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CONTENTS

EFFECT OF NATURE CONSERVATION ON THE SOCIO-ECONOMIC DEVELOPMENT OF MUNICIPALITIES IN THE SOUTH WESTERN BORDER REGION OF THE CZECH REPUBLIC

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ABSTRACT

In this study, we focus on factors affecting the socio-economic development within a protected zone and attempt to elucidate if being in a protected area significantly affects the development or whether other factors also have a role. We focused on population counts recorded in 1991 and 2011 in order to identify the changes in the economy following to the establishment of the Šumava National Park in 1991 and Český les Protected Landscape Area (PLA) in 2005. A total of 39 municipalities of similar size and history were included and 18 socio-economic indicators, which can be broadly categorized in terms of economy, landscape use and municipality income. We performed ANCOVA to determine the association between the size and location (outside or inside protected area) of a municipality and each of the18 socio-economic indicators. They did not vary significantly in 1991. After two decades the demography, economy and landscape usage were significantly different. However, they were not a result of being in a protected area but changes in the sizes of the municipalities. The municipalities located within protected areas may profit from their locality and it has positive rather than negative effects on the socio-economic indicators.

Keywords: border region; Český les PLA; demography; protected area; socio-economic development; Šumava NP

Introduction

One of the most important achievements of nature conservation is protection, implemented by means of specially protected areas, such as national parks (NP) or protected landscape areas (PLA). In the Czech Republic, these protected areas are proclaimed under Act no. 114/1992 Coll. on Nature and Landscape Protection of sites of scientific or aesthetic importance or uniqueness. Such sites are protected because of their biological diversity, unique geology or are typical elements of a particular landscape. The reason for protecting a site is to conserve or improve its preserved state or leave it to spontaneous development.

In the Czech Republic there are no unpopulated landscapes, which imply some restrictions on socio-economic development of municipalities located in protected areas, such as, agriculture practices, building new roads or railways, location of industrial buildings, mining, etc. The management of such areas must take into account the level of protection of the areas in which they are located in order to preserve and create optimum ecological conditions and provide the inhabitants with a good living. Recreational use should be allowed provided it does not adversely affect the natural values of the protected area. Currently, people living in protected territories want to change the legislation and so reduce the level of protection of zones with built-up areas. The strongest argument of the protesters is the incorporation of their municipalities into the Šumava National Park has resulted in a decrease in their socio-economic development.

In this study, we focus on the factors affecting the socio-economic development within a protected zone and attempt to elucidate if being in a protected area significantly affects the development or whether other factors have a crucial role. We focused on population counts recorded in the years 1991 and 2011 in order to identify changes in the economy due to the establishment of the Šumava National Park in 1991.

Methods

Study area

The study area is located on the south-western border of the Czech Republic (Fig. 1) and extends from the northern border of the Český les PLA, across the Šumava NP and PLA, to the eastern border of the future and not yet proclaimed Novohradské hory PLA, which was recently protected by being designated a natural park. There are also many smaller protected areas located here, for example, the national nature reserve (NNR) Terčino údolí, national natural monument (NNM) Hojná voda, Žofínský prales NNR, Čertova stěna–Luč (NNR) and NATURA 2000 network: special protection areas (SPA) designated for birds Boletice, Novohradské hory, Šumava and many sites of community importance (SCI) designated for habitats, plant and animal species. The study area belongs to the following municipalities: České Budějovice, Český Krumlov, Domažlice, Klatovy, Prachatice and Tachov (Albrecht et al. 2003; Zahradnický and

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Mackovčin 2004). From the historical point of view, the study area is in the Sudetes or more specifically the Poor Sudetes (Perlín 1998).

Climate in this region varies depending on altitude with decreasing mean temperature and increasing precipitation from 900 mm to 1,600 mm (Albrecht et al. 2003; Křivancová et al. 2006; Bílá et al. 2018).

Vegetation cover in this area is dense forest of beech mixed with fir at low altitudes and spruce forests in the high mountains along with growths of ash-alder and peat bogs at waterlogged localities, mountain grassland and pastures characteristic of the Šumava NP. At the lowest altitudes in the Český les PLA there are also oak forests (Sofron 1990). There are a number of protected species in the area, but the most valuable are ecosystems unaffected by human activities (Albrecht et al. 2003; Zahradnický and Mackovčin 2004).

Settlement history

The very first human settlements were in the 7th and 8th century when Slavic folk inhabited sites along streams. Population density increased during the Middle Ages and both Czech and German cultures occurred in the study area, whereas Germans prevailed in the central part of the Šumava and Czechs settled more in the Šumava foothills (Nikrmajer 2003; Řezníčková 2003, 2005, 2006; Matušková 2005a; Sassmann 2006).

There were marked changes in 1918 when Czechoslovakia was established and Czechs were moved to sites on the south-western border. In 1938, most of the area studied belonged to Germany but after the Second World War in 1945, Germans had to leave this locality and it was abandoned. There were attempts to recolonise this area with Czechs and emigrants but the number of inhabitants never reached the previous population density (Nikrmajer 2003; Jílek 2005a; Mörtl 2006). From 1948 till 1989, the border was closed and a border zone 2–6 km wide with banned entry was established. During this time, more than a hundred municipalities ceased to exist in this area (Jílek 2003; Jílek 2005b; Matušková 2005b; Klobása 2006).

The most important business in the past and recently is forestry and wood production. In the past, wood production was connected to the paper industry and a smaller part to the local production of glass, which now no longer exists (Lněničková 1996; Procházka 2005; Fröhlich and Lněničková 2006). Agriculture in the Šumava region, in terms of income, was never important, however, it provided all the food for the local people, but more common were cattle breeding or fish farming at the lower altitudes (Český les and Novohradské hory). Mechanical engineering plants and raw mineral material processing are also present in this region (Kočárek jun. 2005; Stejskal 2006). From the time of the establishment of the NP and PLA, tourism provided the major part of income in this area (Bartoš and Čihař 2011; Dickie and Whiteley 2013).

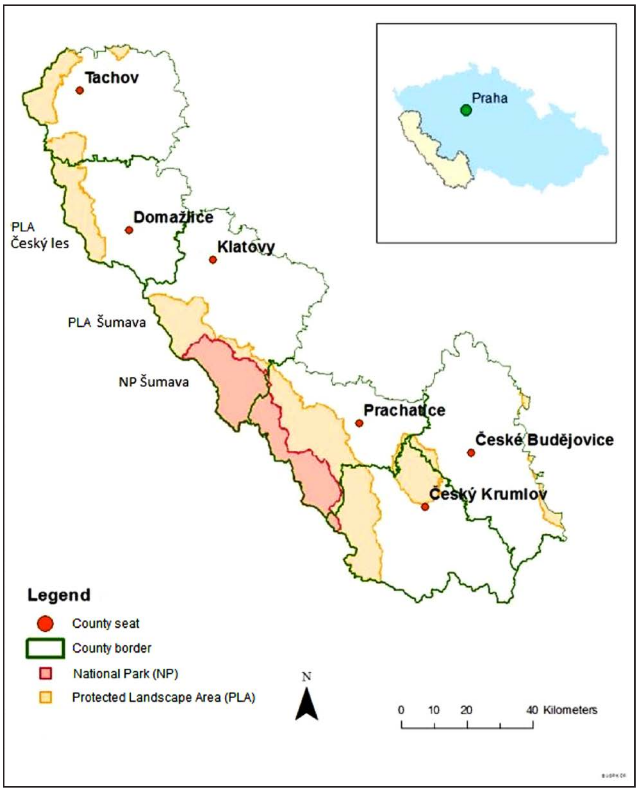

Fig. 1 Map of the south-western border region of the Czech Republic showing the large protected areas: NP – National Park (pink), PLA – Protected Landscape Area (orange).

Municipalities studied

The municipalities studied were either located in the NP or PLA, or outside these protected areas. The number of inhabitants was taken into account using a method used by the Czech Statistical Institute (CSI) in which municipality size is measured in terms of the number of inhabitants per km^2 (CSI 2009; 2013; 2014). This enabled us to select municipalities with very dense populations (e.g. Kubova Huť) and those with high values for the relevant indicators (e.g. Modrava – municipality with a high level of tourism), which resulted in the selection of a total of 39 municipalities (Fig. 2).

Demographic data for the 39 municipalities selected were obtained from the CSI and are for the years 1991 and 2011 when there were censuses of the populations and households in these municipalities. The CSI also provided data on socio-economic factors (years 2003 and 2011) and the information about municipality budgets (years 1994 and 2011) was obtained from the Czech Ministry of Finance. The changes that occurred in land use were obtained from the State Administration of Land Surveying and Cadastre.

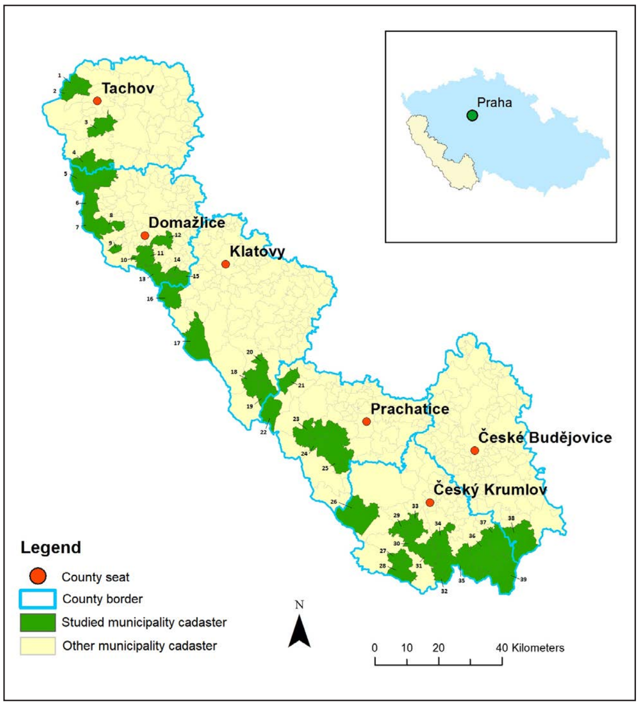

Fig. 2 Cadastral map showing the locations of the municipalities studied (green): 1 – Halže, 2 – Obora, 3 – Staré Sedliště, 4 – Třemešné, 5 – Bělá nad Radbuzou, 6 – Rybník, 7 – Nemanice, 8 – Postřekov, 9 – Pec, 10 – Tlumačov, 11 – Mrákov, 12 – Zahořany, 13 – Všeruby, 14 – Chodská Lhota, 15 – Pocinovice, 16 – Chudenín, 17 – Železná Ruda, 18 – Srní, 19 – Horská Kvilda, 20 – Rejštejn, 21 – Stachy, 22 – Kvilda, 23 – Horní Vltavice, 24 – Lenora, 25 – Volary, 26 – Horní Planá, 27 – Lipno nad Vltavou, 28 – Loučovice, 29 – Světlík, 30 – Malšín, 31 – Rožmberk nad Vltavou, 32 – Horní Dvořiště, 33 – Bohdalovice, 34 – Rožmitál na Šumavě, 35 – Dolní Dvořiště, 36 – Malonty, 37 – Benešov nad Černou, 38 – Horní Stropnice, 39 – Pohorská Ves.

Data analyses

Statistical analyses were performed using STATISTICA 12 and general linear models. We used ANCOVA to identify changes in the factors selected with number of inhabitants (year 1991) as a covariate, independent variable was type of area (0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA) and dependent variable was the change in the factor over time. We used the Shapiro-Wilk test to determine whether the data was normally distributed before ANCOVA and whether a logarithmic transformation log(x + 1) was necessary.

Maps were prepared using ArcGIS 10.4 with map layers from two databases: ArcČR500 and AOPK ČR.

Results

Demographic indicators

Total number of inhabitants in the municipalities studied was 31,439 in 1991 and 31,682 in 2011, with a slightly greater decrease in the number of inhabitants in municipalities located in NP or PLA from 11,214 in 1991 to 10,937 in 2011. Number of inhabitants in Český les PLA slightly increased from 4,813 in 1991 to 4,884 in 2011. There was a slight increase in the number of inhabitants in municipalities outside protected areas from 15,412 in 1991 to 15,861 in 2011. ANCOVA revealed a significant effect of municipality size on the change in the number of inhabitants (p = 0.042) and that location inside or outside protected area had no significant effect (p = 0.905).

Level of unemployment increased from 3.244% in 1991 to 13.047% in 2011. The average unemployment in municipalities outside protected areas was 3.438% in 1991 and 13.702% in 2011 and inside protected areas 2.679% in 1991 and 12.282% in 2011. Similarly, the ANCOVA results revealed a significant effect of municipality size (p = 0.002) and insignificant effect of location (p = 0.112). Regression analysis revealed that there was a higher level of unemployment in municipalities with low numbers of inhabitants.

Percentage of inhabitants of productive age (15–64 years) was 66.141% in 1991 and 70.359% in 2011. The highest increase was recorded in municipalities outside protected areas and the lowest in the Šumava NP and PLA. ANCOVA revealed no significant effect on the percentage of inhabitants of productive age of either of municipality size (p = 0.088) or location (p = 0.167).

ANCOVA of all three above mentioned factors revealed a significant effect of municipality size (p = 0.001), whereas location of the municipality inside or outside a protected area (NP or PLA) has no significant effect on its demographic factors (p = 0.154) (Fig. 3).

Socio-economic indicators

In the municipalities studied there were 7,068 socio-economic indicators in 2003 and 8,229 in 2011. The increase was obvious both inside and outside protected

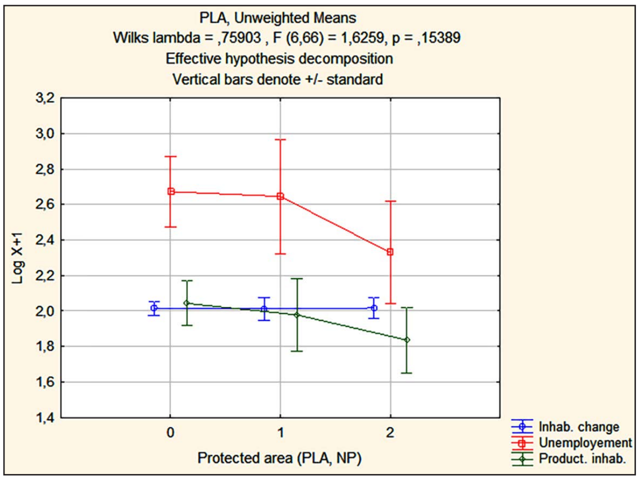

Fig. 3 ANCOVA of the changes in number of inhabitants, level of unemployment and inhabitants of productive age in the years 1991 and 2011. Locality type: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

areas, however, a marginal increase was detected in an area no part of which was ever protected, namely from 2,826 to 3,676. The lowest number of socio-economic indicators was recorded in the NP and PLA. ANCOVA showed no significant association between municipality size and number of socio-economic indicators ($p = 0.097$), but municipality location was significantly associated ($p < 0.001$) (Fig. 4).

Of the total number of inhabitants more than 15 years old, 2.85% obtained a university degree in 1991 and 4.61% in 2011. ANCOVA showed a significant association between municipality size and the percentage obtaining a university degree ($p = 0.022$), but no significant association with municipality location ($p = 0.908$). ANCOVA also revealed that the existence of nursery and primary schools is associated with municipality size ($p < 0.001$) and no significant association between municipality location and nursery schools ($p = 0.338$) or primary schools ($p = 0.444$). ANCOVA of all socio-economic indicators, except economic entities, indicated statistically significant associations with municipality size ($p < 0.001$) and no significant association with locality ($p = 0.604$) (Fig. 5).

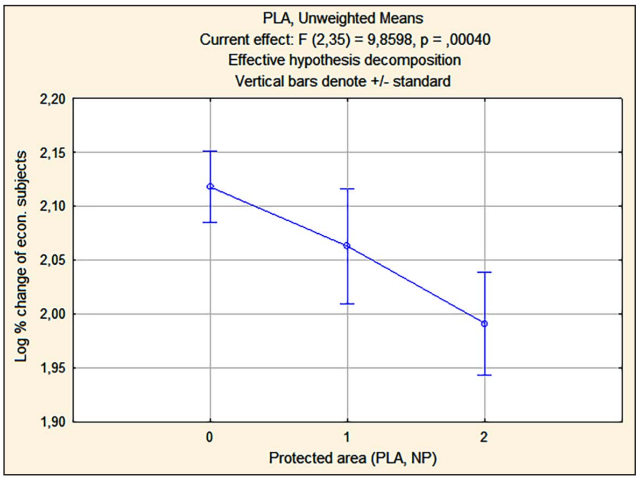

Fig. 4 Results of the ANCOVA of the number of socio-economic indicators in the municipalities studied in 2003 and 2011. Locality type: 0 – outside protected area, 1 – Český les PLA, 2 –Šumava NP and PLA.

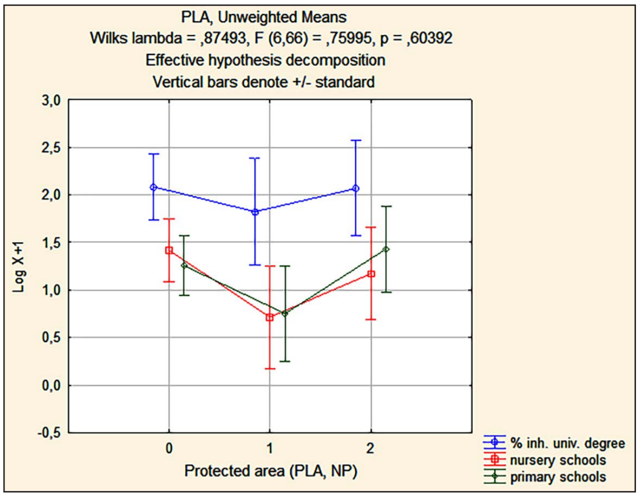

Fig. 5 Results of the ANCOVA of the inhabitants with a university degree and the existence of nursery and primary schools in 1991 and 2011 (in %). Locality: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

Landscape indicators

The area covered by the municipalities studied was 1,611.361 km^2 in 1993 and 1,625.767 km^2 in 2011. The marginal increase in the area covered by the municipality Srní, from 21.823 km^2 to 33.485 km^2 was due to the closure of the military training area Dobrá Voda. Changes in the areas covered by the other municipalities did not exceed 3 km^2.

Number of new houses increased from 7,348 in 1991 to 9,589 in 2011. Most (61%) of these houses were built in localities situated outside protected areas. The highest increase in the number of houses was recorded in the smallest municipalities in 1991. ANCOVA revealed a significant association between municipality size and the increase in the number of houses ($p = 0.011$) and insignificant association with the localities of the municipalities ($p = 0.162$). We also tested the changes in the numbers of unoccupied houses but they were not significantly associated with either municipality size ($p = 0.501$) or location ($p = 0.270$). Similarly, there were no significant associations of changes in terms of recreation (municipality size: $p = 0.163$; municipality location: $p = 0.624$).

ANCOVA revealed significant associations between municipality size ($p = 0.022$) and location ($p = 0.011$), and changes in land use (arable land, forest, grasslands, built-up area). In particular, changes in the area of arable land (municipality size: $p = 0.001$; municipality location: $p = 0.003$) with the highest decrease occurring in the Šumava NP and PLA, changes in the extent of forest (municipality size: $p = 0.647$; municipality location: $p = 0.954$), grassland (municipality size: $p = 0.784$; municipality location: $p = 0.157$) and built-up areas (municipality size: $p = 0.228$; municipality location: $p = 0.903$). ANCOVA summary revealed a significant association of landscape indicators with municipality size ($p = 0.028$) and insignificant association with municipality location ($p = 0.077$) (Fig. 6).

Income indicators

Municipality income in the area studied was 312.141 thousand CZK (Czech crowns) in 1994 and 722.577 thousand CZK in 2011. ANCOVA revealed a significant association between municipality size and income ($p = 0.033$), but no association with municipality location ($p = 0.061$). Average income per inhabitant increased from 9,948 CZK in 1994 to 27,167 CZK in 2011. ANCOVA revealed a significant association between the locality of the municipali-

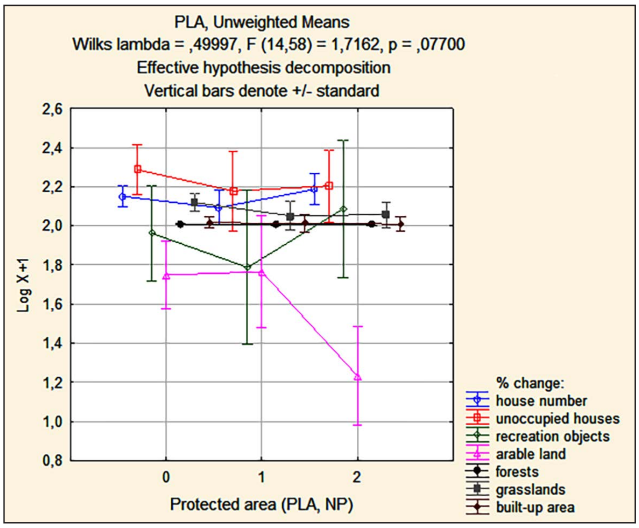

Fig. 6 Results of the ANCOVA of the changes in landscape indicators in 1991 (number of houses), 1993 (type of land use) and 2011 (% change in the number of houses unoccupied houses, recreation objects, arable land, forest, grasslands, built-up area; Locality: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

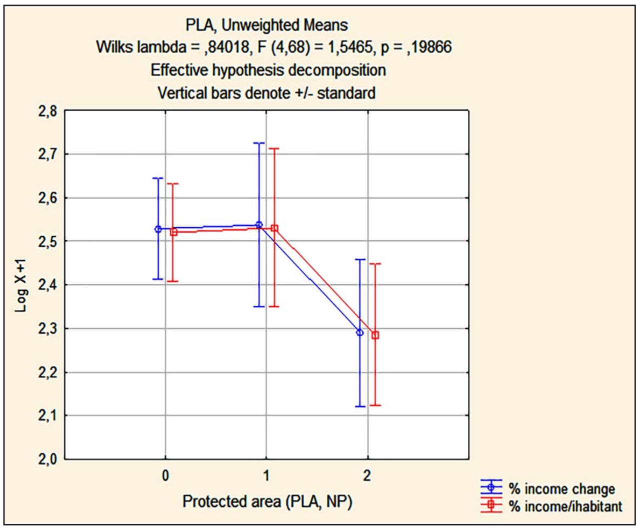

Fig. 7 Results of the ANCOVA of the changes in the incomes per inhabitant in 1994 and 2011, in terms of % income/percentage change in income, % income/inhabitant; Locality: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

ties and income (p = 0.053) and an insignificant association with the size of the municipalities (p = 0.143). The greatest increase was recorded in municipalities located in the Český les PLA. ANCOVA summary showed a significant association with municipality size (p = 0.021) and insignificant association with municipality location (p = 0.199) (Fig. 7). Figures 8 and 9 show incomes per inhabitant.

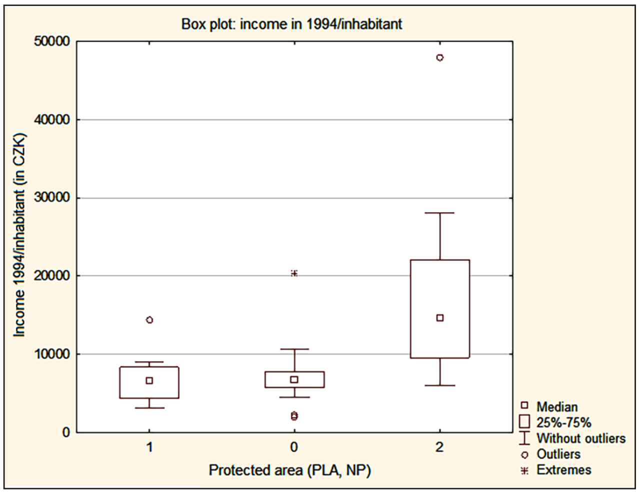

Fig. 8 Box plot showing the income per inhabitant in the different municipalities in 1994. Locality: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

Discussion

Demography of the population in south-western border of the Czech Republic was affected by recent political changes, particularly in the second half of the 20th century when a restricted border zone several kilometres wide was created. This resulted in ecosystems almost unaffected by human activities, which were worth preserving as protected areas (Bláha et al. 2013; Křenová and Vrba 2014). National Park Šumava was established in 1991 (Protected Landscape Area Šumava existed from 1963) and the Protected Landscape Area Český les in 2005. The existence of these protected landscapes and the national park limited their socio-economic development. This comparative study attempts to evaluate in terms of demography, economy and landscape use the effect on municipalities of being inside such protected areas.

This study is unique in considering a large number of factors over a long period of time. Most of the recent literature focuses on one factor, for example, effect of tourism on the economy of the area (Bodnár 2006; Cottrell and Raadik 2008; Mayer et al. 2010). Some of them only consider the effects within protected areas and do not compare it with what is happening in surrounding areas (Dickie and Whiteley 2013). Many papers deal with the

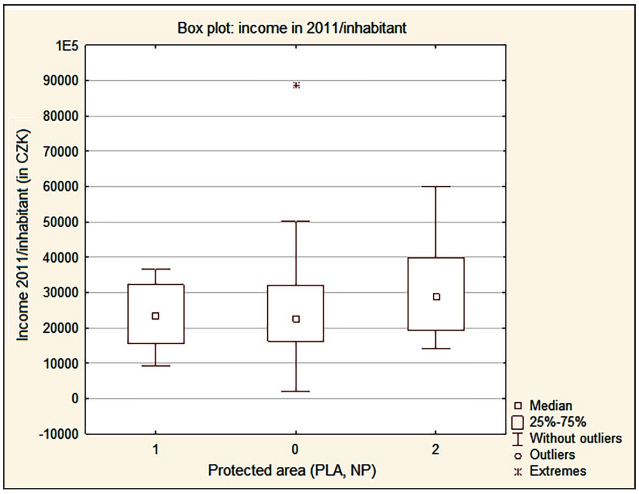

Fig. 9 Box plot showing the income per inhabitant in in the different municipalities in 2011. Locality: 0 – outside protected area, 1 – Český les PLA, 2 – Šumava NP and PLA.

Table 1 Descriptive statistics of demographic indicators.

Variable	Descriptive statistics				
	Valid N	Average	Minimum	Maximum	Stand. Dev.
% unemployment 1991	39	0.03	0.00	0.12	0.02
Number of inhabitants 1991	39	806.13	27.00	3,917.00	761.61
Number of inhabitants 2011	39	812.36	72.00	3,744.00	741.68
Change of inhabitants	39	105.11	71.22	266.67	30.14
% unemployment 2011	39	0.13	0.04	0.39	0.07
Change of unemployment	39	548.31	0.00	2,658.88	473.03
% inhab. in product. age 1991 (15–64)	39	0.67	0.59	0.75	0.04
% inhab. in product. age 2011 (15–64)	39	0.70	0.45	0.76	0.05
Change of inhabitants in product. age	39	125.17	15.83	950.00	150.96

Table 2 Descriptive statistics of socio-economic indicators.

Variable	Descriptive statistics				
	Valid N	Average	Minimum	Maximum	Stand. Dev.
% inhabitants with univ. degree 1991	39	0.02	0.00	0.05	0.01
% inhabitants with univ. degree 2011	39	0.05	0.19	0.19	0.03
Change of inhabitants with univ. degree	39	209.84	0.00	633.64	149.06
Number of economical subjects 2003	39	181.23	35.00	885.00	192.85
Number of economical subjects 2011	39	211.00	34.00	976.00	212.19
Change of economical subjects	39	120.17	53.97	190.08	24.86
Number of nursery schools 1991	39	0.87	0.00	2.00	0.52
Number of nursery schools 2011	39	0.67	0.00	1.00	0.48
% of nursery school persistence	39	57.69	0.00	100.00	48.04
Number of primary schools 1991	39	0.78	0.00	1.00	0.46
Number of primary schools 2011	39	0.64	0.00	2.00	0.58
% of primary school persistence	39	64.10	0.00	200.00	58.43

Table 3 Descriptive statistics of landscape use indicators.

Variable	Descriptive statistics				
	Valid N	Average	Minimum	Maximum	Stand. Dev.
Number of houses 1991	39	188.41	13.00	503.00	136.38
Number of houses 2011	39	245.87	30.00	624.00	167.18
% change in house number	39	148.35	110.48	443.86	69.14
% abandoned houses 1991	39	0.20	0.02	0.89	0.15
% abandoned houses 2011	39	0.31	0.10	0.63	0.13
% change in abandoned houses	39	244.49	70.44	1,900.00	351.08
% of recreation houses 1991	39	0.56	0.00	1.00	0.28
% of recreation houses 2011	39	0.63	0.11	0.96	0.20
% change in recreation houses	39	150.86	0.00	1,463.42	225.94
% arable land 1993	39	0.18	0.00	0.48	0.14
% arable land 2011	39	0.13	0.00	0.44	0.13
% change in arable land	39	58.84	0.00	122.70	35.14
% forests 1993	39	0.54	0.19	0.88	0.18
% forests 2011	39	0.54	0.19	0.88	0.18
% change in forests	39	101.07	94.42	105.05	1.97
% permanent grasslands 1993	39	0.18	0.05	0.28	0.06
% permanent grasslands 2011	39	0.23	0.05	0.49	0.10
% change in permanent grasslands	39	125.35	71.12	201.72	30.78

% built-up area 1993	39	0.01	0.00	0.01	0.00
% built-up area 2011	39	0.01	0.00	0.01	0.00
% change in built-up area	39	103.36	73.15	173.16	15.41

Table 4 Descriptive statistics of municipality incomes.

Variable	Descriptive statistics				
	Valid N	Average	Minimum	Maximum	Stand. Dev.
Municipality income 1994	39	8,003.62	458.82	50,553.04	11,745.47
Municipality income 2011	39	18,527.62	2,156.00	72,716.00	17,421.20
Change in municipality income	39	350.51	33.70	778.88	188.02
Municipality income/inhabitant 1994	39	9,948.14	1,954.09	47,972.42	8,456.95
Municipality income/inhabitant 2011	39	27,166.69	2,013.87	88,715.76	16,295.62
Change in municipality income/inhabit.	39	337.29	33.59	757.80	171.61

same area in the Czech Republic (Hampl 2005; Havlíček et al. 2005), however, none of them compare the effects in protected areas with those in unprotected areas that are similar in terms of natural conditions and history. Although large protected areas have been studied as a whole (NSW department of environment and conservation, 2006) these studies are based mostly on questionnaires, depict the current situation and do not evaluate the changes that might have occurred over time (Bartoš and Čihař 2011).

In contrast to the above, we used a large data set, which included records of demographic, socio-economic, landscape use and municipality income indicators for the years 1991 and 2011. The results indicate that almost every indicator was significantly associated with municipality size, but not with whether they were located within or outside a protected zone. For example, unemployment is 1.5% lower in municipalities located in protected areas than outside such areas. This indicates that jobs are available in national parks and protected landscape areas even though some only seasonal jobs (Picek et al. 2007). Holmes and Hecox (2004) and Job (2008) state that in the areas they studied the level of unemployment decreases with increase in the percentage of wilderness.

Moreover, there was an insignificant association between whether the municipalities were within or without protected areas and the numbers of inhabitants of productive age (15–64 years) as previously reported by Galland (2011) and Perlín and Bičík (2010). The long-term study in the Greater Shoalhaven region in New South Wales (Australia) indicates that the population almost doubled after it was designated a national park and unemployment decreased from 14.9% to 9.0% (NSW department of environment and conservation, 2006). This region is not comparable in terms of the natural conditions with the Czech Republic, but this study does indicate the benefits in terms of demography and socio-economics of being located within a protected area (Carrol and Phillipson 2002; Defra 2011).

In the case of the Šumava NP, the situation might differ because many of the inhabitants have permanent residences elsewhere in the Czech Republic and their income is not included in the budget of the municipality where they operate their business (Dickie and Whiteley 2013). Thus, it is difficult for the local inhabitants to appreciate the benefits of being in a protected area when the income from tourism goes elsewhere (Richardson 2009; Bartoš and Čihař 2011). There are, however, examples of local people appreciating the economic benefits of being in a protected area as in the Biospheric Reserve Etlebuch (Wallner et al. 2007). Similarly, the nearby Bavarian Forest NP profits from nature protection as it has a positive effect on the local population and economy (Job et al. 2004a,b, 2005; Job 2008). According to Thompson and Peepre (2011) and Carrol and Phillipson (2002), only a few extra jobs are necessary for it to have a positive effect on the local economy.

Conclusion

The aim of this study was to determine the effect on the socio-economic development of municipalities within protected areas, which are constrained by the need to protect nature. This was done by comparing similar municipalities inside and outside protected areas over a period of 20 years (1991–2011). The18 indicators measured in the 39 municipalities studied did not differ significantly in 1991. Two decades later there were significant differences in the demography, economy and landscape usage, however, this was not associated with being located in a protected area but with the size of the municipalities. Similarly, in the above-cited publications of studies in other countries and regions, municipalities located within protected areas profit from it or it has more positive effects on their socio-economic indicators than negative effects. There is an urgent need to greatly improve the communication between local people, local offices and the authorities in protected areas.

Acknowledgements

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IS THE ŠUMAVA NATIONAL PARK CHANGING INTO A DESERT? A MINI-REVIEW

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ABSTRACT

The provoking title briefly represents the opinion of a number of politicians and lobbyists. The majority of biologists, however, do not agree with this statement and present several arguments for why such a change will not occur. We attempt to elucidate the current and future situation in the Šumava NP based on available data. We also compare the situation in the Šumava NP with that in the whole of the Czech Republic, where the situation is similar or even worse. The devastated spruce forests are regenerating from young seedlings, biodiversity is increasing and the national park is not threatened by drought any more than the surrounding landscape. Here we dismiss the alarming messages about a desert in the Šumava NP, as the opposite is true, with the drought level there lower than in the rest of the Czech Republic.

Keywords: climate change; Czech Republic, drought; forest regeneration; mountains; Šumava

Introduction

During the last few decades climate change has been a major topic of study and its predicted effects on nature and society are being widely discussed. These changes are natural and occurred in the past; however, human activity has greatly speeded up this process. According to Intergovernmental Panel on Climate Change (IPCC 2018), human-induced warming has reached approximately 1 °C above pre-industrial levels and is increasing at 0.2 °C per decade.

A number of studies already indicate that air temperatures are increasing and precipitation patterns are changing and causing droughts, floods or decreases in snow cover around the world (Boer et al. 2000; Sillmann et al. 2013; IPCC 2018; Pasqui and Di Giuseppe 2019). However, the effects of climate change are not uniform and paradoxical as wet areas are becoming wetter, dry drier, high mountains are losing snow cover, glaciers are melting and sea level increasing. These facts encourage research into the effect fluctuations in weather on built-up areas, cultural landscapes and the remaining islands of untouched nature.

We focused on the situation in the Šumava National Park (NP), in the Czech Republic (CZ), where an extensive drought associated with annual precipitation of 500 mm was recorded in 2015. This low level of rainfall continued for two years and resulted in a low moisture balance, dry soils and a deficit of ground water (Boer et al. 2000; Bečka and Beudert 2016; Bílá 2016). This weather was favourable for the bark beetles infesting the spruce forests within the national park, which resulted many trees dying and stimulated heated debates about the proper management of the trees killed by the bark beetles (Hais and Kučera 2008; Pokorný and Hesslerová 2011; Kindlmann et al. 2012). In opposition to the scientific findings, it was argued the drought was caused by the bark beetle outbreak and death of the trees (Kunštecková 2019; Pokorný 2019). This mini-review attempts to resolve this debate.

Situation in the Šumava NP

National park becoming a desert

This statement is probably based on remote sensing data of the surface temperature measured by satellites or thermal cameras on hot summer days. These results do not take into account changes in habitats over time. However, it is important to consider not only the temperatures recorded, but also the structure of the vegetation, its exposure to sunshine and the number of trees per unit area (Kindlmann et al. 2012; Bílá 2018). Bečka and Beudert (2016) report an increase in summer air temperature of about 2 °C since 1978 in the Šumava NP, which is less than the temperature increase due to warming, presented in the IPCC report.

There was also a relatively low decrease in air humidity (4%) in the core zones of the national park. Even in "dead" forest after bark beetle attacks, higher amounts of precipitation were recorded than in the green forests. This might be due to more of the rain reaching the soil under dead trees than under live trees, where much of the rain falls on the leaves and subsequently evaporates (Hais and Pokorný 2004; Adams 2012; Lamačová et al. 2018; Kopáček et al. 2019). Similar measurements were recorded in the Krušné Mts. and Jizerské Mts. (CZ), which were affected by acidic rain in 80s in the 20th century.

Is the Šumava NP really the most threatened area in the Czech Republic because of the decrease in water level associated with bark beetle outbreaks? Fig. 1 shows the drought situation within the CZ in 2018 and depicts drought threats in a central part of the CZ but definitely not in the south-western border area, where the Šumava

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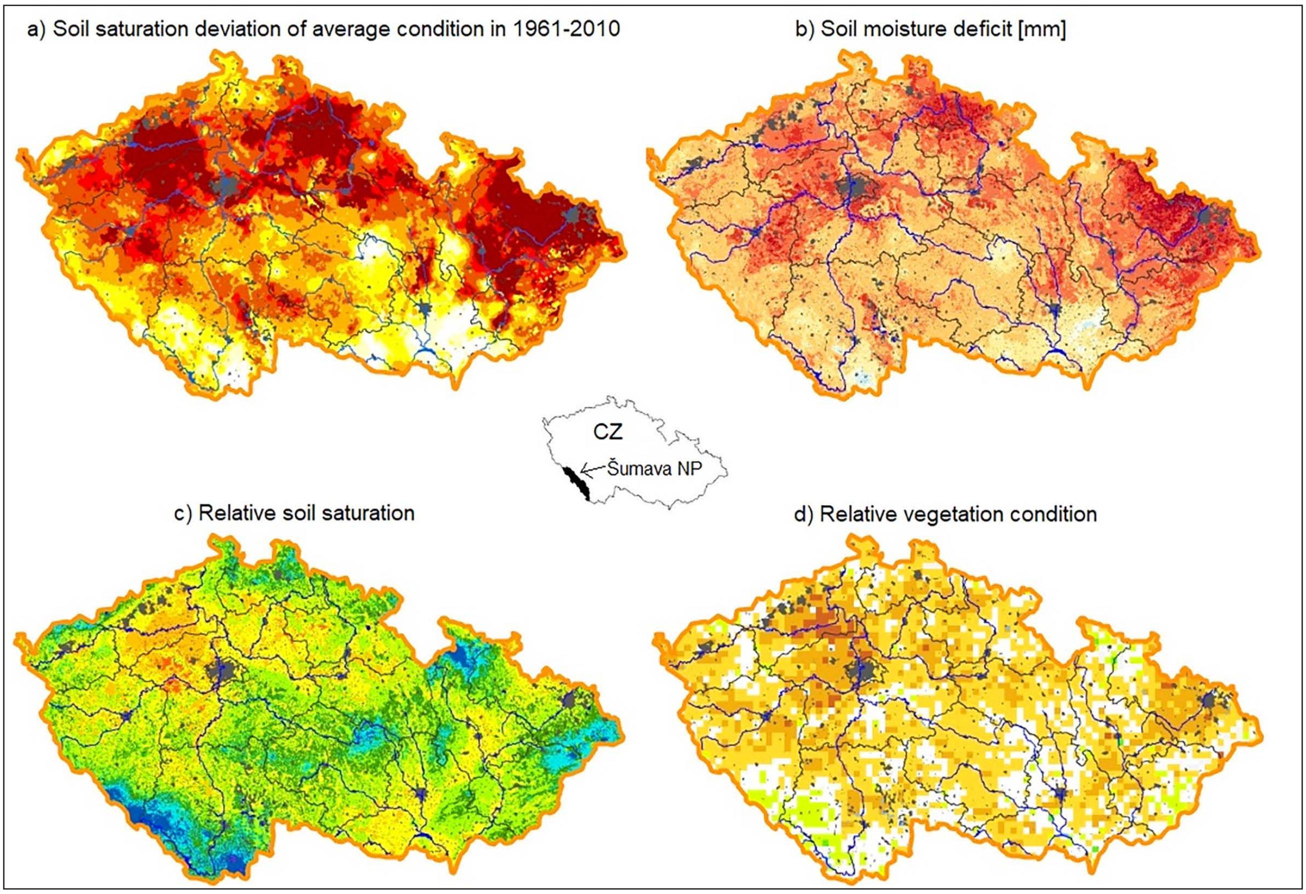

Fig. 1 Map showing the incidence of particular factors associated with drought in the Czech Republic in relation with the reference period 1961–2010 in July 2018 (https://www.intersucho.cz).

NP is located. When we compare soil water content and its saturation in the different mountain and highland areas of the CZ, we obtain very similar results: the Sumava NP is the best of the compared regions in terms of the absolute soil water content (Fig. 2).

End of green forests

Nature conservation is important in western society. It might be seen, however, as of little importance in view of the changes that have occurred naturally over time. The Šumava NP is a good example, as when its spruce forests mature, the old trees are prone to die for several reasons. Bark beetle outbreaks rapidly kill the mature trees and from an aesthetic point of view, Šumava loses its highly valued old forests that have been present for several human generations. It can be viewed as a tragedy or a natural cyclical event followed by regeneration, during which seedlings grow and develop into a young forest (Fig. 3). Disturbance followed by succession has occurred in the south-western border area of the Czech Republic over a very long period of time. Major changes in the characteristics of this forest are recorded in the years 1620, 1690, 1720, 1740, 1780, 1820, 1870 and 1920. Smaller changes in which at least 10% of the trees died were recorded in 41 years and more than 50% in 174 years. The greatest incidences of dying trees occurred at the beginning of the 19th century and recently, both of which can be attributed to the trees being mature and being more prone to being severely damaged by windstorms or killed by bark beetles (Hošek 1981; Kindlmann et al. 2012; Simanov 2014; Kjučukov et al. 2019).

This is also important for biodiversity, which tends to be higher in environments subject to some disturbance (Seidl et al. 2011; Kindlmann and Křenová 2016).

Winners and losers in the forest structure

The Šumava NP is protected because of the extensive forests growing on the mountains and their foothills along its south-western border. Over the last 8,000 years, spruce has been present at localities above 1,100 m a.s.l. and fir-beech forest and beech forests at lower altitudes. This vegetation was artificially modified, starting in the 18th of century, which resulted in the area under beech declining because it was used in the glass industry or because beech was replaced by spruce, which is more profitable. Original and untouched spruce forests occur on the highest summits, because it is difficult to log these areas (Šantrůčková et al. 2010; Kjučukov et al. 2019).

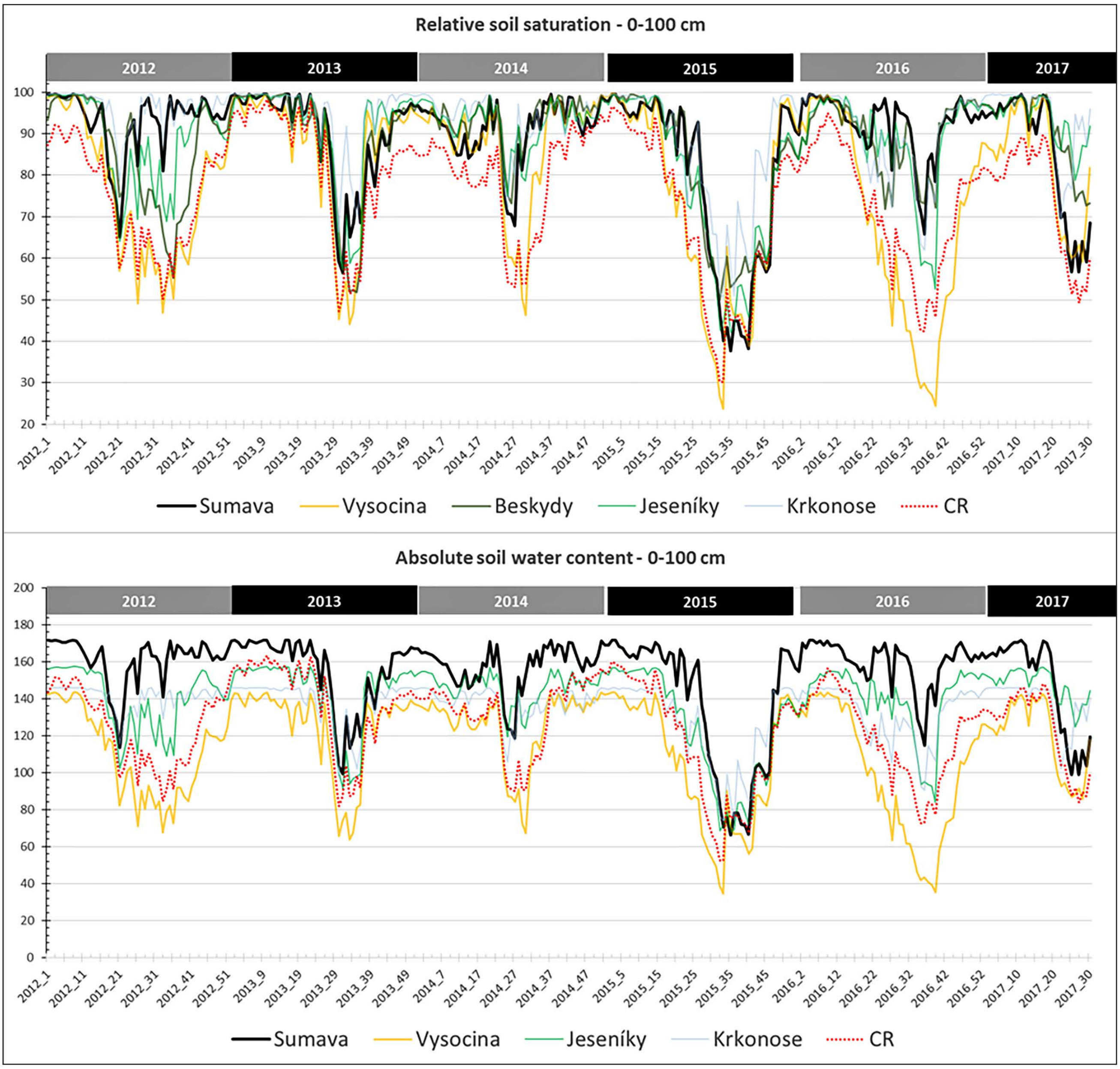

Fig. 2 Relative soil saturation and absolute soil water content at a depth of 0–100 cm in years 2012–2017 recorded in in the different mountain and highland areas in the Czech Republic.

Fig. 3 Growth of spruce seedlings in an area of forest killed by bark beetles.

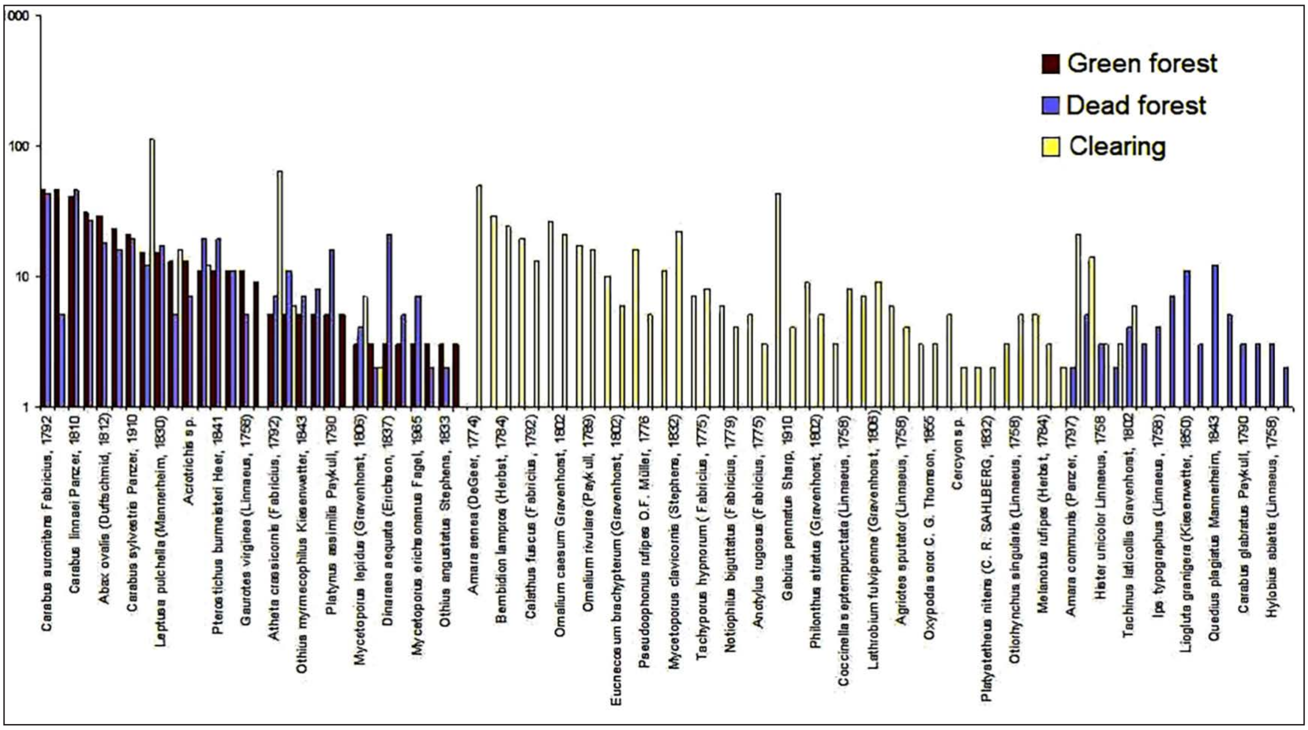

Fig. 4 Comparison of the diversity of carabids in green forest, dead forest and clearings.

With the predicted increase in temperature, it is expected that broadleaf trees will eventually colonize the higher altitudes; however, this currently is not occurring, as there are only spruce seedlings and closed canopy spruce in most of the core area of the Šumava NP. The spruce seedlings grow in clusters in suitable microhabitats, such as those associated with prostrate tree trunks or stumps in the vicinity of dead trees. Natural spruce forest regeneration is characterized by a spatial mosaic of microhabitats. Even dead trees serve as a refuge for many animals, such as carabids: species characteristic for spruce mountain forests remain present even after a bark beetle outbreak, but disappear in clearings – most likely due to change of microclimate there (Fig. 4). With the predicted increase in global temperature, the species composition of ground beetles is likely to change. There will be winners and losers in these forests, some species will profit from the temperature increase and some will be affected negatively (Müller-Kroehling et al. 2014).

Conclusion

The last few years were extremely dry and affected the Šumava NP. Spruce forests there suffered attack by bark beetles that can have more than one generation a year and can spread very fast. Similar situations were recorded in other mountains and highlands in the Czech Republic. Bark beetle outbreaks also occurred in the north of Moravia and in the Bohemian-Moravian Highlands. Suggesting that the core zones in the NP, where the valuable ecosystems are conserved, are the reason for bark beetle outbreak is misleading.

Although the dead trees in these core zones might be considered by most of people to be unsightly, they are important for spruce forest regeneration (Schwarz 2013). Abiotic conditions at these sites are not so hot as to change the Šumava NP into a desert as claimed by Kunšteková (2019) and Pokorný (2019). Differences in the energy balance in dead and green forests is only a temporary phenomenon as the seedlings growing in the dead forest will quickly result in the regeneration of a green forest (Økland et al. 2015). In the case of the Šumava NP spruce forest, this proceeded very fast during the first decade after the bark beetle outbreak and was accompanied by an increase in relative air humidity (Kopáček et al. 2019).

A recent study by the Czech Hydrometeorological Institute confirms there have been no extreme changes in precipitation in the Šumava NP and surrounding foothills (Bílá et al. 2018). Changes probably will occur in terms of vegetation cover and drying out of soil but have not yet started (Fig. 1). On the contrary, the soil beneath dead prostrate tree trunks is damp and the trunk provides shade suitable for the establishment of mosses, which provide favourable conditions for the growth of spruce seedlings (Šantrůčková et al. 2010; Kindlmann et al. 2012; Bílá 2018). There is not the same growth of forest in the Šumava NP as before the bark beetle outbreak, but under current conditions, succession will result in a green forest made up of trees of different ages (Jonášová and Prach 2004). That is, the valuable ecosystems in the Šumava NP will not be replaced by a desert in the near future.

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METHODS FOR MONITORING ALPINE PLANT PHENOLOGY: A PILOT STUDY IN THE LINE CREEK PLATEAU RESEARCH NATURAL AREA, USA

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ABSTRACT

Alpine plant phenological traits are studied and several hypotheses about their latitudinal variation are tested within a comparative research project, which is being conducted on groups of plant species for which relationships are inferred from available phylogenies. The study sites for this project are located in tropical Ecuador, semitropical Bolivia and the temperate Rocky Mountains in the USA. Several temperate alpine species occur near the Rocky Mountains Field Station in Colorado and large populations of three alpine target species (*Caltha leptosepala*, *Castilleja pulchella*, *Gentiana algida*) were found at the Line Creek Plateau Research Natural Area, in the Beartooth Range in Montana. This location was initially investigated in August 2018 to choose the study site and test a methodology for collecting plant trait data. This study site, the target plant species and the results of a preliminary study, together with recommendations for the full season monitoring, are discussed in this paper.

Keywords: alpine plants; *Caltha*; *Castilleja*; climate change; *Gentiana*; reproductive ecology

Introduction

Many measurements as well as various climate scenarios indicate that climate is warmer and drier during last decades. Alpine habitats are one of the most threatened due to their island distribution. Better understanding of the phenology and reproductive strategies of alpine plants growing in alpine areas in both the tropics and temperate regions can be used to determine how functional traits respond to the different selective pressures of alpine environments.

It is suggested that the remarkable plant radiation in alpine areas in the tropics may be related to their "escape" from the stress of a seasonal temperate climate (Pfitsch 1994). Whereas growth of alpine plants in temperate regions is limited to the summer period (Körner 2003), the continuous growing season prevailing at high altitudes in the tropics may have released them from the constraints imposed by a seasonal climate. For instance, there is no need for a rapid development of flowers and fruits, and observations of tropical alpine communities in Ecuador (Kolář et al. 2016; Sklenář 2017) indicate that plant phenology is less constrained temporarily than in the temperate zone. Moreover, given the low probability of encountering severe freezing temperatures (below −10 °C) during the growing season in the humid tropics, selection for pronounced freezing resistance is less likely in tropical alpine plants. This is supported by data on plant tolerance of freezing measured in Venezuela, Ecuador, the Rocky Mountains (USA) and Giant Mountains (Czech Republic) (Sklenář et al. 2012, 2016).

Functional plant traits are defined as morpho-physio-phenological traits that impact the fitness of individual species via their effects on growth, reproduction and survival, the three components of individual performance (Violle et al. 2007). Moreover, functional traits can indicate how a species relates and responds to its environment, which offers a powerful approach to addressing ecological questions (McGill et al. 2006). The alpine plant traits are studied and several hypotheses about their latitudinal variation are tested within the project "Evolution of functional traits of alpine plants in temperate and tropical environments", granted by the Czech Grant Agency (Grant number 17-12420S). The most important and novel point of this project is that the comparative research is carried out on species and species groups of plants for which relationships are inferred from available phylogenies. In addition to the above, the species groups meet the following criteria: i) they have the same growth form and ii) they occur in equivalent habitats in the compared regions. The following plant groups are studied:

- *Caltha* – the North American species *C. leptosepala* is sister to a clade of South American species, including *C. sagittata*, which occurs both in Ecuador and Bolivia (Schuettpelz and Hoot 2004).
- *Cerastium* – the species pair *C. arvense* – *C. arvensiforme* is studied, which are sometimes considered conspecific. The South American species of *Cerastium* are derived from a North American clade, which supports a north-to-south migration (Sheen et al. 2004).
- *Castilleja* – perennial species are sampled, i.e., South American *C. nubigena* along with some North Amer-

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ican perennial *Castilleja* species. The South American species are sister to meso-American species and together they are sister to North American species (Tank and Olmstead 2008).
- *Gentiana* – Perennial *G. algida* from temperate regions is compared with the supposedly perennial *G. sedifolia* and perennial *Gentianella punicea* (Holub 1967) in the Andes.
- *Valeriana* – Herbaceous species forming a basal leaf rosette and growing in similar alpine habitats.

The study sites are located in tropical Ecuador and semitropical Bolivia. Several temperate species occur near the Rocky Mountains Field Station in Colorado, USA (Fowler et al. 2014; Sklenář *pers. comm.*), and large populations of other target species (*Caltha leptosepala*, *Castilleja pulchella*, *Gentiana algida*) were found at the Line Creek Plateau Research Natural Area in Montana, USA, in August 2018. Information on the latter study site and results of a preliminary study, including recommendations for a full season of phenology monitoring, are presented in this paper.

Methodology

Study area

The Line Creek Plateau Research Natural Area (RNA) is one of over 530 RNAs established by the U.S. Forest Service. This national network of protected areas provides sites for research, education, monitoring, and the conservation of biological diversity. The RNAs established to date include a wide range of vegetation types that occur on the national forests and grasslands in the United States (Evenden et al. 2001).

The Line Creek Plateau RNA was established in 2008. The total area is 22,422 acres (9,074 ha), making it the largest RNA established by the US Forest Service. Most of the RNA (19,369 acres) is located in the Beartooth Ranger District, Custer-Gallatin National Forest, Montana, with a smaller part (3,053 acres) in the Clark's Fork Ranger District of the Shoshone National Forest in Wyoming (Fig. 1).

Line Creek Plateau is the easternmost alpine area in the Beartooth Mountains. Elevations in the RNA range from 7,400 feet (2,256 metres) to 10,892 feet (3,321 metres). Gently rolling alpine tundra and turf are predominant on the top of the plateau, and cover approximately 9,750 acres (3,948 ha) in the RNA. Nine alpine plant community types (c.t.) are represented, with three types being the most prevalent: *Festuca idahoensis/Geum rossii* c.t., *Carex elynoides* c.t., and *Carex scirpoidea/Geum rossii* c.t. Most of the remaining acreage in the RNA is dominated by forested plant communities, including *Pinus albicaulis*, *P. flexilis*, *Abies lasiocarpa*, and *Pseudotsuga menziesii*. There are two plant communities dominated by willow species in the Montana portion of the RNA: *Salix glauca/Deschampsia cespitosa* c.t. and *Salix planifolia/Carex paysonis* c.t. Soils of the RNA are derived from hard, coarse-grained metamorphic rocks (mostly gneiss and granite), and range from well-drained loams to sands. The average temperature on Line Creek Plateau is 9–11 °C, with an average low temperature of −2 °C and an average high temperature of 21–23 °C. The plateau receives an average of 1,016 mm of precipitation annually, predominantly in the form of snow (USDA Forest Service 2001).

Part of the study site for the three target species occurs in the community types with *Geum rossii* (*Castilleja pul-*

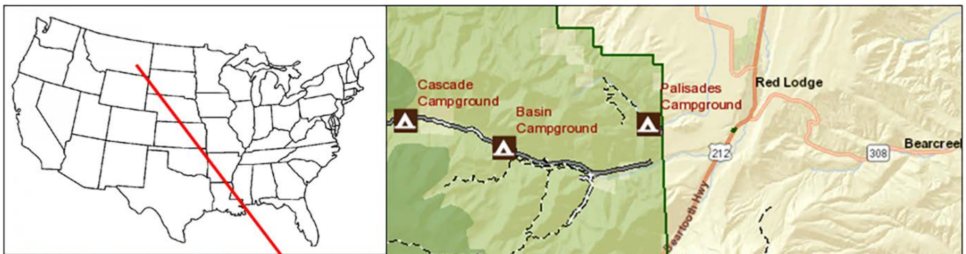

Fig. 1 Study area. The red arrow marks our study site. The map has been modified from the data available on https://www.fs.fed.us.

chella), while the other part (with *Caltha leptosepala* and *Gentiana algida*) occurs with a *Salix* community type.

Study species

We studied three alpine plant species: *Caltha leptosepala, Castilleja pulchella* and *Gentiana algida.*

The white marsh marigold, *Caltha leptosepala* (*Ranunculaceae),* is an erect, perennial herb with hairless and usually leafless stems, 5–10 cm tall, sometimes to 40 cm, growing from short, erect rhizomes (Booth and Wright 1966). Leaves are basal, cordate, up to about 6 cm long, slightly longer than wide, rounded at tip, thick and waxy, almost entire to coarsely toothed with wavy edges. The petioles are shorter than the leaf blades to 2–3 times their length. Flowers are white, sometimes tinged bluish, saucer-shaped, 2–4 cm wide, with 5–15 showy, oblong to oval sepals and no petals. The flowers are usually solitary on stalks 3–10 cm, rarely up to 20 cm tall, usually with a single leaf. Flowering occurs from May-August, depending on the elevation. Fruits are erect clusters of numerous pods (follicles), about 15 mm long. It is distributed from the subalpine to the tundra from 2600 to 3900 m a.s.l. (Lindeberg-Johnson 1981). The species occurs from Alaska to Colorado, Utah, Arizona, and western to north-eastern Nevada (http://montana.plant-life.org). In Montana, this species grows in wet subalpine and alpine places in western and southern parts of the state (Lesica 2012).

The *Caltha leptosepala* species complex is taxonomically unresolved, with authors of various regional floras recognizing different names and numbers of species (Wefferling and Hoot 2017, 2018). Integrating molecular, morphological, cytological, and geographic data, they described three species in the complex, restoring two species names, *C. biflora* and *C. chionophila*, in addition to recognizing *C. leptosepala*. Based on chloroplast and nuclear ribosomal phylogenies, they illustrated key morphological synapomorphies for the three *Caltha* species, assessed the usefulness of previously used morphological characters, and provided a dichotomous key for their field identification. *Caltha leptosepala* is a polyploid species and comprises a complex of hexaploids (6×), rare nonaploids (9×), and dodecaploids (12×). Wefferling et al. (2017) focused on the biogeography and evolutionary history of the complex. They delineated the geographic distribution and contact zones of the cytotypes, and investigated morphologies of cytotypes and subspecies. Genome size estimates from flow cytometry were used to infer cytotypes and a key morphological character, leaf length-to-width ratio, was measured to evaluate whether these dimensions are informative for taxon and/or cytotype delimitation. They found that *Caltha leptosepala* presents clear patterns of cytotype distribution at the large scale. Marked differences in morphology, range, and genome size were detected between the hexaploid subspecies, *C. leptosepala* subsp. *howellii* in the Cascade-Sierra axis and *C. leptosepala* subsp. *leptosepala* in the Rocky Mountains. Sympatry between cytotypes in the Cascades and a parapatric distribution in the Northern Rockies suggest unique origins and separate lineages in the respective contact zones.

Caltha leptosepala is a hermaphrodite pollinated by bees, beetles, and flies (https://pfaf.org). The species is one of the alpine herbs with a developmental preformation, i.e. the initiation of leaves and/or flowers one or more growing seasons before they mature, which is common among alpine and arctic tundra plant species (Mark 1970). Aydelotte and Diggle (1997) studied a developmental preformation of *Caltha leptosepala* in detail and found that all structures in *C. leptosepala* are preformed. Leaves are initiated one or two growing seasons before they mature and flowers are initiated one growing season before maturation. The features of development and architecture in *C. leptosepala*, however, appear to differ from the determinate growth patterns of other exclusively preforming species, and may allow within-season variability in the seasonal development and maturation of structures. Cohorts of leaves initiated are asynchronous with maturation cohorts, and each year the number of leaf primordia per plant at snowmelt exceeds the number to mature aboveground. Therefore, some flexibility in whether leaves complete a 2-yr or 3-yr developmental trajectory might occur. Plasticity in reproductive phenotype might also occur via the process of floral abortion.

Castilleja pulchella, the beautiful Indian paintbrush, is a perennial herb in the family Orobanchaceae (Lesica 2012). Stems are 5–15 cm high, ascending, and more or less villous especially above. Leaves are 1–4 cm long, the lower entire and linear-lanceolate, the upper broader and 3-cleft, puberulent and slightly villous; lobes linear-lanceolate, attenuate; bracts elliptic, usually 3-cleft, the middle lobe broad and rounded, tinged with brownish, villous; calyx yellowish, tipped with brownish, about 18 mm. long; the corolla is slightly exserted, with the galea 7 mm. long and the lip 5 mm. long, its lobes lanceolate, and obtuse. In Montana, *Castilleja pulchella* occurs in the southwest and south-central parts of the state (Lesica 2012). This species is intermediate between *C. lutea* and *C. occidentalis* (Barnhart 1907).

Tank and Olmstead (2008) studied Castillejinae phylogeny and suggested that the perennial habit evolved a single time from an annual ancestral lineage that persisted throughout the diversification of Castillejinae, contrary to classical interpretations of life history evolution in plants. They argued that the prevalence of polyploidy among perennial *Castilleja* species, perenniality may have played an important role in the origin and establishment of polyploidy in *Castilleja*.

As many other *Castilleja* species, flowers of *Castilleja pulchella* are pollinated by bumblebees. Bauer (1983) studied relationships between several alpine species, including *Castilleja pulchella*, and ten bumblebees species during his research conducted on the Beartooth Plateau.

Gentiana algida, the white gentian, whitish gentian or Arctic gentian, is a glabrous perennial plant with short rhizomes (Lesica 2012). They have one to several leafy and hairless stems, usually 5–20 cm tall. Dark green leaves are hairless, about 5–10 mm broad, the basal ones linear- to lance-shaped, 4–12 cm long. Lance-shaped leaves about 3–5 cm long are growing opposite, in 3–5 pairs. Flowers are funnel-shaped, usually in terminal pairs. The corolla is 35–50 mm long, white or pale yellowish, with five short pointed lobes, with a purple-blotched area from the back of each lobe nearly to the calyx and with a lengthwise fold between each lobe. The calyx is narrowly funnel-shaped, about 2 cm long, usually purplish-blotched, the tube squared off between the lobes, which are almost equal, linear to lance-shaped, slightly keeled, from half as long to almost equal the tube length. The species flowers from June-August. Fruits are capsules 3–4 cm long. The species grows in moist but well-drained sites in the alpine zone from Colorado and Utah to Alaska, and also in east Siberia and the Himalayas. In Montana, the species occurs the south-central and southwestern parts of the state (Lesica 2012). Along with many other gentians, secondary metabolites and medical effects of extracts from this species were studied (Tan et al. 1997; Khobrakova et al. 2017).

Thunderstorms with hails and summer snow showers are common in alpine regions where *Gentiana algida* occurs. The closure of the upright, tubular flowers of *this species* were observed during the frequent afternoon thunderstorms characteristic of the central and southern Rocky Mountains (Bynum and Smith 2001). Flowers closed within minutes of an approaching thunderstorm and reopened after direct sunlight returned. The authors experimentally tested this behavior and found that manually opened flowers had substantial losses of pollen and reductions in female fitness also occurred. They supposed that corolla closing and opening in *G. algida* associated with frequent summer thunderstorms may be a behavioral adaptation that improves both paternal and maternal reproductive effort.

Data collection and analyses

We used the USDA plant species database (https://plants.usda.gov) and specimens housed at the Herbarium at the University of Montana, Missoula to preselect the Line Creek Plateau RNA as a suitable site for study of three target species: *Caltha leptosepala, Castilleja pulchella* and *Gentiana algida.* We visited the Line Creek Plateau RNA between August 21–23, 2018 and found sufficiently large populations of all three species.

We randomly selected ten individuals of each species, i.e. we threw a little ball over our head and chose the nearest plant, and recorded the following plant traits to describe their habitat and phenology status.

Each *Caltha leptosepala* plant was characterized by (1) size of the rosette in cm. We collected two diameter measurements and averaged them. We did not press a rosette during measurement, and hence measured only the natural size of each rosette; (2) number of leaves in the rosette; and (3) the maximum width and length (mm) of the youngest well-developed leaf in the rosette. The maximum length of the leaf was measured from the tip of the leaf to the tip of a cordate leaf lobe, not including the petiole; (4) the highest flowering stem in cm; and (5) the number of buds/opened flowers/old flowers/ripe fruits/immature fruits, i.e. phenological phases I–V (Fig. 2). We also measured the height of other stems (if they were present) and recorded the total number of flowers and fruits on each measured stem.

Castilleja pulchella does not have a basal rosette and we measured only (1) height of the highest stem; (2) the maximum width and length (mm) of the most well-developed leaf on the tallest stem; and (3) the number of buds/opened flowers/old flowers/ripe fruits/immature fruits, i.e. phenological phases I–V (Fig. 3). For other stems (if they were present) we measured the height (cm) and recorded the total number of flowers and fruits.

Gentiana algida plants form compact rosettes, or a group of several rosettes, with many overlapping linear-to lance-shaped leaves, and we could not count all of them without causing damage to the plants. We thus measured (1) the size of each rosette or group of rosettes (cm), taking two diameter measurements of each plant and averaging them; (2) the maximum width and length (mm) of the newest well-developed leaf on the highest stem; (3) the highest flowering stem (cm) and (4) counted the number of buds/opened flowers/old flowers/ripe fruits/immature fruits, i.e. phenological phases I–V (Fig. 4). For other stems (if they were present) we measured the height (cm) and recorded the total number of flowers and fruits.

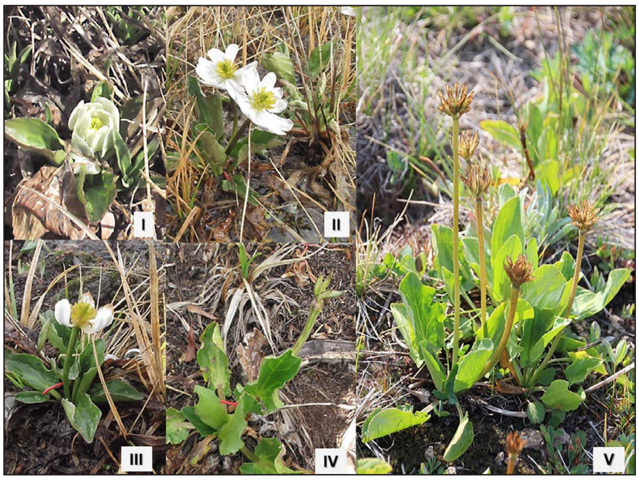

Fig. 2 Five phenological phases of *Caltha leptosepala*. I – a flower bud; II – an open flower; III – an old flower, which has already finished its flowering but fruits are not presented yet; IV – a green, immature fruit; V – a ripe fruit.

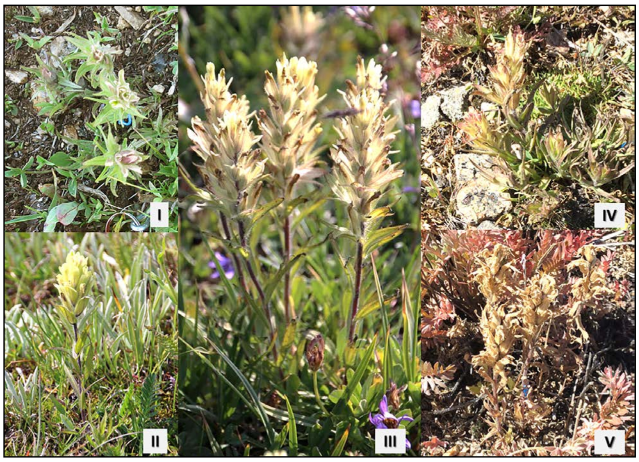

Fig. 3 Five phenological phases of *Castilleja pulchella*. I – a flower bud; II – an open flower; III – an old flower, which has already finished its flowering but fruits are not presented yet; IV – a green, immature fruit; V – a ripe fruit. The pictures show plants with a prevailing proportion of flowers of the selected phenological phases.

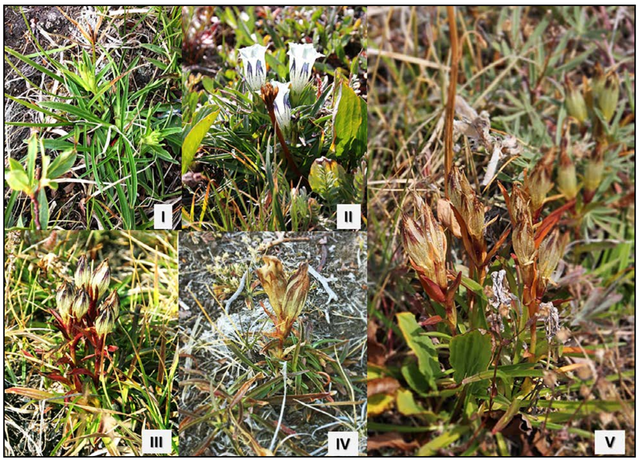

Fig. 4 Five phenological phases of *Gentiana algida*. I – a flower bud; II – an open flower; III – an old flower, which has already finished its flowering but fruits are not presented yet; IV – a green, immature fruit; V – a ripe fruit; with a partly opened capsule. The pictures show plants with a prevailing proportion of flowers of the selected phenological phases.

We also calculated the total number of stems, average height of stems and total number of flowers and fruits on each plant. We used the analysis of variation in STATISTICA 12 (Anonymous 2012) to compare phenological phases of each species and we used MS Excel for testing correlations between measured plant traits. Logarithmic transformation log(x + 1) was used to standardize data.

Results

We visited our study site on the Line Creek Plateau for three days in August 2018 and measured ten individuals of three target species (*Caltha leptosepala, Castilleja pulchella* and *Gentiana algida*) to optimise our methodology. The study species differ in their habits and plant traits (Table 1).

Two of the ten measured *Caltha* plants were sterile, without flowering stems. Four flowering *Caltha* plants had one flowering stem and four others had two flowering stems. Each flowering stem carried only one flower. *Caltha* plants differed in the size of their leaf rosettes. The average size of the main rosette was 11.10 cm in diameter and the plants had an average of 13.2 leaves. The smallest plant had a rosette only 6 cm in diameter and only seven leaves, while the largest plant had a rosette 20 cm in diameter and 15 leaves. We also measured a plant with 26 leaves that had a rosette only 13 cm in diameter. We found a slightly positive correlation between the size of the main rosette and the number of leaves (Fig. 5). The areas of the youngest well-developed leaves varied between 1,080–2,660 mm^2 and there were no correlations between this leaf area and the size of rosettes, nor between this leaf area and the number of leaves in the rosette. We found a positive correlation between plant leaf area, calculated as the number of leaves in the main rosette * leaf area of the newest well developed leaf, and the number of flowers and fruits (Fig. 6).

Ten measured *Castilleja* plants had flowering stems. Some plants had only one flowering stem and the largest plant had seven flowering stems. Several plants also had some sterile stems without flowers. The height of the highest flowering stems varied between 6–16 cm. The

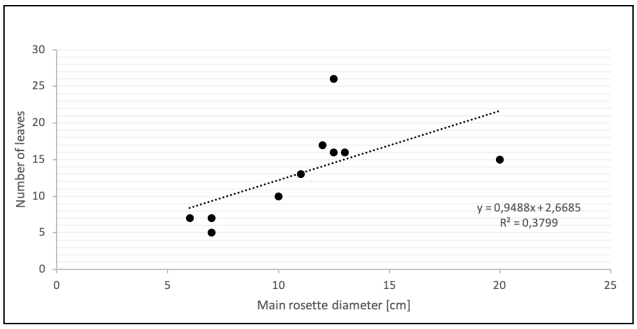

Fig. 5 *Caltha leptosepala*. Correlation between size of the main rosette and the number of leaves.

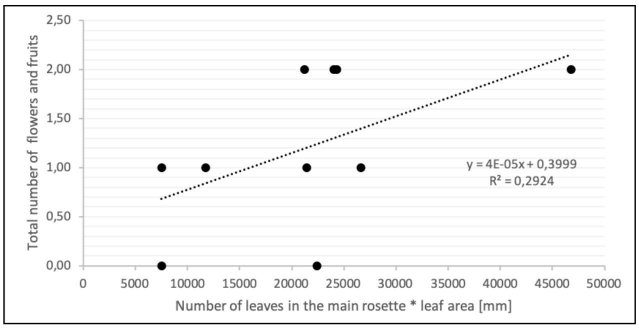

Fig. 6 *Caltha leptosepala*. Correlation between plant leaf area, i.e. number of leaves in the main rosette * leaf area of the newest well developed leaf, and the number of flowers and fruits.

Table 1 The plants traits describing habit and phenology of three study species. n.m. – these traits were not measured.

	Total number of stems	Average stem height [cm]	Total number of flowers and fruits	Main rosette diameter [cm]	Number of leaves in the main rosette	Width of the selected leaf [mm]	Length of the selected leaf [mm]	Leaf area of the selected leaf [mm]	Height of the highest flower stem [cm]	Number of flowers and fruits on the highest stem
Caltha leptosepala										
mean ± SD	1.20 ± 0.79	9.86 ± 5.64	1.20 ± 0.79	11.10 ± 4.06	13.20 ± 6.25	29.10 ± 5.86	55.60 ± 9.41	1,614.00 ± 426.31	10.03 ± 5.75	0.80 ± 0.42
min	0	0	0	6	5	20	40	1080	0	0
mix	2	16	2	20	26	43	75	2666	16	1
Castilleja pulchella										
mean ± SD	2.90 ± 2.08	9.14 ± 3.49	20.10 ± 14.08	n.m.	n.m.	n.m.	n.m.	n.m.	10.58 ± 2.99	9.90 ± 1.97
min	1	3	8	n.m.	n.m.	n.m.	n.m.	n.m.	6	8
mix	7	15	48	n.m.	n.m.	n.m.	n.m.	n.m.	16	14
Gentiana algida										
mean ± SD	1.80 ± 1.48	9.73 ± 5.99	4.60 ± 2.76	10.80 ± 2.11	n.m.	4.90 ± 1.37	51.30 ± 7.53	246.90 ± 56.71	7.28 ± 0.96	2.00 ± 0.94
min	1	0	1	7	n.m.	3	38	160	6	1
mix	5	16	11	14	n.m.	8	60	330	9	4

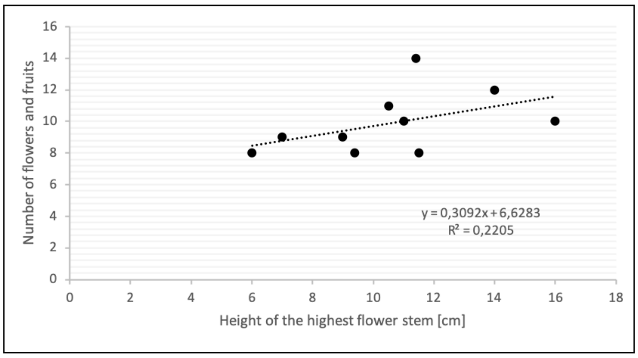

Fig. 7 *Castilleja pulchella*. Correlation between the height of the highest flower stem and the number of flowers and fruits.

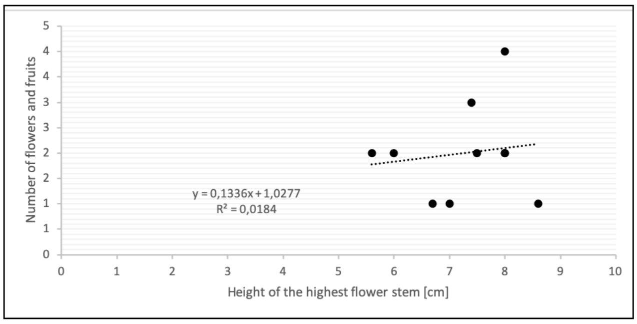

Fig. 8 *Gentiana algida*. Correlation between the height of the highest flower stem and the number of flowers and fruits.

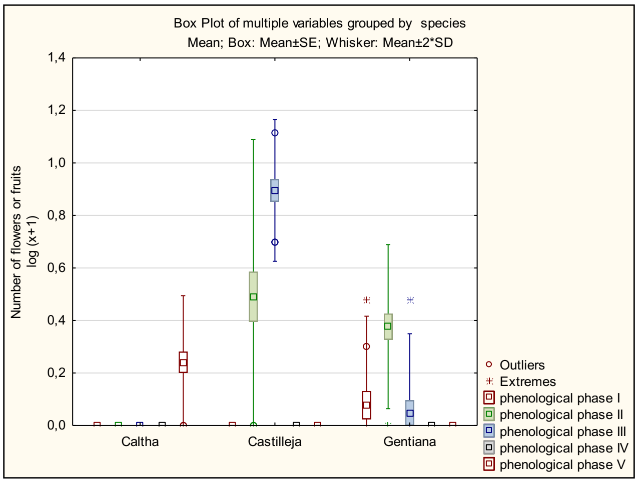

Fig. 9 Flowering phenology of three studied species.

flowering stems had between 7–14 flowers. The leaves of *Castilleja* were hairy and very small. We could not measure them without serious harm to them. We found a positive correlation between the height of the highest flower stem and the number of flowers and fruits (Fig. 7).

All measured *Gentiana* plants also had flowers. Six plants had two flowering stems and two had only one flowering stem. There were also two other *Gentiana* plants with four and five flowering stems, respectively. Flowering stems had between 1 and 4 flowers. We found a slightly positive correlation between the height of the highest flower stem and the number of flowers and fruits (Fig. 8).

The three species were in different phenological phases at the time of this pilot study (Fig. 9). While all eight *Caltha* plants with flowering stems had ripe fruits, i.e. a phenological phase V, the highest stems of *Castilleja* plants had about one third of the flowers open, i.e. a phenological phase II, and two thirds of the flowers had finished their flowering but immature fruits were not present yet, i.e. a phenological phase III. Flowers on *Gentiana* plants were the youngest, least developed ones. The highest stems of two gentians carried buds, i.e. a phenological phase I. Most of the other flowers were opened, i.e. a phenological phase II, and only one flower was characterized as a flower of phenological phase III.

Conclusions and Recommendations

We found some positive correlations between the height of the highest stem and the number of flowers and fruits for two studied species, *Castilleja pulchella* and *Gentiana algida*. For *Caltha leptosepala*, we found a positive correlation between number of flowers and fruits and the plant leaf area, calculated as the number of leaves in the main rosette * leaf area of the newest well developed leaf. These results indicate a positive correlation between the photosynthetic capacity of the plants and their reproductive output. The energy partitioning has been studied and modeled in other plant species, for example in terrestrial orchids (Kindlmann and Balounová 1999).

Positive correlations between our measured plant traits could also indicate the idea to reduce a list of measured traits for each target plants, but the results represent only ten plants of each target species, and we do not support this idea. During testing of our methodology, we found that the time needed for measuring of the planned plant traits was acceptable. With the results from this preliminary study, we can assume that our methodology for monitoring of three target species (*Caltha leptosepala, Castilleja pulchella* and *Gentiana algida*) is appropriate and can be used for full season monitoring in 2019. The field protocols are in Appendix 1–3.

We visited our study site during the last week of August 2018 and found that three target species were in different phenological phases. *Caltha* plants were more or less withered and had ripened fruits, *Castilleja* were in peak flower, and *Gentiana* plants had some opened flowers and even some buds. We can assume that, despite a very short vegetation season, with snow melting about middle of June and the first snow coming again in the middle of September that these alpine species are not actively growing or reproducing throughout the whole season. There can be some phenological shifts within a large study site, because snow melts gradually, but generally, *Caltha* plants are very early and they can start flowering immediately after snow melt. Permanent rosettes of *Gentiana* plants are visible early after snow melt too but the first flowers do not appear until August. *Castilleja* plants begin flowering in the middle of summer (July). To better understand different plant species strategies, the start of the growing season must be defined (Odland 2011) and a full season of phenological data are important. To cover the full phenological variability of the study species, monitoring should be started as soon as possible after snow melt, i.e., the middle of June, and data should then be collected every 3 or 4 weeks. Studied individuals of three target species should be marked with unique markers, and well-developed leaves selected for measuring the leaf traits should be marked with a thin colored wire. The same marked leaf should be measured again during the second and other measurements, if possible. Only if the marker is lost or the leaf destroyed should a new well-developed leaf on the same rosette/stem be found and marked. A color photograph, showing a perpendicular projection of each plant and its label, should be taken during each monitoring visit.

Acknowledgements

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Appendix 1

FIELD PROTOCOL																					
Caltha leptosepala		location:	LINE CREEK		coordinates:				observers:									date:			
			selected leaf				height	flowers on the heighest stem						all other stems							
			leaf 1		leaf 2																
plant No	Main rosette diameter [cm]	Number of leaves in the main rosette	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	Height of the highest flower stem [cm]	No. buds on the plant	No. open flowers on the plant	No. old flowers on the plant	No. ripe fruits on the plant	No. imma-ture fruits on the plant	No. flowers and fruits	Stem height [cm]	No. flowers	Stem height [cm]	No. flowers	Stem height [cm]	No. flowers	Stem height [cm]	No. flowers
G01																					
G02																					
G03																					
G04																					
G05																					
etc.																					

Appendix 2

FIELD PROTOCOL																						
Castilleja pulchella		location:	LINE CREEK		coordinates:				observers:									date:				
	height	selected leaf				flowers on the heighest stem						all other stems										
		leaf 1		leaf 2																		
plant No	Height of the highest flower stem [cm]	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	No. buds on the plant	No. open flowers on the plant	No. old flowers on the plant	No. immature fruits on the plant	No. ripe fruits on the plant	No. flowers and fruits	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]
G01																						
G02																						
G03																						
G04																						
G05																						
etc.																						

Appendix 3

FIELD PROTOCOL																						
Gentiana algida		**location:**	**LINE CREEK**		**coordinates:**				**observers:**											**date:**		
		selected leaf				height	flowers on the heighest stem						all other stems									
		leaf 1		leaf 2																		
plant No	Whole plant diameter [cm]	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	Width of the selected leaf in the stem [mm]	Length of the selected leaf in the stem [mm]	Height of the highest flower stem [cm]	No. buds on the plant	No. open flowers on the plant	No. old flowers on the plant	No. ripe fruits on the plant	No. imma-ture fruits on the plant	No. flowers and fruits	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers	Stem height [cm]	No. Flowers
G01																						
G02																						
G03																						
G04																						
G05																						
etc.																						

MANAGEMENT ZONATION AND ITS IMPLEMENTATION AT A UNESCO WORLD HERITAGE SITE – A CASE STUDY FOR THE PLITVICE LAKES NATIONAL PARK, CROATIA

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ABSTRACT

Plitvice Lakes National Park (PLNP) is the oldest protected area in Croatia (since 1949) and was placed on the UNESCO World Heritage List in1979. It is an area of outstanding universal value consisting of a freshwater ecosystem of 16 lakes divided by tufa barriers. Recently, this area has experienced pressure from visitors and significant infrastructural development. When the previous Management Plan expired in 2017, the PLNP initiated and adopted a new Management Plan. This involved the zonation of management in order to better conserve and use this protected area. Management zonation was based on spatial and other data on the distributions of the species and habitats (Natura 2000 and others); cultural values and geo-localities; visitor experiences; existing and planned infrastructure and settlements. Visitor classes and zones were determined using the Recreation Opportunity Spectrum (ROS) methodology. The new management zonation resulted in an increase of 13.9% in the area of the Park included in the Strict Conservation Zone, while succession and habitat degradation resulted in a decrease in the area of the Park in the Active Management Zone, especially in terms of grassland. Six ROS classes were defined. The established ROS classes and the new management zonation were interconnected, each reflecting the need to manage the protected area in terms of conserving its specific biodiversity and geodiversity, while offering visitors various experience opportunities and meeting the needs of the local community.

Keywords: management plan; Plitvice Lakes National Park; protected area; ROS classes; visitor pressure; zonation

Introduction

A protected area is "a clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values" (Dudley 2008). All protected areas should aim to conserve the composition, structure, function and evolutionary potential of biodiversity and contribute to regional conservation strategies (Dudley 2008). Based on the latest updates on the World Database on Protected Areas (WDPA), there are a total of 242,843 protected areas in 245 countries and territories (WDPA 2019). The coverage of terrestrial protected areas is 14.9%, while that of marine areas was 16.8% in national waters in 2018 (Belle et al. 2018). According to the Croatian Nature Conservation Act (Official Gazette 80/13; 15/18) there are nine protected areas that mainly accord with the IUCN categories. The Republic of Croatia has 408 protected areas, which cover 8.54% of the total surface of this country (CAEN 2019).

The national parks (category II) are large natural or near natural areas set aside to protect large-scale ecological processes, along with the complement of species and ecosystems characteristic of the area, which also provide a foundation for environmentally and culturally compatible spiritual, educational, recreational and visitor opportunities. Among many other objectives, they include the management of use by visitors and most importantly the management of the area in order to maintain the area in as natural a state as possible (Dudley 2008). In South-Eastern European (SEE) countries, the second most widely applied protected area management category is category II (the first is category V) with the largest surface designated under it in Albania (Vasilijević et al. 2018).

Some of the globally most valued and recognized protected areas are designated as World Heritage Sites (WHS) and included on the World Heritage List, for which the key requirement is Outstanding Universal Value (OUV). Natural sites on this List must meet at least one out of four criteria listed from vii to x. Each WHS provides biodiversity and geoheritage conservation benefits and contributes to the well-being of local communities and the wider human society (Osipova et al. 2014). Nevertheless, certain factors affect WHS, and these factors include built up environments (housing and transportation), social/cultural uses of heritage (tourism/visitor/recreational activities) and climate change-related factors (humidity, natural hazards) (Galland et al. 2016).

Management objectives are those that affect the IUCN categories, which means that protected areas in category II are managed mainly for the purposes of ecosystem protection and recreation. Management planning is

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a process, not an event, and the product is a Management Plan (MP). Management Plan is defined as "a document which sets out the management approach and goals, together with a framework for decision making, to apply in the protected area over a given period of time. Management Plans should be concise documents that identify the key features or values of the protected area" (Thomas and Middleton 2003). According to the analysis of information for SEE countries, there are seven adopted management plans for national parks and nine for other protected areas in Croatia, which is far less than in Serbia with 59 protected areas, none of which, however, are national parks (Vasilijević et al. 2018).

Management Plans designate different management zones that are defined as geographical areas within which similar management is applied and similar levels of use permitted and different uses segregated. Zones are identified so that the strategies used for management will best accomplish the objective of achieving the desired future for the protected area (Thomas and Middleton 2003). The establishment of meaningful zones or compartments is based on an analysis of information derived from the management objectives and their associated rationales (Alexander 2010).

Recreational and visitor opportunities are a significant part of protected areas. Tourism in general is an important source of income for many countries and it is estimated that the revenue from international tourism reached USD 1.7 trillion in 2018 (UNWTO 2019). Tourists require beautiful natural areas, healthy wildlife and nature. However, managers of many protected areas are also expected to provide meaningful and educational experiences while at the same time avoiding compromising the environmental integrity of protected areas by overcrowding, overdevelopment or pollution, which tourism can sometimes bring (Leung et al. 2018). The potential benefits from tourism in protected areas include enhancing economic opportunity, protecting natural and cultural heritage and enhancing the quality of life. On the other hand, there are potential risks that tourism will bring financial and economic costs (increased demands for basic services), socio-cultural costs (increased congestion, littering etc.) and environmental costs (to the ecosystems, water, wildlife) (Eagles et al. 2002).

Management of visitors in protected areas has improved significantly since the 1930s, when Parks in the United States were facing challenges of increasing numbers of visitors and it was suggested that the number of people in some wilderness areas must be kept within the carrying capacity (Manning 2011). It was recognized that carrying capacity couldn't be calculated as the maximum number of visitors that an area can accept without negative effects on the area and visitor experience, but that the key questions are what are the desired social and biophysical conditions at a destination (McCool and Lime 2001). There are a number of methodological and conceptual frameworks for planning and managing more appropriate visitor use, including Recreation Opportunity Spectrum (ROS) (Clark and Stankey 1979), Limits of Acceptable Change (LAC) (Stankey et al. 1985), Visitor Impact Management (VIM) (Graefe et al. 1990) and most recently Visitor Use Management (IVUMC 2016). For managers of World Heritage sites the new Visitor Management Assessment Tool will shortly be available and will enable a rapid and efficient assessment of how tourism is being managed in terms of a set of sustainability indicators and identified strategies in order to address the priority issues (WHC 2019a).

This study describes the management zonation used in the development of the 2019–2028 Management Plan for the Plitvice Lakes National Park, which has three main objectives:

i. To compare the differences in management zonation (percentage of zones and subzones, methodology) using both Management Plans (most recent and previous one).

ii. Present the use of ROS classes in visitor management.

iii. Evaluate the effect of zonation on the Plitvice Lakes National Park as a UNESCO World Heritage Site with outstanding universal value.

Plitvice Lakes National Park – site description

This National Park is located in a mountainous part of Croatia at an average altitude of 600 m a.s.l. (Fig. 1). Administratively, around 91% of the Park is located within Ličko-senjska County and 9% in Karlovačka County. There are 20 settlements within the Park. The demographic trends are unfavourable, with an aging population and emigration of young people (CBS 2011). Park area is 29,630.8 ha with predominantly forest areas (81%), grassland (approximately 15%) and areas changed due to anthropogenic activity (around 3%), while the aquatic area only makes up 1% of the surface. Plitvice Lakes National Park was designated a national park in 1949 and is the oldest protected area in Croatia.

This National Park was included on the UNESCO World Heritage List in 1979 as a natural heritage based on criteria (ii) and (iii), which nowadays correspond to criteria (vii), (viii) and (ix) (WHC 2005). The Outstanding Universal Value of the Park is defined through the beautiful and intact series of lakes formed by tufa barriers, which are the result of longstanding and ongoing interaction between water, air, sediments (geological foundation) and organisms (vii), intactness of the tufa formation phenomena as an undisturbed ongoing process (viii) and mosses, algae and bacteria contribute to the creation of these natural barriers (ix) (WHC 2019b). In 1997, the borders of the Park were extended in order to include a wider catchment area and thus enhance the integrity of the site and so safeguard the source of the water and enlarge the area of forest by including unlogged forest (IUCN-WCMC 2000).

Biological diversity in the Park is reflected in its abundant flora with over 1,400 vascular species of plants,

Fig. 1 Map showing the location of the Plitvice Lakes National Park in the Republic of Croatia.

including 5% of strictly protected species, as well as a rich fauna of invertebrates and vertebrates including all three large European carnivores: brown bear (*Ursus arctos*), wolf (*Canis lupus*) and lynx (*Lynx lynx*). There are also various types of habitats in the forest, grassland and freshwater ecosystems. The entire area of the Park is a Natura 2000 site, including 21 wild species of birds, 20 habitats and 27 wild flora and fauna species.

The National Park is managed by a Public Institution and governed by the Ministry of Environment and Energy (MEE), which makes it type A according to the IUCN category of governance (Borrini-Feyerabend et al. 2013). The Institution employs over 1,000 people during the tourist season, while there are around 600 permanent employees. This Public Institution funds its operations and activities from revenue from fees (primarily entry tickets and parking fees), sale of products and goods and provision of hotels, restaurants and other tourism services. It is estimated that slightly under 20 per cent of the Park surface is in private ownership (mainly grassland areas).

The natural beauty of the area has always been an important part of the Plitvice Lakes experience, even before the area was designated a protected area. First accommodation for visitors was built in 1862. Starting from 1893, some of the first examples of visitor infrastructure were constructed. In the second half of the 20th century, facilities for visitors were developed further with the construction of wooden chestnut boardwalks along the lakes and across the tufa barriers, which made the visitor experience even more special. In addition, panoramic vehicles and electric boats were used to transport visitors round the park, but this was only sufficient for a low number of visitors. The highest number of visitors in the period between 1960 and 2000 was recorded in 1985 with 763,590 visitors (Vidaković 2003). Since 2000, the number of visitors has been constantly increasing and in 2018 the National Park was visited by 1,796,670 visitors, who visited less than 1 per cent of the Park surface (Fig. 2).

Methodology

Methodology behind zonation can vary substantially depending on the management of each protected area; however, the natural value or biodiversity of every pro-

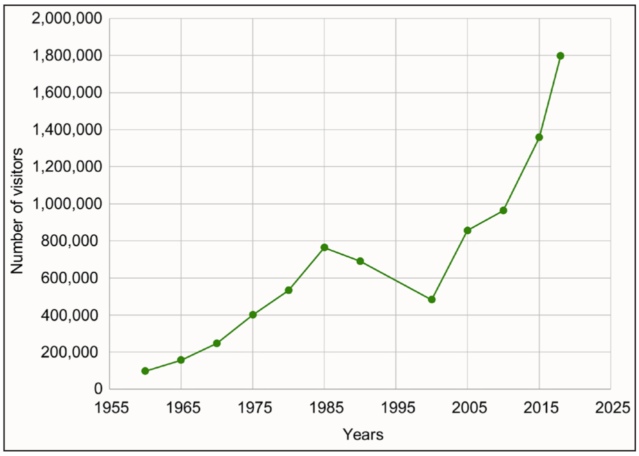

Fig. 2 Changes in the numbers of visitors over the years. Significant decrease in number was observed between 1990 and 2000 due to War and recovery of the management and the area afterwards.

tected area is the main consideration. The process can include various ways of collecting and interpreting data, for instance satellite image data combined with field data (Newman et al. 2007), use of zoning models (Sabatini et al. 2007), Component-Process-Services (CPS) conceptual framework comparing two zoning models (Zeng et al. 2012), or weighting and ranking the priority areas (Soosairaj et al. 2007).

For the Plitvice Lakes National Park, the management zonation is based on the Guidelines for Protected Areas and/or Natura 2000 Site Management Planning (MEE and CAEN 2018). Based on the Guidelines three main zones (I, II and III), with subzones IA and IB were established. Nevertheless, each of the zoned areas does not need to have subzones, and the establishment of subzones will primarily depend on the management needs of an area (Table 1).

The zonation was defined using the ESRI program ArcMap 10.2.2 (ESRI 2014) and topography maps (TM) 25000 from the State Geodetic Administration (SGA). Furthermore, the Croatian cadastre (from SGA) was used, as well as CORINE Land Cover (CLC) that was downloaded from www.copernicus.eu/en as a foundation for Natura 2000 habitats.

The zones mainly consisted of polygons and point localities; however, points are not on the maps due to the resolution and their size. Intermittent streams were presented as polygons with 3 m buffer in order to enable surface calculations, while permanent streams were vectored using orthophoto (taken in 2018) and Light Detection and Ranging (LIDAR, from 2014). State and county roads in subzone IIIB were determined to be 12 m wide and local roads 8 m wide, for the purposes of accurate surface calculations.

The objectives of visitor-use in the new Management Plan were specified using the ROS classes that describe a spectrum of opportunities for visitor experience that the Park can offer. In line with the ROS framework, each of the classes in the spectrum is described by:

– Key elements of experience;
– Biophysical, social and managerial conditions required for the targeted visitor experience;
– Set of indicators, i.e., measurable variables used to monitor the fulfilment of the required conditions;
– Set of standards, i.e., threshold values of indicators for which required conditions are considered fulfilled.

According to the ROS methodology, recreational setting is specified and determined by three types of attributes (biophysical, social and managerial), each varying along a continuum from *none* to *a lot* (e.g. from pristine natural environment with no anthropogenic changes to highly modified environments; from none or few to many and frequent encounters with other visitors; etc.). Several ROS classes were identified, each of which were defined by a combination of attributes, appropriate for targeted visitor experience. They ranged from *primitive* to *modern*, while biophysical, social and managerial conditions varied depending on the class (Fig. 3).

Based on the inventory of the values and opportunities for visitors in the PLNP, as well as sensitivity of particular areas within the Park to the pressures that could result from visitors, the following ROS classes were defined:

– Park areas not open to visitors (zone of prohibited independent entry);
– Class I (direct experience of pristine wilderness with high level of self-reliance);
– Class II (direct experience of conserved nature with medium level of self-reliance);

Table 1 Descriptions of zones and the colours recommended for use in mapping the zones (adopted from MEE and CAEN 2018).

Zone name	Subzone/Zone	Colour		Description
Strict Conservation Zone	IA	Dark green		Areas of natural ecosystems, where habitat conservation status has not changed due to human impact and active management measures are not needed for conservation. Management objective: conservation of natural processes and ecosystem naturalness. Guidelines allowing: research, monitoring or surveillance, emergency interventions (fire, eradication of invasive species) and limited visitation.
	IB	Light green		
Active Management Zone	II	Yellow		Semi-natural ecosystems, geo-localities and cultural landscape areas that require the implementation of active management measures concerning conservation or restoration. Areas where human presence has resulted in ecosystem changes, in history or recent times. Management objective: conservation and/or improvement of the status of biodiversity, geodiversity and cultural landscape. Guidelines allowing: research, monitoring, surveillance and implementation of active measures focused on conservation, visitation, establishment of minimal interpretation and education content and access trails that do not require maintenance.
Sustainable Use Zone	III	Purple		Areas where nature is substantially changed due to the presence of a certain degree of use. Management objective: sustainability of present and planned usage of space, in accordance with protected area management objectives.

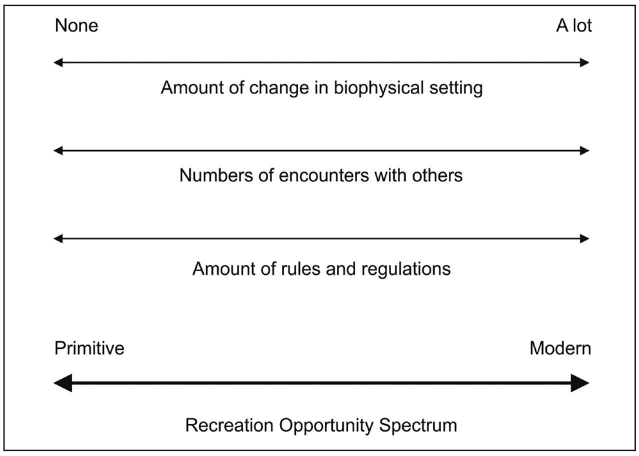

Fig. 3 The Recreation Opportunity Spectrum (adapted from McCool et al. 2007).

- Class III (experience of freshwater ecosystem and opportunity to actively experience Outstanding Universal Value of the Park with low level of self-reliance);
- Class IV (experience of the ambience of traditional settlements and architecture with the possibility of purchasing local products and learning about the cultural heritage);
- Class V (entrances into the Park and tourism services).

Results

In accordance with the characteristics of the area and management needs, all of the 3 main zones with additional division into subzones were determined (Table 2). The highest percentage of the Park (80.7%) is situated in the Strict Conservation Zone (Zone I); 17.1% in the Active Management Zone (Zone II) and 2.2% in the Sustainable Use Zone (Zone III).

Table 2 Management zones and subzones defined in the PLNP Management Plan 2019–2028.

Zone		Subzone	
I	**Strict Conservation Zone**	IA	No visitation
		IB	With limited visitation
II	**Active Management Zone**	IIA	Aquatic ecosystems
		IIB	Grasslands, fens and bogs
		IIC	Cultural landscape
III	**Sustainable Use Zone**	IIIA	Settlement areas
		IIIB	Roads
		IIIC	Built areas with services for visitors
		IIID	Paths, roads and docks managed by the Public Institution

Strict Conservation Zone encompasses areas of natural ecosystems the characteristics and habitat conservation status of which have not been changed by human activities and active management measures are not needed for their conservation. This includes areas of ecosystems that have been changed by human activities but are now recovering naturally. Active management is not needed for the recovery of these areas other than to protect them from human pressure. In line with the Guidelines (MEE and CAEN 2018), the Strict Conservation Zone (Zone I) was further divided into two subzones (Fig. 4a), with the main difference being that in subzone IA visitation is not allowed, while in subzone IB limited low intensity and targeted visitor use is allowed, but only under the supervision and guidance of the Public Institution. Significant changes in the areas of these subzones in Park were recorded, with subzone IB increasing by 53.8% relative to subzone IA. The subzone IB includes forest areas, whereas subzone IA includes the best-conserved natural habitats in the Park (old-growth beech-fir forests (Čorkova uvala), localities of the lady's slipper orchid (*Cypripedium calceolus*), caves and pits, canyon of the river Korana, etc.).

Active Management Zone includes semi-natural ecosystems, geolocalities and cultural landscape areas that need to be actively managed in terms of conservation or restoration for the purpose of long-term conservation. This zone includes areas where human presence has resulted in changes in the ecosystem, historically or recently, and conservation of the biodiversity there requires active management. Active Management Zone (Zone II) consists of three subzones (Fig. 4b). Subzone IIA includes the entire lake system, tufa barriers, most of permanent watercourses, and small aquatic habitats: ponds, wells and wetland. This subzone covers less than 1% of the Park surface. The active management here is focused on the conservation of aquatic ecosystems and key processes (tufa formation), in order to mitigate current anthropogenic effects (e.g. of visitors in the lake area) and/or consequences of previous interventions (e.g. dams, barriers on watercourses etc.). The management measures include the prevention of succession and eutrophication, restoration of habitats for the purposes of conservation of individual species (e.g. brown trout) and possible substantial interventions in space.

Subzone IIB (14.8% of Park surface) includes grasslands, fens, bogs, forest clearings and forested areas along forest roads and paths, including forest areas located within the subzone IB registered as meadows or pastures. Active management is focused on conserving the biodiversity of grasslands, fens and bogs (e.g. by stopping succession) and on restoration of substantially changed habitats of Natura 2000 species or other significant species.

Subzone IIC (1.4% of Park surface) includes human-conditioned traditional landscape around settlements with valuable natural and semi-natural habitats (gardens, crofts, meadows and pastures). Management here includes active management of natural and semi-natural habitats aimed at conserving biodiversity and restoring substantially changed habitats.

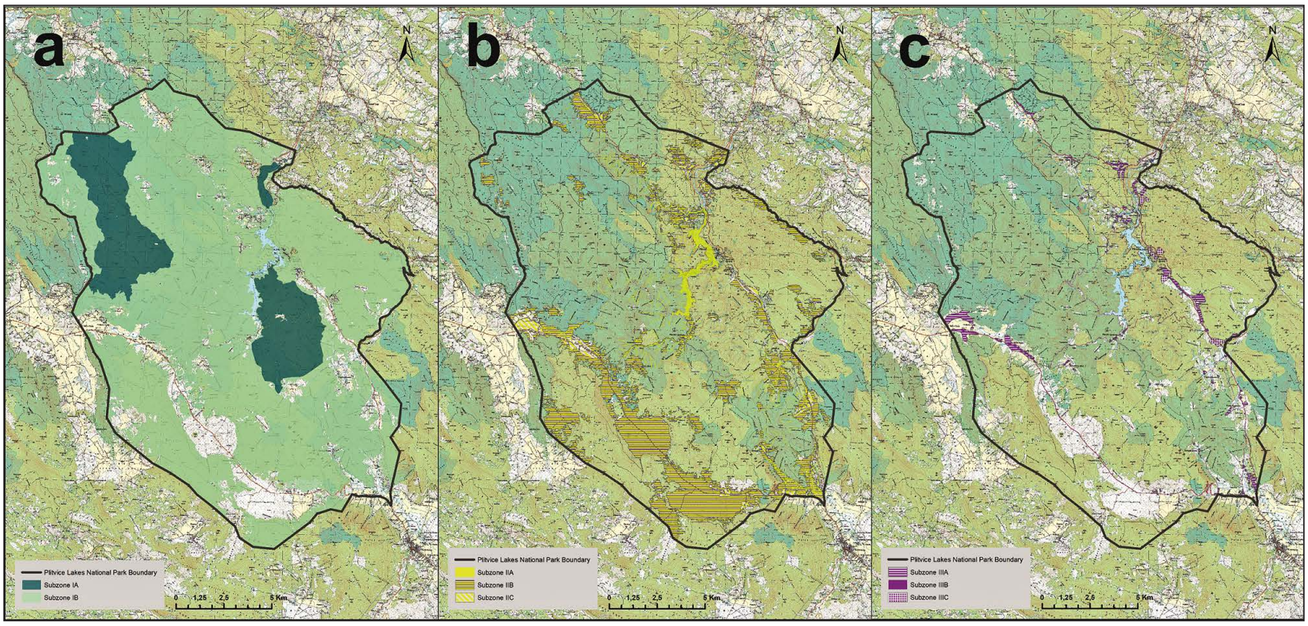

Fig. 4 Map showing the subzones in: a) Strict Conservation Zone, b) Active Management Zone and c) Sustainable Use Zone.

Sustainable Use Zone includes areas where nature has been substantially changed due to use and those designated as the most favourable localities for various permitted forms of high-intensity use, in accordance with the conservation objectives in this zone, which is a compromise between nature conservation and usage. Sustainable Use Zone (Zone III) was further divided into four subzones. In subzone IIIA (Settlements) (Fig. 4c) the primary objective is to ensure adherence to all legal provisions and prescribed nature conservation requirements that prevent settlements and construction in the Park having negative effects on the ecosystems and landscape (e.g. adequate water supply and wastewater treatment, new construction, etc.). Furthermore, every settlement and inhabited area in the immediate vicinity of watercourses must be planned with particular care and situated at least 200 meters away from riverbanks and lake shores.

Subzone IIIB includes all roads within the Park, while the subzone IIIC includes the paths and trails for visitors that require regular maintenance. Subzone IIID includes built up areas outside settlements, with services for visitors (entrances, hotels, restaurants). Management in these subzones is focused on maintenance or reconstruction of existing infrastructure with the purpose of securing the services and security for visitors (installation of fences, removal of trees, etc.), while mitigating the negative effect on ecosystems and landscape. All infrastructure managed by the Public Institution must fulfill the highest energy and environmental efficiency standards.

Management zonation is a decision-making process and its complexity is described in a more detailed map of the area of the Park, with all subzones indicated (Fig. 5). In respect of the special management conditions for each subzone (species, habitats, Natura 2000 sites, etc.), the overlaps had to be consistent and it was not permitted to exclude a single subzone even if it intersects another subzone. The polygon of subzone IA intersects that of subzone IIIB (county road), subzone IIID (forest road) and polygon of subzone IIB. A good example of a different management approach in connection with subzones is evident for subzone IIA (lake area) that intersects subzone IIID (trails).

The comparison of the two Management Plans (previous and recent) reveals not only the changes in zone names, but significant changes in the divisions into subzones and their share of the Park surface. Compared to the previous MP (Šikić 2007a) the Strict Conservation Zone has significantly increased by 13.9% in the new MP. Other significant changes have occurred in Zones II and III. There were only two subzones of Zones II and III in the previous MP, whereas there are three subzones of Zone II and four of Zone III in the new MP. In the new

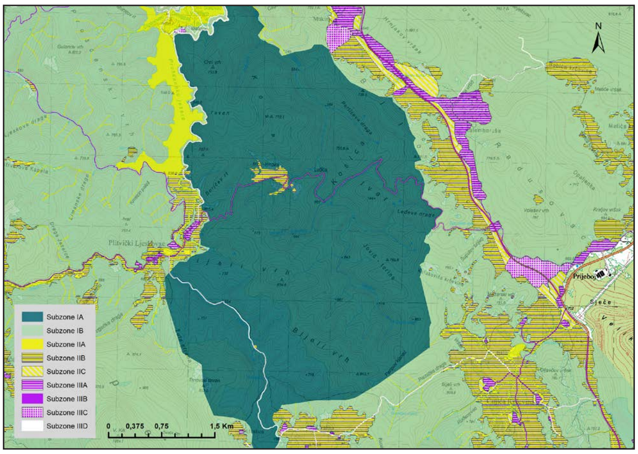

Fig. 5 Detailed map showing the management zones and their subzones of a small area of the PLNP.

Table 3 Comparison of the areas (in ha and %) of the management zones and subzones in previous (2007–2017) and recent (2019–2028) Management Plans.

PLNP MANAGEMENT PLAN 2007–2017						PLNP MANAGEMENT PLAN 2019–2028					
Zone		Subzone		Surface (ha)	Share in Park surface (%)	Zone		Subzone		Surface (ha)	Share in Park surface (%)
I	Strict Conservation Zone	IA	Strictest Conservation Zone	2,480	8.4	I	Strict Conservation Zone	IA	No visitation	3,986.9	13.4
		IB	Very strict conservation zone	17,281	58.4			IB	With limited visitation	19,934.8	67.3
		Total for Zone I		**19,761**	**66.8**			**Total for Zone I**		**23,921.7**	**80.7**
II	Active Conservation Zone	IIA	Active habitat conservation zone	6,729	22.7	II	Active Management Zone	IIA	Aquatic ecosystem	278.3	0.9
		IIB	Active forest ecosystem conservation zone	2,619	8.8			IIB	Grasslands, fens and bogs	4,384.5	14.8
		Total for Zone II		**9,348**	**32.5**			**Total for Zone II**		**5,067.8**	**17.1**
III	Usage Zone	IIIA	Settlement zone	226	0.8	III	Sustainable Use Zone	IIIA	Settlement areas	375.6	1.3
		IIIB	Recreation and tourism infrastructure zone	227	0.9			IIIB	Roads	115.3	0.4
								IIIC	Built areas with services for visitors	79.3	0.3
								IIID	Paths, roads and docks managed by the Public Institution	71.1	0.2
		Total for Zone III		**503**	**1.7**			**Total for Zone III**		**641.3**	**2.2**
OVERALL				29,612	100	OVERALL				29,630.8	100

MP there is a significant decrease of 15.4% in the share of Park surface in the case of Zone II and 0.5% increase in Zone III (Table 3). In addition, the difference in overall Park surface in the two MPs is clear.

Allocation of ROS classes (Fig. 6) for **Zone of prohibited independent entry** overlapped the Strict Conservation Zone (IA), with the addition of the Vrelske bare locality, which is in the subzone IIB (sensitive grassland habitat that requires active management). **Class I** and **Class II** overlapped subzone IB (mainly forest areas), with existing and planned hiking trails belonging to management subzone IIID. The main difference between them is that Class I includes more remote and less frequently visited areas than Class II. **Class III** overlapped subzone IIA area of the lakes, while **Class IV** includes traditional settlements without significant development of tourist infrastructure. **Class V** includes urbanized areas with visitor facilities.

Discussion

Long-term vision and general objectives of the Management Plan, as well as specific objectives and their indicators, define the basic policies of Park management. The previous Management Plan that was adopted in 2007 significantly deviated from ideal planning and was of very limited use in operative planning. This weakness in management was recognized in the recent IUCN World Heritage Outlook report where the National Park was assessed as having deteriorated in terms of its status from good status with some concerns to status of significant concern due to increasing housing development that caters for the ever increasing numbers of visitors. Furthermore, while the ecological values of the site has so far been preserved, protection and management have moved from effective in 2014 to some concern in 2017 (Osipova et al. 2017). Therefore, the adoption of the new MP, including the new zonation, could not have come at a better time.

The most marked difference between the previous and new management zonation is the designation of new subzones within Zones II and III, which is more in line with the management objectives. The difference in overall Park surface in the MPs was a result of incorrect calculations of the total Park surface and its border in the previous MP. However, the Park border and surface was recalculated, and the official Park surface is now 29,630.8 ha.

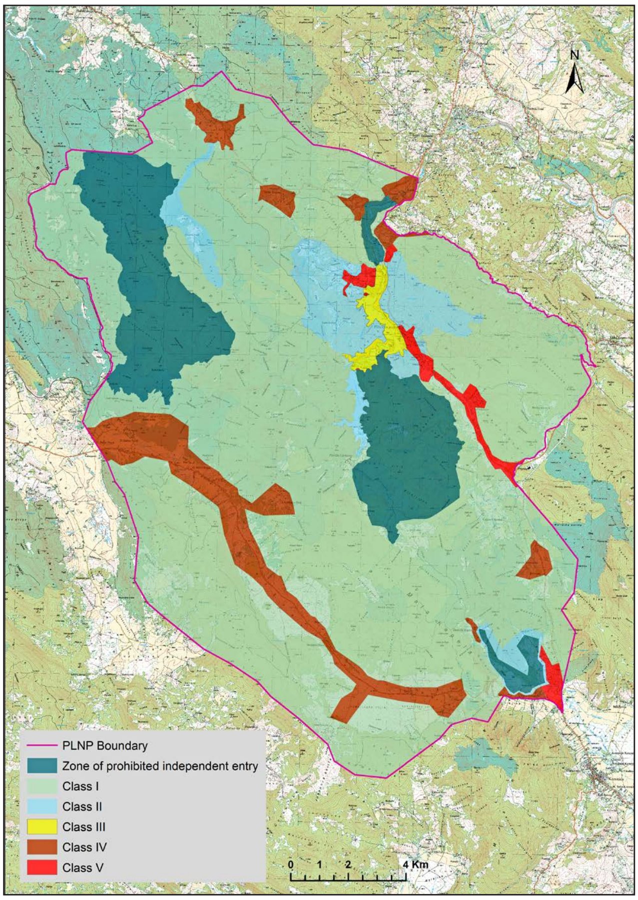

Fig. 6 Map showing the allocation of ROS classes within the Plitvice Lakes National Park.

There are significant changes in the shares of the Park surface in zones and subzones. The highest difference is in Zone II, which was larger in the previous Plan due to subzone IIB, i.e., Active Forest Ecosystem Conservation Zone. In the new zonation, almost the entire former subzone IIB is now included in subzone IB, consequently increasing Zone I. The previous zonation was developed on the basis of the guidelines of the Karst Ecosystem Conservation project, which resulted in the adoption of several MPs for protected areas, including Risnjak, Paklenica and Sjeverni Velebit National Parks. Zonation of Risnjak National Park included the same division into zones and subzones as the PLNP zonation. By comparing the situation with subzone IIB in previous MPs for both Parks, it is concluded that the active forest ecosystem zone implied possible forestry activities such as sanitary logging (Šikić 2007b) while permitted activities in case of PLNP were connected with degraded forest (Šikić 2007a). However, the new concept of management of forest ecosystems in the Park is focused on leaving the forest to natural development, which is the appropriate conservation measures for Natura 2000 habitats.

Increased share of Zone I and decreased share of Zone II of the total Park surface can be explained by succession and loss of grassland to forest. This is addressed in zonation by including all Natura 2000 grasslands in subzone IIB, which requires active management to prevent succession. Furthermore, this Public Institution has the policy of purchasing real estate within Park boundaries from private owners, and this primarily includes agricultural and forest land. This significant activity enables the Park management to actively manage protected habitats, especially grasslands. Grasslands, mires and bogs are reported to be the most degraded habitats across Europe and Central Asia and the biodiversity in temperate grasslands in Eastern Europe exhibit a variable trend in biodiversity (Fischer et al. 2018).

The major difference between the two plans is the transfer of sensitive and unique area around the lakes, with the most prominent OUV, from subzone IIIB (use) to the subzone IIA (active management). This transfer reflects the imperative of conservation, even in the most visited areas.

Zone III was enlarged due to inclusion of roads, which were not included in the previous MP zonation. Paths and hiking trails that are a part of the visitor system are now included in subzone IIID.

This more thorough zonation unlike the previous one opened up more opportunities for better area management. That was not the case for the Šumava National Park (Czech Republic), where Zone I was reduced and Zone II enlarged due to logging (Křenová and Hruška 2012). However, the National Park of Abruzzo (Italy) aimed to increase its Strict Reserve Zone from 6.9% to 14–15% even though the areas to be included do not match the IUCN Protected Area categories (Synge 2004).

Authors McCool and Eagles (2014) recommended PLNP take action and develop a visitor management strategy using ROS methodology. The rationale behind this recommendation was the lack of visitor and tourism management policy in the previous MP. Classification using ROS resulted in the development of classes that were closely in line with the management zonation. Therefore, the establishment of biophysical, social and managerial conditions with their indicators and standards had to be performed with caution, in order to avoid possible inconsistency in further management or decision-making processes. Attention was drawn to the Zone of Prohibited Entry, which was not only allocated to Zone I but also to Zone II in the management zonation. Even though Zone II implies a certain number of visitors, conservation measures were more important in the case of this ROS class. In addition, for the purposes of interpretation of this specific zone, particular in its border areas, Class II was designated based on biophysical conditions determined by hiking trails and trail markings, information and interpretation content. The number of visitors to Class I areas is determined by the use of hiking trails which are in subzone IIID; however, social conditions are such that there is a small probability of encountering other people, while Class II implies the possibility of occasional encounters. Even though the ROS methodology has not been used for Hohe Tauren National Park (Austria), their approach to visitor management is to balance the wishes and expectation of visitors with the need to conserve nature (Synge 2004). For the protected areas in Turkey, it is recognized that long-term development plans are not user-oriented and do not include visitor management (Duzgunes and Demirel 2016).

Conclusions

Management zonation reflects the need to manage the Park in terms of conserving its specific biodiversity, geodiversity and Outstanding Universal Value. It was designated based on available spatial and other relevant data regarding Natura 2000 and other significant species and habitats and their ecological requirements, significant geo-localities, cultural values, visitor experience opportunities and existing and planned infrastructure and settlements. Significant increase in the area in the Strict Conservation Zone is evident, which is a contribution to Aichi Target 11 (CBD 2010).

ROS methodology provided a completely new spectrum of visitor opportunities that enabled the Park management to adopt a new approach to visitor management (together with other visitor-use measures).

The presented management zonation should facilitate the Park management to achieve the vision of Plitvice Lakes National Park as a UNESCO World Natural Heritage Site, a place to experience and learn about the Outstanding Universal Value and other natural and cultural values, with good protected area management in cooperation with the local community, where conserved nature is the foundation of sustainable development.

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IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI AND *PSEUDOMONAS FLUORESCENS* ON GROWTH, PHYSIOLOGICAL PARAMETERS AND ESSENTIAL OIL CONTENT IN *OCIMUM BASILICUM* L.

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ABSTRACT

A pot experiment was performed to see the interactive potential of *Glomus mosseae* and *Acaulospora laevis* alone or in combination with *Pseudomonas fluorescens* on *Ocimum basilicum* L. under glass house conditions. Various morphological and physiological parameters were measured after 120 days. Although, all co-inoculation treatments showed beneficial effects but *G. mosseae* is found to be the most compatible strain found in the rhizosphere of basil plant. *G. mosseae* alone or in combination with other bioinoculants showed maximum increase in all the different parameters studied (plant height, fresh weight, dry weight, leaf number, inflorescence height, root and shoot phosphorus, acidic and alkaline phosphatase and oil content). The overall results demonstrate that the co-inoculation of *P. fluorescens* with AM fungi promotes higher mycorrhizal colonization enhancing nutrient acquisition especially phosphorus (P), producing plant growth hormones resulting in improvement of rhizospheric condition of soil, altering the physiological and biochemical properties of sweet basil.

Keywords: *Acaulospora laevis*; essential oil; *Glomus mosseae*; *Ocimum basilicum*; *Pseudomonas fluorescens*

Introduction

Ocimum basilicum L. (family: Lamiaceae) known as sweet basil, is an important annual herb cultivated all over the temperate regions. Basil is a rich source of phenolic, antioxidant compounds, flavonoids and essential oils. It has been traditionally used for the treatment of many ailments, such as headaches, coughs and diarrhea (Phippen and Simon 1998; Javanmardi et al. 2002). The essential oils mainly found in leaves are volatile and mostly consisting of monoterpenes and sesquiterpenes are commonly utilized in the spice industry (Werker et al. 1993).

Plants generally take nutrients from the soil by simply root soil interaction with the help of various microorganisms. The interaction between the roots of the plant and the soil microorganisms help plants to acquire necessary mineral nutrients from the soil (Prasad et al. 2012; Yadav et al. 2015). Among the different macronutrients, Phosphorus (P) is the most frequent essential mineral occurs in immobilize form in the soil. In the past few decades, Arbuscular Mycorrhizal Fungi (AMF) has emerged as potential biofertilizers and environmentally friendly alternative to chemical fertilizers (Cagras et al. 2000). Plants in association with AM fungi interact to perform the efficient solubilization of these mineral elements in the rhizosphere. During this process, the plant roots intercept with the fungal hyphae establishing a mutual symbiotic association between the roots of the plants and AMF which is very useful in exploration of the larger soil volume thereby making 'positionally unavailable' nutrients 'available' by increasing the surface area for absorption (Tinker and Gildon 1983). On the other hand, the uncontrolled and indiscriminate use of chemical fertilizers causes adverse effects on soil health and environment also.

PGPR (Plant Growth Promoting Rhizobacterium) are naturally occurring soil microorganisms that colonize roots and stimulate plant growth. Such bacteria are applied to a wide range of agricultural crops for the purpose of growth enhancement and yield (Raj et al. 2003). Plant growth promotion in plants as a result of rhizobacterium soil inoculation may increase the production of growth hormones and solubilization of phosphates (Kloepper et al. 1993).

Keeping in view of above information, the present study was carried out to assess the consequent effect of dominant AM fungi (*Glomus mosseae* and *Acaulospora laevis*) alone or in various combinations with *P. fluorescens* to find out the best combination having the maximum capability of increasing plant growth and oil content in basil plant.

Materials and Methods

Mass multiplication of bioinoculants

The two dominant AM species (*Glomus mosseae* and *Acaulospora laevis*) were isolated from the rhizospheric soil of *Ocimum basilicum* L. grown in Botanical Garden of Botany Department, Kurukshetra University by using Wet Sieving and Decanting Technique of Gerdemann and Nicolson (1963) and identified using the key of Schenck and Perez (1990). The starter inoculum for each species were multiplied by "Funnel Technique" of Menge and Timmer (1982) – see also Sharma et al. (2017). The AM species

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were multiplied with maize as host for three months. *Pseudomonas fluorescens* (MTCC NO. 103) was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India and cultured in a Nutrient Broth Medium incubated at 32 °C for 48 hours to obtain a concentration of 1×10^9 colony forming units (cfu) ml $^{-1}$.

Experimental site and setup

The experiment was set up in the polyhouse, Botany Department, Kurukshetra University, Kurukshetra, Haryana during June to September, 2016. The soil used in the experiment consisted of Clay – 3.78%, Silt – 20.8%, Sand – 74.5%, EC – 0.26 dS/m, Organic Carbon – 0.40%, total N – 0.042%, P – 7.30 kg^{-1} acre, K – 88 kg^{-1} acre and S – 14.80 ppm. The experiment was laid out in a randomized complete block design with five replicates per treatment. Top soil (0–30 cm) was collected and sieved through 2 mm sieve, mixed with sand, soil in the ratio 1:3 and sterilized in autoclave for 20 minutes at 121 °C at 15 psi. Earthen pots (25 × 25 cm) were selected having capacity of 2 kg soil. For AM treatment 10% (w/w) of soil of the selected AM inoculum having 845 AM spores approximately and 200g of soil having chopped AM colonized pieces of trapped host barley with the infection level of about 90–95% were added. Before sowing, the roots of the seedlings were dipped in the nutrient broth having *P. fluorescence.* The experiment was set up with the following treatments:

1) Control (C)
2) *Glomus mosseae* (G)
3) *Acaulospora laevis* (A)
4) *Pseudomonas fluorescence* (P)
5) *Glomus mosseae* + *Acaulospora laevis* (G + A)
6) *Acaulospora laevis* + *Pseudomonas fluorescence* (A + P)
7) *Glomus mosseae* + *Pseudomonas fluorescence* (G + P)
8) *Glomus mosseae* + *Acaulospora laevis* + *Pseudomonas fluorescence* (G + A + P)

In the control set no inoculum was added. Plants were watered regularly to maintain humidity. Hoagland Nutrient solution without phosphorus (100 ml/pot) was added to each pot after regular interval of 20 days. Each treatment was replicated five times. After 120 days, five plants from each treatment were analyzed for the various morphological and physiological parameters.

Essential oil isolation

After the flowering, leaves and shoots of basil were harvested and essential oil was extracted by hydro distillation of one-liter water in a Clevenger apparatus for eight hours (Rasooli and Mirmostafa 2003). The essential oil was collected and stored in glass vials prior to determination of chemical compounds.

Harvest and analysis

After 120 days, the plants were uprooted and analyzed for various morphological and physiological parameters. Shoot length (cm) were analyzed. Percentage root colonization was assessed by Rapid Clearing and Staining Technique of Phillips and Hayman (1970). AM spores were isolated by Wet Sieving and Decanting Technique of Gerdemann and Nicolson (1963). Alkaline and acidic phosphatase of fresh roots was estimated by Tabatabai and Bremner (1969). Shoot and root phosphorus were determined by vanado-molybdo-phosphoric acid yellow colour method (1973). Total chlorophyll content was estimated by using Arnon's method (1949) using 80% acetone as solvent.

Statistical analysis

The experimental data was analyzed using analysis of Variance (ANOVA), followed by post hoc test using the Statistical package for Social Sciences (ver. 16). Means were then ranked at ≤ 0.05 level of significance using Duncan's Multiple Range Test (DMRT) for comparison.

Results and Discussion

Significant variation was observed in growth parameters and essential oil content as influenced by different bioinoculant treatments. Results showed that mycorrhizal plants showed significant increase in plant height, shoot and root fresh and dry weight, number of leaves, inflorescence length (Table 1). After 120 days of inoculation highest mycorrhizal colonization and AM spore number was observed in A + G followed by G + A + P (Table 2). Plants inoculated with the different bioinoculants showed higher degree of phosphorus acquisition as compared to control ones (Table 3). Phosphorus content in non-mycorrhizal treatments was lower than that of mycorrhizal treatment. The higher nutrient uptake in mycorrhizal basil plants might be attributed to the combination of fungal external mycelia, which explore a large volume of soil and absorb more nutrients. It was also observed that mycorrhizal treated plants have better root architecture which helps the plants in better uptake of Phosphorus. The main explanation is that AMF developed an extra metrical mycelium, which increased the root phosphate absorbing sites by producing phosphatase enzyme (Bolan 1991). The increase in root phosphorus activity in AM treated plants may be due to higher capacity of AMF hyphae to explore more soil volume beyond the depletion zone and thus triggering the P transport from soil to plant roots.

In the present investigation the effect of AM Fungi on the rhizospheric bacteria was caused by a direct interaction between the fungal mycelia of *Glomus mosseae* and the *Pseudomonas fluorescens.* The nutrient status of plant also influences root exudates composition (Lipton et al. 1987), because of which the rhizospheric population of *Pseudomonas fluorescens* increases which in turn helps in the better absorption of nutrient by exploring phosphorus rich soil areas away from the root surface. In the present study, AM colonization to basil

Table 1 Interaction of AM Fungi and *P. fluorescens* on different growth parameters of *Ocimum basilicum* L. after 120 days.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Leaf number/plant	Inflorescence Height (cm)
Control	29.20^{g}	6.28^{h}	1.48^{h}	5.20^{h}	0.62^{g}	51.00^{e}	7.0^{e}
A. laevis	37.60^{f}	12.80^{g}	2.06^{g}	7.28^{g}	0.79^{f}	67.00^{d}	7.6^{d}
G. mosseae	52.64^{b}	20.90^{b}	4.09^{b}	10.98^{c}	1.22^{c}	86.00^{ab}	8.6^{b}
P. fluorescens	44.80^{d}	16.40^{d}	3.02^{d}	10.22^{d}	0.98^{d}	81.00^{b}	8.2^{c}
A + G	41.60^{e}	13.98^{e}	2.68^{e}	8.78^{e}	0.86^{e}	74.00^{c}	7.8^{cd}
G + P	54.20^{a}	21.40^{a}	4.25^{a}	12.02^{a}	1.47^{a}	90.00^{a}	9.0^{a}
A + P	38.20^{f}	13.22^{f}	2.24^{f}	7.46^{f}	0.82^{ef}	71.00^{cd}	8.0^{cd}
G + A + P	48.20^{c}	17.20^{c}	3.808^{c}	11.88^{b}	1.36^{b}	88.00^{a}	9.2^{a}
L.S.D. ($P \leq 0.05$)	0.725	0.225	0.0615	0.085	0.0515	5.985	0.275
ANOVA ($F_{7,16}$)	986.503	3.572	1.974	5.470	222.16	27.822	16.766

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± Standard deviation
* The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's Multiple Range Test).

Table 2 Effect of AM Fungi and *P. fluorescence* on mycorrhization and oil content (%) of *Ocimum basilicum* L. after 120 days.

Treatments	AMF root colonization (%)	AM Spore number per 20 g of soil	Essential Oil content (%)
Control	0^{e}	0^{e}	0.50^{f}
A. laevis	63^{c}	145^{d}	0.64^{ef}
G. mosseae	76^{b}	200^{b}	1.00^{c}
P. fluorescens	0^{e}	0^{e}	0.74^{de}
A + G	85^{a}	214^{a}	1.05^{bc}
G + P	55^{d}	185^{c}	1.20^{ab}
A + P	63.8^{c}	176^{c}	0.88^{cd}
G + A + P	80^{ab}	212^{a}	1.25^{a}
L.S.D. ($P \leq 0.05$)	7.655	10.515	0.195
ANOVA ($F_{7,16}$)	151.136	543.288	7.694

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± Standard deviation.
* The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's Multiple Range Test).

Table 3 Effect of AM Fungi and *P. fluorescence* on different physiological parameters of *Ocimum basilicum* L. after 120 days.

Treatments	Chlorophyll Content (mg/g FW)			Carotenoid (mg/g FW)	Phosphatase activity (IU/G FW)		Phosphorus Content (P)	
	Chlorophyll a	Chlorophyll b	Total Chlorophyll		Acidic phosphatase	Alkaline phosphatase	Shoot P	Root P
Control	0.368^{f}	0.113^{f}	0.481^{f}	0.186^{e}	0.057^{e}	0.09^{d}	0.43^{f}	0.66^{f}
A. laevis	0.878^{bc}	0.284^{c}	1.162^{bc}	0.391^{b}	0.139^{de}	0.039^{d}	0.67^{e}	0.75^{ef}
G. mosseae	0.815^{de}	0.268^{cd}	1.083^{d}	0.355^{c}	0.18^{cd}	0.221^{b}	0.87^{cd}	1.96^{a}
P. fluorescens	0.782^{e}	0.243^{d}	1.025^{e}	0.32^{d}	0.09^{de}	0.012^{d}	0.46^{f}	0.89^{de}
A + G	0.887^{b}	0.324^{b}	1.211^{b}	0.394^{b}	0.256^{bc}	0.263^{b}	1.07^{b}	1.43^{b}
G + P	0.837^{cd}	0.273^{cd}	1.11^{cd}	0.347^{b}	0.135^{de}	0.128^{c}	0.97^{bc}	1.13^{c}
A + P	0.817^{de}	0.168^{e}	0.985^{e}	0.387^{b}	0.097^{de}	0.054^{cd}	0.71^{de}	1^{cd}
G + A + P	0.956^{a}	0.356^{a}	1.312^{a}	0.46^{a}	0.328^{a}	0.381^{a}	1.89^{a}	2.09^{a}
L.S.D. ($P \leq 0.05$)	.0475	0.0315	0.0555	0.0265	0.082	0.03	0.1639	0.09
ANOVA ($F_{7,16}$)	81.727	50.550	126.235	44.751	6.308	18.043	38.713	50.608

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± Standard deviation,
* The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's Multiple Range Test).

plant showed positive effect on the plant growth and it might have resulted in increased bacterial activity thereby absorbing more nutrients from the soil. Yadav et al. (2015) and Prasad et al. (2012) also noticed growth stimulation effects by using PGPR strains. This specificity appears to be related to different composition of rhizosphere exudates secreted by basil plant affecting the levels of colonization and subsequently the efficacy of the *Pseudomonas fluorescens* or to the specific compounds present in the exudates that may stimulate the synthesis of secondary metabolites in the bacteria used in the plant growth (Mishra et al. 2017).

Essential oils are volatile lipophilic mixture of compounds obtained from plants, mostly consisting of monoterpenes, phenyl propenoids and sesquiterpenes. The biosynthesis of essential oil depends upon the availability of phosphorus content in the plant. In the present investigation, it was found that the essential oil content significantly increased in mycorrhizal treated plant regardless of the AM fungal species. However, plants inoculated with *Glomus mosseae* showed significant increase in essential oil in comparison to *Acaulospora laevis*. There was relatively difference in the percent oil content in the leaves of different bioinoculant treated plants (Table 2). Positive significant relationships were found in inoculated plants between essential oil percent and AM root colonization, AM spore number and biomass. The increases of essential oil content have been linked to greater plant biomass because of mycorrhization (Copetta et al. 2006). When plant growth increases, more photosynthates especially chlorophyll content are produced resulting in higher allocation of fixed carbon source. *G. mosseae* is the most compatible strain found in the rhizosphere of basil plant because where ever *G. mosseae* is present whether alone or in combination with other bioinoculants the growth and essential oil contents were significantly higher. There are many processes that contribute to improved P acquisition by mycorrhizal plants. These include better root architecture for more absorption of nutrients and through solubilization of phosphorus by phosphatases. In our results, mycorrhizal treated plants showed more acidic and alkaline phosphate activity which may be due to increased exploration of soil particles by AM hyphae. Similar results were also reported for *Coriandrum sativum* by Kapoor et al. (2002), for *Citrus jambhiri* by Nemec and Lund (1990), for *Mentha arvensis* by Freitas et al. (2004), Khaliq and Janardhan (1997) respectively. The increased essential oil production is result of the increased production of fresh shoot foliage (Subrahmanyam et al. 1992). Moreover, Copetta et al. (2006) observed that *Glomus rosea* increased essential oil yield was associated with large number of peltate glandular trichomes in the leaves of *Ocimum basilicum*. They further suggested that the greater number of trichomes could be related to alterations in the phytohormonal profile induced by AM fungi.

Conclusions

The results of this study clearly reveal that the inoculation with AM fungi and other bioinoculants is more effective in increasing of growth, biochemical attributes and oil content of basil plant. This increase could be attributed to the increased surface area of roots, better water absorption, enhanced uptake of nutrients and secretion of some enzymes by inoculated microorganisms. *G. mosseae* is the most compatible strain found in the rhizosphere of basil plant because where ever *G. mosseae* is present whether alone or in combination with other bioinoculants the growth and essential oil contents were significantly higher. This combination can be tested further in the field conditions and can be recommended to farmers after proper confirmation.

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EFFECT OF IMPLANTING A PASSIVE INTEGRATED TRANSPONDER TAG IN JUVENILE CHUB, *SQUALIUS CEPHALUS* (L.), ON THEIR CONDITION, GROWTH AND SURVIVAL

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ABSTRACT

The effect of implanting a Passive Integrated Transponder (PIT) tag on the survival, growth and condition of a small cyprinid, juvenile chub *Squalius cephalus* (L.) with a mean weight of 2.4 g was studied in the laboratory. During this experiment, which lasted for 31 days, 80 specimens were tagged. The changes in Fulton's condition factor (K), specific growth rate of mass (G_M) and specific increase in length (L_S) were evaluated. The results showed that implanting PIT tags did not affect the survival or growth of the fish; however, it had a negative effect on their condition. The initial size of the fish had a significant effect on the specific growth rate in terms of mass, but not in terms of length. Survival was 98.8% and tag retention 97.5%, when the tag made up 4.3% of the mass of the fish. These results demonstrate that PIT tagging is an appropriate method for chub heavier than 2 g. For this size category, we recommend that PIT tagging is suitable when the tag makes up 4.3% of the body mass of the individuals.

Keywords: condition; cyprinids; PIT tags; specific growth rate; tagging effect

Introduction

Passive integrated transponder (PIT) tags are used increasingly in both commercial aquaculture and ecological studies on fish (Alanärä et al. 2001; Fischer et al. 2001; Bolland et al. 2009a; Grieve et al. 2018). PIT tagging has become one of the most effective tools for identification of individuals and, due to their relatively low cost and automatic data collection options, they enable a large number of fish to be marked and detected with high reliability. Furthermore, the weight and size of PIT tags make them ideal for studying juveniles or small species of fish (Thorstad et al. 2013; Jørgensen et al. 2017). Although this tagging method is almost universally applicable, size and species-specific adverse effects have been reported (Roussel et al. 2000; Pennock et al. 2016). Careful evaluation of the effects of PIT tagging on a wide range of fish of different sizes is urgently required (Ficke et al. 2012).

Juvenile cyprinids weighing only a few grams have received little attention in this context. For cyprinids, tag retention and the survival of tagged individuals has been evaluated previously for the genus *Squalius* (e.g. Bolland et al. 2009b; Pires et al. 2010). For example, Bolland et al. (2009b) report the effects of PIT tags in terms of mortality and retention in large *Squalius cephalus* (L.). In the study of Pires et al. (2010) survival, tag retention and swimming performance are evaluated for an endangered Iberian cyprinid, Mira chub *Squalius torgalensis* (Coelho et al. 1998). The objective of the present study was to extend the work on small cyprinids, focusing on both retention and survival, and growth, weight and condition of juvenile chub *Squalius cephalus* (L.) after implanting PIT tags.

Materials and Methods

Chub is a common omnivorous cyprinid inhabiting European rivers, with a wide ecological amplitude and known migratory and daily activity patterns (Lucas and Baras 2000). The fish used in this experiment were hatchery-reared juvenile chub, *Squalius cephalus* (L.), obtained from a local fish supplier (Czech Fishery Ltd., Czech Republic). A total of 160 fish of a similar size were randomly distributed among four separate holding tanks (300 l each; natural photoperiod; conditions in all tanks: temperature 19.1 ± 0.5 °C; conductivity 314 ± 6.9 μS cm^{-1}) and acclimated for 3 weeks prior to the start of the experiment. Fish were fed daily with dry fish flake food. In order to ensure good water quality, one third of the water in each tank was changed each week.

Fish from two randomly selected holding tanks (80 specimens) were tagged at the beginning of the experiment, after first anaesthetising them with 2-phenoxy-ethanol (0.2 ml l^{-1}; Merck KGaA, Germany). Fish were measured (L_S; standard length, mean 54 mm, range 47–64 mm), weighed (M; mean body mass 2.37 g, range 1.44–4.24 g), and then PIT tags (Trovan ID100, 0.1 g in air, 12 × 2.1 mm) were inserted into the abdominal cavity using a hypodermic needle attached to a syringe. The tag to body mass ratio varied from 2.36 to 6.95% (mean 4.25%). The fish placed in the two other holding tanks were left undisturbed to serve as controls (Skov et al.

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Table 1 The initial and final lengths (L_S), masses (M) and condition (K) of PIT tagged and control specimens of chub, *Squalius cephalus*.

Treatment	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**	**Mean ± S.E.**
	initial L_S (mm)	**final L_S (mm)**	**initial M (g)**	**final M (g)**	**initial K**	**final K**
Control		56.3 ± 0.4		2.92 ± 0.06		1.63 ± 0.01
PIT tags	53.6± 0.4	56.8 ± 0.4	2.37± 0.06	2.85 ± 0.06	1.53 ± 0.02	1.54 ± 0.01

2005). During the experimental period, which lasted for 31 days, all fish were kept under the same regime with daily monitoring to detect tag expulsion or death. At the end of the experiment, all fish were anaesthetised, measured and weighed. All experimental procedures complied with relevant legislative regulations (Law no. 246/1992, §19, art. 1, letter c).

The specific growth rate (GM) in terms of mass (proportional increase/day) was calculated according to the formula: $G_M = 100(\ln M_2 - \ln M_1)\, t^{-1}$, where M_1 and M_2 are the masses (g) at the start and end of the study period, respectively, and t is the length of the period in days. The increase in L_S (GL) was calculated similarly (Jepsen et al. 2008). The changes and differences in Fulton's condition factor (K) were evaluated as: $K = M\, L_S^{-3}$, where M is mass and L_S is the standard length (mm).

Statistical analyses were performed using the SAS software package (SAS Institute Inc., version 9.2, www .sas.com). Standard length, mass, Fulton's condition factor, specific growth rate and increase in length were analysed using separate mixed models with random factors (PROC MIXED). Random effects were used to account for repeated measures collected for the same experimental units (individual fish) throughout the duration of the study. The significance of an exploratory variable (i.e., tagging treatment) in a particular model was assessed using a F-test. Differences between the classes were tested using a t-test (Tukey–Kramer adjustment for multiple comparisons) and the degrees of freedom calculated using the Kenward–Roger method.

Results

The smallest individual (1.44 g) in which a PIT tag was implanted died the following day. Post mortem examination revealed contusion of the internal organs. The remaining 79 tagged fish survived, resulting in a survival of 98.8%. One fish (1.75 g) expelled its tag during the third week of the experiment, so tag retention was 97.5%.

The PIT tagged fish grew significantly (mass $F_{2,135} = 90.99$, $p < 0.0001$, Fig. 1a; length $F_{2,137} = 149.3$, $p < 0.0001$, Fig. 1b) during the experimental period, and their final size did not differ from that of the control fish (mass *Adj* $p > 0.63$; length *Adj* $p > 0.54$, Table 1), indicating that PIT tagging did not affect the growth of the juvenile chub. The condition of the PIT tagged fish remained the same throughout the experiment ($F_{2,138} = 16.52$, $p < 0.0001$, *Adj* $p > 0.66$, Fig. 1c), but was poorer at the end of the experiment than that of the control fish (*Adj* $p < 0.0001$), which indicates that PIT tagging had a negative effect on fish condition. The tag to body mass ratio had a significant effect on growth in mass ($F_{1,75} = 3.66$, $p < 0.05$, Fig. 2), but not on increase in length ($F_{1,75} = 0.19$, $p > 0.66$); *i.e.* smaller fish had a higher specific growth rate of mass.

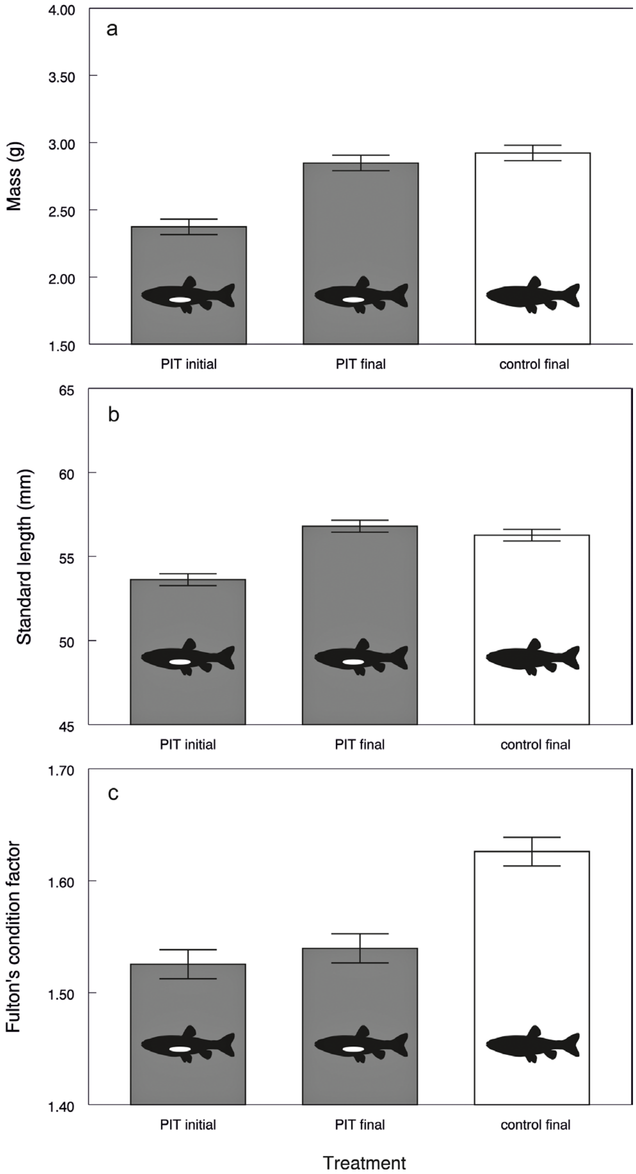

Fig. 1 Initial and final mass (a), length (b) and condition (c) of tagged and control chub. Initial and final values for PIT tagged chub, *Squalius cephalus,* and final values for control fish are adjusted means ± SE.

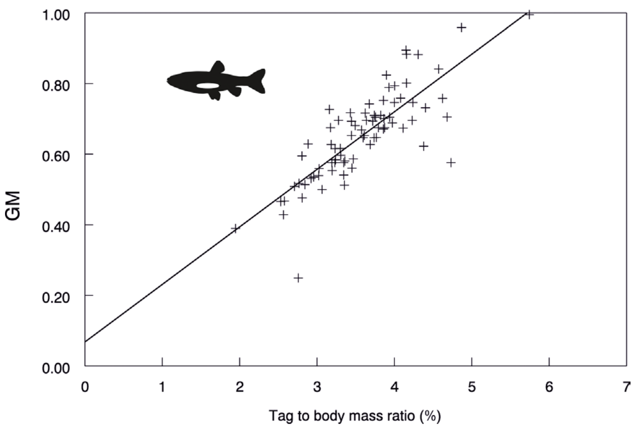

Fig. 2 Relationship between specific growth rate of mass (GM) and the tag to body mass ratio in juvenile chub, *Squalius cephalus*.

Discussion

Small fish are more likely to suffer adverse effects from tagging (Jepsen et al. 2008). Based on the present study, it is recommended that the minimum weight for tagging chub with PIT tags is 2 grams, as no fish above this weight lost the tag or died during the experimental period. Tag retention and survival of tagged fish is closely associated with the tag to body mass ratio (Pennock et al. 2016). In order to minimize the effect of tagging a tag to body mass ratio of less than 2% is often recommended when determining the minimum size of fish to be tagged (Winter 1983; Jepsen et al. 2005). Although this ratio is still used, a number of recent studies have challenged this view and report no or minimal effects of tag to body mass ratios of up to ~10% (Ficke et al. 2012; Ward et al. 2015; Pennock et al. 2016). In this study, juvenile chub implanted with tags that made up 4.3% of their mass were successfully tagged and 98.8% survived with negligible effects. Other studies assessing survival and tag retention by small cyprinids under laboratory conditions report tags that make up to 3.94% of the mass of the fish result in acceptable levels of post-implantation survival (e.g. Skov et al. 2005; Bolland et al. 2009a; Ficke et al. 2012; but see Pennock et al. 2016). All of the flathead chub, *Platygobio gracilis,* implanted with PIT tags that made up 3.94% of their mass survived (Richardson 1836) (Ficke et al. 2012), 96.3% of European chub implanted with tags that made up 2–6% of their mas survived (Bolland et al. 2009a) and 99% of roach, *Rutilus rutilus* (L.), implanted with tags that made up 2.91% of their mass survived. The higher tag to body mass ratios used in some other studies, e.g. 3.8% for creek chub (Bangs et al. 2013), 6.3% for southern redbelly dace, *Chrosomus erythrogaster* (Rafinesque 1820) (Pennock et al. 2016) and up to 12.5% for humpback chub *Gila cypha* (Miller 1946) (Ward et al. 2015), however, are associated with significant reductions in survival (survival between 62.5% and 82%).

At the end of this study, the condition of PIT tagged chub was poorer than that of the controls. This could be a consequence of the energetic costs associated with recovering from the effects of the operation (Jepsen et al. 2008), but the handling and the surgery could also have influenced the observed trends. Capture and handling stress may have different effects on different species of fish. For example, Skov et al. (2005) reports it did affect the condition of PIT tagged rudd, *Scardinius erythrophthalmus* (L.), but not roach *Rutilus rutilus* (L.). The weight of tagged Mira chub (Pires et al. 2010) is also lighter 30 days post tagging. Our results indicate that it is possible to implant PIT tags in small cyprinids weighing more than 2 g. For this size category we recommend the tag should not make up more than 4.3% of the mass of the fish.

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ICHTHYOFAUNAL DIVERSITY OF RANJIT SAGAR WETLAND SITUATED IN THE NORTHWESTERN HIMALAYAS

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ABSTRACT

Diversity of fishes was evaluated at the Ranjit Sager Wetland and its three adjoining streams. Factors like altitude, bed gradient, dominated substratum and habitat types of the streams have also been studied. These streams fall under Type-B category on the basis of habitat variability, gradient and sinuosity. 43 fish species represented by 6 orders have been reported from the study area. Out of all 43 fish species, 13 come under threatened categories of Red List of IUCN, out of which 2, 3 and 8 come under Endangered (EN), Vulnerable (VU) and Low Risk near threatened category (LRnt) respectively. It has been analyzed from the above pattern that maximum fish species reported from this wetland have fallen under different threatened categories.

Keywords: abundance; habitat loss; species richness; threatened species

Introduction

The heterogeneous freshwater habitat in rivers, streams, springs and headwaters like variation in altitude, flow rates, physical substrate and the riparian zones provides good opportunity of food, shade and cover for various fish species. Consequently, freshwater habitats harbor diverse fauna, with fish serving as prime indicators of ecosystem status (Armantrout 1990). Though study of assemblage pattern and partitioning of fish diversity is a challenging subject in fishery science (Ross 1986). Fish research has become an increasingly important study area, as fish population is declining throughout the world due to various anthropogenic activities. The decline of fishes has an adverse impact on aquatic ecosystems as well as a significant impact on human population as it is one of the primary food sources for human. Anthropogenic activities such as modification of the environment, culture, harvesting and effects of modernization have contributed to the pollution of water bodies which serve as habitat for fishes (Plafkin et al. 1989; Siligato and Bohmer 2001; Vijaylaxmi et al. 2010; Tiwari 2011). Due to rise of population in all countries, fishes may play an important role in economic development of countries (Sikoki and Otobotekere 1999; Gharaei et al. 2010; 2011).

In India it is estimated that about 2500 fish species are found within which around 930 species are freshwater. The freshwater fishes are distributed amongst approximately 20 orders, 100 families and 300 genera (Daniels 2000; Kar 2003; Ayappan and Birdar 2004). Fishes are the main component of lake and wetlands ecosystems. They play an important role in energy flows, nutrient cycling and maintaining community balances in these ecosystems. The physical, chemical and biological characteristics of a wetland are major determinants of the type, number and size of fish available (Baker et al. 1993; Abbasi 1997).

Fish biodiversity was studied in Punjab and described as many as 116 fish species (Johal and Tandon 1979; 1980). Dua and Chander (1999) have identified 61 species from Harike wetland. Brraich et al. (2003) identified 3 new fish species from Harike wetland viz., *Nandus nandus*, *Lepidocephalichthys guntea* and *Monopterus chuchia*. Further, Brraich and Ladhar (2005) identified one more species viz., *Badis badis*. Furthermore, they also reported 69 fish species from Harike, 55 from Ropar and 16 from Kanjli wetland.

There are various environmental factors which affect fish communities in freshwaters. The most important are streamflow, water quality, food sources, physical habitat and biotic interactions that affect stream fish and aquatic communities. At the most general level of resolution, channel units are divided into fast and slow-water categories that approximately correspond to the commonly used terms "riffle" and "pool". Within the fast – water category, two subcategories of habitats are identified, those that are highly turbulent (falls, cascade, chutes, rapids and riffles) and those with low turbulence (sheets and runs). Different habitat harbour different verities of fish species (Hawkins et al. 1993).

Ranjit Sagar Wetland and its adjoining streams are one of the great potential (Johal et al. 2002) fishery resources in India. The research on fish species of this wetland has not been conducted earlier. The detailed study on fish diversity observed from this wetland will extend great help to start conservation and management practices in future.

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Materials and Methods

The Ranjit Sagar wetland, also known as Thein Dam, is a fresh water ecosystem situated on the river Ravi (tributary of the Indus river system) near Pathankot city, Punjab, India. This wetland falls into three states i.e. Punjab, Himachal Pradesh and Jammu and Kashmir and spread over an area of 87.60 sq km and catchment area consist of 6086 sq km. There are three major streams (Karnal, Basoli and Behni) feeding Ranjit Sagar Wetland (Fig. 1, Table 1). Fish samples were collected on monthly basis from each stream by selecting three fish collection sites with difference of 1 km each. The fish sample were collected in triplicate with the help of local fishermen by using standard fishing gears like cast, gill and hand nets. The sampling was made from different habitats such as riffles, cascades, ripples and runs (Rosgen 1996). After collection, fish specimen were examined, counted and released into the water. The unidentified specimen were preserved in 5% formalin and brought to the laboratory for further analysis and identification. Fish specimens were identified on the basis of morphological characters and with the help of standard keys and taxonomy text books (Johal and Tandon 1979; Day 1888; Jayaram 2010). Rosgen (1996) classified the hillstreams into three major types Type A, B and F. Type-A streams is high altitude streams with high gradient. These streams have stable bed morphology with boulders or bedrock dominated channels. Type-B streams are with gentle gradient, cobble gravel dominated substrate with variable habitat types. Type-F streams are similar to Type-B except that they are more entrenched in the highly weathered materials.

Table 1 List of study sites of Ranjit Sagar Wetland.

Sr. No.	Site	Location	Altitude (m)	Latitude	Longitude	Substrate	Habitat
1.	Dam (Reservoir)	Punjab	1697	75°45′45″E	32°26′37″N	Rocky	Deep pools and shallow pools
2.	Behni (Stream)	Jammu and Kashmir	1778	75°39′10.88″E	32°31′39.79″N	mostly bedrock, boulders, bravel, cobble and leaf litter	Pools, riffles, cascade, run
3.	Basoli (Stream)	Himachal Pradesh	1768	75°50′46.30″E	32°30′35.95″N	Boulders, gravel, cobble and sand	Deep pools, pools, riffles and cascade
4.	Karnal (stream)	Punjab	2118	75°53′01.53″E	32°27′01.27″N	Mix with big boulders, gravel, cobble and leaf litter	Pools, riffles, cascade and run

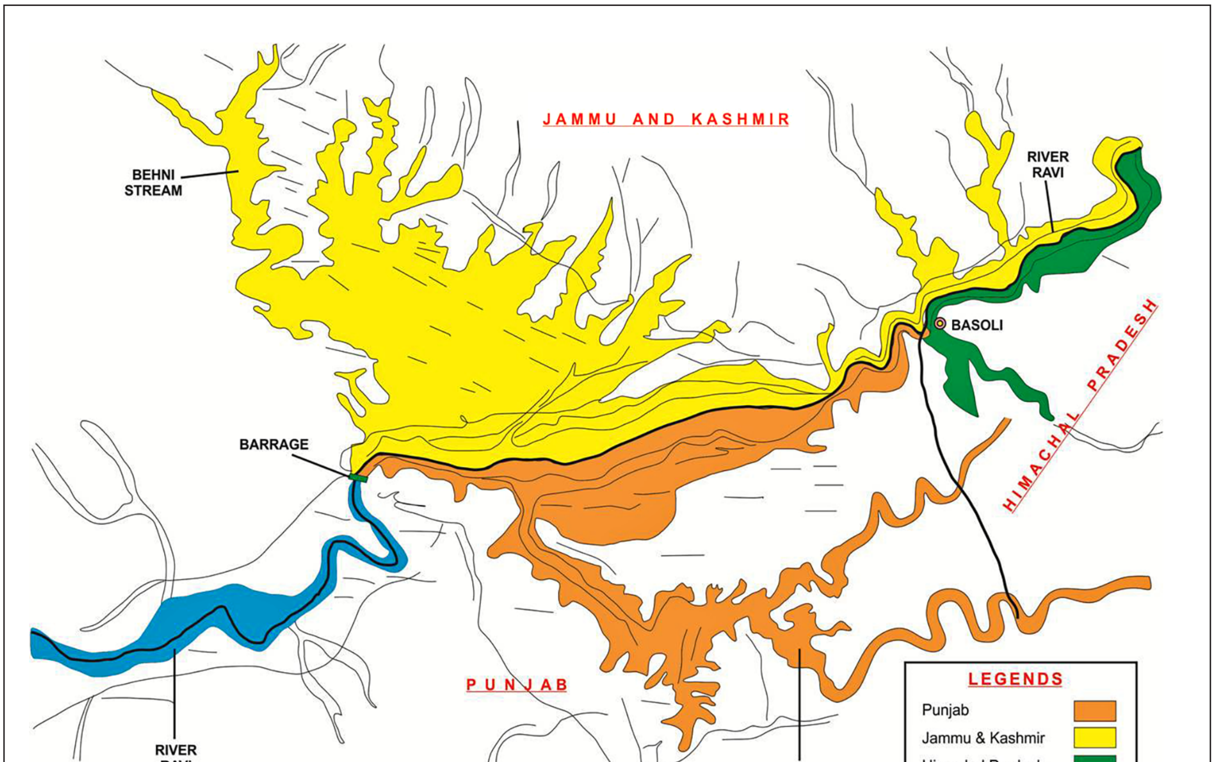

Fig. 1 Map of Ranjit Sagar Wetland.

Results

A total of 43 fish species have been reported from the Ranjit Sagar Wetland and its adjoining streams belonging to 6 orders, 11 families and 27 genera (Table 2). Cyprinidae forms the dominant group among the families with 64% followed by Channidae with 9%, Cobitidae with 5%, Siluridae with 5%, Balitoridae with 5%, and other fami-

Table 2 Classification of the fish species reported from the Ranjit Sagar Wetland and adjoining streams.

S. No.	Name of Fish	English Name	Habitat Preference
I	**Order – Cypriniformes**		
i	Family – Cyprinidae		
	Bangana dero	Kalabans	Inhabit hill-streams in shallow waters. Adults migrate to warmer regions of lakes and streams during winter.
	Barilius bendelisis	Hamilton's Barila	Base of hills in the lotic habitat strewned with pebbles and sandy bottom.
	Barilius shacra	Shacra Baril	Found in streams and rivers.
	Barilius vagra	Vagra Barila	Found in hill streams with gravelly and rocky bottom.
	Cabdio morar	Aspidoparia	Found in streams, rivers and ponds in plains and mountainous regions
	Cirrhinus reba	Reba Carp	Found in large streams, rivers, tanks, lakes and reservoirs
	Devario devario	Devario danio	Fast flowing clear headwater streams.
	Crossocheilus latius latius	Gangetic Latia	Inhabits streams, rivers and lake preferably with gravelly bottom in benthopelagic environment.
	Ctenopharyngodon idellus	Grass Carp	Inhabit large rivers, lakes, and reservoirs with abundant vegetation and relatively shallow waters.
	Cyprinus carpio	Common Carp	Warm, deep, slow-flowing and still waters, such as lowland rivers and large, well vegetated lakes.
	Garra gotyla gotyla	Sucker Head	This species is found in fast flowing streams with boulders and rocks along the Himalayan ranges.
	Hypophthalamichthys molitrix	Silver Carp	It migrates upstream to breed; egg and larva float downstream to floodplain zones.
	Hypophthalamichthys nobilis	Big Head	Inhabits rivers with marked water-level fluctuations, overwinters in middle and lower stretches.
	Labeo bata	Bata Labeo	Found in streams and rivers.
	Labeo dyocheilus	Brahmaputra Labeo	Inhabits clear active currents of large rivers
	Labeo gonius	Kuria Labeo	It inhabits rivers and streams.
	Labeo pangusia	Pangusia Labeo	It inhabits mountain streams, rivers, lakes and ponds
	Labeo rohita	Rohu	It inhabits rivers and streams.
	Pethia conchonius	Rosy Barb, Red barb	Generally inhabits lakes and streams.
	Pethia phutunio	Spotted Tail Barb, Pygmy Barb, Dwarf Barb	Inhibits clear streams and rivers, also muddy waters
	Pethia ticto	Ticto Barb, Firefin Barb, Two-Spot Barb	Inhabits mostly mountain and sub-mountain regions, and flood plains.
	Puntius chola	Swamp Barb, Chola Barb	It inhabits rivers, streams and tanks in the plains
	Rasbora daniconius	Slender Rasbora, Bliack line Rasbora	It occurs in a variety of habitats: ditches, ponds, canals, streams, rivers and inundated fields, but is primarily found in sandy streams and rivers.
	Salmophasia bacaila	Large Razorbelly Minnow	Usually found in slow running streams, but also occurring in rivers, ponds and inundated fields in sub-mountain regions
	Systomus sarana sarana	Olive Barb	It can live in sandy bed mixed with mud and in fairly swift current.
	Tor chylenoides	Dark Mahseer	Inhabits fast-flowing mountain streams
	Tor putitora	Golden Mahaseer, Putitor Mahseer	It inhabits rapid streams with rocky bottom, riverine pools and lakes.
	Tor tor	Tor Mahseer	It grows better in rivers with a rocky bottom.
ii	**Family – Balitoridae**		
	Acanthocobitis botia		Inhabits swift flowing streams in hilly areas with clear water and rocky, pebbly and sandy bottoms.

iii	**Family – Cobitidae**		
	Botia birdi	Botia Loach	Occurs in clear mountain streams.
	Botia lohachata	Y-Loach, Reticulate Loach	Occurs in clear mountain streams.
II	**Order – Siluriformes**		
i	**Family – Clariidae**		
	Heteropneustes fossilis	Stinging Catfish	Inhabits freshwater, rarely brackish waters. This is primarily a fish of ponds, ditches, bheels, swamps and marshes, but it is sometimes found in muddy rivers.
ii	Family – Siluridae		
	Ompok bimaculatus	Indian Butter Catfish	Inhabits plains and sub-mountain regions, and is found in rivers, lakes, tanks and ponds.
	Ompok pabda	Pabdah Catfish	The species inhabits lotic habitats such as rivers and larger streams.
iii	**Family – Sisoridae**		
	Glyptothorax punjabensis		The species inhabits benthopelagic zones of lotic habitats such as rivers and larger streams.
III	**Order – Synbranchiiformes**		
i	**Family – Synbranchidae**		
	Mastacembelus armatus	Tire-Track Spinyeel	Inhabits fresh waters in plains and hills.
IV	**Order- Beloniformes**		
i	**Family – Belonidae**		
	Xenentodon cancila	Freshwater Garfish	Inhabits freshwaters, primarily rivers. It occurs in clear, gravelly, perenni-al streams and ponds.
V	**Order – Perciformes**		
i	**Family – Channidae**		
	Channa marulius	Giant Snakehead	Inhabits large lakes and rivers; prefers deep, clear stretches of water with sandy or rocky bottoms.
	Channa orientalis	Asiatic Snakehead	Species occurring in rivers, lakes, ponds, mountain streams and even brackish water. Found in quiet, shaded, clear, flowing water with silt or gravel substrate.
	Channa punctatus	Spotted Snakehead	Inhabits freshwater streams, rivers, ponds and tanks, generally in the plains. Also found in rice fields and irrigation channels.
	Channa striata	Striped or Banded Snake-head	Inhabits swamps, freshwater ponds, streams and tanks in the plains; prefers stagnant muddy waters and grassy tanks.
ii	**Family – Nandidae**		
	Nandus nandus	Mottled Nandus	It inhabits fresh waters. Found in rivers and in agricultural lands.
VI	Order – Osteoglossiformes		
i	**Family – Notopteridae**		
	Notopterus notopterus	Grey Featherback	It inhabits fresh and brackish waters, and appears to thrive well in lentic waters.

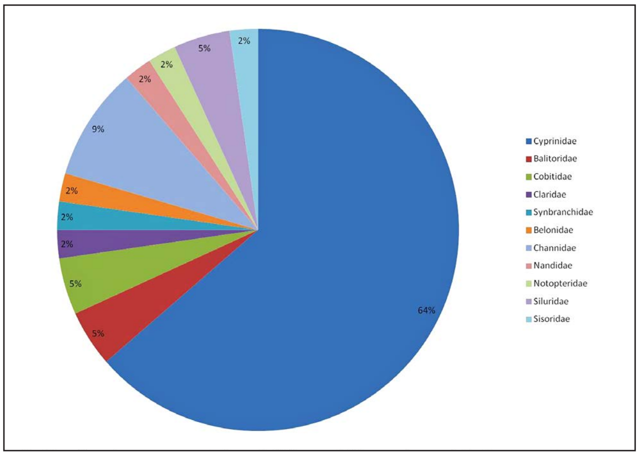

Fig. 2 Family-wise representation of different fish species and their percentage.

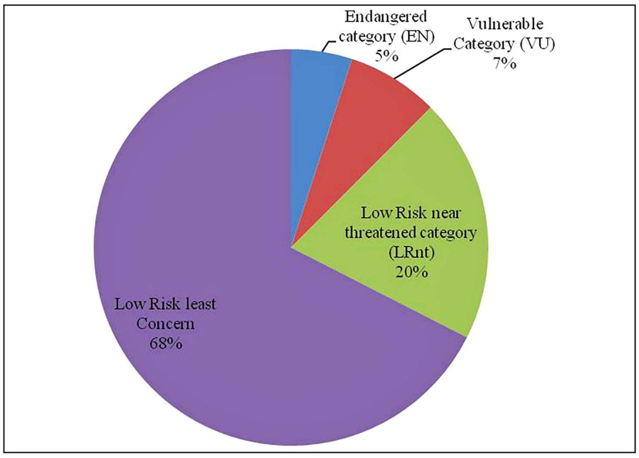

Fig. 3 Percentage-wise IUCN Red List status.

Table 3 Status of fishes from Ranjit Sagar Wetland and adjoining streams based on Red Data list of IUCN (2017) and criteria, threats & research recommendations according to CAMP Report (Molur and Walker 1998).

S. No.	Name of Fish	IUCN Status	Criteria	Sites	Threats	Research Recommended
1.	***Acanthocobitis botia***	**LRlc**	–	Ba, Be, K	Fd, E, F, I, L, Po, Ov, Sn, T(L)	Lh, Hm, M, PP
2.	***Bangana dero***	**LRlc**	(A1acd)	Ba, Be, D, K	Dm, Dr, Fd, F, L, H, I, Lp, Ov, Sn, T (L,D)	S, M, Hm, Lm
3.	***Barilius bendelisis***	**LRlc**	–	Ba, Be, D, K	Fd, F, I, L, Ov, Po, Sn, T (L,C)	M, Lh, Hm, P
4.	***Barilius shacra***	**LRnt**	–	Ba, Be, D, K	I, L, Pu, Sn, T(L)	M, O, P
5.	***Barilius vagra***	**LRlc**	(A1a, 1c)	Ba, Be, D, K	I, L, T(L)	S, M, Hm, P
6.	***Botia birdi***	**LRnt**	–	Be	I, L, Pu, Sn, F, T(L)	Hm, Lh, O, P
7.	***Botia lohachata***	**EN**	(B1, 2c)	Be	Fd, E, I, L, Ov, Po, Sn, T(L)	Lh, Hm, M, P
8.	***Cabdio morar***	**LRlc**	–	Be	I, L, Ov, Pu, T(L,C)	S, M, Lh, T, G
9.	***Channa marulius***	**LRlc**	–	Ba, Be, K	F, L, Ov, T (D, C)	M, H
10.	***Channa orientalis***	**VU**	(A1acd)	Be, K	F, L, T(D)	Hm, S, M, PP
11.	***Channa punctatus***	**LRlc**	–	Ba, Be, D	F, L, Ov, T(D)	H, Hm
12.	***Channa striata***	**LRlc**		D	F, T, (C)	H
13.	***Cirrhinus reba***	**LRlc**	(A1abcd, 2cd)	Ba, Be, D, K	Dm, F, I, L, Ov, Pu, Sn, T(D,C)	S, M
14.	***Crossocheilus latius latius***	**LRlc**	–	Ba, Be, K	L, Fd	Lh
15.	***Ctenopharyngodon idellus***	**DD**	–	Ba, D, K	–	–
16.	***Cyprinus carpio***	**VU**	(A2ce)	Ba, D, K	–	–
17.	***Devario devario***	**LRnt**	–	Be, K	I, Ov, Po, Pu, T(L, D)	S, M, Lr, Hm, Lh, P
18.	***Garra gotyla gotyla***	**LRlc**	(A1acd)	Ba, Be, K	Fd, E, I, L, Ov, Ps, Po, Sn, T(L)	M, Lh, Hm, P
19.	***Glyptothorax punjabensis***	**DD**	–	Be, K	–	–
20.	***Heteropneustes fossilis***	**LRlc**	(A1acd)	Ba, K	F, I, L, T(L,D,C)	S, M, H, Hm, P
21.	***Hypophthalamichthys molitrix***	**LRnt**	–	Ba, D,	–	–
22.	***Hypophthalamichthys nobilis***	**DD**	–	Ba, D	–	–
23.	***Labeo bata***	**LRlc**	–	Be, D, K	L, Ov, Sn, T (C)	M
24.	***Labeo dyocheilus***	**LRlc**	(A1acd)	Ba, D	Dm, Dr, Fd, F, I, H, L, Ov, T(L,D)	S, M, Hm, Lm, PP
25.	***Labeo gonius***	**LRlc**	–	Be, K	I, H, Ov, Pu, T (C)	G, M, S
26.	***Labeo pangusia***	**LRnt**	–	Be,K	I, H, Ov, Pu, T (D)	S, M, Lh, Hm
27.	***Labeo rohita***	**LRlc**	–	Ba, Be, K	L, Ov, Sn, T(C)	G
28.	***Mastacembelus armatus***	**LRlc**	–	Ba, Be, D, K	–	–
29.	***Nandus nandus***	**LRlc**	–	Ba, Be, K	I, L, Po, Pu, Sn, T(L)	S, M, G, P
30.	***Notopterus notopterus***	**LRlc**	–	Ba, D, K	I, Ov, Pu, T(C)	M
31.	***Ompok bimaculatus***	**LRnt**	(A1acd, 2cd)	Ba, D, K	D, Fd, F, I, Po, Ps, Pu, Sn, T(L, D, C)	S, M, G, Hm, Lm, P
32.	***Ompok pabda***	**LRnt**	(A1acd, 2cd)	Ba, D, K	F, I, Pu, T(L, D, C)	S, M, G, Hm, Lm, Lr, P
33.	***Pethia conchonius***	**LRlc**	B1, 2c	Ba, Be, D, K	E, L, Po, Sn, T(L)	Lh, Hm, M
34.	***Pethia phutunio***	**LRlc**	–	Ba, Be, K	T(C)	S, M
35	***Pethia ticto***	**LRlc**	–	Ba, Be, D	F, L, T (L)	Hm
36.	***Puntius chola***	**LRlc**	A1a, 1c, 1d	Be, D, K	I, Pu, T(L)	S, M
37.	***Rasbora daniconius***	**LRlc**	–	Be, K	F, Pu, T (L, D)	S, M, G, Hm
38.	***Salmophasia bacaila***	**LRlc**	–	K	T(L)	Lh, M, S
39.	***Systomus sarana sarana***	**LRlc**	A1acd	Ba, Be, D, K	F, I, L, T (L, D)	S, M, Lr, P
40.	***Tor chylenoides***	**VU**	–	Ba, Be, D, K	–	–
41.	***Tor putitora***	**EN**	A1acd	Ba, Be, D, K	Dm, Dr, Fd, F, I, H, L, Ov, Pi, Sn, T (L, D)	S, M, Hm, Lm, P
42.	***Tor tor***	**LRnt**	A1a, 1c, 1d	Be, D	Dm, Fd, F, I, L, Po, Pu, T(L,D,C)	S, M, G, Hm, Lm, Lr, P
43.	***Xenentodon cancila***	**LRlc**	–	Ba, Be, D, K	F, Pu, T(D)	S, M, Lr, P

Abbreviations

Site: Be – Behni, Ba – Basoli, K – Karnal, D – Dam.

IUCN: EN – Endangered, VU – Vulnerable, LRnt – Lower Risk-near threatened, LRlc – Lower Risk-near least concern.

Threats: I – Human interference, L – Loss of habitat, Lf – Loss of habitat due to fragmentation, Lp – Loss of habitat due to exotic plants, D – Diseases, Dm – Damming, E – Edaphic factors, F – Fishing , Fd – Destructive fishing, H – Harvest, Hf – Harvest for food, P – Predation, Po – Poisoning, Ps – Pesticides, Pu – Pollution, Pi – Powerlines , Sn – Siltation, T – Trade , Ov – Overexploitation, Dr – Drowning.

Research Recommendations: S – Survey search and find , M – Monitoring, H – Husbandry research, Hm – Habitat management, Lr – Limiting factor research, Lm – Limiting factor management, Lh – Life history studies, T – Taxonomic and Morphological genetics studies, G – Genetic Managements, P – Population and habitat viability assessment, PP – PHVA (Pending Further Work), O – Other (Specific to the Species).

lies namely Synbranchidae, Belonidae, Claridae, Nandinae and Notopteridae forming 2% each (Fig. 2). Out of all 43 fish species, 13 come under threatened categories of Red List of IUCN (Table 3), out of which 2, 3 and 8 come under Endangered (EN), Vulnerable (VU) and Low Risk near threatened category (LRnt) respectively (Fig. 3). This wetland also supports four exotic fish species i.e. *Cyprinus carpio, Ctenopharyngodon idellus, Hypophthalmichthys molitrix* and *H. nobilis*. During the study period different type of streams habitats have been studied. All three streams Behni, Basoli and Karnal were dominated by different type of habitats like deep pools, pools, riffles, runs and cascade. Streams banks were stable and covered by riparian vegetation. Different types of stream substrates have also been studied during the course of work in which bedrock type of substrate was predominant, other type of substrates viz. big boulders, gravel, cobble, sand and leaf litter was also present. These types of streams were called Type-B streams which are very productive in nature and support large variety of fish diversity.

Discussion

During the course of study, 25 fish species have been reported from Dam site, 29 from Basoli and 33 each from Behni and Karnal site. A total of 43 fish species have been reported from the Ranjit Sagar Wetland and its adjoining streams were classified under different order and families. Goswami and Goswami (2006) have identified 54 fish species belonging to 36 genera under 22 families in Jamalai wetland in Assam. Sharma et al. (2007) reported 29 species of fishes belonging to six orders from Krishnapura lake, Indore and stated that Cypriniformes was dominant with 15 species followed by Siluriformes with 6 species. Jagatheeswari et al. (2016) also studied the diversity of fish population and their conservation aspects in Kondakarla fresh water lake ecosystem, Visakhapatnam, Andhra Pradesh, India and reported 26 species of fishes.

Occurrence of variety of fish species depends upon the availability of different habitats (Arunachalam 2000). Hence, fishes have also been classified on the basis of their habitat preferences. The dominant fish habitats in the streams were cascade, rapids, riffles and run. Similarly, dam has deep pools in the middle and shallow pools near the banks. Rosenzweib (1995) revealed that number of species increase with an increase in habitat area which supports the phenomenon of species habitat area relationship. Arunachalam (2000) studied the macro and microhabitat of 10 streams of Western Ghats and provide information about the habitat requirement of fish species in different stream/rivers. They stated that habitat diversity is directly related to fish diversity.

Kar and Sen (2007) studied the distribution of fishes on the basis of habitat preference. During the study we study that, the edges of the run habitats have been found to be inhabited mainly by *Puntius chola, Pethia conchonius* and *Barilius shacra* in Behni and Karnal stream while the cascade habitats are colonized by *Labeo pangusia* and *Garra gotyla gotyla* in Behni stream. Dammed pools, backwater pools and deep pool edges with bedrock substratum are the highly preferred habitats for *Botia birdi, Botia lohachata, Tor tor* and *Cirrhinus reba* as found in Basoli stream and main reservoir. *Barilius bendelisis* and *Acanthocobitis botia* are abundant in the riffle-type of habitats in the Basoli, Behni and Karnal stream where the substrata have been found to be mainly dominated by small boulders and cobbles. Among the cyprinids, *Tor tor* and *Tor piutitora* are confined to large deep pools in Basoli stream and main reservoir. Nevertheless, species like *Crossocheilus latius latius, Garra gotyla gotyla* and *Glyptothorax punjabensis* have been recorded from the cascade to riffle regions in the upper gradient zones of rheophilic streams.

In India, the introduction of exotic fish species into Dal lake and Loktak lake has been reported to affect the population of indigenous fish species. The population of native catla and mahseer were depleted considerably in Gobind Sagar reservoir after the introduction of Silver Carp. The freshwater aquatic biodiversity is depleting alarmingly due to introduction of exotic species and other anthropogenic activities (Menon 1979; Molur and Walker 1998; Kumar 2000). The indiscriminate transfers of exotic fishes have brought about a wide array of problems including extirpation of indigenous species. During the present course of work four exotic carps i.e. *Cyprinus carpio*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis* have been reported from the Ranjit Sagar Wetland. It is important to note that these exotic carps are voracious feeders as well as breeders and can pose serious threats to native fauna in future if proper management and research activities will not be initiated about their control (Kumar 2000).

Though this wetland and its adjoining streams were highly productive, but some anthropogenic activities like rampant removal of big and small boulders from the stream bed, mining of sand and gravel by builders or constructor and siltation posing a big threat to the various fish species. Besides, these serious threats, discharge of sewage water and poaching of fishes have also been consider as potential threats to the fish diversity which need to be curbed for their sustainability.

Conclusion

During the study period 43 species have been reported from the Ranjit Sagar Wetland due to availability of variety of habitats. Out of all 43 fish species 13 fish species come under threatened categories of Red List of IUCN. There is a dire need to start conservation and management practices for their sustainability in future. If appropriate steps towards their conservation will not be taken of now, then the day is not far away when these fish species will slip towards extinction. This wetland also supports four exotic carps and these are voracious feeders as well as breeders and can pose serious threats to native fauna in future. Proper management and research activities should be initiated for the conservation of valuable fish fauna of this wetland.

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