (Kranjc, 2010). Moreover, according to Kogovšek (2011), the impact of a specific type of surface pollution on Karst springs can only be shown by special research. Especially in the cases of pollution by water-soluble contaminants, which springs will be polluted and what speed of transfer can be expected can only be deduced on the basis of past research, in particular tracer tests and research regarding the transfer of these contaminants through carbonate rock (Kogovšek & Petrič, 2010). Contaminants are stored in various zones for considerable periods. Therefore, the type of pollution and its degradation products should be known in order to monitor them. Although some data exists about the runoff pollution from motorways in ordinary conditions, little is known about the nature of pollution cause by railway lines in this specific area (Kogovšek, 2011).

8.2.4 INDUSTRIAL POLLUTION

When wastewaters are subject to inadequate water treatment and then go directly into nature they can cause serious pollution of groundwater. The quality of wastewaters is assessed by laboratory tests that measure physical (temperature, solids), chemical (e.g. COD (chemical oxygen demand), BOD (biochemical oxygen demand), nitrogen, chlorine), and biological characteristics (e.g. proximate biological population, pathogenic microorganisms, intestinal pathogens). BOD and COD are typical tests that measure the chemical characteristics of wastewaters. While BOD reflects the biochemical reactions created by bacteria and nutrients, COD reflects reducing chemicals present in wastewaters. Thousands of organic compounds that can be found in wastewater cannot be determined separately but only by category. One of these categories includes AO_x (adsorbable organohalogens). The parameter of AO_x reflects many different compounds such as pesticides, plastics, solvents, lubricants and medicines. It is well known that AO_x like polychlorinated byphenyls (PCBs), hexachlorobenzene (HCB) or dieldrin are toxic, persistent and bioaccumulate in organisms. They can cause severe and varied effects on human health (Cherif *et al.*, 2006). For example the compounds like PCBs can cause acute and chronic damage to the skin, liver and lungs in animals and people. In addition, they cause metabolic disorders and disturbances to the action of the endocrine system, and are associated with loss of bodyweight and immuno-sensitivity. They are mutagenic and teratogenic, and are suspected to be carcinogenic. They can also effect fetal development (Safe, 1994). An example of contamination of the karst environment with such compounds is the Krupa River in SE Slovenia. This river is located near the factory, where tons of waste containing thousands of kilogrammes of pure PCBs had been dumped at various sites. Pollution problems in Krupa river are related to sinking streams that mix with the regional groundwater supply, thus affecting the quality of the groundwater reservoirs. However, it was found that the PCB load was much reduced after the ecological remedial work, as less and less contaminated sediment was being washed into the Krupa from underground (Polič *et al.*, 2000).

Industrial production in the Classical Karst region is not very common and it mainly takes place in the largest settlements. However, the wastewaters of a ham-curing plant registered excessive oil, grease, total phosphorus, chloride, AO_x, chemical oxygen demand (COD) and five-day biochemical oxygen demand (BOD_5). Moreover, the wastewaters of the stud farm and tourist centre exceeded permissible values of COD, BOD_5, total phosphorus, suspended solids, nitrites and total tenzides (Kovačič & Ravbar, 2005). In addition, the glue factory might potentially have a large effect on the pollution of groundwater, since they store tons of dangerous material (Kovačič & Ravbar, 2005).

Moreover, many caves are opened up during quarry work. This happens, because the upper layer is crisscrossed by open and sediment filled fractures, vertical shafts and the remains of former caves. The opening of a cave to the surface, destroys the natural environment in the cave, affects living conditions in the cave and has a negative impact on subterranean biodiversity, destroys various cave forms and sediments and information about geological and speleological history (Zupan Hajna, 2011). According to Zupan Hajna (2011) every cave that is opened up during the working of the quarry should be measured and mapped. In addition, its contents should be registered and its importance should be evaluated from the point of view of conservation, after which its protection or destruction should be incorporated into the development guidelines of the individual quarry (Zupan Hajna, 2011).

8.2.5 ILLEGAL DUMPS

Kovačič & Ravbar (2005) reported 59 illegal dumps in the study area and many of them are still in use. The waste materials found were mainly building and excavation material, agricultural wastes and furniture materials. Among them, however, dangerous materials containing potential groundwater pollutants were also found.

In addition, exceeded permissible levels of COD and BOD_5 were found in wastewaters from the legal landfill of waste material situated in the largest settlement in the area (Kovačič & Ravbar, 2005). However, relatively few identified and reported cases of water pollution exist due to landfills in the Karst. This could be due to the dumping of non-hazardous waste or the influence of dilution with water from other parts of the extensive catchment areas (Hötzl, 1999). Unidentified cases could be the consequence of the lack of suitably planned and longer-lasting monitoring of the quality of groundwater and the corresponding interpretation of such monitoring (Kogovšek & Petrič, 2011). In the study of Kogovšek & Petrič (2011), where tracer was injected on the surface and travelled through the various zones, relatively high flow velocities were identified. They concluded that there is a very high vulnerability and a serious danger of pollution with harmful substances from landfill. However, they noted the concentration of tracer increased after rainfall events. This was noted even one year after injection. In spite of the fact that part of the tracer passes very rapidly along drainage channels, the remainder can remain in the zone for a decade or more (Kogovšek, 2010). In the similar fashion to contaminants, the some of the remainder of the tracer is driven out of the system by each subsequent intense rainfall event. Tracing using artificial tracers enables the selection of the most suitable monitoring points and the elaboration of an effective plan of sampling frequency and distribution in the Karst areas and therefore it is an extremely suitable research method for planning a monitoring program in this study area. As was already mention, Karst springs have large catchment areas within which it is possible that the impacts of various polluters interweave. Kogovšek & Petrič (2011) emphasized that the interpretation of the results of monitoring is not simple and must be based on good knowledge of the characteristics of water flow and the transfer of contaminants through the Karst aquifer towards Karst springs (Kogovšek & Petrič, 2011).

Direct human pollution, by deposition of solid waste in caves could also be a problem in this area. Despite the fact that at least 100 caves in Slovenia have already been cleaned up by cavers, it has been estimated that 90 % of caves in the country are still polluted by solid waste (Prelovšek, 2011). In Slovenia, regular monitoring is only performed at Postojnska jama (cave) and only occasional monitoring takes place in other caves. Therefore, it will be necessary to establish a permanent monitoring of all caves, especially those that are subject to greater pressures (Prelovšek, 2011). The regular monitoring of these specific parameters in these kinds of caves could provide a deeper understanding of natural processes and allow the quantitative evaluation of human impacts (Hamilton-Smith, 2002; Prelovšek, 2011)

8.3 PROTECTION POLICY IN THE KARST REGION

Because karst aquifers are highly vulnerable to contamination, karst water resources require a proper groundwater protection policy and management. In Slovenia many of them are still improperly protected. The reasons for insufficient groundwater protection may be the lack of knowledge on sustainable management of water resources, conflicting interests of different land users and ineffective control of the violations of the provisions (Ravbar, 2007). Although the quality of Karst groundwater is still relatively high (Kraški vodovod Sežana, 2013), some individual cases show the management weaknesses affecting the water resources. The quality of Karst groundwater is discussed in the subchapter 8.5.

Basic legislative provisions concerning ground-water protection policy in Slovenia are part of the Water Act (Uradni list RS 67/02). Based on this

Act, the competent institutions for establishing the protection zones and for ensuring the implementation of the provisions are national institutions. The water protection areas require certain restrictions such as development constraints of urbanization, appropriate public utility arrangements, development of clean industry and moderate use of fertilizers and other means in agriculture. According to *"Rules on criteria for the designation of a water protection zone"* (Uradni list RS 64/2004), the area for the individual protection zone is determined based on the transfer time of a contaminant from the point of injection to the target. However, it should be pointed out that, according to this definition the particularities of water regime in Karst areas (e.g. rapid infiltration, poor filtration, high-speed streaming, links over long distances, streams) are not sufficiently taken into account (Ravbar, 2007).

The current state of protection is also reflected in the cases of individual Karst springs. Although many of them have defined protection zones and within the zone the prescribed permitted, restricted and prohibited activities, in the majority of cases, the adequate legal protection ordinances were not accepted. According to previous legislation in force, the legal protection ordinance was accepted at the municipal level. Therefore, ordinances have not been uniformly accepted and adequate protection of many important Karst aquifers was and still is hindered by administrative borders between communities. The hydrogeological backgrounds of the Malenščica and Vipava springs are only partially protected (Ravbar, 2007; Kovačič & Ravbar, 2005). In this study area the water supply founded on the effective karst groundwater pumping near Klariči is protected by the municipal ordinance. Hubelj and Vipava are two springs supplied from the extensive karst aquifer. Kranjc (1997) used the combined tracer experiments and found that their hinterland mutually intertwined. Therefore, the whole area should be protected. Under the existing legislation, the protection should be directed at the national level and this has not yet been adopted. Overall, adequate legal protection ordinances are only adopted for smaller springs that are easier to protect (Ravbar, 2007).

8.4 THE QUALITY OF KARST GROUNDWATER

The Karst aquifer is very important for the inhabitants of the Karst and the wider surrounding area. This aquifer supplies the entire Karst area and partly the population of Trieste and settlements along the Gulf of Trieste. Moreover, when required, it also helps to supply the population of the Slovenian coast.

More than a billion cubic meters of water a year comes to the surface from the Karst aquifer. This amount of drinking water is more than sufficient for both the local and regional supply (Kranjc, 2010). However, as already mentioned previously, the Karst aquifers are particularly vulnerable to contamination and therefore, the quality of water is very important. Unlike surface waters and groundwaters in the alluvial layers, the Karst underground water is not disinfected by the sun's ultraviolet rays, nor aerated in rapids and waterfalls and not filtered through the tiny pores between the sand grains. Therefore, the quality of water in the springs is the same as it is as it sinks into the ground. Moreover, in situations in which the Karst waters meet another inflow such as wastewaters or even toxic fluids, the Karst water in the spring could be significantly inferior to those at the moment of infiltration (Kranjc, 1999, 2000).

In Slovenia karst ground water supply almost half of population. In order to provide safe drinking water it is necessary to determine microbiological, chemical and indicator parameters (Uradni list RS 19/2004, 35/2004, 26/2006, 92/2006, 25/2006). Microbiological parameters reflect the extent and degree of contamination of drinking water by microorganisms. Regular microbiological testing of drinking water include the determination of the number of microorganisms: *Escherichia coli*, enterococci, total coliforms and total plate count at 22 °C and 37 °C. Within the periodic microbiological testing of drinking water are included regular microbiological tests and the determination of *Clostridium perfringens* (spores). Physical indicator parameters such as colour, conductivity, etc. have a warning function. Different individual chemical parameters (e.g. acrylamide, arsenic, pesticides, selenium copper, nickel, nitrites etc.) determined in drinking water samples show the extent and the degree of the contamination of drinking water with chemicals that may pose a risk to human health. Basic regular physical-chemical testing of drinking water includes the following parameters: temperature, free chlorine, colour, visible dirt, odour, taste, turbidity, pH, conductivity, total organic carbon (TOC), ammonium, nitrite. Extended chemical analyses also include the determination of other parameters such as trihalomethanes, chlorides, iron, total hardness and mineral oils. Within the scope of periodic examinations parameters such as metals and non-metals, pesticides and metabolites or trihalomethanes and hydrocarbons are included as well. Despite the large number of chemicals present in the environment only some of them are included in the norms in order to assess the safety of drinking water. The limit values of indicator parameters are not usually determined based on imminent threat to health, but provide information about the soundness and changes within the entire system. Therefore, when the chemical or biological parameter exceeds the limit vale, the cause and appropriateness of drinking water should be investigated.

Tables 8.1 and 8.2 show the data about the microbiological, chemical and physical parameters of analyzed water samples in 2013, 2012 and 2011 from the Karst water supply (Kraški vodovod Sežana, 2011, 2012, 2013). Out of 559 analyzed samples tested on the microbiological parameters, 15 were inappropriate. The investigated cause of the inadequacy of the samples was in most of the cases due to inappropriate maintenance of the home networks or problems at the sampling site (Table 8.1). In these cases the recommendations for maintenance were given. The percentage of the inappropriate sample decreased over the three years. The percentage of the samples tested found to be inappropriate microbiologically amounted to 3.4 % in 2011, 3 % in 2012 and 1.7 % in 2013. This might be due to implementation of the recommendations for maintenance.

Table 8.1: Results of the Karst water supply internal control and state monitoring of drinking water in the selected area for the year 2013, 2012 and 2011: microbiological parameters (Kraški vodovod Sežana, 2011, 2012, 2013).

Water supply	Year	Microbiological parameters		
		Analyzed samples	Inappropriate	Cause of the inadequacy
Brestovica	2013	105	1	TPC37
	2012	102	2	TCC -home network, made recommendations for maintenance
	2011	97	2	TCC -home network, made recommendations for maintenance
Nanos	2013	17	0	
	2012	22	1	*Cryptosporidium spp.*
	2011	17	0	
Barka (local)	2013	19	0	
	2012	34	0	
	2011	8	0	
Branica (local)	2013	9	0	
	2012	27	1	TCC
	2011	8	2	TPC22, TPC37
Štjak (local)	2013	8	1	TCC- changing the sampling sites
	2012	23	1	TCC
	2011	11	1	*Escherichia coli*, TCC, enterococci - internal network-recommendation given
Vrabče (local)	2013	18	1	TPC22, TPC37, CP
	2012	27	2	TPC22, TPC37, CP (sampling the water from tha transpor tanker)
	2011	7	0	

* TPC22: total plate count at 22 °C; TPC37: total plate count at 37 °C; TCG: total coliform count; CP: *Clostridium perfringens*

Table 8.2: Results of the Karst water supply internal control and state monitoring of drinking water in the selected area for the year 2013, 2012 and 2011: physical and chemical parameters (Kraški vodovod Sežana, 2011, 2012, 2013).

Water supply	Year	Physical and chemical parameters		
		Analyzed samples	Inappropriate	Cause of the inadequacy
Brestovica	2013	55	1	turbidity
	2012	51	1	iron - house network, made a recommendation for the flushing
	2011	56	1	turbidity - pipe was not in function for 4 months
Nanos	2013	12	0	
	2012	13	1	
	2011	13	1	turbidity -impairment at the inlet to the pumping station
Barka (local)	2013	5	0	
	2012	4	1	turbidity
	2011	5	1	turbidity
Branica (local)	2013	5	1	
	2012	2	0	
	2011	4	1	turbidity - heavy rainfall, an emergency action: boiling
Štjak (local)	2013	4	0	
	2012	2	0	
	2011	6	1	turbidity
Vrabče (local)	2013	7	1	turbidity
	2012	5	0	
	2011	4	0	

A lower number of samples were analyzed for the physical and chemical parameters (Table 8.2) compared to samples analyzed for microbiological parameters (Table 8.1). Out of 253 samples 11 samples exceed the values limits. In almost all of the cases the physical parameter reflecting turbidity was inappropriate. The exception was the exceeding of the value for iron in one sample. This was due to inappropriate maintenance of the house network. Therefore, severe chemical contamination has not been encountered so far. As for microbiological parameters, the percentage of inappropriate samples tested on the physical and chemical parameters also decreased over the past three years. The percentage of the inappropriate samples amounted to 5.6 % in 2011, 3.8 % in 2012 and 3.4 % in 2013.

CONCLUSION

Karst areas are more susceptible to environment problems than other areas. Overall, more studies are needed to identify the influence of the use of fertilizers in agriculture, the impact of the traffic and illegal dumps on the quality of Karst groundwaters. Although, the quality of Karst groundwater has been satisfactory, some signs of contamination have already been recorded. According to this review, there is an important need to establish a better groundwater monitoring system in Classical Karst region. Moreover, many important Karst aquifers and springs are improperly protected. There is an important need to better understand the susceptible Karst landscape and try to preserve it. The Classical Karst region is an important part of our natural and cultural heritage.

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PART 2
CONSERVATION AND MANAGEMENT

VAROVANJE IN UPRAVLJANJE

PROTEZIONE E GESTIONE

(EDITOR ALBERTO PALLAVICINI)

CHAPTER 9

THE CONSERVATION OF *APIS MELLIFERA* (LINNAEUS, 1758) IN THE KARST AND ISTRIA

VARSTVO MEDONOSNE ČEBELE *APIS MELLIFERA* (LINNAEUS, 1758) NA KRASU IN V ISTRI

CONSERVAZIONE DI *APIS MELLIFERA* (LINNAEUS, 1758) NELL'AREA CARSICO-ISTRIANA

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ABSTRACT

Genetic analyses conducted on bee samples from Istrian Karst have revealed the presence of a high degree of genetic reshuffling of local populations due to repeated imports of non-native bees for commercial purposes. Nevertheless, genetic traces of two presumably native ecotypes are still present. The first is a hybrid between the yellow Italian bee and the grey Carniolan bee and was found mainly in the area of the Karst above Trieste, the natural boundary between the two subspecies. The second, more abundant in Istria, could be the Istrian-Dalmatian ecotype, which is described in the literature as a coastal form of the Carniolan bee adapted to the warmer drier climate of the coastal Karst. These two ecotypes are morphologically very similar, but distinct from a genetic perspective. The identification and characterization of these local strains is a first step to implementing targeted programmes for the conservation and the restoration of their rearing. It then becomes of primary importance to create regulations that prohibit the introduction of non-native bees in the area to curb the risk of extinction of local varieties.

Key words: honeybees, ecotype, karst, conservation genetics

IZVLEČEK

Genetske analize vzorcev čebel s področja Istre razkrivajo visoko stopnjo hibridizacije lokalnih populacij z neavtohtonimi čebelami, ki jih neprestano vnašajo zaradi komercialnih namenov. Še vedno pa sta na območju prisotna dva avtohtona ekotipa. Prvi je križanec med italijansko čebelo in sivo kranjsko čebelo in je bil najden predvsem na območju Krasa v zaledju Trsta, kjer tudi poteka meja med obema podvrstama. Drugi ekotip, bolj pogost v Istri, je bil prepoznan kot istrsko-dalmatinski ekotip, ki ga v literaturi navajajo kot priobalno obliko kranjske čebele, prilagojeno na toplejše in sušnejše podnebje v priobalnem delu Krasa. Oba ekotipa sta morfološko zelo podobna, vendar iz genetskega stališča jasno ločena. Identifikacija in karakterizacija lokalnih oblik je prvi korak k implementaciji ciljnih programov za varstvo in oživitev njihove vzreje. Zato je zelo pomembno oblikovati pravila, ki prepovedujejo vnos tujerodnih čebel, ki lahko ogrozijo obstoj avtohtonih oblik.

Ključne besede: medonosne čebele, ekotip, kras, varstvena genetika

RIASSUNTO

Le analisi genetiche condotte su un campione di api nell'area carsico-istriana hanno rivelato la presenza di un elevato grado di rimescolamento genetico delle popolazioni locali dovuto alle ripetute importazioni di api alloctone per scopi commerciali. Ciò nonostante sono ancora presenti tracce genetiche di due presunti ecotipi autoctoni. Il primo è un ibrido tra l'ape gialla italiana e l'ape grigia slovena ed è stato riscontrato principalmente nel Carso triestino, zona di confine naturale tra le due sottospecie; il secondo maggiormente presente in Istria potrebbe rappresentare l'ecotipo dalmatico-istriano, descritto dalla letteratura come forma costiera dell'ape carnica adattata al clima più caldo e secco del territorio carsico-costiero. Questi due ecotipi sono morfologicamente molto simili, ma ben distinti dal punto di vista genetico. L'individuazione e caratterizzazione di questi ceppi locali è un primo passo per poter attuare programmi mirati di conservazione e di ripristino del loro allevamento. Diviene quindi di primaria importanza creare normative che vietino l'introduzione di api alloctone nel territorio per frenare così il rischio di estinzione delle varietà locali.

Parole chiave: api, ecotipo, Carso, genetica della conservazione

9.1 INTRODUCTION

The breeding of bees in the twentieth century and the intensive introduction of subspecies considered "superior" in commercial terms have led to a "genetic globalization" of the honeybee and the consequent risk of extinction of local ecotypes in many parts of Europe (Williams *et al.*, 1991). The protection and conservation of native bees should be an urgent priority at the global level because they represent important reservoirs of local adaptation (ecotypes) and are essential for the functioning of ecosystems. Losing part of their biodiversity means interfering with the natural adaptive and evolutionary processes but also jeopardizing the conservation of many plant species that depend on bees for their reproductive success.

In recent years, populations of wild bees in Europe have been strongly influenced by human activities (Barbattini & Greatti, 1995). They are naturally subject to a continuous gene flow due to their peculiar reproductive biology that does not allow complete control over breeding. The area of origin of the drones, in fact, varies from a few tens to several hundreds of kilometres.

It is also assumed that factors such as the parasite *Varroa destructor*, *Varroa*-associated viruses, bacteria, fungi, environmental factors such as drought and the use of neurotoxic neonicotinoid pesticides are involved in the progressive world decline of bees. Their genetic variability, therefore, is further threatened by bottlenecks due to these pathologies.

In Europe, the lack of a legislative policy to regulate beekeeping has allowed the release of subspecies considered superior in terms of productivity and profitability, jeopardizing the survival of local ecotypes that exhibit the ideal characteristics for the ecological environment in which they evolved.

Conservation genetics, using the tools of molecular biology, develops scientific knowledge that supports decisions for the management of population at risk, species or habitats. Through the genetic characterization of a number of individuals of a species of interest, a lot of information that is difficult or impossible to obtain with traditional methods of ecology and field study can be acquired (Frankham *et al.*, 2007). For example, after analysing genetic data with appropriate statistical techniques, it is possible to resolve taxonomic ambiguities, identify population structures, assess risks due to excessive inbreeding between individuals, the presence of hybridization events, or even to select individuals more suitable for reintroduction or expose cases of illegal detention or release. In order to lay down appropriate conservation strategies, an analysis of the genetic variability of a population can be carried out using molecular biology techniques, able to detect the differences (mutations) of the particular regions of DNA in different individuals of the same species. These techniques are based on the identification of specific DNA regions, known as molecular markers.

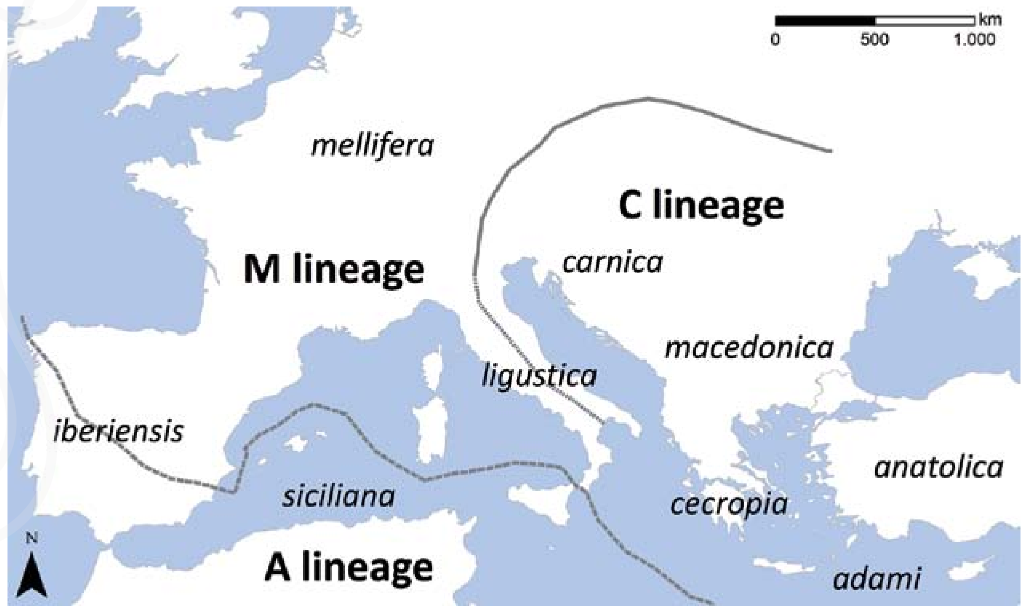

Figure 9.1: Natural distribution of the evolutionary lines and subspecies of *Apis mellifera* in Europe.

9.2 THE PRINCIPAL EVOLUTIONARY LINEAGES OF *APIS MELLIFERA*

Honeybees belong to the species *Apis mellifera* Linnaeus 1758. Bee populations show considerable morphometric and behavioural differences, the result of isolation and adaptation to specific habitats. Many of these biologically distinct populations are recognized as subspecies and grouped on the basis of morphometric studies (Ruttner, 1988), later confirmed by genetic studies (Garnery *et al.*, 1992), into four major lineages (Figure 9.1): Lineage O, in the Middle-East (outside the map); Lineage A, in Africa and the Mediterranean islands; Lineage M, in Western Europe; Lineage C, in Eastern Europe; the subspecies predominant in Europe belong to the lineages M and C.

The main bee subspecies in Europe are: *Apis mellifera mellifera*, *Apis mellifera ligustica* and *Apis mellifera carnica*.

The subspecies *A. m. mellifera* (lineage M), going by the name of the German bee or the "black bee" for its characteristic dark colour, is widespread mainly in the north-west and is adapted to cold climates. It is not very productive and is prone to robbing but it is a big producer of *propolis* (Figure 9.2a).

A. m. ligustica (lineage C), or "yellow Italian bee", is the most popular among honeybees because it has all the qualities making it favourable for rearing, as it is docile, productive, prolific and not overly prone to swarming and robbing. It prefers a Mediterranean climate (Figure 9.2b).

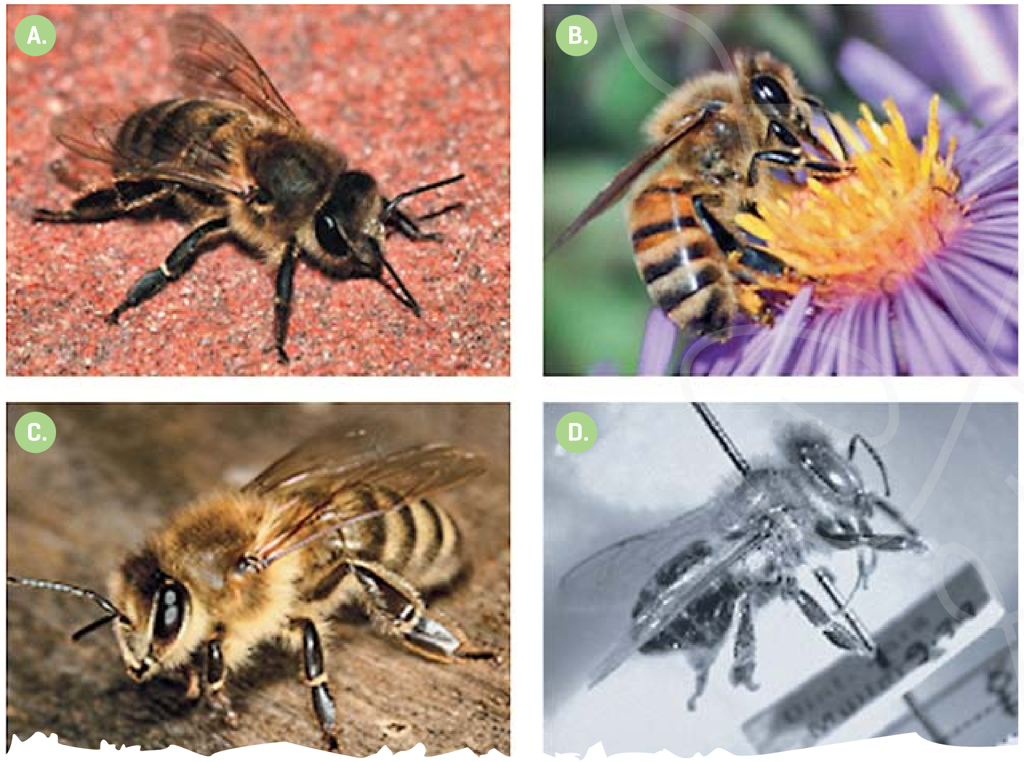

Figure 9.2: Main European subspecies of *Apis mellifera:* A. *Apis mellifera mellifera*; B. *Apis mellifera* ligustica; C. *Apis mellifera carnica;* D. specimen belonging to presumed Istrian-Dalmatian ecotype.

A. m. carnica (lineage C) originates from central Slovenia (Carniola) and it is widespread in Austria, Hungary, Romania and the Balkans. It is brownish-grey in colour and resistant to the rigours of winter and bad weather. The subspecies is adapted to a continental climate with long, cold winters, is docile but is prone to swarming (Figure 9.2c).

The Istrian-Dalmatian (or "Pannonian-Mediter-ran ean") bee has been described since the end of the nineteenth century as a bee with unusual characteristics (Figure 9.2d), differing from the yellow Italian bee but rather like the Carnic subspecies, grey-haired but smaller in size. Its range includes the Dalmatian coast and the island of Cres, up through Istria and the Slovene and Trieste Karst. It is not clear whether it is a subspecies or, rather, a coastal form of *Apis mellifera carnica* adapted to the drier, warmer climate of the coastal karst strip. It is probably an ecotype that, in the absence of barriers, maintained a certain genetic exchange with more internal Carniolan populations, putting a break on a true subspecific separation.

9.3 THE GENETICS OF BEE CONSERVATION IN THE ISTRIA AND KARST

We carried out genetic monitoring using molecular biology techniques with the aim of genetically characterizing the populations of bees in the area of the Karst and Istria, in an attempt to identify the original native bee of these areas.

Between 2012 and 2013, 151 specimens were sampled from 16 localities that include the area of the Karst and some areas of Istria. This area is a natural overlap zone between two subspecies: *A. m. ligustica* and *A. m. carnica*. To compare the local bees with "pure" subspecies we therefore analysed bees coming from the Slovene interior and bees from Reggio Calabria (Italy) as reference groups, respectively, for *carnica* and *ligustica* bees.

We also analysed some museum specimens (Natural History Museum of Trieste), to compare the situation in the past in which this native bee should have represented the dominant form in the Karst's coastal regions, with the current situation in the same areas.

The DNA regions analysed are of two types: the mitochondrial DNA, which is inherited only from the mother and microsatellite DNA, which is inherited from both parents and is characterized by high variability, which makes it particularly suitable to characterize taxonomic units (that is to say a grouping of individuals) that are very close together, such as subspecies or geographical races (ecotypes).

9.3.1 MODERN BEES

The results obtained with both the mitochondrial DNA and microsatellites reveal high levels of genetic variation among individuals within populations. As far as the mitochondrial DNA is concerned, the high number of genetic variants (haplotypes) encountered includes bees belonging to three different evolutionary lineages (C, M, A). This result illustrates a strong genetic reshuffling, mainly caused by the movement of bees being kept by humans, since the only evolutionary lineage that occurs naturally in the areas sampled is C (the eastern Mediterranean line).

Using microsatellite analysis, we estimated the potential number of populations into which the individuals can be divided, without, *a priori*, being assigned to populations to which they belong. The result is a certain number of sets (that define genetic groups or "gene pools") that aggregate the bees on the basis of their genetic similarity. The groups are generated by minimizing the genetic differences within them and maximize those between the groups themselves. Four different gene pools (K) were obtained (Figure 9.3), two of which describe our reference groups (K1 = the *carnica* bee, K2 = the *ligustica* bee). Surprisingly, the local bees seem genetically closer to the Calabrian bees (K2) than they do to those of Slovenia (K1), which is geographically closer. This can be explained only by direct human activity, which for years has actively moved bees from southern Italy into the Karst. Furthermore, the local samples mainly described two other gene pools (K3 and K4).

But what are the latter two groups? We hypothesize that K3, to which the specimens from the Karst above Trieste are assigned, represent a hybrid population, the product of hybridization between the yellow Italian bee (*A. m. ligustica*) and the *A. m. carnica* bee. From our results we are not able to assert to what extent this cross is of natural origin, since we are within an area of spontaneous hybridization, and how much of it, on the other hand, is due to mixing carried out by human agency through the importation of non-native bees, *ligustica* from one side and *carnica* from the other, which have hybridized repeatedly until they have lost the particular characteristics of one or other subspecies.

The fourth gene pool (K4), prevalent in Istrian bees but also present in the Karst above Trieste, however, may be the Istrian-Dalmatian ecotype. It seems that this group is much more diverse than the *carnica* reference group from the Slovene interior, but we are still unable to determine what it constitutes from a taxonomic perspective, a geographical race of the *carnica* bee or a sub-species in itself?

We also hypothesize that the distribution of the Istrian ecotype may overlap in the northern Adriatic area with the bees assigned to the hybrid *carnica-ligustica* gene pool (K3). A final consideration should be made regarding the phenotypic characteristics of bees assigned to groups K3 and K4, having all been assigned to the variety termed the "local hybrid" due to the presence of characteristics intermediate between the Italian and *carnica* bee subspecies.

In our area there is, therefore, a *carnica-ligustica* hybrid (K3) and another variety, which happens to be the Istrian-Dalmatian ecotype (K4), which is morphologically very similar, but in reality is quite distinct from a genetic perspective.

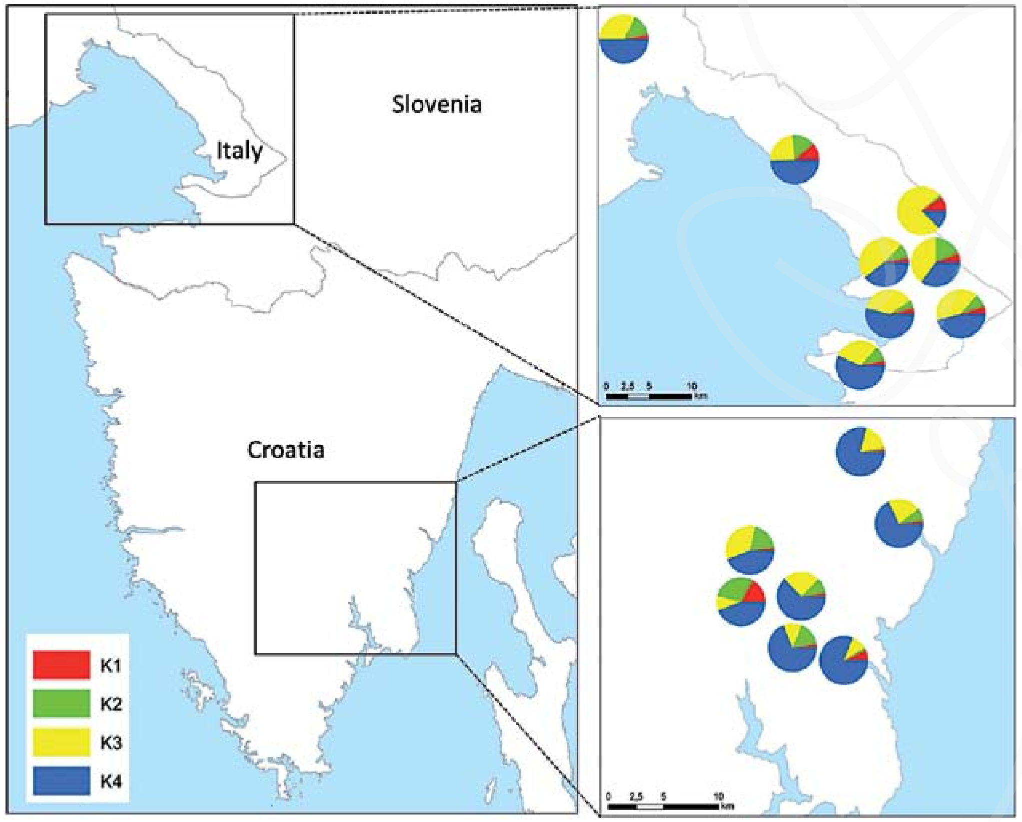

Figure 9.3: The two sampling areas (Istria and the Karst above Trieste) are shown. Each pie chart represents a location and the circles represent the four genetic groups identified, as indicated in the legend. Cartography: Peter Glasnović.

9.3.2 THE MUSEUM BEES

To extract DNA from museum specimens, we developed a non-destructive method to preserve the integrity of the specimens, so that they can be placed back in the collections (Figure 9.4). Samples from Trieste (1934) and Friuli (1966) are strongly attributed to the hybrid *carnica-ligustica* group (K3), supporting the hypothesis that the presence of this hybrid can be considered typical of an area that represents a natural boundary between the two subspecies.

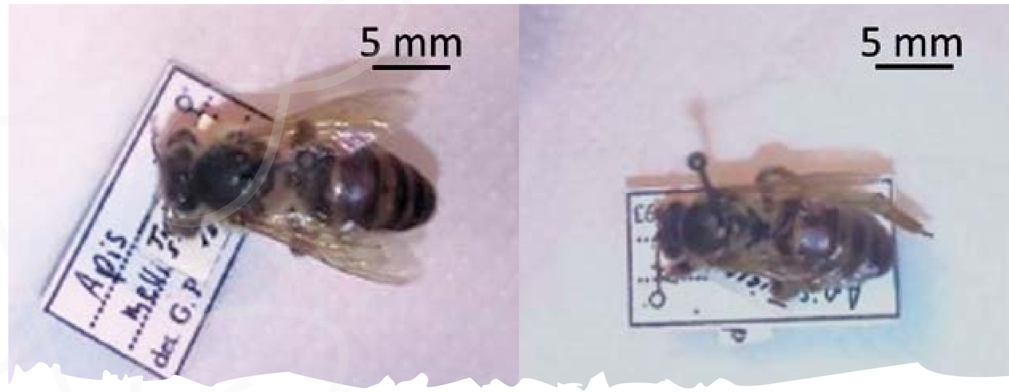

Figure 9.4: Queen bee (Trieste 1934) from the collection of the Museum of Natural History in Trieste. Before (left) and after (right) the extraction of genomic DNA. Photographs: authors.

One can also note a clear temporal gradient from the 1930s through to the present of old bees, which become closer to the bees currently present (Figure 9.5). Unfortunately, the small number of museum specimens and the difficulty of analysing ancient DNA render this data for guidance only. To validate the preliminary results a greater number of museum specimens will be needed, which also includes ancient specimens from Istria, to compare the past situation of both areas with the current one.

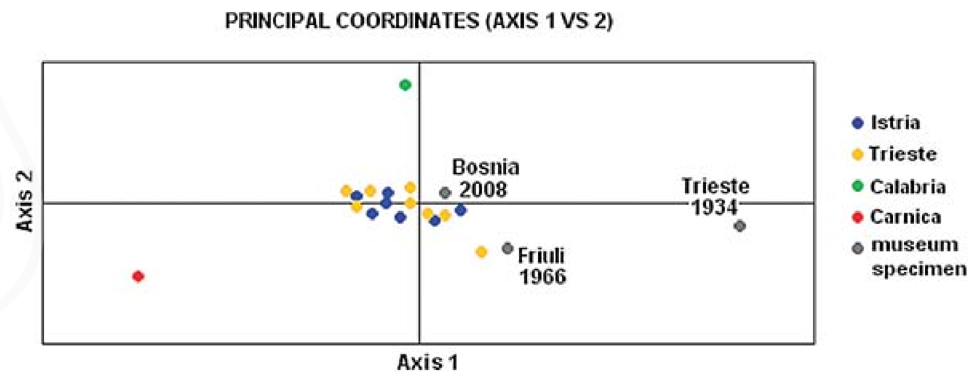

Figure 9.5: Genetic distances between the samples of different populations and three museum specimens. Each circle corresponds to a population.

CONCLUSIONS

Using conservation genetics it is now possible to investigate and characterize many aspects of genetic variability present in bee populations, to provide an information base for the development of programmes for the conservation of local ecotypes and the restoration of their rearing. The identification and characterization of local strains is therefore the first step to implementing conservation programmes.

At this point it becomes necessary to introduce regulatory measures to protect these ecotypes. In Italy there are legislative provisions relating to beekeeping activities as an animal rearing process, but there are no laws regarding the preservation and protection of the genetic resources of native bee stocks, in contrast to countries such as Slovenia, which, since 1991, has made the protection of the native *carnica* bee a priority, prohibiting the introduction of other bee subspecies.

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CHAPTER 10

ANALYSIS OF HEMATOLOGICAL PARAMETERS OF *APIS MELLIFERA LIGUSTICA* (SPINOLA, 1806) IN A POLLUTED SITE

ANALIZA HEMATOLOŠKIH VREDNOSTI PRI PODVRSTI *APIS MELLIFERA LIGUSTICA* (SPINOLA, 1806) Z ONESNAŽENEGA OBMOČJA

ANALISI DI PARAMETRI EMATOLOGICI DI POPOLAZIONI *APIS MELLIFERA LIGUSTICA* (SPINOLA , 1806) IN UN SITO INQUINATO

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ABSTRACT

The main immune defences of the honeybees are the cellular responses represented by phagocytosis and melanisation. There are a number of factors that could impact on the honeybees' immune system and, therefore, increase their susceptibility to disease and lower their survivorship, such as: exposure to pesticides in the air, pollen, nectar and water; fungicides from both field and in-hive treatments; varroacides; the pest *Varroa destructor*; antibiotics used in in-hive treatments; fungal pathogens such *Nosema apis* and the emerging *Nosema ceranae*; bacterial and viral infections. More important, we highlight the interactions among different chemicals and their synergistic effects with diseases in the immune suppression of individuals and on the colony. The present study uses methodologies that can describe the health status of the honeybees, such as total hemocyte counts (THCs), the activities of the plasmatic phenoloxidase (PO) and its inactive form (proPO), to assess the immune competence of individuals. Specimens of *Apis mellifera ligustica* were collected in summer and early autumn from beehives located in S. Giovanni (Trieste, "control site") and from hives placed in Domio (Trieste, "polluted site"). In both seasons the statistical comparison showed a greater number of circulating hemocytes in honeybees from Domio compared to the numbers recorded in those from S. Giovanni. The Domio's honeybees showed a lower PO and proPO activities than those from S. Giovanni. It should be noted that 52% of the bees collected from the hives in Domio had one or two individuals of *Varroa destructor* on the tergites of the thorax. The higher PO and proPO activities recorded in the bees from S. Giovanni site are probably due to the different quality of the environment in the two sampling sites indicating a depression of non-specific immune competence and an increased susceptibility to *Varroa destructor* parasites in the honeybees from the polluted site of Domio.

Key words: honey bees, phenoloxidase, immunology, pollution, *Varroa destructor*

IZVLEČEK

Glavni imunski odziv pri medonosnih čebelah predstavlja celični odziv s fagocitozo in melanizacijo. Na imunski sistem čebel vpliva več dejavnikov, ki lahko povečajo njihovo dovzetnost za bolezni in znižajo njihovo preživetje. To so: izpostavljenost pesticidom v zraku, cvetnem prahu, nektarju in vodi; fungicidom v okolju in njihovim ostankom v panju; zdravilom za zdravljenje varoze; pršici Varroa destructor; antibiotikom, ki se uporabljajo pri zdravljenju v panju; glivičnim patogenom, kot sta *Nosema apis* in *Nosema ceranae*; ter bakterijskim in virusnim infekcijam. Pričujoča študija podaja učinke interakcij med različnimi kemikalijami in boleznimi, ki privedejo do imunosupresije pri posameznih čebelah in v celotni čebelji družini. Za oceno imunokompetence čebel opisujemo njihovo zdravstveno stanje s preprostimi in jasnimi parametri, kot so skupno število hemocitov, aktivnost plazemske fenoloksidaze (PO) in njene neaktivne oblike (proPO). Osebke podvrste *Apis mellifera ligustica* smo pridobili poleti in zgodaj jeseni iz panjev v kraju S. Giovanni (Trst, "kontrolno območje") in v kraju Domio (Trst, "onesnaženo območje"). V obeh sezonah je statistična primerjava pokazala večje število hemocitov pri čebelah iz onesnaženega kraja Domio v primerjavi s čebelami iz kraja S. Giovanni. Čebele iz kraja Domio so imele nižjo aktivnost PO in proPO kot tiste iz S. Giovanni. Poleg tega je imelo 52 % čebel iz kraja Domio na torakalnih tergitih eno ali dve pršici *Varroa destructor*. Višje aktivnosti PO in proPO pri čebelah iz kraja S. Giovanni so verjetno posledica različne kakovosti okolja, kar kaže na zmanjšano nespecifično imunokompetenco in večjo dovzetnost za parazite *Varroa destructor* pri čebelah iz onesnaženega mesta Domio.

Ključne besede: medonosne čebele, fenol oksidaza, imunologija, onesnaženje, *Varroa destructor*

RIASSUNTO

Le principali difese immunitarie delle api da miele sono le risposte cellulari rappresentate dalla fagocitosi e dalla melanizzazione. Un certo numero di fattori che possono influire sul sistema immunitario delle api e, di conseguenza, aumentare la loro suscettibilità alle malattie e ridurre la loro sopravvivenza come: esposizione ai pesticidi, in aria, nel polline, nel nettare e in acqua; fungicidi sia da campo che nei trattamenti in alveare, varroacidi, il parassita *Varroa destructor*, gli antibiotici usati nei trattamenti in alveare; patogeni fungini come *Nosema apis* e l'emergente *Nosema ceranae*, infezioni batteriche e virali. In particolare, vanno evidenziati gli effetti sinergici derivanti dall'interazione tra le diverse sostanze chimiche e le malattie che portano all'immunosoppressione dei singoli individui e dell'intera colonia. Il presente studio utilizza metodi semplici e affidabili in grado di descrivere in modo chiaro lo stato di salute delle api, come la conta totale emocitaria (THC) e le attività della fenolossidasi plasmatica (PO) e la sua forma inattiva (proPO) per valutare l'immunocompetenza dei singoli individui. Esemplari di *Apis mellifera ligustica* sono stati raccolti da alveari situati a S. Giovanni (Trieste, "sito di controllo") e da alveari collocati a Domio (Trieste, "sito inquinato") in estate e all'inizio dell'autunno. Sia in estate che in autunno il confronto statistico ha evidenziato un maggior numero di emociti circolanti nelle api del sito di Domio rispetto ai numeri registrati nelle api di S. Giovanni. L'analisi statistica ha mostrato una tendenza verso una differenza significativa nel mese di luglio e una differenza altamente significativa nel mese di ottobre. Le attività della PO e della proPO delle api di Domio erano significativamente inferiori rispetto a quelle di S. Giovanni. Va notato che il 52 % delle api raccolte da alveari di Domio ha presentavano 1 o 2 individui di *Varroa destructor* sui tergiti del torace. Le attività più elevate di PO e proPO nelle api del sito di S. Giovanni sono probabilmente da attribuire alla diversa qualità dell'ambiente nei 2 siti che provoca una depressione dell'immunocompetenza aspecifica e una maggiore suscettibilità ai parassiti *Varroa destructor* nelle api di Domio.

Parole chiave: api, fenolossidasi, immunologia, inquinamento, *Varroa destructor*

10.1 BEE BIOLOGY

There are four commonly recognised species of honeybees in the world, *Apis florea* (India, Borneo, Burma), the larger and more aggressive *Apis dorsata*, (India, Philippines), the meek but not very hard-working *Apis cerana* (Asia, China, India, Japan, Siberia) and the honey bee, *Apis mellifera*, the most active and productive, present in Europe, Africa and Asia but also in the Americas and imported into Australia and New Zealand.
Primarily size, colour, the "hairs" and the structure of the veins in the wings distinguish the races of *Apis mellifera* that have formed.

- *Apis mellifera mellifera* is dark and characteristic of Western and Northern Europe.
- *Apis mellifera carnica* is also dark and characteristic of Austria, Slovenia and the Balkans.
- *Apis mellifera ligustica*, the Italian bee, has the first segments of the abdomen pale yellow alternating with dark segments and short gold yellow "hairs".
- *Apis mellifera sicula* is black, a little more aggressive and found in Sicily.

Linnaeus described the family Apidae in 1758 as a matriarchal monogynic society, lasting several years. It is formed by a number of individuals belonging to three castes, the queen and the fertile drones and sterile workers. A larva turns into a worker bee when it is fed with royal jelly for the first three days of life and, for the remaining period of growth, with a mixture of pollen and honey. The larva that becomes queen is fed exclusively on royal jelly throughout its life cycle. Therefore, nutrition has effects on the morphology, anatomy and physiology of an individual within the society.

In *Apis mellifera* sizes vary depending on the different subspecies, those of Italian worker bees ranging from 12-13 mm in body length while in India they reach larger sizes (16-18mm). Depending on its age the worker bee takes on different tasks: from the 1st to 3rd day it cleans and prepares cells for laying; from the 4th to 5th it nourishes the older larvae with pollen and honey; from the 6th to the 9th, following the development of the hypopharyngeal glands that produce royal jelly, it feeds the younger larvae, while from the 10th to the 16th day, after the wax glands begin to function, it builds honeycombs. From the 17th to the 19th day it stores honey in the cells as well as being responsible for the ventilation and cleaning of the hive. On the 20th day it defends the hive and only on the 21st does it turn to foraging, collecting nectar, pollen and propolis. In the event of an imbalance in the composition of the "family", a condition arising as a result of a swarming event, the bees may perform functions not strictly related to their age. The drone has a body length of 15 mm and compared to the worker it is stocky with compound eyes that touch at the top of the head, lacks a sting and apparatus for collecting pollen on the legs and its proboscis is very short. It develops over 24 days from unfertilized eggs inside cells with a diameter 6.2-6.4 mm. After hatching it requires a nutritional intake four times higher than that of a worker. The life of the drone, lasting 50 days, and its presence in the hive coincides with the seasonal period during which the bees raise queens. The presence of the drones ensures the fertilization of the young queens. When they are flying, they form true swarms in well-defined areas, within which mating takes place. At the end of the swarming, the workers drive them out of the nest or kill them. The queen has a body 17-20 mm in length, with a proportionally larger abdomen. She has legs without pollen baskets, a very short ligula and a sting with a smaller number of hooks. The queens originate from fertilized eggs and develop in special royal cells. Three days after laying the egg hatches and the larva is fed with an abundant supply of royal jelly. The life expectancy of the queen bee is five years.

10.2 THE IMMUNE SYSTEM IN BEES

Bees are particularly vulnerable to infection by pathogens because of their social lifestyle, with a very high population density within their hives. They spend most of their lives inside the hives in close contact, feeding continuously with each other. It is believed that these extreme living conditions have led to the evolution of very effective strategies to combat pathogens and parasites, collectively referred to as social immunity.

Like all insects, bees are without a classic adaptive immune system. Instead they have evolved several lines of defence mechanisms to cope with microbial infections: (1) the cooperative behaviour of individual members of the social group to fight the disease transmission within the colony (Cremer *et al.*, 2007), (2) physical barriers such as the cuticle and the epithelium of the intestine, and (3) cellular and humoral immune responses that constitute the innate immune system (Randolt *et al.*, 2008).

Under the pressure of pests and pathogens, social insects have evolved both individual and group strategies to combat disease (Evans *et al.*, 2006). Grooming, hive hygiene and other behavioural traits that are found in social insects can reduce the impact of pathogenic bacteria, fungi and parasitic mites. For example, the "hygienic behaviour", described for the first time in honeybees (Rothenbuhler, 1964), is now a classic example of a social defence, where the worker bees identify and remove infected larvae from within a healthy brood (Spivak & Reuter, 2001). Other defences favouring this social insect include the construction of combs with antimicrobial materials (Christe *et al.*, 2003), the placing of larvae in sterile nurseries (Burgett, 1997) and social "fever" in response to illness (Starks *et al.*, 2000).

The powerful innate immune responses benefit not only individuals but also reduce the transmission of diseases among the members of the colony (Evans and Pettis, 2005). The cellular defences are mediated by haemocytes (blood cells) including coagulation, phagocytosis and the formation of capsules and nodules (Strand, 2008). The humoral immune defences, on the other hand, include antimicrobial peptides, proteins and enzymatic pathways (Schmid-Hempel, 2005). During the enzymatic pathways of the prophenoloxidase system (proPO), defined as the phenoloxidase cascade, the phenoloxidase enzyme is produced, responsible for the biosynthesis of melanin, which is involved in cuticle sclerotization, wound healing, nodule formation, encapsulation and the stimulation of phagocytosis (Mason, 1955; Ratcliffe *et al.*, 1984; Cerenius *et al.*, 2008). These responses are always present but non-specific and not always maintained at the highest levels. In contrast, induced responses, including antimicrobial peptides (AMPs) and lysozyme-like activity, require the presence of pathogens to stimulate their production.

Recent work has highlighted that, for the immune system of worker bees and of drones, from the larval stage to the adult, in both their constitutive (PO) and induced responses (AMP), the larvae exhibit low levels of PO activity while adult workers and drones have stronger immune responses (Laughton *et al.*, 2011). On the other hand, the immune response causes a reduction in PO in adult workers, probably due to its rapid use, being impossible to reconstruct the constitutive phenoloxidase. Both worker bees and drones have an immune response induced by pathogens that produces high levels of AMP, but the cost of this response prevents a constant maintenance and both castes show signs of senescence of the immune system in the AMP response.

10.3 PESTS AND PESTICIDES

Pesticides (insecticides, fungicides, nematicides, acaricides and aphicides) are used to protect crops from diseases and pests. The introduction of pesticides, even at sub-lethal levels, especially systemic ones, has challenged the synergy and balance between agriculture and the survival of bees through a very diverse and complex system of action that includes 1) damage to non-target species that although not being the target are adversely affected; 2) bioaccumulation in ecosystems; 3) persistence in the environment and in the food chain; 4) high toxicity, which may change bee behaviour and physiology; 5) systemicity, which involves the spread of the pesticide within the plant to parts that have not been directly treated and through which the bee may come into contact.

Among the most common systemic products there is the entire class of neonicotinoids (e.g. Imidacloprid, Clothianidin and Thiamethoxam) and Fipronil. These chemicals have most of described above. They are the most widely used insecticides in the world in recent years. In 2013, as a result of new evidence and scientific data, the EU authorities have officially recognized that these products have harmful effects on bees. These products have been partially banned from the EU market for a period of two years from December 2013 to December 2015. At the end of this period, the EU authorities will assess the situation and decide what additional measures can be taken.

Bees have natural enemies that are represented almost exclusively by insectivorous birds and by other insects. The insectivorous birds preying on bees include swallows, swifts, martins, titmice, thrushes, bee-eaters, etc. while insects preying on bees include wasps and hornets. Bees also suffer external parasites such as the mite *Varroa destructor*. There are also a number of microbial diseases such as American foulbrood (*Bacillus larvae* White), European foulbrood (*Bacillus pluton*, *Bacillus alvei* and *Streptococcus apis*), the viral sacbrood disease and nosemosis caused by the microsporidian *Nosema apis*.

Varroosis is a parasitic disease caused by the *Varroa destructor* mite and is responsible for the majority of bee die-offs that have occurred in Italy over the last twenty years. This parasitic disease is native to eastern Asia, and was identified for the first time in 1904 as *Varroa jacobsoni* on *Apis cerana* by Oudemans. With this species of bee the *Varroa* mite lives without causing much damage, both as a result of the cleaning behaviour of the bee, which is more, pronounced in *V. cerana*, (including grooming and the removal of parasitised brood) and for the shorter duration of the operculation phase. It was the movement of bee colonies (nomadism) and the trade in swarms of bees with queens, starting in the 1950s, which led to contact between *Apis cerana* and *Apis mellifera* and allowed the spread of the parasite. It became widespread in Europe in the late 1970s and in Friuli 1981, as the first outbreaks in Italy. Only Australia and certain parts of Africa remain *Varroa*-free. The *Varroa destructor* mite lays its eggs in the cells in which the brood is placed as it feeds on bee larvae. Its development begins in the cells, especially the male ones that are larger and filled in the spring, and then proceeds outside of the cells, feeding directly on the bees, to which it attaches itself, sucking the lymph and causing them to die before their time. The strategies in the fight against *V. destructor* can be either chemical or biological in nature.

10.4 CASE STUDY

There are a number of factors that can affect the immune system of bees and, consequently, increase their susceptibility to disease and lower their survival, such as exposure to pesticides in air and water or in pollen and nectar; fungicide treatments in the field and in the hive; the parasite *Varroa destructor* and the acaricides used against it; antibiotics used for in-hive treatments; microsporidian pathogens such as *Nosema apis* and the emergent *Nosema ceranae* as well as bacterial and viral infections (Botías *et al.*, 2013). It is also worth highlighting the synergistic effects arising from the interaction between different chemicals and diseases, leading to immunosuppression in individuals and in the colony. It would appear to be very difficult or even impossible to investigate all possible causes of immunosuppression in an individual or colony and it is more convenient to use simple and reproducible methods that can clearly describe the overall health status of the animals. In recent years, research on the immune system of insects using total haemocyte counts (THC) in plasma as well as phenoloxidase (PO) activity and its inactive form (proPO) have been used to assess the immune competence of individuals. In general, THC measurements are influenced by environmental conditions such as humidity, temperature, photoperiod and stress diseases (Pandey & Tiwari, 2012), while a recent review of phenoloxidase enzyme and its inactive form have underlined that the insects in best condition produce high levels of proPO and / or PO, and that proPO and PO are influenced by a series of environmental factors (González-Santoyo and Córdoba-Aguilar, 2011).

In the case of the study in question foraging specimens of *Apis mellifera ligustica* were collected from hives located in San Giovanni (Trieste, the "control site") and hives placed at Domio (Trieste, "contaminated site"), the latter about 2.5 km 'as the crow flies' from the industrial area of Trieste and the infamous Servola Steelworks. The bees were anaesthetized at 0°C for at least 30 minutes prior to collection of 5µl of haemolymph, both for total haemocyte counts (THC) and to verify the phenoloxidase (PO) enzymatic activity and its inactive form, proPO (Figure 10.1).

Figure 10.1: Anesthetized forager of *Apis mellifera ligustica* during the hemolymph sampling in the laboratory.

The THC foresees the counting of haemocytes and is expressed as number of cells per ml. The cells are counted under a microscope in interference contrast at 40 X magnification. For enzyme activity, plasma diluted in buffer was added to the DL-DOPA substrate (a melanin precursor) and the activity measured in a spectrophotometer at 492 nm for 30 min at 5 min intervals as absorbance units per minute. The values for THC obtained were evaluated with non-parametric statistics (Figure 10.2), the linear models obtained from the analysis of the PO and the proPO being tested with analysis of covariance (ANCOVA) (Figure 10.3). The effects of environmental stress were assessed on the two populations of *Apis mellifera ligustica* that were the object of study in early summer (July) and early autumn (October). In both periods the statistical comparison showed a significantly higher number of circulating haemocytes in bees at the polluted site of Domio.

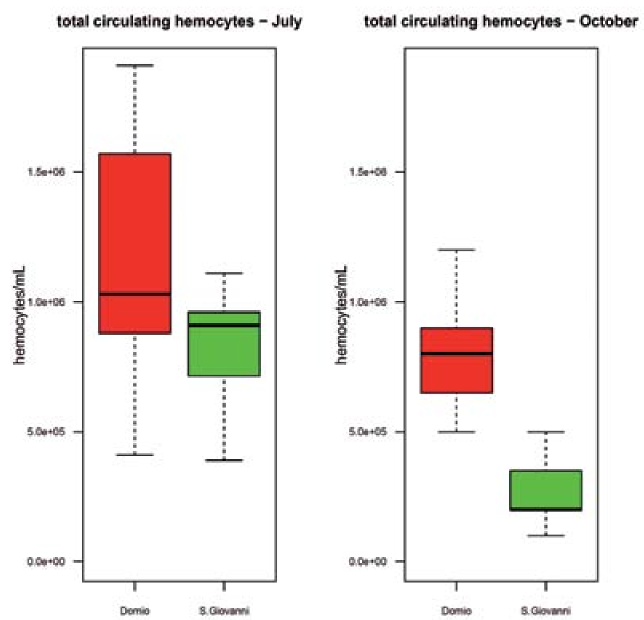

Figure 10.2: Boxplot diagrams of the number of total circulating haemocytes in foragers collected from the site of San Giovanni and that of Domio in summer and autumn.

Bees from the polluted site also exhibited significantly lower enzymatic activity of both PO and proPO. It should be mentioned, however, that 52 % of the bees collected from hives at Domio had one or two individuals of *Varroa destructor* on the thoracic tergites. The higher activities of PO and proPO in bees at the San Giovanni site may be due to the differing environmental quality of the 2 sites and this indicates a nonspecific depression of immunocompetence and increased susceptibility to the parasite *Varroa destructor* in honeybees from Domio.

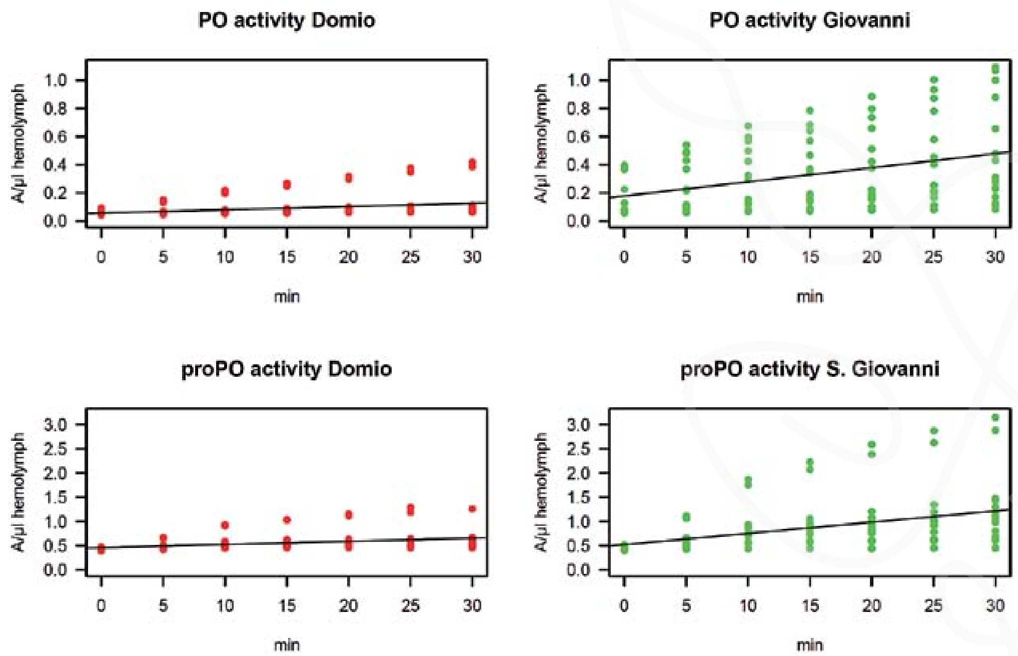

Figure 10.3: Linear models of the enzymatic activity for PO and proPO of the bees from the site of Domio and San Giovanni. The activity is indicated as absorbance per µl of haemolymph for 30 min at 5 min intervals.

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THE TECHNICAL AND SCIENTIFIC MANAGEMENT OF THE STEPPE GRASSLANDS OF THE ITALIAN AND SLOVENIAN KARST FOR THE CONSERVATION OF BIODIVERSITY AND HABITATS

TEHNIČNO IN STROKOVNO UPRAVLJANJE SUHIH TRAVIŠČ NA ITALIJANSKEM IN SLOVENSKEM KRASU Z NAMENOM OHRANJANJA BIOTSKE RAZNOVRSTNOSTI IN HABITATOV

LA GESTIONE TECNICO SCIENTIFICA DELLE PRATERIE STEPPICHE DEL CARSO ITALIANO E SLOVENO AI FINI DELLA CONSERVAZIONE DELLA BIODIVERSITÀ DEGLI HABITAT

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ABSTRACT

The aim of the research is to produce a syntaxonomical framework of Karst dry grasslands (habitat of *Natura 2000* network) and provide cognitive tools that can be used for their restoration through good management practices. The methodological approach is based both on a definition of the optimum state of reference of flora and of vegetation (using phytosociological models) and on the association between phytosociological and agronomic information. The research was conducted in two steps: a. update of taxonomic nomenclature and definition of a theoretical syntaxonomical reference framework; b. study of the relationships between the agronomic aspects and aspects of the flora and vegetation for meadows and pastures for specific sampling sites in the Italian and Slovenian Karst.

a. The unpublished (Poldini; Zanatta *ex schedis*) and published relevés of the dry grasslands (class *Festuco-Brometea*, ordo *Scorzonero-Chrysopogonetalia*) of the Karst were classified along with similar but more thermophilic (alliance *Chrysopogono grylli-Koelerion splendentis*) and more mesophilic coenoses (class *Molinio-Arrhenatheretea*). The indicator species indices calculated for associations have permitted the objective identification of groups of character and differential species for association. The distribution of dry grasslands in the Karst landscape is outlined and the taxonomic framework is updated via a nomenclatural and phytogeographical revision. The standard parameters (chorotype and life form spectrum) were calculated. The conservation value is defined by the calculation of standard indices of diversity.

b. This floristic-vegetation information represents the baseline for the assessment of changes in the vegetation and the status of the habitat. For each site phytosociological relevés were repeated in the years 2012-13. In order to define their syntaxonomic affiliation, the collected data was compared with the phytosociological models. Almost all the sites fall within the *Danthonio-Scorzoneretum*. For each site the standard parameters are calculated, in order to assess the degree of deviation from the theoretical models and analyze the relationships between the community, management and productivity. The study highlights the trend towards a significant change in structure and function of the karst lands and the negative correlation between floristic richness and productivity. This raises the need to implement new strategies to bring the system moor towards the desired state.

Keywords: Karst dry grasslands, phytosociology, *Scorzonero-Chrysopogonetalia*, assessment of the conservation status, cultural landscape

IZVLEČEK

Cilj raziskave je bil določiti sintaksonomski okvir suhih kraških travnikov (habitatnih omrežij *Natura 2000*) in pridobiti kognitivna orodja za obnovo suhih travišč preko dobrih praks upravljanja. Metodološki pristop je temeljil na opredelitvi optimalnega stanja vegetacije in flore (na podlagi fitocenoloških modelov) ter povezavi fitocenoloških in agronomskih podatkov. V raziskavi smo uporabili dva metodološka koraka: a. posodobitev taksonomske nomenklature in opredelitev teoretičnega sintaksonomskega referenčnega okvira; b. študija agronomskih vidikov in vidikov ohranjanja biodiverzitete suhih travišč in pašnikov na izbranih vzorčnih mestih na italijanskem in slovenskem Krasu.

a. Iz neobjavljenih (Poldini; Zanatta *ex schedis*) in objavljenih fitocenoloških popisov lahko suha travišča Krasa (razred *Festuco Brometea*, rodov *Scorzonero-Chrysopogonetalia*) uvrstimo k podobnim, vendar bolj termofilnim združbam (alliance *Chrysopogono grylli-Koelerion splendentis*) ter k mezofilnim združbam (razred *Molinio-Arrhenatheretea*). Indeksi indikatorskih vrst izračunani za te združbe omogočajo objektivno identifikacijo na podlagi skupnih značilnosti in razlik med vrstami v združbah. Taksonomski okvir razporeditve suhih travišč na Krasu je posodobljen z nomenklaturnimi in fitogeografskimi revizijami. Standardne parametre smo izračunali s pomočjo klorotipa in spektra življenjskih oblike. Naravovarstveno vrednost smo določili s standardnimi indeksi raznolikosti.

b. Podlago za oceno sprememb v vegetaciji in stanja habitata predstavljajo floristično – vegetacijske informacije. Za vsako vzorčno mesto je bil opravljen fitocenološki popis v letih 2012 in 2013. Za opredelitev sintaksonomske pripadnost vzorčnih mest smo zbrane podatke primerjali z fitocenološkimi modeli. Skoraj vse proučevane lokacije smo definirali v združbo *Danthonio-Scorzoneretum*. Za vsako vzorčno mesto smo izračunali standardne parametre in določili stopnjo odstopanja od teoretičnih modelov in analizirali razmerja med združbami, upravljanjem in produktivnostjo. Študija poudarja trend statistično značilnih sprememb v strukturi in funkciji kraških zemljišč in negativno korelacijo med flornim bogastvom in produktivnostjo, kar sproža potrebo po izvajanju novih strategij za ohranjanje želenega stanja.

Ključne besede: suhi kraški travniki, fitosociologija, *Scorzonero-Chrysopogonetalia*, ocena stanja ohranjenosti, kulturna krajina

RIASSUNTO

Scopo della ricerca è produrre un quadro sintassonomico aggiornato delle lande carsiche (Habitat della rete *Natura 2000*) e fornire strumenti conoscitivi utili al loro recupero mediante buone pratiche gestionali. L'approccio metodologico si basa sulla definizione di uno stato floristico-vegetazionale ottimale e di riferimento teorico (deducibile dai modelli fitosociologici) e sull'associazione delle informazioni fitosociologiche con quelle agronomiche.

Fasi: a. Aggiornamento della nomenclatura tassonomica delle specie e definizione del quadro sintassonomico di riferimento teorico; b. studio della relazione tra aspetti agronomici e aspetti di flora e vegetazione dei prati e dei pascoli di specifici siti campione del Carso italiano e sloveno.

a. I rilievi fitosociologici inediti (Poldini; Zanatta *ex schedis*) e pubblicati delle lande carsiche (classe *Festuco Brometea*, ordine *Scorzonero-Chrysopogonetalia*) sono stati classificati assieme alle fitocenosi floristicamente simili, ma più termofile del Carso litoraneo (alleanza *Chrysopogono grylli-Koelerion splendentis*) e più mesiche (classe *MolinioArrhenatheretea*). Il successivo calcolo del valore indicatore delle specie per ogni associazione ha consentito l'identificazione oggettiva di gruppi di specie caratteristiche e differenziali di associazione. Sono stati calcolati i parametri standard (spet-tro corologico e biologico). Il valore conservazionistico viene definito dal calcolo di indici standard di diversità.

I risultati sono dati dall'aggiornamento critico del quadro sintassonomico e dalla descrizione della distribuzione delle associazioni nel paesaggio carsico.

b. Queste informazioni floristico-vegetazionali rappresentano la baseline per valutare le modificazioni della vegetazione attuale rispetto i modelli fitosociologici, quindi dello stato di conservazione dell'habitat. Per ogni sito sono stati effettuati i rilievi fitosociologici ripetuti negli anni 2012-13. Al fine di conoscere l'appartenenza sintassonomica, i dati raccolti sono stati confrontati con i modelli fitosociologici. Tutti i siti ricadono nel *Danthonio-Scorzoneretum*. Per ogni sito sono stati calcolati i parametri standard, al fine di valutare il grado di deviazione dal modello teorico e analizzare la relazione tra comunità, gestione e produttività.

Viene evidenziata la tendenza verso una sensibile alterazione strutturale e funzionale delle lande carsiche e la correlazione negativa tra ricchezza floristica e produttività. Ciò solleva l'esigenza di mettere in atto nuove strategie in grado di riportare il sistema landa verso uno stato desiderato.

Parole chiave: praterie aride del Carso, fitosociologia, *Scorzonero-Chrysopogonetalia*, alutazione dello stato di conservazione, paesaggio culturale

11.1 INTRODUCTION

The grasslands of the Northern Adriatic Karst are plant communities rich in species coming mostly from the eastern steppes whose origin can be ascribed to the pastoral practices associated with transhumance and periodic hay-making that are thousands of years old. They represent elements of the landscape from at least 2000-2500 years ago when the ancient Romans carried out extensive deforestation in the Karst territories of Istria and Dalmatia (Kaligarič *et al.*, 2006). Since then their alternating phases of reforestation and deforestation have followed in a dynamic landscape dependent on human history and the socio-economic models of such time. Today, after decades of neglect of traditional agro-pastoral practices resulting in a recovery of the dynamic processes, there is a trend towards a return to farming in marginal areas. This may well open up a new scenario in which there is a regression in woodland and the recovery of areas of *landa carsica* grassland more generally. However, while this process may allow the recovery of grassland formations, the conservation of the diversity of the flora typical of these grasslands is not equally certain. In fact, the shift from a collective extensive land management model towards private or corporate ways of farming with permanent or semi-permanent pastoralism could limit or cancel out the syngenetic ecological factors and lead to a shift in the original floristic – vegetation status towards a state of lower conservation value or even towards banalization of the flora. In this context it is necessary to establish effective methods of assessment of the conservation status of meadow/pasture ecosystems under different management methods, in order to monitor the inevitable changes in the community and find an acceptable compromise between conservation and production, including landscapes of memory with an identity value and future socio-economic needs.

11.2 WHY DEFINE A REFERENCE STATE USING MODERN PHYTOSOCIOLOGICAL CRITERIA?

The *landa carsica* grasslands have been recognized by the EC Habitats of Community Interest criteria with the code 62A0 (Eastern sub-Mediterranean dry grasslands) (*Scorzoneratalia villosae = Scorzonero-Chrysopogonetalia*) in accordance with the "Habitat" Directive 92/43/EEC. This fact implies significant repercussions on the nomenclatural plane and the methods to be adopted in order to comply with the requirements of the legislation governing the conservation of their species and habitats. In fact, with the enactment of the Directive and the prescribed Habitats Manual, a language and methodology common to all Member States has been officially recognized, that of modern phytosociology.

Since Member States have the obligation to maintain these ecosystems in a Favourable Conservation Status to ensure their functionality, it follows that knowing the status of the *landa carsica* as much as possible as it was before the abandonment, means having a term of reference to establish the limits beyond which the changes in the plant communities are such as make them no longer recognizable and functional as required by law. The legislative aspect and consequently the syntaxonomical reference is therefore the first operational reference. In other words, the habitat 62A0, as conceived as an ecosystem, becomes the reference point with an *ad hoc* legislative foundation.

The study is part of a methodological framework designed over a long period, which provides assessments of the state of conservation, monitoring and actions with retroactive value aimed at achieving a "favourable conservation status" (Figure 11.1). This work is based on the definition of a reference state (phytosociological models) and on a first assessment of the evolutionary trends of some sample cenoses of dry grassland in the Italian and Slovenian Karst (real state) from the perspective of their productivity.

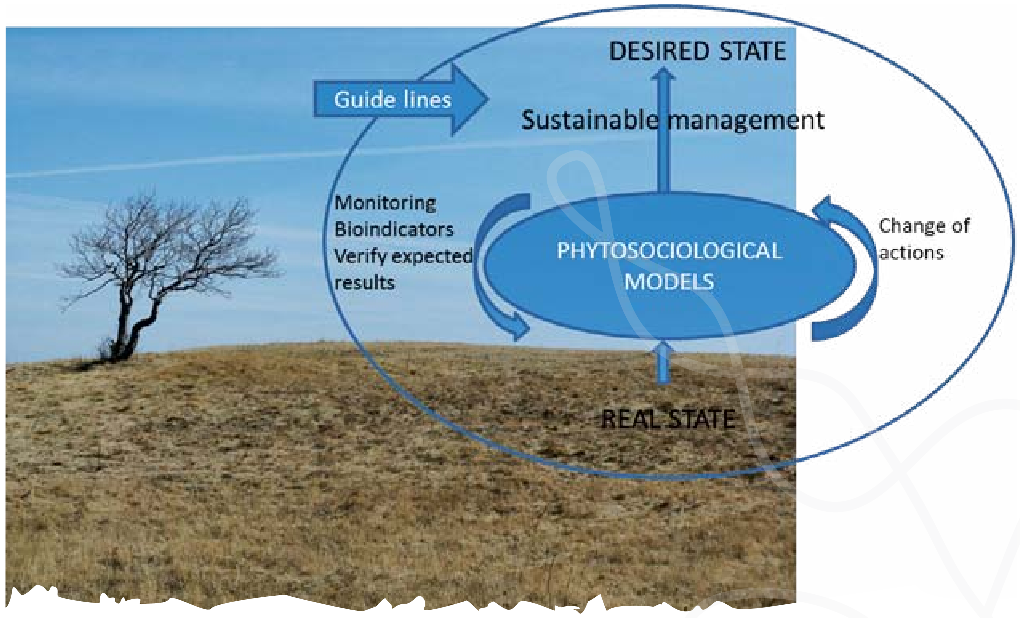


Figure 11.1: Methodological scheme (author: Katia Zanatta).

11.3 VARIABILITY IN THE *LANDA CARSICA* IN THE LANDSCAPE CONTEXT

The phytosociological models are based on plant associations (the basic units). According to the Braun-Blanquet approach, were processed and analyzed 422 relevés and 583 species using multivariate statistics (cluster analysis). The unpublished (Poldini; Zanatta in prep.) and published relevés (Ferlan & Giacomini, 1957; Lausi & Poldini, 1962; Poldini, 1964; Poldini, 1975; Poldini, 1978; Poldini, 1980; Poldini, 1989; Poldini & Kaligarič, 1997) of the dry grasslands (class *Festuco-Brometea*, ordo *Scorzonero-Chrysopogonetalia*) of the Karst were classified along with similar but more thermophilic (alliance *Chrysopogono grylli-Koelerion splendentis*) and more mesophilic coenoses (class *Molinio-Arrhenatheretea*). According to the analysis carried out the areas of landa in the northern Adriatic Karst are made up of 8 associations.

SYNTAXONOMIC CLASSIFICATION: SYNOPTIC TABLE

(Class) *FESTUCO-BROMETEA* Br.-Bl. & Tx. Ex Klika & Hadač 1944

(Ordo) *SCORZONERO VILLOSAE-CHRYSOPOGONETALIA GRYLLI* Horvatić *et* Horvat in Horvatić 1963

(Alliance) *CHRYSOPOGONO GRYLLI-KOELERION SPLENDENTIS* Horvatić 1973

Salvio officinalis-Euphorbietum fragiferae Lausi & Poldini 1962

Lactuco vimineae-Bothryochloetum ischaemum Poldini 1975

(Alliance) *SATUREJION SUBSPICATAE* (Horvat 1974) Horvatić 1975

Seseli gouanii-Artemisietum albae Poldini 80

Genisto-Caricetum mucronatae Horvat in Horvat, Glavač *et* Ellemberg 1974

Genisto sericeae-Seslerietum tenuifoliae Poldini 1980

Centaureo rupestris-Caricetum humilis Horvat 1931

Centaureo cristate-Chrysopogonetum grylli Ferlan & Giacomini 1955 nom. inv. hoc loco

(Alliance) SCORZONERION VILLOSAE Horvatić 1963

Danthonio alpinae-Scorzoneretum villosae Horvat *et* Horvatić (56) 58

ECOLOGICAL CLASSIFICATION: PARASTEPPIC THERMO-XEROFILE ASSOCIATIONS OF THE TRIESTE COASTAL STRIP

1. *Salvio officinalis-Euphorbietum fragiferae* Lausi & Poldini 1962 (Sa-Eu): Pioneer association on accumulations of limestone scree.
2. *Lactuco vimineae-Bothryochloetum Ischaemum* Poldini 1975 (Lac-B): Parasteppe of the northern Adriatic Karst.

PRIMARY GRASSLANDS ON ROCKY FRINGES

3. *Seseli gouanii-Artemisietum albae* Poldini 80 (Se-Art): Community of the ridge-edges of arid Mediterranean-montane environments.
4. *Genisto-Caricetum mucronatae* Horvat in Horvat, Glavač *et* Ellemberg 1974 (Ge-Ca): Association of poor dry montane grasslands with *Genista holopetala* and *Carex mucronata*.
5. *Genisto sericeae-Seslerietum tenuifoliae* Poldini 1980 (Ge-Se): Association found on windy ridges.

PASTURES AND MEADOWS: SEMI-NATURAL XERO-THERMOPHILIC AND SUB-MESOPHILIC SECONDARY STEPPE GRASSLANDS OF THE ITALIAN-SLOVENIAN KARST (LANDA)

6. *Centaureo rupestris-Caricetum humilis* Horvat 1931 (Ce-Ca): Dinaric Karst sub-steppe grassland.
7. *Centaureo cristate-Chrysopogonetum grylli* Ferlan &t Giacomini 1955 nom. inv. hoc loco (Ce-Chry): Chrysopogonetums of the Gorizian Karst.
8. *Danthonio alpinae-Scorzoneretum villosae* Horvat *et* Horvatić (56) 58 (Dant-Sc): Sub-steppe meadows of the Dinaric Karst.

11.3.1 PHYSICAL ENVIRONMENT AND DIVERSIFICATION OF PLANT COMMUNITIES

The natural geographical limits of the Northern Adriatic karst are not clear. To the northwest it is limited by the flood plain of the River Isonzo/Soča, fading out to the northeast beyond the *massifs* of Nanos, above Illirska Bistrica, Pivka and Čičarija, while to the southeast it continues on through Istria as far as the Velebit *massif* in the extreme north West of Croatia, therefore extending within Italy, Slovenia and Croatia. Existing across this geographical area are highly variable soil and climatic conditions that determine a unique floristic richness and complexity (Pignatti, 1980). The main geomorphological elements that affect the diversification of the dry grasslands can be assigned to four main categories: the coastline above Trieste, the edges of the karst ridges, the limestone plateau and the flysch-like sandstones.

- The coastal strip lying between Duino and Grignano (Trieste) is a landscape of great importance as a refuge for species of Mediterranean environments. This represents an area of high densification in terms of vegetation diversity with the sea playing a crucial factor in climate mitigation. In addition, the area's south-southwesterly exposure, along with the presence of limestone cliffs, *grize* (limestone rubble) and scree, bring about extreme environments, which, in the past, have not been excluded from grazing by sheep and goats.
- The edges of the ridges are areas where environmental conditions become extreme and very selective. The limiting ecological factors include wind (the *bora*) and drought due to high water percolation rates, often accompanied by shallow soils with rocky outcrops. These conditions have led to the formation of strongly adapted plant communities, in part of a primary nature and of high conservation value.
- Limestone plateau (Cretaceous-Eocene limestone), rising gradually from the northwest to the southeast, passing from a montane elevation to a hilly one. It is characterized by xero-rendzina soils with surface and underground karst phenomena, high permeability to fissuring and a neutral-basic reaction. To this very arid and mostly unproductive substrate, with the exception of the sinkholes and the plots of land that, for centuries, humans have de-stoned for use in cultivation or as hay-meadows. Before reforestation, it featured large areas of rough sub-steppe grazing, xerophile in character and particularly rich in endemic species.
- Flysch-like sandstones (Eocene marls and Flysch sandstones) are present in the strictly coastal area and extend towards northern Istria. The substrate consists of deep layers of not very compact and alterable rock and by superficial layers of clay-rich soil that is slightly acidic in reaction. These are soils with better water retention properties and therefore are more favourable to the growth of both wild and cultivated plantlife. Along with the red earth of the Karst above Gorizia, these rock types form the substrate of the sub-mesophilic steppe meadows and pastures, characterized by high species richness, particularly orchids.

11.3.2 ESSENTIAL FEATURES OF THE VEGETATION

The large quantity of phytosociological relevés collected and processed has produced a sort of "photograph" of a floristic-vegetation structure that is believed to be closer to that which existed before the Second World War. The syntaxonomical framework that it is derived from therefore represents the reference state. It is considered that this arrangement is not necessarily the best but is simply the only one available. The same frame of reference may not function in a future of climate uncertainty and in socio-economic scenarios, which may prove unpredictable.

To define the characteristic and differential species of the associations an objective method

has been applied that returns statistically representative groups of species (=Indicator Species Analysis) (ISA - program developed by Michele Scardi, 2005 according Dufrêne & Legendre, 1997). In terms of its application, the knowledge of these species facilitates the recognition of various associations in the field.

THERMO-XEROFILE PARASTEPPE ASSOCIATIONS OF THE TRIESTE COASTAL STRIP

Salvio officinalis-Euphorbietum fragiferae

Pioneer association on accumulations of limestone scree. Indicator species: wild sage *Salvia officinalis*, strawberry spurge *Euphorbia fragifera*, yellow germander *Teucrium flavum*.

A thermo-xerophile Dalmatian - Northern Adriatic association locally distributed along the coast of Trieste between Duino and Grignano on *grize* and limestone screes. The environment in which the association is found is typical of relict stations of the holm oak *Quercus ilex*. This association is strongly characterized by chamaephytic stenomediterranean (strictly Mediterranean) species such as *Salvia officinalis* and *Teucrium flavum* and the Illyrian chamaephytic element provided by *Euphorbia fragifera*.

The associated flora is rich in species of grasslands of the plateau above the Karst edge, highlighting the influence of nearby *Carici-Centauretum*. Compared to the similar association in Croatia, it is missing markedly Mediterranean species such as spiny spurge *Euphorbia spinosa* and *Helichrysum italicum*, pointing out the marginal position of the plant communities of the coast of Trieste, within the distribution and therefore their high vulnerability.

Lactuco vimineae-Bothriochloetum ischaemum
Parasteppe of the Northern Adriatic Karst. Indicator species: yellow bluestem *Bothriochloa ischaemun*; the fescue *Cleistogenes serotina*; dwarf morning glory *Convolvulus cantabrica*; pliant lettuce *Lactuca viminea*.

An association distributed along the northern Adriatic coast from the hill above Medea (Gorizia), probably as far as northern Istria (the valley of the Dragonja river). The stations are markedly xeric, featuring south-facing slopes, mineral soils with wide variation in their stoniness, generally poor in organic matter and therefore subject to summer desiccation, resulting in typical summer fissuring with a lozenge-shaped surface network. The environment in which the association tends to be found are those of the relict stations of holm oak woodland and the more thermophilic ones featuring downy oak *Q. pubescens*.

An important diagnostic factor is provided by the association's chromatic characteristics, typically determined by the autumn-flowering reddish-brown *Bothriochloa ischaemun* and associated species. During edaphic summer drying one can see the short stolons of the *Bothriochloa ischaemun*. It is a secondary association derived initially from deforestation and edaphic drying out, with plausible primary situations in correspondence to the edges of the ridge, probably maintained in its secondary form by grazing activities, especially by sheep. *Bothriochloa ischaemun* has an apophytic invasive tendency and can therefore be encountered a little throughout the ruderalised northern sub-Mediterranean habitats.

PRIMARY GRASSLANDS OF ROCKY EDGES

Seseli gouanii-Artemisietum albae

The community is found on the edges of the ridge of arid Mediterranean-montane environments. Indicator species include round-headed leek *Allium sphaerocephalon*, gouan's carrot *Seseli gouanii*, white wormwood *Artemisia alba*, branched bastard toadflax *Thesium divaricatum*, the junegrass *Koeleria splendens*.

The life form structure of the association is characterized by high coverage and frequency of hemicryptophyte species, followed by chamaephytes. The chorotype spectrum highlights the Mediterranean and eastern footprint of these formations. In particular, the thermophilic aspect is evident from the stenomediterranean chorotype (e.g. *Teucrium flavum*) while the eastern chorotypes are south-east European, Pontic and Illyric. These communities play host to important endemic species such as white knapweed *Centaurea alba/splendens*, crested knapweed *Centaurea cristata* and Illyrian iris *Iris cengialti/illyrica* and rare species such as the south-east European orophyte Genoa broom *Genista januensis*, highlighting an important and significant role in contributing to the biodiversity of the Karst landscape.

The association is distributed from the Valley of the Judrio to hilly parts of Istria, at stations mainly on south-facing ridges.

Genisto-Caricetum mucronatae

An association of dry montane grassland with the broom *Genista holopetala* and the sedge *Carex mucronata*. Indicative species: Kerner's spurge *Euphorbia triflora/triflora*, *Carex mucronata*, *Genista holopetala*, Clusius's gentian *Gentiana clusii*.

An association of high conservation interest widely described in the literature for the only station of the Northern Adriatic Karst (M. Čaven, Slovenia). The stations are located on the montane plateau at between 800 and 1200 m above sea level, in full light with frequent cloudiness, the cool climate of the upper montane plateau, on arid, sub-basic or neutral-basophilic soils that tend to be oligotrophic.

This is a relict post-glacial endemic association and well separated from the Croatian part of its distribution. Although not among the coastal Karst formations, this association deserves to be mentioned for its connection to the *Carici-Centaureetum*, which lies at lower altitudes (600 m a.s.l) exhibiting a clear vertical substitution.

Genisto sericeae-Seslerietum tenuifoliae

An association of windy ridges. Indicator species include silky broom *Genista sericea*, narrow-leaved moor grass *Sesleria juncifolia* (=*S. tenuifolia*), candy carrot *Athamantha turbith*, common houseleek *Sempervivum tectorum*, Gouan's carrot *Seseli gouanii*. It thrives even at low altitude (as low as 250-300 m a.s.l.) on the Karst edge on rocks, sometimes on scree, but always on very poor land. The habitat is characterized by extreme conditions in terms of water availability, lack of soil and nutrients and is always exposed to strong winds. This association is found in small areas, generally in connection with the *Carici-Centaureetum rupestris*. On the Karst rocks in the Slovenian part of Čičarija it is often linked with large areas occupied by *Carici-Centaureetum seslerietosum* (a sub-association with a high coverage of *Sesleria juncifolia*).

The physiognomy of the association is determined by the dominance both in terms of the coverage and the presence of hemicryptophytes, largely provided by *Sesleria juncifolia*. The shrubby chamaephytes represent the second component determining the character of the association, especially *Genista sericea* that is the key recognition species especially in terms of its colour, given its bright yellow blooms in spring. From an adaptive perspective, it is interesting to note the structure of the leaf of *Sesleria juncifolia*, with a flexible lamina but simultaneously resistant which as a result of its slim form and convoluted offers no resistance to the wind but is an emblematic example of adaptation to extreme conditions.

The habitat is home to a species of special conservation interest, *Athamantha turbith*, a relict species from the Tertiary period, distributed in the southeastern Alps and northern Illyria, present in Italy only in Veneto and Friuli Venezia Giulia. The relict stations in the Karst extend its northern Illyrian distribution.

PASTURES AND MEADOWS: SECONDARY SEMI-NATURAL XERO-THERMOPHILIC AND SUB-MESOPHILIC STEPPE GRASSLANDS OF THE ITALIAN AND SLOVENIAN KARST (*"LANDA"*)

Centaurea rupestris-Caricetum humilis

Sub-steppe Dinaric Karst pastures - Indicator species: dwarf sedge *Carex humilis*, rock knapweed *Centaurea rupestris*, jurinea *Jurinea mollis*, the plantain *Plantago holosteum*, mountain pasque-flower *Pulsatilla montana*, honewort *Trinia glauca* and the saw-leaved moon-daisy *Leucanthemum platylepis*.

This is an association with very wide distribution, which covers the entire Dinaric mountain chains from Montenegro to the Karst above Trieste. Along these same mountainous and hilly chains the snaking paths of the transhumance routes, activities which, over the millennia have played a fundamental syngenetic role, both as a factor in migrations, and therefore in gene flow between populations, and as a factor of disturbance (the trampling and browsing of animals), thus determining the maintenance of these communities. These are cenoses of calcareous soils characterized by the presence of rocky outcrops indicative of thin and unproductive soils with no agronomic uses other than grazing.

The physiognomy of the *Centaurea-Caricetum* is determined mainly by the dominant hemicryptophytes (*Carex humilis* and *Bromopsis erecta*) in both its montane and hill forms. At higher altitudes *Sesleria juncifolia* is present, dominant in the *Seslerietosum* subassociation and accompanying species of considerable conservation interest including great yellow gentian *Gentiana lutea/symphyandra* and the aesthetically and landscape-important poet's narcissus *Narcissus radiiflorus*. Descending in altitude the observer encounters e.g. hairy melic *Melica ciliata*, savory *Satureja*, *Jurinea mollis*, *Koeleria splendens*, all species that contribute to the appearance of the association particularly with their showy blooms.

Centaurea cristate-Chrysopogonetum grylli

The *Chrysopogonetums* of the Karst. Indicative species include crested knapweed *Centaurea cristata*; Zimmeter's cinquefoil *Potentilla zimmeteri*; scented grass *Chrysopogon gryllus*; common globularia *Globularia punctata*; the hawkbit *Leontodon crispus*; tansy ragwort *Jacobaea vulgaris*.

The association was described in 1956 and since then is considered typical of the Isonzo Karst pastures. However, the results of the research indicate that currently an impoverished form of *Danthonio-Scorzoneretum* prevails and the stations that exhibit true centaureo-crisopogonetums seem to be rare. Their sinsystematic position therefore remains open to further research.

The current ecological distribution of these lands is dependent on the micro-morphology of the land:

- Convex morphology with thin soil (10-40 cm) with a variable slope and rich in skeleton. The thermo-xerophile variant with *Centaurea cristata*, corresponding to the actual centaureo-crisopogonetums.
- Flat-concave morphology with accumulated soil that is flat, deeper (> 40 cm) poor in skeleton, grazed/mowed. The species present (Common quaking-grass *Briza media*, dropwort *Filipendula vulgare*, the umbellifer *Peucedanum oreoselinum*, brown knapweed *Centaurea jacea/weldeniana* indicate a convergence towards the dantonietums.

In general, the endemic component is an important part of the flora's composition. This geo-element is mainly represented by *Centaurea cristata* and, sporadically, by *Centaurea alba/splendens* and *Iris cengialti/illyrica*. Within the Mediterranean-Montane geoelement it is worth recalling *Potentilla zimmeteri*, whose range is restricted to the north-east sector of the Mediterranean and therefore can be considered endemic to the Karst area as far as its Italian side is concerned.

Danthonio alpinae-Scorzoneretum villosae

Sub-steppe Dinaric Karst meadows. Indicator species: villous viper's-grass *Scorzonera villosa*, *Centaurea jacea/weldeniana*, *Briza media*, Illyrian scabious *Knautia illyrica*, Siberian sainfoin *Onobrychis arenaria/tommasinii*, cut-leaf self-heal *Prunella laciniata*.

In synecological terms, the dantonietums are communities with accentuated edaphic determinism, related to flat or slightly concave soils, marl, sandstone or *terra rossa*, overlying carbonate rocks, of medium depth and free of rocky outcrops. The water availability is variable and dependent on the relief. The meadowy character of the *Dantonietums* present on carbonate substrates has been accentuated in the course of time by the de-stoning work of the soil, aimed at favouring mowing and haymaking operations.

The information provided by the abundance of data processed has allowed a better definition of the internal variability of the plant communities and the relationships with the linked floristic cenoses. The innovative aspect is provided by the identification of a transition group towards the mowed and fertilized meadows (association *Anthoxantho-Brometum* (Ant-B), *class Molinio-Arrhenatheretea*) and is important as it can help to describe and explain the high variability observed in the field. This is characterized by a group of forage species common in the meadows of the *Anthoxantho-Brometum*: false oat-grass *Arrhenatherum elatius*, sweet vernal grass *Anthoxantum odoratum/odoratum*, smooth meadow-grass *Poa pratensis* and cock's-foot *Dactylis glomerata*. These are differentiated by: rampion bellflower *Campanula rapunculus*, dropwort *Filipendula vulgaris*, shining bedstraw *Galium lucidum*, everlasting sweet pea *Lathyrus latifolius*, spiny restharrow *Ononis spinosa s.l.*, southern scabious *Scabiosa triandra*, *Scorzonera villosa*, *Bromopsis erecta* (aggr.) *Brachypodium rupestre* sl, *Briza media*.

SUMMARY OF LIFE FORM AND CHOROTYPE SPECTRUM

A reading of the adaptive response of plants to different environmental characteristics and to degree of human action exercised is given by life forms, functional characters aimed at the protection of embryonic tissues (buds or seeds) during the adverse season. The percentage of the various biological forms present in a given plant formation or in an area is known as biological spectrum.

The life form spectrum (Figure 11.2) of the Karst grasslands (*landa*) highlights their structural organization and reflects the adaptation to the environment and management pressure. To better highlight the link with the more mesophilic meadows the *Anthoxantho-Brometum* (Ant-B), a mown and fertilized meadow of the class *Molinio-Arrhenatheretea*, also known as "Karst *Arrhenateretum*", is also included. In all the cenoses the hemicryptophytes represent the predominant life form, followed by chamaephytes. The latter decrease in pasture and meadow (Ce-Ca, Ce-Chry, Dant-Sc), with minimum values in the setting of pastures and meadows, although therophytes increase with the increase in mesophilic conditions and mowing. This life form may also indicate excess of disturbance due to an intensification of management practices or alteration of the soil. The presence of shrubs and trees (NP/P) indicates the evolutionary potential, which is the spontaneous return of the forest with the cessation of mowing and grazing activities. This phenomenon affects all plant communities with the exception of hay meadows (Ant-B), which are better managed.

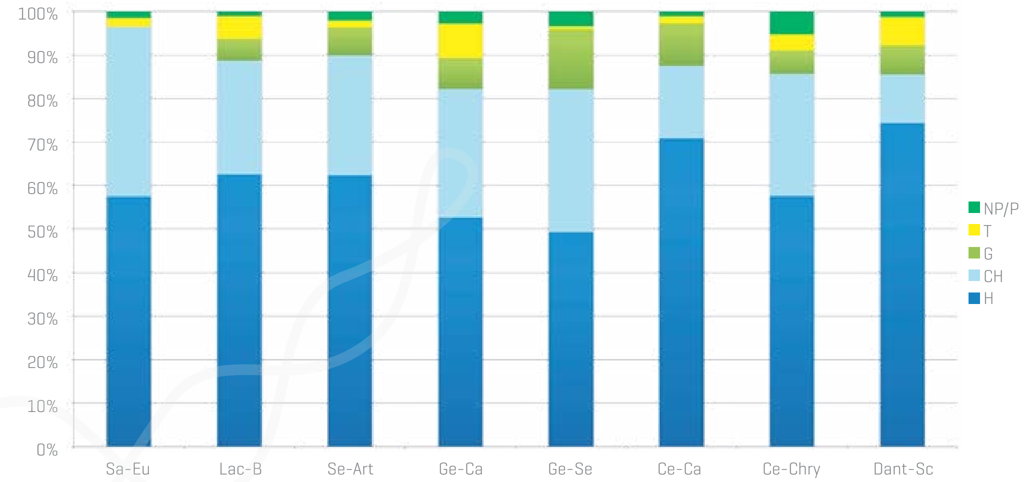


Figure 11.2: Life form spectrum of karst lands. NP (Nanophanerophytes): woody plants no more than 2m tall; P (Phanerophytes): woody perennials with buds more than 30 cm above the ground; T (Therophytes): annual plants that pass the adverse season as seeds; G (Geophytes): perennials with underground buds bulbs or rhizomes; CH (Chamaephytes): perennials, woody at the base, with buds at 20-30 cm above the ground; H (Hemicryptophytes): perennial plants with buds at ground level. Sa-Eu: *Salvio officinalis-Euphorbietum fragiferae*; Lac-B: *Lactuca vimineae – Bothryochloetum ischaemum*; Se-Art: *Seseli gouanii-Artemisietum albae*; Ge-Ca: *Genista sericeae – Seslerietum tenuifoliae*; Ge-Se: *Genista caricetum mucronatae*; Ce-Ca: *Centaurea rupestris-Caricetum humilis*; Ce-Chry: *Centaurea cristate – Chrysopogonetum grylli*; Dant-Sc: *Danthonio alpinae – Scorzoneretum villosae*; Ant-B: *Anthoxantho-Brometum*.

The complexity and floristic diversity of arid grasslands is also explained by the geographical origin of the species. This is described by the chorological spectra, or the current percentage configuration of the various geoelements evaluated according to the distribution of origin of the species in a given area or plant formation. The configuration of the distributions is the result of ecological factors and the phytogeographic history of the migration of floras, especially from the post-glacial period through to the present.

Figure 11.3 presents Principal Component Analyses (PCA) of eight different associations and proportion of chorotypes. The horizontal axis PCA 1 separates the Ge-Ca (C4) from all the others because of the presence of high levels of endemism in terms of species specifically related to montane environments. The vertical axis PCA 2 below separates a contingent of dry and hot environments linked to the Mediterranean basin together with a more mesic European one, which includes Sa-Eu (C1) and Lac-B (C2). The part above the horizontal axis is characterized by contingents from Asia and South-Eastern Europe with Atlantic elements indicative of atmospheric humidity typical of the northern Adriatic. To this group belong the Ge-Se (C5) and Ce-Chry (C7).

The Se-Art (C3), the Ca-Ce (C6) and the Dant-Sc (C8) are placed between the two quadrants described above, that is, between that coming from the Mediterranean and the eastern contingent and are characterized by the prevalence of chorotypes from arid continental areas beyond the Urals, Siberia and the Far East, demonstrating their connection with the vast steppes of continental origin.

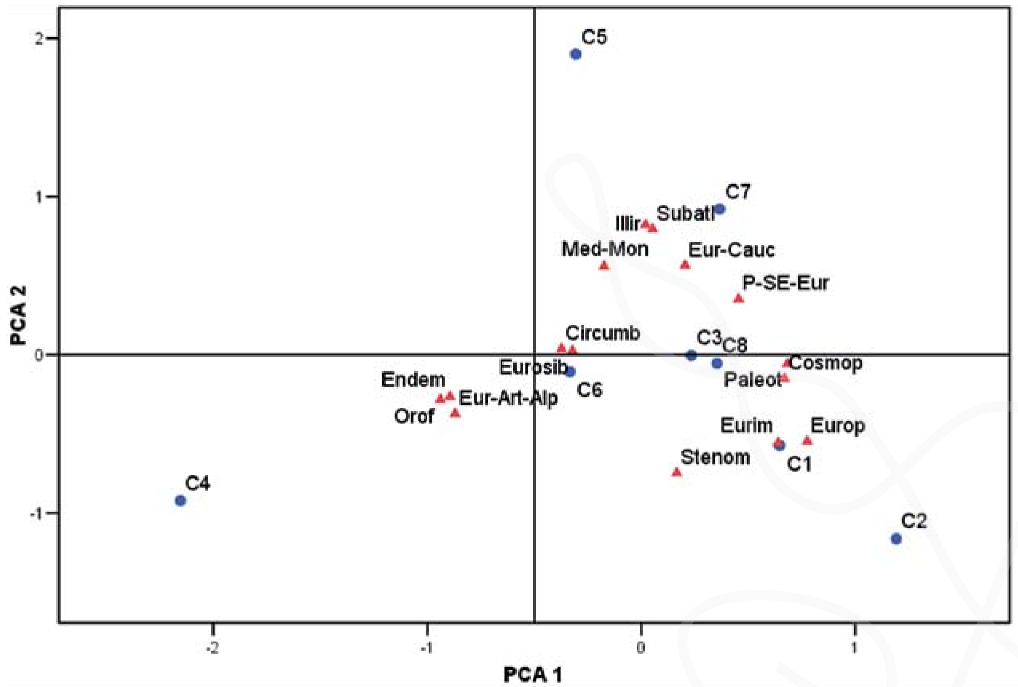


Figure 11.3: Principal Component Analyses (PCA) of of associations of the *landa carsica* and proportion of chorotypes. Legend: C1: *Salvio officinalis-Euphorbietum fragiferae*; C2: *Lactuco vimineae – Bothryochloetum ischaemum*; C3: *Seseli gouanii-Artemisietum albae*; C4: *Genisto sericeae – Seslerietum tenuifoliae*; C5: *Genisto-Caricetum mucronatae*; C6: *Centaureo rupestris-Caricetum humilis*; C7: *Centaureo cristate – Chrysopogonetum grylli*; C8: *Danthonio alpinae – Scorzoneretum villosae*. Endem (Endemic): northern Adriatic Karst; Orof (Orophyte): strictly mountainous and rocky environments; Eur-Art-Alp: Alps; Eurosib (Eurosiberian) cold-temperate and cold regions of Eurasia; Stenom (Stenomediterranean): the Mediterranean coasts; Eurim (Mediterranean): Eurasia influenced by the Mediterranean; Europ (Europe): Europe; Paleot (Paleotemperate): Eurasian and North Africa; Circum (Circumboreal): Eurasia and N America; P-SE-Eur (Pontic/SE-European): Ukraine, Pannonia, north of the Black Sea/south-eastern Europe; Med-Mon (Mediterranean-Montane): mountain ranges of south-east Europe; Illir (Illyria): Former Yugoslavia-SE-Alps; Subatl (Subatlantic): Europe and western Siberia; Eur-Cauc (European-Caucasian): Europe with significant presences to the CaucasusCosmop (Cosmopolitan): a large part of the globe.

11.4 CASE STUDIES (REFERENCE SITES AND SYNTAXONOMIC MEMBERSHIP)

Project sites were selected to be representative of a sample of meadows and pastures under different modes of management. The sites are distributed in the Italian Karst above Gorizia (Alture di Polazzo - Gorizia) and the Slovenian karst (Hrastovlje, the Podpeč uplands and Rakitovec, all in the administrative district of Koper). The choice was made according to geomorphological and vegetational criteria. Except for two meadows above Podpeč, for all other sites phytosociological relevés were made in the years 2012-13, for a total of 25 relevés (21 of pastures, 4 of meadow). The collected data was compared using cluster analysis and analysis of the similarity matrix with the analytical tables of the *Centaureo rupestris-Caricetum humilis, Centaureo cristate – Chrysopogonetum grylli, Danthonio alpinae – Scorzoneretum villosae* (phytosociological models) and parameterized in order to make them comparable with the reference model. Two sites

are not attributable to any association of *landa* (an area with permanent sheep and goat grazing in Hrastovlje and a formerly-cultivated sinkhole at the Alture di Polazzo). The remaining sites, however, fall within the *Danthonio-Scorzoneretum,* which then becomes the *model association* with which to compare the case studies.

11.4.1 ASSESSMENT OF THE CONSERVATION STATUS AND THE EVOLUTIONARY TREND

The state of preservation and the evolutionary trend of the meadows and pastures is evaluated by comparison with appropriate statistical tests of some descriptive parameters of the reference model (*Danthonio-Scorzoneretum*) with the sample sites (real state). Only the significant results of the following parameters are reported:

- Shannon diversity index, the sum of the coverages of species (N);
- number of indicator species (S_{si});
- the sum of the cover of the indicator species (N_{si});
- certain biological forms and chorological types;
- Species indicative of the integrity of the floristic cortege.

The floristic-vegetational integrity of a site is evaluated based on the number of indicator species present. In Table 11.1 shows the average values of the number of indicator species (S_{si}) and their coverage (N_{si}). The comparison between the model and the actual state indicates a marked decrease in both the number of indicator species and their abundance in the sites studied. It follows that the sites considered indicate a change in the floristic cortege compared the association historically present.

Table 11.1: Parameters of significant indicator species.

	MODEL	REAL STATE
S_{si}	11.5	7.8
N_{si}	108.1	55.5

The variation of these two parameters alone was also evaluated in relation to the allocation of sites to: (1) sites that are not attributable to any *landa* association; (2) mown meadows; (3) pastures (Table 11.2). From their comparison one can note that the lowest values of frequency and coverage of indicator species are found in the sites that cannot be classified in phytosociological terms. The values increase for pastures and meadows, but with values still far below those of the model. The sites with most the intact flora are the mown meadows.

The results clearly indicate that the permanent pasture and mixing the soil can affect the floristic-vegetational structure.

Table 11.2: Variation of parameters for indicator species in sites with different management.

	REAL STATE		
	pasture not determinable	pasture	meadows
S_{si}	4.8	8.38	8.86
N_{si}	25.8	55.08	77.36

- Life forms: structural integrity

The information provided by the statistical analysis trace a trend towards a change of the structural organization in relation to hemicryptophytes and therophytes (Table 11.3).

Table 11.3: Average frequency of statistically significant life forms. Legend: H: Hemicryptophytes; T: Therophytes.

	MODEL	REAL STATE
H	30	24.3
T	2	5.9

- Chorotype spectrum: chorological integrity

The values reported in Table 11.4 show a downward trend in the current states of the chorotypes typical of grasslands (Figure 11.3) and an increase of the Mediterranean component compared to the model. The increase in the eurimediterranean chorotype is linked to the entry of eurimediterranean therophyte species such as rough marsh-mallow *Althaea hirsuta*, sterile brome *Bromus sterilis*, woolly distaff thistle *Carthamus lanatus*, the hawksbeard *Crepis neglecta*, button medick *Medicago orbicularis* and rough clover *Trifolium scabrum*. These species settle in gaps of bare soil created by the hooves of animals under conditions of overgrazing and may damage the quality of the grazing (particularly *Carthamus lanatus*).

Table 11.4: Average frequency of statistically significant chorotypes.

	MODEL	REAL STATE
Circumboreal	1.5	0.5
Eurimediterranean	5.6	8.4
Eurosiberian	2.6	0.8
European - Caucasan Orophyte	1.4	0.7
Eurasiatic-Caucasian	7.7	5.6

THE RELATIONSHIP BETWEEN DIVERSITY AND PRODUCTIVITY

The values on productivity (Chapter 14) of the sites sampled were correlated with the corresponding parameters of species' diversity. The only significant negative correlation relationship was that between total productivity (DM (dry matter)/ kg/ha) and floristic richness (S) shown in the scatterplot in the Figure 11.4. The fields with higher values for productivity, have fewer species while the pastures with low levels of productivity possess high levels of floristic richness, in part due to their natural floristic and vegetational characteristics and in part due to the increase in therophyte species extraneous to the floristic structure of the original pastures.

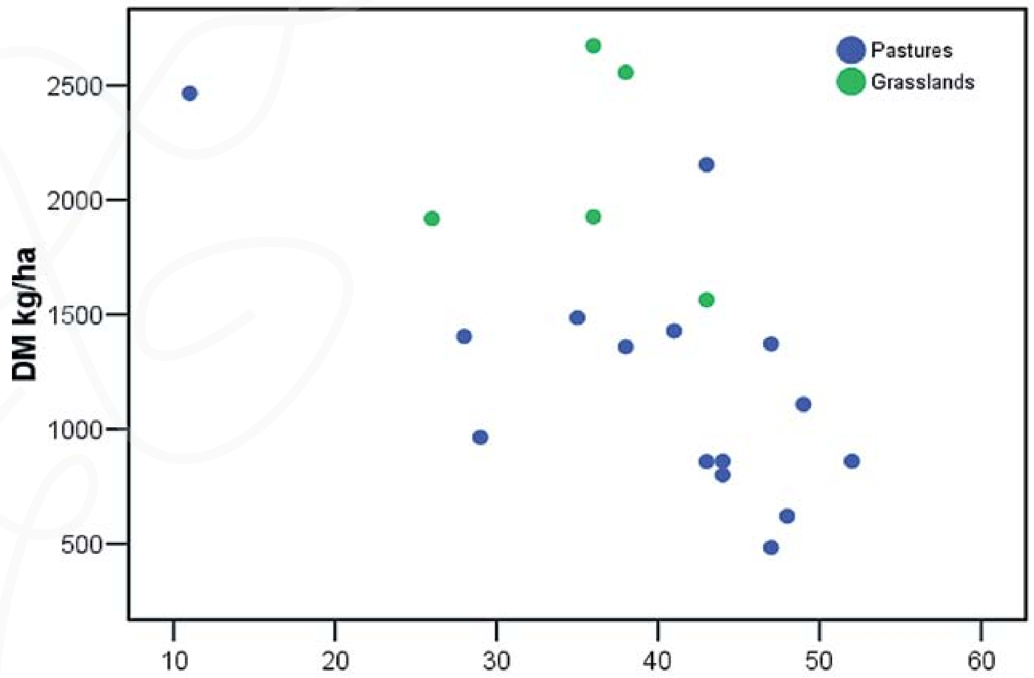


Figure 11.4: Correlation between total productivity (DM kg/ha) and floristic richness (S).

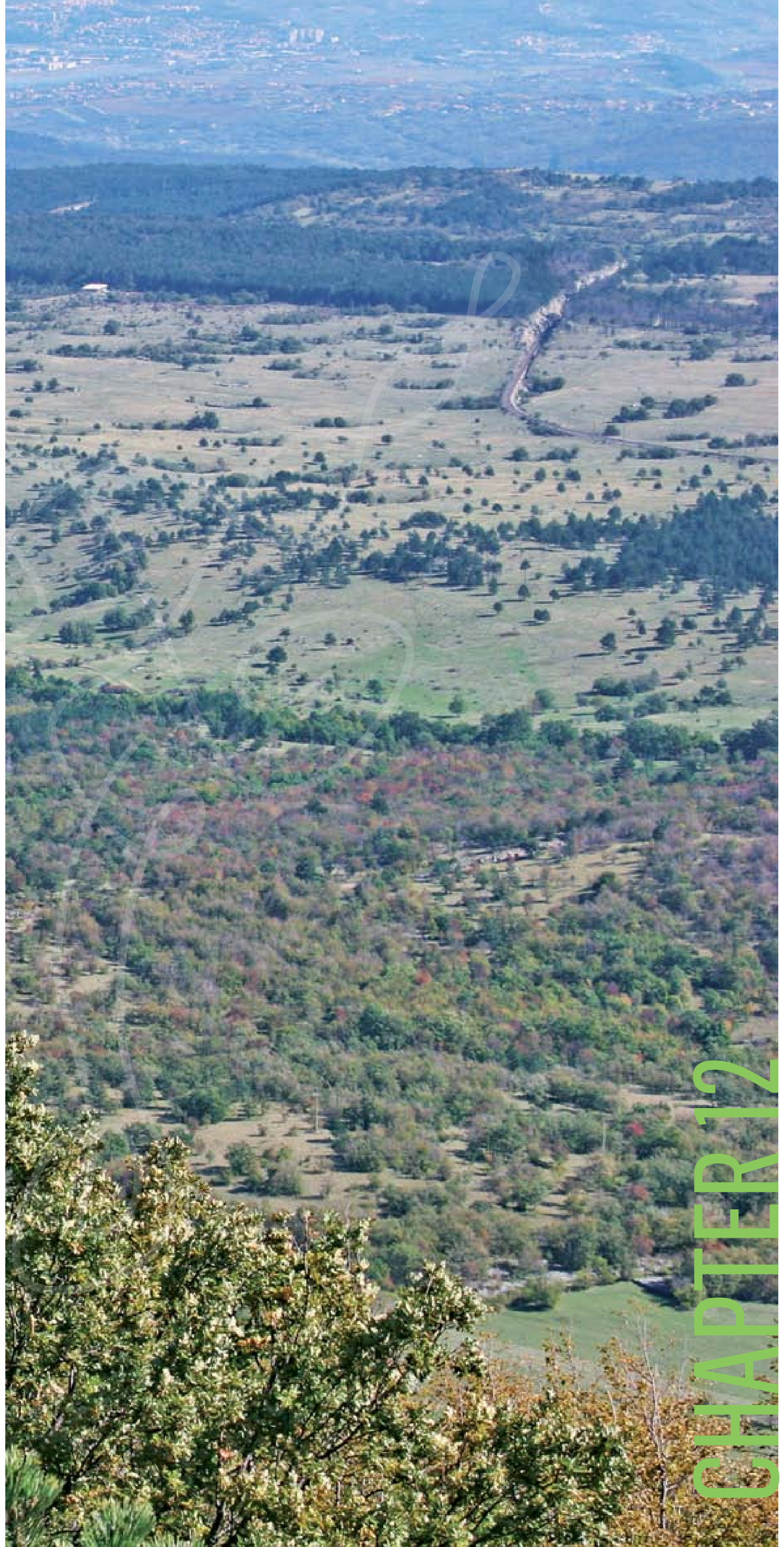
CONCLUSIONS

The alteration of the floristic and vegetational structure of the various types of *landa carsica* towards a state of lesser conservation value or even a banalization of the flora is now a widespread process. The comparison between the model and the actual state of affairs shows a significant decrease of the species typical of the association and a general structural and chorotypical deterioration. The typical *landa carsica* ecosystems are extraordinarily rich in species, that for their beauty and diversity represent high value elements of the natural and cultural landscape. Their value should not be understood solely in terms of productivity, but in terms of their multiple ecosystem services. In order to initiate strategies for "sustainable conservation" an "enhancement of biodiversity in the supply chain", should increasingly be pursued, a path that cannot be separated at its root from an in-depth knowledge of the flora and vegetation of the *landa*. It will also be of paramount importance to understand the environmental and human processes that have generated them, in parallel with the alteration processes taking place.

Only on the basis of this knowledge - and public awareness of the importance that these environments have for the Karst - will it be possible to conserve and restore this shared resource.

SUGGESTED READING

• Poldini, L. (2001): La landa carsica quale luogo di biodiversità. In: Atti Convegno "Landa carsica – Luogo d'incontro tra natura, cultura ed economia". Trieste, 27. 9. 1997. Trieste, Regione autononoma Friuli Venezia Giulia – Direzione Regionale Ambiente, WWF Sez.



CHAPTER 12

PROCESSING OF IMAGES OBTAINED USING UAV/RPAS TO ASSESS THE DEGREE OF SCRUBBING OVER OF DRY GRASSLANDS IN THE GORIZIAN KARST

OCENA ZARAŠČENOSTI POKRAJINE NA GORIŠKEM KRASU NA PODLAGI FOTOGRAFIJ IZ BREZPILOTNEGA ZRAČNEGA PLOVILA (UAV/RPAS)

ELABORAZIONE DI IMMAGINI OTTENUTE DA UAV/RPAS PER VALUTARE IL GRADO DI INCESPUGLIAMENTO DELLA LANDA CARSICA GORIZIANA

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ABSTRACT

During the project an aerial survey from a UAV (Unmanned Aerial Vehicle) mini-drone was carried out to identify the areas most suitable for the restoration of the *landa carsica*. The area covered by the research involves the part of the Karst above the River Isonzo falling within the municipality of Ronchi dei Legionari (Gorizia, NE Italy). The high spatial resolution of images (3 cm) that reveal a level of detail far superior to satellite images and aerial photos, even allowed the identification in the area of individual plant species. Using the digital model of the objects (DSM: Digital Surface Model), produced by photogrammetric reconstruction of 3D images taken by the same UAV, the height of the vegetation was obtained. With this parameter a classification with high accuracy was obtained of the study area that allowed us to distinguish the meadow areas, the overgrown ones and those covered by karst scrub. On the basis of the density of the scrubbing-over, (defined as the ratio of the scrubbed-over areas per unit area - OGU: Operational Geographic Units -, suitably identified by a grid), the areas most suitable to the restoration of the *landa carsica* were identified.

Keywords: Karst grassland, remote sensing, drone, digital surface model, scrubbing

IZVLEČEK

Z namenom, da bi opredelili najprimernejša področja za obnovo kraške pokrajine, smo na projektnem območju pripravili posnetke iz ptičje perspektive (uporabili smo zračno plovilo brez posadke, UAV). Območje raziskave je zajemalo del Krasa nad reko Sočo v okolici naselja Ronchi dei Legionari (Gorica, SV Italija). Visoka prostorska ločljivost posnetkov (na 3 cm natančno) razkriva podrobnosti in presega ločljivost satelitskih in letalskih posnetkov ter hkrati omogoča prepoznavo posameznih rastlinskih vrst na preiskovnanem področju. S pomočjo digitalnega modela predmetov (DSM: Digital Surface Model), smo pripravili tridimenzionalne rekonstrukcije in izračunali višino vegetacije. Ta informacija je omogočila razlikovanje med travniškimi, že zaraščenimi površinami in površinami v zaraščaju. Najbolj primerna območja za obnovo kraške pokrajine smo tako natančno definirali na podlagi zaraščajočih površin, ki smo jih definirali z razmerjem zaračajočih površin na enoto - OGU: Operational Geographic Units -, ter jih ustrezno označili v mreži).

Ključne besede: kraška travišča, zaznavanje na daljavo, digitalni model površine, brezpilotno zračno plovilo

RIASSUNTO

Nell'ambito del progetto è stata eseguita una ripresa area da mini-drone UAV (Unmanned Aerial Vehicle) per individuare le aree più adatte al recupero della landa carsica. L'area interessata dalla ricerca riguarda la parte del Carso isontino ricadente nel Comune di Ronchi dei Legionari (GO). L'elevata risoluzione spaziale (3 cm) delle immagini ha permesso di avvalersi di un livello di dettaglio nettamente superiore alle immagini satellitari e alle ortofoto aeree e ha consentito l'identificazione sul territorio anche di singole specie vegetali. La possibilità di usufruire inoltre di un modello digitale degli oggetti (DSM: Digital Surface Model), prodotto mediante ricostruzione fotogrammetrica 3D dalle stesse immagini UAV, ha permesso di ottenere l'altezza della vegetazione. Questo parametro ha contribuito ad ottenere una classificazione dell'area di studio ad elevata accuratezza che ha permesso di distinguere le aree prative da quelle incespugliate e dalla boscaglia carsica. La densità di incespugliamento, definita come rapporto tra aree incespugliate per unità di territorio, ha permesso di individuare le zone più idonee al recupero della landa.

Parole chiave: landa carsica, telerilevamento, aeromobile a pilotaggio remoto (APR), incespugliamento

12.1 INTRODUCTION

In the field of plant ecology the identification and description of a vegetation's spatial patterns plays a key role in an area's ecological classification. For several decades now, plant ecology, and landscape ecology more generally, has used two powerful analytical tools for the study of areas, remote sensing (Malatesta *et al.,* 2013) and Geographic Information Systems (GIS), the latter tool being more representative of the current geographical information technology (Poldini *et al.,* 2014).

The recent advent of proximal or low altitude remote sensing using small drones (UAV/RPAS) opens up new perspectives for the study of the vegetational landscape. The advantage of these aircraft lies mainly in the high spatial resolution and their operativity, which is significantly simplified, compared to traditional aerial and satellite platforms. It is in this context that the research presented in this work takes place with the aim of assessing the degree of scrubbing-over of the *landa carsica* in order to plan targeted restoration actions.

12.2 STUDY AREA

The Karst is a limestone plateau, sedimentary in origin and very broad. The western part of the Isonzo Karst is bounded to the north by the rivers Isonzo and Vipacco and to the south by the depression of the Vallone, the area covered by the research concerns the part falling within the municipality of Ronchi dei Legionari (Gorizia, NE Italy) located on Monte Sopra Selz. The vegetation of the area consists of the xero-thermophilous grasslands typical of the *landa carsica* and its dynamic phases of vegetational succession.

12.3 *LANDA CARSICA*

The *landa* is one of the most interesting herbaceous (grassland) formations of the Karst landscape and its rich flora, up to 60–70 species per 150–200 square metres (Poldini, 2009), is such as to be included in the European network of protected areas known as *Natura 2000*. The *landa* is a secondary zoogenic prairie, formed as a result of grazing sheep (in ancient times) and cows (more recently) on deforested areas. This deforestation became particularly evident about 4,000 years ago, roughly in the Bronze Age and early Iron Age when the '*Castellieri*' (=hill forts) civilization flourished. The abandonment of grazing, a widespread activity in the Karst until the middle of the last century, has led to a phenomenon of vegetational succession ('scrubbing over') and the spontaneous recovery of the forest (karst scrub), greatly reducing the areas of *landa carsica*, with a major threat of biodiversity loss.

Today, the recovery of the *landa*, as well as the supporting biodiversity, would also reduce the risk of severe fires that affect the Karst area, thus creating a huge saving on emergency response operations.

12.4 MICRO-UAVS (DRONES)

In recent years, the rapid advances in the field of sensors combined with the high-performance batteries and motors, have allowed the development of a new class of aircraft known as UAV (Unmanned Aerial Vehicles), also known by the acronym RPAS (Remotely Piloted Aircraft Systems) or more commonly called "drones". UAVs are aircraft designed to operate without a human pilot on board, and are remote-controlled from the ground. The drone we used, provided by the AIRMAP company of Trieste, is a quadricopter with a autonomy of about 100 minutes.

The photos of the study area were carried out on the morning of June 19th 2013. The total area surveyed covered 700,000 square metres obtained by performing 350 photographs along eight 1400-metre strips flying at a height of 80 metres and with a spatial resolution on the ground of 3 cm per pixel. The digital camera (a Canon S100) mounted on the drone filmed the area in the three visible components of the electromagnetic spectrum: blue, green and red.

12.5 CLASSIFICATION OF THE IMAGES FROM THE UAV

The image processing was performed on a sample area of 12,800 square metres, representative of the area covered by the study. The image shown in Figure 12.1 was obtained by georeferencing and mosaicking of the original UAV photo.

Figure 12.1 : Mosaicking of UAV images from the sample area studied.

To distinguish between the different types of vegetation (grassland areas, areas undergoing scrubbing-over and karst scrub), three methods of supervised classification of the UAV images were applied and compared. This technique, unlike the unsupervised classification, is based on knowledge of certain areas of the sample (termed 'training areas' and 'validation areas') that are representative of the thematic classes to be obtained. The methods used were as follows:

1) Maximum likelihood classifier with a Gaussian model (Maximum Likelihood, ML);

2) SMAP classifier (Sequential Maximum *A Posteriori*) with a Gaussian mixture model;

3) Non-parametric Random Forest classifier (RF), which is one of the 'tree classifiers' or 'decision trees'.

The first and the third use only the spectral information of each pixel of the image (pixel-based) while the second also exploits the spectral information of the contour (object-based). The first two classifiers used (SMAP and ML) are part of the GIS GRASS software package (Geographic Researcher Analysis Support System) (Neteler & Mitasova, 2008) while the Random Forest package is part of the statistical software "R". All of these programmes are free to download from the internet.

For each classification the accuracy was tested using the *kappa* coefficient applied to the confusion matrix that allowed us to identify the classification more accurately. Higher accuracy was obtained with use of the contextual classifier (SMAP) for which the value of the *kappa* coefficient was 0.8 (Table 12.1).

Table 12.1: Percentage of overall accuracy and kappa values for three classifiers.

Classifier	% Overall accuracy	Kappa
ML	84.74	0.78
SMAP	86.03	0.80
RF	79.33	0.71

12.6 3D MODEL OF THE GROUND SURFACE (DSM) AND POST-CLASSIFICATION

Aerial photogrammetry is a technique that allows the acquisition of important metric data for an object (shape and position) through the analysis of pairs of frames using the principle of stereoscopy. On the basis of the photographs acquired by the drone and as a result of the partial overlap of the frames it was possible to create a three-dimensional model of the ground surface (DSM) of the area of interest (Figure 12.2). The DSM (Digital Surface Model) represents, in digital form, the level of the land with all the objects found on a given plot including buildings, infrastructure and vegetation.

The difference between the altitudes measured between the digital terrain model (DTM), which only measures the trends encountered in the geodesic surface, and the DSM allowed the height of the vegetation to be derived. The model of the heights was validated, in the sample, by means of a series of ground measurements.

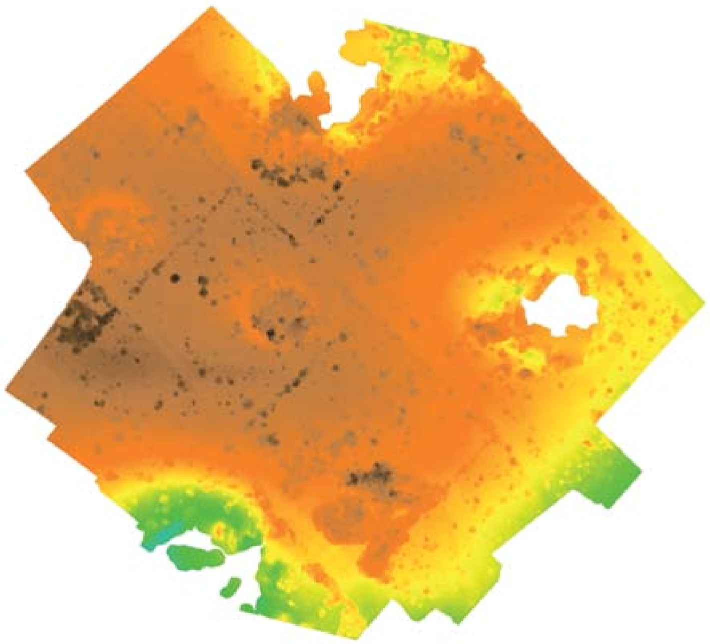

Figure 12.2: Digital Surface Model (DSM) obtained from the analysis of the photos taken by the drone.

12.7 POST-CLASSIFICATION

The height of the vegetation allowed for the establishment, in the GRASS GIS environment, of a set of decision rules for post-classification to check the veracity of the membership classes of the pixels. For example, the smoke tree (*Cotinus coggygria*) with a reddish inflorescence and the typical red soil of the Karst are often not distinguished by the classifier due to their very similar chromaticity. Taking advantage of this parameter, it was possible to thematise the two objects well.

After post-classification (SMAPost), applied to the classified map using the SMAP method, the value of the accuracy is also increased (Table 12.2).

Table 12.2: Percentage of overall accuracy and kappa values for the two classifiers (SMAP, SMAPost).

Classifier	% Overall accuracy	Kappa
SMAP	86.03	0.80
SMAPost	88.22	0.83

Figure 12.3 shows the classified map with the eight classes of land cover for which the percentage coverage is shown in Table 12.3.

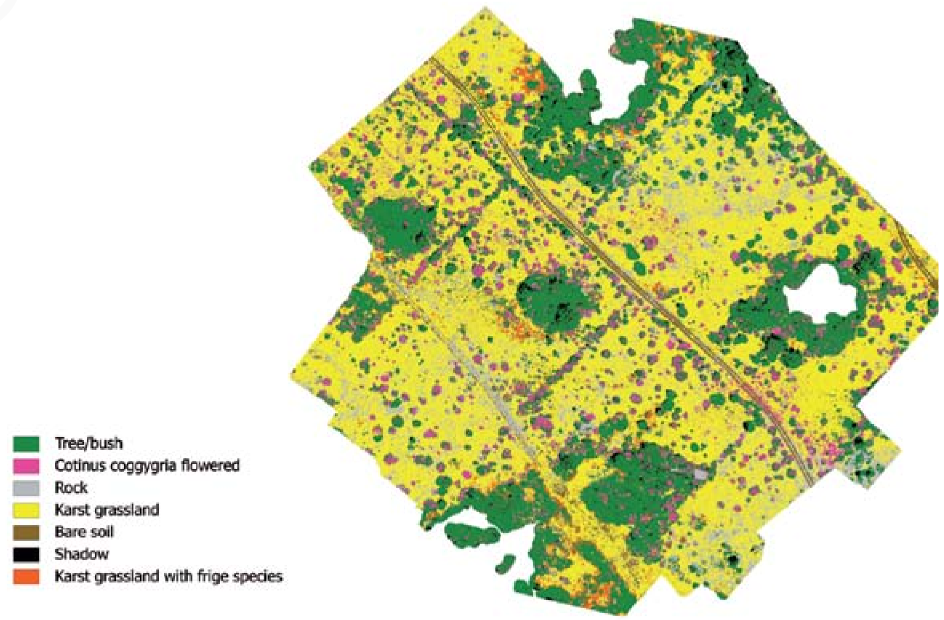

Figure 12.3: Classified map produced using the SMAP (*Sequential Maximum A Posteriori*) classifier following post-classification.

Table 12.3: Percentages of land cover for seven classes.

	Classes	% Cover
1	tree and shrub species	28.08
2	smoke tree in flower	6.85
3	bare rock	9.8
4	*landa carsica*	49.09
5	bare soil	1.94
6	shadow (attributable to the *landa*)	1.39
7	*landa* with old 'thatch' (*karst grassland with fringe species*)	2.85

The percentage cover of the classes, grouped by the main types encountered, are as follows: 53.3 % of *landa* (including *landa* with thatch), 34.9 % of tree / shrub species (including smoke tree in bloom) and 11.7 % of un-vegetated areas (rock and bare soil).

12.8 ESTIMATION OF THE DEGREE OF SCRUBBING-OVER OF THE *LANDA CARSICA*

The density of scrubbing-over (DI) expressed as a percentage was evaluated in the context of quadrats covering 400 square metres (20 x 20 metres) obtained by superimposing a regular grid of cells (OGU: Operational Geographic Unit) (Altobelli *et al.*, 2001) on the classified map. In the GRASS GIS environment, for each OGU, the DI was derived from the percentage of overall coverage of the classes related to the species of trees and shrubs. All the OGU were grouped into five classes by scrubbing-over density obtained by subdividing the percentage of DI according to the Braun-Blanquet scale (Table 12.4).

Table 12.4: Distribution of OGU in five classes of density on the basis of the percentage of scrubbing-over.

DI Class	Density limits (%)	Freq. OGU	Rel. freq. (%)	Accum. rel. freq. (%)
1	0 - 5	1	0.7	0.7
2	>5 - 25	64	46.4	47.1
3	>25 - 50	47	34.1	81.2
4	>50 - 75	18	13.0	94.2
5	>75 - 100	8	5.8	100.0
Totals		138	100	

The cells belonging to classes 1 and 2, with the lowest values of scrubbing over and/or afforestation, constituting about 50 % of the study area (Figure 12.4) were considered to be the most economically advantageous for *landa carsica* restoration. The entire area of the sample assessed can be considered suitable for conversion to pasture.

Figure 12.4: UAV images with the quadrats (20 x 20 metres) superimposed in red with a scrubbing-over density less than or equal to 25 %.

CONCLUSIONS

The advantages offered by the use of the drone for the study of vegetation are as follows: the flexibility of the programming of the flight, the simplicity of the aircraft's operation and the high level of detail of the pictures taken. By contrast, the major limitation was, in our case, the use of a camera only able to take photos in the visible and not in the infrared part of the spectrum. This part of the electromagnetic spectrum is of fundamental importance in obtaining a physiognomic-structural classification of the vegetation.

This limit was overcome by the possibility of obtaining a three-dimensional model of the vegetation with its height using stereoscopic processing of the original images. This allowed a good separation of the different classes of land cover in the post-classification phase. Once the classified map had been produced it was possible to derive the scrubbing-over density and identify the most economically advantageous areas for the restoration of the *landa carsica*.

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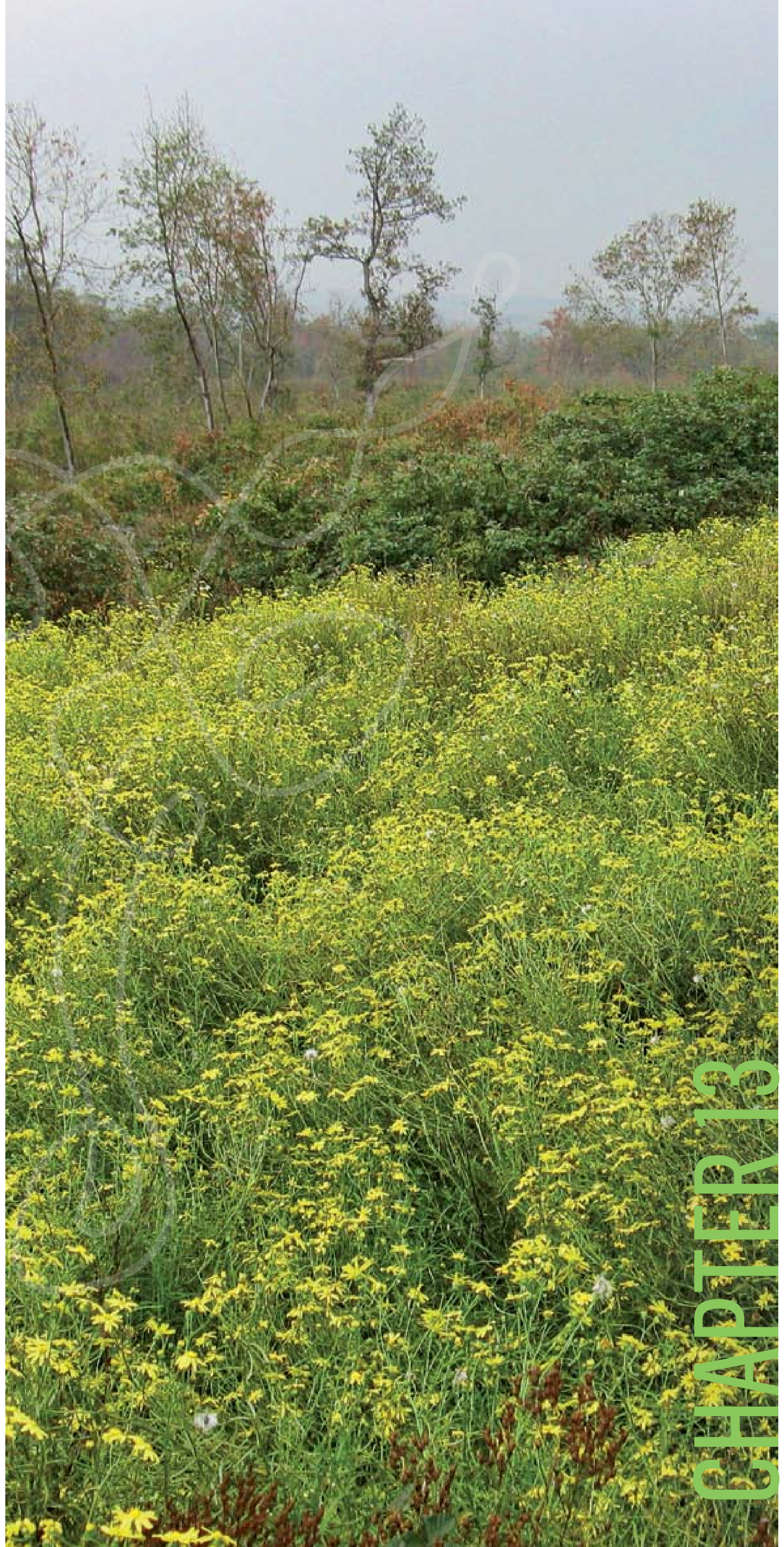

CHAPTER 13

CONSERVATION OF PLANT DIVERSITY OF KARST DRY GRASSLANDS BY THE REINTRODUCTION OF GRAZING. THE CASE STUDY OF BASOVIZZA/ BAZOVICA IN THE ITALIAN KARST

VAROVANJE RASTLINSKE RAZNOLIKOSTI KRAŠKIH SUHIH TRAVIŠČ S PONOVNO VZPOSTAVITVIJO PAŠE. PRIMER IZ BAZOVICE NA ITALIJANSKEM KRASU

CONSERVAZIONE DELLA DIVERSITÀ VEGETALE DELLE PRATERIE A RIDE CARSICHE MEDIANTE REINTRODUZIONE DEL PASCOLO. L'ESEMPIO DI BASOVIZZA / BAZOVICA SUL CARSO ITALIANO

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ABSTRACT

Floristic and vegetation studies were conducted in an experimental area of the Italian Karst at Basovizza/Bazovica included in the *Natura 2000* site "Carso Triestino e Goriziano" to assess the effects of the reintroduction of grazing on plant species and communities. In the area, partially deforested in 2006, cattle, sheep and goats were introduced in the summer of 2007. A monitoring programme for plant populations was performed focusing on four target species typical of the karst dry grasslands: Rock Knapweed *Centaurea rupestris*, Green-winged Orchid *Orchis morio*, Toothed Orchid *Orchis tridentata* and Mountain Pasqueflower *Pulsatilla montana*. Three exotic invasive species, detrimental to grazing, were also included: Tree of Heaven *Ailanthus altissima*, Narrow-leaved Ragwort *Senecio inaequidens* and Common Ragweed *Ambrosia artemisiifolia*. For each species, the GPS position was taken both for individuals and large groups of plants; a count of the individuals present at each measured point was performed too. In order to verify the process of re-colonization by species of calcareous dry grasslands, phytosociological relevés were carried out in areas previously occupied by Black Pine (*Pinus nigra*) plantations, woods with Downy Oak (*Quercus pubescens*) and Hop Hornbeam (*Ostrya carpinifolia*) and thermophilous scrub of Common Juniper (*Juniperus communis*), documented from vegetation maps made in 2005. For each vegetation type 6 permanent plots were selected, in which phytosociological relevés were carried out to. The study shows that in the areas of the original karst dry grassland more widely distributed species typical of the habitat remain. In overgrazed areas the grassy turf is discontinuous, segetal terophytic species of the class *Stellarietea mediae* entering into the gaps. Cover of the Musk Thistle (*Carduus nutans*) has increased significantly, as spiny species are not palatable to animals. The occasional presence of neophytes such as Daisy Fleabane (*Erigeron annuus*) and Canadian Horseweed (*Conyza canadensis*) was also found. In undergrazed areas, however, the natural processes of succession and scrubbing over are taking place. In the areas formerly occupied by pine plantations and woods with *Quercus pubescens* and *Ostrya carpinifolia*, Autumn Moor Grass (*Sesleria autumnalis*) and Rock Tor-grass (*Brachypodium rupestre*) are still able to become covered by a compact herbaceous sward. An abundant presence of *Carduus nutans* and the entry of *Senecio inaequidens*, the latter especially abundant on wood chips derived from tree clearing, were encountered as well as the spread of *Ailanthus altissima* favoured by the opening up of the scrubland.

Key words: Karst dry grasslands, grazing, invasive plants, monitoring

IZVLEČEK

Znotraj poskusnega območja italijanskega Krasa v bližini Bazovice, ki je del SCI območja "Tržaški in Goriški Kras" smo izvedli floristično/vegetacijske raziskave s katerimi smo želeli ugotoviti vpliv ponovne vzpostavitve paše na rastlinske vrste in združbe. Na območju, ki je bilo deloma očiščeno gozda med letoma 2006 in 2007, je bila v odboju pomlad-poletje 2008 ponovno vpeljana paša govedi in drobnice. Izvedli smo popise ciljnih indikatorskih vrst kraških travišč (*Centaurea rupestris*, *Orchis morio*, *Orchis tridentata* in *Pulsatilla montana*) in nekaterih tujerodnih invazivnih vrst (*Ailanthus altissima*, *Senecio inaequidens* in *Ambrosia artemisiifoli*), ki zavirajo pašo. Vsaki izbrani vrsti smo določili GPS položaj osebka ali populacijskih jeder ter prešteli vse osebke na določenem območju. Izvedli smo fitocenološke popise na območjih, ki jih je nekoč prekrival nasad borovega gozda, gozdovi puhastega hrasta in črnega gabra ter termofilna grmišča z navadnim brinom, ki so bila dokumentirana v okviru kartografskih popisov leta 2005; za vsako tipologijo vegetacije smo izbrali 6 lokacij znotraj katerih smo izvedli fitocenološki popis z namenom ugotovitve procesa ponovne kolonizacije travniščnih vrst. Rezultati raziskave kažejo, da na površinah, ki so jih prvotno prekrivala travišča ostajajo vrste habitatov s širšim arealom. Na prepašenih površinah je bila travna ruša pogosto prekinjena, prekinitve pa so zasedli ruderalni terofiti iz razreda *Stellarietea mediae*; znatno se je povečalo število *Carduus nutans*, trnate zeli, ki jo živina ne popase. Gre omeniti tudi pojavljanje posameznih primerkov vrst *Erigeron annuus* in *Conyza canadensis*. Na območjih kjer paša ni bila intenzivna, smo lahko opazili naravni proces zaraščanja. Na območjih, ki jih je nekoč prekrival nasad črnega bora in gozdovi puhastega hrasta in črnega gabra, vrsti *Sesleria autumnalis* in *Brachypodium rupestre* še vedno prevladujeta v travni ruši. Opazili smo množično prisotnost vrste *Carduus nutans*, močan vdor vrste *Senecio inaequidens*, predvsem na območju poseke, in širjenje vrste *Ailanthus altissima* kot posledice odstranitve gozda.

Ključne besede: kraška travišča, paša, invazivne vrste, monitoring

RIASSUNTO

In un'area sperimentale del Carso italiano presso Basovizza/Bazovica, ricadente nella ZSC "Carso triestino e goriziano" sono stati condotti studi floristico-vegetazionali per valutare gli effetti della reintroduzione del pascolo su specie e comunità vegetali. Nell'area, parzialmente disboscata nel 2006 sono stati introdotti bovini, ovini e caprini nella primavera-estate 2008. Sono stati effettuati campionamenti di specie target indicatrici del tipo di landa (*Centaurea rupestris*, *Orchis morio*, *Orchis tridentata* e *Pulsatilla montana*) e delle specie avventizie invasive *Ailanthus altissima*, *Senecio inaequidens* e *Ambrosia artemisiifolia*, detrattori del pascolo. Per ogni specie vegetale selezionata è stata rilevata mediante GPS la posizione dei singoli individui o dei nuclei di popolazione ed effettuata la conta degli individui per ogni punto rilevato. Sono stati eseguiti inoltre rilievi fitosociologici in aree precedentemente occupate da pinete d'impianto, querco-ostrieti e cespuglieti termofili a ginepro comune documentate dalla cartografia realizzata nel 2005; per ogni tipologia vegetale sono state selezionate 6 stazioni nelle quali sono stati effettuati rilievi fitosociologici per verificare il processo di ricolonizzazione da parte delle specie di landa. Dallo studio emerge che sulle superfici originarie di landa carsica si mantengono specie tipiche dell'habitat ad areale più ampio. Sulle superfici sovrapascolate il cotico erbaceo risulta fessurato e nelle lacune sono entrate specie terofitiche ruderali di *Stellarietea mediae*; è aumentata sensibilmente la copertura di *Carduus nutans*, specie spinescente non appetita dagli animali. Si segnala inoltre la sporadica presenza delle neofite *Erigeron annuus* e *Conyza canadensis*. Nelle aree sottopascolate sono invece ripresi i processi di incespugliamento naturale. Sulle aree un tempo occupate da pineta d'impianto e dell' ostrio-querceto, *Sesleria autumnalis* e *Brachypodium rupestre* tendono a creare ancora un cotico compatto. E' stata rilevata abbondante presenza di *Carduus nutans*, ingressione di *Senecio inaequidens* abbondante soprattutto sul cippato derivato dall'attività di disbosco, e diffusione di *Ailanthus altissima* favorito dall'apertura della boscaglia.

Parole chiave: landa carsica, pascolo, specie invasive, monitoraggio

13.1 INTRODUCTION

Since World War II, grazing and animal husbandry have undergone a major decline due to changes in socio-economic conditions, with negative consequences for land management. As a result, secondary succession began its course leading to the progressive scrubbing-over of the karst dry grasslands (*landa carsica*), re-establishing forest cover through a series of intermediate stages. This has led to the current situation characterized by forests extending over more than 60 % of the Karst of Trieste, on the one hand, and the reduction of semi-natural herbaceous communities to less than 20 % land cover, on the other (Poldini, 2009).

An experimental area for restoration and maintenance of the karst dry grassland is located near the village of Basovizza/Bazovica, in the Municipality of Trieste, NE Italy, next to the Basovizza/Bazovica – Lipizza/Lipica border crossing, below the southern slopes of Mount Cocusso/Kokoš (674 m a.s.l). It falls within the SPA - IT3341002 "Aree carsiche della Venezia Giulia "/SAC-IT3340006 "Carso Triestino e Goriziano". The area had already been identified as part of a previous project for the conservation of the dry calcareous grassland habitat (62A0 - Eastern sub-Mediterranean - dry grasslands (*Scorzoneretalia villosae*), in Annex I of the 92/43/EEC "Habitats" Directive) through the reintroduction of extensive grazing, carried out by the Province of Trieste in cooperation with the Universities of Trieste and Padua from 2005 to 2009. This part of the Karst area is characterized by a high biodiversity of flora (cca. 750 species) with a high concentration of endemic and/or rare species (Poldini, 2009).

Research was conducted on the area between 2005 and 2006 to define the situation *ex ante* in terms of floristic diversity. The actual vegetation map made in 2005 (scale 1:5,000) shows 21 vegetation types including woodlands, mantels, hedgerows, hems, karst dry calcareous grasslands, meadows, ruderal vegetation and anthropic elements (Poldini, 1982, 1989; Poldini & Vidali, 1995; Poldini *et al.*, 2002), grouped into the two main following series.

- Edapho-xerophilic woodland composed of hop hornbeam (*Ostrya carpinifolia*) and downy oak (*Quercus pubescens*) of a mesophilic type with cornelian cherry (*Cornus mas*) (*Aristolochio-Quercetum pubescentis cornetosum maris*) (Poldini, 2008), which grows on calcareous xeric soils, from shallow to medium depth on south-facing slopes.
- High oak forest (*Seslerio-Quercetum petraeae*), predominantly sessile oak (*Quercus petraea*) and turkey oak (*Quercus cerris*) on more mature soils with a moderate north-facing slope (colluvial soils, decalcified *terra rossa* soils).

Alongside these two main types of vegetation are the polycormic Hop Hornbeam karstic scrub (*Seslerio-Ostryetum carpinifoliae*) and plantations of black pine (*Pinus nigra*), mainly exhibiting the renewal of local broadleaf species.

The downy oak forest gives rise to moderately thermophile karst pasture on relatively deep soils (*Carici-Centaureetum rupestris leucanthementosum liburnici*), with the sessile and Turkey oak high forest yielding the meadow-pasture (*Danthonio-Scorzoneretum*) (Poldini, 1989; Poldini & Kaligarič, 1997) and hay meadows (*Anthoxantho- Brometum erecti*).

To restore areas for reuse as pasture, at the end of

2006 an initial plot of *c.* 15 hectares was cleared, where, during the summer of 2007, cattle, sheep and goats were introduced. From 2007 to 2009 an initial set of measurements were carried out on this site in order to evaluate the effects of the reintroduction of grazing on the species and plant communities. Later, in 2010, a more substantial intervention was carried out with the acquisition of additional land to be used as grazing for a total of 43.1 hectares.

The research was re-started in the same area in the context of the BioDiNet cross-border project - (WP 4.3 "Monitoring of biodiversity following the restoration of grazing in an area of karst grassland in the Italian Karst") - to test the effects of grazing on biodiversity by monitoring plant species and vegetation. In particular, the following data were recorded:

- boundaries of the areas currently grazed;
- monitoring of the floristic composition within 6 permanent plots of pre-existing dry grassland (10 x 10 m) where, in the previous project with the Province of Trieste, analyses were carried out on the phytomass and productivity of the pasture;
- phytosociological relevés on the areas mapped as downy oak woodland, pine plantations or thermophile scrubland in 2005, before the deforestation work and grazing;
- monitoring of the nemoral grasses *Sesleria autumnalis* and *Brachypodium rupestre* and other dry grassland species using two continuous belt transects positioned within grazed areas located in formerly wooded areas, in order to assess the influence of grazing and/or deforestation on their distribution;
- monitoring of populations of selected target species (toothed orchid *Orchis tridentata*, green-winged orchid *Orchis morio*, mountain pasqueflower *Pulsatilla montana* and rock knapweed *Centaurea rupestris*) using GPS (position measurement and counting of individuals);
- monitoring of the neophytes invasive species tree of heaven (*Ailanthus altissima*), narrow-leaved ragwort (*Senecio inaequidens*) and common ragweed (*Ambrosia artemisiifolia*) using GPS (position measurement, counting of individuals and/or measuring of the areas, estimating percentage cover of the individual plants).

Figure 13.1: Study area near Basovizza/Bazovica (Trieste). Highlighted in blue are the boundaries of the grazed areas. Symbols: yellow squares = phytosociological relevés of xerophilous grassland (*Carici-Centaureetum rupestris*), orange square = phytosociological relevés of former scrub surfaces of juniper and smoketree, square brown = phytosociological relevés of former downy oak woods surfaces, green squares = phytosociological relevés of former Black Pine woods surfaces, blue star = transect dominated by *Brachypodium rupestre*, purple star = transect dominated by *Sesleria autumnalis*

The grazed area was re-assessed using GPS (cca. 58 hectares), given that since 2005 the plots have been gradually enlarged and the grazing activity was not started at the same time in each of them but staggered over time. There are four main areas (Figure 11.1), each divided into sub-units for the rotational grazing of cattle, sheep or goats. Given that certain areas are predestined to degradation due to essential infrastructure (barn, paddocks, mobile watering tanks, feeding areas), the following changes to both the herbaceous sward of the pasture and the target and neophyte species were observed.

13.2 CHANGES IN THE PASTURE

To monitor the effects of grazing and to assess the recovery of the karst grassland species in the areas that were once forested a range of methods were used.

13.2.1. PHYTOSOCIOLOGICAL SURVEY OF PRE-EXISTING GRASSLAND AREAS

In 6 10 x 10 m permanent plots on pre-existing areas of grassland, corresponding to the moderately thermophile karst grassland of the association *Carici-Centaureetum rupestris*, already sampled before the introduction of grazing, phytosociological relevés were repeated in 2013 to check for any changes resulting from such activity. The grazing load capacity for this type of grassland, calculated by the University of Padua's Department of Agronomy, Animal Nutrition, Natural Resources and Environment (DAFNAE), is between 0.41 and 2.72 LU ha^{-1} (livestock units per hectare) for dry grassland with an abundance of rock outcrops, while for those with few rocky outcrops varies between 0.71 and 4.74 LU ha-1. This analysis was used to define the number of grazing animals in the experimental area.

One can note a reduction in narrow-leaved flax (*Linum tenuifolium*), an increase in species favoured by the discontinuity in the herbaceous sward produced by trampling including the vernal sandwort (*Minuartia glaucina*), bulbous meadow-grass (*Poa bulbosa*) and thyme-leaved sandwort (*Arenaria serpyllifolia*). Some species regarded as "weeds of pastures", such as common ragwort (*Senecio jacobaea*), had increased and one can note the entry of segetal species such as long-stalked crane's-bill (*Geranium columbinum*), the nitrophilous long-headed clover (*Trifolium incarnatum* subsp. *molineri*) and an increase in cover of the spiny musk thistle (*Carduus nutans*).

13.2.2. PHYTOSOCIOLOGICAL SURVEY OF GRAZED AREA AFTER TREE AND SCRUB CLEARANCE

On areas that were once occupied by Downy Oak woods, by Black Pine plantations with deciduous renewal and by the Common Juniper and Smoketree scrubland, a resumption of the process of re-colonization took place by forage species such as upright brome (*Bromopsis erecta* aggr.) (with a coverage of 21 – 60 % in areas of former scrubland), dwarf sedge (*Carex humilis*), Tommasini's cinquefoil (*Potentilla acaulis* subsp. *tommasiniana*), Mediterranean creeping thyme (*Thymus longicaulis*) and the prostrate canary clover (*Dorycnium germanicum*). The two grasses *Sesleria autumnalis* and *Brachypodium rupestre* remain on the areas of the former pine forest and oak woodland sometimes with more than 60 % ground cover, while they are almost absent on the areas of former scrubland.

13.2.3. MONITORING OF NEMORAL GRASS SPECIES BY TRANSECTS

In formerly wooded areas subjected to grazing for seven years, monitoring was also carried out using continuous belt transects to assess the two main groundcover species of the karstic scrub and woodland, the grasses *Sesleria autumnalis*, typical of the well-lit woodlands of downy oak, and *Brachypodium rupestre*, a herbaceous species that dominates in the herbaceous fringes. These two large herbs represent poor forage for grazing animals and reproduce vegetatively, forming a compact herbaceous layer, which is competitive in relation to other species. The two transects, one dominant in *Sesleria* and the other featuring large quantities of *Brachypodium*, are 7 m long, divided into 7 subplots of 1 x 1 m, and were positioned within formerly wooded areas (respectively in downy oak woodland and a black pine plantation dominated by broadleaf trees) and grazed. With this sampling, the different behaviour over time of *Brachypodium* and *Sesleria* following the variation of two limiting factors, light (increased at ground level as a result of deforestation) and grazing (effects of the browsing, trampling and manuring) was analyzed. Within each 1 x1 m subplot the percentage cover of all the occurring species was estimated. In addition, the areas occupied by the two grass species as well as shrubs and thorny species were also recorded to scale.

Figures 13.2 and 13.3 show the projections of the selected analyzed species within the two transect over time: it can be seen that both the grasses undergo a considerable reduction. Looking at the measurements on these surfaces from 2007 to 2013, *Brachypodium rupestre* and *Sesleria autumnalis* suffer a significant reduction in favour of some potential forage species such as *Potentilla acaulis* subsp. *tommasiniana*, *Thymus longicaulis*, *Dorycnium germanicum* and *Bromopsis erecta* (aggr.). On the other hand there is an increase of spiny species, favoured by negative grazing selection such as *Carduus nutans* and, to a lesser extent, Amethyst Eryngo (*Eryngium amethystinum*).

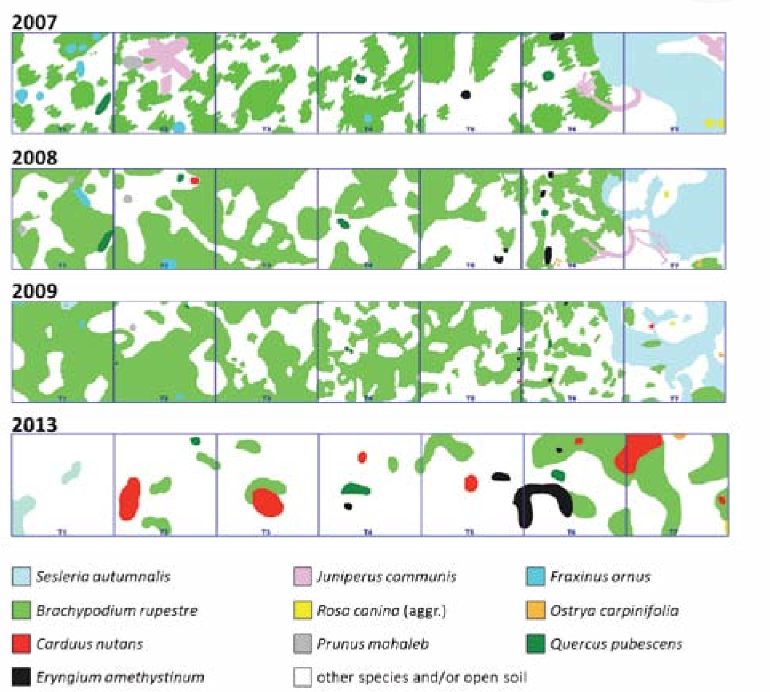

Figure 13.2: Transect dominated by *Brachypodium rupestre* detected from 2007 to 2013.

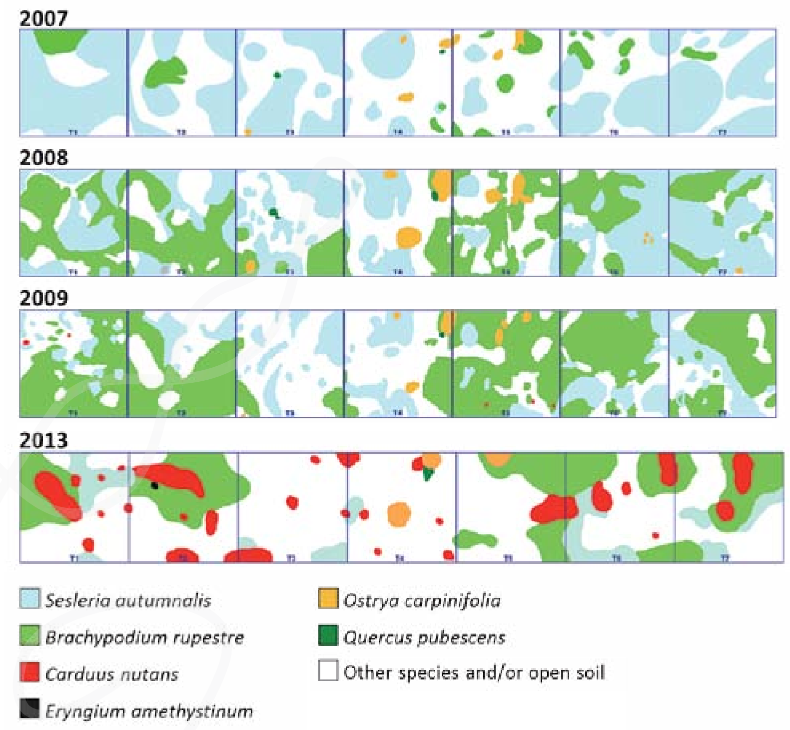

Figure 13.3: Transect dominated by *Sesleria autumnalis* detected from 2007 to 2013.

13.3 TARGET SPECIES VARIATIONS

Target species are those defined as indicator species or which have an ecological optimum in a particular habitat and that respond to changes in environmental parameters. In this work four species were selected from those typical of karst grassland and meadow-pasture: *Centaurea rupestris* L. (Figure 13.4a), *Pulsatilla montana* (Hoppe) Rchb. (Figure 13.4b), *Orchis morio* L. (Figure 13.4c) and *Orchis tridentata* Scop. (Figure 13.4d).

Figure 13.4: Target species: *Centaurea rupestris* L. (a), *Pulsatilla montana* (Hoppe) Rchb. (b), *Orchis morio* L. (c) and *Orchis tridentata* Scop. (d). Photograph: Cristiano Francescato.

Data on populations of these species were collected using GPS to identify the location of the plants. For each species, the individuals within a radius of about 1.5 m were counted, which corresponds to the error of the instrument, in order to evaluate any fluctuations in populations (increase and number of individuals) resulting from the activity of deforestation and grazing. Figures 13.5 and 13.6 show the distributions of target species detected across the study area before the intervention (2006) and after seven years of grazing (2013).

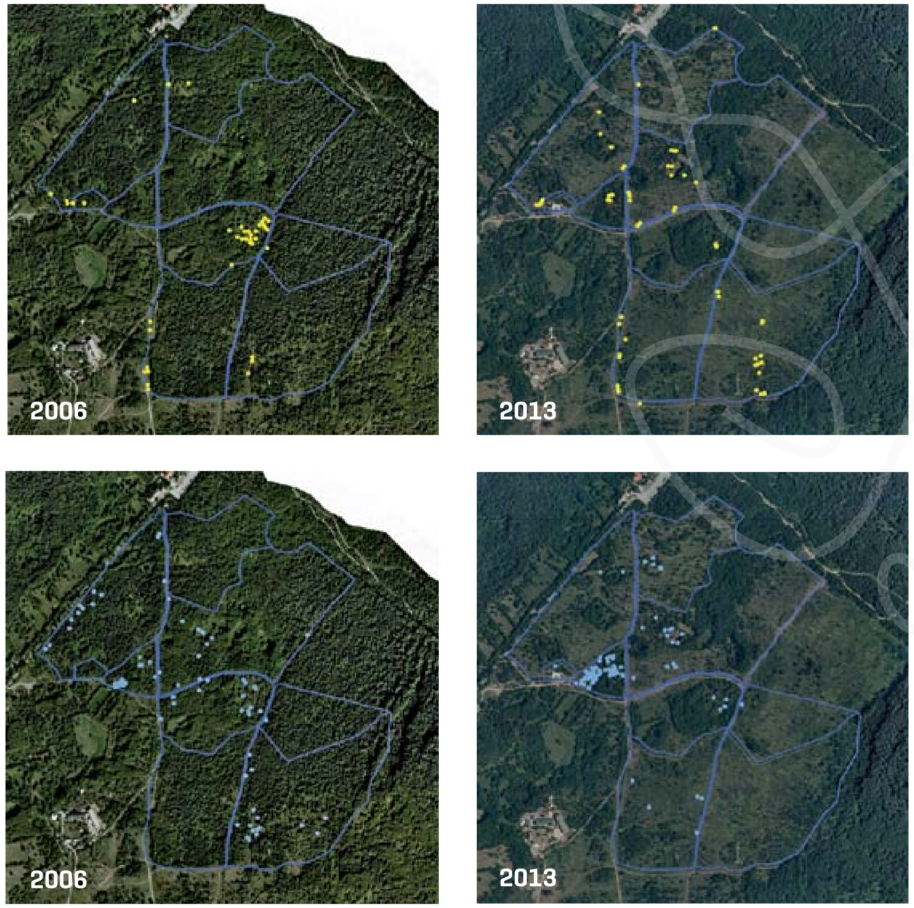

Figure 13.5: Distribution of *Centaurea rupestris* L. (up) and *Pulsatilla montana* (Hoppe) Rchb. (down) throughout the study area before the tree and scrub clearance and the reintroduction of grazing (2006), and after seven years of grazing (2013).

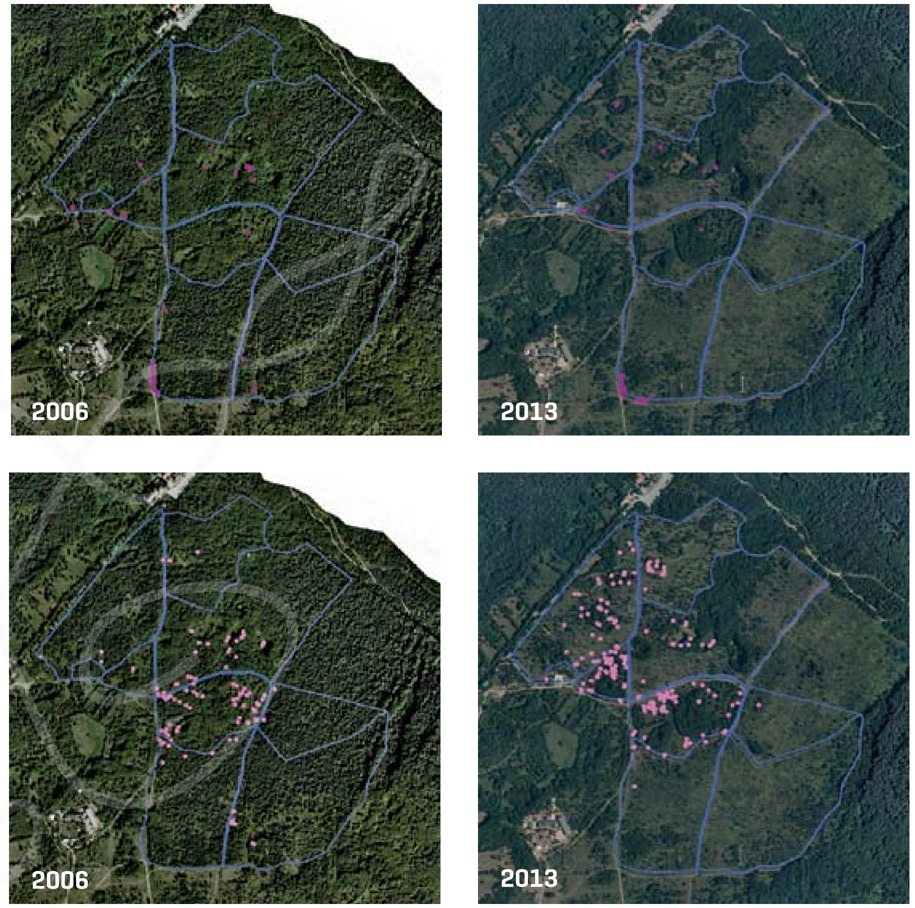

Figure 13.6: Distribution of *Orchis morio* L. (up) and *Orchis tridentata* Scop. (down) throughout the study area before the tree and scrub clearance and the reintroduction of grazing (2006), and after seven years of grazing (2013).

A statistical analysis of the data sampled was performed only within the subunit where the grazing activities lasted for seven years (2007-2013). From this it was found that three of the four selected target species (*Orchis morio, Centaurea rupestris* and *Pulsatilla montana*) had declined significantly.

The greening of the deforested area by many species of the karst dry grassland was certainly favoured by the activation of the seed bank pre-established by the accumulation over time of the seeds from the fragments of dry grassland occurring in adjacent areas. The response of the species to the grazing factor, however, is species-specific and cannot be generalized to draw conclusions.

13.4 CHANGES IN ALIEN SPECIES NUMBERS

The two neophytes present in the study area, *Ailanthus altissima* Mill. (Figure 13.7), originating from China, and *Senecio inaequidens* DC. (Figure 13.8), introduced from South Africa, were monitored over time.

Figure 13.7: Young plants of *Ailanthus altissima* Mill. that invade the dry grassland. Photograph: Cristiano Francescato.

These are two invasive species with high environmental impact, the presence of which must be controlled and their distribution limited as far as possible (see also the LR 17/2010 of the Friuli Venezia Giulia region, art. 64 which includes actions to combat pest plant species that are harmful to human health and the environment).

Figure 13.8: Population of *Senecio inaequidens* DC. which developed out of material resulting from the tree and scrub clearance (wood chips). Photograph: Cristiano Francescato.

The sampling methodology is identical to that used for the target species, although in cases of a high presence of the species it was preferred to assess the total surface area occupied, thus obtaining an estimate of the coverage. Indeed, especially with regard to *Ailanthus altissima*, it is difficult to understand what constitutes a single individual because of its vegetative reproduction.

The covers in terms of area (m^2) and percentage area occupied (%) of the two alien species *Ailanthus altissima* and *Senecio inaequidens* were monitored from 2005 to 2013 as reported in two separate diagrams to highlight the trend towards expansion over time (Figures 13.9 and 13.10).

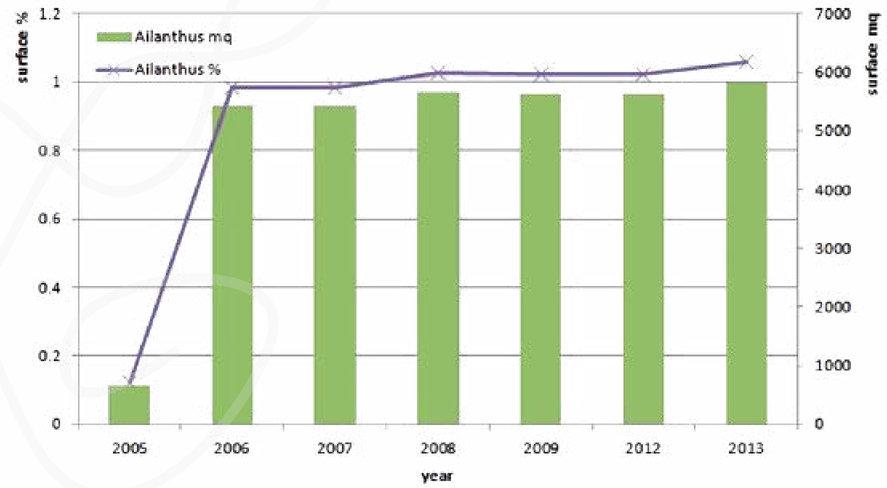

Figure 13.9: Spread of *Ailanthus altissima* Mill. in the study area from 2005 to 2013. The values of coverage of the areas occupied are expressed in square meters and in percentage.

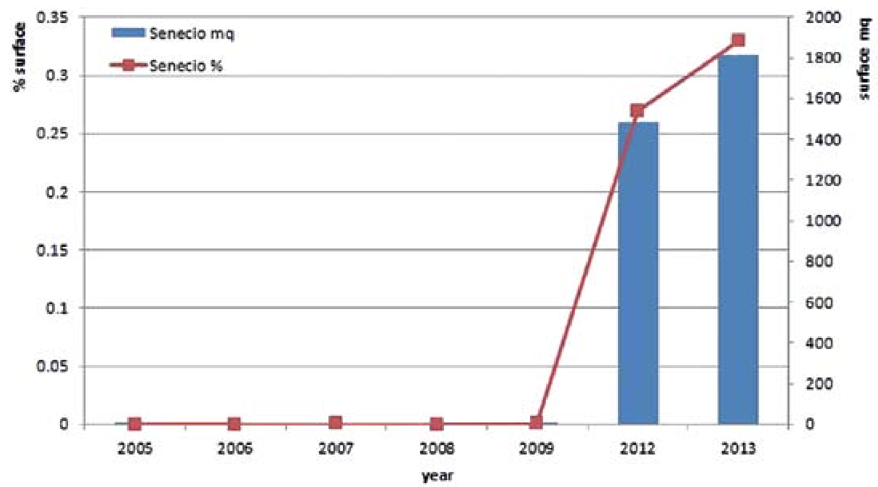

Figure 13.10: Spread of *Senecio inaequidens* DC. in the study area from 2005 to 2013. The values of coverage of the areas occupied are expressed in square meters and in percentage.

Figure 13.11 shows the distribution of *Ailanthus altissima* from 2005 (before the intervention of deforestation and grazing) through to 2013. One can note the increase in this species distributed mainly along the roadside leading to the border-crossing where one can encounter both single individuals and large blocks completely occupied by this species. A total of 40 punctiform stations were measured, for a total of 165 trunks, and 3 stations with coverage of between 60 and 75 %. The spread of this species, already present in the study area, has been favoured by the tree clearance. As it is not palatable to animals, in order to contain it, a continuous and repeated cutting of the stems would be necessary (at least twice a year during the growing season to prevent the re-growth of shoots and to weaken the plant), especially those bearing seeds before they reach maturity, as well as the eradication of seedlings with the removal of the biomass.

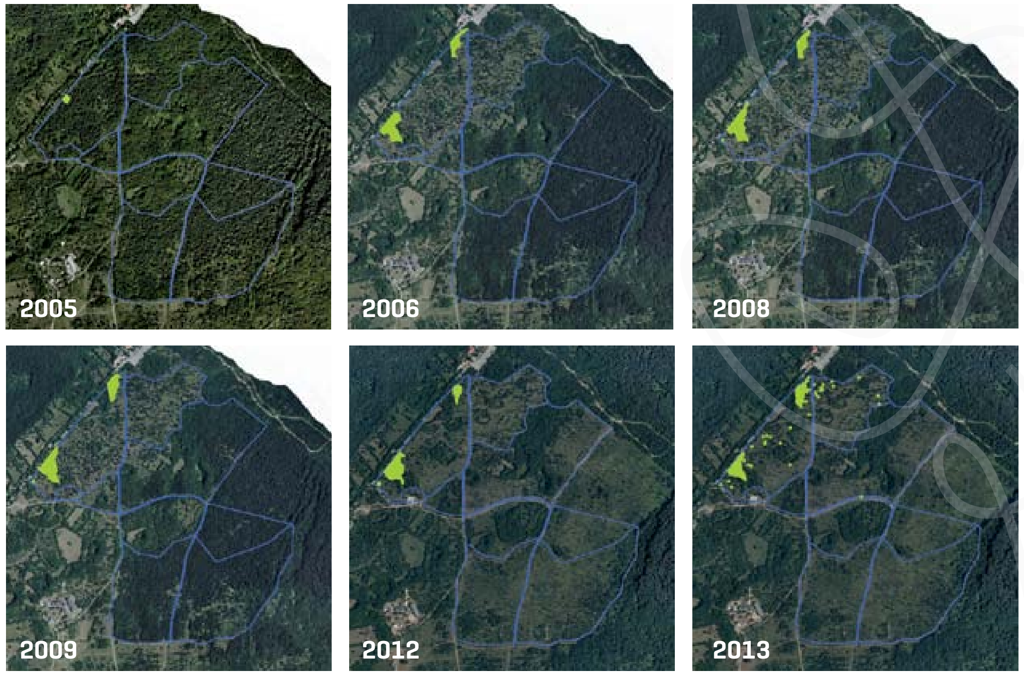

Figure 13.11: Monitoring of *Ailanthus altissima* Mill. from 2005 to 2013 in the study area.

Senecio inaequidens, with very few individuals present in 2005, has spread throughout the study area (Figure 13.12). It is most abundant at the points where materials were accumulated during the tree clearance phase and which constitute a very fertile substrate. 86-point stations were sampled for a total of 624 individuals and two areas with covers of between 85 and 90 %. To limit the expansion of this species the mowing of the large areas should be carried out with the eradication of individuals before flowering and the removal of the biomass. This should be done several times over the course of the year, from spring to late autumn.

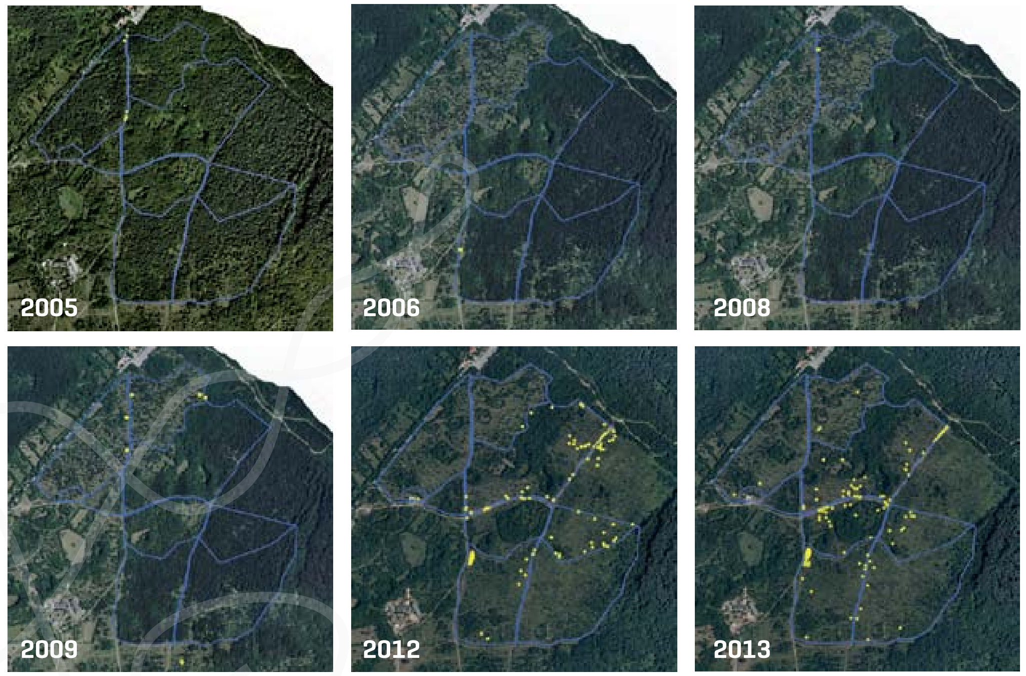

Figure 13.12: Monitoring of *Senecio inaequidens* DC. from 2005 to 2013 in the study area.

In 2012, close to the cowshed, *Ambrosia artemisiifolia* L. was also observed (Figure 13.13a and 11.13b). This is an invasive neophyte included in the LR 17/2010 and native to North America, which causes significant harm to human health, being highly allergenic. The individuals of this species can easily be eliminated by annual eradication and must be removed and disposed of as waste.

Figure 13.13: *Ambrosia artemisiifolia* L. (a) and its presence (b) detected in 2012 near the cowshed of Basovizza/Bazovica. Photograph: Cristiano Francescato.

CONCLUSIONS

The type of grazing carried out in the area exhibited significant imbalances in the animal load needed to ensure a favourable conservation status. On overgrazed surfaces the grassy sward is broken and, into the gaps created, terophytic segetal nitrophilous species of the *Stellarietea mediae* association have entered. There has been a significant increase in the coverage of *Carduus nutans*, and to a lesser extent of *Eryngium amethystinum*, both spiny species that are unattractive to grazing animals. The occasional presence of the neophytes *Erigeron annuus* and *Conyza canadensis* were also noted.

Many ruderal and segetal nitrophilous species are also found in areas where animals are held for extended periods, along the routes used by the stock and the routine feeding and watering points.

In undergrazed areas the natural processes of scrubbing-over are underway. It would be advisable to cut out the suckers that have grown since the first deforestation measures were undertaken, together with the removal of scrub and the immediate reintroduction of cattle grazing to contain the natural dynamics.

There is also a lack of control and containment of invasive alien species favoured by tree and scrub clearance as well as by the accumulation of organic material resulting from the forestry work and points where the soils have been disturbed.

The introduction of grazing as a conservation tool for grassland habitats requires a great attention to management methods and practices, which must always consider the consequences of grazing on vegetation structure and dynamics.

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CHAPTER 14

PRODUCTIVITY AND FORAGE QUALITY OF KARST MEADOWS UNDER A RANGE OF MOWING MANAGEMENT

DONOS IN KAKOVOST KRME KRAŠKIH TRAVNIKOV GLEDE NA NJIHOVO UPRAVLJANJE S KOŠNJO

PRODUTTIVITÀ E QUALITÀ DEL FORAGGIO DEI PRATI CARSICI IN RELAZIONE ALLA MODALITÀ DI SFALCIO

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ABSTRACT

Biodiversity is strongly related to the number and the type of habitats present in a given territory. The human activity plays a determining role in these habitats. Among these, grasslands play an important role due to their natural function mainly linked to biodiversity, resulting from the fact that these communities harbour a large number of species. Mowing is the most important cultural practice in the management of the meadows. The lawns of forage crops are able to provide one or more crops a year, the production of which is removed and used to feed animals. The productivity and quality of forage depend on agronomic and environmental factors, including vegetation types, climatic conditions and geomorphological aspects of the site. Taking these factors in consideration, the grasslands of the Karst region falls under the category of dry grasslands. These grasslands are generally cut once or twice a year: the first cut is made between the end of May and the beginning of June, the second in early September. The experiments conducted under the project BioDiNet aimed to determine the production of dry matter (DM) and the quality of the forage crop at different times in 10 fields of the Karst region. The results showed that the lawns of the Karst meadows provide a low production and are strongly dependent on climatic conditions and in particular on precipitation. Since the quality of the forage is linked to productivity (for example, the crude protein decreases with increasing the dry matter, while the NDF increases), the more productive meadows behave in a different way compared to less productive. For this reason it is crucial the choice of when to perform the cut. Cuts at the beginning of flowering season, especially in more productive fields, contributes to a better forage quality without loss of dry matter product.

Key words: meadow, dry matter yield, forage quality, management strategies, harvest date

IZVLEČEK

Biotska raznovrstnost je močno povezana s številom in vrsto habitatov na določenem območju. Človekova dejavnost ima odločilno vlogo pri ohranjanju habitatov. Med temi imajo travišča pomembno vlogo, zaradi naravne funkcije predvsem v povezavi z raznovrstnostjo in dejstvom, da so te združbe vrstno izredno bogate. Košnja je najpomembnejša kulturna praksa pri upravljanju travnikov. Travniki zagotavljajo enega ali več pridelkov letno, ki se odstranjujejo in uporabljajo za krmo živali. Donos in kakovost krme je odvisna od agronomskih in okoljskih dejavnikov, vključno z vrstami vegetacije, klimatskimi pogoji in geomorfološkimi značilnostmi območja. Ob upoštevanju teh dejavnikov travišča na Krasu lahko uvrstimo v kategorijo suhih travnikov. Ti travniki so na splošno košeni enkrat ali dvakrat letno: prva košnja se izvaja med koncem maja in začetkom junija, druga v začetku septembra. Namen aktivnosti izvedenih v okviru projekta BioDiNet je bil določiti proizvodnjo suhe snovi (SS) in kakovost pridelka krme v različnih obdobjih na 10 območjih Krasa. Rezultati so pokazali, da kraški travniki zagotavljajo nizek donos, ki je močno odvisen od vremenskih razmer in še zlasti od padavinah. Ker je kakovost krme vezana na produktivnost (na primer, količina surovih beljakovin se zmanjšuje s povečanjem suhe snovi, medtem ko NDV raste), se bolj donosni travniki obnašajo drugače v primerjavi z manj donosnimi. Zato je ključnega pomena izbira časa, ko se izvaja košnja. Košnja na začetku cvetenja, še posebej bolj produktivnih travnikov, prispeva k boljši kakovosti krme brez izgube suhe snovi.

Ključne besede: travniki, proizvodnja suhe snovi, kakovost krme, strategije upravljanja, čas košnje

RIASSUNTO

La biodiversità è fortemente dipendente dal numero e dal tipo di habitat presenti in un determinato territorio. L'attività antropica svolge un ruolo determinante nell'aumentare tali habitat. Tra questi rivestono un ruolo importante le praterie, data la loro funzione naturalistica, legata soprattutto alla biodiversità derivante dal fatto che queste cenosi possono ospitare un elevato numero di specie. Lo sfalcio è la pratica colturale più importante nella gestione dei prati. I prati sono delle colture foraggere in grado di fornire uno o più raccolti l'anno, la cui produzione viene rimossa e usata per alimentare gli animali. La produttività e la qualità del foraggio dipendono da fattori agronomici e ambientali, tra cui i tipi vegetazionali, le condizioni climatiche e gli aspetti geomorfologici del sito. Considerando queste caratteristiche, il Carso ricade nella categoria produttiva dei prati magri. I prati del carso sono generalmente tagliati una o due volte l'anno: il primo taglio viene effettuato tra la fine di maggio e l' inizio di giugno, il secondo all'inizio di settembre. La sperimentazione condotta nell'ambito del progetto BioDiNet si proponeva di determinare la produzione di sostanza secca (DM) e la qualità del foraggio raccolto, in epoche diverse, in 10 prati del Carso. I risultati hanno mostrato che i prati del Carso forniscono una produzione scarsa e fortemente dipendente dalle condizioni climatiche ed in particolare dalle precipitazioni. Dal momento che la qualità del foraggio è legata alla produttività (ad esempio la proteina grezza diminuisce all'aumentare della sostanza secca, mentre l'NDF aumenta), i prati più produttivi si comportano in modo diverso rispetto ai meno produttivi. Per questo motivo risulta fondamentale la scelta del momento in cui eseguire il taglio. La raccolta ad inizio fioritura, specialmente nei prati più produttivi, contribuisce ad ottenere un foraggio di miglior qualità senza diminuzione della sostanza secca prodotta.

Parole chiave: prati, produzione di sostanza secca, qualità del foraggio, strategie di gestione, periodo di sfalcio

14.1 MEADOWS AND HUMAN ACTIVITIES INVOLVED IN THEIR MANAGEMENT

Meadows are forage crops maintained for two or more years, which provide every year one or more harvests. They are managed through phytomass cutting, its collection and removal as fodder for herbivores, not used *in situ* and usually after it has undergone transformation (e.g.: haymaking, drying, etc.). Since meadows are cut at least once a year, their vegetation is made up exclusively of herbaceous species.

Figure 14.1: Meadow at Podpeč, Municipality of Koper, Slovenia.

Meadows can be classified according to their origin or duration. They can be *natural* – when present at levels above the tree-line, *spontaneous* - if growing spontaneously below the tree-line, or *artificial* - when they are created and maintained through human intervention (such as seeding, reseeding or overseeding). They are *permanent*, when their duration exceeds 10 years, *multi-year*, when they provide production for about 3-5 years, or *biennial* when their lifecycle is completed in two years.

Together with production, meadows have other environmental and landscape functions. The environmental functions include the reduction of the surface soil erosion due to wind or water and promotion of a stable soil structure formation. This improves water filtration and purification and reduces losses due to nutrient leaching. The landscape function mainly derives from their capacity to diversify the landscape, highlighting the contrasting colours and shapes. Furthermore, meadows are particularly appreciated for their naturalistic function, as they increase the biodiversity of communities of animals and plants.

Figure 14.2: Amethyst eryngo *(Eryngium amethystinum)*, a characteristic species flowering between July and September on the karst grasslands.

As a result of environmental variability and management techniques there are different types of meadows, differing in numerous features, including their productivity profile (Table 14.1).

Table 14.1: Principal characteristics of stable or permanent grassland in northern and central Italy.

Type of meadow	No. of species in 100 m²	No. of cuts per year	t ha⁻¹ of dry matter per year
Perennial ryegrass (*Lolium*)	6-12	4-6 (7)	12-16 (20)
Oatgrass (*Arrhenatherum*) lowland	15-20	3-4 (5)	8-11
Oatgrass (*Arrhenatherum*) montane	20-25	2-3	7-9
Yellow oatgrass (*Trisetum*)	30-45	1-2	4-6
Bromes (*Bromus*)	35-60	1-2	3-4
Sedges (*Carex*)	3-7	0-1	1-3

In addition to differences in terms of productivity, meadows can also be distinguished by their forage quality, with consequences for their use in animal feeding. Herbage nutrient contents are taken into consideration to define forage quality (Figure 14.3), in particular regarding fibre (crude fibre, neutral detergent fibre [NDF], acid detergent fibre [ADF] and acid detergent lignin

[ADL]], crude protein, ether extract (crude lipids) and ash content. The ingestion and digestibility of fodder is limited by the fibrous component (cellulose, hemicellulose, lignin, minerals and other indigestible substances); proteins improve forage palatability and are important for their energy component. For ruminant animals, fibres provide over 50% of the nutritional value, but the release of this energy depends entirely on the activity of the rumen microflora on the fibrous components in the food. For those animals the cellular content (proteins, sugars, starches, fat) has a digestibility of 98%, the cell wall (NDF) 62%, hemicellulose 79% and ADF (cellulose, lignin) 30%. Lignin is rather indigestible. In the production of mountain meadows, the crude protein content can vary from 5 to over 20% of the dry matter, the NDF content from 40 to 70%, the ADF from 20 to 50%, and ADL from 3 to 9%.

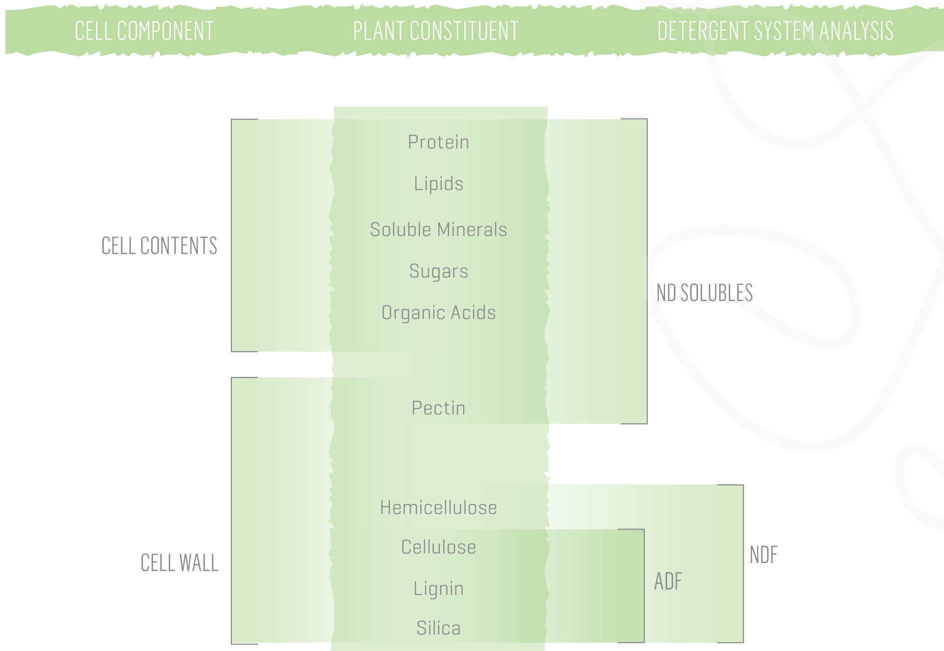


Figure 14.3: Plant constituent of forage dry matter divided into cell contents and cell walls. The analytical system on the right (Detergent System Analysis) describes the fibre extraction with Van Soest method (1960): neutral detergent solubles (ND Solubles) are primarily the cell contents, neutral detergent fibre (NDF) and acid detergent fibre (ADF) are cell walls (Barnes, 2003a).

14.2 MANAGEMENT OF DRY GRASSLANDS

The potential production of a meadow depends primarily on the environmental conditions, soil fertility and its water status. These factors together determine the length of the vegetative period and the intensity of nutrient assimilation. The most favourable situations are encountered in temperate climates, with regular and abundant rainfall, with deep, well-structured, soils rich in nutrients and with water constantly available. Moving from this ideal situation, production capability decreases, reaching minimum values in cold sites with thin dry soils.

Generally, where the situation is not ideal, meadows are watered and fertilized, where possible, to increase productivity. However, dry grasslands are

considered important not only for their production but also for their natural function, especially for their contribution to biodiversity and territorially-specific plant and animal communities. Good management is essential for the maintenance of these functions.

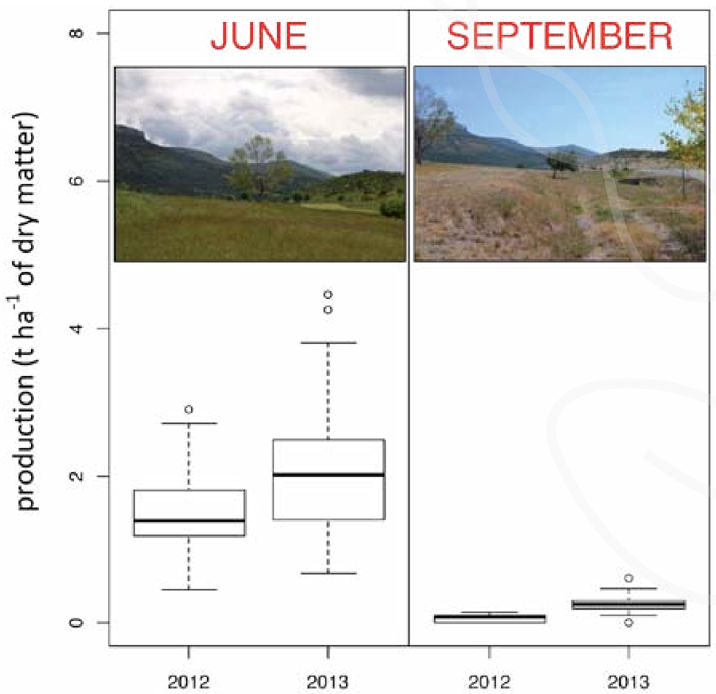
It appears from Table 14.1 that an increase in productivity corresponds to a decrease in number of species. This is a consequence of the different species response to fertilization and irrigation used to increase productivity. Under conditions of high fertility, where species benefitting from such situations will be favoured, other species will be overwhelmed and eventually eliminated through competition. Therefore, where meadows are managed for preserving biodiversity, it is appropriate to restrict fertilizers and irrigation.

Figure 14.4: Dry meadow on terraces at Podpeč, municipality of Koper, Slovenia.

14.3 MANAGEMENT OF KARST MEADOWS

Due to its climatic and geo-morphological characteristics the grasslands of the Karst area fall into the productivity category of dry grasslands. The soils are shallow with low water retention, precipitation is not well distributed throughout the year and high summer temperatures induce summer dormancy of the vegetation. Given the characteristics of these meadows, it is reasonable to intervene in support of agronomic production. Fertilization, a practice commonly used on dry grasslands but not very widespread in the Karst, could have negative effects on biodiversity and aquifers in this type of territory. In fact, the number of species and type of vegetation present, and therefore the plant communities, may be strongly influenced by the increase of nutrients provided with fertilization. Many studies have shown that the botanical composition of a meadow is closely related to the nitrogen availability. In addition, the geomorphology of the Karst and in particular the water movement towards the aquifer increases the vulnerability of this area. For the maintenance of such natural environment, so unique and fragile, fertilization is a practice that has to be used with great caution.

According to our data and other studies carried out on the Karst meadows, we concluded that local farmers practice one or at most two cuts per year. The first harvest is done between the end of May and the beginning of June, while the second is done in early September. It has been observed that after the first cut, as a consequence of high temperatures and low rainfall, the vegetation enters in a state of summer dormancy that effectively impairs the production of biomass for the second cut. In a study conducted on some meadows in the municipality of Basovizza, the second cut is always performed, even if production is low. In contrast, our data from the municipality of Koper (Figure 14.6) showed that the second cut is not performed regularly due to the lack of productions that in some fields even approaches 0.



Site	June 2012	June 2013	September 2012	September 2013
1	1.7 ± 0.6	2.6 ± 0.5	0	0
2	1.6 ± 0.4	2.0 ± 0.4	0	0.3 ± 0.06
3	1.5 ± 0.6	1.7 ± 0.2	0.09 ± 0.04	0.3 ± 0.04
4	1.6 ± 0.04	1.9 ± 0.4	0	0
5	1.5 ± 0.04	1.4 ± 0.4	0.09 ± 0.04	0.2 ± 0.07
6	1.4 ± 0.4	2.7 ± 0.5	0	0.3 ± 0.05
7	1.2 ± 0.3	3.6 ± 0.6	0.07 ± 0.03	0.5 ± 0.20
8	1.1 ± 0.4	2.0 ± 0.4	0.07 ± 0.06	0.3 ± 0.09
9	2.3 ± 0.5	2.5 ± 0.5	0.10 ± 0.02	0.2 ± 0.03
10	1.4 ± 0.3	1.0 ± 0.3	0	0

Figure 14.6: Production (t ha^{-1} dry matter) of 10 sites in Podpeč (municipality of Koper, Slovenia). In the graphs the boxes define the interval that encloses half of the values and are internally divided by the median, while the segments are the minimum and maximum values. The graph on the left shows the production of the first harvest (June) in 2012 and 2013, while the graph on the right shows the production of the second harvest (September) in 2012 and 2013. The table gives the production of each site for the same harvests with their relative standard errors.

Based on these results it is clear that the productivity of Karst meadows is closely dependent on the weather conditions, with production being affected by spring and summer precipitation and temperatures throughout the growing season. Therefore, the productivity of these meadows varies greatly from year to year, particularly regarding the second harvest.

The experiment conducted within the BioDiNet project showed that the Karst meadows have limited productivity but high ecological importance and an extraordinary intrinsic naturalistic value. To encourage the retention of these plant communities it is necessary to maximize the production capacity of the meadows. Excluding the possibility of applying fertilizer, which, as mentioned above, may negatively interfere with biodiversity, it is more appropriate to exploit the forage produced from a qualitative point of view. To achieve this goal the timing of the first cut is crucial.

14.4 PRODUCTION OF THE MEADOWS IN THE KARST

As previously mentioned, the morphological structure of the Karst landscape leads to a series of different microclimatic situations over a short distance. This heterogeneity favours the presence of a wide variety of plant communities; therefore meadow productivity is also very variable. To evaluate and characterize the production of the Karst meadows, 10 sites were selected in the locality of Podpeč (in the municipality of Koper, Slovenia) as representative of the Karst area. The choice was based principally on the floral composition and characteristics of the terrain - these fields are indicated by a number from 1 to 10. The reported results involve data collected for the growing seasons of 2012 and 2013. The meadows belong to the *Festuco-Brometea* class, order *Scorzonero villosae-Chrysopogonetalia grylli.* All were classified as the association *Danthonio alpinae-Scorzoneretum villosae* although some had a greater presence of xeric species, while mesic species were dominant in others, due to special conditions and site management.

The studied sites had productions that in most cases did not exceed 1 t ha^{-1} of dry matter, with some exceptions that reached a production of more than 2 t ha^{-1}. Figure 14.7 provides the production values for the first harvest (mean values of three harvests in June). In fact the production from the second harvest (September) did not achieve values that were justifiable economically in either of the two study years. As shown in Figure 14.7, the production was very variable, not only from meadow to meadow, but in some cases even from year to year. In particular, the production of sites 6, 7 and 8 resulted very different during the two years covered by the experiment. The productivity of these sites appeared to be strictly dependent on the rainfall trends occurring during vegetative growth in spring (March to June): production was significantly higher in the year with more plentiful rainfalls.

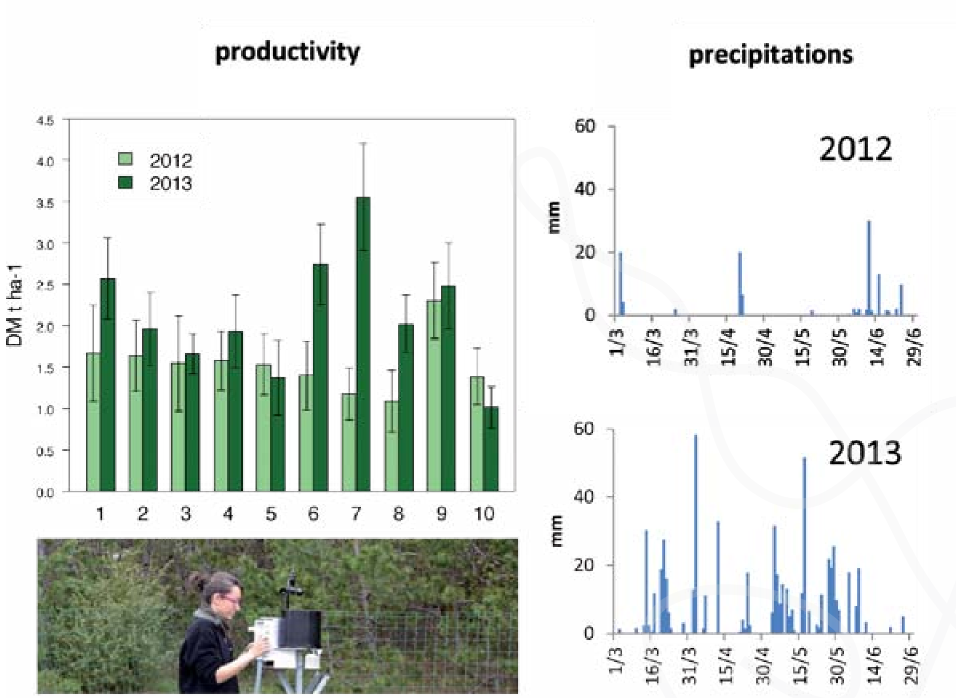


Figure 14.7: The graph on the left shows the dry matter (DM) yield at first harvest of 10 meadows in Podpeč (municipality of Koper, Slovenia) in 2012 and 2013. The graphs on the right show the daily rainfall recorded between March and June for the same locality in 2012 (top) and 2013 (bottom).

The explanation for the strong dependence of production on rainfall can be found in the floristic composition of the studied site. Mesophilic meadows are more sensitive to variations in water availability. On the other hand, in the meadows with an abundant presence of xeric species, production remains unchanged even if rainfall is abundant. The mesic species take advantage of the favourable climatic conditions, growing more than the species typical of xeric environments that do not have this ability and remain small despite water availability.

As shown in Table 14.2, the sites with higher productivity, such as 6 and 7, are composed of species typical of mesic meadows (with higher indices of moisture and nutrients indicating more favourable conditions), with greater forage value. Site number 10, which had a limited production in both years, has species more typical of xeric situations.

Table 14.2: Species from the studied sites with their relative abundance, forage value, moisture index and nutrient index. Sites of meadows, specifying mesic or xeric conditions, are also indicated.

Species	Abundance (%)	Meadows	Xeric meadows	Mesic meadows	Forage value	Moisture index	Nutrient index
Dactylis glomerata	>30	6 & 7		X	7	3	4
Vicia cracca & sativa	>1	6 & 7		X	6	3	3
Festuca arundinacea	10	6		X	4	4	4
Trifolium pratense	5	6 & 7		X	7	3	3
Poa pratensis	30	7		X	8	3	3
Bromopsis erecta (agg.)	10	10	X		5	2	2
Salvia pratensis	5	10	X		2	2	2
Carex caryophylla	1	10	X		2	2	2
Chrysopogon gryllus	5	10	X		-	1	2
Festuca stricta & valesiaca	5	10	X		-	1	2

With regard to the forage quality, as observed from the dry matter production, high variability was recorded. This is related to the site and collection period (June or September). Specifically, NDF (neutral detergent fibre) varies from 39 to 79%, ADF (acid detergent fibre) from 28 to 51% and finally ADL (acid detergent lignin) from 4 to 10%, with average values of 59, 38 and 6%, respectively. Crude protein varies from 4 to 15%, with an average of 9%. Lower crude protein values and higher fibre values generally corresponded to the second harvest (September), and in particular for the sites with lower production. This is due to the slow regrowth observed in these meadows after the summer dormancy. In September, for the most part, the vegetation here is still largely dry and low.

As an example, Figure 14.8 reports the quality values of two sites at the extremes with regard to their productivity. The first is characterized by the presence of *Dactylis glomerata* and composed of ruderal nitrophilic mesophile species - typical of enriched cultivated lands with species from Karst meadows. The other exemplifies a xeric meadow rich in species from the *landa* grazing lands, important for the presence of several species of *Orchidaceae*. The two sites differ by the availability of water and nutrients (higher in the first site) and by the high species richness and natural values (second site).

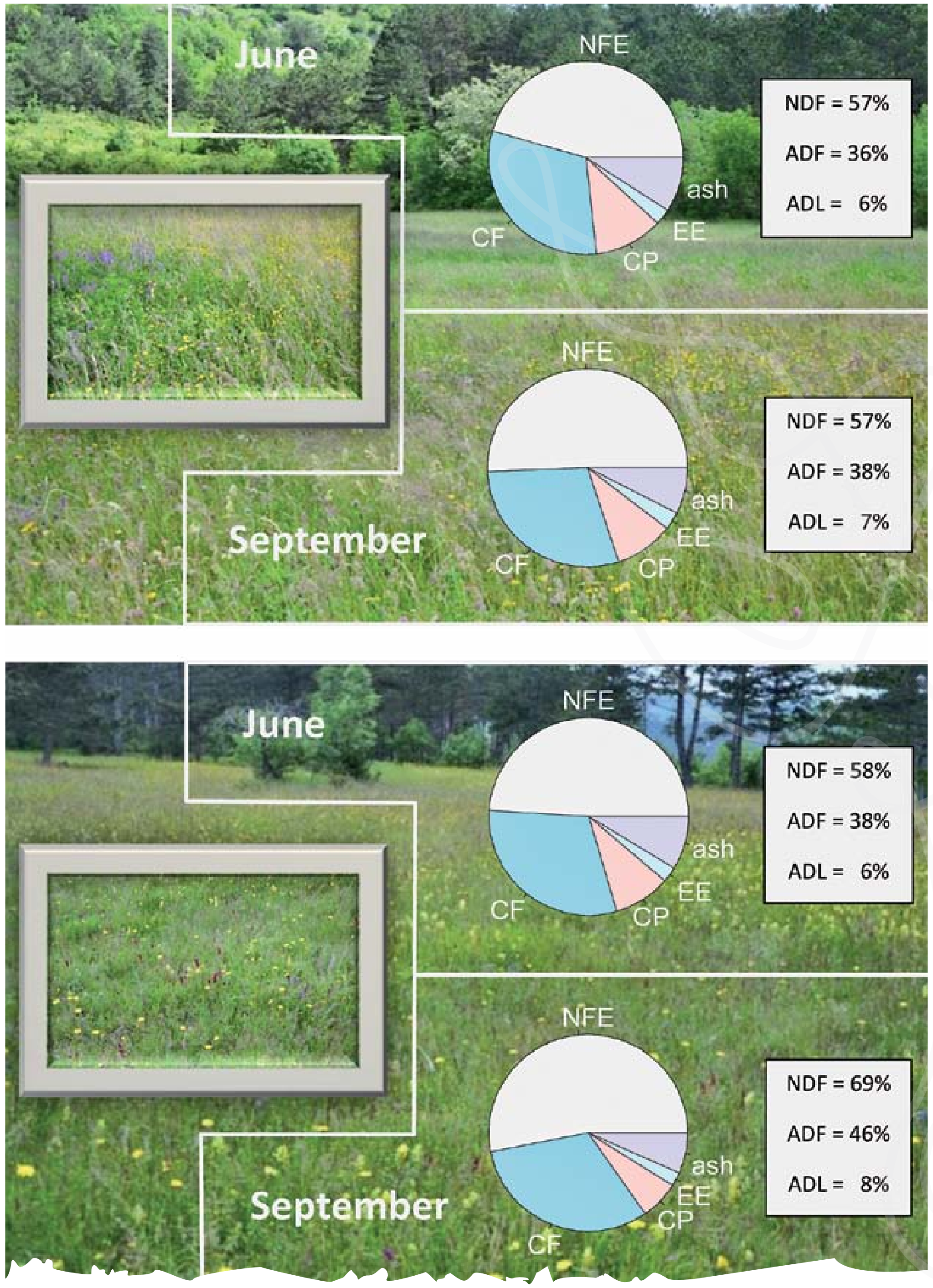


Figure 14.8: Values of the nitrogen-free extract (NFE), crude fibre (CF), crude protein (CP), ether extract (EE), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) for two sites in Podpeč (municipality of Koper, Slovenia) for the first and second harvest, respectively (June and September). The pictures represent a meadow with mesic species (above) and a meadow with xeric species (below).

14.4 THE EFFECT OF THE TIMING OF CUTTING

Figure 14.9. Dry meadow on terracing at Podpeč, Municipality of Koper, Slovenia.

The studied sites did not exhibit particularly high production or quality values. They are managed for family use and are probably of little importance for the local economy. However, the positive effects they bring to the area make a necessity to maintain them. In order to optimize the harvest from these fields and enhance the product, we studied the quality of their forage on three different dates. They were chosen in the two weeks before the harvesting was done, usually in the first week of June and September.

As might be expected, more productive sites behaved differently from less productive ones. The protein and NDF contents, for example, showed significant changes in relation to the dry matter yield. On the other hand, the ADF and ADL contents were influenced by neither cutting date nor yield.

Figure 14.10 shows these relationships (we show only the results of the June harvest, since no September cut was done): the protein content decreases with a rise in productivity, however this decrease is less pronouncedin the first cutting date. On the contrary, NDF content is higher in more productive meadows than in less productive ones and seems to be little affected by timing of cutting. These results suggest that anticipating the cut, when the majority of the species have not yet reached full bloom, provides better forage quality and palatability, especially from the more productive meadows, because of the higher crude protein content.

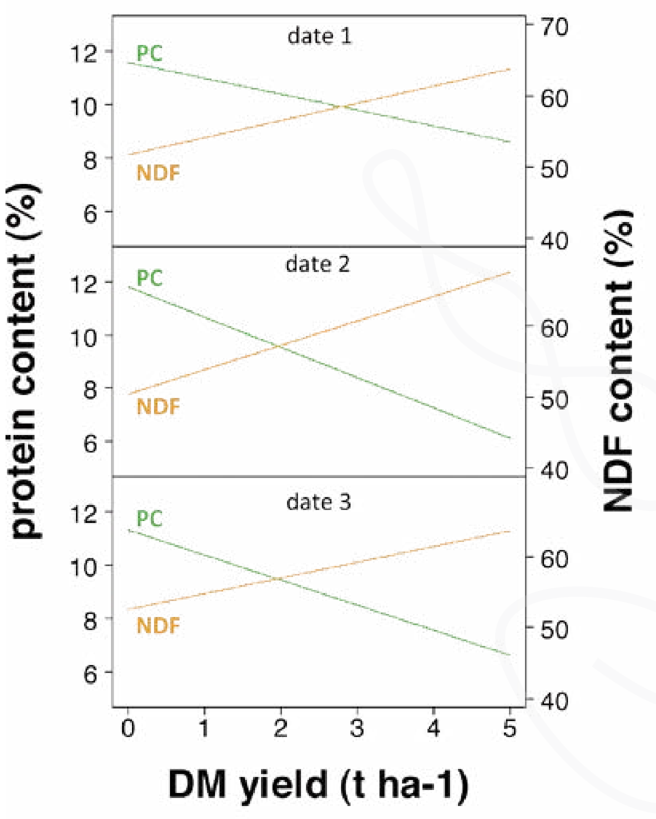


Figure 14.10: Percentage of crude protein (PC) and neutral detergent fibre (NDF) from the first harvest of 10 meadows in Podpeč (municipality of Koper, Slovenia) as a function of the productivity of the meadows. Dates 1, 2 and 3 identify the sampling dates when herbage was collected: 3. First week of June (traditional management); 2. One week earlier; 1. Two weeks earlier.

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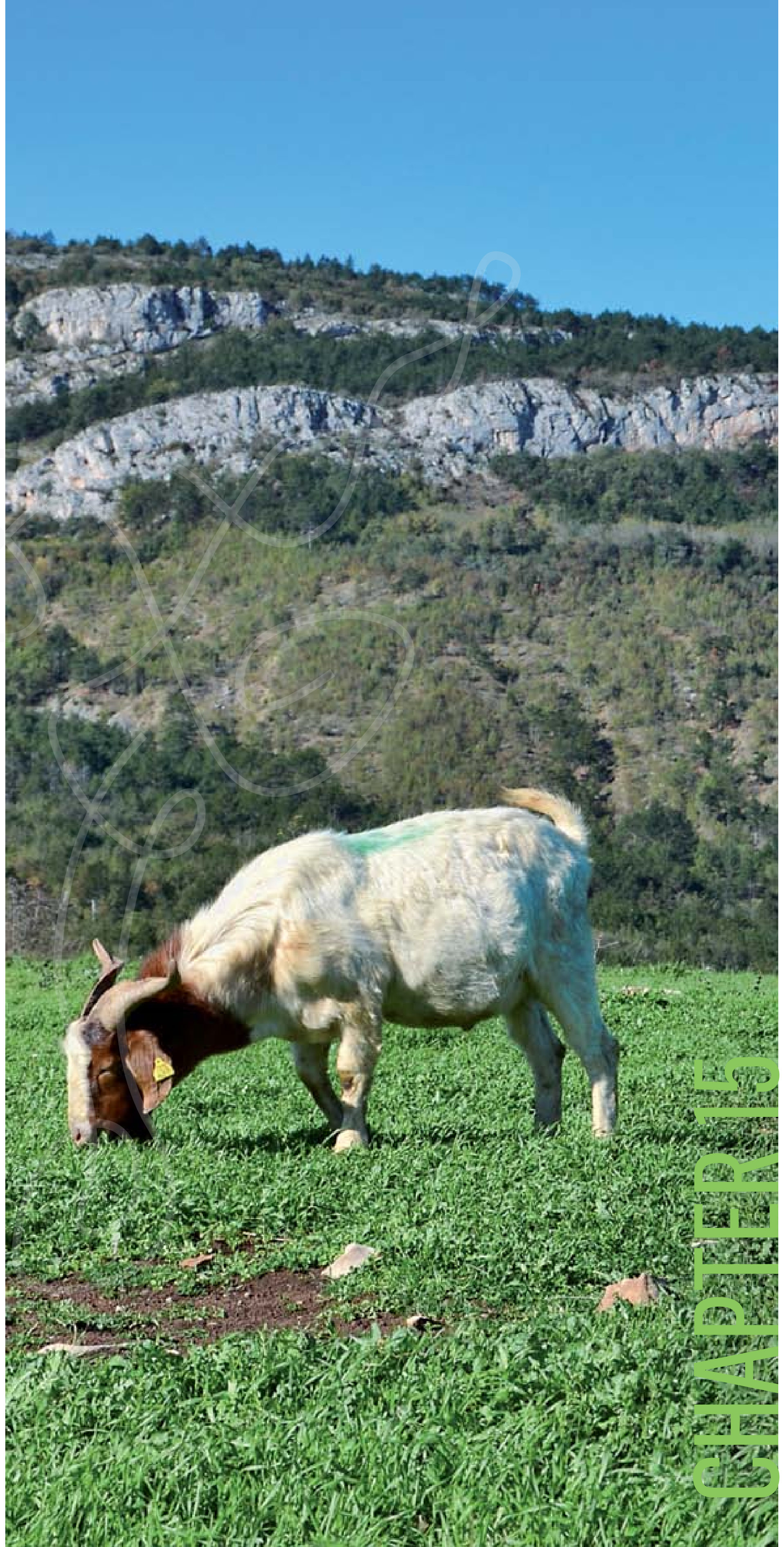

CHAPTER 15

SPATIAL AND SEASONAL VARIATION OF HERBAGE YIELD AND QUALITY OF SOME KARST PASTURES

PROSTORSKO IN SEZONSKO NIHANJE PRIDELKA IN KAKOVOSTI NEKATERIH KRAŠKIH PAŠNIKOV

VARIAZIONE SPAZIALE E STAGIONALE DELLA PRODUZIONE E DELLA QUALITÀ DEL FORAGGIO DI ALCUNI PASCOLI DEL CARSO

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ABSTRACT

In this chapter we focus in pastures that along with meadows represent the primary agricultural activity in the Karst area. It is a review of traditional management of pastures with focus on management strategies. Due to the climatic and topographic characteristics of the karst areas, the productivity of grassland varies according to very specific spatial and temporal patterns. Therefore it is essential to understand the seasonal changes in herbage yield and quality of these pastures. The seasonal changes in daily DM yield in the karst pastures can be described by a two-phase model, with a maximum in May, followed by a rest in August, and a slight recovery between September and October. Higher-quality herbage occurs at the maximum DM yield. These results can help to optimize grazing management strategies. For a sustainable utilization of these pastures, the differences found within sites suggested that in a morphologically complex landscape as the karst, such complex structure have great influence on management decisions.

Key words: pastures, dry matter yield, seasonal production, forage quality, stocking rate

IZVLEČEK

V poglavju predstavljamo raziskave na pašnikih, ki skupaj s travniki predstavljajo primarno kmetijsko dejavnost na Krasu in podajamo pregled njihovega tradicionalnega upravljanja s poudarkom na strategijah upravljanja. Zaradi podnebnih in topografskih značilnosti kraških območij, produktivnost travišč se spreminja glede na zelo specifične prostorske in časovne vzorce. Zato je bistveno razumeti sezonske spremembe v donosu krmnih rastlin in kakovosti teh pašnikov. Sezonske spremembe v dnevnem donosu (DM) na kraških pašnikih lahko opišemo z dvofaznim modelom z viškom v maju, nato sledi počitek v avgustu, in rahel višek med septembrom in oktobrom. V višku je tudi kakovost trave najvišja. Podani rezultati lahko pomagajo optimizirati strategije upravljanja s pašo. Za trajnostno rabo pašnikov v morfološko kompleksni kraški krajini kot je Kras krajinske posebnosti vodijo odločitve pri upravljanju.

Ključne besede: pašniki, pridelek suhe snovi, sezonska proizvodnja, kakovost krme, stopnja skladiščenja

RIASSUNTO

Il capitolo si focalizza sui pascoli che insiemi ai prati rappresentano l'attività agricola primaria nella zona del Carso. Si tratta di una revisione della gestione tradizionale dei pascoli con particolare attenzione alle strategie di gestione. A causa delle caratteristiche climatiche e topografiche delle aree carsiche, la produttività del pascolo varia secondo schemi spaziali e temporali molto specifici. Perciò è essenziale comprendere i cambiamenti stagionali di rendimento e la qualità di questi pascoli. I cambiamenti stagionali di rendimento quotidiano della SS nei pascoli carsici possono essere descritti da un modello a due fasi, con un massimo a maggio, seguito da una pausa nel mese di agosto, e una leggera ripresa tra settembre e ottobre. La qualità superiore del pascolo avviene durante il massimo rendimento della SS. Questi risultati possono aiutare a ottimizzare le strategie di gestione del pascolo. Per un utilizzo sostenibile di questi pascoli le differenze individuate all'interno dei singoli siti suggeriscono che in un paesaggio morfologicamente complesso come il Carso, queste hanno una grande influenza sulle decisioni di gestione.

Parole chiave: pascoli, resa di sostanza secca, produzione stagionale, la qualità del foraggio, tassi di stoccaggio

15.1 GENERAL ASPECTS OF THE PASTURES

Figure 15.1: Animals grazing on meadows in the Italian and Slovenian karst.

Pastures are forage crops used by domestic and wild herbivores; the animals feed by consuming the herbage directly in the place of production. For this reason, grazing is considered the cheapest and simplest way to feed herbivores. Grazing also seems to be the most efficient way to exploit the herbage mass, as shown in Figure 15.2. Some differences in forage quality between meadow and pasture depend on the herbage utilization: compared to meadows, which require cutting and the subsequent removal of hay, grazing entails a lower forage yield loss and a greater use of the leaves, the more nutrient-rich component of the forage (a description of the quality can be found in subchapter 15.1).

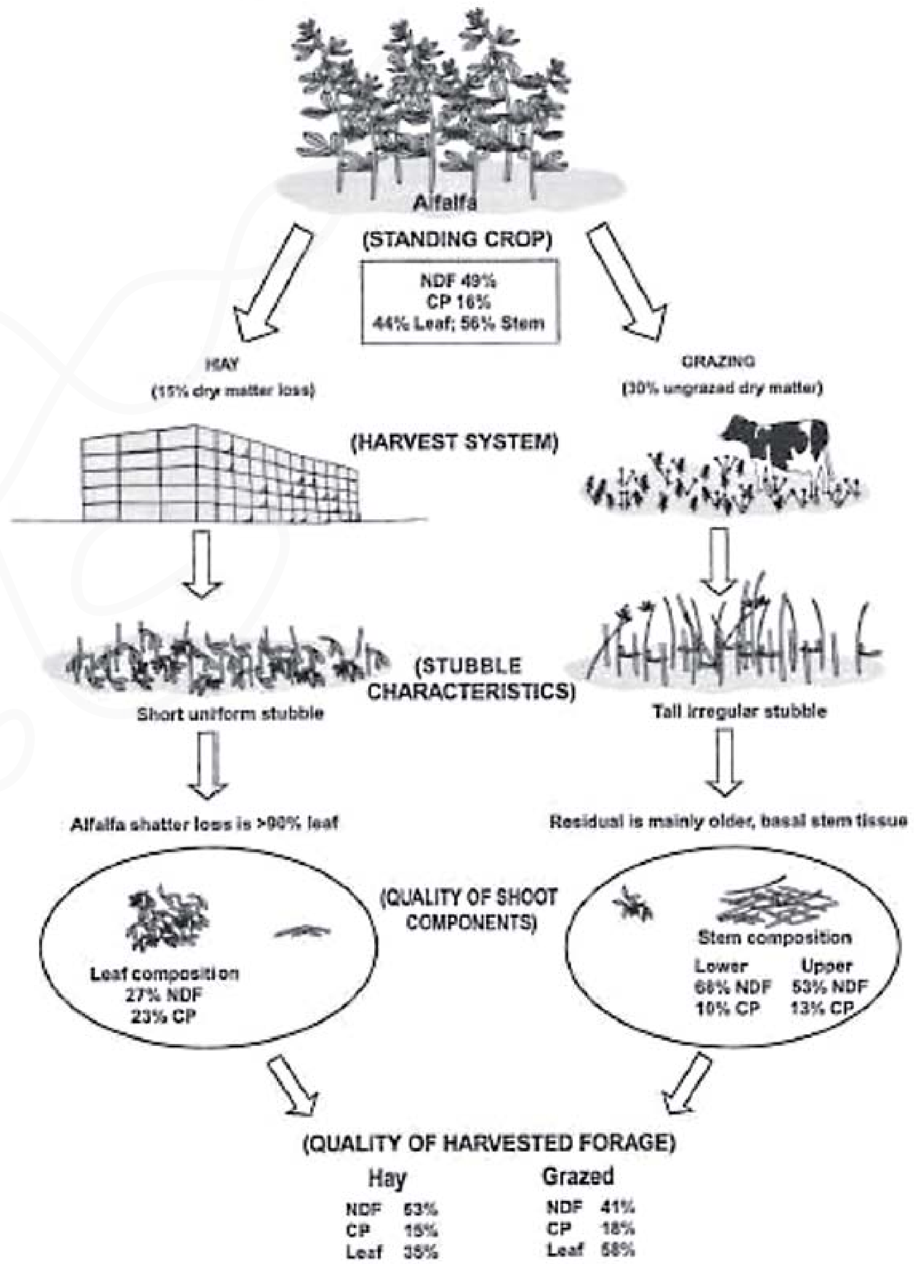


Figure 15.2: A comparison between the utilization efficiency of a crop through cutting (left) and grazing (right) (Barnes *et al.*, 2003).

Pastures are classified according to their origin as *natural (or primary)* if present above the treeline, *spontaneous (or secondary)*, if their vegetation formed spontaneously in a previously deforested area, or *artificial*, if initiated by a human intervention such as seeding, re-seeding or overseeding.

Pastures also differ according to their duration (*annual*, *pluriannual* or *perennial*), species number (*monophytic*, *oligophytic* or *polyphytic*) and type (*herbaceous*, *shrubby* or *arboreal*). For example, natural pastures located just above the treeline are composed of herbaceous species mixed with shrubs, while at higher elevations the plant communities are entirely constituted by herbaceous species. In spontaneous pastures along with herbaceous species, there may often be shrubs and trees and their encroachment is closely dependent on the management strategy of the area.

The herbivores and plants productive cycle are therefore strictly linked in a grazing system (Gusmeroli, 2012). The botanical composition of vegetation as well as the quantitative and qualitative characteristics of herbage mass produced before, during and after grazing, are strongly influenced by the grazing system used (Muller, 2002). The herbage mass removal by grazing is proportional to the size of the herbaceous species (Díaz *et al.*, 2007; Kahmen & Poschlod, 2008). Due to this, under intensive grazing, the competitive ability of taller species is greatly reduced to the benefit of low and medium-sized species. Grazing is thus essential in forming and maintaining a species-rich vegetation.

Pastures are a key component of the mountain landscape where, in addition to providing noteworthy forage production, they perform many other environmental, landscape and ecological functions (as mentioned in Chapter 14). As a result of the progressive depopulation of mountain areas and a progressive reduction in traditional agro-pastoral activities many pastures have been abandoned or not properly used, with the following consequences: 1) spontaneous pastures undergrazed (a consequence also of the use of concentrates in the livestock diet) or abandoned are easily invaded, initially by shrubs and/or large forbs and subsequently by woodland; 2) pastures maintained with a correct stocking rate but grazed by animals fed with concentrates and held for long periods on the same area are subjected to eutrophication, resulting in the spread of a nitrophilous vegetation; 3) overgrazed pastures, subjected to a higher stocking rate than they can support, result in land degradation leading to desertification (Da Ronch & Ziliotto, 2010; Lasanta-Martínez *et al.*, 2005).

15.2 CLASSIFICATION OF MOUNTAIN PASTURES

Environmental factors, especially temperature and substrate characteristics, are primarily responsible for the differentiation of plant communities (Klimek *et al.*, 2007). On the basis of temperatures, pastures can be defined as *macrothermal*, *mesothermal* or *microthermal*, while, with regard to the availability of nutrients in the soil they are divided into *highly-productive pastures* (typical of fertile conditions with abundant availability of mineral elements in the soil) and *dry* ones (with poor quality soils).
In relation to the wide environmental variability characterising mountainous areas, the classification of pastures is quite complex and involves the following types: 1. rich, 2. dry macro- or mesothermal on neutral or alkaline soils, 3. dry meso- or microthermal on neutral or alkaline soils 4. poor on acid soils, 5, cacuminal or subnival areas, 6. nitrophilous herbaceous vegetation (Ziliotto *et al.*, 2004) (Table 15.1).

Table 15.1: Simplified typology of mountain pastures.

	Altitude [m a.s.l.]	No. of species	Days of grazing	LU ha^{-1} load
Highly-productive pastures				
Low altitude *Lolio-cynosuretum*	200-1000	20-30	130-180	2.0-3.0
Med. altitude *Lolio-cynosuretum*	1000-1300	20-30	105-135	1.8-2.7
Festuco-cynosuretum	900-1600	30-65	90-135	1.0-2.0
High mountain-subalpine	1400-2400	28-35	ca. 90	1.5-2.5
Dry macro-mesothermal pastures on neutral or alkaline soils				
Mesophile *Brometum*	300-1600	40-70	ca. 150	0.4-1.5
Alternating humiditymolinietum	0-1800	30-60	ca. 150	0.2-1.0
Brachipodietum	400-1800	40-65	90-150	0.2-1.0
Dry meso-microthermal pastures on neutral or alkaline soils				
Evolved xeric *Seslerietum*	1200-2300	40-70	60-80	0.2-1.0
Mesophile *Seslerietum*	1600-2400	30-60	60-80	0.2-1.0
Caricetum-ferrugineae	1700-2300	35-60	60-80	0.5-2.0
Dry pastures on acid soils				
Montane *Nardetum*	1000-1600	30-65	ca. 100	0.4-1.0
Subalpine *Nardetum*	1600-2300	30-50	60-80	0 2-0.9
Curvuletum	2100-2700	20-30	60-65	0.1-0.9
Cacuminal or subnival zone pastures				
Elynetum	2200-2700	12-25	ca. 60	0.1-0.6
Snowy gulley basiphile cenoses	2000-2700	20-30		0.1-0.2
Snowy gulley acidophile cenoses	2000-2700	10-20		0.1-0.6
Nitrophilous herbaceous vegetations				
Dock-beds (*Rumex*)				
Thistle-beds (*Cirsium*)				
Goosefoot-beds (*Chenopodium*)				
Nettle-beds (*Urtica*)				

Karst pastures fall into the category of dry macro-mesothermal grasslands on neutral or alkaline soils (Pipenbaher *et al.*, 2011). They are usually growing on shallow soils with basic, neutral or slightly acidic reaction and high permeability. Their maximum distribution reaches up to 1600-1800 m a.s.l. Their production and pastoral value are low resulting in a low stocking rate with a maximum of 1.5 LU (Livestock Units) h-1. As a result of this low productivity these pastures are among the grazing areas at greatest risk of abandonment (Aguiar, 2005).

Figure 15.3: Grazing land in use in the Italian Karst (Alture di Polazzo, Gorizia).

From a perspective of environmental sustainability these pastures represent an irreplaceable resource because, despite their low productivity, they support a wealth of flora and fauna (Klimek *et al.*, 2007). They also carry out some important environmental functions, including anti-erosion and water filtering services.

Sustainable management must therefore guarantee the maintenance of all characteristic functions of these pastures. First, they must be used continuously and uniformly to preserve the grazing surface. The utilization must also be targeted at the conservation of botanical richness (Muller, 2002). Lastly, the management impact on the environment should take into account (soil and water).

15.3 THE MANAGEMENT OF PASTURES IN THE KARST

In the karst, livestock are grazed on less productive grasslands with shallow soils, often stony or with rocky outcrops and difficult to use for other purposes. Traditionally these areas are grazed throughout the year and the animals are let loose on the entire available area. However, with increasing attention to this environment and the growing awareness of farmers, free grazing is increasingly being replaced by rotational grazing on different areas of the grazing surface. In addition, hardy breeds of cattle are being used more often given their capability to adapt to the environment and reach even the most marginal areas.

Figure 15.4: Grazing land in use in the Slovenian karst (Hrastovlje, Koper).

Regarding the knowledge required to sustainably manage these pastures, calculation of the proper stocking rate is essential (Škornik *et al.*, 2010; Orlandi *et al.*, 1996). Livestock units are calculated using the following formula:

where: $$C = \frac{P}{Ig \times d} \times K$$

C = the number of LUs (livestock units);

P = the productivity of the pasture during a growing season (t ha^{-1}dry matter, DM);

Ig = daily intake of DM per LU;

d = length of the grazing period in days;

K = utilization parameter ($0 < K < 1$).

Together with the stocking rate, the occurrence of phenological phases of dominant species has to be taken into account for a correct management plan (Carlassare & Scotton, 2011). This supports the choice of grazing techniques for a better use of the available biomass.

When the free grazing technique is applied the grazing surface and stocking rate remain constant throughout the season. For this reason the stocking rate is commensurate with the forage yield available during less favourable periods. This involves the formation of uneaten biomass during the period of maximum growth that will be used later during less productive periods. This grazing technique doesn't permit the selection of pasture species by the animals to be controlled and induces the alternation of undergrazed and overgrazed areas (for the LUs formula K is 0.3-0.4).

Among the various grazing techniques, the one that allows the best use of biomass (K = 0.5-0.7), and also involves no great expenditure of time or money, is rotational grazing. With this technique the grazing surface is divided into a number of sections; the animals are stationed in each of them until they have utilized the available forage. With rotational grazing a rest period from grazing of at least 25-30 days is planned to allow plant regrowth.

To obtain a sustainable management of pastures, it is important to avoid grazing during periods of summer or winter dormancy. The presence of grazing animals in a quiescent period on surfaces already utilized causes pasture deterioration, because plants are unable to recover from browsing. This leads to the use of supplementary feed in the livestock diet and a return of mineral elements to the soil exceeding those removed. The result is eutrophication of the area and a progressive loss of biodiversity.

In the karst area there are also pastures abandoned for many years, in which the vegetation differs from regularly managed pastures. The restoration of these grasslands represents an important issue that should be carefully evaluated. The results of a study conducted to determine the effect of abandonment on the productivity of some ungrazed pastures in the karst (Table 15.2) showed that the herbage mass produced during the first year following the restoration reached 2 t ha-1 y-1 of dry matter, with an applicable stocking rate of slightly more than 0.5 LU ha-1 in the case of lactating cows and 1.2 LU ha-1 in the case of dry cows. Regularly utilized pastures, however, provide 1 t ha-1 y-1 of dry matter and are therefore able to support about 0.3 or 0.6 LU for lactating or dry cows respectively.

Table 15.2: Total annual yield ($t\ ha^{-1}$ DM) and stocking rates (LU) of some karst pastures in Bazovizza (Trieste, NE Italy) from 2005-2009.

site	year	abandoned	in use	annual production ($t\ ha^{-1}$)	(LU) lactating cows	(LU) dry cows
A	2005	X		2.1±0.12	0.59±0.03	1.23±0.07
B	2005	X		2.2±0.14	0.61±0.04	1.31±0.08
C	2005	X		1.0±0.05	0.28±0.01	0.60±0.03
D	2006	X		3.0±0.14	0.83±0.04	1.77±0.08
E	2006	X		2.1±0.13	0.59±0.04	1.26±0.07
F	2006	X		2.0±0.12	0.53±0.03	1.13±0.07
G	2007	X		2.0±0.14	0.53±0.04	1.14±0.08
H	2007	X		2.5±0.15	0.69±0.04	1.46±0.09
I	2007	X		1.1±0.11	0.29±0.03	0.63±0.06
L	2008		X	0.8±0.04	0.22±0.01	0.47±0.03
M	2008		X	1.5±0.07	0.42±0.02	0.90±0.04
N	2008		X	1.2±0.05	0.34±0.01	0.72±0.03
O	2009		X	0.7±0.02	0.19±0.01	0.40±0.01
P	2009		X	1.2±0.03	0.32±0.01	0.68±0.01
Q	2009		X	0.8±0.03	0.21±0.01	0.44±0.02

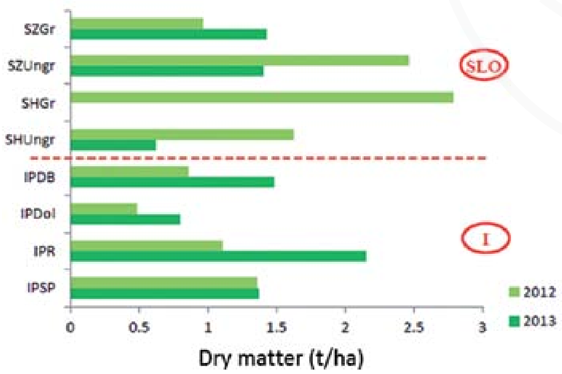
15.4 ANALYSIS OF KARST PASTURES PRODUCTION

The determination of pasture productivity is the basis for the stocking rate calculation and the choice of grazing techniques. One of the aims of the BioDiNet project was to evaluate the biomass produced by some pastures in the Italian and Slovenian karst. The Corrall-Fenlon method (Corall & Fenlon, 1978) was used for the determination of the daily dry matter production, involving the weekly biomass removal from randomly selected plots set out on the grazing surface (Figure 15.5). This method permits reconstruction of the seasonal pattern of daily productivity and quantification of the total annual production.

Figure 15.5: Data collection phases for the study of pasture productivity: a) subdivision of the area into plots, b) measuring the vegetation height, c) cutting the plot, d) biomass collection.

Eight typical karst grazed areas, including four sites located in the Alture di Polazzo (Gorizia, Italy) and four in the municipality of Koper (Slovenia), were identified to study their productivity. A two-year study was conducted from spring 2012 to autumn 2013. The sites choice was made based on their vegetation and geomorphological characteristics. From a phyto-sociological perspective the grazing areas fall within the *Scorzonerion villosae* alliance and are attributable to the *Danthonio alpinae - Scorzoneretum villosae* association. These plant communities are associated with soils on flat or slightly concave ground, of a marly - arenaceous (study area in the Slovenian karst) or terra rossa type overlying carbonate rocks (study area in the Italian Karst), with varying water availability and moderate soil depth. Only one site in Alture di Polazzo (reported in Figure 15.6 as IPDol) is situated in a doline once used for horticultural crops; this site was chosen as representative of favourable conditions for grazing, but it is composed of heterogeneous vegetation not attributable to the types listed above. The remaining sites are located on slopes or ridges at the edge of dolines. Finally, two sites in the municipality of Koper were selected as representative of abandoned pastures in order to evaluate the response to a possible grazing restoration.

As shown in Figure 15.6, the annual forage production of the studied sites was in general low. With the exception of two sites, the annual yield did not exceed 1.5 t ha^{-1} of dry matter. The sites with higher values are eutrophicated (SHGr) or abandoned (SZUngr), or rather sites where the vegetation takes advantage of the abundant nutrients or the absence of previous grazing. Significant differences in production were recorded between the first and second year of experimentation. In the Slovenian study area production was higher in 2012 than 2013, while the opposite occurred in the Italian one. For a first analysis of these results the differences in production between the two years may depend on climatic conditions, as described in Chapter 14, with rainfall events seeming to play a decisive role. The different production capacity shown by the sites does not seem to be related to differences in the vegetation, but rather to the geo-morphological and soil characteristics.



Site (acronym)	Locality	Characteristic
SZGr	Zazid (Koper, Slovenia)	- altitude 401 m; - utilized
SZUngr	Zazid (Koper, Slovenia)	- altitude 390 m; - abandoned
SHGr	Hrastovlje (Koper, Slovenia)	- altitude 99 m; - utilized
SHUngr	Hrastovlje (Koper, Slovenia)	- altitude 96 m; - abandoned
IPDB	Alture di Polazzo (Gorizia, Italia)	- altitude 105 m; - on doline ridge
IPDol	Alture di Polazzo (Gorizia, Italia)	- altitude 100 m; - within doline
IPR	Alture di Polazzo (Gorizia, Italia)	- altitude 101 m; - on marginal ridge, facing the plain
IPSP	Alture di Polazzo (Gorizia, Italia)	- altitude 117 m; - on ridge used as holding area

Figure 15.6: Total pasture production (t ha^{-1} DM) of eight sites in the Italian and Slovenian karst studied in 2012 and 2013. For the abbreviations in the figure and site characteristics see the table below the figure.

In addition to dry matter yield, the forage quality was also evaluated. Nutrient concentrations were determined via near infrared reflectance spectroscopy (NIRS) analysis and revealed an NDF (neutral detergent fibre) content between 40 and 72% of dry matter with an average of 56%, ADF (acid detergent fibre) between 26 and 53% with an average of 39% and ADL (acid detergent lignin) between 3 and 15% with an average of 7%. Lastly, the dry matter crude protein content had a mean of 10%, ranging between 4 and 22%.

The great variability in nutrient concentration depends on many factors, including climatic conditions, phenological stage of vegetation at harvest and geo-morphological and soil characteristics of the site.

15.5 PRODUCTION AND SEASONALITY OF KARST PASTURES

The productivity measured as daily dry matter yield (kg ha-1) of karst pastures follows a trend characterized by two growing periods (Pornaro *et al.*, 2014). Described trend (Cavallero *et al.*, 2002) is a typical for Mediterranean pastures (Figure 15.7).

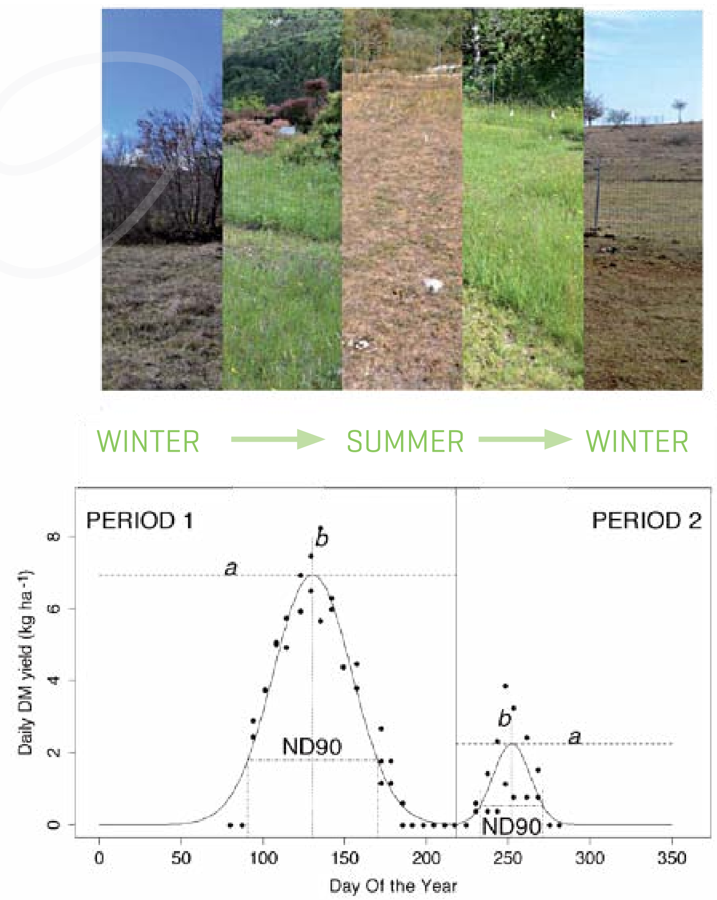


Figure 15.7: Seasonal pattern of daily productivity (kg ha^{-1} DM) of typical karst pastures (IPDol site). The letters *a* and *b* indicate the maximum daily production for that period and the day of the year on which maximum production is reached respectively, "ND90" indicates for each period the number of days in which 90% of the production takes place.

The first peak (period 1) occurs between April and May, the second (period 2) in September. Thus, together with a vegetative stasis in winter, a summer dormancy period of 4-6 weeks was also observed from mid/late July to the end of August. The daily production trend can be described by the parameters of the curve, as shown in Figure 15.7. It varies from year to year and especially among sites. The parameters "a" and "b" indicate the maximum daily production for each period and the day of the year on which the maximum production is reached respectively, while "ND90" indicates for each period the number of days in which 90% of the production takes place. Table 15.3 shows the parameter values for all the analyzed sites in both years.

Table 15.3: Parameters (*a* and *b*) of the curves explaining the daily productivity of pastures (Fig. 4.7) and number of days required to reach 90% of production (ND90), calculated for periods 1 (spring) and 2 (late summer-autumn) on the eight sites studied in the Slovenian and Italian karst.

	2012						2013					
	Period 1			Period 2			Period 1			Period 2		
Sites	*a1*	*b1*	ND90	*a2*	*b2*	ND90	*a1*	*b1*	ND90	*a2*	*b2*	ND90
SZGr	15.2	132	80	1.89	257	34	21.5	132	88	0	0	0
SZUngr	32.1	113	100	1.00	252	30	17.1	117	108	0	0	0
SHGr	37.3	130	74	19.0	255	50	-	-	-	-	-	-
SHUngr	25.2	108	84	1.41	261	36	11.3	115	72	0.31	243	34
IPDB	9.30	120	80	2.26	252	38	17.5	117	104	3.78	266	44
IPDol	6.94	130	114	2.25	252	34	21.6	119	50	0.06	258	36
IPR	13.4	122	104	1.76	258	36	26.1	105	106	2.57	267	42
IPSP	13.4	124	116	5.83	258	42	15.0	127	104	6.62	249	42

It is clear that the studied sites are very different, not only for the maximum production (parameter *a*), but also for the date on which it is reached (parameter *b*). The use of these parameters can have an important practical implication when combined with the total production. For example, in 2013, two sites (SZGr and SZUngr) in the Zazid area had the same dry matter yield (about 1.5 t ha^{-1}). However, the first site (SZGr) reached its maximum production in mid-May, and had a growing period of 88 days (ND90), while the second (SZUngr) reached its peak production at the end of April and had a growing period of 108 days.

Forage quality also follows a seasonal trend. The fibre (NDF, ADF, ADL) and crude protein concentrations varied depending on the time of forage collection, moreover fibres did not show notable differences were found between sites. For all sites, and in both years an increase of protein concentration was observed from the beginning of the growing period until the end of May; this date occurred from 20 to 50 days (depending on the site) after the maximum production of period 1. The concentrations of structural carbohydrates (NDF, ADF and ADL) were similar for all pastures, with significant seasonal variation and higher percentages found in the more productive year (2013). It therefore follows that the sites differ more in their productivity than in the forage quality. For this reason, the management of these grazing surfaces should be based primarily on the production capacity of the different sites.

The results suggest that the sustainable management of pastures in the karst must take into account how the vegetation reacts to changes in environmental factors. Furthermore, the vegetation behaviour is heavily influenced by the geomorphological conditions of the site. For example, the site located in the doline (IPDol) showed a good reaction capability to abundant precipitations such as occurred in 2013. In fact, during the second year of experimentation the production was double that in 2012, and it was produced in a shorter period of time. The site located on the ridge bordering the doline (IPDB) also produced more in 2013 than in 2012, with an extended growing period, and the site on the ridge (IPR) produced more in 2013, increasing the maximum production (peak value). This knowledge is important and should be adequately evaluated by local farmers for a better exploitation of these plant communities, reducing production loss through non-grazing and reducing the alternation of under- or over-grazed areas.

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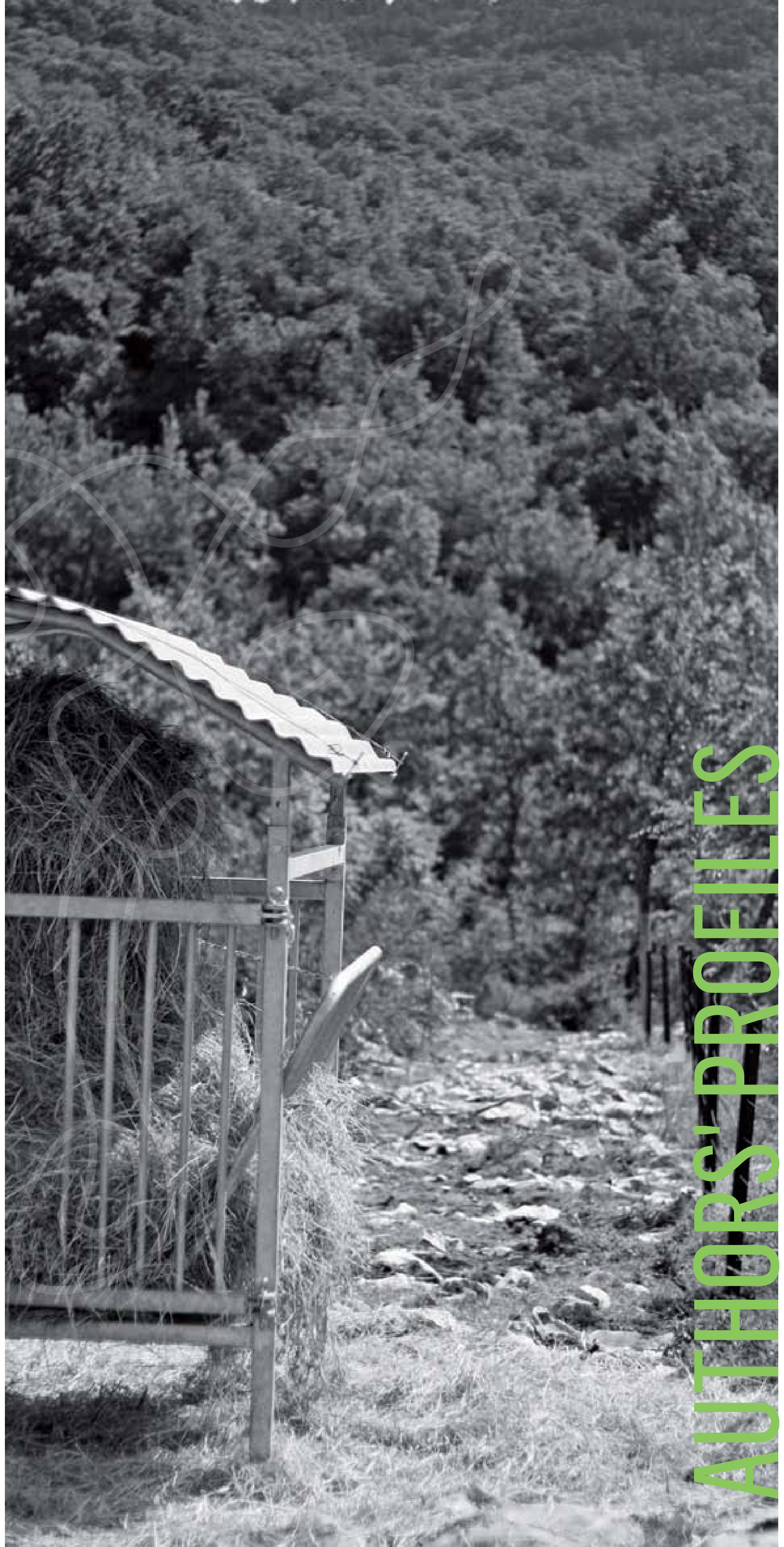

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PAOLA GANIS: Graduated in Natural Sciences in 1980, she is currently technical officer at the Department of Life Sciences (University of Trieste) where she worked since 1982. Within the research group "Quantitative Ecology and Processing Data" she developed methods and software specifically for the quantitative ecology. She developed relational databases for vegetational, climatic, ecological and agricultural data and computerized analytical keys. She has participated in more ministerial research projects on ecological and environmental issues and is the author of more than 60 scientific papers mainly concerning the study and analysis of ecological data using multivariate analysis techniques.

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NATAŠA KOPRIVNIKAR: graduated in Conservation Biology at the University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies on dung beetles (Coleoptera: Scarabeoidea) ecology and diversity from the Karst edge. Presently she is doing her Master´s study in Nature Conservation (University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies).

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DR. MARTINA LUŽNIK studies newts and other amphibians using diverse approaches: from population genetics, phylogenetics to population ecology. Her PhD was focused mainly on newt populations in ponds of the Karst edge.

DR. STEFANO MACOLINO graduated in 1996 in Forestry Science at Faculty of Agriculture of Padova University. In 2003 he achieved the PhD in Environmental Agronomy at Padova University with dissertation on Mixtures and construction systems for high-intensity turfgrasses in transition zones. Since September 2007 he is researcher at Department of Agronomy Food Natural resources Animals and Environment (DAFNAE) of Padova University. His fields of expertise are: forage quality and plant response to cultural practices, strategies for grasslands conservation and turfgrass management. He participated as collaborator or responsible to research projects funded by Italian Ministry for Education, University and Research, Italian Ministry of Agriculture. He teaches the following courses at Padova University: Turfgrasses for sports fields (Bachelor in Forestry and Environmental Sciences) and Grass restorations, turfgrasses and green roofs (Bachelor in Land-use Planning and Landscape Protection).

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DR. ANA MIKLAVČIČ VIŠNJEVEC: BSc in Food science and Technology, PhD in environmental health, 5 years working experience at Department of Environmental Science at the Institute of Jozef Stefan, 9 months working experience at the Institute of Epidemiology and at the University of Udine, has acquired expertise on: work on research projects regarding environmental health, statistical data evaluation, biomonitoring of metals in water, microbiology testing of food and water at the Institute of Public Health Koper.

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PROF. LIVIO POLDINI: Professor emeritus at the Department of Life Sciences (University of Trieste) from 2003. His research work is concerned mainly with floristic, phytogeographic, phytosociologic and phytochemical studies, with vegetation mapping and naturalistic land assessment. Among his numerous papers (more than 300 papers), there are a monograph on the vegetation of the north-Adriatic Karst and two chorological atlases of the flora of Friuli Venezia Giulia and of the book of plant diversity of the Karst between Trieste and Gorizia in order to evaluate the state of the environment. He is correspondent member of the Slovenian Academy of Sciences, Lectern and Arts of Ljubljana, regional adviser for Hegi "Illustrierte Flora von Mitteleuropa" and for "Atlas Flora Europaea", part of the editorial board of several journals.

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KATIA ZANATTA: Graduated in Biological Sciences in 2001 at the University of Trieste, with thesis on the vegetation of the north-Adriatic Karst, tutor prof. Livio Poldini. 2007-08: title of Master's Degree in "Governance of Protected Areas" at the University of Molise (IS-IT). She worked in the field of teaching in high school; of the scientific research as Temporary Research Fellow at the department of environmental sciences-University of Venice and in the field of environmental design with public and private entities. She is currently a PhD student in Environmental Biology at the University of Trieste, where I work for the "Recovery of the 'landa carsica'".

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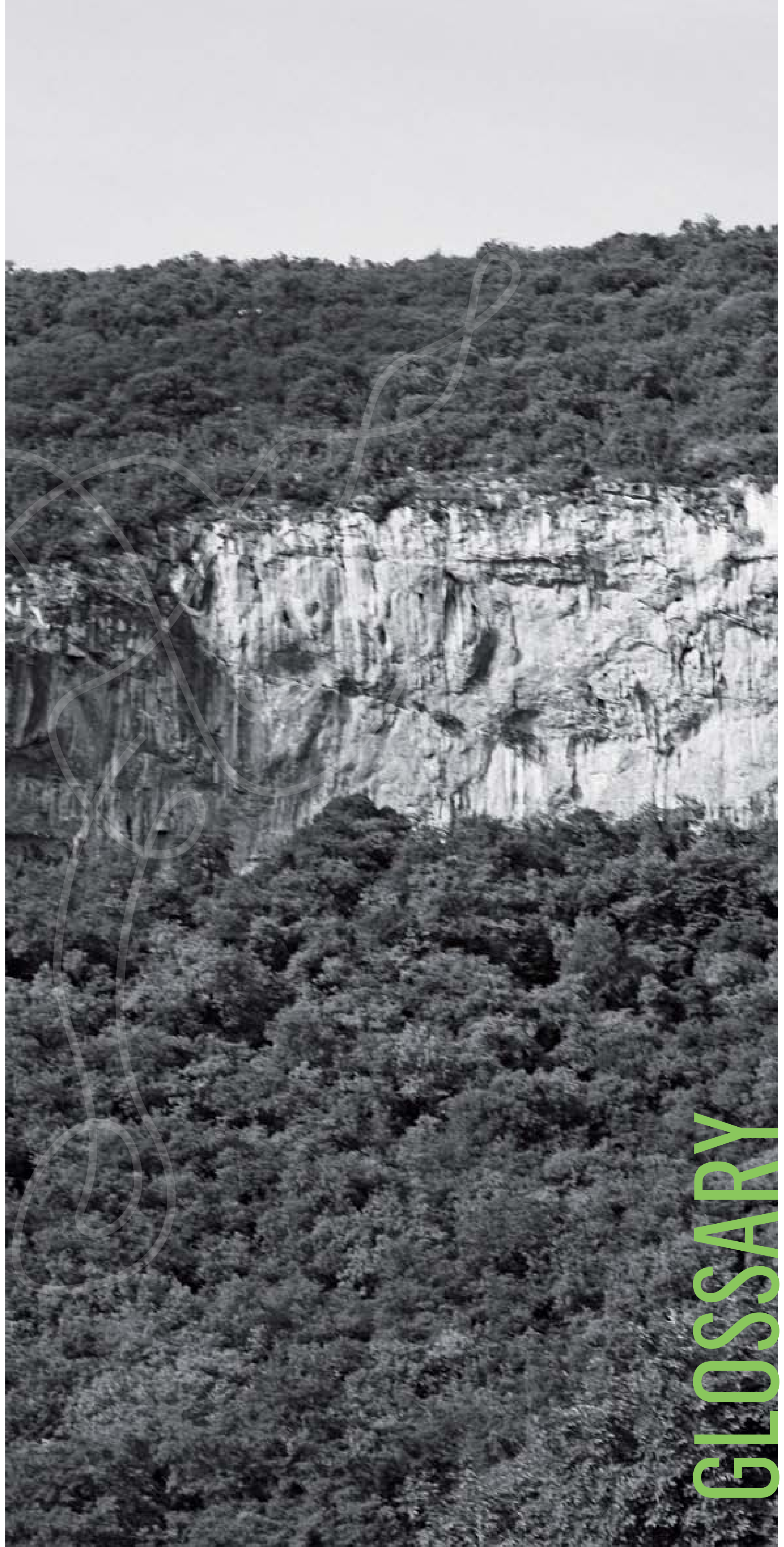
PROF. UMBERTO ZILIOTTO gained his degree in Agronomy Science in 1964. He had teaching experiences in Agronomy,General and Systematic Botany, and Mountain Agriculture. From 1993 to 1999 he was Dean of the Agriculture Faculty at Padova University, and from November 2000 to October 2003 he was the Head of the Department of Environmental Agronomy and Crop Production of the Padova University. Since 1982 he is full professor of Forage Crops at Padova University. His main research interests are on factors affecting yield, forage quality and stand persistence of forage crops, mainly alfalfa, strategies for grasslands conservation (meadows and pastures) and turfgrass management. He is the author and co-author of numerous papers published in scientific journals, books and Proceedings of Italian and international Conferences. He has been in charge of various projects at regional, national and European levels.

SARA ZUPAN is currently a PhD student at University of Primorska in the field of biogeography and involved in research of molecular genetics (evolutionary genetics, phylogenetics, and population genetics) of different vertebrate and invertebrate species.



GLOSSARY

AFLP: short for Amplified Fragment Length Polymorphism. It is a PCR-based technique that uses selective amplification of digested DNA fragments to generate and compare unique fingerprints for genomes of interest.

ALIEN SPECIES: (synonyms: allochtonous, introduced, non-indigenous, exotic, xenophytes) organism in a given area whose presence is due to intentional or unintentional human involvement. They are divided into casual, naturalized or invasive organisms.

AQUIFER: a saturated geologic formation (rock or sediment) capable of storing, transmitting and yielding reasonable amounts of groundwater to wells and springs.

AQUIFER VULNERABILITY: the sensitivity of groundwater quality to an imposed contaminant load, which is determined by the intrinsic characteristics of the aquifer.

BAYESIAN ANALYSIS: Probabilistic analysis defined as levels of confidence in the occurrence of a specific event or to describe a particular structure.

CAVE: A natural cavity in rock. May be water filled. May also be blocked partly or fully by sediment or ice. Commonly formed by solution in limestone. Caves also form in many other rock types, and by many other processes.

CHASMOPHYTE: a plant that grows in the crevices of rocks.

CLUSTER ANALYSIS: identification of groups of samples similar to each other but distinctive from other groups of samples.

COPROPHAGOUS: feeding on excrements, e.g. in mammal excrements (dung).

CRETACEOUS: a geologic period and system from circa 145 ± 4 to 66 million years ago. In the geologic timescale, it follows the Jurassic period and is followed by the Paleogene period of the Cenozoic era. It was a period with a relatively warm climate, resulting in high eustatic sea levels and creating numerous shallow inland seas. These oceans and seas were populated with now extinct marine reptiles, ammonites and rudists, while dinosaurs continued to dominate on land. At the same time, new groups of mammals and birds, as well as flowering plants, appeared. The Cretaceous ended with a large mass extinction, the Cretaceous–Paleogene extinction event (K-T extinction), in which many groups, including non-avian dinosaurs, pterosaurs and large marine reptiles, died out.

CRUDE PROTEIN (CP): the nitrogen in forage multiplied by 6.25.

DIAPAUSE: a physiological state of dormancy, comprising the delay in development in response to recurring adverse environmental conditions.

DIGESTIBLE ENERGY (DE): the energy apparently absorbed from the digestive tract (energy in the food minus energy lost in the faeces).

DNA BARCODING: a method that uses a short genetic marker in an organism›s DNA to identify it as belonging to a particular species.

DOLINE (SINKHOLE): a basin- or funnel-shaped hollow in limestone, ranging in diameter from a few meters up to a kilometre and in depth from a few to several hundred meters.

DRY MATTER YIELD: the total dry weight of forage per unit area of land above a defined reference level, usually ground level, at a specific time.

DSM: Digital Surface Model, represents, in digital form, le altitudes of the upper part of the ground including buildings, infrastructure and the vegetation.

DUNG BEETLES: coprophilous members of the families Scarabaeidae, Aphodiidae and Geotrupidae.

DUNG: animal feces. Commonly used as a synonym of cattle excrements.

ECOTYPE: organism that is closely linked to its ecological characteristics of the environment in which they live.

ELAIOSOMES: fleshy structures rich in lipids and proteins attached to the seeds of many plant species. Many plants have elaiosomes that attract ants.

ENDEMIC SPECIES: a species which is only found in a confined region or location and nowhere else in the world.

ENVIRONMENTAL MONITORING: describes the processes and activities that need to take place to characterise and monitor the quality of the environment.

ENVIRONMENTAL POLICY: refers to the commitment of an organization to the laws, regulations, and other policy mechanisms concerning environmental issues and sustainability. These issues generally include air and water pollution, solid waste management, biodiversity, ecosystem management, maintenance of biodiversity, the protection of natural resources, wildlife and endangered species.

FAULT: a fracture separating two parts of a once continuous rock body with relative movement along the fault plane; it results from tectonic stresses on the rock mass.

FIBRE CONCENTRATION: a nutritional entity that is relatively resistant to digestion and is slowly and only partially degraded by herbivores.

FLYSCH: A sequence of sedimentary rocks that is deposited in a deep marine facies in the foreland basin of a developing orogen, typically during an early stage of the orogenesis (syn-orogenic sediment); it is characterized by thinly interbedded sandy shale, mudstone, sandstone and conglomerate.

FORAGE QUALITY: a description of the degree to which forage meets the nutritional requirements of a specific kind and class of animal.

GENE POOL: the set of all alleles of the entire set of genes that belong to all the individuals that make up a population.

GLACIAL REFUGIA: areas which were ice-free during the Pleistocene, which served as places where biodiversity persisted and acted as areas of recolonization in interglacial periods. In the present day they are recognised high genetic and biotic diversity.

GRASSLANDS: vegetation including grasses, legumes and other forbs, and at times woody species.

GRAZING STRATEGIES: the manipulation of the soil–plant–animal complex of the grasslands in pursuit of a desired result.

GRAZING: grazing is a type of feeding, in which a herbivore feeds on plants (such as grasses), but the grazed organism usually not killed.

GROUNDWATER CONTAMINATION: an introduction of certain contaminant(s) into the groundwater which reduces the quality of groundwater making its use very limited, or in some cases impossible. Many different chemicals, and various synthetic products, applied to the soil or left on it, are usually the main causes.

HAPLOTYPE: combinations of allelic variants along a chromosome or in the same sequence.

HARVEST: forage defoliated by a single grazing or cutting, or over a series of grazing or cuttings. It may be reported as daily amount, a single harvest, or seasonal or annual totals.

HARVEST DATE: the date in which herbage biomass of a meadow is harvested.

HEMOCYTES: blood cells (e.g. of honeybees).

HERBAGE: the above-ground biomass of herbaceous plants, other than separated grain. Grasses, grass-like species, herbaceous legumes and other forbs collectively; the foliage and edible stems of herbs.

IMMUNOCOMPETENCE: the ability of the body to produce a normal immune response following exposure to pathogens.

IMMUNOSUPPRESSION: reduction in the activation or efficacy of the immune system.

INDICATOR SPECIES (TARGET SPECIES): a species which is a good indicator of the living conditions in a particular habitat and that responds to changes in environmental parameters.

INDICATOR SPECIES ANALYSIS: technique aimed at the objective assessment of a species that characterize a series of reliefs divided into groups (clusters) default.

INVASIVE SPECIES: a non-native species whose introduction or spread threatens biodiversity and/or cause serious damage, including economic, to the activities of man and/or health.

IUCN: The International Union for Conservation of Nature. It is the world's oldest and largest global environmental organisation, a leading authority on the environment and sustainable development. One of its tasks is assessing the conservation status of species, subspecies, varieties, and even selected subpopulations on a global scale in order to highlight taxa threatened with extinction, and thereby promotes their conservation.

KARST: Terrain with special landforms and drainage characteristics due to greater solubility of certain rocks in natural waters than is common. Name derived from the province in Slovenia, where this type of landform was first described.

KARST AQUIFER: a body of soluble rock that conducts water principally via conduits formed by the dissolution of the rock by the water itself. It is commonly structured as a branching network of tributary conduits, which connect together to drain a groundwater basin and discharge to a perennial spring. It is generally characterized by high heterogeneity and important groundwater storage, apart from high vulnerability to pollution.

KARST (DRY) GRASSLANDS: meadows and pastures rich in species from the Ponto-Caspian region and of the SE-Europe, co-evolved with the activities of transhumance and mowing.

KARST PONDS: manmade water bodies in limestone areas where natural superficial water is absent. Ponds were used in traditional farming mainly for watering cattle and are nowadays endangered biodiversity reservoirs.

KARSTIFICATION: A periodic or cyclic process where phases of active solutional development of karst are followed by infilling of karst conduits and voids.

LIMESTONE: A sedimentary rock consisting mainly of calcium carbonate ($CaCO_3$) derived from the deposition of the calcareous remains of animals.

LOCUS CLASSICUS: the site where the type of a species was originally collected.

MANAGEMENT STRATEGIES: the manipulation of the soil–plant complex of the grasslands in pursuit of a desired result.

MARK-RELEASE-RECAPTURE: a method used frequently in ecology to estimate animal population's size. It is based on proportions of marked and unmarked individuals in successive sampling occasions.

MEADOW: A natural or semi-natural grassland often associated to hay or silage for animal feeding.

MELANIZATION: defense reaction against pathogens through the production of the melanin pigment that forms a barrier around the pathogen's body.

MICROSATELLITES: sequence of non-coding DNA consisting of very short repeating units (1-5 bp) arranged in tandem, can be extremely effective as molecular markers.

MITOCHONDRIAL HAPLOTYPE: a set of mitochondrial DNA sequences having the same single nucleotide mutations. Similar haplotypes share a common ancestor and are clustered in haplogroups or lineages using phylogenetic analyses.

MITOCHONDRIAL MOLECULAR MARKERS: fragment of mitochondrial DNA that is associated with a particular species.

MYRMECOCHORY: seed dispersal by ants. Myrmecochorous plants produce seeds with elaiosomes.

NATURA 2000: an European network of nature protection areas established in order to assure the long-term survival of Europe's most valuable and threatened species and habitats. It is comprised of Special Areas of Conservation (SAC) designated by Member States under the Habitats Directive, and also incorporates Special Protection Areas (SPAs) designated under the 1979 Birds Directive.

NUCLEAR MOLECULAR MARKERS: nuclear DNA fragment that is associated with a particular species / organism.

OROGENESIS: a great range of geological processes involved when a large structural deformation of the Earth's lithosphere (crust and uppermost mantle) occurs, due to the engagement of tectonic plates, resulting in the formation of long tracts of highly deformed rock called orogens or orogenic belts.

ORTOLAN BUNTING – *Emberiza hortulana*, an endangered species of bunting typical of dry karstic meadows, unlike the other species of bunting present in the study areas it is a trans-Saharan migrant.

PASTURES: a type of grazing management unit enclosed and separated from other areas by fencing or other barriers and devoted to the production of forage for harvest primarily by grazing.

PCR: Polymerase chain reaction, PCR, is an efficient and cost-effective way to amplify small segments of DNA or RNA.

PHAGOCYTOSIS: process by which certain living cells called phagocytes ingest or engulf other cells or particles.

PHYTOSOCIOLOGY: a branch of science that studies the vegetation classification and plant communities; the phytosociological nomenclature is the basis of the definition of habitats according to EU Habitat Directive (43/92/EEC).

PLASM PHENOLOXIDASE: enzyme responsible for the synthesis of the dark pigment melanin, involved in the defense against pathogens. The plasma component is present in the blood (not inside the emocytes).

POPULATION ECOLOGY: studies the dynamics of species populations over time and space; and their interaction with the environment.

POPULATION INDEX: percentage of population recorded with the same method, relative to the starting point in time.

PROPHENOLOXIDASE (PROPO): inactive form of the phenoloxidase enzyme, activated by the presence of pathogens.

REMOTE SENSING: the practice of deriving information about the Earth's land and water surface using images acquired from an overhead perspective using electromagnetic radiation in one or more regions of the electromagnetic spectrum, reflected or emitted from the Earth' surface.

SCORZONERO-CHRYSOPOGONETALIA: xeric grasslands of the sub-Mediterranean zones of NE-Italy, Istria and the Balkan peninsula; it is habitat N2000 62A0.

SEASONAL PRODUCTION: the total dry matter yield of a grazed surface, throughout the year.

SMAP: Sequential Maximum A Posteriori algorithm attempts to improve imagery classification accuracy by segmenting the image into regions rather than segmenting each pixel separately.

SPA: Special Protection Area is an area designated according to the European Union Birds Directive.

SPRING: a natural discharge of groundwater at the land surface.

STENOENDEMIC SPECIES: an endemic with a narrow distribution range.

STOCKING RATE: the number of animals on a given amount of land over a certain period of time, generally expressed as animal units per unit of land area.

STYGOBIONT (STYGOFAUNA): a species or population strictly that live in ground water systems or aquifers (below the water table), such as some caves, fissures and vugs.

SUMMER DORMANCY: is a period of arrested plant growth, it is a survival strategy exhibited by many plant species.

TERTIARY: the first geologic period of the Cenozoic era, between the Cretaceous (after K-T extinction) and Quaternary periods, and comprising the Palaeogene and Neogene sub-periods. It lasted from 65 to 1.8 million years ago. Many mammals developed then, including primitive whales, rodents, pigs, cat, rhinos, etc.

THRUST: a type of fault in which rocks of lower stratigraphic position are pushed up and over higher strata, often recognized because they place older rocks above younger. Thrust faults are the result of compressional forces.

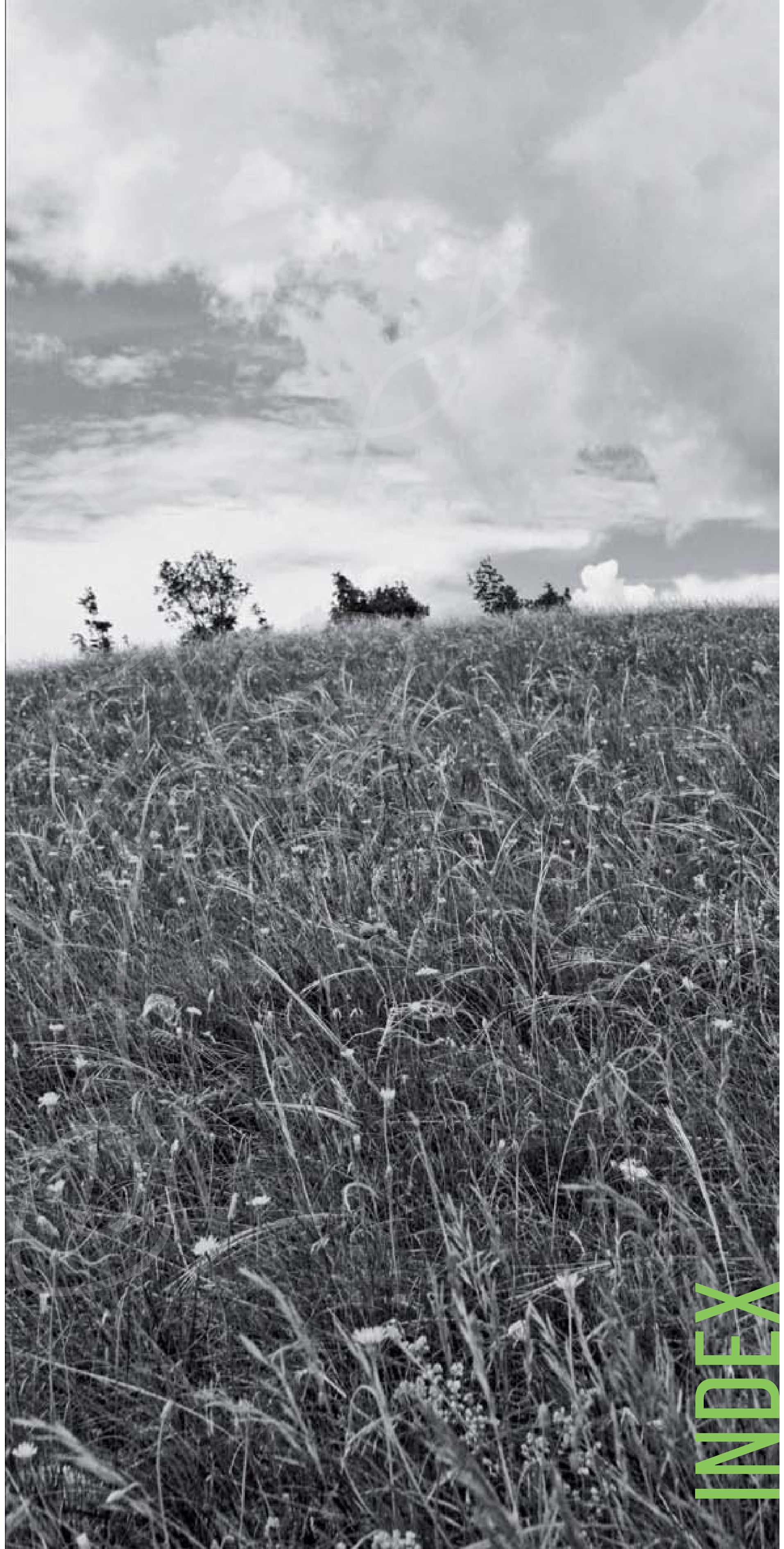
TOTAL HEMOCYTE COUNT: honeybee emocyte count done with a special graduated microscope slide called hemocytometer.

TRANSECT COUNT: count of animals, especially birds along the line of walking; transect counts enable the calculation of relative breeding densities of birds.

TRANSECT: is a path along which one counts and records occurrences of the phenomena of study (e.g. plants). Belt transects are used in biology to investigate the distribution of organisms in relation to a certain area.

TROGLOBIONT (TROGLOFAUNA): a species or population strictly limited to a hypogean habitat, above the water table.

UAV: Unmanned Aerial Vehicle (also known by the acronym RPAS or Remotely-piloted Aircraft System), is a vehicle designed to operate without a human pilot aboard.



INDEX

Biodiversity and conservation of karst ecosystems is a synthesis of knowledge about geological and geomorphological and biological aspects, which clarifies the required conditions for maintaining biodiversity on the karst and Karst edge, a broad region, located along the border of Italy and Slovenia.

In this book we provide brief scientific summaries of the research work founded by European program of cross-border collaboration Slovenia-Italy 2007-2013. The Guidelines for the biodiversity conservation of karst ecosystems are designed for stakeholders and local public administration.

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REPUBLIKA SLOVENIJA
SLUŽBA VLADE REPUBLIKE SLOVENIJE ZA RAZVOJ IN EVROPSKO KOHEZIJSKO POLITIKO

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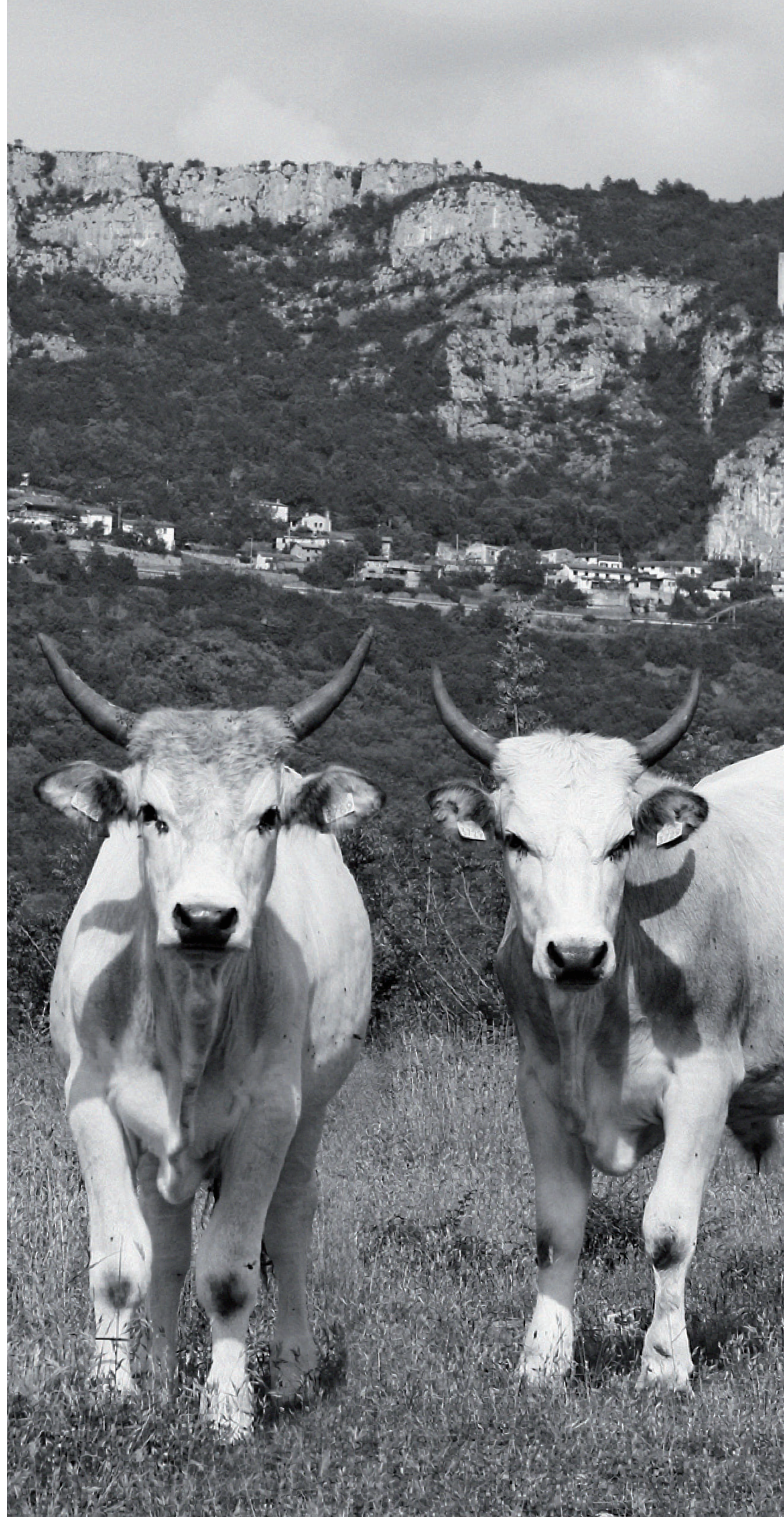

BIODIVERSITY AND CONSERVATION OF KARST ECOSYSTEMS