



A review of wildlife ecotourism in Manaus, Brazil

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Abstract

The Amazon's ability to draw tourists is thought to be strongly associated with the opportunity to have sight of and interact with iconic wild animals. Tourism leaders are calling for the private and public sectors to develop wildlife focused ecotourism in this region. However, specific information regarding current practice and their impact on wildlife is lacking. Although wildlife ecotourism here remains in its relative infancy, our study demonstrates that a wide variety of wildlife-focused activities are already being promoted and provided to tourists who visit the city of Manaus in Brazil. Issues of potential wildlife conservation and animal welfare concern include wildlife-baiting, swim-with free-ranging pink river dolphin activity, the use of captive wild animals as photo props and the sale of wildlife body parts as souvenirs. We found that tour guides actively promoted these activities on 77% of excursions attended, which involved a range of different wild animals, representing at least 10 different species from three different taxonomic classes. From a legal perspective, despite the potential risks imposed to wildlife and tourist well-being, there are still no specific laws regulating feeding, touching and swimming with pink river dolphins in Brazil. However, the illegality of advertising and providing direct physical contact wildlife 'photo prop' tourism is demonstrated by enforcement action taken by wildlife authorities during our study. We suggest that tourist focused human behavior change initiatives should become a critical component of a wider holistic approach to effectively balance wildlife protection goals and any expansion of wildlife ecotourism in the Amazon.

Keywords

Animal welfare, Bradypus variegatus, Conservation, Inia geoffrensis, Sustainability

Introduction

The Amazon's reputation and ability to draw tourists is thought to be strongly associated with the natural environment and with tourist's ability to have sight of and interact with iconic wild animals (Alves et al. 2011, Tortato and Izzo 2017). Although tourism is only estimated to contribute about one per cent to the Brazilian Amazon region's overall GDP (Filho 2006), given the predicted continued growth of wildlife based tourism globally (UNTWO et al. 2014), studies have drawn attention to the potential of developing forest based ecotourism products. These products are centered not only on the regions unique fauna, but also its natural landscapes, indigenous culture and heritage, particularly in older cities such as Manaus and Belém (e.g. Lohmann and Dredge 2012). These cities are surrounded by the rainforest and act as 'gateways' to wildlife based tourism activities such as river tours and jungle lodges (Unibanco Guides Amazon 2009, Lohmann and Dredge 2012).

In some cases, wildlife-focused ecotourism can be profitable for rural people living in or near wilderness areas because they possess first-hand knowledge of local land-scapes and native flora and fauna (Hoefle 2016). For example, working as guides or by providing transportation and accommodation, they can receive payment for environmental services in lieu of previous unsustainable hunting practices (Hoefle 2016). This in turn complements modern wildlife conservation and animal welfare policy which has generally shifted from removing long-resident people from new designated protected areas, and/or penalizing those who practice unsustainable activities in buffer zones, to that of creating sustainable alternative community-based activities with the aim of both promoting forest preservation and alleviating rural poverty (Hoefle 2016).

However, although wildlife ecotourism can and does have net positive impacts on wildlife (Brockington and Duffy 2010), recent research has highlighted that wildlife focused ecotourism can also have net negative impacts on both the conservation and the welfare of wild animals (Moorhouse et al. 2015, 2016). For example, regular close proximity of tourists with free-ranging wildlife can have an adverse impact on an array of animal behaviors such as breeding (Jacobson and Lopez 1994) and foraging (Meissner et al. 2015). Direct physical contact with wild caught wildlife can also lead to the unintentional death of individuals belonging to threatened species. For example, in 2016 media attention focused on a La Plata river dolphin (*Pontoporia blainvillei*) that died after beachgoers in Argentina hauled the dolphin out of the water to pose with the dolphin for photos (National Geographic 2016).

When wildlife focused ecotourism operators decide to place specific emphasis on achieving net positive outcomes for individual wild animals and remaining wild populations, some trade-offs in the values of conservation, animal welfare, visitor satisfaction and profitability may occur (Fernandez et al. 2009). However, the outcomes of these trade-offs, particularly the negative impacts on wild animal conservation and welfare, are difficult to detect, especially by tourists themselves (Moorhouse et al. 2015). Different attitudes and societal expectations can often complicate such decision-making even further (Moorhouse et al. 2016). As can the myriad of different legal situations

involved, which can include absent, ambiguous, inaccessible and or conflicting legislation (TRAFFIC 2008).

Tourism leaders have called for the private and public sectors to develop domestic and international wildlife ecotourism in the Amazon region, yet specific information regarding current practice and impact is lacking (Lohmann and Dredge 2012). To help address this situation, herein we provide a case study review using the Amazon 'gateway' city of Manaus as a geographic area of focus. Specifically, we asked: (1) what types of wildlife ecotourism activities are currently being provided; (2) how prevalent are they; (3) what taxonomic groups are most commonly involved; (4) what is their legal status; and (5) what potential impact are they having on wild animals. We hope the information gathered will help to guide existing efforts to develop wildlife ecotourism in a manner that safeguards animal welfare and conserves wild animal populations.

Methods

Study site

Amazonas is the largest state of Brazil, with a total area of 1.6 million km² (Divino and McAleer 2009). Around 77% of the Amazonas state forest remains intact and includes scenic natural landscapes, that not only serves as habitat for wild fauna but also as an enticing tourist attraction (Divino and McAleer 2009). The capital of Amazonas state is Manaus [estimated population, 2,094,391 (IBGE 2016)], which is at the confluence of the two main tributaries of the Amazon River, where the black water of the Rio Negro and the yellowish-brown water of the Rio Solimoes join to form the Amazon River (Divino and McAleer 2009). Located close to intact Amazonas state forest, Manaus has an airport that can receive full-size jet aircrafts and is connected to wildlife tourist destinations by paved highways and larger riverboats (Divino and McAleer 2009).

Operating model

Tourists typically arrive by air in Manaus, are taxied to the port, and taken by boat to floating hotels or hostels located on the river edge (Figure 1; Hoefle 2016). Wildlife ecotourism excursions can be booked before and after arrival in Manaus, through tour agents that operate indirectly online and directly via local branches (Figure 1). Tour guides and boat operators are employed to accompany tourists on excursions with the size of transport crafts ranging from covered outboard-powered boats up to small cruise ships (Figure 1; Hoefle 2016). Luxury jungle lodges are also located along the Negro River in Iranduba and Novo Airão municipalities, upstream from Manaus (Hoefle 2016). This route has been described as one of the most scenic of the Amazon tributaries with forests that are largely intact and black water that produces 'beautiful reflections' (Hoefle 2016, Lohmann and Dredge 2012).

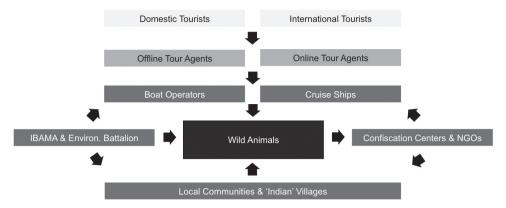


Figure 1. Stakeholder map / operating model.

Fieldwork

We used online search engines to identify boat tour companies operating from Manaus that specifically advertised wildlife ecotourism. We conducted fieldwork in Manaus between October 4th and November 18th 2016. We gave each wildlife boat tour a unique identification code noting: the date of the tour; the name of the tour company; the price of the tour [(in Brazilian Reals (BRL)]; the number of other tourists present; and documented the types of wildlife ecotourism activities provided. Whenever opportunities for close and or direct physical contact with wildlife were provided, we recorded the geographic location (via GPS Garmin model GPS-MAP64), species; estimated age class (juvenile or adult); and the number of animals involved. We also recorded whether these types of activities were actively encouraged or discouraged by the official tour guide, taking photographic images and other qualitative observations.

Legislative review

We identified relevant legislation to fully understand the legal status of any close and or direct contact opportunities observed during our fieldwork via online search engines and consultation with relevant government agencies. This included legislation relating, but not limited, to wildlife management, conservation, national red lists of endangered species, animal welfare, tourism, environmental crime, and jurisprudence. As part of our subsequent review, we included national legislation such as constitutions, laws, decrees, resolutions, and regulations, normative instructions in addition to any international treaties and relevant case studies of legal precedence. Using these sources, following our fieldwork we specifically evaluated three types of activity: (1) baiting of wild animals for tourists (i.e. provision of food to attract); (2) capture (and subsequent captivity) of live animals for tourists; and (3) handling by tourists.

Tour ID	Tour start	Duration	Price (\$BRL	No. of	Contact provided	Contact encouraged
number	date	(days)	per day)	tourists	(yes or no)	(yes or no)
1	04.10.16	1	200	22	Yes	Yes
2	06.10.16	1	200	50	Yes	Yes
3	08.10.16	1	150	29	Yes	Yes
4	09.10.16	1	200	49	Yes	Yes
5	11.10.16	1	200	61	Yes	Yes
6	13.10.16	1	350	6	Yes	Yes
7	15.10.16	1	200	24	Yes	No
8	16.10.16	1	150	50	Yes	Yes
9	18.10.16	1	220	38	Yes	No
10	20.10.16	1	180	44	Yes	Yes
11	22.10.16	1	200	52	Yes	Yes
12	23.10.16	1	260	13	Yes	No
13	24.10.16	1	150	24	No	N/A
14	27.10.16	1	200	54	Yes	Yes
15	29.10.16	1	180	14	Yes	Yes
16	03.11.16	3	250	5	Yes	Yes
17	06.11.16	1	350	4	Yes	Yes
Total	_	19	_	515	_	_

Table 1. Information about tours attended during our 2016 fieldwork in Manaus.

Results

Fieldwork involved active participation on 17 different wildlife boat excursions provided by 17 different tour agencies. Excursion duration ranged between 1 to 3 days, prices ranged from 150 to 350 BRL (approximately 48 to 112 \$ USD per day), and tourist attendance ranged between six and 61 individuals (Table 1). The opportunity for direct contact with live wild animals (i.e. to touch baited free-ranging or handle captive individuals) was provided to tourists on 94% (n = 16) of excursions at six different locations (Table 1, Figure 2). Official tour guides were observed actively encouraging these types of tourist activity during 77% of excursions. The opportunity to touch free ranging baited pink river dolphins [Inia geoffrensis (2–10 individual animals; on 82% tours)] was most commonly provided, followed by the opportunity to handle captive brown-throated three-toed sloths [Bradypus variegatus (2–4 animals; on 71% tours)], to handle captive common caiman [Caiman crocodilus crocodilus (1-2 animals; on 65% tours)] and to touch free-ranging baited squirrel monkeys [Saimiri sciueus sciueus (> 50 animals; on 6% tours)] (Table 1, Figure 3).

Wildlife baiting

We observed four aggregations of free-ranging pink river dolphins that have been conditioned to human contact through provisioning of fish (Figure 4). Tourists were able to feed, touch, and swim with these animals with access provided via four floating

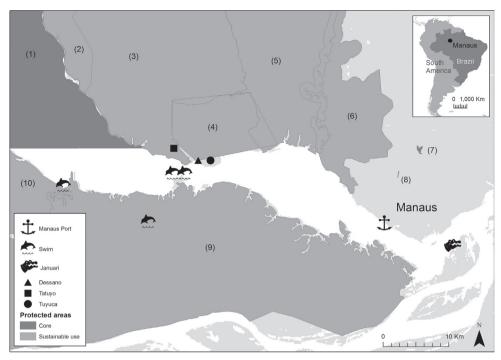


Figure 2. Map of key boat tour locations. Protected area names are numbered as followed I PN (Parque Nacional) de Anavilhanas 2 APA (Área de Proteção Ambiental) Margem Esquerdo Rio Negro Setor Aturiá_Apuauzinho 3 RDS (Reserva de Desenvolvimento Sustentável) Puranga Conquista 4 RDS Tupé 5 APA Margem Esquerdo Rio Negro Setor Tarumá Açu-Tarumá Mirima 6 APA Taruma/Ponta Negra 7 PE (Parque Estadual) Sumaúma 8 APA Parque Linear do Bindá 9 APA Margem Direito Rio Negro Setor Paduari-Solimões I 0 RDS Rio Negro. Map was made in ArcMap 10.2.2 using publicly available data from the Ministry of Environment (Available at http://mapas.mma.gov.br/i3geo/datadownload.htm).

structures (Figure 2). During all dolphin interactions, we observed local staff baiting dolphins so that they could hold them in position out of the water for tourist photo opportunities; (Figure 4). On two of these floating structures we observed tourists feeding adult pirarucu (*Arapaima gigas*) with fish tied to the end of bamboo poles. We also observed one troop of squirrel monkeys that had been conditioned to human contact through provisioning of food so that tourists could feed, touch and have photo opportunities with these animals.

Wildlife photo props

We observed captive live animals being made available for handling by tourists, particularly for use as photo props, on and around three floating structures that serve as debarkation points for access to an unprotected area of forest known locally as 'Januari Ecological Park' (Figure 2). Here, we observed two adult captive green anacondas in

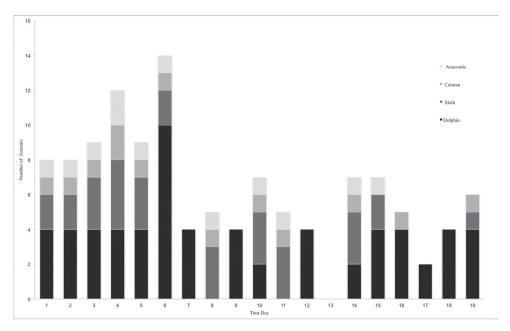


Figure 3. Number of animals provided for direct contact per tour.

general poor body condition showing specific signs of dehydration and bearing wounds indicating prior physical trauma. During all excursions, we observed handlers restraining the snakes by tightly gripping their neck whilst they were either touched or held by tourists (Figure 4). Both captive common caiman (n=2) observed during excursions were restrained using a rubber band around their jaws (Figure 4); with one animal kept in a small broken fridge when not being handled by tourists. Similarly, we observed a total of 9 individual captive brown-throated three-toed sloths (2 adult and 7 juveniles) two of which were tied to a tree with rope when not being handled by tourists.

Wildlife souvenirs

Although not a primary focus of our study, we also observed several different wildlife based products being sold as souvenirs to visiting tourists during these boat tours. Key points of sale include craft markets located at access points to Januari Ecological Park, on floating restaurants, and floating platforms used for pink river dolphin interactions (Figure 5). Three craft markets are operated by individuals belonging to Tatuyo, Tuyuca and Dessano tribes, typically visited by tourists before and after performances and photo opportunities involving individuals wearing traditional dress (Figure 2). We observed preserved fish [including catfish (e.g. *Pterygoplichthys anisitsi*) and piranha (*Serrasalmus* spp.) being commonly sold as ornaments at these craft stalls. On one occasion, we also observed a pink river dolphin, two common caiman skulls and an ocelot (*Leopardus pardalis*) skull on sale at a Tuyuca craft stall (Figure 5).



Figure 4. Example images of species provided to tourists for photo prop opportunities when visiting Manaus, Brazil. **A** Common caiman (*Caiman crocodilus crocodilus*) **B** Brown-throated three-toed sloth (*Bradypus variegatus*) **C** Green anaconda (*Eunectes murinus*); and **D** Pink river dolphin (*Inia geoffrensis*). Image by: Neil D'Cruze / World Animal Protection.

Relevant legislation

We found no specific laws regulating the baiting of free-ranging wildlife in Brazil. However, national legislation relating to environmental crimes [Federal Law 9,605/1998 (Appendix II)] states that it is illegal to pursue, capture, or kill any specimen of wild fauna in Brazil without due permit, license or authorisation from a competent authority (Suppl. material 1). Additionally, The Hunting Act [Federal Law 5,197 (Appendix II)] also strictly forbids any such activity 'involving wildlife living naturally outside of captivity' which are deemed property of the State (Suppl. material 1). Designated



Figure 5. Example images of wildlife products available for purchase by tourists when visiting Manaus, Brazil. **A** Pink river dolphin (*Inia geoffrensis*) and ocelot (*Leopardus pardalis*) skull **B** Floating Structure **C** Catfish (*Liposarcus anisitsi*); and **D** Common caiman skull (*Caiman crocodilus crocodilus*). Image by: Neil D'Cruze / World Animal Protection.

fines per specimen are 500 BRL (150 USD), however, this can reach up to 5,000 BRL (1,500 USD) per specimen if they are included on the Brazilian list of endangered species [Federal Decree 6,514 (Appendix II)] (Suppl. material 1). With regards to the species observed during our study, only the pink river dolphin is currently included on the Brazilian list of Threatened species; where the species is classified as 'Endangered' [Federal Law 6,938 (Appendix II)] (Suppl. material 1).

However, there are a few notable exceptions. For example, a Normative Ruling [(IBAMA 26/2002 (Appendix II)] establishes rules for the sustainable use (capture

and killing) of non-endangered indigenous Brazilian wildlife conventionally used by 'traditional populations' in a minority of 'Nature Conservation Units' specifically designated as 'Extractive Reserves' (a type of 'sustainable-use' protected area, Figure 2) (Suppl. material 1). The Hunting Act also enables the federal government to issue species-specific time-bound permits for such activity where 'regional peculiarities accommodate hunting activities' [Federal Law 5,197 (Appendix II)] (Suppl. material 1). Additionally, another normative ruling (IBAMA 07/2015) permits the private ownership and commercial use of certain species, provided that the wild animals have been bred in captivity and their owners can provide adequate proof of origin to the relevant authorities (Suppl. material 1). Another ruling [394/2007 (Appendix II)] aims to establish the criteria needed to determine the wild species that can be utilised in this manner (Suppl. material 1).

It is worth noting that none of the tour boat destinations visited during our study were in the 'Extractive Reserves' mentioned above. The destinations were surrounded by protected areas of stricter sustainable-use categories (areas numbered 2–10, Figure 2) and several were located within 10km of one 'core' or fully protected area, the most stringent class of 'Nature Conservation Units' (Proteção Integral, 1, Parque Nacional de Anavilhanas, Figure 2).

Discussion

Although wildlife ecotourism at key sites in the Amazon remains relatively underdeveloped (Lohmann and Dredge 2012), our study demonstrates that a wide variety of activities are already being promoted and provided to domestic and international tourists who visit via the city of Manaus. Specific activities involving direct physical contact with wild animals include wildlife baiting, swimming with free-ranging pink river dolphins, and the handling of captive wild animals as photo props that are available throughout the year irrespective of season. These activities involve a range of different wild animals, representing at least 10 different species from three different taxonomic classes (fish, mammals and reptiles). Although tourism leaders are increasingly calling for increased investment, with several studies citing wildlife eco-tourism in Manaus as an exemplary model (e.g. Lohmann and Dredge 2012, Hoefle 2016), to date none have provided a detailed review of the associated conservation, animal welfare and legal implications.

Conservation implications

The majority of species that we observed being used for wildlife ecotourism activities in Manaus are not currently considered to be of high conservation status, from either an international or a national perspective. None are currently considered as Threatened according to the IUCN Red List (IUCN 2017) and only the pink river dolphin is cur-

rently listed (as Endangered) on the Brazilian Red List of Threatened Species (Portaria No 444, 2014). However, it is important to note that internationally the pink river dolphin is currently considered as Data Deficient, the green anaconda has not yet been assessed, and it is acknowledged that existing common caiman assessment requires updating for the IUCN Red List (IUCN 2017). As such, at least from a local population perspective, the conservation impact of this emerging commercial activity on these species should not be completely disregarded as their use may already be or could become a potential threat to the survival of wild populations in future.

The unregulated killing of wild animals and subsequent sale of their body parts as tourist souvenirs (e.g. pink river dolphin skulls) is of potential concern in terms of its sustainability and negative conservation impact. However, photo prop tourism in Manaus also appears to involve the repeated long-term removal of individual animals from wild populations and associated mortalities. Taking the use of brown-throated three-toed sloths as a case in point; a total of six sloths were observed being used in this manner during our main fieldwork, however none of these same animals were observed just five months later during a brief reconnaissance in April 2017. Although it was not possible for us to specifically determine the fate of these animals, mortalities are a distinct possibility given the relatively low reported survival rate of this species at rescue and rehabilitation facilities (Moreno and Plese 2006) and the technical challenges associated with successful wild release (IUCN 2017).

Animal welfare implications

From an animal welfare perspective, the handling and use of captive wild animals as 'photo props' has become a particularly controversial tourism activity (Idfwru 2013). Prior research has already highlighted how this type of practice can severely compromise the physiological and psychological well-being of wild animals during capture, restraint and subsequent use (e.g. Baker et al. 2013). Our field observations also support existing concerns regarding how such repeated handling, combined with poor husbandry, exposure to continual flash photography, and unnatural surroundings could lead to stress, disease, injury and associated mortalities (Idfwru 2013). For example, given the observed frequency of use and key traits of their natural history [e.g. cryptic, arboreal behavior and relatively low metabolic rate (Fowler and Cubas 2001, Munaó Diniz and Oilveria 1999)] the use of brown-throated three-toed sloths for this type of tourism is perhaps of particular concern.

Although it involves free-ranging individuals, human baiting and associated direct contact (e.g. touching) with wildlife such as dolphins and primates has also become a controversial tourist activity (Orams 2002, Moorhouse et al. 2015). Despite the economic benefits, the baiting of pink river dolphins has already been identified as being of potential animal welfare concern (Alves et al. 2011). Escalation of pink river dolphin behavior from habituation to increased confidence, assertiveness, 'pushiness' and potential eventual aggression, towards conspecifics has been observed during previous

studies (Alves et al. 2011). Similarly, cases of potentially harmful human behaviour have also been observed (for example, attempting to restrain or ride the dolphins, striking the dolphins and feeding inappropriate objects) (Alves et al. 2011). Consequently, because of poor management, such activity could result in net negative impacts on the welfare of the animals involved (Alves et al. 2011).

Legal implications

Despite the potential risks imposed to dolphins, there are no specific laws regulating baiting, touching and swimming with wild pink river dolphins in Brazil (Alves et al. 2011). In fact, the official tourism website for Brazil actively promotes such wildlife ecotourism activity; citing that it generates income and encourages cetacean protection amongst fishermen in the region (Visit Brazil 2017). However, Federal decree number 6514 article 30 states that the intentional disturbance of any species of cetacean in Brazilian waters is forbidden with lawbreakers subjected to fines. Also, more broadly speaking, while no specific legislation prohibits wildlife baiting within Brazilian conservation units, this type of activity is prohibited by the internal regulations of some national parks [e.g., Serra dos Órgãos National Park (IBAMA 2010)]. In these cases, those who ignore these regulations may be subject to fines. When taking this legislation into account, the pink river dolphin tourism activities observed in Manaus could be interpreted as legal infringements (Alves et al. 2011).

In contrast, the illegality of advertising and providing captive wildlife for handling and associated 'photo prop' tourism in Manaus is demonstrated by 'Operação Teia', an enforcement action taken during the course of our study. In November 2016, following complaints against tour operators and evidence obtained from social media platforms, intelligence agents from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) and police from the Environmental Battalion, issued six tourism companies fines totalling \$R 1.3 million (\$\approx\$425,000 \$USD) using Federal Law 9,605 and Federal Decree 6,514. As part of this operation the authorities confiscated six captive wild animals from January Ecological Park, directly returning five of them to the wild [two green anaconda, two common caiman and one boa constrictor (*Boa constrictor*)]. A juvenile sloth was also forwarded to IBAMA's Centre for wild animals [(Centro de Triagem de Animais Silvestres (CETAS)] for rehabilitation (Acritica 2016, IBAMA 2017). But, despite this enforcement operation we observed photo-prop tourism, using these same species, at this same location five months later during a reconnaissance visit in April 2017.

Recommendations

Our fieldwork was limited to a select number of tours in Manaus, during two months of one dry season in 2016, and a brief reconnaissance in 2017. Therefore, this repre-

sents only a momentary glimpse into a complex and varied industry. It is reasonable to assume that additional wildlife-focused ecotourism activities, involving a wider range of wild animal species, at additional geographical locations, are currently being offered that went unobserved by us. Standards and practices will inevitably vary between tour operators, and our research methods restricted our ability to make a full and detailed assessment of the impacts on individual animals and species. However, our study provides an important initial insight into wildlife-focused ecotourism in Manaus that can serve as the foundation for further research. Based on our field observations and concerns already raised in the existing literature, we suggest that longer and more detailed animal welfare and conservation impact assessments of species-specific activities (such as pink river dolphin swims and brown-throated three-toed sloth photo-prop tourism) are required. Increased information on how wild animals are being sourced, kept and disposed would prove particularly useful.

Our study also highlights existing legislation relating to wildlife ecotourism can be ambiguous, inaccessible and/or conflicting (TRAFFIC 2008). For example, the legal status of wildlife ecotourism activities in Brazil can vary, depending on how a wild animal is sourced, how they are used, who is using them, and where (Table 1). Increased clarity regarding the legal status of baiting pink river dolphins with food for close interactions is particularly needed to help regulate and mitigate any negative impacts both on these Endangered cetaceans. Although there is more clarity regarding the illegal status of captive wildlife handling and 'photo-prop' tourism in Manaus, it is apparent from on-going activity, post Operação Teia, that enforcement action alone will not be enough to halt this illegal use of wildlife. Rather, a wider and more holistic approach that includes education and human behaviour change focused initiatives targeting both local communities, operators and in particular, tourists is required to prevent potential negative impacts from inevitable ecotourism expansion in the Amazon.

Increased research focused on the attitudes of tourists is required to inform the development of effective public awareness initiatives aimed at reducing demand for harmful wildlife ecotourism both in Manaus and elsewhere in the Amazon region. In the absence of global regulatory authorities, and given their wide global audience (e.g. TripAdvisor, 2016), the dissemination of relevant information via international online travel websites could also prove highly impactful in achieving positive human behaviour change in this regard (Moorhouse et al. 2016). On a national level, Brazil already has a National Tourism Plan [Plano Nacional do Turismo, 2013 (Federal Decree 7,994/2013]. However, we note that neither the current plan nor its associated guidelines (Ministério do Turismo, 2016) appear to contain any specific guidance regarding the proper regulation of wildlife ecotourism in Brazil. Given that the National Tourism Plan is revised every four years, we recommend that such information should be included in future plans to help balance and manage growing tourist interest in wildlife ecotourism and wider wildlife protection goals.

Conclusion

Wildlife tourism can and does have positive impacts on wildlife (Brockington and Duffy 2010), but can also have neutral and negative impacts (Higginbottom 2004, Moorhouse et al. 2015). In the absence of global regulatory authorities (Moorhouse et al. 2016), independent ground-level audits, using direct observations and interviews with staff focused on welfare and conservation aspects (e.g. Alves 2011, Arena et al. 2012, Schmidt-Burbach et al. 2015, Carder et al. 2016) like this study can provide important insights to help reduce the prevalence of ecotourism activities with negative impacts and increase those with positive impacts on wildlife. Arguably, this task is set to become more urgent and challenging in the future; globally wildlife tourism is growing because of increasing disposable incomes, improved accessibility for urban citizens and greater publicity for, and generation of public interest in, wild places and species (Karanth 2012).

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Supplementary material I

Relevant Legislation

Authors: Neil D'Cruze, Fernando Carniel Machado, Neil Matthews, Margaret Balaskas, Gemma Carder, Vanessa Richardson, Roberto Vieto

Data type: Table

Explanation note: Relevant legislation regarding the legal status of any close and or direct contact opportunities with wildlife observed during our fieldwork. This included legislation relating, but not limited, to wildlife management, conservation, national red lists of endangered species, animal welfare, tourism, environmental crime, and jurisprudence.

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Systematic Raptor Monitoring as conservation tool: 12 year results in the light of landscape changes in Dadia-Lefkimi-Soufli National Park

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Abstract

Dadia-Lefkimi-Soufli National Park forms part of the Natura 2000 network in a region of Greece and represents one of the most diverse landscapes for raptors (birds of prey) breeding in Europe. It is adjacent to Bulgaria and Turkey and is a renowned biodiversity hotspot. WWF Greece established a Systematic Raptor Monitoring scheme in this area in 2001. This study summarises the results of the first 12 years of monitoring in the National Park. Overall, 25 to 27 raptor species were recorded by pooling data, of which 20 species reproduced in the National Park. Raptors with continuous presence in the National Park exhibited stable, species-specific inter-annual variation. An average of 348±15.4 raptor territories were distributed throughout the National Park for all species. The Common buzzard (Buteo buteo) and the Shorttoed eagle (Circaetus gallicus) were the most common species year-round, followed by the Lesser-spotted eagle (Clanga pomarina) and Booted eagle (Aquila pennata). The Long-legged buzzard (Buteo rufinus), Honey buzzard (Pernis apivorus) and Egyptian vulture (Neophron percnopterus) exhibited a noticeable drop in population numbers over the study period. A significant new entry was the re-appearance of the Whitetailed eagle (Haliaeetus albicilla), which was recorded breeding again in the region after a 21-year absence. Species trends, along with their ecological traits, are discussed with respect to landscape changes in Dadia NP and minimum viable population and territory thresholds are proposed to outline essential conservation issues. Although a multi-year balance of the total number of occupied territories for all species was recorded, the number of common species increased compared to specialist species which had smaller, declining populations. The abandoning of traditional livestock farming, which induces an increase in closed-canopy forest coverage, might have led to the decline of the Lesser-spotted eagle, Long-legged buzzard and Honey buzzard numbers. Additional pressure is added from specialist forest dwelling raptors which are favoured by this change in habitat. The results of this study are expected to provide useful insights to facilitate conservation and management decisions about raptors and their habitat in this region.

Keywords

birds of prey, population trends, modelling, GAM, Greece, conservation

Introduction

Although Dadia-Lefkimi-Soufli National Park (Dadia NP) was established in 2003, it has had protected reserve status since 1980 as a result of its high ornithological value (Adamakopoulos et al. 1995, Kati et al. 2004a, 2004b, Catsadorakis and Källander 2010). It is a specially protected area (SPA) within the Natura 2000 network of the European Union (EU) as it supports a highly diverse raptor assemblage, hosting breeding pairs of both rare and endangered species. Examples include the last breeding colony of the Cinereous vulture (*Aegypius monachus*) in the Balkans, in addition to colonies of the Lesser-spotted eagle, the Short-toed eagle and the Booted eagle (Poirazidis et al. 2004, Skartsi et al. 2008, Vasilakis et al. 2008). Out of all known European raptor species, 90% have been observed in Dadia NP (Hallmann 1979), highlighting the importance of this region for Greece and Europe. This region is also a renowned biodiversity hotspot for other taxa (Kati et al. 2004a, 2004b, Kati and Sekercioglu 2006, Kati et al. 2007, Zografou et al. 2015).

Although the landscape of Dadia NP remains heterogeneous and diverse (Schindler et al. 2008), it has changed significantly after 1970, due to both reforestation and land abandonment (Triantakonstantis et al. 2006). This land-use change affected the availability of suitable habitats for many species of raptors, as well as other taxa (Poirazidis et al. 2007, Poirazidis et al. 2010). Therefore, a Systematic Raptor Monitoring (SRM) scheme was established in Dadia NP to evaluate and improve conservation measures at regular intervals and to establish precise management actions (Witmer 2005). Many countries, at least within the EU, have established long-term raptor monitoring schemes as conservation tools (Kovacs et al. 2008). In contrast, in Greece and specifically within Dadia NP, an SRM scheme has only been established since 2001 by WWF Greece. This scheme aimed at exploring population trends by monitoring variations in the number of raptor territories. This scheme is unique as it is the only region in the whole of Greece where the whole raptor bio-community is monitored rather than just for specific species.

Therefore, the current study aimed to: (i) assess the six monitoring surveys (March to August 2001–2005 and in 2012) completed in Dadia NP since 2001 by exploring the relative variation in the number of raptor territories in this region and (ii) model population trends of raptors from 2001 to 2012, to identify key conservation issues

and explore which species are in need of immediate action. These results were expected to show the importance of SRM as a conservation strategy in the study region and as a protocol for evaluating variations in raptor numbers from which to develop focused processes to assess the drivers of these variations.

Methods

Study area

Dadia NP is located in the Evros Prefecture of Greece (N40°59" to 41°15"N, E26°19" to E26°36'), forming part of the south-eastern Rhodope mountains, bordering with Turkey in the east. The area includes two strictly protected core areas, encompassing a total of 7290ha. The altitude of the area ranges between 10m and 654m. Including the surrounding buffer zone, the area encompasses 35170ha (Catsadorakis and Källander 2010). The landscape is characterised by small and large valleys crossed by a hydrological network of both small and large watercourses. Dadia NP is dominated by forests of pines mixed with broadleaf forests. Maquis scrublands are also present in the vegetation mosaic. The most common tree species is the Calabrian pine (*Pinus halepen*sis subsp. Brutia), while the Corsican pine (Pinus nigra) forms smaller forest patches, usually adjacent to riparian habitats. Four species of oak (Quercus spp.) are also present in the ecosystem. Riparian vegetation is mainly composed of Common alder (Alnus glutinosa) and, to a lesser extent, species like Willow (Salix sp.), the Black poplar (Populus nigra) and Tamarisk (Tamarix spp.) (Adamakopoulos et al. 1995, Poirazidis et al. 2004). Grazing lands, fields and villages disrupt the continuity of these forested areas, creating a characteristic habitat-mosaic that favours high landscape and biological diversity in the Dadia NP (Schindler et al. 2008).

Field methodology

In general, three main sampling methods are used for the census of breeding raptors: (i) line transects for surveying small areas on either side of a road; (ii)point count surveys in specified areas around fixed points and (iii)territory mapping (Fuller and Mosher 1987). In this study, all three methods were combined through (i) the surveillance of fixed areas from permanent vantage points, from which observations were mapped and (ii) surveillance from a vehicle along predetermined transects in which raptor activity was documented along both sides of the roads with hand-held GPS units (Schindler et al. 2011). Binoculars and telescopes were used according to standard methodology (Bibby et al. 2000, Vorisek et al. 2008, Gilbert et al. 2011). Following, Millsap and Le Franc (1988), permanent (rather than random) vantage points were selected. Twenty-four vantage points and 10road transects (see Supplementary File 1: Map of Dadia NP vantage points and line transects) were selected throughout the entire study area to

monitor the raptor population in detail and to secure the viable, long-term reproducibility of the methodology for future monitoring efforts.

Due to the topography of the area, good vantage points were limited and definitive vantage points were selected using the following criteria: (i) the point ensured the best and widest view of all neighbouring hillsides; (ii) the total area surveyed from vantage points included all main habitat types in proportion to their availability; (iii) the points were distributed equally over the entire study area, without habitat-bias towards plots with already known high raptor presence; (iv) access time to vantage points from the nearest road was short and (v) Black vulture colonies were avoided to reduce disturbance. Road transects were selected based on the following criteria: (i) how they complemented vantage points and, especially, for coverage of valley areas, where the positioning of adequate vantage points was not possible and (ii) to obtain the maximum coverage of the reserve by the two methods. Each survey was completed by two observers who alternated at sampling sites to reduce observer bias.

Details of the SRM scheme, describing both spatial and temporal parameters, are provided in Poirazidis et al. (2011). For a detailed map of the study area, transect and vantage points' position, as well as landscape and vegetation analysis, National Park limits and core areas' extension, see Schindler et al. (2008, 2015) and Poirazidis et al. (2009).

Data analysis methods

The total number of raptor species, along with the total number of individuals of each species, was recorded each year, along with details on the vantage point, transect and monitoring season. The total number of observations for each species was also recorded in a similar manner. To standardise differences in raptor numbers between years, the percentage of all recorded observations for each raptor species was calculated in relation to the total raptor observations in each monitoring year.

The number of territories was estimated using three standard steps to allow meaningful comparison. In Step 1, the observation data were entered in seven different ArcGIS layers: general flights, territorial observations, landings, synchronous observations, nest areas, meeting points and meeting point flights. The number of individuals, species, age, sex and different raptor activities were also recorded in an Access database that was interconnected with the GIS layers of Dadia NP. In Step 2, each territory was estimated independently for each vantage point and each road transect (representing 34 discrete sampling plots). When territories extended beyond the boundaries of discrete plots (continuing on to neighbouring plots), further analysis reshaped the polygon limits, creating new ones in a progressive process in which the recordings of each subsequent month in the same year were used to correct previous estimations (for details, see Poirazidis et al. 2009; Schindler et al. 2011). In Step 3, in order to define the final limits of a breeding pair's exploited territory, the total observations for each year were used for the whole breeding season. Therefore, "territory centres" (which were derived from the polygons) should not be used in a deterministic context, but in a

relative spatial context. To avoid potential bias in counting the same individual in more than one territory (and not confusing one large territory with smaller neighbouring ones), simultaneous observations from observers in different locations over the whole region were used as the tool for separation. At the time of evaluation, major raptor activities were included, such as displays and landings.

Breeding territories were classified as: "confirmed" or "possible." The classification "possible" was used when it was not possible to confirm with absolute certainty that the observations were obtained from separate individuals that maintained a separate territory. At the end, the final number of territories for each species was calculated by adding 50% of possible territories to the confirmed values (Palma et al. 2004).

Inter-annual variation in the relative number of raptor territories was explored by using the first SRM year as the starting point, appointing it with a value of 1. Then, for all subsequent monitoring years, a number was produced showing the relative difference in the number of territories per species in relation to the baseline value of 1 (Siriwardena et al. 1998).

To explore the population trends of raptors, two approaches were implemented. First, the non-parametric and distribution-free test of Mann-Kendall (MK) was applied to the annual SRM values for the number of raptor territories to statistically determine whether there was a monotonic upward or downward trend of the variable of interest over time (Baldwin et al. 2012). A monotonic upward/downward trend means that the variable consistently increases/decreases through time. The MK test is used in place of a parametric linear regression analysis, as the residuals from the fitted regression line do not need to be normally distributed. MK "tau" and "p" values were calculated, along with the Sen Slope value which is the median slope joining all pairs of observations and is expressed both by quantity per unit time and percent of the mean quantity per unit time. MK tests were applied with R programming language (R Core Team 2017) and the Kendall package (McLeod 2015). Second, the annual values of the number of territories per species from the SRM scheme were tested progressively against time with Generalised Linear Models (GLMs) with Poisson and Negative Binomial distributions and then with Generalised Additive Models (GAMs), with and without smoothing terms, in terms of cubic and cyclic cubic regression splines. GAMs were the best fitting models for these data, based on the criterion of AIC and BIC (Guisan et al. 2002).

GAMs are actually flexible extensions of GLMs. In GAMs, the linear equation predictors that are associated with the GLMs are replaced by a more general additive predictor which allows the change in abundance over time to follow any smooth curve and not just a linear form (Fewster et al. 2000). In order to capture the non-linear form of data over time, GAMs use a variety of smoothing techniques which were originally developed for smoothing scatterplots. Such smoothers are the kernel smoothers, weighted regression smoothers and running-median smoothers (Fewster et al. 2000). In the present study, smoothing splines were used which satisfy a penalised least squares criterion. The penalty criterion is applied by using cubic and cyclic cubic polynomials, the roughness of which is actually penalised by the number of degrees of freedom.

Specifically, as the degrees of freedom in a model increase, the smoothing functions increase the model's flexibility, with more turning points and gradients appearing. In GAMs, predictor variables are specified as non-parametric smooth functions (Hastie and Tibshirani 1986). GAMs were applied with R programming language (R Core Team 2017) and more specific with the R package mgcv (Wood 2012).

GAMs are also used to separate actual underlying trends from short-term fluctuations. However, the precise point where a signal is interpreted as an irregular fluctuation, rather than a long-term trend, is poorly defined; thus, for each point, a framework to delineate noise from trends must be generated (Fewster et al. 2000).

A number of diversity indices was also calculated for the raptor community in Dadia NP, following Magurran (2004). Shannon, Simpson and Invert-Simpson diversity indices were calculated, these being three of the most commonly used diversity metrics in bio-community analysis (Magurran 2004).

The Osprey (Pandion haliaetus), Hen harrier (Circus cyaneus) and Red kite (Milvus milvus) were observed in Dadia NP, but were excluded from the analyses because they are currently, at least, non-breeders in the area. Similarly, the Black kite (Milvus migrans) was excluded from population trend analyses as it occupied territories close to Dadia NP, but did not breed inside the NP. The Western marsh harrier (Circus aeruginosus) was also excluded from the analysis, as it was absent during the first year, only establishing territories in the final year of monitoring. The colonial vulture species Griffon vulture (Gyps fulvus) and Cinereous vulture (Aegypius monachus) were also observed, but excluded from all analyses, as they form colonies and undertake common and long distance flights for foraging and do not present a strict territorial behaviour similar to other raptors. Finally, species that were sporadically recorded across years (Imperial eagle (Aquila heliaca), Lanner falcon (Falco biarmicus) and White-tailed Eagle were not included in this analysis.

Results

Presence and relative abundance

Dadia NP was monitored from March to August 2001–2005 and in 2012 (i.e. totalling six censuses) within the framework of the SRM. A total of 23 raptors that form territories were recorded over all breeding seasons (Table 1).

The number of Egyptian vulture territories was counted and evaluated in the SRM. Due to the rapid population decline of this species in the study region (and also in SE Europe), specific additional monitoring schemes were implemented from 2009 to 2017 in Dadia NP. The aim was to secure the Egyptian vulture survival in Greece and Bulgaria. More details are included in the official project's website The Return of Neophron (http://www.lifeneophron.eu/en/index.html).

One-way ANOVA tests indicated that in a species-specific context, the presence of raptors was stable between monitoring years (25.17 \pm 1.47, $F_{(6.1)}$ =2.172, p=0.465).

Table 1. Relative frequency of raptors recorded during each Systematic Raptor Monitoring year in Dadia-Lefkimi-Soufli National Park. Percentages are calculated from the total of individuals observed from March to August 2001–2005 and in 2012. Data are shown in a decreasing value-order with respect to the first year of observation.

S	1st SRM year	2 nd SRM year	3 rd SRM year	4th SRM year	5th SRM year	6th SRM year
Species of raptors	(2001)	(2002)	(2003)	(2004)	(2005)	(2012)
Buteo buteo	40.07	27.64	26.55	27.11	29.39	27.58
Circaetus gallicus	20.61	25.07	26.19	24.07	22.90	21.47
Clanga pomarina	4.49	5.79	4.18	5.75	4.81	5.13
Neophron perncopterus	4.23	4.88	3.87	5.87	5.01	1.89
Aquila pennata	3.97	3.49	3.78	3.19	2.99	4.65
Pernis apivorus	3.25	3.49	2.63	1.17	2.11	2.46
Accipiter nisus	2.73	3.03	2.41	1.21	1.52	2.74
Falco tinnunculus	1.83	1.55	0.91	2.04	1.40	1.09
Accipiter gentilis	1.80	2.29	1.08	0.91	1.60	1.96
Aquila chrysaetos	1.53	1.39	1.06	1.72	1.84	0.96
Buteo rufinus	1.01	0.61	0.53	0.64	0.72	0.28
Falco subbuteo	0.60	0.63	0.27	0.34	0.30	1.30
Aquila heliaca	0.37		0.11	0.09	0.01	
Falco biarmicus	0.37		0.00			
Milvus migrans	0.49	0.83	0.22	0.77	0.81	1.09
Falco peregrinus	0.26	0.04	0.18	0.58	0.41	0.28
Accipiter brevipes	0.26	0.26	0.13	0.04	0.21	0.24
Circus aeroginosus		0.04	0.09	0.03	0.17	0.72
Circus cyaneus					0.01	0.04
Circus pygargus				0.01	0.01	0.09
Haliaeetus albicilla			0.04		0.21	0.74
Milvus milvus						0.02
Falco vespertinus		0.09				

This pattern was similar in the whole NP and at vantage point plots $(24.83\pm1.47, F_{(2.3)}=0.316, p=0.750)$ and along road-transect plots $(20.67\pm1.37, F_{(3.2)}=0.549, p=0.697)$.

The most common species were the Common buzzard, Short-toed eagle and Less-er-spotted eagle, with an average relative abundance of 29.72%, 23.38% and 5.02% of all raptor species per monitoring year. All other species had a relative abundance of less than 3% per monitoring year (Table 1).

The relative abundance of the Egyptian vulture was 4.8% (range: 3.87–5.87%) in 2001–2005, but dropped to 1.89% in 2012, indicating a rapid decline in the number of breeding territories over the course of a decade. In addition, the presence of the Long-legged buzzard decreased by 70% from 2001 to 2012 (Table 1). The Lanner falcon was only recorded in 2001. The relative abundance of the Imperial eagle declined over the observation period, with this species being absent in both 2005 and 2012 (Table 1).

Relative variation in territory number and diversity

The Booted eagle and Golden eagle (*Aquila chrysaetos*) displayed negative values in 2004 (SRM year 4); however, the population had re-established by 2012 (SRM year 6) to similar levels as in 2001 (SRM year 1) (Figure 1). A positive relative increase of 4.9% was documented for the Booted eagle. In comparison, the Golden eagle had a similar number of territories in 2012 (SRM year 6) as in 2001 (SRM year 1). The number of Lesser-spotted eagle territories increased from 2001 to 2004 (SRM year 1–4); however, the number of territories in 2012 (SRM year 6) was similar to that in 2001 (SRM year 1) (Figure 1). The only eagle for which the number of territories increased from 2001 to 2005 and was maintained in 2012 was the Short-toed eagle, with a 22% relative increase (Figure 1).

Buzzards presented two opposing trends in the relative number of territories. The Common buzzard occupied more territories across all survey years, with a relative increase of 11.5% (Table 2). In comparison, the relative number of territories of the Long-legged buzzard and the Honey buzzard strongly declined by 57% and 45%, respectively (Figure 2). Falcons also exhibited contrasting trends. For instance, after a continuous increase from 2002 to 2005, the number of Eurasian kestrel (*Falco tinnunculus*) territories noticeably declined, reaching a negative value of 29% with respect to the baseline value (Figure 3). In comparison, the number of Peregrine falcon (*Falco peregrinus*) and Hobby (*Falco subbuteo*) territories strongly increased by 75%

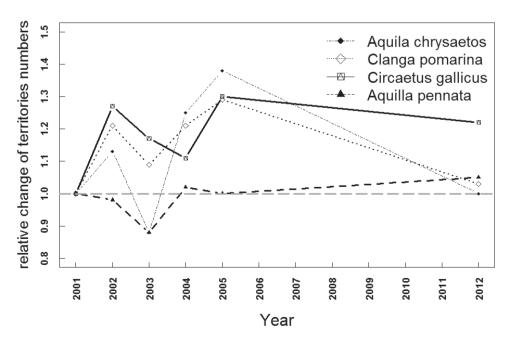


Figure 1. Relative variation in the territory numbers of eagles in Dadia-Lefkimi-Soufli National Park during the Systematic Raptor Monitoring surveys (March to August 2001–2005 and in 2012).

Table 2. Relative variation in the number of raptor territories in the Dadia-Lefkimi-Soufli National Park during the Systematic Raptor Monitoring surveys from March to August 2001–2005 and in 2012. Calculations were made in relation to a "baseline value," with the number of territories in year 1 being assigned the value of 1. Data are shown in decreasing value-order with respect to total variation (%).

Species of raptors	1st SRM year (2001) "baseline value"	2 nd SRM year (2002)	3 rd SRM year (2003)	4 th SRM year (2004)	5 th SRM year (2005)	6 th SRM year (2012)	Total Variation (%)
Falco peregrinus	1	0.25	1.50	1.50	1.50	1.75	75.0
Falco subbuteo	1	1.38	0.54	1.31	1.08	1.46	46.2
Accipiter brevipes	1	2.40	1.60	0.40	1.20	1.40	40.0
Accipiter gentilis	1	0.97	0.87	1.03	1.26	1.24	23.7
Circaetus gallicus	1	1.27	1.17	1.11	1.30	1.22	22.2
Buteo buteo	1	1.17	1.14	1.02	1.11	1.11	11.4
Aquila pennata	1	0.98	0.88	1.02	1.00	1.05	4.9
Clanga pomarina	1	1.21	1.09	1.21	1.29	1.03	2.9
Aquila chrysaetos	1	1.13	0.88	1.25	1.38	1.00	0.0
Accipiter nisus	1	0.81	0.86	0.76	0.90	0.78	-22.2
Falco tinnunculus	1	0.90	1.00	1.26	1.45	0.71	-29.0
Pernis apivorus	1	0.95	0.81	0.65	0.86	0.54	-45.6
Neophron percnopterus	1	1.05	0.86	1.09	0.86	0.45	-54.5
Buteo rufinus	1	0.86	0.86	0.86	1.14	0.43	-57.1
Circus aeruginosus	0.00	1.00	1.00	0.00	2.00	6.00	500.0
Total territory variation	1	1.07	1.04	1.01	1.12	1.02	2.1

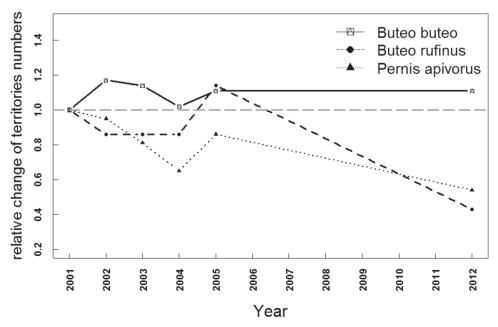


Figure 2. Relative variation in the number of territories of buzzards in Dadia-Lefkimi-Soufli National Park during the six Systematic Raptor Monitoring surveys (March to August 2001–2005 and in 2012).

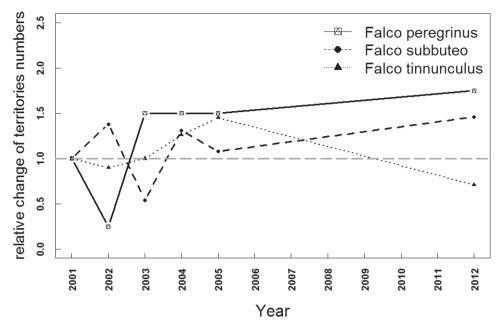


Figure 3. Relative variation in the number of territories of falcons in Dadia-Lefkimi-Soufli National Park during the six Systematic Raptor Monitoring surveys (March to August 2001–2005 and in 2012).

and 46.2%, respectively (Table 2), with this trend being noticeable in comparison to all other raptors.

With respect to hawks, the number of Sparrowhawk (*Accipiter nisus*) territories decreased from 2001 (SRM year 1) to 2012 (SRM year 6) (Figure 4). The number of Levant sparrowhawk (*Accipiter brevipes*) and Northern goshawk (*Accipiter gentilis*) territories increased by the year 2012 (SRM year 6); however, the Levant sparrowhawk exhibited major fluctuations in territory number across the years (Figure 4). The Egyptian vulture exhibited the largest relative decrease in territory number of all species, reaching a negative value of 54.5% in 2012 (Table 2, Figure 5), holding just five active territories.

The diversity of the raptor assemblage in Dadia NP decreased in the region over the study period based on all three indices (Shannon, Simpson and Invert-Simpson) (Figure 6). However, all indices presented generally high values of diversity overall (Simpson: 0.834±1.01, Shannon: 2.194±0.04, Invert-Simpson: 6.048±0.39).

Overall percentage change between the first and last year of the SRM

Three species exhibited noticeable overall increases in their presence in Dadia NP from 2001 to 2012; namely, the Common buzzard, the Short-toed eagle and the Northern goshawk. In contrast, four species exhibited noticeable decreases; namely, the Common kestrel, the Egyptian vulture, the Eurasian sparrowhawk and the Honey buzzard.

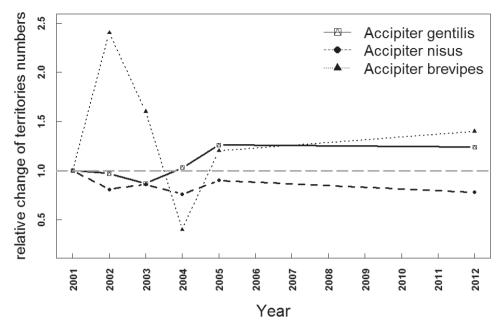


Figure 4. Relative variation in the number of territories of hawks in Dadia-Lefkimi-Soufli National Park during the six Systematic Raptor Monitoring surveys (March to August 2001–2005 and in 2012).

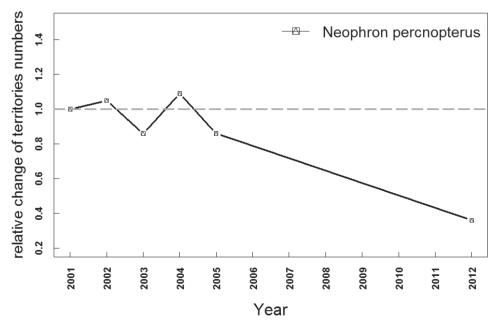


Figure 5. Relative variation in the number of territories of Egyptian vultures in Dadia-Lefkimi-Soufli National Park during the six Systematic Raptor Monitoring surveys (March to August 2001–2005 and in 2012).

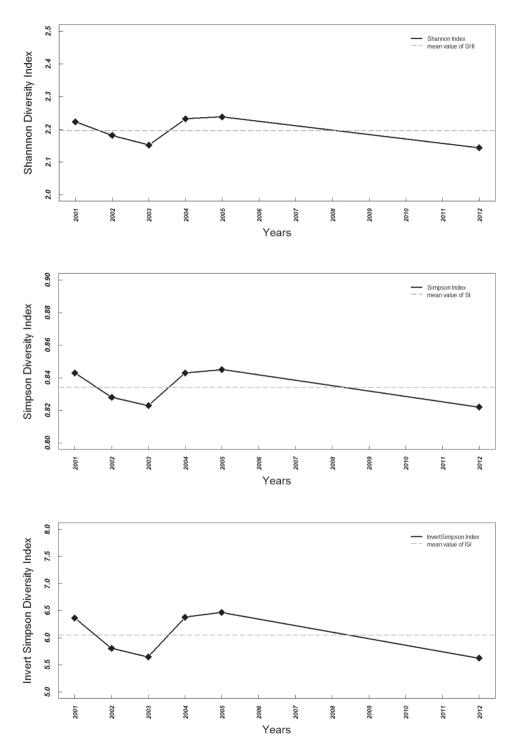


Figure 6. Diversity of raptor assemblages in Dadia NP from 2002 to 2012. Variation in Shannon, Simpson and Invert-Simpson indices is shown.

A further two species displayed moderate increases; namely, the Western marsh harrier and the Eurasian hobby. Four species showed marginal upward trends; namely, the Peregrine falcon, the Levant sparrowhawk, the Booted eagle and the Lesser-spotted eagle. Two species displayed moderate decreases; namely, the Golden eagle and the Long-legged buzzard. (See Table 4 for details)

Models of population trends

The population trends of 14 raptors were modelled. These species had a continuous presence, with active territories in the study area across all survey years (Table 3).

Although the MK test did not reveal statistically significant monotic trends, it provided valuable information on species trends (positive or negative), depending on the sign of the Sen slope value. This result was obtained due to the time series being short. Furthermore, a monotonic line cannot always fit significant fluctuations. In comparison, the more flexible GAMs confirmed that time had a significant effect on population change, with the preliminary trend for the study period being graphically presented using the smoothing technique.

The Short-toed eagle was the only eagle with an increasing trend. The MK test showed an upward trend (though not significant), whereas the GAM showed a significant relative change in the number of territories over time, with a good fit (Table 3, Figure 7a, b). The Golden eagle occupied the same number of territories at the first and last census (Figure 7c, d), presenting very little overall change in variation with respect to the other eagles (Tables 2, 3 and 4). The Lesser-spotted eagle and Booted eagle had slightly higher relative variance in the number of territories by 2012 (Tables 2 and 4), attaining positive M\K slopes. GAMs for the two species of eagles (t=33.291, p<0.0001 and t=45.54, p<0.0001 respectively) indicated significant variation over time. The Lesser-spotted and Booted eagles (Figure 8a, b, c, d) had a relatively stable population, even when considering the small overall change in variation (Table 4). However, the Lesser-spotted eagle exhibited a noticeable decline in 2012 (Figure 8a).

With respect to buzzards, only the Common buzzard had a stable trend (MK: tau=0.066, p=1). The Long-legged buzzard and Honey buzzard showed decreasing MK slopes (Sen slope: -0.166 and -2.6, respectively). The variation in the number of Buzzard territories versus monitoring years was highly significant, with a good fit when using GAMs (Table 3, Figure 9a, b). The Honey and Long-legged buzzards displayed decreasing population trends, with a strong decline being detected for the Honey buzzard (Figure 9c–f).

The trend recorded for hawks was significant when using GAMs (Northern goshawk: t=69.240, p<0.01, Eurasian sparrowhawk: t=24.292, p<0.0001, Levant sparrowhawk: t=14.990, p<0.05), with a robust fit (Table 3) which was attributed to the significant effect of time on their inter-annual variation (Figure 10a–f). Only the Northern goshawk had a positive upward slope (Sen slope: 1.25) in its population trend (tau=0.467, p=0.259) with a robust fit in GAM (Figure 10a, b). The Levant sparrowhawk had no clear trend after 2005, leading to a negative, non-significant MK slope,

Table 3. Generalised Additive Models fitting the Systematic Raptor Monitoring values for each survey year of the number of raptor territories in Dadia-Lefkimi-Souffi National Park (March to August 2001–2005 and in 2012). Results of Linear GAMs and GAMs with cubic and cyclic cubic smoothing splines are shown.

						GAM cr	I cr			GAM ccr	ccr	
		Linear GAM Family: Gaussian	GAM saussian		Cubic	Cubic regression smoothing splines	moothing sp	olines	Cyclic cu	Cyclic cubic regression smoothing splines	n smoothin	g splines
Species of raptors		Link function: Identity	n: Identity			ramity: Gaussian Link function: Identity	saussian on: Identity			ramity: Gaussian Link function: Identity	saussian m: Identity	
	R^2adj	Deviance explained	ADD	AIC	R^2 adj	Deviance explained	ADD	AIC	R^2 adj	Deviance explained	ADD	AIC
EAGLES												
Circaetus gallicus	-0.072	14.2%	19.729	36.05	0.998	100%	0.180	-2.74	966.0	%6.66	0.2685	-0.34
Aquila chrysaetos	-0.25	0.02%	1.015	18.25	0.131	43.1%	0.861	16.32	0.244	54%	0.807	15.51
Clanga pomarina	-0.215	2.82%	7.045	29.87	0.477	%9.79	3.921	25.09	0.375	61.6%	4.728	26.16
Aquila pennata	0.078	26.3%	2.039	22.43	0.078	26.3%	2.039	22.43	0.032	3.25%	1.712	22.06
BUZZARDS												
Buteo buteo	-0.2	3.99%	99.656	45.77	0.688	92.99%	91.541	35.85	0.676	92.7%	95.525	36.05
Buteo rufinus	0.47	92.6%	0.556	14.64	0.911	%8.96	0.209	3.52	0.911	%2'96	0.198	3.63
Pernis apivorus	0.629	70.4%	13.879	33.94	0.852	96.1%	17.003	27.13	0.629	70.4%	13.879	33.94
SPARROWHAWKS												
Accipiter gentilis	0.369	49.5%	8.303	30.861	0.942	98.5%	2.406	15.132	0.629	70.4%	13.879	33.943
Accipiter nisus	0.078	26.3%	14.327	34.13	0.078	26.3%	14.329	34.13	-0.019	-1.95%	12.683	34.08
Accipiter brevipes	-0.243	0.58%	5.157	28.00	0.893	%8.76	1.713	11.13	0.887	%9.76	1.808	11.48
FALCONS												
Falco tinnunculus	-0.092	12.6%	27.32	38.00	0.998	%6.66	0.231	-0.66	0.998	%6.66	0.210	-0.70
Falco subbuteo	0.015	21.2%	7.189	29.99	0.015	21.2%	7.189	29.99	0.005	0.5%	5.805	29.39
Falco peregrinus	0.24	39.2%	1.368	20.04	0.24	39.2%	1.368	20.04	0.249	40.6%	1.367	19.99
VULTURES												
Neophron perncopterus	0.801	84.1%	2.551	23.78	0.819	%9.98	2.495	23.32	0.813	85.8%	2.526	23.50
TOTAL TERRITORIES												
Total number of territories in Dadia NP	-0.241	0.71%	436.1	20.04	0.903	%86	130.97	37.16	0.897	97.8	139.82	37.55

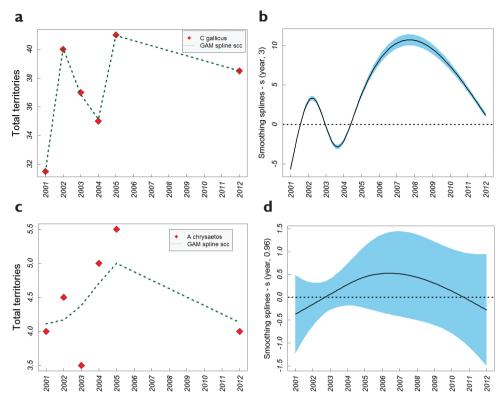


Figure 7. Inter-annual variation in the numbers of territories for Short-toed eagle and Golden eagle in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trend modelling is also shown for each species, with cyclic cubic regression (cc) splines used as smoothing terms. **a** Variation in the number of Short-toed eagle territories fitting a GAM **b** Predictive GAM for the Short-toed eagle with smoothed terms (cc splines) **c** Variation in the number of Golden eagle territories fitting a GAM **d** Predictive GAM for the Golden eagle with smoothed terms (cc splines).

but it had a robust fit in GAM of R²adj=0.893. This equation defined the effect of time in this short variation cycle, with uncertain fluctuations (Figure 10e, f). The number of Eurasian sparrowhawk territories decreased from 36 to 28 (Figure 10c, d).

The Common kestrel had a negative population trend. The MK slope was not significant, but positive for this species (tau= 0.138, p=0.848). This result was possibly due to a strong increase during the initial years of monitoring (Figure 11a, Table 3), followed by the lowest value being obtained in the final year, indicating a possible negative population trend (Figure 11a, b). The Eurasian hobby showed a positive MK slope (tau= 0.333, p=0.452). This trend was probably due to the highest value being obtained in the final monitoring year (Tables 2 and 4). The Peregrine falcon had a positive MK slope that was marginally significant (tau= 0.745, p=0.069) and a significant GAM (t=6.428, p<0.05). A low adjustment GAM R² value fitted through the Peregrine falcon data, demonstrating an increasing trend for the species. The estimated number of Peregrine falcon territories increased from two in 2001 to 3.5 in 2012 (Figure 11e, f).

Table 4. Relative frequency in the number of raptor territories in Dadia-Lefkimi-Soufli National Park in 2001 and in 2012. Percentages were calculated from the total number of territories per year. Percentage change variation was based on the difference between the first and the last year of the Systematic Raptor Monitoring scheme. Absolute territory numbers per species are shown for both 2001 and 2012. Data are shown in decreasing value-order with respect to the overall percentage variation change.

Birds of prey species	Absolute number of territories in 2001	Absolute number of territories in 2012	Relative territory frequency in 2001 (%)	Relative territory frequency in 2012 (%)	Percentage (%) variation change between 2001 and 2012
Buteo buteo	110	122.5	32.93	35.92	2.99
Circaetus gallicus	31.5	38.5	9.43	11.29	1.86
Accipiter gentilis	19	23.5	5.69	6.89	1.20
Circus aeruginosus	0	3	0.00	0.88	0.88
Falco subbuteo	6.5	9.5	1.95	2.79	0.84
Falco peregrinus	2	3.5	0.60	1.03	0.43
Accipiter brevipes	2.5	3.5	0.75	1.03	0.28
Aquila pennata	20.5	21.5	6.14	6.30	0.17
Clanga pomarina	17	17.5	5.09	5.13	0.04
Aquila chrysaetos	4	4	1.20	1.17	-0.02
Buteo rufinus	3.5	1.5	1.05	0.44	-0.61
Falco tinnunculus	15.5	11	4.64	3.23	-1.41
Neophron percnopterus	11	5	3.29	0.47	-1.83
Accipiter nisus	36	28	10.78	8.21	-2.57
Pernis apivorus	28.5	15.5	8.53	4.55	-3.99

Out of all documented raptor species, the Egyptian vulture demonstrated the greatest population decline (Tables 2–4, Figure 12a, b). This species had a negative slope that was marginally significant (MK: tau = -0.412, p=0.07). When the time series was extended to include 2000 to 2016 (WWF Greece, unpublished data), the Egyptian vulture showed a highly significant MK negative trend (tau=-0.62, p< 0.001).

With respect to the overall number of raptor territories, a small overall increase was detected (Table 2). This increase was possibly influenced by an increase in the number of Short-toed eagles and Common buzzards. Overall, the territories fit a significant GAM model vs time, with high R² adjustment (Table 3). The overall trend for 2001–2012 is shown in Figure 13a, b.

Discussion

Raptor monitoring as a conservation tool

Monitoring is an essential tool for effective nature conservation and management. Both long-term trends and the present status of populations allow sound management programmes to be formulated (Kirk and Hyslop 1998, Saurola 2008). In particular,

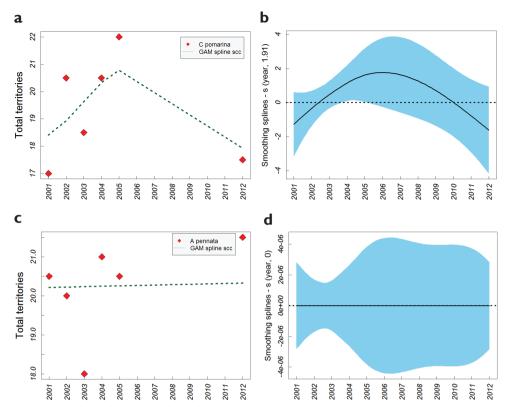


Figure 8. Inter-annual variation in the numbers of territories for Lesser-spotted eagle and Booted eagle territories in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trend modelling is also shown for each species with cyclic cubic regression (cc) splines used as smoothing terms. **a** Variation in the number of Lesser-spotted eagle territories fitting a GAM **b** Predictive GAM for the Lesser-spotted eagle with smoothed terms (cc splines) **c** Variation in the number of Booted eagle territories fitting a GAM **d** Predictive GAM for the Booted eagle with smoothed terms (cc splines).

forest-dwelling raptors are difficult to monitor, as they are widely dispersed and are difficult to detect, due to vegetation, forest canopy and land topography (Fuller and Mosher 1987). Thus, monitoring the population dynamics of raptors involves high requirements in personnel, time and cost (Catsadorakis 1994). Yet, the early detection of causes in population fluctuation provides the opportunity for "on-time" management decisions (Vos et al. 2000). The EU has set as one of its key objectives "to conserve most important species and habitats," including a large proportion of Europe's raptor species (Kovacs et al. 2008). At present, 64% of the 56 raptor and owl species that occur in Europe have an unfavourable conservation status (Kovacs et al. 2008).

Globally, the monitoring effort of raptors tends to focus on nest location and the observation of individuals (Fuller and Mosher 1987), this being a highly resource demanding process. In comparison, WWF Greece organised a different SRM approach which has been implemented in Dadia NP since 2001. This scheme involves monitor-

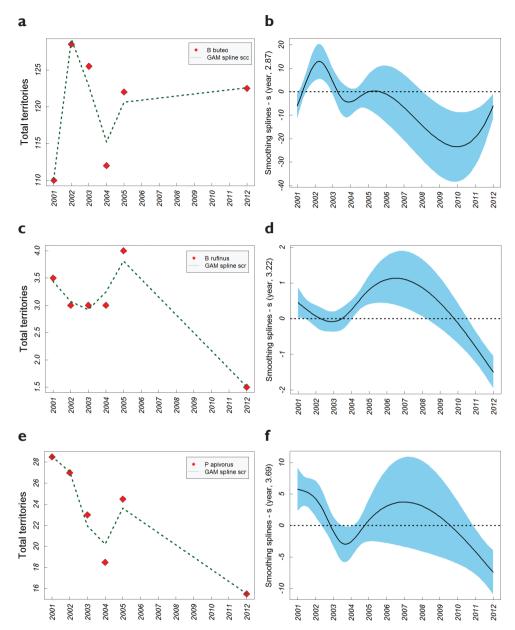


Figure 9. Inter-annual variation in the number of buzzard territories in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trends modelling is also shown for each species with cubic regression (cr) or cyclic cubic regression (cc) splines used as smoothing terms, depending on lowest AIC in each case. **a** Variation in the number of Common buzzard territories fitting a GAM **b** Predictive GAM for the Common buzzard with smoothed terms (cc splines) **c** Variation in the number of Longlegged buzzard territories fitting a GAM **d** Predictive GAM for the Long-legged buzzard with smoothed terms (cr splines) **e** Variation in the number of Honey buzzard territories fitting a GAM **f** Predictive GAM for Honey buzzard with smoothed terms (cr splines).

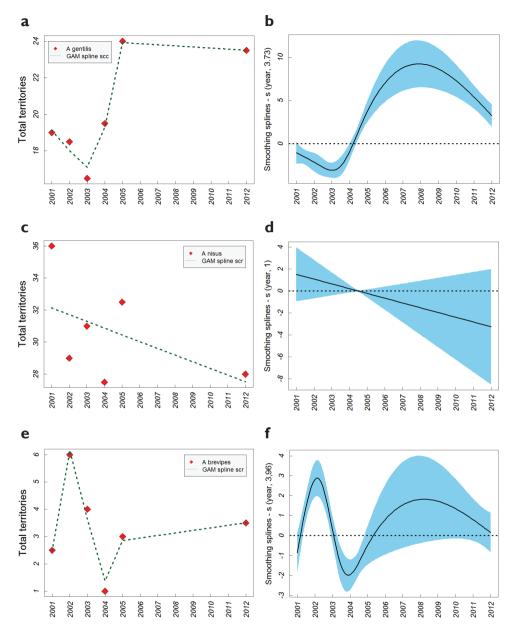


Figure 10. Inter-annual variation in the number of hawk territories in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trend modelling is also shown for each species with cubic regression (cr) or cyclic cubic regression (cc) splines used as smoothing terms, depending on lowest AIC in each case. A Variation in the number of Northern goshawk territories fitting a GAM **b** Predictive GAM for the Northern goshawk with smoothed terms (cc splines) **c** Variation in the number of Eurasian sparrowhawk territories fitting a GAM **d** Predictive GAM for the Eurasian sparrowhawk with smoothed terms (cr splines) **e** Variation in the number of Levant sparrowhawk territories fitting a GAM **f** Predictive GAM for the Levant sparrowhawk with smoothed terms (cr splines).

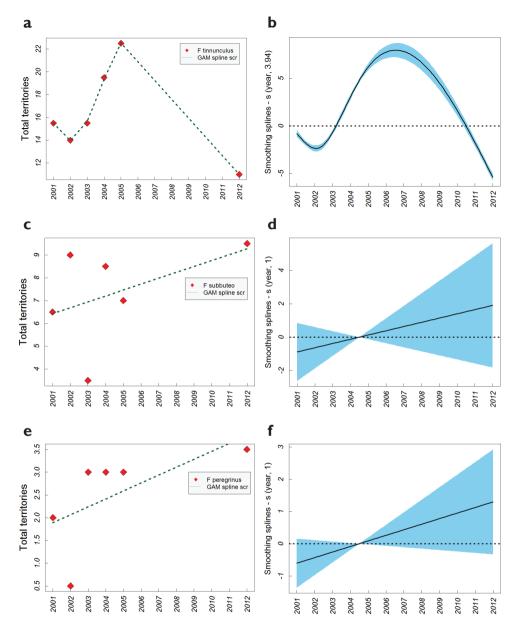


Figure 11. Inter-annual variation in the number of falcon territories in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trends modelling is also demonstrated for each species with cubic regression (cr) splines used as smoothing terms. **a** Variation in the number of Common kestrel territories fitting a GAM **b** Predictive GAM for the Common kestrel with smoothed terms (cr splines) **c** Variation in the number of Eurasian hobby territories fitting a GAM **d** Predictive GAM for the Eurasian hobby with smoothed terms (cr splines) **e** Variation in the number of Peregrine falcon territories fitting a GAM **f** Predictive GAM for the Peregrine falcon with smoothed terms (cr splines).

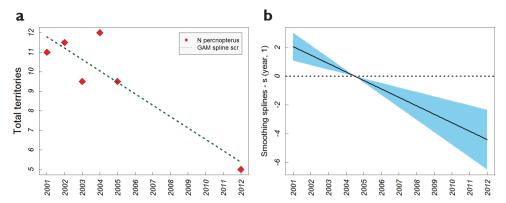


Figure 12. Inter-annual variation in the number of Egyptian vulture in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trend modelling is also shown for the species with cubic regression (cr) splines used as smoothing terms. **a** Variation in the number of Egyptian vulture territories fitting a GAM **b** Predictive GAM for the Egyptian vulture with smoothed terms (cr splines).

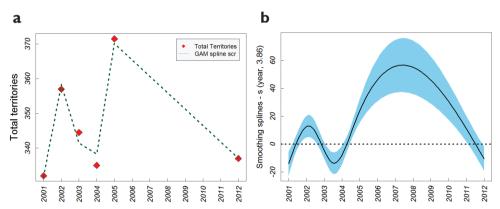


Figure 13. Inter-annual variation in the number of overall raptor territories in Dadia-Lefkimi-Soufli National Park fitting Generalised Additive Models. Population trends modelling is also demonstrated for the whole number of territories with cubic regression (cr) splines used as smoothing terms. **a** Variation in the number of overall raptor territories fitting a GAM **b** Predictive GAM for overall raptor territories with smoothed terms (cr splines).

ing and analysing inter-annual variations in breeding territories and is a useful and cost-effective tool for tracking raptor population trends (Katzner et al. 2007, Carrete et al. 2002, Poirazidis et al. 2009). Furthermore, the current study establishes a reproducible, long-term evaluation protocol for the raptor population status. The SRM was conducted from March to August (breeding period) in 2001–2005 and in 2012. Thus, the monitoring effort in Dadia NP is at an early stage when compared with other long-established European monitoring schemes (Crick et al. 1990, Gil-Sanchez et al. 2004, Stjernberg et al. 2006, Saurola 2008, Aradis and Andreotti 2012, Vrezek et al. 2012, Gamauf 2012, Galushin 2012, Spasov et al. 2012, Urcun 2012, Sanchez-Zapata 2012, Palma 2012, Vermeersch and Paquet 2012, Velevski et al. 2015).

Diversity of the raptor assemblage

A total of 27 diurnal raptor species were recorded in Dadia NP during the breeding season, 20 of which breed in the NP regularly. Three different diversity indices calculated for the raptor assemblage (Shannon, Simpson, Invert-Simpson) showed that raptors exhibited high diversity in the NP. These values had the highest ranking compared to values for raptors in other areas (Colwel 2009), reaffirming the high value of Dadia NP for raptors, where 36 of the 38 raptor species in Europe have been observed (Hallman 1979, Catsadorakis and Källander 2010).

However, over the 12-year period (from SRM1 to SRM 6), raptor diversity declined. All diversity values reached their lowest point in the final SRM year (2012) (Figure 6). Over this period, the number of the dominant Short-toed eagle and the Common buzzard (Figures 1 and 2), represented more than 50% of observed individuals and territories, along with a parallel decrease in less abundant species, such as the Golden eagle, Long-legged buzzard, Honey buzzard and Eurasian sparrowhawk (Tables 1 and 4). These trends are probably explained by changes in environmental factors, such as the landscape homogenisation (Adamakopoulos et al. 1995, Triantakonstantis et al. 2006), negatively impacting less competitive species that are dependent on specialised spaces in the forest ecosystem, leading to their being replaced by more common (competitive) species. Moreover, other anthropogenic factors are also driving the decline in raptor numbers in this region, examples including illegal poisoning and collisions with wind turbines (Kafetzis et al. 2017, Skartsi et al. 2014, Saravia et al. 2016, Ntemiri and Saravia 2016, Kret et al. 2016, Vasilakis et al. 2017).

However, it is also important to determine whether this decline in overall raptor diversity is actually due natural fluctuation processes or whether there is actually a consistent decline in rare and more specialist species requiring management action. A definite answer could only be provided following the long-term implementation of the SRM scheme. Such information would clarify whether the natural fluctuation process will be reversed or whether the decrease in diversity will continue due to intra-specific competition, landscape change and anthropogenic pressure. In particular, species with smaller numbers, less competitive mechanisms and more specialised habitat requirements might be at risk (Sanchez-Zapata and Calvo 1999, Juliard et al. 2006).

Overall, Dadia NP has a generally stable number of territories, with a small annual increase (Figure 13a, b). A total of 346–350 breeding territories was documented for all species combined in Dadia NP, with a fluctuation of ±40, possibly defining the limits of a natural fluctuation across the years. The Short-toed eagle and the Common buzzard (Figures 7a, b, 9a, b) were the most common (and abundant) raptor species in the region, with the trends for these two species shaping the detected variation of all breeding territories in the NP. Thus, this measure must be treated with caution, as the overall stability in territories for all species combined fails to account for the detected decline in rare and more specialist species. Of note, 19 of the 23 raptor represent less than 3% relative frequency of the records with respect to all recorded observations per year (Table 1).

Raptor population trends and possible effects of land use and landscape change

The Short-toed eagle is a specialist predator with a narrow niche diet of reptiles (Bakaloudis 1998), along with specific habitat needs for nesting (Bakaloudis 2001). Within the NP, forests have begun encroaching on abandoned agricultural land, increasing the extent of closed canopy (Adamakopoulos 1995, Poirazidis et al. 2011) and negatively impacting biodiversity (Zakkak 2014). This phenomenon could negatively impact species that need open areas to forage. Yet, the Short-toed eagle has maintained a stable population (even with a small increase) in this study area (Figure 7a, 7b). A possible reason for this might be that edge-habitats, which are necessary for reptile diversity and successful hunting (Sanchez-Zapata and Calvo 1999), have not reached critical levels for the species due to the presence of a network of tertiary roads that intersect the area. The Short-toed eagle population was generally stable, with a natural variation of 37±6 territories which might represent the stability threshold for the species in Dadia NP (Figure 7b).

With respect to eagles, the Booted eagle demonstrated robust population stability, whereas the Golden eagle and Lesser-spotted eagle exhibited contrasting trends. Both species exhibited overall stability, with similar values being obtained in the first and last SRM years; however, values fluctuated irregularly in the intervening years. In particular, 2003 was a poor year for eagle abundance in Dadia NP (Figures 7a, c, 8a, c), possibly due to climatic factors influencing the availability of prey for the raptors (Terraube et al. 2011, Wichmann et al. 2013). Alternatively, this decline might be a regular repetitive pattern which could only be detected through long-term monitoring. The Booted eagle had overall high abundance values for all years, except 2003 and an increase in 2012 (Figure 8c). If 2003 were excluded from the model, the population trend for this species would be clearly positive. The stability threshold of Booted eagles in the region is probably around 20 occupied territories on average. This raptor is generally scarce; yet, its population has somehow increased in Western Europe. This species is particularly sensitive to the management of forest ecosystems and co-existence with agricultural land (Suárez et al. 2000).

The Golden eagle and Lesser-spotted eagle presented identical trends (Figures 7d and 8b), with the numbers of both declining in 2003. These increasing fluctuations over the first 5 years, along with the large decrease in 2012, create an uncertainty about whether this is a regular fluctuating trend or if the decrease in 2012 requires immediate mitigating action.

A recent, noticeable, change in modern agricultural practices in the region of Dadia NP is the monoculture of Helianthus (*Helianthus annuus*), due to contract agriculture. Contract farming involves agricultural production being carried out on the basis of an agreement between the buyer and farm producers, where the buyer specifies the quantity required and the price, with the farmer agreeing to deliver at a future date. Similar changes in Estonia have caused problems for eagles that forage in open areas during the breeding period, these eagles decreasing due to Helianthus farming, including the Lesser-spotted eagle (Väli et al. 2017). Thus, the large decrease in the Lesser-spotted

eagle population between 2005 and 2012 (Figure 8a, b) might reflect the negative effect of landscape changes in relation to this crop in Dadia NP. Poirazidis et al. (2010) showed that the Lesser-spotted eagle population had been stable over the last 25 years in Dadia NP; however, there was a marked change in the elevation at which it nested. For instance, only 50% of pairs bred below 100m in the 1970s (Hallmann 1979), whereas this number had risen to 67% by 2001. Habitat change is the major driver behind this shift; thus, the change in the distribution of Lesser-spotted eagles in Dadia NP might be related to the decline in open and semi-open habitats in the interior of the forest since the 1950s (Triantakonstantis 2006, Poirazidis et al. 2015). This decline in forest heterogeneity might have also caused the abundance of reptiles and amphibians, which represent an important food source for the Lesser-spotted eagle in DNP, to decline (Vlachos and Papageorgiou 1996).

The Common buzzard is a generalist predator with a broad habitat niche (Rooney and Montgomery 2013, Jankowiak et al. 2015). Large fluctuations in territory numbers were detected in the initial SRM years; however, an increase was detected in 2012 (Figure 9a). The irregular initial fluctuations, along with the gap between fifth and sixth surveys generated high variability (Figure 9b), with more monitoring being required to determine whether this is a regular population trend. Overall, in Dadia NP, Common buzzards occupy an average number of 120 territories.

The Honey buzzard and Long-legged buzzard are more specialist species and demonstrated negative population trends. Interestingly, in the bordering regions of Bulgaria, the Long-legged buzzard has not undergone any major decline for 20 years (Iankov 2007). In contrast, in Dadia NP, the species probably reached low enough numbers for the population to crash in 2012 (Figure 9c). This raptor occupied very few territories in the NP (Table 4). Nonetheless, from 2001 to 2005, its numbers regularly fluctuated around three breeding territories which might be the minimum viable threshold for the species. However, the decrease in territories by 2012 probably indicates a negative population trend driving the Long-legged buzzard below its survival limits in Dadia NP (Figure 9d). Thus, continued monitoring is required to determine whether the population can recover. This decline might be associated with the noticeable decline in nomadic livestock farming between 2000 and 2012. Decreased grazing induced the lower use of open area patches within the forest ecosystem, altering the form of the heterogeneous mosaic in Dadia NP, leading to a parallel increase in the homogeneity index in the region. Consequently, open area foraging raptors lack adequate hunting space.

The Honey buzzard showed the largest population decrease of all the buzzard species in Dadia NP (Table 4). Even though it is a strict forest nesting species, without specific habitat requirements for its nesting sites (Gamauf et al. 2013), the increase in forested area in the region does not seem to have benefitted it. This fact is difficult to explain. Other factors might be suppressing the population, particularly in relation to its migratory habits. For instance, juvenile mortality of up to 50% is thought to occur during the "Sahara crossing" (Strandberg et al. 2009). Furthermore, this species has been subject to more than 30 years poaching in regions of the Mediterranean (Barca et al. 2016). As the species is exhibiting clear negative population trends in the study area

(Figure 9e, f), it is not possible to determine the minimum viable population threshold for this site. Although an average of 24 territories might be a representative threshold, further confirmation is needed.

In contrast to the Eurasian sparrowhawk (Figure 10c, d), hawks, which are also strictly forest dwelling species (Rutz 2006), exhibited stable to increasing populations in Dadia NP. Specifically, the Northern goshawk showed the highest overall percentage variation increase, in terms of occupied territories (Table 4). This increase might have contributed to the decline in the Honey buzzard, due to inter-specific competition. While Honey buzzards do not have specific habitat criteria for nesting sites, they do locate their nests away from Northern goshawks to avoid their young being subject to predation (Gamauf et al. 2013, Hakkarainen et al. 2004). The increase in the closed canopy forest in Dadia NP might have allowed the more agile and competitive Northern goshawk to out-compete the Honey buzzard from optimum forest sites, allowing Northern goshawk numbers to increase to the detriment of Honey buzzard numbers. Twenty territories on average might be the stability threshold for the Northern goshawk in Dadia NP.

The Levant sparrowhawk has a much lower population size in the study region (Table 1), with a relatively stable fluctuation curve (Figure 10f). However, it is difficult to monitor this species because of its cryptic nature and preference for difficult-to- access terrain. Therefore, the short-term monitoring results are not sufficient to determine whether this species demonstrates natural population fluctuations (Figure 10e, f). Only part of this population actually breeds in the NP, as its main breeding territories occur along the riparian ecosystem of the Evros River (Poirazidis, unpublished data) which forms the natural eastern border of the NP on the border with Turkey. The decrease in the numbers of Eurasian sparrowhawk in the region (Figures 10c and 10d) might be explained by several factors, such as abiotic environmental conditions, resource availability, population density and inter/intra-specific interactions (Nielsen and Moller 2006). Competition and resources partitioning might be the primary causes for the population decline of this species, as optimum forest habitat appears to be increasing in Dadia NP.

The Common kestrel was the only falcon to exhibit decreasing trends in the Dadia NP (Figure 11a, b). However, the time-series is too short to make clear conclusions. The Common kestrel is quite abundant in Greece and is one of the most common raptors in the EU; thus, it is not expected for this species to be under pressure. One potential source of pressure might be the increase in Northern goshawks in Dadia NP, due to inter-specific competition and predation on the young (Petty et al. 2003). In comparison, the population trends of the Peregrine falcon and Eurasian hobby are clearly positive (Figure 11c, d, e, f), with numbers increasing in Dadia NP.

One of the most important and emblematic species for the region, the Egyptian vulture, is being subject to distinct decreasing population trends (Figure 12a, b). Successful conservation projects have been implemented throughout the distribution of the species range in Greece and abroad (Oppel et al. 2016) with the support of EU and national funding. However, the future of the species in Dadia NP is uncertain,

with only three breeding pairs remaining at Dadia NP. This decrease has been primarily attributed to illegal poisoning which is a pan-European issue (Hernández and Margalida 2009, Skartsi et al. 2014, Ntemiri and Saravia 2016). In the Balkans, the species has fragmented into six sub-populations, with major problems being poisoning, electrocution, direct persecution and changes in food availability (Saravia et al. 2016). These factors operate at large spatial scales, affecting birds both on breeding grounds and during migration and wintering (Oppel et al. 2017, Velevski et al. 2015). Recent research on radio-tagged birds in the Balkans has demonstrated that high mortality exists in first-time young migrants which die over seas during migration. In this species, inexperienced juveniles follow experienced adults to the foraging grounds; however, as the population shrinks, the number of experienced adults is declining, forcing juvenile birds to migrate without conspecific guidance (Oppel et al. 2015).

Conservation implications for management

A common concept in wildlife populations is that, under situations of pressure, limitation of resources, competition and habitat/landscape change, specialist species are the first to pay the price, due to their inability to adapt to rapid changes and have narrow niches (Ferrer and Negro 2004). In Dadia NP, slow but continuous landscape change has been recorded. The homogeneity index of the forest ecosystem has increased, with the slow disappearance of forest openings, due to the abandonment of traditional and open livestock farming (Triantakonstantis 2006, Poirazidis et al. 2015). In addition, traditional agricultural practices are converting to Helianthus crops which benefit contract-farmers. These two changes are negatively affecting raptors that require openarea habitats to hunt, as well as other species that preferentially inhabit land mosaics. In particular, this change is impacting both specialists in the region and biodiversity in general. While deforestation is a major problem in many countries (Carrara et al. 2015), the ecosystem of the Dadia NP is based on the balanced co-existence of man, forest, land use and livestock farming through a traditional framework that maintained forest clearings. This combination is the key to the success of this region in supporting a highly diverse landscape and biodiversity.

Specialisation is an expected evolutionarily response to habitat stability (in space or time), whereas the generalist strategy is a response to the lack of stability of the environment (Futuyma and Moreno 1988). For instance, Julliard et al. (2006) showed that, within a given habitat category, generalist species tend to aggregate at certain sites, while specialist species tend to aggregate at others. The authors suggested that specialists prefer more stable sites, while generalists the more unstable sites. Thus, the slow homogenisation of the Dadia NP forest is causing the previously balanced ecosystem to enter an unstable situation; consequently, generalists have been increasing in the forest, whereas specialists have been driven away in search of more stable ecosystems. The additional pressures of poisoning, electrocution and collisions with wind turbines also exert an influence on the relative survival of different species.

The complex ecosystem of Dadia NP, along with its complex raptor assemblage, creates a highly dynamic system where raptors are influenced by competitive interactions and various environmental parameters at different ecological scales (Hakkarainen et al. 2004, Sánchez-Zapata and Calvo 2004). This region is highly sensitive, making it difficult to balance all components and maintain high population numbers for all species through management (i.e. one action that benefits one species might hinder another). Yet, by combining the Systematic Raptor Monitoring in combination with species-specific ecological studies, it is possible to identify key issues allowing the timely implementation of mitigation measures.

Conclusions

At present, five major conservation issues exist in the Dadia NP raptor assemblage:

The total number of occupied raptor territories in the NP appears to be stable over time, showing the viability of the ecosystem to host raptors; however, a parallel increase in common and generalist species has been observed and this may be causing a reduction in the number of territories occupied by the more specialist species with smaller populations.

The increase in homogenisation through forest encroachment on forest clearings that are no longer grazed and the change in the type of agricultural crops being planted through contract-farming, might be contributing to the decline in certain species, like the Lesser-spotted eagle, Long-legged buzzard and Honey buzzard.

Furthermore, the increase in certain forest dwelling species that favour the increase in the closed-canopy environment, such as the Northern goshawk, might also be placing more pressure on these declining species, including the Eurasian sparrowhawk and the Common kestrel, due to its greater competitive ability.

Except for raptor species with increasing and decreasing trends, some raptors exhibited relative stability, but with noticeable fluctuations, such as the Levant sparrowhawk, the Booted eagle and the Golden eagle. To determine whether the observed trends in these and all other raptor groups are indicative of normal population fluctuations or deviations from regularity, long-term monitoring is required to remove "noise." Therefore, without doubt, the SRM in Dadia NP must be continued without interruption to enrich the time-series data and to optimise management in the decades to come.

The important anthropogenic pressure which has been recently identified by other studies, denoting the impact of illegal poisoning and collisions with wind turbines is an additional limiting factor on raptor populations.

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Supplementary material I

Map of Dadia-Lefkimi-Soufli National Park, vantage points and line transects

Authors: Konstantinos Poirazidis Data type: PNG image file

Explanation note: Map of Dadia-Lefkimi-Soufli National Park, indicating the 24 different monitoring vantage points and 10 different line transects, along with the borders of the Special Protected Area core within the National Park.

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Joining of the historical research and future prediction as a support tool for the assessment of management strategy for European beech-dominated forests in protected areas

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Abstract

European beech-dominated forests are crucial for maintaining biodiversity in forested mountain land-scapes of the European temperate zone. This paper presents the results of research and assessment of management strategy for mountain beech-dominated forests in the Jeseníky Mountains (Czech Republic). Our approach is based on combining research on historical development of the forest ecosystem, assessment of its current state, and predictions of future dynamics using a forest growth simulation model. Using such a method makes it possible to understand the current state of the mountain beech-dominated forest ecosystem and predict its future development as a response to specific management strategies. The application of this method is therefore appropriate for assessing the suitability of selected management strategies in mountain protected areas. Our results show that a non-intervention management for mountain beech forest in the next 80 years complies with the Natura 2000 requirement to maintain the existing character of the forest habitat. Thus, the current management plan for the beech-dominated forests in the Jeseníky Mountains does not require significant corrections in the context of its conservation targets (i.e. maintaining biodiversity and current character of the forest ecosystem dominated by beech). The results of this study suggest that combining the knowledge on historical development with forest growth simulation can be used as a suitable support tool to assess management strategies for forest habitats in protected areas.

Keywords

Beech-dominated forest, biodiversity, forest history, forest management plan, growth simulation model, Natura 2000

Introduction

Because most of the forests in Europe have been influenced by human activity, primeval forests currently account for less than 1 % of the total area of European forests (Vanbergen et al. 2005). Biodiversity and dynamics in these forests should be paid more attention as concluded by ministerial conferences on forest conservation in Europe, which have also declared the need of arresting the loss of biological diversity and supporting sustainable management (Parviainen et al. 2007). Primeval forests – thanks to their long history – are ideal research subjects for studies on spatial structure related to biodiversity (Nagel et al. 2013) and for studies on methods of sustainable forest management, which are aimed at maintaining forest biodiversity (Holeksa et al. 2009).

Primeval European beech forests consist of a mosaic of sub-stands which can be typified to the developmental stage (phase) by the structure of the tree layer (Fischer 1997). Thus the key to understanding the natural dynamics of primeval European beech forests is the concept of the small development cycle (Standovár and Kenderes 2003). Natural cyclic regeneration of primeval European beech forests mainly includes the tree species of the terminal phases, especially the beech itself. Changes of tree composition within the cycle are the exception – in European beech forest light-demanding tree pioneer species seem to be restricted to rather small patches under natural conditions (Yamamoto 2000), in contrast to the big developmental cycle in boreal spruce forest, where the role of pioneer tree species in forest natural dynamics is very important in large areas (Angelstam and Kuuluvainen 2004).

European beech and fir-beech forests are the predominant types of natural potential vegetation from planar to montane vegetation zones of temperate Europe (Bohn et al. 2002). For these forest ecosystems, the theory of the small development cycle of temperate mixed forest was conceptualized (Schmidt-Vogt 1985). The three phases of this development cycle (growth, optimum and disintegration stage) were defined based on analyses of the forest stand structure, which is considered to be an important biodiversity indicator generally used to support forest management decision-making (Kenderes et al. 2008).

The theory of the small development cycle has a long history. In 1959 Leibundgut developed the former idea of Rubner (1925) about different structural characteristics of sub-stands of European beech forests by documenting the different physiognomy of beech forest patches and analysing the growth behaviour of the trees in detail. Numerous authors used this idea to analyse virgin deciduous forests in temperate zones of Europe with special attention to European beech forests. Remmert (1992) postulated the following sequence of events and tree species concerning the European beech forests:

(1) A fraction of a beech forest stand becomes disturbed (e.g. by windfall).

- (2) The first phase of the tree layer regeneration is dominated by pioneer trees (e.g. *Betula* sp.) in open space of the disturbed patch, while the old beech trees along the border of the open disturbed area are going to die off owing to "sunburn" (the cambium of the exposed beech trunks dies).
- (3) Regeneration starts in the shadow of the pioneer trees and a new forest stand, again dominated by beech, builds up. Remmert finished the description of this cycle with the words: "Very often there is a short-cut in the cycle, and beech follows beech".

It is interesting, that Remmert's former idea about European beech maintaining dominance across the full forest development cycle has been confirmed in current studies (e.g. Glatthorn et al. 2017).

In 1995 Korpel published extensive results regarding the primeval European beech dominated forests in Slovakia (Appendix 1: fig. A1). The numerous examples presented in this study document the lack of pioneer-dominated phases in the regeneration cycle; beech, fir and spruce dominate all the regeneration phases. These results are supported by modelling of the tree species composition during the regeneration process. The most important parameters in competition amongst trees are height increment and shading capacity combined with shade tolerance. The European beech is a shade tree, and middle-aged and old growth beech have high rates of annual height increment. Models based on these parameters (e.g. Roloff 1992) predict a pure beech forest after 150 years of cycling. Thus it can be stated that cyclic regeneration of primeval European beech forests predominantly includes the tree species in the terminal phases and those that are shade tolerant, especially beech and fir. Under natural conditions for beech forest regeneration cycles (e.g. in protected areas without human activity) light-demanding pioneers seem to be restricted to small forest patches in short time episodes.

Currently the theory of the small development cycle is, in literature, also known as the concept of forest gap dynamics (Rugani et al. 2013). Investigation of gap characteristics and tree regeneration patterns is central for our understanding of beech forest dynamics (Vacek et al. 2017). Fine-scale gap-phase dynamics is a main characteristic feature of primeval beech-dominated forests in temperate Europe (Splechtna et al. 2005). Gaps are important in maintaining plant species diversity in beech forests (Degen et al. 2005). The size, shape, age and temporal changes of gaps in beech forests influence the regeneration patterns of tree species, due to different ecological traits of the particular tree species and the effects on the herbaceous layer in the history of soil conditions (Modrý et al. 2004).

Gap dynamics now only exist in strictly protected areas, because the most of European beech dominated forests have been managed in line with the paradigm of the Central European forestry (Hahn and Fanta 2001). This paradigm is based on very intensive treatment of forest stands by age classes, on prescriptive forest planning and on sophisticated forest management techniques. Natural processes in these forests have been largely ruled out in order to keep timber production. In the past three decades a criticism of this forest management practice has been formulated in relation to the ecological risks and the loss of biodiversity caused by the uniformity and simple homogenous structure of commercial forests. A growing interest for sustainable forest

management (which is aimed at maintaining forest biodiversity) is increasing. The implementation of sustainable forest management should be based on the knowledge of natural processes in primeval forests. It plays an essential role in strictly protected areas of forested landscapes. For example, most parts of the Kékes Forest Reserve (which protects 63 ha of primeval European beech montane forest ecosystem in Hungary) show the characteristic fine-scale mosaic of forest developmental stages sensu Korpel (1995). Standovár (et al. 2017) found the very intensive fine-scale dynamics of the beech forest, determined by natural stand dynamics in this strict forest reserve. The above mentioned authors proved that extinction and colonisation episodes even out at the stand-scale, implying an overall compositional stability of the herbaceous vegetation at the stand- scale after 17 years (whereas the abundance of vegetation changed considerably in relation to the partial closure of the canopy). They discovered that fine-scale gap dynamics, driven by natural process or applied as a management method, can warrant the survival of many closed forest specialist species in the long-run. An important decrease in herbaceous species cover in relation to light deficiency from a denser canopy during the process of forest gap dynamics was published by Lysik (2008) in a primeval beech forest in Poland. The author connected these changes with the massive recruitment of beech regeneration in the frame of natural forest dynamics. The denser canopies effect was observed as a decrease in light-demanding species and an increase in shade-tolerant species (Hédl et al. 2010). The species diversity of the herbaceous layer can be also influenced by invasive species, even in primeval forests, as noted by Lysik (2008). Ujházy et al. (2005) found relatively low species turnover in primeval fir-beech forest compared to commercial forests in Slovakia in all three developmental stages of forest dynamics - the growing, optimum and decay stages were differentiated rather by the value of abundance and dominance then by changes in diversity. Applying this theoretical knowledge in the practice of sustainable forest management (using only small regeneration areas) can support the conservation of the diversity of the herbaceous layer and prevent invasive or ruderal species with strong competitive abilities (Kelemen et al. 2012). Thus, the application of retention forestry in the frame of conservation planning for forested protected areas can be considered as a good support tool for integrating conservation targets to forest management practice (Fedrowitz et al. 2014).

Primeval European beech forests have higher stand diversity (at the level of structural and tree species diversity) than commercial forests on comparable sites (Král et al. 2010). All the presented results above obviously support this statement relating to the key importance of knowledge in forest dynamics in stand-scale for conserving forest biodiversity.

The structure of managed and unmanaged European beech-dominated forests (Bílek et al. 2011) and their treefall gap dynamics (Schliemann and Bockheim 2011) have long been subject to ecological research (Kenderes et al. 2008b) that has resulted in defining relevant principles for sustainable forest management (Angelstam et al. 2004). In recent years, research methods have shifted from simple visual estimation (Leibundgut 1993) to the utilization of maps of repeatedly measured trees (Vrška et al. 2001) and dendrochronological analyses (Podlaski 2004), and include studies of

natural regeneration (Barna 2011). Currently, forest growth models are also being increasingly used (Pretzsch 2010).

The near-natural and virgin ecosystems of European beech and fir-beech montane forests are characterized by a long-term cyclical alternating dominance of two main species: Silver fir (*Abies alba* Mill.) and European beech (*Fagus sylvatica* L.), (Saniga 1999). Some studies show that this phenomenon of long-term cyclical changes is rather a steady trend of the gradual replacement of fir by beech (Janík et al. 2014). A possible explanation of this trend may be the long-term response of forest ecosystems to past human activity, such as the medieval colonization of mountains associated with intensive local exploitation of forests by grazing and litter raking (Samonil and Vrska 2007). Understanding the historical development of mountain forest ecosystems is therefore of great importance for understanding their current state (Agnoletti and Anderson 2000).

Preserved segments of European beech-dominated forests represent valuable natural laboratories (Schultze et al. 2014, Standovár et al. 2017) that are often incorporated into national ecological networks (Jongman 1995) and international systems of conservation areas, such as the Natura 2000 network (Miko 2012). The high environmental value of these conservation areas requires a multidisciplinary approach to develop and assess forest management plans and policy instruments (Brukas and Sallnäs 2012), to evaluate management regimes (Torres-Rojo et al. 2014) and to seek specific forest management alternatives (Götmark 2013).

The main objective of this paper is to show the importance of integrating historical research of forest ecosystems for the assessment of forest management strategy, using an example of protected mountain beech and fir-beech forests of temperate Europe. The particular targets of this paper are:

- a) to explain the current state of the beech-dominated forest ecosystem in two study sites using historical analysis of their past,
- b) to predict the likely future trend in the dynamics of forest stands using a growth simulation model,
- c) to review the current forest management plan and decide whether it ensures maintaining the current character of the habitat and thus complies with the Natura 2000 conservation objectives for forest habitats.

Materials and methods

Study sites

The research was conducted at two mountain study sites (Fig. 1) in the 5th and 6th forest vegetation zones of the Czech Republic (Machar 2012). Both study sites are part of the core zone of the Jeseníky Mountains Protected Landscape Area (JMPLA).

The Jelení Bučina (JB) study site (45.84 ha) is located at 50°06'N, 17°17'E, 740–920 m above sea level on a mostly steep north-west slope with a gradient of 23 %.

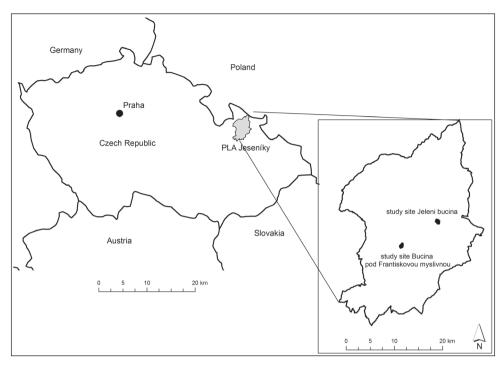


Figure 1. Location of Protected Landscape Area Jeseniky and both study sites (Jeleni bucina and Bucina pod Frantiskovou Myslivnou) in the Czech Republic.

The local bedrock geology consists of paragneiss. The soil is very stony with debris, mostly Ranker mesotrophic cambisols with the moder humus type. Most of the study site (91%) is covered by natural beech forest of an old-growth character with small patches of *Tilio-Acerion* ravine forest and minor fragments of *Caricion remotae* forest springs. This forest stands can be considered as forests sensu Korpel (1995) with natural dynamics and high biodiversity. A small part of the study site (9%) is covered by an even-aged, artificial monoculture of Norway spruce (*Picea abies* (L.) Karsten). The forest ecosystem of the JB study site belongs to the *Aceri-Fagetum sylvaticae* phytocoenological association.

The Bučina pod Františkovou Myslivnou (BFM) study site (25.49 ha) is located at 50°03'N, 17°11'E, 1050–1105 m above sea level. This site represents the highest-elevation beech forest in the entire Jeseníky Mountains. At higher altitudes, the beech forest naturally transforms into a natural spruce forest ecosystem (Machar et al. 2014). The BFM study site is located on a highly steep north-east slope with a gradient of 32 %. The local bedrock geology consists of highly weathered paragneiss and migmatites with amphibole. The soil is mostly modal mesotrophic cambisols with the moder humus type. Most of the study site (95%) is covered by natural beech forest admixed with spruce, sycamore maple (*Acer pseudoplatanus* L.), Scots elm (*Ulmus glabra* Hudson) and, very rarely, with fir. This part of the study site can be considered as forest stands with natural dynamics sensu Korpel (1995), which have typical high biodiversity. Adjacent to

the study site are artificial spruce monocultures. The forest ecosystem of the BFM study site belongs to the *Aceri-Fagetum adenostylosum* phytocoenological association.

According to the classification system of the Natura 2000 network, both study sites are classified as "Medio-European subalpine beech woods with *Acer* and *Rumex arifolius*" (habitat code 9140). According to the Habitat Catalogue of the Czech Republic (Chytrý et al. 2010), this habitat type is classified as "Montane sycamore-beech forests" (code L5.2). The borders of both study sites are identical to the borders of nature reserves of the same name. The forest management plan of both reservations for the period of 2013–2022 prescribes a non-intervention protection regime, allowing a spontaneous succession of the forest ecosystem.

Data collection, verification and processing

Data sources and analysis of historical development of forest ecosystems in the study sites

To study the historical development of forests in the JB and BFM study sites in the period of 1621–1947, we used the historical documents of the Teutonic Order, a former owner of the studied forests. The set of original documents is stored in the State Regional Archive in Opava in the "Central Administration of the Teutonic Order – Bruntál Estate" collection numbered 1.477–1.543 and forestry maps numbered 5.799–5.802, 5.945, 5.948 and 5.955 in scale 1:2880 In addition, we used several archive materials from the State Regional Archive in Janovice from the collections "Loučná Estate" and "Velké Losiny Estate". Since 1947, the forests in both study sites have been owned by the state. Valuable historical data for this period were found in the forest management plans deposited in the archives of the Forest Management Institute in Brandýs nad Labem. Recent data necessary for the analysis were taken from the forest management records deposited at the JMPLA administration office.

Prediction of future dynamic of forest ecosystems in the study sites

To predict the future forest development in the JB and BFM study sites, we used the SIBYLA growth simulation model (Fabrika and Dursky 2006) modified for the specific conditions of the Czech Republic based on a previously created climatic model (Simon 2007).

At both study sites research plots were defined in such a way that they reflected the characteristic conditions of the forest in the study sites and their typical tree species composition (Table 1). The centre of each research plot was recorded with GPS to allow a potential research repetition. The research plots (100×100 m) were selected using the FieldMap software (IFER-Monitoring and Mapping Solutions Ltd.).

All trees wider than 5 cm of DBH were located and marked in a rectangular coordinate system. The following parameters were measured: diameter of the tree trunk at 1.3 m (diameter at breast height, $d_{1.3}$), total height of the tree (m), height of green tree top setting, and social position. We employed the dendrometric measurements from

Study site	GPS coordinates	Type of forest ecosystem	Tree species composition (% proportion in the forest stand)		
JB	50.06633°N, 17.17945°E	Highly heterogeneous mountain beech forest with patches of natural regeneration	Canopy layer in the disintegration stage: BK 60, KL 20, JL 10, SM 10 Understory formed by isolated patches of natural beech regeneration: BK 100		
BFM	50.03446°N, 17.11800°E	Highly heterogeneous mountain beech forest with extensive natural regeneration and admixture of fir	Canopy layer in the optimum stage: BK 94, KL 3, JL 1, SM 1, JD 1 Understory formed by patches of natural regeneration diffused over the entire site: BK 96, JD 4		

Table 1. Forest stand characteristics for the Jeleni Bucina (**JB**) and Bucina pod Frantiskovou Myslivnou (**BFM**) study sites as used for the growth simulation model.

Used abbreviations of tree species: $BK - Fagus \ sylvatica$, $KL - Acer \ pseudoplatanus$, $JL - Ulmus \ glabra$, $SM - Picea \ abies$, $JD - Abies \ alba$.

all research plots to create stand height curves, using the non-linear Naeslund regression height function.

In 2012, we carried out visualization and simulation of the future forest development under a non-intervention management (i.e. spontaneous forest development in relation to conservation targets of Natura 2000 sites) using a growth simulation model. The growth simulation was based on a mortality model, consisting of two components: probability of tree necrosis (Dursky 1997) and competition threshold (Pretzsch et al. 2002). We simulated future forest development for the periods of 25, 50 and 80 years, and calculated the development of the leaf area index (LAI), an important indicator of natural beech regeneration.

For both study sites we predicted changes in tree diameter diversity of the beech stands (excluding individuals originating from natural regeneration) and changes in their standing tree volumes (m³.ha⁻¹). Further, we evaluated the development of tree species composition, representation, and horizontal and vertical structure using the following structural indices: Clark-Evans aggregation index (Clark and Evans 1954), standardized Arten-profile index (Pretzsch 2005) as a relative rate of diversity, and Pielou segregation index (Pielou 1977).

Linking historical research with growth simulation model to assess forest management strategies in the study sites

The assessment of forest management strategies currently implemented in the JB and BFM study sites is based on combining research of historical development of the studied ecosystem and interpretation of future development of main edificators predicted by the growth simulation model. The historical research helps to objectively explain the current state of the forest ecosystem determined by the forest management and other human activity carried out in the study sites. The growth simulation model of future forest development predicts the structure of woody vegetation over defined time periods in the future based on specific management strategies delineated in the protected areas management plan.

The final synthesis based on the results of both analyses (historical research and growth simulation) allows the assessment of whether the spontaneous (succession) development of the ecosystem resulting from the current forest management plan ensures maintaining the existing character of the mountain beech forest habitat as defined by Natura 2000 (Moravec et al. 2000). This synthesis then allows the suggestion of possible adjustments in the forest management, in order to comply with the protected area mission that is, retaining defined habitat character and biodiversity as defined by the Habitats Directive (Roth 2003).

Results

History of forest ecosystems in the JB and BFM study sites

Until the 12th century, the Jeseníky Mountains were part of the "borderline forests", an unpopulated and forested border mountain chain that formed a natural defense of lowland areas of the Bohemian Kingdom, intensely inhabited since the Neolithic period (Bouzek 2011). The first colonization efforts in the Jeseníky Mountains associated with anthropogenic impacts date back to the 15th century, when the exploitation of iron ore and precious metals started (Hosek 1970). At the end of the 17th century, the alpine meadows above the tree line were first used for cattle and sheep grazing during the summer months (Anonymous 1689). Sheep grazing on the alpine pastures was most intense in the 19th century on the eastern slopes of the Bruntál Estate, as evidenced by the name of today's recreational site – Ovčárna ("sheep stable"). Mowing and harvesting of hay in the 19th century was another significant human activity that also extended into the naturally sparse forest of the tree line areas. For instance, 50–70 car loads (circa 250-280 quintals) of hay were harvested annually on the grounds of the Bruntál Estate (Anonymous 1866). In contrast, the alpine beech forests and climax spruce forests below the tree line were only affected by selective logging and had the character of an old-growth forest by the mid 18th century. In 1750, the forests on the Loučná Estate (which included the area of today's BFM study site) were described as being full of decaying wood and many fallen trees, making the passage for both man and livestock dangerous and, in some places, completely impossible. Moreover, these forests allegedly provided a safe shelter for large wild animals such as bears, wolves and lynxes (Anonymous 1750). The alpine forests of the Jeseníky Mountains began to be more significantly affected by the selective logging only at the end of the 18th century due to the high demand for wood needed for the intensively developing iron industry (ironworks and forges) in the lower areas of the mountain region. Exploitation of the alpine forests by logging had a great impact as the forest stands did not have large timber volumes - the "Josefsky Cadastre" from 1786 (Oprsal et al. 2016) shows that the forests had about 180 m³ of timber in harvest age per hectare. The high demand for wood by the iron industry led to a gradual transition from selective logging to clearcut logging. The original old-growth forests on the Bruntál Estate (including the area

of today's JB study site) were completely harvested by selective logging around 1750, but they regenerated naturally. Between the years 1778–1808, a repeated harvest took place throughout the Bruntál Estate with almost no subsequent artificial restoration, leaving the stands to be spontaneously renewed by natural regeneration. Based on the economic interests of the Estate owner (the Teutonic Order) in timber harvesting, a first forest management plan was formulated in 1803 by Jan Vavřinec Knappe (Knappe 1803). Forest districts were divided in smaller units in order to reduce the annual harvest volumes. In 1803, stand No. 401 B, forming today's nature reserve and the JB study site, was described as a 50-year old forest stand of uneven stocking level, with beech as a predominant species (one third of all tree individuals). One sixth of the vegetation consisted of sycamore maple, another sixth of spruce, and the remaining third consisted of goat willow. Fir was present only as a rare admixture. The next forest management was carried out in 1827 following identical principles (Krones 1827). There was no more evidence of the goat willow on the site in the later forest management plan in 1862 (Anonymous 1862). When the forest reached its harvesting age, a harvest plan was created to schedule timber extraction using the shelter-wood cutting method (to support natural regeneration). However, the harvest plan was probably never fully implemented due to a strong windstorm that substantially damaged the forest in 1868, and uprooted mainly the spruce trees (Anonymous 1875). Frequent wind-throws in the following years slowly lowered the forest stocking level down to 0.6–0.8 in 1884. In 1910, the given forest stand was attached to the adjacent forests dominated by spruce (partly outside of the JB study site). The newly formed timberland (marked as No. 60) had an area of 34.4 ha, was 140 years old, and consisted of beech (60 %) and spruce (40 %) (Anonymous 1910). Between 1910 and 1919, a significant number of spruces were removed due to health reasons, so the species practically vanished from the site. In the subsequent period up until the Second World War, the forest management plans prescribed harvesting large volumes of beech. In fact, however, such harvests were rarely carried out, with the exception of stand thinning in 1939 (harvest volume of mere 300 m³) to make space for the naturally regenerating beech that forms the basis of today's JB study site. From this year on, no other planned harvests took place. But after 1950, fir trees were selectively harvested in the JB study site and the adjacent areas. In 1960, the forest in the JB study site was ranked as an overaged stand dominated by beech, with 95 % canopy cover in the southern part (Anonymous 1960). At that time, the establishment of a nature reserve was discussed and thus the forest management plan prescribed "conservation management activities" (i.e. non-intervention management) that have been enforced on the site up to the present. The forest stand in the Jelení Bučina (JB) study site was officially proposed for legal protection in 1970 (as a "beech old-growth forest"), but the reserve was not declared until 2001. In terms of historical development, the current forest ecosystem in the study site is not a primary old-growth forest, but, for the most part, a remnant of the first generation forest following such a primary old-growth forest, resulting mostly from natural regeneration.

Historical development of the BFM study site was essentially similar. At the turn of the 19th century the site was covered by an old forest stand dominated by beech,

admixed with spruce, sycamore maple and elm. Until the end of the 18th century, selective logging was applied to support natural regeneration of beech. Around 1800, the old trees were almost completely harvested but it is not known whether it was a deliberate timber extraction or a forced harvest after a windstorm (Pechanec et al. 2015a). At the beginning of the 19th century, only few isolated free-standing trees remained from the original forest. The understory, which naturally regenerated after the radical harvest, later formed the canopy layer of today's forest stand in the BFM study site. In 1856, the regenerated saplings already formed an extensive young beech stand. Beech has regenerated naturally in gaps created by fallen old trees. Sporadically, the open gaps were also used for sowing and planting spruce, using seeds of local origin. Spruce was being introduced for economic reasons and its proportion in the forest was gradually increasing. In 1894, the forest in the BFM study site consisted of beech (70 %) and spruce (30 %), admixed with sycamore maple. However, spruce was being greatly affected by the local abiotic factors (especially windstorms), so its proportion in the study site had reduced to a mere admixture by 1952. In 1955, the Ministry of Culture declared the "Bučina pod Františkovou Myslivnou" a nature reserve to protect the 160-180 year old "sycamore-beech old-growth forest". Since that time, no deliberate timber extraction and artificial regeneration has been carried out, with the exception of a thinning harvest in the spruce stands, which were attached to the reserve due to land consolidation. The current forest in the BFM study site has a visual character of an "old-growth forest", although it is in fact a second generation forest after the primary old-growth forest. The forest is of high local genetic value, as it resulted from natural regeneration - similarly as in the JB study site.

The main findings from the historical analyses of both study sites can be generalized for Central European mountain beech forests as follows:

- A) A decrease in the occurrence of fir and a stable dominance of beech in natural forests is obvious during the long-term history of forest stands.
- B) Despite the influence of human activity, most beech forests in protected areas can be considered as natural forests with natural dynamics.
- C) In fully protected areas we can identify the first generation forest stands following former primary old-growth forest, resulting mostly from natural regeneration with a high potential for maintaining natural forest dynamics (and biodiversity) under the theory of the small development cycle.

Predicting future dynamics of forest ecosystems in the JB and BFM study sites

Visualization of the growth simulation results for the JB study site shows that "non-intervention management" induces significant changes in the forest structure (compared to the current state) already in the first forecast horizon (25 years) (Fig. 2): The canopy layer of the mountain beech forest gradually thins out. For the second forecast horizon (50 years), the growth simulation shows a development trend to-

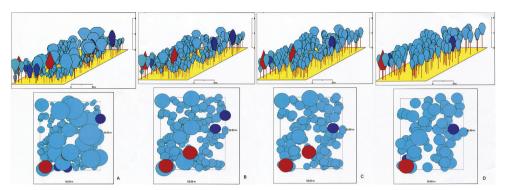


Figure 2. Growth simulation model for the Jeleni Bucina study site: current state (**A**) and future forest development for the periods of 25 years (**B**), 50 years (**C**), 80 years (**D**) (light blue: *Fagus sylvatica*, dark blue: *Acer pseudoplatanus*, red: *Picea abies*).

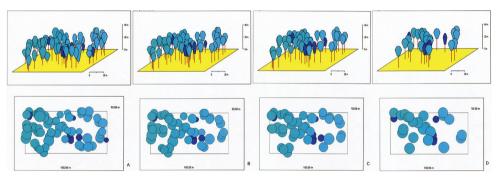


Figure 3. Growth simulation model for the Bucina pod Frantiskovou Myslivnou study site: current state (**A**) and future forest development for the periods of 25 years (**B**), 50 years (**C**), 80 years (**D**) (light blue: *Fagus sylvatica*, dark blue: *Acer pseudoplatanus*).

wards reduction of the forest stocking level and expansion of vacant bare gaps that provide space for natural regeneration of beech. The forest transitions from the disintegration stage to the growth stage, manifests clearly in the forecast horizon of 80 years. The growth simulation for the BFM study site shows a very similar trend (Fig. 3). The results of growth simulations for the diversified mountain beech forests in both study sites identically indicate that the trend of the gradual forest development leads to a partial shift in forest stages. This corresponds with the theoretical model of the small forest development cycle. The non-intervention management regime in both study sites advances the dominance of beech (Figs 2 and 3). In contrast, an increase in the proportion of sycamore maple, elm or fir does not seem very likely. The changes in leaf area index (LAI) for both study sites are shown in Figure 4. The initial values of LAI for the JB and BFM study sites calculated by the growth simulation model are 2.2 and 5.6, respectively. The long term development of LAI on both sites follows an identical trend – in 50 years, the LAI is equal for both sites as they reach the

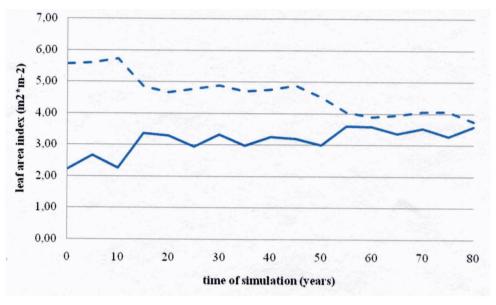


Figure 4. Time development of the leaf area index in study site Jeleni Bucina (solid line) and Bucina pod Frantiskovou Myslivnou (dashed line) for prediction period of 80 years.

growth stage with a dominance of beech which is tolerant to reduced light conditions for natural regeneration (Fig. 4).

Figure 5 shows a graphical representation of the Naeslund height function for both study sites. Forest stands in the study sites have slightly different spatial structure (forest canopy cover in the BFM study site is more open and trees are higher than in the JB study site), which is reflected in the slight phase shift of the height function curve between the two sites (Fig. 5). Under the non-intervention regime, the proportion of trees with the smallest DBH in the BFM study site decreases (these individuals "move" to classes with higher DBH – see Fig. 6). In contrast, the proportion of individuals with DBH >50 cm remains remarkably stable in time (Fig. 6), which is important both in terms of maintaining the existing habitat character and stable state of the forest stand, as well as in terms of the potential natural regeneration. In the JB study site (Fig. 7), the prediction indicates a decrease of trees in the understory as compared to the status quo. Similarly as in the BFM study site, the proportion of large trees remains stable.

The long-term trend of spontaneous transition of forest ecosystems on both study sites from the disintegration stage to the growth stage is documented by the timber stocks prediction model (Fig. 8). Over the entire simulation period of 80 years, the total timber stocks in the JB and BFM study sites will decrease by 20.7~% ($34.5~\text{m}^3$) and 25.2~% ($45.7~\text{m}^3$), respectively.

Table 2 provides an overview of three structural indices used in the study. The horizontal structure of the forest stands according to the Clark-Evans aggregation index has

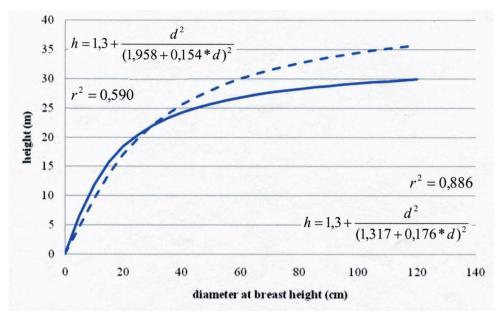


Figure 5. Naeslund height function for both study sites: Jeleni Bucina (solid line) and Bucina pod Frantiskovou Myslivnou (dashed line).

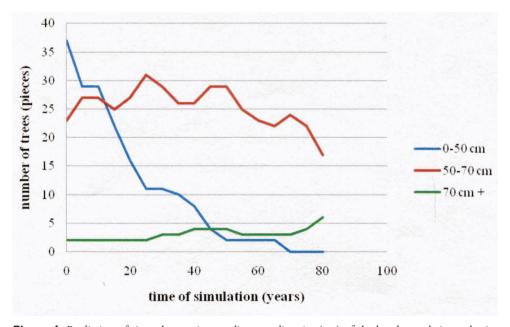


Figure 6. Prediction of time changes in tree diameter diversity (cm) of the beech stands in study site Bucina pod Frantiskovou Myslivnou.

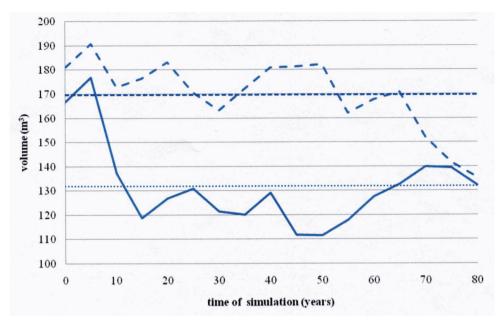


Figure 7. Prediction of time changes in tree diameter diversity (cm) of the beech stands in study site Jeleni Bucina.

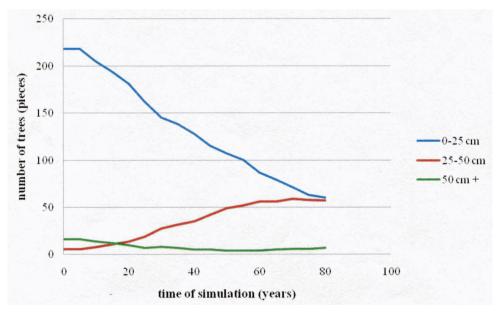


Figure 8. Prediction of time changes in standing tree volumes (m³.ha⁻¹) for both study sites: Jeleni Bucina (solid line) and Bucina pod Frantiskovou Myslivnou (dashed line).

	Index						
Time of simulation	Clark-Evans index		Arten-profil index		Pielou segregation index		
	JB	BFM	JB	BFM	JB	BFM	
0	0,880	0,852	0,574	0,463	0,437	0,442	
5	0,880	0,852	0,578	0,467	0,434	0,448	
10	0,886	0,865	0,615	0,489	0,483	0,498	
15	0,923	0,855	0,623	0,469	0,533	0,515	
20	0,942	0,860	0,618	0,474	0,527	0,522	
25	0,953	0,864	0,592	0,470	0,492	0,508	
30	0,967	0,897	0,613	0,498	0,525	0,545	
35	0,963	0,897	0,600	0,498	0,536	0,548	
40	0.960	0.897	0.588	0.488	0.527	0,548	

Table 2. Indices prediction on the Jeleni Bucina (JB) and Bucina pod Frantiskovou Myslivnou (BFM) study sites after spontaneous development.

a slight tendency towards lower aggregation. The trend is rather clear for the JB study site, but indistinct for the BFM study site. The spatial diversity of the forest stands (according to the Arten-profile index) on both study sites is medial with a very slight increase as a result of the decline of the parent stand followed by natural regeneration in the process of transitioning from the disintegration to the growth stage. The Pielou segregation index indicates that the spontaneous forest development of beech stands on both study sites tends to lead to a more regular arrangement of trees (Table 2).

The main conservation target of the management plan for both nature reserves as well as Natura 2000 sites (comprising the JB and BFM study sites) is to maintain the current character of the habitat, as required by the European Union Habitat Directive No. 92/43/EEC. From this perspective, it is notable that the growth simulation model predicts changes in the spatial structure and shifts in the development stages of modeled forest ecosystems under the non-intervention regime for the next 80 years (see 3.2), but it does not assume any significant changes in the character of the habitat code 9140 as defined by the Natura 2000 classification system.

Discussion and conclusion

As a consequence of changing climatic conditions in Europe (CO₂ content, air temperature, precipitation, heat waves and drought episodes) it is expected that European beech forests will change in the future (Bošela et al. 2016; Machar et al. 2017a). According to present knowledge, beech forests will probably remain the most important natural forests in temperate Europe during this century (Lindner et al. 2014). Some of the most important predictions regarding European beech forest under climate changes are the following: (1) The geographical area of European beech will shift northwards and will reach to higher altitudes in the mountains (Garamvoelgyi and Hufnagel 2013). (2) The dominance of European beech in temperate European deciduous for-

ests will be broken in some cases, because European beech tends to retreat in upland and submontane landscapes where the summer seasons will become drier in the future (Saltré et al. 2015). (3) Increasing CO₂ content can increase the biomass (and wood production) of beech stands in newly occupied areas at higher altitudes (Machar et al. 2017b). Králíček et al. (2017) confirmed these results by founding of strong correlation between the radial increment of European beech and the temperature in mountain areas. Long-term simulations of climate change's impact on forest dynamics in Silver fir-European beech stands in Dinaric Mountains in Slovenia (Mina et al. 2017) revealed that European beech will be favoured by higher temperatures in contrast to drought-induced growth reduction in Silver fir. But generally there is a knowledge gap on the details of the floristic structure in the future beech forest communities under changing climate conditions.

Predicting future forest dynamics in stand-scale is an essential component of sustainable forest management (Thurnher et al. 2017). The general shift from forest management aimed at pure forest stands (coniferous monoculture) to sustainable forest management aimed at multifunctional uneven-aged mixed-species forests requires modern tools for yield projections which predict future stand development for different management regimes, including conservation targets (Simon et al. 2015). As a result, single-tree growth simulators have been developed. Tree growth simulators predict the future growth of forest in stand-scale and can be used as support tools for conservation forest biodiversity. An important advantage of tree growth simulators are there flexibility, based on using forest inventory data as inputs for modelling of silvicultural management scenarios in order to produce management plans, which can be used for forested protected areas. A combination of routine forest inventory data and the set of functions implemented in the transparent forecast system can be an important support tool for conservation planning in the frame of conservation forest biodiversity. Growth simulation models for the prediction of future forest development represent a promising tool for sustainable management of forest ecosystems (Pretzsch et al. 2015, Simon et al. 2014). However, growths models have not yet been fully integrated into the forest management practice (Porté and Bartelink 2002, Machar et al. 2016) and are rarely used to assess the forest management strategies in conservation areas (Sodtke et al. 2004). The advantage of growth simulation models lies in reasonably accurate predictions of the future character of forest stands under variable growth conditions and types of forest management (Kolström 1998). We believe that the combination of a growth simulation model with the analysis of historical forest development could be more widely used, especially to assess management plans for conservation areas that consist of forest ecosystems (Idle and Bines 2005, Machar 2010). Understanding the historical development of forest stands can significantly improve our understanding of their current state (Honnay et al. 2004).

The multidisciplinary combination of the two different methods (from social and natural sciences) helps to make the assessment of forest management strategies in conservation areas more objective, as it enables the prediction of likely development of forest stands under specific management plans in a specific nature reserves (Peng 2000).

This is particularly important in the Natura 2000 conservation areas (Parviainen and Frank 2003) because the growth model enables the prediction of the likely future development of forest ecosystems based on the conservation targets of a particular forest management plan (Villard and Jonsson 2009).

Based on the combined results of the growth simulation model and the historical analysis, it is possible to evaluate the current management strategy and suggest potential adjustments in the forest management plan, in order to comply with the mission of a protected area, i.e. retaining the defined habitat character and biodiversity (Pechanec et al. 2015b).

Simulation results for both study sites support the theory that the anthropogenic influence is a major cause of fir decline in European mountain beech forests (Paluch 2007). Our results from historical analyses in both of the study sites confirmed this fact. This founding is important in the frame of historical background of European mountain forests, where generally old-growth forests originally co-dominated by fir and beech appear to be transitioning to forests dominated by beech, regardless of the disturbance history, which suggest that beech expansion may be a robust process (Jaloviar et al. 2017). From the nature conservation point of view, the return of fir as a natural component of mountain beech forest in the nature reserve would be desirable (Kral et al. 2014). As shown by the growth simulation model, increasing the proportion of fir in both study sites would only be possible under a targeted intervention by forest practitioners into the current non-intervention management regime. The fir could be reintroduced in these ecosystems, provided that a planting material of local origin is available and individual protection of seedlings against ungulates is secured (Vacek et al. 2014).

The relative value of LAI depends on the character of assimilation organs, that is, on a forest type, as demonstrated by empirical measurements by Jarvis and Leverenz (1983). The high LAI value for the BFM study site reflects a significant reduction in solar radiation as it passes through highly differentiated vegetation. The calculated initial (i.e. current) LAI value for the JB study site is also relatively high. The LAI value for European temperate forests ranges from 0 (bare areas with no vegetation) to 6 (dense forest stands) (Fabrika and Pretzsch 2011).

The computed shape of stand height curves is characteristic only for a particular stand age, and the curves shift with stand age (Laar and Akca 2007). This fact corresponds with findings presented in this paper for both study sites (Fig 5.) in the Jeseníky Mountains (Senfeldr and Madera 2011).

Differences in altitude occurrence of European beech can be considered as influences of past silvicultural management (Štefančík and Bošela 2014). Also the long forest continuity is an important factor in supporting the forest's specialist organisms (Ódor and Standovár 2001). The relationship between the richness of ancient forest indicator plants and other biodiversity in the case study of macrofungi was presented by Hofmeister et al. (2014). Forest stand structure in Europe is generally the result of the environmental history of a phytocoenosis and includes anthropic influences. The study of the disturbances and structural dynamics of forests in the past is very important for

conservation efforts in fully protected areas, as shown by Bianchi et al. (2011) in beech forests in the Apennines. Fully-protected natural forest reserves with long-term forest continuity are living nature laboratories, where the process of forest dynamics can be investigated over long time periods. Thus historical reconstructions of past changes in forest dynamics and human management activities in forest stand-scale can be very a important tool for forest management that attempts to emulate natural forest dynamics in order to conservation forest biodiversity, as proved Firm et al. (2009) based on study of the disturbance history of mountain forests in the Slovenian Alps.

The historical analysis of forest development on both study sites revealed that the current structure of forest stands is strongly influenced by the former management. If the modification of the tree species composition is desired for conservation purposes, in order to get closer to the theoretical assumption of potential vegetation, then some form of management intervention will be necessary – e.g. the artificial reintroduction of fir that disappeared due to anthropogenic activities in the past (see section 3.1).

For both study sites, the growth simulation model indicates changes in forest development over an 80-year time horizon. These future growth changes correspond with the theoretical model of the small development cycle of European temperate forests. They are significant in terms of biodiversity protection, as the long-term spontaneous development of forest ecosystems leads to the creation of valuable habitats for numerous endangered species (Sebkova et al. 2011). In the long term, non-intervention forest management will undoubtedly contribute to an increase of dead wood material in the ecosystem, and thus affect the biodiversity of organisms dependent on various forms of decomposing wood and dying old trees (Vandekerkhove et al. 2009).

Based on the synthesis of the historical research and the growth simulation model, we conclude that in the next 80 years the current non-intervention forest management, which is based on spontaneous (succession) development of the ecosystem, does not contradict the Natura 2000 requirement of protecting this habitat type. From this perspective, the forest management plan for the JB and BFM study sites (and the reserves in which they are located) does not require any corrections in the context of their conservations goals, that is, maintaining the habitat character and biodiversity.

The historical research revealed that both study sites (although having a visual character of an old-growth forest and therefore being protected as nature reserves and Natura 2000 sites) are in fact a second-generation forest following the previous primary old-growth forest. The growth simulation model for both study sites predicts a partial shift in forest stages, corresponding with the theoretical model of the small development cycle of European temperate forest. These future growth changes in the forest ecosystem are significant in terms of biodiversity protection, as the long-term spontaneous development of forest ecosystems leads to the creation of valuable habitats for numerous endangered species.

The studied forest ecosystems are part of the European network of nature protection areas – Natura 2000. The forest management strategy applied in both of the study sites, resulting from the categorization of protected areas by the IUCN (Dudley 2008), is a non-intervention management (IUCN Category Ia – Strict

Nature Reserve). The presented results show that the non-intervention management for mountain beech forest in the next 80 years complies with the Natura 2000 conservation targets to maintain the existing character of the habitat. The multidisciplinary research helps to make the assessment of forest management (in any geographical and environmental conditions) more objective, provided that basic historical and dendrometric data about the studied forest ecosystem are available. The application of this multidisciplinary approach is therefore particularly appropriate for assessing the suitability of selected management strategies in protected areas. The results of this study suggest that combining the research on historical development with a forest growth simulation can be used as a suitable decision-support tool to assess management strategies for forest habitats in protected areas worldwide.

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Appendix

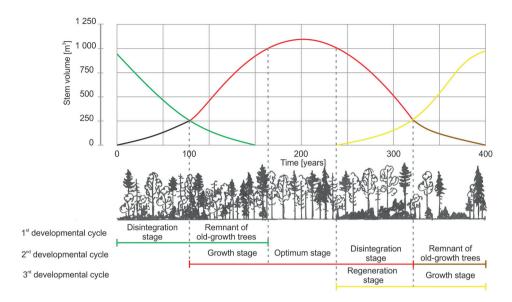


Figure A1. Mosaic of small development cycles in primeval beech-fir mountain forest – adapted from Korpel (1995), Natural Reserve Dobrocsky Prales, Slovak Republic.





Connecting the social and the ecological in the focal species concept: case study of White Stork

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Abstract

In this article we provide an overview of five case studies of initiatives using the image of White Stork as a focal species. Our case studies are preceded by a short overview of existing approaches to achieve broader environmental goals through species conservation and a review of the social, ecological and social-ecological importance of White Stork. With the use of the above, we investigate linkages, complementarity and friction between the ecological, social and social-ecological perspectives on focal species, and eventually propose a framework for a more multi-targeted approach. The proposed concept of a social-ecological keystone species recognises social-ecological system complexity and goes beyond traditional divisions into ecological and social. Our approach extends the cultural keystone species concept to tie into new spheres – modern societies with more indirect connections to nature as well as indigenous communities, and all forms of human relationships with other species, not just for consumption – and to explicitly include the ecological significance of a species. Apart from serving as a potentially highly useful conservation proxy, a social-ecological keystone species emerges as a vehicle for ecological literacy, expanding from an interest in a species to learn more about the system of which it is part. White Stork, with its long history of coexistence with humans and many linkages with specific cultural practices offers an excellent example for discussing the broader social-ecological relevance of species in establishing meaningful connections to nature.

Keywords

Social-ecological keystone species, keystone species, flagship species, cultural keystone species, social-ecological systems

Introduction

Conservationists select certain species as a focus of their activities – or at least for public relations or visibility purposes (Tisdell and Swarna Nantha 2007; Clucas et al. 2008). They compete for attention and funds with other messages that are also promoted in a similar way, using key, spectacular, sometimes shocking pieces of news (Kotler et al. 2002). The selection of species is often based on emotions or ad hoc reasoning, rather than a broad understanding of these species by the addressees of these campaigns (Home et al. 2009; Żmihorski et al. 2013), not to mention their ecological importance. However, for this approach to be effective, these species need to be selected with due care to ensure the conservation of habitats and many other species connected to them (Kontoleon and Swanson 2003). Interestingly, species "importance" has also been argued based on a deeper understanding of the social-ecological interactions that have led to the prominence of certain species for certain social groups, as reflected in the concept of cultural keystone species (Cristancho and Vining 2004; Garibaldi and Turner 2004). In this article, our objective is to investigate the linkages between the ecological, social and socialecological approaches to the focal species idea, and to highlight the potential of a more comprehensive social-ecological approach to address multiple targets simultaneously.

We provide an overview of the different perspectives on what constitutes particularly important species present in environmental conservation and social sciences, and eventually bring these different perspectives together to scrutinise the White Stork (Ciconia ciconia). The example of White Stork helps us identify outstanding issues that need to be addressed in future discussions on focal species. White Stork holds a particularly high social/cultural status in most areas where it is present (Kronenberg et al. 2013) and it is also recognised as an umbrella species and used as an ecological indicator (Tobolka et al. 2012). Thus, this species provides an interesting case for studying the different ways in which a single species can be conceived of and used in specific circumstances and to achieve various outcomes. We investigate five campaigns that either focused on White Stork conservation or relied on the special status of this species among the general public as an argument for broader nature conservation. Eventually, we analyse the focus on White Stork in each case study campaign: whether it was its social/cultural appeal only, or an understanding of its broader social-ecological importance. In the discussion section, we suggest that the previously considered ecological, social and social-ecological approaches to focal species could be broadened to cover additional aspects of the species' importance. We highlight the merits of using the more inclusive concept of social-ecological keystone species in the broader context of environmental conservation and conclude with some suggestions on how and why species should be selected for conservation purposes.

Different perspectives on the selection of particularly important species

Several concepts have been proposed to denote focal species, organisms particularly important from the point of view of nature conservation, each with its own "myriad of

definitions and applications" (Zacharias and Roff 2001: 60). Some of these concepts focus on the ecological importance of species (keystone, umbrella and indicator species in particular) while others refer principally to their social perception (flagship, symbolic and iconic species in particular) (see Tables 1 and 2 for exemplary definitions). Although these concepts focus on single species, these species are meant to represent broader conservation purposes. Many of these concepts are often used interchangeably and academic discussion continues with regard to which of them are the most accurate as proxies for nature conservation (Simberloff 1998; Kontoleon and Swanson 2003; Caro et al. 2004; Roberge and Angelstam 2004; Favreau et al. 2006).

Some authors (e.g. Carignan and Villard 2002) suggest that for scientific reasons ecological criteria for selecting focal species seem to be more relevant for conservation purposes than social criteria. Indeed, as a social construct, flagship species can be selected and promoted with the use of creative processes involving conservationists, artists, media specialists and other stakeholders. They are often selected in response to widespread ecological illiteracy where keystone, indicator and umbrella species may not necessarily be known to or appreciated by the wider society. Besides, people may be more concerned with the loss of a charismatic species than with the loss of habitat, and they may not necessarily be able to see the link between habitat loss and threats to the species (Entwistle et al. 2000).

However, to some extent, all of these concepts involve social and ecological aspects, albeit to a varying degree. The perceived ecological importance of species is based on social knowledge, cultural norms and values. Conservation projects are built on the knowledge of their authors and preferences of their sponsors. Modern approaches to conservation often draw on local traditional knowledge, citizen science and many other forms of stakeholder involvement (Berkes 2009).

Meanwhile, the socially oriented selection frameworks involve at least some level of ecological knowledge, at the very least on the part of those who promote certain species as socially important. Flagship species may be selected based on their conservation status (Rodrigues et al. 2006), population size, ecological importance (Home et al. 2009), although - probably most often - what counts is their appearance, charisma and utility (Walpole and Leader-Williams 2002; Serpell 2004; Martín-López et al. 2008; Veríssimo et al. 2009; Ullmann and Stachowitsch 2015). Appearance is mostly related to large body size, physical attractiveness and likeability which involve some kind of similarity to humans (Gunnthorsdottir 2001; Tisdell et al. 2006) or relatively babyish looks which trigger affection in adult humans (Gould 1980). Charisma may be related to appearance (aesthetic and corporeal charisma) or it may relate to broader detectability and distinctiveness of species (Lorimer 2007). Utility refers to people's perceptions of the benefits related to those species or their instrumental value (Serpell 2004). Human preferences towards species also depend on broader social and cultural values, the physical and behavioural characteristics of species, people's knowledge of those species, and finally "past and present interactions with particular species, including cultural factors, such as religiosity or traditional practices, and social factors, such as property relationships or recreational use" (Martín-López et al. 2007: 68). In any case, selection criteria depend on context and purpose, e.g. at which level (local, national, global) a species is going to be presented

Concept	Definition/understanding	
Keystone species	Species that has a particularly significant impact on the state of a community or an ecosystem (disproportionate to the species' abundance or biomass) (Mills et al. 1993; Power et al. 1996; Simberloff 1998).	
Foundation species	Highly interactive and abundant species, playing a dominant role in an ecosystem, a less restrictive variant of the keystone concept (Soulé et al. 2003).	
Umbrella species	Species which have relatively large habitat/area requirements and saving them automatically saves many other species (Simberloff 1998: 249).	
Indicator species	Used to monitor environmental conditions and community/habitat/ecosystem composition (Carignan and Villard 2002). Similarly, sentinel species, usually used as biomarkers (Lower and Kendall 1990; Bossart 2006), and signal species (Uliczka and Angelstam 2000) have been used to signal environmental change.	

Table 1. Concepts focusing on the special ecological importance of a species.

Table 2. Concepts focusing on the special social importance of a species.

Concept	Definition/understanding				
	"Popular charismatic species that serve as symbols and rallying points to stimulate				
Flagship species	conservation awareness and action" (Leader-Williams and Dublin 2000: 56; based on:				
	Heywood 1995: 491).				
Charismatic species	"Immediately recognizable and identifiable by name, () commonly associated with				
Charismatic species	a particular geographic location or habitat" (Kontoleon and Swanson 2003: 483).				
High-profile species	Often used in exchange for flagship, widely recognised species (e.g. Scott et al. 1995).				
D	A species which qualifies to be used on a poster promoting a given conservation				
Poster species	campaign (Jacquet and Pauly 2008).				
Iii	Reflecting the "wider social, economic and ecological roles" of those species (Everard				
Iconic species	and Kataria 2011: 101).				
C	Holding a symbolic, cultural, religious value, e.g. an animal serving as a national				
Symbolic species	emblem (e.g. Sattout et al. 2007).				
	Bearing special, totemic importance for indigenous communities (Descola and				
Totemic species	Palsson 1996); but also – more broadly – totemising or totemisation of certain species				
roteinic species	equals attributing special features to those species in public discussions – highlighting				
	our moral and ethical obligations towards those species (Kalland 1993).				
	Used to 'sell' conservation ideas or objectives, attracting funds to conservation				
Species that sells	(Kontoleon and Swanson 2003), but sometimes also used by companies to sell market				
	goods.				
Tourism flagship A species of particular importance from the point of view of attracting to					
species	(Walpole and Leader-Williams 2002; Veríssimo et al. 2009).				
Species with a high	Quantification of relative interest levels for different species based on pair-wise				
wow-factor	comparisons. A measurement of an observer's relative level of enjoyment derived from				
wow-factor	spotting such species in the wild (Heydinger 2014). Closely linked to species rarity.				
Bambi effect	Cute and cuddly, anthropomorphic and sentimentalised, activating "protective instinct				
	which prevents humans wanting to kill or consume animals" (Ferreday 2012: 88).				
Local flagship species	Species which are important to local people and are used purposefully to promote				
Local hagship species	conservation within local communities (Bowen-Jones and Entwistle 2002).				

(Jepson and Barua 2015). Eventually, at the far end of the socially informed concepts, species are disconnected from their conservation context and used purely for branding or attention grabbing (e.g. species that sell, tourism flagship species).

Many authors have already argued that flagship species would serve their purposes better, had they not only reflected their social importance but also the ecological importance (Heywood 1995; Simberloff 1998; Home et al. 2009). Connecting these two approaches to particularly important species would seem essential for engaging with coevolving social-ecological systems. Several authors realised that the perception of different focal species has to be considered not only from the point of view of those who are going to sponsor conservation but also related to those who are most closely linked to those species, i.e. local communities which co-exist with those species on an everyday basis (Entwistle 2000; Bowen-Jones and Entwistle 2002). Local perception of the importance or value of those species emerged as an important success factor for effective conservation efforts, ensuring the participation of local communities (Veríssimo et al. 2009). Entwistle (2000) and Bowen-Jones and Entwistle (2002) highlighted the importance of local values, perceptions and attitudes, and made connections to cultural symbolism and self/community identity. Bowen-Jones and Entwistle (2002) and Walpole and Leader-Williams (2002) focused mostly on the risk that a species of high symbolic value for people willing to pay for conservation may not be equally desirable to those living next to this species (who may see this species as a nuisance), e.g. a tiger or a lion.

These ideas are quite comprehensively reflected in the concept of cultural keystone species (Cristancho and Vining 2004; Garibaldi and Turner 2004; Platten and Henfrey 2009). Cultural keystone species embrace the special utilitarian but also spiritual and symbolic importance of species to different cultures, "essential to the stability of a cultural group over time" and whose "withdrawal from the culture's context would entail significant cultural disruptions" (Cristancho and Vining 2004: 155). The main focus here is on the importance of such species for indigenous societies, and on how those societies depend on such species – for food, energy, medicine, building materials but also in the psycho-socio-cultural sphere. Indeed, in some cases these species have been used to define the identity of specific human communities. Eventually, the keystone role is not only performed by a species alone, but by a complex of a species and its ecological relationships, social artefacts, knowledge, practices, beliefs, ideas, norms and values (Platten and Henfrey 2009).

Although cultural keystone status does not necessarily coincide with the ecological prominence of those species, it often reflects the traditional ecological knowledge. As a result of the strong dependence of indigenous communities on nature, ecologically "valuable" species tend also to be socially valuable. However, this is not always the case and it does not have to reflect the specific keystone, indicator or umbrella status of a species. It does, however, underline the co-evolutionary character of social-ecological systems and again suggests that any understanding of a species' importance is a social construct.

Methods

Based on a literature review, we describe the importance of a selected well-known species, which often serves as a conservation conduit – the White Stork. The selection of literature for this review was aided by our previous work on this species. In line with

the overview of the different perspectives on particularly important species presented in the previous section, we divided the results of our review into the ecological, social, and social-ecological aspects.

To provide an in-depth analysis of the articulation of the focal species concept in the case of White Stork, we pay special attention to five case examples, mostly drawing from recent conservation practice. For each case study, we investigated the involved stakeholders, the time of a campaign, its objectives, addressees (and what was expected from them), activities involved, communication channels used to reach the relevant audiences, and outcomes. We also analysed in which way the White Stork was used in a given campaign, and what it symbolised.

The existing frameworks for selecting focal species serve as the basis for our social-ecological account and the case descriptions reflect the most important issues addressed in the concepts developed so far, complemented with a broader social-ecological systems thinking. The description of each case study is based on the review of publicly available resources, combined with additional information sought from the relevant project representatives.

The social and ecological importance of White Stork

Ecological context

Among farmland birds, the White Stork seems to be a good indicator of both environmental conditions and habitat diversity (Tobolka et al. 2012). First of all, it is an icon of nature conservation in Europe and elsewhere (Creutz 1985) and data on stork population size, and even breeding success, have been collected in some places since 1890 (Bairlein 1991). It is charismatic and easy to detect – it builds huge, easily located nests on electricity poles, high chimneys and roofs of buildings, and it is easy to find a sufficient sample size (Tobolka et al. 2012). For these reasons, its presence is often used as an argument for habitat conservation, especially wetlands, and the cultural character of changing farmland (Tryjanowski et al. 2006). On a more detailed level, White Storks can be used as environmental indicators based on the concentration of heavy metals in their blood and feathers (Tryjanowski et al. 2006; Kamiński et al. 2008). Additionally, White Storks are highly visible during their migration and can be used as indicators of environmental problems along the migratory route. According to the IUCN, the White Stork is of Least Concern in terms of its conservation status globally, which means that it enjoys low extinction risk.

The presence of White Stork provides benefits to other species. White Stork nests are known as good nesting sites for other bird species (Indykiewicz 1998; Kosicki et al. 2007), and they may be of key importance for the survival of some species in winter (Tobolka 2011). White Stork nests are also important as seed banks for seed plants, including rare vascular plant species (Czarnecka and Kitowski 2013), and the soil in the nest is a habitat for saprophagous mites (Błoszyk et al. 2005).

From the point of view of agriculture, White Stork probably plays an important pest regulative role as it feeds, among other species, on the Common Vole *Microtus*

arvalis, which can cause serious damage to crops, especially in meadows, pastures and perennial crops (clover, alfalfa). Indeed, in the so-called murine years the number of pairs of storks and their reproductive success increase (Tryjanowski and Kuzniak 2002). However, the stork is unlikely to be a key species for predatory control of vole populations and it has not been shown to what extent the stork contributes to reducing them. In Africa, in wintering grounds, the White Stork is a valuable predator on Brown Locusts *Locustana pardalina* in the Grassy Karoo where swarms occur (Vesey-FitzGerald 1959; Milstein 1966). Similarly, in grassland and woodland habitat, the White Stork is attracted to caterpillar outbreaks (Herremans and Herremans-Tonnoeyr 1993).

Social context

Several accounts of the special cultural importance of White Stork have been published in national languages in countries where White Stork is present, indicating the prominent cultural role of this species (Bense 2006, 2014; Kronenberg et al. 2013). White Storks are present in folklore, as reflected in numerous beliefs and traditions, but also modern social norms. Their close connections with people (nesting and feeding close to human settlements) and the resulting familiarity as well as their relatively anthropomorphic look and the qualities frequently ascribed to them (such as fidelity, wisdom, caregiving, nobility) make them almost unequivocally considered friendly and close to humans.

White Storks are widely represented in art, which is not only restricted to literature, paintings and folk arts, but extends to applied arts and industrial design, including toys, jewellery, household appliances, clothes, souvenirs and various collectible goods (post-cards, stamps, phone cards, beverage bottles etc.). They serve as important symbols, being considered one of the key 'national birds' by the Belarusians, Danish, French, Germans, Hungarians, Lithuanians, and Polish. For example, they are featured in national promotional campaigns in Poland, and in local coats of arms in Poland and elsewhere (e.g. the Hague). Furthermore, the image and symbolism of White Stork is also exploited for commercial purposes by companies ranging from infertility treatment clinics, through restaurants and hotels located close to storks' nests, an on-line shop offering Polish produce to Polish immigrants in the UK, to many companies offering products and services seemingly unrelated to storks (see our case study 5 which solely relied on the symbolic meaning of the stork, without any consideration of environmental facts and factors). Finally, because of their familiarity, White Storks are frequently used in ecological education projects, with one database featuring several hundreds of such undertakings in Poland alone.

Social-ecological context

Although White Stork is a particularly prominent species, especially from a cultural perspective, its significance is different than in the case of species traditionally used as examples of cultural keystones (Cristancho and Vining 2004; Garibaldi and Turner

2004). However, inasmuch as a species can have a cultural keystone status in a modern, industrialised society, the White Stork meets the relevant criteria (Table 3). Although we agree with Platten and Henfrey (2009: 496) that "No approach of this type, based on lists of features, can be usefully diagnostic of cultural keystones, which in the absence of a formal systems model can be identified only in reference to contingent features of any particular case," we still find it useful to check how White Stork fares with regard to the cultural keystone criteria put forward so far.

Here, we focus on the role of White Stork in European countries where this is a widely known and common species (such as Poland, Lithuania, Hungary or Germany). We see the national level as the most relevant for discussing the concept of a social-ecological keystone species in a modern, industrialised society, although we also admit that such an analysis could be performed for a distinguishable cultural group. Clearly, in a modern society, dependence on a species is more indirect, not necessarily involving the direct, instrumental or use value related to consumption. White Storks are charismatic enough that almost everyone derives satisfaction from observing them as part of a traditional rural landscape. So the criteria from Cristancho and Vining (2004) and Garibaldi and Turner (2004) which emphasise the instrumental value related to direct, physical use or trade in the species are less relevant.

Additionally, as already indicated, the social-ecological perspective should not only suggest that the species is important because of co-evolutionary interdependence between the social and the ecological, it should also reflect a more objective ecological role of a species in question. Although the White Stork is not a keystone species, it is still important enough in an ecosystem, either as an indicator or – at least to some extent – as an umbrella species, that it fits into the broader concept of a social-ecological keystone species. This indicates the relevance of the local flagship species concept (Bowen-Jones and Entwistle 2002), which incorporated both social and ecological criteria and therefore was used in our comparison in Table 3.

Case studies: White Stork as a focal species

Case study 1: White Stork and illegal hunting in Lebanon

Bird hunting in the Mediterranean countries have long been a source of concern for European bird conservation organisations (BirdLife International 2010). Recently, with the development of social media, conservation organisations have discovered the photos, which illegal hunters had posted on their profiles of themselves and their trophies, and – based on these new proofs – demanded concerted European action and political pressure to be exerted on countries such as Lebanon to protect migrating birds more effectively (CABS and LEM 2013). This campaign had little resonance in Poland until the pictures of White Storks killed by Lebanese poachers and displayed in their social media profiles were shown to the public (Kozera 2014). The images of piles of White Storks shot illegally in Lebanon roused public opinion, creating a window

Table 3. Does the White Stork meet criteria for a cultural keystone species? Criteria from major articles on cultural keystone species and the related concept of a local flagship species have been collated in rows according to their relative proximity.

	Criteria		
Cristancho and Vining (2004)	Garibaldi and Turner (2004)	Bowen-Jones and Entwistle (2002)	In what way does the white stork meet these criteria?
The story of the species' origin is tied to the myths, the ancestors, or the origin of the culture.	Role in narratives, ceremonies, or symbolism.		Prominent status in legends, myths, folklore.
The species is either related to or used in activities intended to supply the basic needs of the community such as getting food, constructing shelters, curing illnesses, etc.	Intensity, type and multiplicity of use.	Cultural significance – folklore, arrs, food etc.	According to historical records, because of respect and various beliefs, storks were consumed only as the extreme last resort. Material from storks' nests as well as parts of stork's body were used as medicaments until eighteenth century. Not relevant any more. Today, non-material use of storks as a widdy recognised symbol dominates.
The species is central to the transmission of cultural knowledge.	Persistence and memory of use in relationship to cultural change.	Traditional knowledge.	Only through folklore.
The species is indispensable in the major rituals on which the community's stability depends.	Naming and terminology in a language.	Common names – without negative connotations or interpretations in local languages.	The coming of spring – expected sign.
The species has significant spiritual or religious value for the culture in which it is embedded.	I	Positive associations.	Highly respected, featuring only positive associations.
The cultural group refers to the species as one of the most important species.	Level of unique position in culture.	Charisma.	Prominent role as a national symbol, as well as in arts and design. Symbol of traditional rural landscape.
The species exists physically within the territory that the cultural group inhabits or to which it has access.	1	Geographical distribution – present in the area of interest.	Common in many countries.
ı	Extent to which it provides opportunities for resource acquisition from beyond the territory.	I	n/a

	Criteria		
Cristancho and Vining (2004)	Garibaldi and Turner (2004)	Bowen-Jones and Entwistle (2002)	In what way does the white stork meet these criteria?
ı	I	Recognition – known to the rarget audience.	Widely known worldwide.
ı	I	Existing usage – avoiding conflicting symbolism.	Widely used to symbolise different things, none of which is in conflict with conservation.
ı	I	Conservation status – not necessarily rare, may be common for better recognition.	Common, widely recognised and respected.
ı	1	Ecological role – central role in the ecosystem preferably.	Not central, but important.

Table 4. Summary of our case studies.

	White Stork and illegal bird hunting in Lebanon	European Stork Villages Network and other stork villages	White Stork reintroduction programme in Sweden	"Almost gone" campaign by NABU	Commercial use of the White Stork image by Atlas
Coordinator	Polish NGOs led by Grupa Ekologiczna	NGOs in several countries led by EuroNatur	Swedish NGOs: Swedish Society for Nature Conservation in Scania and the Scanian Ornithological Society	German NGO: Nature and Biodiversity Conservation Union (NABU)	Private company: Atlas
Objectives	Migratory bird conservation	Habitat conservation, local development	Habitat and bird conservation, local branding	Nature conservation, ecological education	Marketing products (not related to conservation)
Addressees	Broader society	Donors, tourists, broader society	Broader society	Broader society	Consumers
Use of white stork	Images, symbolism, rhetoric	Information, symbolism	Information, symbolism	Images, information, symbolism	Images, symbolism
Focal species concept	Flagship species, umbrella species, social-ecological keystone species	Flagship species, umbrella species, tourism flagship species, species that sells	Flagship species, umbrella species, social-ecological keystone species	Flagship species, species that sells, iconic species, indicator species	Species that sells

of opportunity which Polish conservation organisations have successfully exploited to pursue further activities in Lebanon (for an overview of our case studies, see Table 4).

In 2013, referring to the importance of White Stork for the Polish society, several environmental NGOs (led by Grupa Ekologiczna) started to put pressure on Lebanese authorities to take action. Simultaneously, in cooperation with the Polish embassy in Beirut and Lebanese conservation organisations, they launched an educational campaign in Lebanon, highlighting that the Lebanese society has important responsibility to protect storks on their way to and from Poland. The involvement of the diplomatic mission stressed that killing migratory birds (storks in particular) negatively affected the image of Lebanon in Poland. In Lebanon posters, leaflets and a video documentary were distributed in schools and research organisations, at public events, and among other interested stakeholders, along with some organised presentations on this topic. In 2015, a photo contest was organised for the best photo featuring White Stork, with a trip to Poland to take part in ringing storks as the first prize. In Poland additional awareness raising activities were carried out through the use of traditional and social media, with several opportunities for the general public to get involved in this campaign (e.g., sending emails and postcards to the prime minister of Lebanon). Satellite tracking of one stork during its migration – especially when crossing the territory of Lebanon – attracted additional attention to this campaign in both countries. The campaign has continued in subsequent years; in 2017 Polish activists showed a 10-minute animated cartoon in Lebanese schools, and distributed 1000 copies to Lebanese children. Over time, this campaign has raised interest in Lebanese and Polish media. It has led to regulatory changes improving conservation and a higher determination to execute the law in Lebanon (level of government), and hopefully increased awareness among the general public.

As a charismatic, flagship species, White Stork was able to attract broad attention to the problem of illegal bird hunting in Lebanon (or to "sell" the broader conservation objectives). However, most prominently, this case study presents White Stork as a social-ecological keystone species, or at least a local flagship species.

Case study 2: European Stork Villages Network and other stork villages

Since 1994, EuroNatur awards the European Stork Village title to a village that protects a White Stork nesting colony and in this way helps to reverse the loss of traditional agricultural habitat. Local stakeholders in European Stork Villages are expected "to enhance the living conditions of the White Stork, such as preserving or rewilding large open wet meadows or erecting artificial stork nests" and to increase environmental awareness of inhabitants and visitors (Wiesehomeier et al. 2014: 2). European Stork Villages are places of cultural activities focused on the topic of White Stork and its conservation, as well as of interaction between conservation organisations and local communities (Ferger and Schwaderer 2016). Several other stork villages undertake similar activities, without being part of the Network which only features one village per country.

In Central and Eastern Europe White Storks currently usually nest solitarily. Although the several dozens of colonial nesting sites of White Stork in Poland are seen as spectacular natural spots, few have been successfully marketed as tourist attractions and even fewer have become the focus of large-scale conservation activities. Zywkowo in the north-east of the country provides the best example of a combination of both objectives. Conservation and tourism promotion activities have been carried out there since 1997 by the Polish Society for the Protection of Birds (PTOP) within the "Stork Village" project.

As a conservation organisation, PTOP has been primarily interested in protecting natural habitats and biodiversity, which have been increasingly exposed either to development pressure (especially intensive agriculture) or to changes related to land abandonment (afforestation, secondary succession). To counteract these pressures, which would have resulted in the loss of the typical landscape mosaic and the storks' foraging grounds, PTOP attracted significant funding from international and national donors to buy and lease land, renovate buildings, maintain an organic farm with traditional breeds of horses and cows, and create tourist infrastructure. Subsequent projects were carried out not only in Zywkowo, but also in several other "stork villages" in northeast Poland, all of which aimed at the conservation of natural foraging grounds for the

storks (increasing water retention and improving water quality, supporting extensive agriculture, improving the storks' nesting conditions etc.). Seven villages have been connected under the Warmian-Masurian White Stork Tourist Trail, with some national scale promotional activities, including brochures, posters, website, basic tourist infrastructure, ecological education activities and infrastructure, and national media coverage. Zywkowo has remained the most popular village within the trail, annually visited by 2000–5000 tourists (c.f. Czajkowski et al. 2014), and a prominent conservation project which continues until today.

Local stakeholders in stork villages are expected to contribute to nature conservation and to increase environmental awareness of inhabitants and visitors. Thus, White Stork serves as an umbrella species (for other species, for landscape mosaic, for the whole ecosystem) and a tourism flagship species. The different projects carried out by PTOP in this area have been successful in preventing the degradation of the stork habitats, and they have also attracted tourists to stork villages. This further indicated that the potential of birdwatching tourism is higher than usually assumed, because it involves not only specialised tourists, but also casual ones who are lured to attractions such as stork villages (Kronenberg 2016). Hence, part of the effort has been directed at creating and maintaining the stork village brand (Kronenberg 2015; Ferger and Schwaderer 2016).

Case study 3: White Stork reintroduction programme in Sweden

The first half of the 20th century saw the decline and final disappearance of White Stork from Sweden, with the last unsuccessful attempt at breeding in 1954 (Nilsson 1989). The decline was probably driven by several factors, most prominently landscape change. Wetlands were drained and converted into agricultural fields and the extent of semi-natural grasslands was reduced, effectively removing much of the habitat on which the stork depends. This development, overall landscape change as well as the disappearance of storks, was seen as undesirable by many and in 1989 the Swedish Society for Nature Conservation (SSNC) in Scania (the southernmost province of Sweden) and the Scanian Ornithological Society (SkOF) initiated a reintroduction programme for White Stork.

The programme aims to bring back free, breeding and migrating storks to Sweden and much of the media attention and public focus is on the breeding programme (storks were first brought in from Algeria via Switzerland, later Poland). However, the reintroduction programme is paralleled by landscape restoration efforts, primarily aimed at restoring wetlands and it was early progress here that convinced SSNC and SkOF that it was time to start with the Swedish Stork Programme (Rimberth 2013). During the last decades, however, the work of restoring wetlands has intensified, at least partly in response to the reintroduction programme. The landscape has been very much influenced by people over millennia (Karlsson 1989) and the White Stork is for many people an expression of this history (Cavallin 1997). The possibilities to

reconstruct the "landscape of the stork" and thereby bring back the stork constitute a significant driving force in many ongoing restoration projects, such as the Swedish Stork Programme.

The stork is described as a flagship species in text written by the Stork Programme and in information relating to the programme. Depending on audience and objective we contend that the stork, as used in the region, can be considered a flagship species, an umbrella species or an indicator species. Participation in the restoration programme has also been used by municipalities and organisations to brand their environmental engagement. The stork is the main attractor for tourism, guided tours, environmental education and public engagement in nature conservation, all of which fit in well under the flagship identity. However, because of the stork's role in maintaining the culturally meaningful landscape, the stork bears a deeper social-ecological meaning. Indeed, the success of the reintroduction programme is also used as an indicator of the progress of the landscape restoration efforts, and by implication (and often explicitly stated) biodiversity conservation or promotion.

Case study 4: "Almost gone" campaign by NABU (Germany)

"Almost gone" campaign was launched in 2009 to attract interest and funding for nature conservation, focusing on the problem of disappearance of seemingly common and widely known species. Using examples of species that have experienced significant population declines in Germany in the past decades, the campaign informed that extinction may also concern familiar, national species, which most people do not consider threatened. These included White Stork, Wolf, Tree Frog, Apollo Butterfly and Cod, each of which could only be seen in part, disappearing from the picture.

The campaign included over 4700 posters displayed in billboards in all major German railway stations and along major roads, plus online banners, flyers and postcards, and short commercials shown in regional television channels.

The stork was selected as one of the flagship species because it is familiar to everyone and yet the risks that it faces are not so well-known. Although the stork typifies many animal species that are threatened with extinction (at least in Germany), its importance was reinforced by the fact that this species is featured in NABU logo (i.e. an iconic or totemic species). Along with other species, the stork was also partly considered an indicator species because its presence was connected to the condition of its habitats (and to habitat loss).

Case study 5: Commercial use of the White Stork image by Atlas

Atlas, the largest Polish manufacturer of construction chemicals, is widely recognised not only for its products but also because of its association with White Stork, which the company has been using as its symbol and mascot. The company was established in

1991, in the early period of Polish socio-economic transformation, and it started to use White Stork in a large scale outdoor advertising campaign already in 1993. Since then, about 1500 advertisement poles featuring the company's logotype and a laminate White Stork on a nest have been planted all over the country (about 1250 remain until today). This campaign was so successful in making the brand widely recognised and popular that White Stork has become the central concept in subsequent promotional activities ("everything else was thought around the storks" (Adam Masiulanis, personal communication)). This has started to change relatively recently when the company decided that even though the association with White Stork is genuinely positive and has been very successful in terms of creating the company's image, it is not promising enough in terms of building the brand further. Thus, the main focus has been placed on other activities, such as trainings for key customers and business partners and a trade magazine. Nevertheless, the White Stork has remained a prominent symbol and mascot, widely used in various promotional activities, especially those involving sports and children.

Interestingly, the selection of this species was a pure coincidence and its use has never been backed up with proper market analyses. In the early 1990s, the advertising market in Poland was poorly developed and any original idea had huge chances of becoming a success. Only over time – once the campaign started to bring effects – various commentators have developed deeper meaning for the use of White Stork by Atlas. The species which had first been seen as an impressive crowning of an advertisement pole, became a symbol of the Polish origin of the company, of the good constructor (referring to the storks' nests), and a source of many other positive emotions and connotations. Clearly, the ecological connotations of White Storks were not considered in this case and the company did not sponsor White Stork conservation projects, except for some incidental instances.

Interestingly, recent survey among construction professionals revealed limited potential to capitalise on this project for conservation purposes (Bocian 2017). Twenty-six percent of survey respondents associated Atlas with the image of White Stork, however only fifteen percent considered such associations as an important argument for choosing the company's products. Most importantly, 91% of respondents suggested that Atlas should sponsor White Stork conservation projects, and most commonly they indicated that the company should spend 1–2% of its revenue in this way. Interestingly, in 2013 the company's revenues exceeded 560 million PLN (181 million USD), and it only spent 49,200 PLN (16,000 USD) sponsoring one project related to White Stork conservation between 2012 and 2015.

Although this case study does not represent a nature conservation project, it illustrates the broader social connotations of focal species, and especially the 'species that sells' concept (selling commercial products rather than environmental conservation). To some extent, it has become a totemic species for Atlas. Although the company did not reveal willingness to pay for White Stork conservation in this case, this does not preclude other more witty opportunities to capture the hidden potential of capitalising on such corporate practices. Indeed, through its extractive activities, the company negatively influences White Stork habitats, which might provide further arguments to generate funds for White Stork conservation.

Discussion

Insights from the case studies

Three of our case studies demonstrate the use of White Stork as an umbrella species – at least to some extent. Most prominently, the stork villages (CS2) and the Swedish reintroduction programme (CS3) illustrate how conservation organisations attempt to achieve broader conservation objectives by focusing on a single species. To a lesser degree, in the White Stork and illegal bird hunting in Lebanon (CS1), White Stork served as an umbrella species for other migratory birds – in the sense that it drew attention also to the hunting of other migratory species and the importance of safe migratory routes (interestingly, migratory species are usually not considered good umbrella species [Zacharias and Roff 2001]). Finally, species selected for the "Almost gone" campaign (CS4) served as indicators of broader biodiversity loss.

The social importance of White Stork was the uniting theme in all five case studies, each of which demonstrated strong emphasis on stork images and symbolism. In each of them, White Stork was used as a flagship species that was meant to 'sell' broader conservation objectives, attract tourists to bird habitats, or – in CS5 – commercial products. As a charismatic, flagship species, White Stork was able to attract broad attention to the problem of illegal bird hunting in Lebanon (CS1). In all other case study campaigns, White Stork served as a brand for broader objectives. In stork villages (CS2) and the Swedish reintroduction programme (CS3), White Stork is not only branding conservation but also tourism and local development (tourism flagship species). In Germany (CS4), the stork was selected to illustrate the risks that it faces which are not so well-known.

Among our case studies, CS1 most prominently presents White Stork as a social-ecological keystone species, or at least a local flagship species – *sensu* Bowen-Jones and Entwistle (2002). This campaign involved a message from the Polish society to the Lebanese society – "storks are a key part of our culture, and our connection to nature; please respect this", thus reflecting the social-ecological importance of this species. Interestingly, according to previous reports by BirdLife International (2010), Lebanese hunters preferred shooting migratory species to resident ones among other reasons because they felt no connection to them. To some extent, the stork bears a deeper social-ecological meaning also in stork villages (CS2) and in Sweden (CS3), because of its role in maintaining the culturally meaningful landscape.

Our case studies help to identify the main opportunities and challenges related to the use of various focal species approaches. Only three case studies resonate with our suggested social-ecological perspective (CS1, and to some extent CS2 and CS3), but all five demonstrate both the social and ecological importance of this species to various extents. Additionally, CS2, CS3 and CS5 indicate that the social importance of this species extends beyond environmental conservation. In each case study, White Stork was used as a focal species because storks have positive connotations and attract attention and interest. However, this was not always related to their ecological significance or needs.

Indeed, any social or ecological concept of a focal species alone is not relevant enough from the point of view of broader conservation purposes. There are many border-line cases, making it impossible to distinguish between the social and the ecological within the various terminological distinctions put forward so far. We see the concept of a cultural keystone species as an initial combination of ecological and social, and we propose to extend the understanding and application of this concept to an even more comprehensive social-ecological keystone species. This is in line with the most recent developments of the cultural keystone species "as having essential roles in maintaining any level of complexity within a social–ecological system" (Platten and Henfrey 2009: 491).

From ecological and social to a social-ecological perspective

Scientists increasingly realise the social-ecological context of the world around us. Indeed, the social and the ecological are hardly separable, even if many people still think they are (Berkes et al. 2003). The society and the environment mutually affect each other, but people can make (partly) informed decisions shaping these relationships. Nevertheless, our perception of the world around us is bounded by ignorance. The setting of conservation priorities and even broader conservation approaches is based on our interpretations of the environment and these interpretations have increasingly been considered subjective, reflecting the dominant socio-economic paradigms and priorities of those in power (Ernstson 2013).

So far, the concept of the local flagship species by Bowen-Jones and Entwistle (2002) most comprehensively captured the social-ecological importance of a focal species, explicitly suggesting that – to make it relevant from the point of view of ecosystem management – a flagship species should be ecologically significant. Meanwhile, the related cultural keystone species concept, conceived of as "a convergence point for interdisciplinary collaboration" (Cristancho and Vining 2004: 161), emphasised the human physical and symbolic *use* of species, which reflected their ecological importance only to a limited extent (Platten and Henfrey 2009). Indeed, some authors have already suggested that "future approaches should seek flagships that are also good keystone (or umbrella and indicator) species [...] or that act as surrogates for landscapes" (Leader-Williams and Dublin 2000: 81).

Thus, our proposal is that the concept of cultural keystone species should be extended to new spheres: not only indigenous communities but also modern societies who are more indirectly connected to nature, and not only consumption but all forms of human relationships with other species. Furthermore, it should explicitly address the environmental and ecological significance of the species. This in recognition of a commonly occurring situation in which a species is so important for people that the conservation of many other ecosystem components hinges on the protection of this species. In spite of the commonly held view that people are mostly willing to conserve species that are particularly physically attractive – charismatic, totemic etc. – some studies have found that conservation attributes (information on the status of a certain

species) may rank even higher in terms of the public's support for conservation of those species (Tisdell et al. 2007; Veríssimo et al. 2009).

Our proposed concept is different from the traditional keystone species, which only reflected ecological importance, and from the flagship species which were only meant to "operate in the public relations and fundraising spheres" (Walpole and Leader-Williams 2002: 543; c.f. Andelman and Fagan 2000; Caro et al. 2004; Caro and O'Doherty 1999). As argued by Perry (2010), conservation efforts should focus on key endangered interactions between species, not just on endangered species (which is why we consider the White Stork as a relevant example despite its low extinction risk). Finally, we need to acknowledge that even the ecological keystone species is still vague and empirically unsound (Mills et al. 1993) and that it can only serve as a general guidance to which species are the most relevant for targeted conservation efforts.

We see the concept of a social-ecological keystone species as a comprehensive way to depict these complex interactions and interests involved in bringing the different aspects of species prioritisation into a single indicator. A social-ecological keystone species is likely to be more meaningful for broader conservation objectives because it complements the ecological importance with the social perception of a species, thereby opening an opportunity to connect various dimensions of social/cultural value that people attribute to nature to ecological quality and dynamics. The focus on different interactions, not least relations between humans and other species, supports a different worldview where species and nature conservation is not just for nature's sake but also for our own. The more ways a species, or conservation more broadly, can be linked to people and the values they hold or could cultivate, the more likely it will be that conservation efforts are sustained and successful. The social-ecological keystone species highlights our own role as stewards, with the ultimate target of not only preserving species and ecosystems but also the different avenues for meaningful human interactions with them.

Selecting social-ecological keystone species

The selection and use of social-ecological keystone species can be aided by cultural creation and branding. The status of a social-ecological keystone species may result from its contribution to the maintenance of selected types of landscapes, which may be culturally important, as in the case of White Stork and rural landscapes in Southern Sweden and Central and Eastern Europe, or – similarly – in Japan in the case of another stork species (Naito et al. 2014). In such a case, the ecological importance of a social-ecological keystone species refers to the preservation of a human-dominated ecosystem where the species have closely interacted with people and human land uses.

Selection principles matter. They are particularly relevant for engaging with the broader society and in education, and they can help to achieve broader conservation objectives beyond the support for particular conservation projects. For these reasons

social-ecological keystone species is also better than the traditional single species approaches, which have been deemed insufficient to ensure the conservation of broader biodiversity (Williams et al. 2000; Andelman and Fagan 2000). Meanwhile, the main advantage of using separate approaches would be not to overload the audience with too much information regarding one species. However, we argue that communicating the social-ecological connections is important enough to warrant the additional effort and to more accurately select the focal species, especially in a modern, industrialised society.

A limitation might be that for many ecosystems we may not be able to readily find species that would be recognised as socially and ecologically important by modern societies. However, again this provides excellent opportunity for more comprehensive education than in the case of pure flagship species. It maintains the appeal of a flagship species (Barua 2011), but narrows down the selection of potential species to those that are ecologically relevant. Such education and awareness-raising can be extended to international contexts, as in our CS1 on illegal bird hunting in the Mediterranean within which an attempt was made to inform one society about the importance of some species for the other.

Bees constitute another good example, where more and more lay people are concerned about their situation (Brown and Paxton 2009). The fact that such a broad attention has been paid to bees recently illustrates the cultural creation of a social-ecological keystone species (or a group of species in this case). In fact, the example of the bees indicates the rediscovery of the social-ecological importance of a (group of) species, because they have always been important to people. Only recently the deteriorating environmental conditions that have led to their decline have brought them to broader attention and have successfully contributed to broader calls for environmental protection (Moore and Kosut 2013).

To this point, Platten and Henfrey (2009: 496) suggested that "Formulating the cultural keystone concept in systemic terms demands that it refer not to biological species, but to complexes of interconnected material and subjective factors." This approach, which was not reflected in the original cultural keystone species concept, well illustrates the social-ecological extension that we are advocating in the present article. In the case of yet another pollinator, the monarch butterfly, the special status was co-produced principally through partnerships among various actors: those already involved in conservation, and others who associated potential involvement with their personal interest (e.g. to demonstrate corporate social responsibility) (Gustafsson et al. 2015). However, although many examples of social-ecological keystone species are available, there is no simple prescription on how to promote such an approach, especially with regard to non-charismatic species. Additionally, some social-ecological keystone species are not necessarily unanimously accepted by people, especially when they are considered problematic by some groups, such as the abovementioned tiger and lion or wolf and other large predators. Still, highlighting the ecological component within a social-ecological approach may add much importance to those species, the social appeal of which is not sufficient to ensure their special status and conservation.

Conclusions

White Stork provides an important example of a focal species used in different conservation campaigns and in broader social communications, addressing a range of targets. It is most often used because of its social appeal, and while its ecological importance is not similarly spectacular, it is still a valid argument for conservation. This species has a long history of coexistence with humans, and many linkages with specific cultural practices. Hence, White Stork offers an excellent example for discussing the broader social-ecological relevance of species.

Although we do not expect the concept of a social-ecological keystone species to replace the ecological concepts of a focal species, which have their well-established uses in biological and ecological science, we argue that a social-ecological keystone species in principle should be more relevant than a typical social focal species approach (such as flagship species). The added value of a social-ecological keystone species compared to traditional socially-defined focal species approach relates to the higher transparency of the former. A social-ecological approach avoids the specific focus or hidden agendas of specific groups of interested stakeholders who might favour the conservation of certain species over the other, it ensures that these species are relevant to broader social spheres, and it ensures that the selected focal species will indeed help to solve broader environmental problems and will not only serve as conservation mascots. It also demands that that the inherent ecology is easily understood and related to in a way that is meaningful to people.

The social-ecological keystone species concept, where selected species have clear connections to ecological qualities and dynamics can make people (understood as a diverse group of stakeholders) aware that their "preferred" species is a product of ecosystem processes and interactions (and often ecosystems where humans are a dominating force). Thus, conservation projects referring to the idea of a social-ecological keystone species highlight the importance of the connections between the social and the ecological, providing a very good opportunity for ecological education and nurturing a sense of responsibility for nature conservation and additional benefits of biodiversity (Andersson et al. 2015). Many of the other concepts and traditional approaches rely on the mediator, the conservation agency or similar, to make sure that the public image and understanding of the species is translated into relevant action. This means there is a potential disconnect between public awareness and interest, and the actual on-theground conservation work (Schlegel and Rupf 2010). Hopefully, the social-ecological keystone species concept would bridge and integrate these two sides more smoothly and engage people more directly, which would also take some of the weight off (and dependence on) the conservation agents.

Nevertheless, the social-ecological keystone species is still not likely to solve some important challenges related to previously used concepts of ecological and social focal species. The different focal species concepts are frequently used in an imprecise manner (Barua 2011) and we cannot be certain that a new – and even more complex concept – will not add ambiguity to current discussions. Also, as our environmental management practices are bounded by ignorance regarding ecosystem functioning, a focus on

selected keystone species may miss other species that are at least as important even if we are not aware of them or their importance. While this problem may become even more severe with further environmental change, it also affects all other environmental management decisions.

In short, a social-ecological keystone species can be used in practice to define conservation objectives and to further ecological education, showing how people and the environment are interlinked through selected relatively easily recognised species. It can be used as part of broader systems of indicators used for environmental management that incorporate social and ecological contexts to "evaluate the state of cultural wellbeing associated with different ecosystems and address the conditions which threaten or enable these conditions to thrive" (Poe et al. 2014: 173). The social-ecological keystone species builds on non-monetary approaches to the identification of important species, drawing on social psychology, conservation psychology, ecological anthropology, human ecology, folkbiology, enthnobiology and to the notion of biocultural diversity and biocultural importance, all of which can provide insights into the complex social-ecological background of different species.

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Biodiversity, threats and conservation challenges in the Cerrado of Amapá, an Amazonian savanna

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Abstract

An Amazonian savanna in northern Brazil known as the Cerrado of Amapá is under imminent threat from poor land-use planning, the expansion of large-scale agriculture and other anthropogenic pressures. These savannas house a rich and unique flora and fauna, including endemic plants and animals. However, the area remains under-sampled for most taxa, and better sampling may uncover new species. We estimate that only ~9.16% of these habitats have any kind of protection, and legislative changes threaten to further weaken or remove this protection. Here we present the status of knowledge concerning the biodiversity of the Cerrado of Amapá, its conservation status, and the main threats to the conservation of this Amazonian savanna. To secure the future of these unique and imperilled habitats, we suggest urgent expansion of protected areas, as well as measures that would promote less-damaging land uses to support the local population.

Keywords

Brazil, Protected areas, Development policy, Land clearing

Introduction

Tropical savannas are dynamic systems of grassland and open woodland that cover 15 to 24.6 million km² of South America, Africa and Asia (Silva and Bates 2002), occupying one-fifth of the Earth's surface (Scholes and Archer 1997). A large part of the human population lives and works within these ecosystems, which house more pastoral land and wild herbivores than any other in the world, and which have a significant impact on the global economy (Sankaran et al. 2005; Scholes and Archer 1997). Tropical savannas are also recognised globally for their rich and unique biodiversity, and this, together with high levels of anthropogenic disturbance, has led to increasing conservation concern (e.g. Klink and Machado 2005). In South America, the largest savanna complexes are the Cerrado in Brazil, Bolivia and Paraguay, and the Llanos in Venezuela and Colombia (Silva and Bates 2002). However, further islands of savanna of varying size occur throughout the Amazon biome, known as Amazonian savannas (Carvalho and Mustin 2017; Prance 1996). The Amazonian savannas represent a distinct ecosystem within the large Amazonian region (see Prance 1996), different also from the white-sand ecosystems that further pepper the Amazon biome (see Adeney et al. 2016). These Amazonian savannas have generally been under-studied (see Carvalho and Mustin 2017) and are highly threatened, principally as a result of land-grabbing and the advance of cultivation of *grãos* (grains and pulses, mainly soybeans and maize), plantations of exotic woody species (eucalyptus and acacia), and un-controlled fires (Barbosa et al. 2007; Carvalho and Mustin 2017; Aguiar et al. 2014).

It has been estimated that, in Brazil, 12.3% of Amazonian savannas are within Strictly Protected areas (IUCN categories I-IV), 5.1% in Multiple Use areas (IUCN categories V-VI) and 40.3% in Indigenous Lands (Carvalho and Mustin 2017). Indigenous Lands have been shown to be effective in preventing deforestation (Nepstad et al. 2006), however, the effectiveness of protected areas in preventing degradation depends not only on protection type but also on the level of anthropogenic pressure to which the areas are exposed, and the intensity of enforcement among other factors (Nolte et al. 2013; Pfaff et al. 2014). Furthermore, some types of multiple use areas can offer protection of both biodiversity and local communities in the face of large-scale development, but it should not be assumed that local needs, expectations and attitudes toward conservation are easily compatible with conservation goals (Kohler and Brondizio 2017).

In recent years, a lack of protection of less isolated areas of Amazonian savannas (Cerrado of Amapá, Lavrados of Roraima and smaller fragments including those at Humaitá, Santarém and Monte Alegre), has facilitated the opening up of new areas to plantations of *grãos* and associated degradation of savanna areas (Barbosa 2013;

Barbosa and Campos 2011; Carvalho and Mustin 2017; Vidal 2017). Since 2006, deforestation of Amazonian forests to make way for soybean plantations has declined considerably, thanks largely to the implementation of a moratorium on soya (Gibbs et al. 2015). However, the expansion of soybean cultivation in Brazil has shifted the agricultural frontier to other areas such as the region between the states of Maranhão, Piauí, Tocantins, and Bahia, known as MAPITOBA, and has maintained its expansion across the Cerrado (Gibbs et al. 2015). The moratorium on soya is not applicable in the Cerrado biome, and is also seemingly not being applied to savanna habitats within the Amazon, opening up these areas to large-scale production of *grãos*. This can lead to deforestation and degradation, conservation conflicts and conflicts over land, increased burning, and displacement of traditional populations (Barbosa 2013; Barbosa et al. 2007; Domingues and Bermann 2012; Fearnside 2006; Gibbs et al. 2015; Vidal 2017).

The Cerrado of Amapá

The Cerrado of Amapá is one of the largest, least protected and arguably the most threatened complexes of Amazonian savanna in Brazil at present (see Carvalho and Mustin 2017). It covers approximately ~7.2% of the total area (~140,012 km²) of the state of Amapá, in the far north of Brazil. Amapá has no road connections to the rest of the country and has very limited connections with neighbouring French Guiana. These facts have doubtless contributed to protecting the Cerrado of Amapá from large-scale agricultural activities until now. However, the state does have a port at the mouth of the Amazon River that allows for export of *grãos*, wood and minerals to China (via the Panama Canal) and Europe (Monteiro 2015).

The Cerrado of Amapá is characterised by a mosaic of areas with open woody vegetation, areas with a denser woody shrub layer, and open grassy areas with sparser shrubs and trees, and by seasonally flooded areas in the transition zone with floodplains (Castro 2009; Mochiutti and Meirelles 1994; Oliveira 2009). This ecosystem is also intersected by gallery forests (Castro 2009; Mochiutti and Meirelles 1994). The Cerrado of Amapá can be subdivided in to areas dominated by one of four main vegetation types: shrub savanna (savana arborizada), woodland savanna (savana florestada), grass savanna (savana gramíneo-lenhosa) and park savanna (savana parque) (Figures 1 and 2). There is clear variation in the composition and number of species that can be encountered in different locations in the Cerrado of Amapá. For example, the woody species Salvertia convallariodora is not found in the savanna areas to the north of the Araguari River, despite being one of the most abundant species in the savannas to the south of this same river (GEA et al. 2016).

The Cerrado of Amapá is among the most threatened ecosystems in the state (IBGE 2004b) and is subject to high human pressure containing both the largest urban centres and the majority of the network of highways of the state (see Figure 3). Since 2004 it has been recognised as a "very high" conservation priority for Brazil (Brazil 2004). Despite this recognition, and despite representing ~7% of the area of the state,

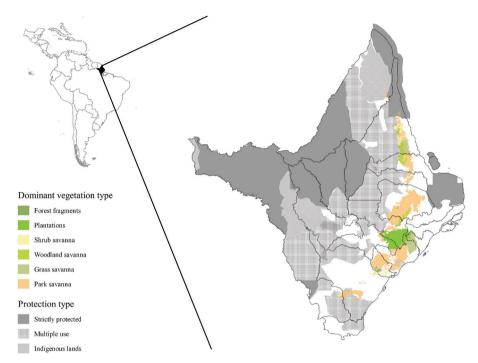


Figure 1. Spatial distribution of dominant savanna types and protected areas in the state of Amapá. Distribution of the four dominant savanna vegetation types (based on the Brazilian Institute of Geography and Statistics (IBGE) vegetation cover map for the Amazon (IBGE 2004a), and the three categories of protected areas (based on the shapefiles of Conservation Units and Indigenous Lands available from the Amapá State Environmental Secretariat (SEMA-AP 2016) and the World Database on Protected Areas (IUCN and UNEP-WCMC 2016) in the state of Amapá. Solid grey lines show the limits of the sixteen municipalities that make up the state. The shapefile of boundaries of the municipalities was also obtained from the Amapá State Environmental Secretariat.

as well as a significant percentage of its economically productive area, the Cerrado of Amapá has not received the attention that we argue it deserves. Here we present the status of knowledge concerning the biodiversity of the Cerrado of Amapá, its conservation status, and the main threats to the conservation of this Amazonian savanna. We further suggest pathways necessary to conserve this unique ecosystem and to secure its future in the face of mounting anthropogenic threats.

Biodiversity

To date, at least 378 plant species, 350 species of invertebrates, 200 bird species, 108 mammals (including 38 bat species), 26 species of fish, 41 amphibian species and 26 reptile species have been reported in the Cerrado of Amapá (Aguiar and Naiff 2010; Avila-Pires 1995; Azevedo 1967; Barbosa and Souto 2011; Boss 2009; Boss and Silva

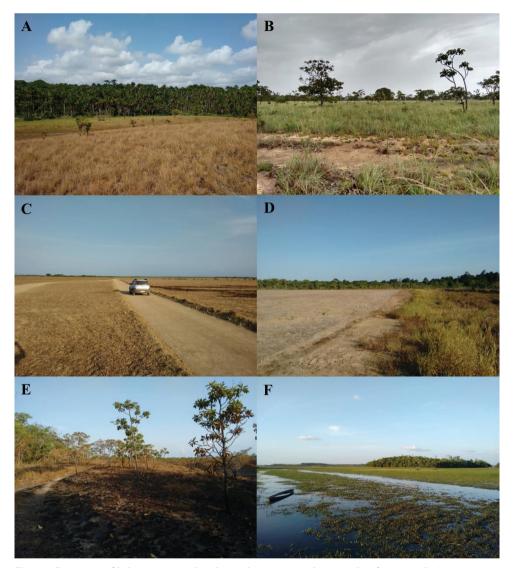


Figure 2. Images of habitat types and soybean plantations in the Cerrado of Amapá. **A** Grass savanna with gallery forests in the background, showing characteristic presence of palms **B** Area of park savanna **C** Area of park savanna converted to plantations of soybeans and maize **D** The right-hand side of the image shows what is left of an area of park savanna, the left-hand side shows an area prepared for planting with soybeans and maize, and in the background are natural forest fragments that occur within the Cerrado of Amapá **E** An area of park savanna after being burned; and **F** flooded savanna with grass savannas and a natural forest fragment behind.

2015; Cantuáriua 2012; Carvalho et al. 2009; Castro 2009; Costa-Campos 2015; Costa-Neto 2014; Costa-Neto et al. 2017; Deus et al. 2013; França et al. 2006; Galardo et al. 2013; Hamada et al. 2003; Jardim Botânico do Rio de Janeiro 2016; Jesus-Barros et al. 2012; Lima et al. 2017; Melo 2006; Mendes-Júnior 2013; Mesquita 2005; Mes-

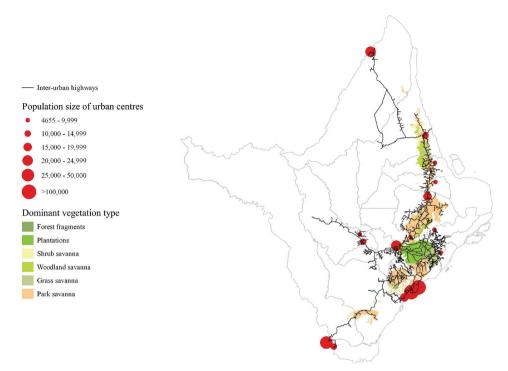


Figure 3. Highways and conurbations in the Cerrado of Amapá. The network of main highways (black lines), and municipal limits (grey lines) in the state of Amapá obtained from the Amapá State Environmental Secretariat (SEMA-AP 2016). The total length of highways in the state is 3,578.5 km, of which 1,999.9 km (55.9%) is within the Cerrado of Amapá. Of the 16 urban centres, 11 are within 10 km of the Cerrado of Amapá (points, with the size of the point proportional to the population – information obtained from IBGE (2016)).

quita et al. 2007; Nunes 2001; Pereira-Junior et al. 2013; Rocha et al. 2014; Saraiva et al. 2011; Schunck et al. 2011; Silva et al. 2013; Silva et al. 1997; Silva et al. 2006; Silveira 2003; 2006). Of the plant species reported, two are endemic to the state of Amapá – the carpet grass Axonopus amapaensis G. A. Black and the herb Borreria amapaensis E. L. Cabral & Bacigalupo (Jardim Botânico do Rio de Janeiro 2016; Rocha et al. 2014). Furthermore, the species' Appendicularia thymifolia (Bompl.) DC and Chamaecrista desvauxii var. saxatilis (Amshoff) H.S.Irwin & Barneby (Collad.) Killip occur only in the Guianan shield (Jardim Botânico do Rio de Janeiro 2016; Silva et al. 2015). The species Philodendron carinatum E.G.Gonç., also found in the Cerrado of Amapá, is considered rare in Brazil (Temponi et al. 2009). Amphibian species richness is high relative to other Amazonian savannas, likely maintained by the complex mosaic of savanna, forest patches, swamps and temporary ponds (Lima et al. 2017). Two fish species have also been described from the Cerrado of Amapá – the Amapá tetra Hyphessobrycon amapaensis (Zarske and Géry 1998), and Melanorivulus schuncki (Costa and De Luca 2010). The Amapá tetra has a very restricted distribution, and as such maintaining the integrity of the streams in which it is found is extremely important for

the conservation of this species (Nogueira et al. 2010). At least two of the mammals that have been recorded are endemic to the Amazonian savannas – Alston's cotton rat *Sigmodon alstoni* and a recently discovered species of opossum *Cryptonanus* sp. (Silva et al. 2013; Voss 2015). However, the area remains under-sampled for most taxa, and new state records of species of amphibians, birds and mammals have been made in the Cerrado of Amapá (Costa-Campos and Freire 2015; Schunck et al. 2011; Silva et al. 2013; Silva et al. 1997), highlighting the possibility of encountering new species and/or extending the ranges of existing species with better sampling of the region.

Owing to its geographic isolation and therefore relatively well-preserved state, Amapá is particularly important for the conservation of some species. For example, the only known populations of red-handed howler monkey (Alouatta belzebul) to the north of the Amazon River are found in the state of Amapá, where preliminary surveys show that occurrence of this species is predominantly limited to riparian forest within the Cerrado of Amapá (R. Hilário, unpublished data). To the south of the Amazon River, most populations of this species are highly threatened by the arc of deforestation, with just 10 small populations of this species remaining outside of the main area of deforestation pressure, in the Northeast Region of Brazil (Veiga et al. 2008). The species is listed as vulnerable by the IUCN (IUCN 2016). The Cerrado of Amapá is also recognised as an Important Bird Area (IBA) by Bird Life International, owing largely to the presence of large numbers of two declining bird species – the shrike-like tanager Neothraupis fasciata and the rufous-sided pygmy tyrant Euscarthmus rufomarginatus (De Luca et al. 2009). Both species are listed as near threatened in the IUCN Red List, with the principal threat being destruction and degradation of their Cerrado habitats (IUCN 2016). Importantly, E. rufomarginatus was previously listed as vulnerable, and was down-graded specifically due to its presence outside the highly threatened Cerrado biome, in the Amazonian savannas (IUCN 2016). As such, the massive conversion of the Cerrado of Amapá for agricultural production would represent a substantial loss of important habitat for *E. rufomarginatus*, and the species would almost certainly be up-graded once more in the IUCN Red List.

Conservation status

While the state of Amapá has ~72% of its territory covered by protected areas (Dias et al. 2016), these areas are almost entirely made up of *terra firme* (lowland tropical forest) and *várzea* (floodplain) forests, and flooded areas, and just 917.69 km² (~9.16%) of the Cerrado of Amapá has protection in strictly protected areas, multiple use areas and Indigenous Lands (Table 1). At least an additional 68.9 – 274.9 km² of savanna habitats fall within quilombos, traditional lands of the descendants of escaped African slaves, which are recognised under Brazilian Federal law as protected areas (Brazil 2006). However, biodiversity conservation is not usually a primary objective of these areas and their effectiveness in protecting against degradation has not been well documented. The vast majority of protected savanna habitats in Amapá are within multiple

Table 1. Total area and area protected of the four savanna types, and area of exotic plantations. Total area and area protected in each of three protection categories, of each of the four main vegetation types in the Cerrado of Amapá, and the total recorded area of plantations of exotic woody species. All values were calculated in ArcGIS v10.4.1 (ESRI 2011) using the Brazilian Institute of Geography and Statistics' vegetation cover map for the Amazon (IBGE 2004a), and the shapefiles of Conservation Units and Indigenous Lands available from the Amapá State Environmental Secretariat (SEMA-AP 2016) and the World Database on Protected Areas (IUCN and UNEP-WCMC 2016).

Vegetation type	Total area (km²)	Strictly Protected (IUCN I-IV) (km²)	Indigenous Lands (km²)	Multiple Use (IUCN V-VI) (km²)	Total area protected (km²)
Park Savanna	6048.76	$40.24^{1,2}$	27.03^{3}	414.54,5,6	481.77 (8%)
Grass Savanna	930.22			11.095	11.09 (1.19%)
Woodland Savanna	835.36			247.195	247.19 (29.6%)
Shrub Savanna	549.6			177.645,6	177.64 (32.32%)
Plantations	1657.46				NA
TOTAL	10,021.4	40.24 (0.4%)	27.03 (0.27%)	850.42 (8.49%)	917.69 (9.16%)

Numbers indicate the following protected areas: ¹Cabo Orange National Park; ²Seringal Triunfo Private Reserve; ³Uaça Indigenous Lands; ⁴Curiaú Environmental Protection Area; ⁵Amapá State Forest; ⁶Rio do Cajari Extractive Reserve.

use or sustainable use areas (IUCN category V-VI; 850.42 km², 8.49% of the total area) (Table 1).

Protection also varies across the four dominant vegetation types. For example, the grass savannas, the second most common savanna type in the Cerrado of Amapá, are the least protected, with just 1.19% of their 930.22 km² falling within the Amapá State Forest, a multiple use area (Table 1). This PA covers a total of ~403 km² of the Cerrado of Amapá, including areas of each of the four dominant savanna vegetation types. However, there have been proposals to revoke the act of creation of the Amapá State Forest (Euler 2016), originally established to consolidate the Biodiversity Corridor of Amapá (GEA 2010). This PA contains significant stretches of savanna-forest transition zones, and is incorporated in the mosaic of protected areas of the state (see Dias et al. 2016), in recognition of its importance for biodiversity conservation (Euler 2016). The Amapá State Forest also protects the only stretches of woodland savanna found within PAs, and about two-thirds of the shrub savannas that have protection (Table 1). As such, were it to be down-sized or degazetted, the grass savannas and woodland savannas of the Cerrado of Amapá could lose all protection, and shrub savannas could have their protection substantially reduced.

Park savannas represent ~60% of the total area of the Cerrado of Amapá, and are the only dominant vegetation type to be protected outside of multiple use areas (Table 1). However, most of their protection is still offered by multiple use areas including the Amapá state forest, the Rio do Cajari Extractive Reserve, and the Curiaú Environmental Protection Area (Table 1). Environmental Protection Areas (APAs), such as Curiaú, are not, however, subject to the same environmental licensing requirements for activities that have the potential to be polluting or to cause environmental degradation that govern other conservation units in Brazil (Brazil 2011). Instead, the large-scale

planting of crops is controlled by the same legislation (Resolution CONAMA 237/97) that governs environmental licensing of crop plantations and other activities in any part of the country (CONAMA 1997). Furthermore, Curiaú does not currently have a management plan, meaning that specifics of what is or is not permitted within the PA limits have not been made official.

Approximately 40 km² of park savanna is also protected by strictly protected areas – the Cabo Orange National Park and the Seringal Triunfo Private Reserve (Table 1), and a further ~27 km² fall within the Uaça Indigenous Land (Table 1). There is an overlap between Cabo Orange National Park and the Cunani Quilombo and owing to conflicting legislation, negotiations are on-going regarding the limits of the two protected areas which could eventually lead to a reduction in the amount of savanna habitat under strict protection. In Brazil, Indigenous Lands offer very effective protection (Nepstad et al. 2006), but the Brazilian federal government is currently considering modifications to legislation that would allow for exploitation of natural resources within Indigenous Lands (Fearnside 2016). As such, should the legislation be approved, Indigenous Lands could be opened up to mining and large-scale planting of crops (Fearnside 2016). Taking all of this into consideration, we conclude that the Cer rado of Amapá does not currently have effective long-term protection.

Major threats: present and future

While there is no good estimate available of the total area of the Cerrado of Amapá that has been cleared and/or degraded, estimates suggest that at least 450 km² have been cleared (Sano et al. 2017), and one estimate does suggest that up to ~1,949 km² (~19.5% of the total area) have been altered for use in silviculture, mechanized agriculture, livestock production and exploration of mineral resources (GEA et al. 2016). This includes at least 148.6 km² planted with soybeans in 2016 (an increase of almost 70% from 2013) (see Figure 2 C, D; IBGE 2017), with an expected increase to ~4,000 km² planted with soybeans by 2026 (almost 40% of the total area of the Cerrado of Amapá) (Silva 2016), with export facilitated by improvements to the Port of Santana (Monteiro 2015). Approximately 1,657.5 km² of the Cerrado of Amapá is also already planted with plantations of eucalyptus (Table 1). Massive silvicultural plantations in the Cerrado of Amapá were planned in the 1990s as a contribution to migrating global carbon emissions (Ab'Sáber et al. 1990; Fearnside 1998), but have not been planted as planned. However, such plans could be revived as Brazil's Intended Nationally Determined Contribution (iNDC), announced at the 2015 Paris Conference of the Parties under the climate convention, calls for "restoring and reforesting 12 million hectares of forests by 2030, for multiple purposes" (Brazil 2015).

Increases in infrastructure, including the construction of a new port, are acting to increase interest in use of the Cerrado of Amapá for plantations of crops and woody species, and the area has been recently referred to as Brazil's "final frontier" of soybean production (Silva 2016). *Grilagem* (land grabbing, or the illegal appropriation

of public land) with subsequent 'legalisation' of land-ownership rights is a further key contributing factor to the increase in area planted with eucalyptus and soybeans in the Cerrado of Amapá (CPT 2015; Silva 2014). With deeds of ownership in hand, future owners will be able to sell their lands or to acquire financing for investments in equipment and infrastructure for planting soybeans and eucalyptus (Gallazzi 2016; Silva 2016). Despite legislative tools to control deforestation (e.g. permanent preservation areas, legal reserves, and the Rural Environmental Register or CAR – *Cadastro Ambiental Rural* in Portuguese), and promotion of zero deforestation, land-grabbing continues in Amapá and other parts of the Amazon, contributing to on-going deforestation and conflicts (Benatti et al. 2006; Hill 2016; Oliveira 2013; Silva 2014; Tinoco and Sá 2016). This situation may be aggravated by the passing of Federal Lands to the state of Amapá, which occurred on 15th April 2016 (see decree in Brazil 2016), though land grabbing, land conflicts and the expulsion of local farmers have been on-going in the state since 2002 (Silva 2014).

The Cerrado of Amapá and other Amazonian savannas are further threatened by un-controlled burning over large areas that occurs as a result of poor fire management practices in areas where fire is used to clear areas for plantation and for livestock production (see Figure 2 E; Barbosa et al. 2007). Indeed, thousands of fire outbreaks have devastated the Cerrado of Amapá each year (Figure 4), mainly in the dry season (Figure 5). The number of "hot pixels", or 1-km² areas on a MODIS satellite image that contain one or more fires, has been on the increase since 2007 with dramatic increases in more recent years, with the number more than doubling between 2014 and 2015, and remaining very high in 2016 (Figure 4). In general, the number of fire outbreaks is higher in municipalities with a greater area of savanna habitats, including Tartarugalzinho and Macapá, where the highest numbers of fires occur and which are also the two municipalities with the largest areas of savanna habitats (Figure 4). Obvious exceptions to this pattern are Ferreira Gomes and Porto Grande, which is probably a reflection of the replacement of much of the savanna habitats in these municipalities with plantations of eucalyptus and acacia (see Figures 1 and 4), where fires are controlled by the companies that administer these plantations. While fire in Amazonian savannas has been present since pre-Columbian times, as indicated by charcoal in the soil (e.g. Turcios et al. 2016), these clear increases in recent years could be driven by climatic influences or by the expansion of commercial agriculture in some municipalities. Indeed, expansion of agriculture and livestock production across the state is likely to aggravate this threat, and in turn lead to a loss of biodiversity from the Cerrado of Amapá. The presence of roads, and consequent ignition sources, dramatically increases the frequency of fires in Amazonian savannas (Barbosa and Fearnside 2005b) and the Cerrado of Amapá contains more than half of the inter-city highways in the state (Figure 3).

The Cerrado of Amapá, other Amazonian savannas and forest areas that are still well-preserved in the states of Amapá and Roraima, and to the north of the Amazon River in the state of Pará, also face increased threats from the potential completion of the BR-210 Highway. The BR-210, if completed, would link Boa Vista in the state of Roraima with Macapá in the state of Amapá, crossing the state of Pará (G1 - GLOBO 2016). The stretch of the BR-210 that has already been constructed in Roraima has led

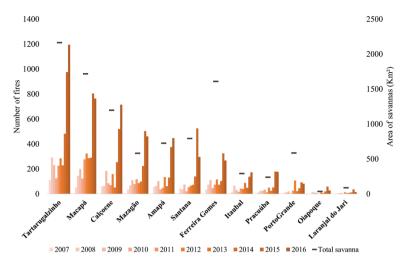


Figure 4. Fire in the Cerrado of Amapá, 2007 – 2016. Total number of hot pixels between 2007 and 2016, and total area of savanna habitats per municipality, in order of total number of outbreaks between 2007 and 2016. The hot pixels were quantified from shapefiles of the occurrence of burns obtained from the databases of the *Instituto Nacional de Pesquisas Espaciais* (INPE) (https://prodwww-queimadas.dgi. inpe.br/bdqueimadas/). Based on these shapefiles, and using ArcGIS v10.4.1 (ESRI 2011) the total number of hot pixels per year and per month within the Cerrado of Amapá were quantified in accordance with the Brazilian Institute of Geography and Statistics vegetation cover map for the Amazon (IBGE 2004a) and overlaid with the municipality boundaries available from the Amapá State Environmental Secretariat (SEMA-AP 2016). The area of savanna habitats was quantified as described in the legend of Figure 1.

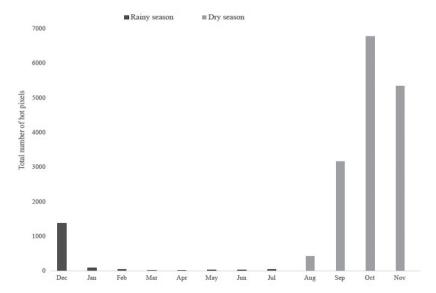


Figure 5. Total fires per month in the Cerrado of Amapá, 2007 – 2016. Total number of hot pixels in the Cerrado of Amapá between 2007 and 2016 in the rainy and dry seasons (summed across all municipalities and all years). The hot pixels were quantified as described in the legend of Figure 4. The classification of months in to the rainy and dry seasons followed Tavares (2014).

to the loss of large areas of forest along the highway (Barni et al. 2015), and in Amapá, practically all the deforestation that has occurred in recent years has occurred immediately adjacent to highways (SEMA-AP 2014). This construction, and the subsequent advance of illegal mining, land occupation and conflicts led to the organisation and ultimate recognition of the land rights of the Waiāpi indigenous people in 1996 (Gallois 1998). However, if completed, the planned highway would cross well-preserved areas and pass through Indigenous Lands, including those of the Waiāpi, and other protected areas. The threat to these areas would increase even further should legislation currently awaiting approval in the Brazilian National Congress be passed, thereby authorising mining within Indigenous Lands, and transferring the power to create new protected areas and Indigenous Lands to the legislative branch, where representatives of large landholders are a dominant influence (see Fearnside 2016).

The way forward

The current network of protected areas is insufficient to ensure the protection of the Cerrado of Amapá in the face of looming threats from large-scale planting of soybeans, plantation trees and other crops. Plans are already underway for zoning of the area for these economic activities (GEA et al. 2016), and we assert that this process must be open, equitable and participatory, involving local researchers, conservationists and crucially the rural, traditional and indigenous populations living in and around the Cerrado of Amapá, following the rules established in Brazilian Federal law concerning Ecological-Economic Zoning (Brazil 2002). To allow for the identification of representative areas for the protection of the region's biodiversity, investment of resources in research is now urgently required. Indeed, while many parts of the Cerrado of Amapá remain under-sampled, it is already clear that much heterogeneity exists in the flora and fauna of these savannas. As such, implementation of new protected areas within the Cerrado of Amapá must now be guaranteed, and these PAs must be positioned to be representative of the savanna ecosystem, taking in to account this heterogeneity and the social value of the different areas (Fearnside 2015; Fearnside and Ferraz 1995). Without such a process, there is a risk of losing a unique and important biodiversity before it has been properly documented.

Other priorities for sustainable development of the Cerrado of Amapá should include implementation of sustainable management practices, including appropriate management of the fire regime (Borges et al. 2016), soil conservation measures (Hudson 1995) and reduced pesticide and herbicide usage (Grovermann et al. 2017). Mechanisms should also be put in place to protect the many freshwater springs that originate in, or feed into, the Cerrado of Amapá. Crucially, proper monitoring will be required to evaluate the impacts of all activities in these unique savanna habitats, and particularly to ensure that negative biodiversity and social impacts of large-scale intensive agriculture and plantations are minimised. Indeed, sustainable development in the region will be impossible without a planning approach that generates income to

sustain local rural populations (Dias et al. 2016). Mechanisms that support and favour the continued development of markets and incentives for small-scale producers will be crucial for the sustainable development of the state of Amapá. At a broader scale, we further highlight the need to recognise the Amazonian savannas as a distinct and important habitat type, different from others large areas of savannas (i.e. Brazilian Cerrado), which would allow specific conservation initiatives to be implemented as part of a broader conservation policy. For example, Brazil's soya moratorium, which requires that soybeans not be produced in Amazonian forest areas deforested after July 2006 (Gibbs et al. 2015), could and should now be extended to require the same of soybeans produced in areas of Amazonian savanna (see Carvalho and Mustin 2017).

Productivity could be increased through integrated crop-livestock-forest systems (ICLFS) in areas already planted with eucalyptus. ICLFS contribute to soil conservation, using the soil more intensively but in concert with ecological management tools such as direct planting (Moraes et al. 2014). This type of approach to increase productivity has been promoted as a form land sparing to prevent the loss of stored carbon under the assumption that new areas will not be opened for agriculture (Pacheco et al. 2013). Brazil's voluntary pledges at the 2005 15th Conference of the Parties of the climate convention (COP-15) include the use of technologies such as ICLFS to help cut carbon emissions (Kichel et al. 2014). Brazil's Low-Carbon Agriculture (ABC) programme was launched in 2010 (Brazil 2010), and this programme further incentivizes implementation of ICLFS through provision of low-interest loans, although uptake has been slow (Angelo 2012; Strassburg et al. 2014). However, land sparing is a difficult conservation strategy because financial success of more productive commercial agriculture leads to more, rather than less, clearing for agricultural expansion (e.g. Fearnside 1987; 2002; Kaimowitz and Angelsen 2008).

Avoiding the conversion of Amazonian savannas to agriculture would contribute to maintaining climatic stability at local and regional scales (Butt et al. 2011) and benefit biodiversity conservation. The roots of savanna vegetation store a significant quantity of below-ground carbon (Barbosa et al. 2012). Without an effective programme to avoid savanna conversion, the quantity of carbon released by the savannas of Amapá could reach 8.15 t ha⁻¹ [estimate based on data from arboreal vegetation near Macapá collated by JJT and estimates available for Roraima according to Barbosa and Fearnside (2005a) and Barbosa et al. (2012)]. This release would represent a loss of US\$ 27 ha⁻¹, since each ton of carbon could be sold for US\$ 3.30 in the Voluntary Carbon Market (Hamrick and Goldstein 2016).

Here we draw attention to the Cerrado of Amapá, a biodiverse and highly threatened ecosystem that has to date received very little attention and almost no protection, compared with forested parts of the state. We have shown that there is an urgent need to implement protected areas, with local communities, scientists, conservationists and policy-makers working together to construct a sustainable and equitable plan for their management. By doing so, we can ensure the sustainable development of this isolated state in the far north of Brazil, providing solutions that result in positive social, economic and biodiversity outcomes— the so-called 'triple bottom line' for conservation.

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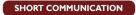
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Ecosystem services in a peri-urban protected area in Cyprus: a rapid appraisal

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Abstract

Protected areas around the world are increasingly being recognized for their potential to protect various ecosystem services in addition to biodiversity. We carried out an ecosystem services (ES) assessment at the Rizoelia National Forest Park, a biodiversity hotspot in Cyprus. For ES assessment we used TESSA v.1.1 and an ES matrix-approach to map the capacity of habitat types in the area. According to TESSA the most important ES provided by the study area are aesthetic benefits, recreation/ tourism, biodiversity, global climate regulation, and environmental education. Total Carbon stock was estimated to 14247.327 tonnes and the total number of annual visits was 14471. There were no statistically significant differences in the number of visits among visitation periods but there were statistically significant differences among National Holidays, Weekends and Weekdays. We identified that plantations had the highest capacity for most groups of services particularly where their understory comprises semi-natural habitat types rich in biodiversity. This is the first study in Cyprus which provides a preliminary quantification of ES in a protected area context using widely employed tools. The paper discusses how these findings can help decision-makers to plan direct future restoration and management actions to the benefit of a wide range of stakeholders.

Keywords

ES matrix-approach, island, mapping, Natura 2000, TESSA

Introduction

Worldwide the designation of protected areas aims to separate the components of biodiversity from natural or anthropogenic processes that threaten their persistence (Margules and Pressey 2000). This notion has been expanded over the years (see IUCN 1994, MA 2005) and while safeguarding biodiversity remains a Protected Area's (PA) primary aim, increasingly they are also considered to play a key role in the maintenance of ecosystem processes and the ecosystem services they provide (Bastian 2013; Watson et al. 2014). It is thus vital to assess the extent to which existing protected area systems represent those services. Local protected areas are an important resource for policy makers since they can be a real benefit to local populations. In an urban / periurban setting, the role of PAs is inextricably linked to quality of life, while at the same time they remain under intensive pressure from urban expansion and land-use/cover changes (Grimm et al. 2008). The paradox is that the negative effects of these changes to biodiversity result in turn in deterioration of ecosystem services (MA 2005), but at the same time human-induced actions may increase biodiversity of urban nature (Savard et al. 2000). The provision of ecosystem services (ES) by urban nature is considered part of a high-quality living environment (Tzoulas et al. 2007), while access to recreational services is important for public health (e.g. de Vries et al. 2003; Korpela and Ylén 2007). Thus, by identifying and assessing those benefits (ecosystem services), local policy makers can provide motivation for the establishment of protected areas beyond conservation – that of enhancing local human well-being.

ES have been defined differently by many authors (Boyd and Banzhaf 2007; Fisher and Turner 2008; Fisher et al. 2009; Seppelt et al. 2011, Burkhard and Maes 2017), with a number of classification frameworks proposed over the years (MA 2005; Haines-Young and Potschin 2013). In addition, there has been significant progress in the development of methods on ES quantification and mapping (Chan et al. 2006; Naidoo et al. 2008; Egoh et al. 2009; Eigenbrod et al. 2010; Vihervaara et al. 2010). However, most of the approaches developed for ES quantification use model-based proxies (Schulp et al. 2014) relying heavily on land cover-based assessments (Burkhard et al. 2009; 2012; Kandziora et al. 2013) in the absence of direct monitoring data on ES, and often produce maps that are too coarse in resolution to be useful at the local scale.

PA management is practised at the site-scale and thus requires practical inexpensive tools that can provide baseline information for biodiversity and ES assessment and monitoring. Although lately there has been a proliferation of ES assessment tools, site-based assessments, which are more useful to managers and practitioners, usually rely on theoretical scenarios or extrapolations from global models or require much greater technical skill and resources and are designed more for the academic user (Peh et al. 2013). As a result real site-scale approaches to ES assessment remain limited since the ES are often technically difficult and expensive to measure at that scale. The aim of this paper was to evaluate ES in a peri-urban national park in Cyprus and test the applicability of two widely used approaches namely:

a) the Toolkit for Ecosystem Service Site-based Assessment (TESSA) (Peh et al. 2013) and b) the 'ES matrix' approach (Burkhard et al. 2009) to map the potential/capacity of habitat types within the park. The specific objectives were to identify and assesse key ecosystem services provided by the park, map their spatial distributions and provide nature conservation authorities with a rapid methodology which can be applied in site-based ES assessments on the island.

Methods

Study area

Rizoelia National Forest Park (RNFP), a peri-urban Natura 2000 site close to the city of Larnaca, Cyprus (Fig. 1) is a biodiversity hotspot which has suffered intense human-related pressure over the years. Afforestation with non-native species, leisure activities and a dense road network has led to a decrease in size of two priority habitats at the European level: 1520* Iberian gypsum vegetation (Gypsophiletalia) and 5220* Arborescent matorral with *Ziziphus*.

TESSA based - Ecosystem Services Assessment

For ecosystem services (ES) assessment we used TESSA v.1.1 (Peh et al., 2013). TESSA is not computer intensive, it is inexpensive, easy to follow and easy to use for training purposes particularly for non-specialists. It focuses on the site level, is ideal for sites of biodiversity conservation importance and addresses the need to bring ecosystem services approach down to the operational scale using locally gathered information. This makes it ideal for assessing ES delivered by protected areas, producing results relevant to local decision-making and, when scaled up, for wider communication (Peh et al., 2013). Since different groups of beneficiaries value services differently, diverse stakeholder groups, i.e. local users, site managers and experts were involved. Based on a recent vegetation map (Andreou and Christodoulou 2014) of the site the mapped vegetation types were classified into broad habitat types according to TESSA (Table 1). In order to determine the most important ES delivered by the RNFP, we conducted an initial identification of its potential ES, taking into account historical information, literature data and the expert judgement by the Department of Forests (DF) officers (Table 2). We identified all the ES and their benefits delivered by the site using a table provided by TESSA. The provided benefits were all scored from 0-5, where 0=not relevant and 5= highly important (indicates the number of people benefiting). Then, the different stakeholder groups discussed and agreed on the five priority benefits (based on their scores). We used the MA (2005) framework for grouping services in order to be consistent with the chosen ES mapping method as described in the sections below.

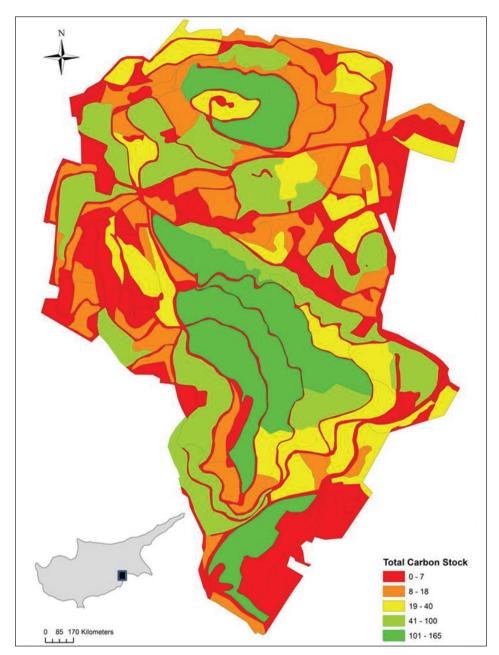


Figure 1. Location of the study area and Total Carbon Stock (tones of Carbon) derived from TESSA estimates.

Methods applied for global climate regulation assessment

Different habitats and land uses/cover have different potential influences on service of global climate regulation i.e. the exchange of carbon dioxide and other greenhouse

Table 1. Main habitat types at Rizoelia N.F.P, the corresponding habitat classification proposed by the TESSA toolkit and their extent in hectares.

	Habitat type	TESSA habitat classification	Area (ha)
Tree-dominated habitat	Needleleaf Plantation		14.57
free-dominated nabitat	Broadleaf Plantation	Temperate scrub/woodland	14.09
Cl 1	Sarcopoterium spinosum phrygana (5420)	f Plantation f Plantation f Plantation f Plantation sum phrygana (5420) Is with Ziziphus (5220) s and annuals of the Thero - classification 14 14 15 16 17 18 19 19 10 10 11 11 11 11 11 11 11 11 11 11 11	14.48
Shrub-dominated habitat	Arborescent matorrals with Ziziphus (5220)	scrub/woodland - Temperate	0.38
Grass Dominated	Pseudo-steppe with grasses and annuals of the Thero - Brachypodietea (6220)		0.19

Table 2. Potential ecosystem services at the current state of the site.

ES category (according to MA 2005)	ES	Benefits	Current state (score 0-5) 5= highly important	Top five services in the current state
	Global climate regulation	carbon storage in trees	4	V
	Local climate and air quality regulation	Providing shade, removing pollutants, influence rainfall	2	
Regulating		Water for human use	0	
0 0	Water-related services	Water flow regulation	0	
		Water quality improvement	3	
	Erosion control	Avoiding landslides	2	
		Foods	2	V
	II	Fibre	0	
	Harvesting wild goods	Natural medicines	0	
Provisioning		Energy	0	
		Food	1	
	Cultivated goods	Fibre	0	
		Energy	0	
Supporting	Biodiversity		5	V
		Scientific		
Cultural	Cultural/intellectual and	Educational	5	V
	representatives interactions	Entertainment	,	
		Aesthetic		V

gases between the atmosphere and the plants, animals and soils within ecosystems. Therefore, we treated separately each habitat type that was identified during the rapid appraisal stage. Field-based measurements were not possible due to lack of resources, as well as due to the protection/management regime of the area, which restricts interventions as required by the TESSA measurements. Therefore, we relied on the estimates provided in the TESSA manual for the study area which they were derived from credible values from similar sites or reliable sources mainly from the reports of the Intergovernmental Panel on Climate Change (IPCC) (Table 3). For each habitat type

Table 3. Summary of TESSA methods used for the estimation of carbon stock components

Habitats	Decision	Relevant Section	Climate section 6 Estimating total carbon stock	TESSA method	Method description
			Above-Ground live Biomass (AGB) carbon stock	IPCC- tier 1 - Method climate method 2 Estimating above-ground live biomass carbon stock using IPCC tier 1 estimates	To calculate the total above-ground live biomass of each habitat at the site, multiply above-ground live biomass by the area (ha) of the habitat. To calculate the total above-ground live biomass of each habitat at the site, multiply above-ground live biomass by the area (ha) of the habitat. To calculate the total above-ground live biomass carbon stock (t C) of your habitat, multiply the total above-ground live biomass by a conversion factor of 0.5 for tree-dominated, forest plantations, woody savannahs, perennial crop-dominated habitats and urban parks, or by 0.47 for grass dominated habitats, wetlands and urban lawn.
Tree- dominated habitats	Climate section 2	Trees were planted- Complete Climate sections 6-10	Below-Ground Biomass (BGB) carbon stock	IPCC conversion factors – Climate Method 5: Estimating below- ground biomass carbon stock using IPCC conversion factors	IPCC conversion factors – Climate Below-ground biomass carbon stock was estimated using a ratio of below ground-ground biomass to above ground biomass (R) for particular vegetation types (IPCC 2006): Temperate conifers (TC; 0.40), Temperate Bucahptus spp., (TE; 0.44), semi-arid grassland (SAG; 2.8) and Shrubland (S; 2.8).
			dead organic matter carbon stock	IPCC tier 1 estimates-Climate Method 6. Estimating dead organic matter (litter and dead wood) carbon stock using IPCC tier 1 estimates.	IPCC tier 1 estimates-Climate Method 6. Estimating dead organic The default value for the Needle leaf evergreen plantation is 20.3. To calculate the total litter carbon stock of each habitat, this value is audion stock using IPCC tier 1 multiplied by the area (in ha) of the habitat (14.566).
			soil organic carbon stock in mineral and organic soils –	Climate Method 7: Estimating soil organic carbon stock in mineral and organic soils. This section provides information on how to calculate soil carbon stocks in either organic or mineral soils.	Climate Method 7: Estimating soil soil on the Rizoelia site is mineral. The default mineral soil classification organic carbon stock in mineral soils. This section provides information on how to realculate soil carbon stocks in either the IPCC (2006), for the default reference soil organic carbon stock = 38 tonnes C ha-1. Total area size = 47.8632ha

Habitats	Decision	Relevant Section	Climate section 6 Estimating total carbon stock	TESSA method	Method description
			Above-Ground live Biomass (AGB) carbon stock	IPCC. tier 1 - Method climate method 2 Estimating above-ground live biomass carbon stock using IPCC tier 1 estimates	To calculate the total above-ground live biomass of each habitat at the site, multiply above-ground live biomass by the area (ha) of the habitat. To calculate the total above-ground live biomass of each habitat at the site, multiply above-ground live biomass by the area (ha) of the habitat. To calculate the total above-ground live biomass carbon stock (t C) of your habitat, multiply the total above-ground live biomass by a conversion factor of 0.5 for tree-dominated, forest plantations, woody savannahs, perennial crop-dominated habitats and urban parks, or by 0.47 for grass dominated habitats, wetlands and urban lawn.
Grass- dominated habitats	Climate section 3	Complete Climate sections 6,7,9,10	Below-Ground Biomass (BGB) carbon stock	IPCC conversion factors – Climate Method 5 Estimating below- ground biomass carbon stock using IPCC conversion factors	Below-ground biomass carbon stock was estimated using a ratio of below ground-ground biomass to above ground biomass (R) for particular vegetation types (IPCC 2006): Temperate conifers (TC; 0.40), Temperate Eucalyptus spp., (TE; 0.44), semi-arid grassland (SAG; 2.8) and Shrubland (S; 2.8).
			dead organic matter carbon stock	IPCC tier 1 estimates-Climate Method 6. Estimating dead organic matter (litter and dead wood) carbon stock using IPCC tier 1 estimates	IPCC tier 1 estimates-Climate Method 6. Estimating dead organic No existing tabulated data from IPPC (2006). It is assumed that there matter (litter and dead wood) are no significant litter and dead wood carbon pools for grass-dominated carbon stock using IPCC tier 1 habitats.
			soil organic carbon stock in mineral and organic soils –	Climate Method 7: Estimating soil organic carbon stock in mineral and organic soils. This section provides information on how to calculate soil carbon stocks in either organic or mineral soils.	
			Soil Organic Carbon Stock in Mineral soils	inventory	The first step is the definition of the climate domain of the sites. According to the Annex 3A.5 in Chapter 3 of IPCC (2006), for the default climate classification scheme maps, Cyprus is classified as warm temperate dry. The second step concerns the soil type of each habitat type. The FAO Soils Map of the World was used to identify the soil types on the site as cambisols. Next step is the definition of the habitat types in the area and to multiply the area of each habitat type with the default reference soil organic carbon stock for the area, which is 38 tonnes C ha ¹ .

Estimation of the greenhouse gases (CO ₂ , N ₂ O, CH ₄) emitted by the plants, soil and animals over time (positive flux).	TESSA method	Results
1. Carbon Dioxide (CO ₂) emissions	Climate Method 9	CO ₂ soil emissions from the site can be considered insignificant because the Rizoelia site has mineral soils. Therefore the emission of carbon dioxide from the Rizoelia site is negligible.
2. Methane emissions	Climate Section 10	Methane emissions from the site can be considered insignificant because the Rizoelia site has no grazing.
3. Nitrous oxide emissions	Climate Section 11	Nitrous oxide emissions from the site can be considered insignificant because the Rizoelia site has no fertilisers added, is not a drained peatland and is not grazed.
The carbon sequestered (taken in from the atmosphere) over time by the plants and soil (negative flux)	Climate section 6	Represented as C sequestered in above ground biomass vegetation over 1 year.

Table 4. Summary of TESSA methods used for the estimation of greenhouse gasses emissions and results.

Table 5. Summary of total carbon estimation results per habitat type.

	RNFP Habitat Type	TESSA Habitat Classification	Area (ha)	C_AGB	C_BGB	C_Soil	C_dead	Total Carbon stocks (tC)
	Needle-leaf Plantation	Temperate scrub/ woodland	14.56	349.58	139.83		295.69	
	Other Trees Plantation	Temperate scrub/ woodland	14.08	338.05	148.74		no data	
	5420	Temperate scrub/ woodland	14.47	347.47	972.92		no data	
	5220	Temperate scrub/ woodland	0.38	9.19	25.73		no data	
	6220	Grass- Dominated/ Temperate grassland	0.18	0.20	0.57		0	
	Non- vegetated	n/a	45.60	no data	no data		no data	
	Synanthropic	n/a	1.42	no data	no data		no data	
Total			90.73	1044.51	1287.81	11619.30	295.69	14247.32

(tree-dominated and grass- dominated) we assessed factors, which might affect global climate regulation: the carbon stored in the plants (above-ground biomass; below-ground biomass), dead organic matter and soil and the greenhouse gases emitted by the plants, soil and animals over time. The methods given by TESSA and used for carbon stock estimation and gas emissions are given in Table 3 and Table 4 respectively, while the results are summarized in Table 5. The methodology used by TESSA provides with decision trees to help users determine the most appropriate method for estimating carbon stock and gas emissions based on a number of questions related to study area characteristics and data availability.

Methods applied for Nature-based tourism and recreation

TESSA methodology takes into account two different aspects of recreation and nature-based tourism ecosystem services delivery i.e., the total number of visits to the site, and the associated expenditure. Census Recreation Method 1, is given as a means to measure the volume of nature-based tourism and recreation, while Recreation Method 2 for the economic value.

Recreation Method 1 was used to measure the volume of nature-based tourism and recreation, (Peh et al. 2013). Particularly, Recreation Method 1 is essentially a census of visitors, where a count of the people visiting the site (at the visiting entrances) over an entire year takes place (Peh et al. 2013). The initial assumption was that visits are over 100 per year so a census was conducted in order to estimate the total number of annual visits. Counting of visits took place from April 2014 until February 2015 divided in three periods (March-May, June-October and November-February) to examine the effect of seasonality on visitation. In each period, seven census surveys (21 in a total) were conducted during weekends, weekdays and national holidays. Counting points were located at RNFP's entrances (North and South) and surveys took place from 7:00 to 19:00. In order to identify people which were visiting the site for nature-based tourism or recreation we asked all visitors directly. At the same time we used Recreation Method 2 (Peh et al. 2013) for estimating tourism and recreation economic value. A short questionnaire was prepared to capture visitors' spending on the area and was distributed to 246 visitors. The Questionnaire requested information regarding gender, ethnicity, frequency of site visits, duration of trip and amount spent during travel and on the site.

ES 'matrix' approach

We used the ES 'matrix' approach (Burkhard et al. 2009; 2012), which links ES to appropriate geo-biophysical spatial units, to map the potential/capacity of habitat types to deliver ecosystem services. The ES matrix approach is based on a normalisation of ES indicator values to a relative scale ranging from 0–5 for their supply, flow and/or demand ranking. "0" represents no relevant ES supply or demand, while at the other end of the scale, "5" represents the maximum ES indicator value (Burkhard and Maes 2017). We assessed the maximum potential quantity of a service provided per unit area of each habitat type/land cover type in the RNFP under natural conditions using the scale of 0–5 (Appendix 1).

The scores assigned to each habitat and function were derived through brainstorming sessions (experts judgement) between local experts from the Department of Forests (DF) and the Department of Environment (DE), experts from two national Universities and literature searches. Scores retained represent the consensus of these sessions. We did not evaluate provisioning services since activities related to these services are not allowed or in the area encouraged. However, we have accounted for occasional biomass resulting from fuel wood extracted during invasive species removal.

Mapping ES

In order to map the spatial extent of carbon related components at the RNFP, we used the habitat types as spatial units within which we attributed the values for each of the four carbon components and the total carbon stock, as derived from TESSA estimates (Fig. 1). In addition, we mapped supporting services, regulating and maintenance, and cultural services per habitat type based on the ES 'matrix' approach.

Results

The most important habitat types at the site and their current extent are given in Table 1. According to the Rapid Appraisal analysis the most important ecosystem services delivered by the study area refer to Aesthetic benefits/inspiration, Recreation/tourism, Biodiversity, Global climate regulation, and Environmental education. Aesthetic benefits/inspiration and Recreation/tourism are included in the broad category of Nature-based recreation and Global climate regulation referred mainly to the Carbon related services.

TESSA based ES assessment

Global climate regulation

The total carbon stock at the RNFP was calculated by adding the carbon stocks for each habitat at the site [Above-Ground Biomass (C_ABG), Below-Ground Biomass (C_BGB), Soil (C_Soil) and Dead organic matter (C_dead)] to derive the total carbon stock at the site (expressed as tonnes of carbon) (Table 5). The total annual greenhouse gas flux for the site was insignificant and it was excluded from the calculations (Table 4).

Nature-based recreation - tourism and recreation economic value

The total number of annual visits (TANV) in RNFP was 14471. Particularly, TANV for periods A (March-May), B (June-October) and C (November-February) was 3834, 4734 and 5903 respectively. There were no statistically significant differences (One-way ANOVA; Sig. = 0.459) in the number of visits among visitation periods (March-May, June-October, and November-February). On the contrary, there were statistically significant differences (one way ANOVA; Sig. = 0.009) among National Holidays, Weekends and Weekdays. Specifically, multiple comparisons using Least Significant Difference t test (LSD) showed that the highest differences in the mean number of visitors are between National Holidays and Weekdays. There were also significant differences between National Holidays and Weekends. The results indicated that the number of visits increased significantly during public holidays. The economic survey revealed that

75.4% of the respondents spent less than 5 euros (mainly for fuel) during their trip to the study area. However, most of the respondents stated that there would be willing to spend more should there be facilities provided in the park.

Mapping ecosystem services

In terms of supporting ES we identified that plantations, despite considered of low biodiversity value, (Needle-leaf Plantation, Temperate scrub/woodland) have the highest capacity (compared to the natural and semi-natural habitats of the site) to provide ES, particularly in places with priority habitat types to their understory. Mapping regulating services capacity corroborated the importance of plantations compared to other habitat types due to the dominant life-form. Apart from roads, habitats comprising exclusively synanthropic vegetation communities scored low in general, except for the cases that these occur in mixture with the *Ziziphus lotus* habitat type (higher score). In addition, the conifer plantations of RNFP were perceived more important for recreation in a peri-urban setting; hence, scored higher at the cultural services compared to other habitat types (Fig. 2). When the sum for all groups of services was calculated, the results demonstrated that some plantations within the RNFP attained the highest value in places where their understory comprises one or more of the habitat types 1520*, 6220* and 5420.

Discussion and conclusions

This is the first study of a complete site-based ES assessment in a protected area setting in Cyprus and as such, it has the potential to support environmental management and policy. The results corroborated the importance of RNFP for ES provision, in addition to biodiversity support, with direct and indirect benefits to the local community. Among the most important findings of this study are the results on recreation as a service in the study area, since it is higher than ever recorded in the past with annual visits (TANV) reaching a total number of 14471, with most of them during days off work in the period November to February. Recreation activities in the study area are less associated with nature-based activities like wildlife and forest appreciation. Only 21% of the respondents gave as main reason of their visit the appreciation of nature.

High visitor numbers may result in conflict between nature conservation and recreation in peri-urban parks (Borgstrom 2008; Sterl et al. 2008; Wagner et al. 2005; Zai-kanov and Kiseleva 2008). Recreational services have economic importance (Tyrväinen and Miettinen 2000) while they are also important for tourism, as nature-related activities often influence the decisions of foreign tourists to travel to Cyprus. Protected areas, if properly managed, can provide, among other benefits, a source of income to local communities. In the case of RNFP visitors live and work locally, therefore contribute relatively small amounts to the local economy while there are no extra facilities

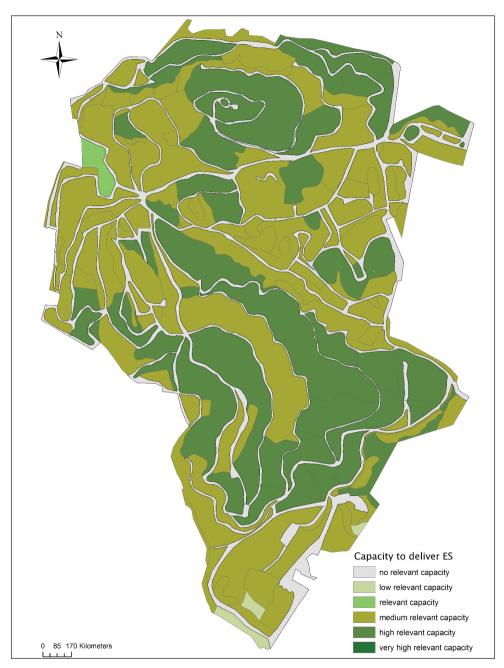


Figure 2. Capacity of habitat types in the study area to deliver ES (based on ES matrix approach described in the text).

on site where money could be spent on. Within a peri-urban park, green areas and tree plantations can function as carbon sinks (McHale et al. 2007), although urban vegetation only sequestrates a small part of annual CO₂ emissions of a city (Jansson and

Nohrstedt 2001; Lebel et al. 2007). In fact, urban parks can function as carbon sources because management and the use of parks produce multiple amounts of CO₂ emissions compared to the carbon sequestration capacity of a green area (Oliver-Sola et al. 2007). Although urban carbon sinks, do not necessarily have a significant impact on the global carbon balance, urban green areas can have local importance as carbon sinks.

In the study area, apart from the Mediterranean grass-dominated habitat (6220*), the above-ground carbon stock measurements did not show variability. On the other hand, the highest below-ground carbon biomass was estimated in the Mediterranean scrub habitats (5220* and 5420). The lack of tabulated data for scrubs or broadleaf evergreen woodland was the main limitation for the implementation of the TESSA approach, which led to the underestimation of litter and dead organic material carbon stocks for all habitat types, except the Needle-leaf plantation. Another underestimation refers to the contribution of the extensive root systems, particularly in the cases of known phreatophytes like *Ziziphus lotus* (Gorai et al. 2010). Despite this limitation, in the absence of resources for extensive fieldwork, TESSA provides a consistent, inexpensive, rapid tool for ecosystem services appraisal at local level, which can be easily applied by practitioners and understood by stakeholders (Peh et al. 2014; Blaen et al. 2015). The tool becomes even more valuable in protected areas, including Natura 2000 sites, where often restrictions apply for land use and conservation management, which might influence the provision of services.

The second part of this assessment included an expert-based evaluation and mapping of the capacity of various habitat types to support a range of services which is now widely accepted in ES science (Jacobs et al. 2015), despite its reported challenges (Burkhard et al. 2009; 2012; Eigenbrod et al. 2010). Although, Rizoelia NFP is a biodiversity hotspot hosting three priority habitat types of European importance, the study highlighted that plantations in the area, despite their low biodiversity value, are equally important in terms of their capacity for ecosystem services supply. Therefore, in addition to biodiversity conservation, competent authorities should also manage the area in the future in a way that maximizes the provision of these services. This is now facilitated by the present first evaluation of the area's potential, and the explicit spatial nature of the approach for visualizing the extent of services in the park (Tallis and Polasky 2009). What is equally important in terms of management is shifting from solely biodiversity delivery to ES delivery and the need to identify trade-offs between ES. The relationship between biodiversity and ecosystem services is a complex one (Mace et al. 2012) and has sparked a debate among conservationists on whether management interventions can achieve both ES and biodiversity related targets (see Reyes et al. 2012). In order to quantify the complex inter-relations between ecosystem services, drivers and pressures there is a need to take into account these different components and identify trade-offs (Haines-Young and Potschin 2007; Haines-Young et al. 2012; Haines-Young 2012; Sharp et al. 2016). The ES matrix approach is an evolving one and since it is quite general, there is a lot of scope for improvement. However, this rapid expert driven approach is a good early indication of what it can be supported by the park in terms of ecosystem services, their general patterns and how they can be assessed given more time and resources. It is interesting to note that the

mapping results for carbon sequestration using TESSA, corroborates experts-based assessment; the latter aiming at assessing the magnitude of provision/capacity and therefore the importance of certain habitat types (like plantations) for regulating and maintenance services delivery in the study area, was carried out independently.

The study demonstrated the importance of site-based assessment for ecosystem services delivered in protected areas, pointing out a gap at the national level for a rigorous approach in such assessments in parallel to other national studies at the European level and in accordance with the obligations of Cyprus under the EU biodiversity strategy for 2020. These types of assessments are precursors to economic valuation of ecosystems services and the identification of their direct financial benefits to the local communities (Defra 2007, TEEB 2010, UK-NEA 2011).

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Appendix I

Matrix for the assessment of the different habitat types capacities to deliver selected ecosystem goods and services (adapted from Burkhard et al. 2009). The assessment scale ranges from 0-5 as described below. In the rows between the assessments (yellow colour), sums for the individual ecosystem services groups were calculated.

Equivalent Land Cover Types	Habitat Types / Land cover types	Supporting Services	Abiotic heterogeneity	Biodiversity	Biotic waterflows	Metabolic Efficiency	Exercy Capture (Radiation)	Reduction of nutrient loss	Capacity Storage	Provisioning Services	Crops	Livestock	Fodder	Wild Food	Timber	Fuel Wood	Energy (Biomass)	Biochemical/Medicine	Regulating Services	Local climate	Global climate	Flood protection	Groundwater recharge	Air quality	Erosion	Nutrients	Water Purification	Pollution	Cultural services	Recreation & Aesthetic values	Intrinsic Biodiversity Value
Industrial or commercial units	Buildings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Road and rail networks	Roads	4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Plantations	21	2	1	3	3	4	4	4	2	0	0	0	0	0	2	0	0	23	3	1	4	2	2	4	4	1	2	6		1
	Plantations+ 1520	24	2	4	3	3	4	4	4	2	0	0	0	0	0	2	0	0	23	3	1	4	2	2	4	4	1	2	9		4
Green urban areas	Plantations+ 5420	23	2	3	3	3	4	4	4	2	0	0	0	0	0	2	0	0	23	3	1	4	2	2	4	4	1	2	8		3
areas	Planta- tions+6220	24	2	4	3	3	4	4	4	2	0	0	0	0	0	2	0	0	23	3	1	4	2	2	4	4	1	2	9		4
	Plantations+ synanthropic	22	2	2	3	3	4	4	4	2	0	0	0	0	0	2	0	0	23	3	1	4	2	2	4	4	1	2	7		2
Non-irrigated arable land	Cultivation	17	2	1	3	3	3	1	4	0	0	0	0	0	0	0	0	0	5	2	1	1	1	0	0	0	0	0	1	0	1
NY 1	1520	24	3		2	2	2			0	0	0	0	0	0	0	0	0	16	2	3	1	1	0	3	3	3	0	6	1	5
Natural grassland	6220+1520	20	3		2	2	2	3	3	0	0	0	0	0	0	0	0	0	16	2	3	1	1	0	3	3	3	0	6	1	5
5143314114	6220+5420	20	3		2	2	2	3	3	0	0	0	0	0	0	0	0	0	16	2	3	1	1	0	3	3	3	0	6	1	5
	5420	21	3	4	2	3	3	4	2	0	0	0	0	0	0	0	0	0	16	2	1	1	1	0	3	3	3	2	6	2	4
	5420+1520	20	3	5	2	2	3	2	3	0	0	0	0	0	0	0	0	0	19	2	1	1	2	0	4	4	3	2	7	2	5
	5420+5220	22	3	5	4	2	3	2	3	0	0	0	0	0	0	0	0	0	20	2	1	2	3	0	3	3	4	2	7	2	5
Sclerophyllous	5420+6220	18	3	3	2	2	3	2	3	0	0	0	0	0	0	0	0	0	19	2	1	1	2	0	4	4	3	2	5	2	3
vegetation	5420+ Plantation	22	4	3	3	3	4	2	3	0	0	0	0	0	0	0	0	0	20	2	2	2	2	1	3	3	3	2	5	2	3
	6220+1520	19	3	4	2	2	3	2	3	0	0	0	0	0	0	0	0	0	17	2	1	1	2	0	3	3	3	2	6	2	4
	5420+ synanthropic	17	3	3	2	2	2	2	3	0	0	0	0	0	0	0	0	0	14	2	1	1	2	0	2	2	2	2	5	2	3
	Synanthropic	10	3	2	1	1	1	1	1	0	0	0	0	0	0	0	0	0	3	1	0	1	1	0	0	0	0	0	1	0	1
Sparsely	Synanthropic+ 5220	20	3	5	2	2	3	2	3	0	0	0	0	0	0	0	0	0	23	2	2	2	3	0	4	4	4	2	7	2	5
vegetated areas	Synanthropic+ 5420	18	3	3	2	2	3	2	3	0	0	0	0	0	0	0	0	0	16	2	1	1	2	0	3	3	2	2	5	2	3
	Synanthropic+ 6220	18	3	3	2	2	3	2	3	0	0	0	0	0	0	0	0	0	16	2	1	1	2	0	3	3	2	2	5	2	3
0	no relevant ca	ipad	city	of	th	e h	abi	tat	typ	e t	ор	rov	vide	e th	is	par	ticu	ılaı	r ec	osy	ste	m s	erv	rice							
1	grey green = l	ow	rel	eva	nt	cap	aci	ty,			•																				
2	light green =	rele	var	ıt c	apa	cit	y																								
3	yellow green :				_			ca	pa	city	ζ,																				
4	blue green = l	nigł	ı re	elev	ant	ca	pao	city	an	d																					
5	dark green = 1	vers	, hi	iah	rel	eva	nt	car	200	tx																					





Terrestrial mammal assemblages in protected and human impacted areas in Northern Brazilian Amazonia

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Abstract

Mammal communities in the vicinity of human settlements are often subject to subsistence hunting and retaliatory killings. We used fourteen digital camera traps equipped with infrared triggers to sample the medium-sized and large mammal communities for ca. 34 (±1.64) days per site. Diversity was measured as both Shannon entropy and Fager's number of moves (NMS), and dominance was quantified using the Berger-Parker index. We used Kruskall-Wallis tests to investigate if there were statistically significant differences in richness, diversity and dominance among the sites. At an overall sampling effort of 1,946 trap days we recorded 216 independent observations of a total of 20 species belonging to 17 genera and 15 families. Richness and diversity appeared to be determined by forest structure, since, independent of the level of human impact, the richest areas were those closest to the ombrophilous forests of southern Guyana shield, closest to central Amazonia, whereas the poorest were at those sites closest to the vegetation mosaics of central Guyana shield. The disappearance of *Tayassu pecari* from the impacted areas as well as higher relative abundances in the protected areas, albeit not significant, foresees a possible bleak future for the mammalian assemblages in the near future.

Keywords

Medium-sized and large terrestrial mammals, Amazonia, Richness, Relative abundance, Fishbone human settlements

Introduction

Studies in South America, India, and Africa (Hill et al. 1997, Naughton-Treves et al. 2003, Rist et al. 2009, Levi et al. 2009, 2011, Pillay et al. 2011, Rovero et al. 2012) have shown that deforestation and hunting have been the major drivers of mammalian assemblages decline and ultimately mass extinction (Mendes Pontes et al. 2016). This can change the overall composition of the assemblage, but can be attenuated by the presence of a continuous forest matrix, which allows an efficient source-sink balance with recolonization of the depleted areas, such as in southeastern Peru (Ohl-Schacherer et al. 2007), and Ecuadorian Amazonia (Zapata-Rios et al. 2009).

Richness and diversity of terrestrial large mammals in the Neotropics is higher in strictly protected areas that are surrounded by other undisturbed forests than in those subject to deforestation and hunting in its surroundings, and is lowest in highly impacted areas (Tobler et al. 2008, Ahumada et al. 2011, Pickles et al. 2011, Botelho et al. 2012, Santos and Oliveira 2012, Carvalho et al. 2014, Melo et al. 2015, Meyer et al. 2015). In strictly protected areas that are surrounded by other undisturbed forests, Carnivora and Artiodactyla are among the richest mammalian orders (Santos and Oliveira 2012, Carvalho et al. 2014), whereas in protected areas that are subjected to anthropogenic impacts in its surroundings, although Carnivora may still be the most speciose order, Rodentia and Xenarthra may replace Artiodactyla and become the most species-rich orders (Ahumada et al. 2013, Michalski et al. 2015, Lizcano et al. 2016). This could be the result of a time-lag between deforestation and/or hunting, and species extirpation, or result from location, resources availability, species resilience and proximate benefits of secondary vegetation (Tobler et al. 2008, Pickles et al. 2011, Botelho et al. 2012, Bovendorp and Guevara 2015, Galetti et al. 2015, Melo et al. 2015, Meyer et al. 2015).

The first to become rare or extinct from the impacted areas are the large-bodied, terrestrial mammals such as white-lipped peccary, *Tayassu pecari*, jaguar, *Panthera onca*, giant ant-eater, *Myrmecophaga tridactyla*, tapir, *Tapirus terrestris*, and puma, *Puma concolor*, with *T. pecari* being one of the most sensitive, and therefore, the first to go locally or, regionally, extinct (Azevedo and Conforti 2008, Peres 1996, Reyna-Hurtado and Tanner 2007, Naranjo and Bodmer 2007, Richard-Hansen et al. 2014, Melo et al. 2015, Meyer et al. 2015). This is due to their higher energetic demands, larger home ranges, slower reproductive rates, and densities (Peres 2000, Brown and Brown 1992, Grelle et al. 2006).

The impacts of hunting, nevertheless, may also depend on the ethnic origin and dietary preferences of the local assemblages of hunters (e.g. indigenous; *caboclos*, which are descendants of Indigenous peoples with Europeans, among others) (Peres 2000).

In the northern Brazilian Amazonia, where the Government provides incentives for non-Amazonians of European and African ancestry to immigrate to the newly created human settlements (as in this study), long-term monitoring of their taboos and hunting practices is critical for assessing the impacts of hunting on primate assemblages (see Levi et al 2009, 2011).

Studies of medium-sized and large mammals in the Neotropics with the use of camera-traps has become increasingly popular in the last decades. It has been used to determine richness and diversity, abundance, habitat preferences and activity patterns, and has been suggested to be among the best methods for conducting minimally invasive mammal surveys (van Schaik and Griffiths 1996, Maffei et al. 2005, Goulart et al. 2009, Ahumada et al. 2011, Harmsen et al. 2011, Munari et al. 2011, Gonçalves 2013, Michalski et al. 2015, Lizcano et al. 2016).

Camera-trap studies have also shown that the most abundant species in strictly protected areas that are surrounded by undisturbed forests in Central Amazonia is normally a large mammal, such as *Tapirus terrestris* or *Tayassu pecari* (Santos and Oliveira 2012, Reyes 2013), whereas in protected areas that are subjected to anthropogenic impacts in its surroundings and in those highly impacted areas throughout the Amazonia *Dasyprocta* spp. is normally the most abundant (Tobler et al. 2008, Botelho et al. 2012, Michalski et al. 2015, Lizcano et al. 2016). Studies of medium-sized and large mammals in the State of Roraima are relatively recent and the focus has been mainly on the mammals of protected areas, such as Maracá Ecological Station (Mendes Pontes 1997, 1999, Fragoso 1998, Mendes Pontes et al. 2007), and the Waimiri-Atroari (Mazurek et al. 2000), Yanomami and Macuxi Indian reserves (Fragoso 2004). Studies around the fishbone human settlements are almost completely absent, with the only exception of our previous study (Melo et al. 2015). Thus, the diversity of mammals, the impact of hunting and the types of wildlife used in these settlements of Roraima remain almost completely unknown.

In this study we aimed at determining richness, diversity and abundance of medium-sized and large mammals in the Guyana shield of the Brazilian Amazonia, in protected areas and also in fishbone human settlements surrounded by undisturbed forests where no previous camera-trap studies were available.

Methods

Study area

The study was carried out in the Guyana shield of the Brazilian Amazonia, in two protected areas (PA) and two impacted areas (IA), as follows: (1) PA Maracá Ecological Station (3°25'28"N; 61°39'21"W) (Figure 1): an area of 104,000 ha, located in the centre of the Guyana shield, in Brazililan Amazonia, with a typical vegetation comprised of a mosaic of seasonally-dry terra firme forest, campinarana forest, campina and savannas. Mean annual temperatures range between 24°C and 31.6°C (Mendes Pontes 2004, ICMBio 2010, SEPLAN 2010a).

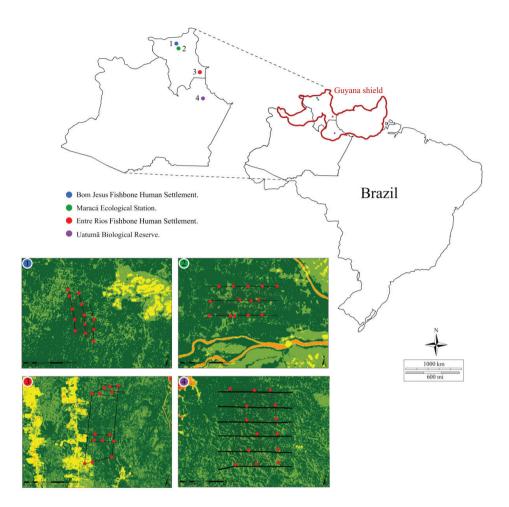


Figure 1. The study area in Northern Brazilian Amazonia showing the four study sites with the study transects and trapping stations. Guyana shield is according to Hoorn et al. (2010).

(2) PA Uatumá Biological Reserve (1°48'24"S; 59°14'16"W) (Figure 1): It has an area of 9,500 ha, is located in southern Guyana shield, and has a vegetation typical of southern Guyana shield, central Amazonia, comprised of dense ombrophilous terra firme forests (ICMBio 2013). Mean annual temperature is around 28.5°C (Governo do Estado do Amazonas 2013).

The impacted areas are formed by two fishbone human settlements, which are forest clearings that appear in satellite images in a fishbone pattern, with forest clearance extending along secondary roads from the main road (Figure 1). The settlements were founded in the 1990's by the Government of the State of Roraima in what was once undisturbed forests (Governo do Estado de Roraima 2005). It is primarily populated

by non-indigenous settlers (and their descendants) of European and African ancestry who came from regions outside the Amazonia (mostly north and northeast of Brazil) attracted by free land offered by the Federal Government as part of an Amazonian colonization program initiated under the initiative ,Cut it or Lose it'.

The settler families have a low income, and frequently practice deforestation and forest burning both inside and outside their plots to clear the land for pasture and to plant crops. Because these plots are located within the forest, this is easily accessed and this facilitates a form of hunting which is practiced indiscriminately and without any control by the local government (as in Melo et al. 2015; R.B.L. personal observation). Additionally, settlers are entitled to 'subsistence hunter permits' granted on request by the local office of the Federal Police (Melo et al. 2015; A.R.M.P. personal communication). The settlements are:

- (3) IA Bom Jesus human settlement (3°37'53"N; 61°42'29"W) (Figure 1): It covers 15,000 ha, and is located in the centre of the Guyana shield. Natural vegetation is a mosaic of seasonally-dry terra firme forest, campinarana forest, campina and savannas (ICMBio 2010). Mean annual temperature is around 26°C (SEPLAN 2010a). Population is approximately 900 non-indigenous settlers whose main activities are livestock and smallholder farming, but they also practice subsistence agriculture.
- (4) IA Entre Rios human settlement (0°48'1"N; 59°25'41"W) (Figure 1): It covers 1,206 ha, and is located in the south of the Guyana shield. Natural vegetation is seasonally-dry to dense ombrophilous terra firme forests (IBGE 2005). Mean annual temperature is around 27°C (Governo do Estado de Roraima 2005, SEPLAN 2010b). Population is 1,100 non-indigenous settlers whose main activities are livestock and smallholder farming, but they also practice subsistence agriculture.

Vegetation types present at the study sites and recognisable on RADAM Project (RADAMBRASIL 1975, IBGE 2005) satellite images, are classified as:

- (1) Dense ombrophilous terra firme forest: continuous closed-canopy forests with trees of up to 50 m tall that occur in alluvial soils, which are or have been subjected to seasonal flooding. In this study they occurred in southern Guyana shield;
- (2) Seasonally-dry terra firme forest: closed- and open-canopy forests with trees of up to 40 m tall that occur on flat terrain that is not subjected to flooding. In these forests many trees lose their leaves seasonally due to water stress which can last for up to six months. In this study they occurred in central and northern Guyana shield;
- (3) Campinarana forest: open canopy forests with thin-trunked trees of up to 15 m tall on hydromorphic soils with many shallow pools that flood during the wet season. In this study they occurred in central and northern Guyana shield;

- (4) Campina: Sparse short trees of up to 5 m tall on sandy oligotrophic soils. Occasional stands of palms occur along streams, and some areas are covered by grasses and sedges. In this study they occurred in central and northern Guyana shield;
- (5) Savannas: open field formed mainly by grasses, an herbaceous layer and widely spaced contorted trees. In this study they occurred in central and northern Guyana shield.

Experimental design

Fieldwork in all areas was carried out during the dry season (Figure 2). In PA Maracá field work was carried out between 3rd November and 7th December 2012. In PA Uatumã, between 21st August and 25th September 2012. In IA Bom Jesus, between 9th March and 9th April 2013. In IA Entre Rios, between 15th February and 21th March 2012. We sampled the medium-sized (≤5 kg, excluding mice and marsupials) and large (>5 kg) terrestrial mammals of the selected areas via digital camera traps (Tigrinus Digital 6.5D, Timbó, Santa Catarina, Brazil), equipped with infrared triggers. Fourteen cameras were placed in each of the 14 pre-established trapping stations, fixed 30 to 40 cm from forest ground, and maintained working uninterruptedly for 24 hours a day for a mean of 34 (±1.64) days. We set cameras to take one picture at every 3 min., and each picture was considered an independent record of the same species in the same camera after a period of one hour from the previous one. We visited the cameras every eight days when to conduct maintenance, replace memory cards and download data.

In the PAs, surveys were carried out in the 25-km² RAPELD grids of the PPBio program of the Brazilian government (www.ppbio.inpa.gov.br), which consists of six north-south and six east-west 5-km trails alphanumerically marked every 100 m. We aimed to place the 14 camera-traps within the grid in the most homogeneously order possible so as to cover the greatest part of the 25-km² PPBio grid. In the IAs the surveys were carried out in freshly opened parallel trails, each one located behind and perpendicular to one of the inhabited plots of the fishbone human settlements.

Field constraints (locals denying permission to access their plots, camera traps thefts, and threats to the lives of the researchers) determined the size, location and number of trails, and camera trap points in the IA (six 4-km trails in IA Entre Rios; three 3-km trails IA Bom Jesus). Following Ahumada et al. (2011), Munari et al. (2011) and Botelho et al. (2012), once we determined the camera trap points in the four areas, we subsequently searched for spots close to animal trails, footprints and mud holes to maximize image capture. Additionally, we also had to avoid those trails that were seasonally or eventually flooded to prevent camera damage. Mean distance between camera traps in Maracá Ecological Station was 1,033 m (±463), in Uatumã Biological Reserve it was 1,820 m (±249), in Bom Jesus fishbone human settlement it was 529 m (±121), and in Entre Rios fishbone human settlement it was 1,338 (±197).

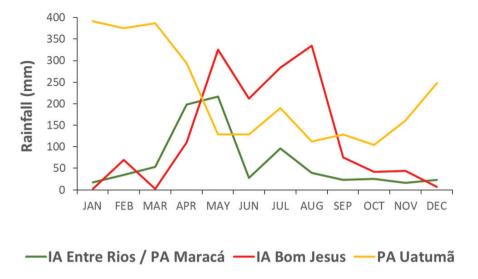


Figure 2. Rainfall in the study areas during the study.

Data analysis

Sample effort for each area was calculated as number of trapping stations per area multiplied by number of sampling days and trapping success was calculated as number of records per area divided by sample effort, multiplied by 100 (Srbek-Araújo and Chiarello 2007). We used Shapiro-Wilk (*W*) test of data normality. Diversity was measured as Shannon entropy (*H*) and Fager's number of moves (*NMS*), dominance was quantified using Berger-Parker index (*d*) (Hubálek 2000). We used Kruskall-Wallis tests (*K*) to detect statistically significant differences between sites. Moreover, we report effect sizes to facilitate both interpretation and inclusion of our results in meta-analysis (Morris and DeShon 2002).

Relative abundance was calculated as number of species individual records multiplied by 100, and divided by sample effort (Jenks et al. 2011). Kruskall-Wallis test was used in order to verify it there was any statistically significant difference in mean relative abundance among the areas, and Mann-Whitney to verify if there was any statistically significant difference in mean relative abundance between protected and impacted areas. All statistical tests were performed using the software R (R Core Team 2012), version 3.2.

Results

Species richness and diversity

Overall total sample effort was 1,946 trap-days, and total sample effort per study area varied between 448 trap-days in IA Bom Jesus and 504 trap-days in IA Entre Rios

Areas	Sample (days)	Camera traps per area	Number of independent records	Camera traps per day	Capture success (%)
IA Bom Jesus	32	14	19	448	4.24
PA Maracá	35	14	86	490	17.55
IA Entre Rios	36	14	27	504	5.36
PA Uatumã	36	14	54	504	10.71

Table 1. Sample size in the protected areas and in the fishbone human settlements in northern Brazilian Amazonia.



Figure 3. Medium-sized and large terrestrial mammals recorded during this study in the Northern Brazilian Amazonia. **a** *Panthera onca* **b** *Eira barbara* **c** *Tayassu pecari* **d** *Tapirus terrestris* **e** *Leopardus pardalis* **f** *Puma concolor.*

and PA Uatumã (Table 1). This resulted in an overall total of 216 independent species records (11.1% capture success), with a minimum of 19 records (4.24% capture success) in IA Bom Jesus and a maximum of 86 records in PA Maracá (17.55% capture success) (Table 1).

During this study 20 species were recorded, from 17 genera and 15 families (Table 2; Figure 3). PA Uatumã had the highest number of species (n = 15), followed by PA Maracá and IA Entre Rios (n = 10), and IA Bom Jesus (n = 9) (Table 2). No significant difference were detected in species richness ($\chi^2 = 3.68$, df = 3, p = 0.298) among the areas.

The most speciose orders in PA Uatumã and PA Maracá were Carnivora (n = 6 and n = 4), Artiodactyla (n = 3 and n = 2), and Xenarthra (n = 3 and n = 2). In IA Entre Rios and IA Bom Jesus most species belonged to Carnivora (n = 5), Artiodactyla (n = 3), and Rodentia (n = 3).

The highest Shannon diversity was registered in PA Uatumã (H = 2.4; NMS = 41.31), and the lowest in PA Maracá (H = 1.2; NMS = 13.06) (Figure 4). PA Uatumã

Table 2. Medium-sized and large terrestrial mammals relative abundance in the study areas in northern Brazilian Amazonia.

Species	PA Uatumã	PA Maracá	IA Entre Rios	IA Bom Jesus
Artiodactyla				
Cervidae				
Mazama americana	0.4	0.41	1.39	0.67
Mazama nemorivaga	_	_	0.2	_
Tayassuidae				,
Pecari tajacu	2.38	_	0.79	_
Tayassu pecari	0.2	12.04	_	_
Carnivora				
Felidae				
Leopardus wiedii	0.4	_	_	_
Leopardus pardalis	1.39	0.41	0.4	0.22
Panthera onca	1.98	1.02	0.2	
Puma concolor	0.6	0.2	_	0.22
Procyonidae			*	
Nasua nasua	0.4	_	_	0.22
Procyon cancrivorus	_	_	_	0.22
Mustelidae				
Eira barbara	0.2	0.41	_	_
Cingulata		1		
Dasypodidae				
Dasypus novemcinctus	1.19	_	_	_
Priodontes maximus	0.2	0.2	_	_
Perissodactyla				
Tapiridae				
Tapirus terrestris	0.6	2.45	2.58	0.22
Pilosa				,
Myrmecophagidae				
Myrmecophaga tridactyla	0.4	_	0.2	_
Tamandua tetradactyla	_	0.2	_	_
Rodentia				
Cuniculidae				
Cuniculus paca	_	0.2	0.2	0.45
Dasyproctidae				
Dasyprocta leporina	1.59	_	3.17	1.12
Myoprocta acouchy	-	_	_	0.89
Didelphimorphia				
Didelphidae				
Didelphis sp.	0.79	_	0.2	_
Total	12.72	17.54	9.33	4.23

had the lowest Berger-Parker index (d = 0.2), and PA Maracá, the highest (d = 0.7) (Figure 4). No significant differences, however, were detected in Shannon entropy (χ^2 = 5.82, df = 3, p = 0.121), or dominance (χ^2 = 5.53, df = 3, p = 0.137).

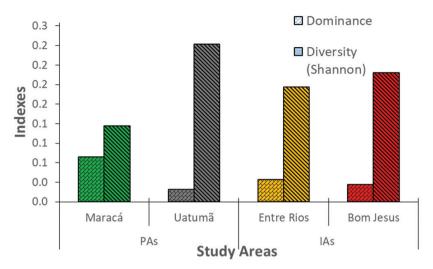


Figure 4. Diversity and dominance indexes for the four study sites in the Northern Brazilian Amazonia.

Relative abundance

No statistically significant differences were detected in mean relative abundance among the areas (χ^2 = 5.32, df = 3, p = 0.150) (Table 2). At PA Maracá the overall relative abundance was 17.55 ind./trap-days, with *Tayassu pecari* as the most abundant species (12.04 ind./trap-days), followed by *Tapirus terrestris* (2.45 ind./trap-days), and *Panthera onca* (1.02 ind./trap-days) (Table 3). At PA Uatumá the overall relative abundance was 12.7 ind./trap-days, with *Pecari tajacu* as the most abundant species (2.38 ind./trap-days), followed by *Panthera onca* (1.98 ind./trap-days), and *Dasyprocta leporina* (1.59 ind./trap-days (Table 3).

At IA Entre Rios the overall relative abundance was 9.33 ind./trap-days, with *Dasy-procta leporina* as the most abundant species (3.17 ind./trap-days), followed by *Tapirus terrestris* (2.58 ind./trap-days), and *Mazama americana* (1.39 ind./trap-days) (Table 3). At IA Bom Jesus the overall relative abundance was 4.24 ind./trap-days, with *Dasyprocta leporina* as the most abundant species (1.12 ind./trap-days), followed by *Myoprocta acouchy* (0.89 ind./trap-days), and *Mazama americana* (0.67 ind./trap-days) (Table 3).

No statistically significant differences were detected in mean relative abundance between protected and impacted areas (U = 142, p = 0.121). Overall relative abundance, however, appeared to be higher in the PAs (15.09 ind./trap-days) than in the IAs (6.93 ind./trap-days) (Table 4). In the PAs the most abundant species in this study was *Tayassu pecari* (6.04 ind./trap-days), followed by *Tapirus terrestris* (1.51 ind./trap-days), and *Panthera onca* (1.51 ind./trap-days), whereas in the IAs the most abundant species was *Dasyprocta leporina* (2.21 ind./trap-days), followed by *Tapirus terrestris* (1.47 ind./trap-days), and *Mazama americana* (1.05 ind./trap-days) (Table 4). Failing to detect any statistically significant results should most probably be due to sample size, which, nevertheless, is a common problem with large terrestrial mammals, many of which are rare and highly secretive.

Table 3. The three most abundant medium- and large-sized terrestrial mammals in the study areas in Northern Brazilian Amazonia.

Study areas	Total relative abundace (ind./trap-days)	Species	Relative abundance (ind./trap-days)
		Tayassu pecari	12.04
PA Maracá	17.55	Tapirus terrestris	2.45
		Panthera onca	1.02
		Pecari tajacu	2.38
PA Uatumã	12.7	Panthera onca	1.98
		Dasyprocta leporina	1.59
		Dasyprocta leporina	3.17
IA Entre Rios	9.33	Tapirus terrestris	2.58
		Mazama americana	1.39
		Dasyprocta leporina	1.12
IA Bom Jesus	4.24	Myoprocta acouchy	0.89
		Mazama americana	0.67

Table 4. Overall relative abundance of the medium- and large-sized terrestrial mammals in protected *vs.* fishbone human settlements in northern Brazilian Amazonia.

Species	IAs	PAs
Artiodactyla	, ,	
Mazama americana	0.74	0.4
Mazama nemorivaga	0.11	0
Pecari tajacu	0.42	0.7
Tayassu pecari	0	6.54
Carnivora		
Eira barbara	0	0.3
Leopardus pardalis	0.21	1.01
Leopardus wiedii	0	0.1
Nasua nasua	0.11	0.1
Procyon cancrivorus	0.11	0
Panthera onca	0.11	1.11
Puma concolor	0.11	0.3
Cingulata		
Dasypus novemcinctus	0	0.6
Priodontes maximus	0	0.3
Perissodactyla		
Tapirus terrestris	0.95	1.61
Pilosa		
Myrmecophaga tridactyla	0.11	0
Tamandua tetradactyla	0	0.1
Rodentia		
Dasyprocta leporina	1.37	0.3
Cuniculus paca	0.32	0.2
Myoprocta acouchy	0.42	0.1
Didelphimorphia		
Didelphis sp	0.11	0.3
Total	5.15	14.08

Discussion

Determinants of the structure of the mammalian assemblages

The number of species recorded during this study, between nine and 15 terrestrial species, is higher than expected. Literature has shown that mammal species richness in the Guyana Shield has been recognized as one of the poorest in the Brazilian Amazonia, and is poorest in its central zone (between two and 10) where this study took place (Eisenberg and Redford 1979, Emmons 1984, Janson and Emmons 1990, Hoorn et al. 2010, Mendes Pontes et al. 2010). As shown by Zuquim et al. (2014) and Tuomisto et al. (2014), this should be a result of poor soil quality and comparatively drier climates, with longer dry season, which is positively correlated with plant diversity, and ultimately, with the availability of food resources for the mammalian assemblage. Regarding only our study areas, Maracá and IA Bom Jesus, with the poorest soils (Thompson et al. 1992) were the ones with the lowest richness, whereas Uatumã, with the richest soils (Zuquim et al. 2014, Tuomisto et al. 2014), was the one with the highest.

In this study Carnivora was the most speciose order, followed by Artiodactyla, in both PAs and IAs, suggesting that the structure of the mammalian community is preserved, although some of the species are generalist and matrix/fragmented tolerant species, such as Eira barbara. Studies in the Neotropics have shown that Carnivora is the most speciose order of large terrestrial mammals in protected areas, followed by Artiodactyla (Tobler et al. 2008, Munari et al. 2011, Springer et al. 2012, Santos and Oliveira 2012, Ahumada et al. 2013, Burton et al. 2015, Michalski et al. 2015), whereas in protected areas that are surrounded by human disturbances, Carnivora was followed by Rodentia and Xenarthra, which could represent an intermediate conservation status (Pickles et al. 2011, Carvalho et al. 2014, Lizcano et al. 2016), or Rodentia could even be the most speciose order (Michalski et al. 2015). In impacted areas, however, the most speciose order seems to be Xenarthra, followed by Rodentia (Michalski and Peres 2007, Botelho et al. 2012). Different patterns, however, may be found in highly impacted areas, in which most medium-sized and large mammals are extirpated or driven to such low numbers that the most abundant species becomes the smallerbodied rodent, Dasyprocta spp. (Tobler et al. 2008, Botelho et al. 2012, Michalski et al. 2015, Lizcano et al. 2016).

In this study in the Guyana shield of the Brazilian Amazonia no statistically significant differences were detected between PAs and IAs. In our previous studies (Mendes Pontes 2004, Mendes Pontes et al. 2007, Mendes Pontes et al. 2012, Melo et al. 2015), we showed that in the highly heterogeneous vegetation mosaics of the northernmost Guyana shield the structure of the terrestrial mammalian assemblages was shaped by forest type and heterogeneity, which, in turn, is directly connected with resource availability. Species richness and diversity may vary between protected and impacted areas, with the latter in most studies presenting a lower number of species due to hunting (Lopes and Ferrari 2000, Peres and Nascimento 2006, Michalski and Peres 2007, Sampaio et al. 2010, Ahumada et al. 2011, Melo et al. 2015, Meyer et al. 2015).

Accordingly, the richest area was PA Uatumã, located south of the Guyana shield, closest to central Amazonia, the natural vegetation cover for which is dense ombrophilous forests, followed by IA Entre Rios, which is located in the transition between the dense ombrophilous terra firme forests of the south of the Guyana shield, and the seasonally-dry forest mosaics of central Guyana shield, and the poorest areas were PA Maracá and IA Bom Jesus, in the northernmost Guyana shield, where the vegetation is mainly seasonally-dry forest mosaics interspersed with extensive areas of savannas (See Hoorn et al. (2010)). The highest NMS value in Uatumã (41.31) could be related to the fact that this is one of the most ombrophilous and productive forests, besides being a protected area, whereas the lowest NMS (5.49) in Bom Jesus settlement could be related to the fact that it is a highly degraded, impacted area, besides having a very open, discontinuous forest.

Our previous studies (Mendes Pontes 2004, Mendes Pontes et al. 2007, Mendes Pontes et al. 2012, Melo et al. 2015), also showed that the more heterogeneous and shorter the vegetation types (e.g., the mosaics of terra firme forest, campinaranas, campinas, and savannas of northernmost Guyana shield), the higher the abundance of *Tayassu pecari*, which decreases considerably the diversity of the mammal assemblage. PA Maracá, in the northernmost Guyana shield, therefore, had the lowest species diversity due to the very high dominance of *Tayassu pecari*, whereas PA Uatumã, in the south of the Guyana shield, closest to central Amazonia, had the lowest dominance, and therefore, the highest species diversity. At the fishbone human settlements IA Bom Jesus and IA Entre Rios, due to the fact that *Tayassu pecari* has been extirpated, dominance was lower than in PA Maracá (and diversity higher), but was higher still than PA Uatumã (and diversity lower). In these impacted areas the medium-sized *Dasyprocta leporina* had the highest dominance, possibly replacing *Tayassu pecari*, as seems also to be the case in Meyer et al. (2015) where agoutis, (*Dasyprocta leporina*) were the most abundant, causing a decline in evenness and dominance.

Hunting has been shown to be the major driver of the decline and extirpation of the mammalian abundance in human impacted areas around the world (Hill et al. 1997, Naughton-Treves et al. 2003, Rist et al. 2009, Levi et al. 2009, 2011, Pillay et al. 2011, Rovero et al. 2012). In the neotropics, *T. pecari* was one of the first to disappear due to its extreme sensitivity to hunting in areas close to human settlements (Peres 1996, Reyna-Hurtado and Tunner 2007, Naranjo and Bodmer 2007, Meyer et al. 2015, Reina-Hurtado et al. 2015).

In some cases, however, no differences were detected in the structure of the mammalian assemblages between protected and impacted areas, in which case, hunting was considered sustainable (Peres and Nascimento 2006, Ohl-Schachereret al. 2007, Zapata-Rios et al. 2009), because large contiguous tracts of surrounding primary forests allowed an efficient source-sink balance and recolonization of the depleted areas. Hunting may be sustainable also if the forest is remote and the assemblages have very low human population density, if settlements are relatively young, small, and have a low consumer population (De Thoisy et al. 2005, Demmer et al. 2002, Mazurek et al. 2000, Alvard et al. 1997).

In this study, relative overall abundance of medium-sized and large mammals did not present any statistically significant differences between PAs and IAs. When we considered the areas separately, PA Maracá was the area with the highest overall relative abundance, mainly due to the very high abundance of *Tayassu pecari*, which, in these highly seasonal forests of the Guyana shield, are among the most abundant terrestrial mammals. For instance, its relative abundance in PA Maracá is ~70% of the overall abundance of the area, and is almost the total relative abundance of the second most abundant area, PA Uatumã. As expected, the latter does not have such a high *Tayassu pecari* abundance as most areas of the Guyana shield (Munari et al. 2011, Negrões et al. 2011, Botelho et al. 2012, Santos and Oliveira 2012).

The scenario of this study comprises PAs and IAs that are surrounded by large tracts of undisturbed forests that although not legally protected and totally accessible, are protected from human presence by remoteness. Additionally, the IAs are comparatively younger (~25 years since decreed), are among the smallest in the state of Roraima (around 4,000 and 15,000 ha, compared to 220,000 of the largest), and, consequently, have fewer families (around 200, compared to a maximum of around 2,000 in the largest settlement) (Governo do Estado de Roraima 2017).

Additionally, the settlers normally obtain their necessary animal protein intake from domestic livestock (cattle, pig, goat, chicken, duck, turkey) (R.B.L., pers. comm.), and these IAs are only a few miles from the nearest town where they can obtain additional food supplies from the market, especially that most of them have their own means of motorized transport. As in Melo et al. (2015) in Novo Paraíso, a similar fishbone human settlement also in the Guyana shield, subsistence hunting does not play a significant role in the diet of the settlers in these fishbone human settlements in the Guyana shield of the Brazilian Amazonia.

Thus, the structure of the mammalian assemblages here studied were determined by the structure of the vegetation, whether it was the highly heterogeneous vegetation mosaics of the northern part of the Guyana shield, the ombrophilous forests of the southern part, or a transition between them, and in the case of the IAs, the integrity of the mammalian assemblages were also maintained by the large tracts of surrounding undisturbed forests that allowed an efficient source-sink balance and recolonization of the depleted areas.

How sustainable were the hunted-mediated impacts of these fishbone human settlements (IAs)?

Despite the fact that the structure of the mammalian assemblages did not present any statistically significant differences between PAs and IAs, one major change, the local extirpation of *Tayassu pecari* from the IAs, predicts their bleak future. The structure of the mammal assemblage also appears to be changing in these settlements because in the protected areas the most abundant species are the three largest mammals, namely,

Tayassu pecari, Panthera onca, and Tapirus terrestris, whereas in the impacted areas the most abundant species are smaller: a rodent, Dasyprocta leporina, followed by Tapirus terrestris, and Mazama americana. D. leporina was also the most abundant mammalian species in the nearby Novo Paraíso human settlement (Melo et al. 2015).

Deforestation and hunting are among the main causes of species extirpation in the tropics, and have been responsible for what is considered the sixth major extinction event of the planet (Brook et al. 2003; Thomas et al. 2004; Asfora and Mendes Pontes 2009; Mendes Pontes et al. 2016). Some species may even be driven to extinction before they are known by scientists, or described, such as was the case of a primate and a deer in northeastern Brazil (Mendes Pontes et al. 2016). The only alternative to avoid such catastrophic extinctions is to prevent the first stages of the anthropogenic impacts in the environment, such as those that are occurring in the impacted areas of this study. It should be a matter of time until we can witness its devastating impacts on the mammalian assemblages.

Conclusion

This study confirmed that the northernmost Brazilian Amazonia, in the Guyana shield, is one of the poorest regions in mammal species, and is poorest in those sites that are drier and that have a longer dry season, such as Maracá Ecological Station. No significant differences were detected between protected and impacted areas, possibly because the fishbone human settlements are relatively young and surrounded by vast tracts of source forests, allowing the structure of the mammalian assemblage to be maintained even under the human pressure exerted by these settlements. The local extirpation of *Tayassu pecari* in these impacted areas, however, and lower abundances of other large mammals such as *Panthera onca*, suggest imminent changes to the mammalian assemblage structure.

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Conservation biology of the last Italian population of Cistus laurifolius (Cistaceae): demographic structure, reproductive success and population genetics

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Abstract

Isolated populations are usually subject to low fitness and reduced genetic diversity, both of which may negatively affect their survival and adaptive potential. Hence, these issues cannot be neglected when planning conservation actions for isolated populations. The Italian population of *Cistus laurifolius* subsp. *laurifolius* is extremely isolated. Furthermore, it is affected by fragmentation, being constituted by a single larger subpopulation, surrounded by three much smaller subpopulations, a few hundred metres to a few kilometres apart. In order to fill gaps in demographic and genetic knowledge concerning the Italian population, its area of occupancy, size, age-stage structure and phenology were investigated and its reproductive fitness, pollination strategies and genetic variability were assessed. The population was inferred as fully xenogamous and showed good reproductive performance. Despite this, its genetic variability was low and it showed relatively high levels of inbreeding depression (F_{IS}), seemingly not affected by sub-population size. These results suggest that the Italian population recently suffered fragmentation and reduction in size. The low genetic diversity observed could be explained by the high percentage of mature individuals found in the population, possibly established before fragmentation. For these reasons, the Italian population of *C. laurifolius* subsp. *laurifolius* should be monitored and concrete actions aimed at its conservation planned.

Keywords

Conservation biology, genetic diversity, reproductive biology, Italy

Introduction

The knowledge of relationships amongst population size, age-stage structure, fitness and genetic diversity is of crucial importance in plant ecology and conservation, in order to gain an insight into population dynamics and evolutionary potentialities (Stockwell et al. 2003; Rodriguez-Perez 2005; Leimu et al. 2006).

Population size strongly affects local adaptation (Leimu et al. 2008), whereas population structure can influence breeding systems and, eventually, reproductive outcomes (Carta et al. 2016a). Population dynamics are usually age-stage-dependent and related to the survival potential of a species (Harper 1977).

Small outcrossing plant populations may suffer disadvantages due to the Allee effect, i.e. an individual fitness reduction caused by a decrease in population size/density (Forsyth 2003). Populations with a low density may be affected by an insufficient pollinator service and consequently express a reduced fruit and seed set (Kunin 1997; Forsyth 2003; Ashman et al. 2008).

According to Ghazoul (2005), the vulnerability of plant species to the Allee effect depends upon their mating system. Self-incompatible species are more likely to experience pollen limitation than self-compatible plants, as the former cannot compensate for reduced pollinator services through selfing (Aizen and Feinsinger 1994; Knight et al. 2005; Xia et al. 2013). In addition, when peripheral and isolated plant populations (PIPPs) (Abeli et al. 2009) display reduced genetic variability, they may fail to effectively cope with environmental changes, especially in the light of dramatic shifts imposed by global climate change (Willi and Hoffmann 2009, Hoffmann and Sgrò 2011). The Italian peninsula hosts several examples of such populations, highlighting the importance of case studies for planning proper conservation actions (Gargano et al. 2007, 2009; Rossi et al. 2009; Carta et al. 2016b; D'Antraccoli et al. 2016).

The laurel-leaved rock rose, *Cistus laurifolius* L. subsp. *laurifolius*, can be regarded as a notable case for studying relationships between genetic diversity and conservation in the context of PIPPs. This species shows a distribution scattered across the Mediterranean, the main populations being located in the Iberian peninsula and south France in the west and Anatolia in the east (Fernández-Mazuecos and Vargas 2010). In between, *C. laurifolius* survives in a single population in Italy, near Santa Brigida village (Tuscany) (Roma-Marzio et al. 2016a, 2016b and literature cited therein). A single eastwards migration event may have been the source of a recent dispersal of the species from putative refugial areas in the western Mediterranean (Fernández-Mazuecos and Vargas 2010). According to the latter authors, narrow ecological requirements (altitude and edaphic conditions) and low germination rates may explain the current distribution pattern of this species.

No information about the genetic diversity, demographic structure and reproductive traits is available for the Italian population of laurel-leaved rock rose. This population has been considered as a relict, resulting from fragmentation (Dansereau 1939; Rizzotto 1979; Fernández-Mazuecos and Vargas 2010), due to the stenoecy of this species (Rizzotto 1979). The dramatic reduction of the Italian population during the last centuries

was also determined by human impact (Astuti et al. 2015; Roma-Marzio et al. 2016b). According to the latter authors, two out of five sub-populations are much depleted and, taking into consideration the threats recorded in the area, this taxon has been evaluated as *Vulnerable* at Regional level, according to the IUCN criteria (IUCN 2014).

The aim of this study is to accumulate information useful for the conservation of this species, including: i) area of occupancy of the Italian population, ii) number of immature, virginile and mature individuals, iii) phenology, iv) reproductive fitness, v) pollination strategies and vi) genetic variability. The obtained results will be a framework to design a conservation programme for the species.

Methods

Study species

Cistus laurifolius L. subsp. laurifolius is a shrub with large, white, hermaphrodite flowers, pollinated by generalist insects (e.g. beetles, bees and flies), flowering from May to June (Astuti et al. 2015). This taxon has relatively superficial roots and nutrient-poor leaves, mainly adapted to oligotrophic and degraded soils, as other Cistaceae (Moro et al. 1996). Cistus laurifolius typically occurs in open and dry habitats of non-coastal Mediterranean areas (Rizzotto 1979; Grossoni and Venturi 2009; Roma-Marzio et al. 2016b). It shows relatively small seed mass (about 1 mg/seed), a feature related to easier penetration and accumulation of seeds in the soil (Fenner 1985; Thanos et al. 1992). Seed dispersal is seemingly barochorous (Thanos et al. 1992) but, despite this feature, long-distance dispersal events have been documented in the genus (Rizzotto 1979).

Concerning the native geographic distribution, *C. laurifolius* occurs in the western (Morocco, Portugal, Spain, France) and eastern Mediterranean basin (north-eastern Greece and Turkey). An isolated population is found in Central Italy (Tuscany) (Warburg 1968; Rizzotto 1979; Fernández-Mazuecos and Vargas 2010, Dimopoulos et al. 2013).

Study area

The only Italian population of *Cistus laurifolius* L. subsp. *laurifolius* is located in Tuscany, near the village of Santa Brigida (Firenze). The mean annual temperature and mean annual rainfall of the area, measured between 1992 and 2010 by a thermopluviometric station located in Pontassieve (WGS84: 43.812324, 11.399167; 120 m a.s.l.) are 13.7°C and 856.2 mm, respectively (http://agrometeo.arsia.toscana.it/).

With the exception of a single individual surviving in the near proximity of the village of Santa Brigida, the population is fragmented into four sub-populations (Astuti et al. 2015; Roma-Marzio et al. 2016b) (Figure 1). Three of them (C5, D and FOR in Figure 1) are located in an open habitat (garrigue) dominated by *Cistus salviifolius* L., *Erica arborea* L., *E. scoparia* L. subsp. *scoparia* and *Cytisus scoparius* (L.) Link subsp.

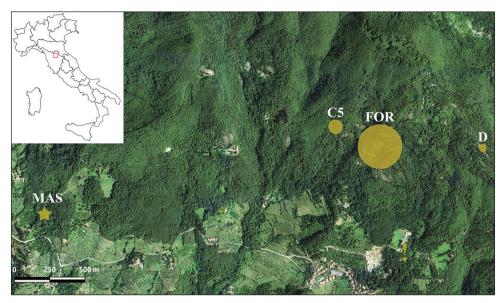


Figure 1. Distribution map of the Italian population of *C. laurifolius* L. subsp. *laurifolius*. Yellow symbols indicate the location of each sub-population. Circle size is proportional to the area occupied by the sub-populations of Fornellaccio (FOR), Fontassenzio (D) and west of Fornellaccio (C5). The yellow star, not proportional to the area of occupancy, refers to the small sub-population of Masseto (MAS). The exclamation mark (bottom right) indicates the single individual surviving in the near proximity of the village of Santa Brigida (not investigated in this study). In the top left corner, the location of the study area (red square) in Italy is indicated.

scoparius. The remaining one (MAS in Figure 1) is located in the underbrush of a *Pinus nigra* L. subsp. *nigra* plantation, together with sclerophyllous vegetation, mainly composed of *Cistus salviifolius* L., *Quercus ilex* L. and *Rubus ulmifolius* Schott (Roma-Marzio et al. 2016b).

This fragmentation has most probably been caused by human induced landscape transformation, as is suggested by the ongoing disappearance of the sub-population in the near proximity of the village of Santa Brigida and by the occurrence of buildings (e.g. farms) and a network of roads surrounding the population (Figure 1).

Population size and structure

To estimate the population size (number of individuals), its density and demographic structure, an evaluation of the area occupied by each sub-population was carried out. To this end, preliminary data published by Grossoni and Venturi (2009) were verified and adjusted by field surveys, using a GPS receiver. Thereafter, each sub-population's area of occupancy was determined within a GIS environment (QGIS software v. 2.18; QGIS 2016), by delimiting a minimum convex polygon. All individuals were counted in the smaller sub-populations (C5, D, MAS), while, for the largest sub-population (FOR), all the individuals occurring in fifteen randomly sampled 10×10 m plots were

counted. The number of individuals in FOR (I_{FOR}) was estimated according to the following formula:

$$I_{FOR} = {}^{I_p} \times A_{FOR} / {}_{A_p}$$

where I_p is the total of individuals occurring in all the plots, A_{FOR} is the total area occupied by the sub-population FOR and A_p is the sum of the areas of each plot (1500 m²).

The age in shrub plants is hardly detectable and plant growth is highly dependent upon environmental and ecological parameters (Chapman 1986). Therefore, to describe the demographic structure of each sub-population, three practical "age-stage" classes have been arbitrarily selected, based on the presence/absence of flowers and the number of leaves. According to the terminology proposed by Gatsuk et al. (1980), the three classes were defined as following: class I) immature plants (lacking flowers, showing less than six leaves and with a plantlet-like appearance; class II) virginile plants (lacking flowers, but showing more than six leaves; class III) mature plants (showing flowers).

It was not possible to take into account the very small and ephemeral cotyledon phase (seedling), due to difficulties in detecting this stage in the soil and leaf litter and also because its presence could be easily affected by exceptional weather conditions (Alonso et al. 1992).

Reproductive fitness

For each sub-population, reproductive fitness was evaluated by means of seed set (number of seeds/number of ovules) and seed mass. The number of ovules was averaged on ten randomly selected flowers in each sub-population (for a total of 40 flowers). The ovaries were dissected along their septa using a razor blade in order to count the ovules under a 60× magnification stereomicroscope.

Since no difference was found for mean ovule numbers amongst sub-populations (ANOVA, p > 0.05), then the mean ovule number at population level (94.72) was used as the reference to calculate the seed set for all sub-populations. In the case of single fruits showing a seed number exceeding the mean ovule number, a seed set of 100% was assigned by default. To calculate the seed number, 50 fruits (capsules) were randomly collected for each sub-population and the seed number was counted for each capsule. Aborted (i.e. showing a seed-shape, but lacking embryo) seeds were not taken into account. To evaluate the seed set, the data were averaged at the sub-population level as follows:

$$\frac{1}{n} \sum_{i=1}^{n} x_i$$

where n (=50) is the number of sampled fruits for each sub-population and x is the seed set calculated for each fruit.

To evaluate the seed mass, ten replicates, each consisting of a group of 50 randomly selected seeds, were weighed (± 0.001 mg accuracy), for a total of 500 seeds per sub-population.

Floral sex allocation

To characterise the floral sex allocation and to infer the breeding system of the species at the population level, flowers were collected at an early developing stage (showing mature, but still not-dehiscing anthers), in order to evaluate the flower biomass (mean dry weight) and the mean number of pollen grains produced per flower (P).

According to the regression formula proposed by Herrera (1985), the dry flower weight was used as an indirect measure of the daily nectar secretion rate (Bosch 1992). Dry weight was calculated on 34 flowers (ten from the sub-population FOR, ten from C5, ten from MAS and four from D). After their collection, the flowers were dried at 60°C for 24h and then weighed (± 0.001 mg) (Kay and Picklum 2013).

The total pollen production per flower was estimated according to the dilution method proposed by Galloni et al. (2007). Pollen grains were estimated on seven flowers (two from the sub-population FOR, two from C5, two from MAS and one from D). Anthers were taken from fresh flowers and placed in vials with 1 ml of ethanol/glycerinated fuchsine-glycerol solution (3:1). For effective pollen release, vials were sonicated for 1 min at 14 kHz using a Sonoplus Ultrasonic Homogeniser GM 2070. A known volume (1 μ L) of suspension was mounted on a microscope slide within 10 seconds from sonication, to guarantee the homogeneity of suspension.

Pollen grains were then counted using a light microscope (250× magnification) and recorded with the help of a manual cell counter. The number of pollen grains was finally multiplied by the dilution factor and then by the number of anthers to obtain the total number of pollen grains estimated for a whole flower. Finally, according to the values indicated by Cruden (1977), the P/O ratio was used to infer the breeding system of the species. For the number of ovules, the same mean value already used for the seed set was referred to, as explained in the previous section.

Statistical analysis of demographic structure and reproductive traits

To evaluate the overall effect of three single predictors (logarithm of area of occupancy; sub-population density and % of adults) on the seed set, each of them was fitted with a single Generalised Linear Model (GLM), with a logit link function and a binomial error structure, followed by a likelihood test. The logistic regression was selected since the seed set is a binomial phenomenon.

The values of the area of occupancy were subjected to logarithmic transformation to reduce the large differences amongst sub-populations.

Differences in seed mass, flower mass and ovule number amongst sub-populations were tested by means of an ANOVA test, followed by Tukey's pairwise comparisons, after checking normality and homoscedasticity of the data. Differences in seed set amongst sub-populations were tested by means of χ^2 test.

For all statistical tests, significance was accepted at $p \le 0.01$. All analyses were performed using R 3.3.1 software (R Core Team 2016).

Microsatellite markers

Microsatellite markers have been developed according to Albadejo (2010). About 1500 clones from a non-enriched genomic library were sequenced. The sequencing reads were assembled with CodonCode Aligner in 1348 unique sequences. Sequences were checked for the occurrence of di-, tri- and tetra-nucleotide repeats with the online software Sputnik (available at http://wheat.pw.usda.gov/ITMI/ EST-SSR/LaRota). Although microsatellite motifs were detected in 25 sequences (1.8%), ten were discarded as the microsatellite motifs were too short, nucleotide repeats were too close to the vector for primer design or the clones showed high sequence homology. Subsequently, 15 primer pairs were designed by using Primer3 software (Rozen and Skaletsky 2000). In a preliminary screening of the markers' variability, 30 individuals from two sub-populations were examined. Out of the 15 primer pairs, nine were discarded, as they failed to amplify, produced multibanding patterns, were monomorphic or showed too pronounced stuttering. The presence of null alleles in the remaining six markers (Suppl. material 1) was examined following the Expectation Maximisation (EM) algorithm (Dempster et al. 1977) using FREENA (Chapuis and Estoup 2007). The estimated frequency of null alleles ranged from 0.13 to 0.23 with the exception of cislau11 (frequency = -0.01). Due to the excess of null alleles (frequency = 0.78) the marker cislau5 was excluded from further analyses.

Genetic diversity analysis

The total DNA from 189 plants, sampled from the four sub-populations, was isolated using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) from 80–100 mg of leaf dry tissue. Amplifications were performed by polymerase chain reaction (PCR) in 10 μ L volumes, containing 10–50 ng of template DNA, 1× reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4; Invitrogen), 0.5 U of Taq polymerase (Invitrogen), 0.5 μ L of 1% W-1 solution (Invitrogen), 2 mm of MgCl₂, 1 μ M of each primer, 60 μ M of dNTP mix.

Reactions were performed in a Gene Amp PCR system 9700 (PE Applied Biosystems), with the following programme: an initial denaturation step of 3 min at 94° C, followed by 10 touchdown cycles of 30 s at 94 °C, 40 s at 60 °C (1 °C lower per cycle) and 30 s at 72 °C and 25 cycles of 20 s at 94 °C, 20 s at 50 °C and 30 s at 72 °C with a final extension step of 8 min at 72 °C. A final extension of 6 min at 72 °C was performed in all programmes. Amplified fragments were run in an ABI 3130xl automatic sequencer (Applied Biosystems). Electropherograms were analysed using GeneMapper version 4.0 (Applied Biosystems). Linkage disequilibrium between loci and deviations from Hardy-Weinberg (HW) expectations were tested using Fisher's exact tests based on Markov chain procedures in GENEPOP ver. 3.4 (Rousset 2008). Basic statistics were calculated using the software GENALEX 6.2 (Peakall and Smouse 2006), to

determine: allele frequencies, mean observed heterozygosity (H), unbiased expected heterozygosity (H₂), the number of alleles at each SSR locus (N₂), the effective number of alleles (N_r) and the inbreeding coefficient (Fis). GENALEX 6.2 was also used to perform the analysis of molecular variance (AMOVA). Significance levels were determined using 1,000 permutations. To test whether any of the sampled sub-population experienced bottlenecks in the recent past, the Bottleneck programme version 1.2.02. (Piry et al. 1999) was used to compute the difference (averaged over loci) between actual heterozygosity and the heterozygosity expected for a population in mutationdrift equilibrium. The Bottleneck software allowed the testing of the bottleneck hypothesis, taking into account two possible models: a stepwise mutation model (SMM) and a two-phase mutation model (TPM). The significance of heterozygosity excess was determined using the Wilcoxon signed-rank test, which has been demonstrated to be the most accurate test in case of both low number of polymorphic loci (< 20) and small samples sizes (< 30) (Piry et al. 1999). The genetic structure of the four subpopulations was analysed by the Bayesian algorithm implemented in STRUCTURE v. 2.3.3 (Pritchard et al. 2000), which assigns individuals to a K number of genetically homogeneous groups, based on allele frequencies at each locus. For the analyses with STRUCTURE, a burn-in period of 50,000 and a posterior number of Markov Chain Monte Carlo (MCMC) of 100,000 permutations was used.

Fifteen replications (runs) were performed for each value of K ranging from K = 1 to K = 10. An admixture and allele frequencies correlated model was used. The most likely number of genetic clusters (K) was estimated following Evanno et al. (2005), which uses an *ad hoc* parameter (Δ K) to estimate the rate of change of likelihood values amongst successive K values. Ten runs for each simulation were averaged using algorithms found in CLUMPP (cluster matching and permutation programme; Jakobsson and Rosenberg 2007) and represented as bar graphs using DISTRUCT (Rosenberg 2004). The membership probability of each individual in every cluster was assessed by the value of Q and each individual was assigned to a specific cluster taking into account a threshold of Q > 0.75 (Atiqur et al. 2016).

Results

Demographic structure

The estimated number of individuals within the population was 9,962, occupying an area of 86,145 m². The largest sub-population FOR hosted the vast majority of the plants (Table 1). Overall, mature plants appeared in the greatest numbers, followed by virginiles and immatures. Percentages similar to the overall values were found in the sub-populations FOR and MAS, whereas C5 and D showed a higher number of immature and a lower number of mature individuals. In addition, D showed the highest percentage of virginile plants (Table 1).

Table 1. Demographic structure of the Italian population of *C. laurifolus* subsp. *laurifolius*. In square brackets: % values of area of occupancy and individuals with respect to the whole population. In round brackets: % age-stage classes with respect to the whole number of individuals for each sub-population. * = mean value.

Subpopulation	Area (m²)	Density (individuals/m²)	Mature individuals	Virginile individuals	Immature individuals	Total individuals
FOR	77,215 [89.7%]	0.12	8,185 (86.4%)	1,132 (12%)	154 (1.6%)	9,471 [95.1%]
C5	6,680 [7.7%]	0.05	261 (74.4%)	45 (12.8%)	45 (12.8%)	351 [3.5%]
D	2,100 [2.4%]	0.04	69 (75.0%)	17 (18.5%)	6 (6.5%)	92 [0.9%]
MAS	150 [0.2%]	0.31	40 (85.1%)	6 (12.8%)	1 (2.1%)	47 [0.5%]
Population	86,145	0.11*	8,556 (85.9%)	1,200 (12.0%)	206 (2.1%)	9,962

Reproductive fitness and floral sex allocation

According to χ^2 test, the seed set of FOR (mean value 87.5%) and D (mean value 38.9%) showed the highest and the lowest values, respectively (Table 2). Regarding the seed mass, differences were significant only between FOR and MAS, with the latter sub-population showing the lowest values (Table 2), yet not significantly different from C5 and D.

The mean P/O value calculated for the population was 5,138.72 \pm 4,310. Concerning dry flower mass, no differences were found amongst sub-populations (p > 0.05): the mean value for the entire population was 102.05 \pm 0.02 mg. According to Herrera's regression formula, the daily nectar's production was estimated as 5.18 \pm 0.78 mg per day per flower.

Concerning factors affecting seed set, a significant positive effect was found of the sub-population's area of occupancy, density and frequency of class III (mature plants) (Table 3).

Population genetics

A total of 66 alleles was detected for five loci (Suppl. material 2) in the 189 individual genotypes sampled in this analysis. The overall diversity in all sub-populations was very low (Table 4); the mean number of alleles per locus (N_A) was 3.3 ± 0.5, the observed heterozygosity (H_O) was 0.25 ± 0.05, whereas expected heterozygosity (H_E) was 0.32 ± 0.06. No sub-population showed a significant excess of heterozygotes compared with the equilibrium expectation based on data modelling (one-tailed Wilcoxon signed-rank test, p > 0.80 for all sub-populations under both the SMM and TPM). Instead, all sub-populations showed a significant excess of homozygotes (as indicated by a positive F_{IS}), with the exception of MAS, where the F_{IS} value was slightly negative.

Linkage disequilibrium (LD), the non-random association of the alleles at different loci, was analysed for all pairs of SSR markers within each sub-population and across the whole population. Only one locus (cislau12) showed a significant depar-

Table 2. Reproductive features of the Italian population of *C. laurifolius* subsp. *laurifolius*. For each subpopulation, mean values and standard deviations are reported. Different letters indicate growing ranking significant differences amongst groups (ANOVA test for ovule number, seed number and seed mass; χ^2 test for seed set).

Sub-population	N. ovules (N = 10)	N. seeds (N = 50)	Seed set (N = 50)	Mass of 50 seeds (mg) (N = 10)
C5	95.9 ± 27.3ª	55.28 ± 37.2 ^b	58.36% ^b	42.43 ± 1.7 ^{a, b}
D	100.4 ± 24.7 ^a	36.84 ± 27.0 ^a	38.89%ª	43.28 ± 1.5 ^{a, b}
FOR	102.6 ± 28.0 ^a	82.90 ± 39.5°	87.52%°	44.24 ± 1.4 ^b
MAS	80.0 ± 20.7 ^a	56.96 ± 40.0 ^b	60.14% ^b	41.15 ± 2.0 ^a

Table 3. Overall effect of the three predictors on the seed set, estimated by three single GLM analyses. log(area) = natural logarithm of the area occupied by each sub-population, III% = percentage of the class III stage-age individuals, SE = standard error, DE% = percentage of the deviance explained by each model.

	Intercept	Estimate	SE	p value	DE%
log(area)	-0.530	0.108	0.007	< 0.01	1.98
Density	-0.008	3.031	0.148	< 0.01	3.20
III%	-7.154	9.407	0.270	< 0.01	9.19

ture from equilibrium (5% level) in the C5 sub-population. The population structure determined by AMOVA showed that approximately 4% of the total variation was attributable to variation amongst sub-populations and 96% of the total variation was attributable to differences amongst individuals within sub-populations. All pairwise F_{ST} values differed significantly from zero (p < 0.05), except between C5 and MAS (p= 0.252) and D and MAS (p = 0.076). The estimate of overall F_{ST} was significantly different from zero, but very low ($F_{ST} = 0.050$; 95% CI 0.022-0.083), suggesting strong inter-subpopulation gene flow. Concerning this result, the mean value of N_M (number of migrants) was estimated to be 7.25. The optimum cluster number inferred from the STRUCTURE analysis (Suppl. material 3) was K = 3. Further analyses were performed based on K = 3, to investigate the composition of each individual and each sub-population with respect to the three inferred genetically homogeneous groups. In agreement with the low levels of genetic diversity, the genetic structure was also very weak. The proportion of membership of each inferred genetically homogeneous group was, in all cases, lower than 52%; the major component of genetic composition is attributable to the first K group (51%) for D, to the second K group for FOR (41%) and to the third K group for MAS (50%) (Figure 2). This result demonstrates that each gene pool shows a high degree of admixture, pointing towards extensive gene flow or common ancestry.

Table 4. Genetic diversity parameters for the four Italian sub-populations of C. laurifolius. N = sample size; $N_A = \text{number of alleles}$; $N_E = \text{effective number of alleles}$; H_0 = observed heterozygosity; H_E = unbiased expected heterozygosity; F_{IS} = inbreeding coefficient; * = p < 0.05.

		FO	FOR (N = 7	75)			C	C5 (N = 62)	2)			D	D(N = 42)	a			MA	MAS $(N = 10)$	(01	
	z	$\mathbf{Z}_{_{\mathrm{H}}}$	H_{0}	$\mathbf{H}_{\scriptscriptstyle{\mathrm{E}}}$	F _{IS}	z	Z	\mathbf{H}_{o}	$\mathbf{H}_{\scriptscriptstyle\mathrm{E}}$	F _{IS}	z	Z	$H_{\rm o}$	$H_{\scriptscriptstyle E}$	F _{IS}	Z	Z	$H_{\rm o}$	$\mathbf{H}_{\scriptscriptstyle \mathrm{E}}$	F _{IS}
Cislau1	3	1.03	0.03	0.03	-0.01	3	1.14	90.0	0.13	0.55*	1	1.00	0.00	0.00	0.00	1	1.0	0.00	0.00	-0.00
Cislau7	4	2.65	0.45	0.63	0.28*	2	1.83	0.40	0.46	0.11	3	2.98	0.52	0.67	0.21*	3	2.38	09.0	0.61	-0.03
Cislau11	3	1.10	0.10	60.0	-0.04	1	1.00	0.00	0.00	0.00	2	1.13	0.12	0.12	90.0-	2	1.34	0.30	0.27	-0.18
Cislau12	8	2.38	0.26	0.64	0.59*	8	4.32	0.65	0.78	0.16*	9	2.90	98.0	99.0	0.45*	4	1.70	0.50	0.44	-0.20
Cislau14	3	1.14	0.10	0.12	0.17	1	1.00	0.00	0.00	0.00	5	1.66	0.29	0.40	0.27*	3	1.87	0.30	0.49	0.35*
Mean	4.2	1.73	0.19	0.30	0.20*	3	1.86	0.22	0.27	0.27*	3.4	1.93	0.26	0.37	0.18*	5.6	1.6	0.34	0.32	-0.02

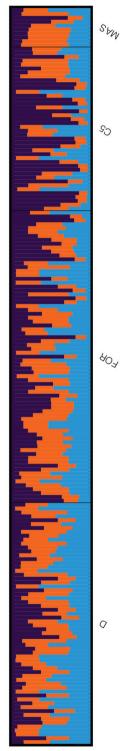


Figure 2. Estimated genetic clustering (K = 3), obtained with STRUCTURE analysis of 189 individuals from the Italian population of Cistus laurifolius. Each individual is represented by a vertical line, which is partitioned into coloured segments, indicating the individual's estimated membership fraction in K clusters. Different sub-populations are separated by a vertical black line.

Discussion

Demography, reproductive fitness and genetic structure

The survival chance of the Italian population of the laurel-leaved rock rose is mostly dependent upon the fate of the largest sub-population (FOR), including the vast majority of the individuals. The demographic structure of the population, as well, clearly parallels that of the largest sub-population, where a high percentage of mature plants and a low percentage of virginile and immature plants was observed (Table 1). The sub-populations of intermediate size and low density, namely C5 and D, show higher percentages of virginile and immature plants compared to the largest and to the smallest sub-populations (FOR and MAS, respectively). Accordingly, C5 and D may provide an important source of recruitment, albeit the seed set of these two sub-populations was relatively low, possibly due to the Allee Effect. As a consequence, C5 and D sub-populations can be regarded as patches of a fragmented habitat, surrounding the largest sub-population (FOR). Taking into account the smallest and most isolated sub-population (MAS), its peculiar environmental conditions should be underlined. As reported by Roma-Marzio et al. (2016b), MAS is subjected to severe threats (e.g. canopy closure and alien species) which are significantly affecting its ecological asset. Only 40 mature plants are surviving in this sub-population and only one immature plant was observed during this survey. Despite the low number of individuals in MAS, the seed set value was not low, probably due to the high density of mature individuals, as highlighted by the GLM results. Although there are relatively high values of the seed set, the low germination rates of this species (Fernández-Mazuecos and Vargas 2010) could however represent an additional threat.

According to the categorisation of P/O values made by Cruden (1977), the studied population seems fully xenogamous. This is also confirmed by the estimated daily nectar production which is consistent with a xenogamous breeding system, according to the data presented by Bosch (1992). The mating system is reported to have a significant impact on the distribution of genetic variability in a plant population (Duminil et al. 2007) and this genetic study indicates that 96% of the detected genetic variation is maintained within each sub-population. This value complies with that which is expected for an outcrossing plant (Hamrick and Godt 1989). Given that outcross seems the only way to produce new individuals in this species, its capability to cope with environmental shifts is strictly dependent upon the reproductive fitness of mature plants. The number of mature plants and the seed set measured in the population are quite high (Tables 1 and 2). In the light of these data, it seems that the Italian population of *C. laurifolius* shows a good reproductive performance. However, further investigations on the mating system using molecular markers could provide useful information (Glémin et al. 2006).

Compared with values reported for other outcrossing species ($H_O = 0.63$; $H_E = 0.65$) and for long-living perennials ($H_O = 0.63$; $H_E = 0.68$) (Nybom 2004), these results suggest that genetic diversity is low within the population as a whole (mean H_O)

= 0.25 \pm 0.06; mean H_E = 0.31 \pm 0.03). Low genetic diversity is commonly observed in endangered and rare species, with a reduced number of individuals in their populations (Hughes et al. 2003; Zhang et al. 2005), albeit different molecular markers can yield different diversity estimates (Nybom 2004). Although there are no concrete experimental data supporting a bottleneck hypothesis, the low genetic diversity, coupled with a relatively high F_{IS} (averaged value over all sub-populations: 0.21 \pm 0.08), suggest that the sub-populations could have suffered a reduction in size (Frankham 2005; Wright et al. 2009). Furthermore, the loss of habitat and its fragmentation (Ellstrand and Elam 1993), as well as multi-generation isolation (Keller and Waller 2002), are also known to have significant effects on gene flow and genetic diversity, as a result of both drift and increased inbreeding levels (Aguilar et al. 2008). In particular, outcrossing species containing most of their genetic variability within sub-populations, are subjected to genetic erosion through habitat fragmentation (Frankham 2005). Furthermore, the proportion of short-distance mating events has been shown to increase as a consequence of an increased fragmentation process (Ismail et al. 2012).

There was evidence for inbreeding in all the sub-populations with the exception of MAS: the F_{1S} value of this small sub-population did not differ from Hardy-Weinberg expectations (Table 4). Although the F_{IS} value of MAS could be partially influenced by the low number of sampled individuals (N = 10, however representing 25% of mature plants), this further confirms the absence of a relationship amongst the sub-population size and the inbreeding coefficient, as already shown by other authors (e.g. Leimu et al. 2006; Honnay and Jacquemyn 2007; Aguilar et al. 2008). Age-structure of a sub-population is known to affect the Hardy-Weinberg expectations (Jacquemyn et al. 2004), therefore estimates of the inbreeding coefficient such as F_{1S} should also be considered with caution. Indeed, this sampling strategy involved only adult plants. Although no information is available in literature about the life span of *C. laurifolius*, it is speculated that the oldest individuals possibly established before habitat fragmentation (Evanno et al. 2005). This could explain the observed absence of effects of the sub-population size on the inbreeding coefficient. In this sense, minor sub-populations hosting more virginile and immature plants may play a significant role with respect to the survival of the last Italian population of C. laurifolius, despite no genetic differences amongst (mature individuals of) sub-populations were found. The lack of genetic structure could be explained by two main factors acting together: (i) a recent fragmentation of the population (F_{ST} has been shown to respond slowly to the fragmentation process) (Landguth et al. 2010) and (ii) xenogamous species behaviour. These two factors might have maintained gene flow amongst sub-populations, and gene flow is a very important factor counteracting loss of genetic diversity and inbreeding depression. Moreover, the long-living cycle of the species could be further delaying the genetic differentiation amongst subpopulations (Hamrick and Godt 1996; Colling and Matthies 2006).

Gene flow may be insufficient to counteract the effects of drift, especially at low levels of population density (Kettle et al. 2003; Rosas et al. 2011). For example, Ismail et al. (2012) have shown that pollen dispersal occurs over large distances in highly

fragmented agro-forest landscapes. However, as forests become more fragmented, inbreeding due to non-random mating amongst related individuals, as well as genetic drift, are likely to be exacerbated. The low inbreeding in MAS may reflect the mating system preceding the fragmentation when the population was more continuous, so that extensive gene flow could take place. On the other hand, several additional factors could affect the relationship between F_{IS} and population size (Honnay and Jacquemyn 2007). The absence of homozygotes for rare alleles could bias downwards the F_{IS} value in small sub-populations (Young et al. 1999), whereas F_{IS} in large sub-populations could be biased upwards as a result of the Wahlund effect.

Whether recent or not, habitat fragmentation is one of the most important factors invoked to justify the low seed set values found in many natural plant populations, mostly due to its influence on pollination and genetic erosion (Ågren 1996; Young et al. 1996; Severns 2003; Ghazoul 2005; Leimu et al. 2006; Ouborg et al. 2006; Aizen et al. 2007; Aguilar et al. 2008). Indeed, as demonstrated in other species (Honnay et al. 2005), a negative effect of small areas of occupancy on the seed set was also detected, as well as a positive effect of plant density. High seed set values in all sub-populations point towards a good reproductive performance, but no data are available for the effective establishment of seedlings. Probably, the low number and density of mature individuals in the sub-population D accounts for the lower seed set observed (Allee effect). On the other hand, the high level of seed set shown by the large sub-population FOR parallels a low percentage of immature and virginile plants. In long-living perennials, the population fitness can be related to growth and survival rates which may reduce seed production (Silvertown et al. 1993, 1996). The low presence of immature and virginile plants in the largest subpopulation FOR may be potentially deleterious for the population as a whole, since the lack of recruitment could lead to genetic depletion. In this perspective, immature and virginile plants supplied by the sub-populations C5 and D may be crucial for the long-term survival of the Italian population of laurel-leaved rock rose. On the other hand, a population structure characterised mostly by aged plants, in the worst scenario, may lead to local extinctions (García et al. 1999; Brys et al. 2003; Jacquemyn et al. 2003). The smallest sub-population (MAS) may experience such a trend within a relatively short period.

Conservation approaches

As population size, breeding system and genetic structure of *C. laurifolius* in Italy were completely unknown, the results of the present study provided relevant new knowledge, crucial for designing a programme for species management and conservation. Despite habitat fragmentation seeming to have no effect on the reproductive fitness, it is argued that this species in Italy could be affected by an ongoing process of population size reduction, linked to inbreeding depression, loss of genetic variation and fixation of deleterious alleles. All these factors play a role in reducing the adaptive potential of a population (Del-

mas et al. 2014; Theodorou and Couvet 2015). This local reduction and impoverishment is congruent with a general range contraction and fragmentation, already highlighted in this species by Fernández-Mazuecos and Vargas (2010). Without active conservation actions and also taking into account low genetic diversity (Frankham et al. 2002), the Italian population of *C. laurifolius* will face increasing risks of extinction.

Gene flow amongst sub-populations may partially compensate for losses of genetic diversity. This could reduce the mating between relatives, avoiding the increase of homozygosity and inbreeding depression (Hardner and Potts 1995).

To cope with habitat fragmentation, often due to canopy closure, the following *in situ* conservation actions are needed: a) coppicing, to reduce competition and to provide adequate light intensity for the seedling growth (Jacquemyn et al. 2008) and b) filling the spatial gaps amongst the sub-populations as much as possible, by means of targeted translocations, to contribute in maintaining gene flow. For translocation activities, there is no particular need to prefer any sub-population as a source of material, given the general homogeneity of the genetic asset. Concerning *ex situ* conservation, seeds from each sub-population are available at Pisa Germplasm Bank (Bedini and Carta 2010; Hay and Probert 2013). Studies about seed viability and ecology (Baskin and Baskin 2014) and phenology of radicle emergence (Carta et al. 2014) in this species will provide basic knowledge to establish a nursery of immature and virginile plants, to be translocated *in situ*. This may alleviate the lack of generation turnover in the natural population. Finally, a population genetics study using the same markers, but applied to populations collected all across the distribution range, may provide further insights for the conservation of this species.

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Supplementary material I

Characteristics of the 6 polymorphic microsatellites markers developed for *Cistus laurifolius*

Authors: Giovanni Astuti, Francesco Roma-Marzio, Marco D'Antraccoli, Gianni Bedini, Angelino Carta, Federico Sebastiani, Piero Bruschi, Lorenzo Peruzzi

Data type: molecular data

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Supplementary material 2

Genetic diversity parameters for the 4 stands of C. laurifolius

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Data type: molecular data

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Supplementary material 3

Results of STRUCTURE analysis

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Data type: molecular data

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Integration of species and ecosystem monitoring for selecting priority areas for biodiversity conservation: Case studies from the Palearctic of Russia

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Abstract

At the start of the third millennium, new opportunities have arisen in biogeographical research, namely in the generalisation, visualisation and cross-spectrum analysis of biological and geographical information and in the compilation of biogeographical maps and innovative models for regions that differ in the availability of distribution data. These tasks include long-term monitoring of plants and animals which are in danger of extinction, geographical analysis of biodiversity distribution and development of effective wildlife conservation strategies for specific regions. The studies of the Department of Biogeography of Moscow University on geography and biodiversity conservation are based on long-term field expeditions. The examples of the Asian Subarctic Mountains, the steppes of Central Kazakhstan and the urbanised north-west of Russia are used to illustrate Russian approaches to the use of biogeographical monitoring for the identification of priority areas for biodiversity conservation. The species populations of the higher plants and vertebrates listed in the Red Books have been considered as the basic units of biodiversity.

Keywords

biodiversity, biogeographical mapping, monitoring, wildlife conservation

Introduction

Biodiversity analysis is an actively developing method for assessing the Earth's living state. Biodiversity analysis affects not only relevant fields of scientific knowledge, but also the scope of the states' international obligations to preserve the diversity of life in their territories. Preservation of biodiversity is currently regarded as one of the priorities of the states' sustainable development. The contribution of Northern Eurasia, a territory of Russia and its bordering countries, to the global biodiversity of the planet is exceptionally large. The global role of the states in this area is estimated first by assessing the biosphere functions and ecosystem services provided by the respective biota and ecosystems. Due to the preservation of natural landscapes, the number of the functions and services existing in Russia constitutes about 10% of the world's entire quantity of such functions and services (Tishkov 2002).

As previous attempts to protect the species listed in the Red Books have shown, patronising protection or cultivation of these species in artificial conditions without attempts to protect and recover the ecosystems do not give the desired result (Velasco et al. 2015).

One stage of biodiversity conservation strategy is *quantitative and comparative assessment* in natural ecosystems at different levels. Mere inclusion in the federal and regional Red Books is insufficient; without identifying rare species, establishing their status and range boundaries, defining factors that have a negative effect on their populations, organising habitat protection and undertaking regular monitoring, the majority of the species in the Red Books would become extinct. Therefore, an essential responsibility of conservation programmes for rare species is to monitor the status of their various regional groups (i.e. the populations of the species) within the range of the state. For the next stage – the cartographic stage – *biogeographical maps* are created of various subject matters; these maps spatio-temporally integrate the different scales and types of information (Ogureeva 2012).

There are many articles in the non-Russian literature on the global and regional aspects of the assessment and conservation of biodiversity. These articles are devoted to species distribution modelling (Franklin 2010, Gallien et al. 2010, Scoble and Lowe 2010), defining priorities for networks of protected areas (Cadotte and Davies 2010, Ferrier and Drielsma 2010, Kraft et al. 2010, Proença and Iknayan 2014), identifying threats to biodiversity from invasive species (Gallien et al. 2010, Leung et al. 2010, Thuiller et al. 2010) and determining biodiversity responses to climate changes (Ackerly et al. 2010, Franklin 2010, Thomas 2010). The criteria for identifying the conservation value of the territories have been studied in the articles of Drechsler and co-authors (Drechsler 2005, Drechsler et al. 2009). However, the experience of Russian biogeographers in the Palearctic has received little attention.

The aim of this study is to summarise the experience of long-term monitoring, mapping and assessment of rare and protected plant and animal species at various spatial scales and levels in Russia. The basic units of biodiversity considered were species, families and populations of protected plant and animal species. Different parts of the

Palearctic were selected on the basis of the area's biogeographic zoning: the Russian Subarctic (Putorana Plateau), the steppe zone of Central Kazakhstan and the urbanised north-west of Russia (Kaliningrad region).

Case study I: Putorana Plateau

The Putorana Plateau is a remote and under-explored region of the Russian Arctic that is located almost entirely north of the Arctic Circle. This is one of the few vast regions of the Central Palearctic that has unusually diverse northern taiga fauna and an admixture of tundra and mountain elements. The Putorana Plateau is a significant region that ensures biodiversity of the entire Palearctic. In 2010, its territory was designated as a UNESCO world cultural and natural heritage site.

The great extent of the plateau in both latitudinal and longitudinal directions and its clearly defined vertical zoning have resulted in great diversity and a unique combination of animal communities that are prevalent throughout the Palearctic (Romanov et al. 2014). Rare and endangered fauna species that are amongst the typical representatives in the plateau are included in the Red Books of the International Union for the Conservation of Nature (Walter and Gillett 1988) and the Russian Federation, but the current state of these species' populations is largely unknown. The specific natural conditions and lack of a permanent human population significantly impede conducting regular monitoring in this area. Existing data are sparse and fail to adequately describe the distribution, abundance and current state of the protected species' populations in the area. In 1989, the state wildlife preservation organisation "Putoranskiy" initiated ornithological research and made it possible, for the first time, to generate summarised results on two species that are under international protection: the lesser white-fronted goose (*Anser erythropus*) and the white-tailed eagle (*Haliaeetus albicilla*). The results obtained for both species are presented in this study.

The lesser white-fronted goose (*Anser erythropus* (Linnaeus, 1758)) is an endangered species with a continuously and drastically decreasing population and it is included in the Red Book of Russia (Pavlov 2001) and in the IUCN Red List (Walter and Gillett 1988). It is a category 2 threatened species. The range of the species in the Palearctic is highly fragmented and represents numerically insignificant, isolated, small-scale areas that are scattered along the river valleys and lake basins from the tundra of the Kola Peninsula to Chukotka.

Putorana Plateau is one of the largest and most under-explored parts of the species' range, including its borders and the number of breeding pairs. Over the last 35 years, the population has decreased to one-sixth of its original size – from 100,000 to 18,000. Of the remaining 18,000 animals, about 5,000 inhabit Taimyr which forms the southern boundary of the Palearctic (Morozov and Suroechkovskiy Jr. 2002).

The white-tailed eagle (*Haliaeetus albicilla* (Linnaeus, 1758)) is a widespread Palearctic species. Its range includes the entire territory of Russia, but no more than 2,500 pairs remain (Pavlov 2001). The species is included in the Red Book of Russia and the IUCN Red List-96. It is a category 3 rare species.

Haliaeetus albicilla is distributed widely throughout the vast area of the Eurasian territory. A similar pattern can also be seen in Siberia, where *H. albicilla* has always been the most common large bird of prey, with its greatest numbers in the northern taiga subzone (Rogacheva 1988). In the taiga, the boundaries, within which *H. albicilla* is located, almost completely coincide with the boundaries of the Putorana Plateau. On this plateau, there is the largest area of its breeding range which is also the area with the highest number of breeding individuals (Dorogov 1988, Volkov 1988, Zyryanov 1988, Romanov et al. 2007, Romanov and Rupasov 2009). *Haliaeetus albicilla* is very rare north and south of the Putorana Plateau, with only a few breeding pairs existing outside this area (Kozhechkin and Polushkin 1983, Kuznetsov et al. 2007, Kharitonov et al. 2007, Pospelov 2007).

Environmental conditions

The Putorana Plateau is located at the extreme north-western tip of the Central Siberian Plateau (north of the Krasnovarsk territory; 65°00'-71°00N; 90°00'-100°00E; (Fig. 1). The plateau is an array of basalt with flat tops and with an average elevation of 900-1200m above sea level (asl). The landscape is predominantly lowlands, with a maximum height of 1701m asl in the eastern part of the region. The altitudinal zonation comprises the northern-taiga (up to 600m asl) and the subalpine (600-800m asl) and alpine (800m asl) zone (Norin 1986). The climate of the Putorana Plateau is subarctic, with average January temperatures ranging from -32°C to -36°C and average July temperatures ranging from +8°C to 12°C. Summer is short; the polar day lasts less than one month (22 days) in the south and about 2 months (64 days) in the north. Winter is long, with a polar night from 22 to 60 days (Zemtsova 1976). The annual amount of precipitation ranges from 300mm in the northeast to 600mm in the southwest of the region. Due to the Putorana Plateau including subarctic areas of excessive moisture, as in other provinces, favourable conditions are created to maintain a dendritic and full-flowing water network. The surplus water in subarctic landscapes fills all concave forms of relief (potholes, bowls), leading to the formation of large tectonic oligotrophic-type lakes. The volume of water mass in the lake area of the plateau is the greatest in the Russian Subarctic. As the hydrography of Putorana is mainly determined by a tectonic snap system, the valleys and potholes from modern reservoirs form narrow and deep gorges and canyons and most rivers are mountain-type rivers: they are turbulent and full-flowing with many knickpoints. In winter, ice blisters form on the largest of the rivers; in summer, there are frequent high-level protracted floods. While the central and southern parts of Putorana Plateau are located in the northern taiga subzone, most of its territory is above the tree line.

Therefore, widespread mountain—tundra landscapes within the belt occupy about half of the territory in the south and most of the territory in the central part. Amidst the mountain landscape, integral, separate and unique fauna complexes have been formed, such as forest (mountain—northern taiga), golets (mountain—forest tundra)

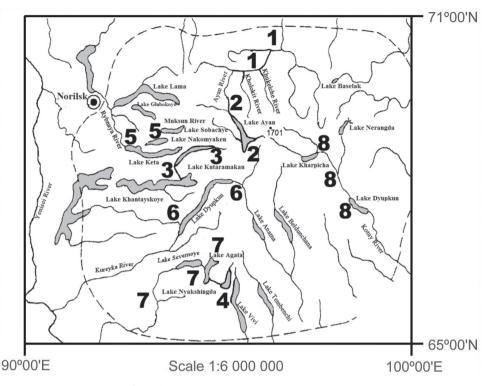


Figure 1. Areas and years of ornithological research on the Putorana Plateau: I Northern regions (Lake Bokovoye; rivers: Ayan, Ambar, Munil, Nerakachi, Dakit, Kholokit, Khukelche), 1989 2 Central regions (Lake Ayan, Lake Kapchug; rivers: Amnundakta, Gulyami, Bolshoy Khonna-Makit, Kapchug), 1988 3 Western regions (lakes: Kutaramakan, Kapchuk, Khantayskoye; rivers: Verkhniy Kutaramakan, Kutaramakan, Kapchuk, Bogadil, Irkinda), 1990 4 Southern regions (Lake Nyakshingda, Lake Vivi; rivers: Amundykan, Verkhnyaya Nyakshingda, Nyakshingda, Irbukon, Morktakon, Sengan), 1991 5 Western regions (lakes: Keta, Nakomyaken, Sobachye, Glubokoye; rivers: Nahta, Mikchangda, Muksun), 1999, 2004, 2008 6 South-west (Lake Dyupkun Kureiskiy; rivers: Kureyka, Yagtali), 2001, 2006 7 South-western regions (lakes: Agata Verkhnyaya, Agata Nizhnyaya, Severnoye; rivers: Oron, Epekli-Sen, Severnaya), 2003 8 Eastern regions (lakes: Kharpicha, Dyupkun Kotuiskiy, Lyuksina; Kotuy River), 2007. Traditional border of Putorana Plateau.

and sub-golet (mountain-tundra) belts (Nikolaev 1999). These areas do not have identical counterparts in the other mountain systems in Northern Eurasia and, therefore, are independent, valuable objects of research and protection. Expeditionary research on the Putorana Plateau from 1988–2008 is presented in Figure 1.

Materials and methods

Recording and monitoring of the populations of rare and endangered Palearctic avifauna were undertaken from 1988 to 2008 (during 13 summer seasons from May

to August). Field parties of two to four people were organised to walk overland and to navigate water routes by boat. The expeditionary groups' equipment consisted of navigational aids, special optical equipment, individual telemetry tracking devices for birds, items for labelling and standard field equipment for field research in the Arctic. During this period, an area of about 200,000km², including 11 large tectonic lakes, was investigated. All the material was collected using survey routes. The total length of the overland survey routes was 8,617km and that of water routes was 1,516km. While traversing the routes, the researchers visually assessed all species of birds and their status (such as nomadic, breeding, hunting). The investigation also included assessing the borders of territorial pairs as well as areas potentially suitable for the birds to breed. Fixed surveillance of the birds' flight during the migration season and daily monitoring of the nests during the breeding season were undertaken.

The breeding accuracy was estimated according to the criteria recommended by the European Ornithological Atlas Committee (EOAC) (Hagemmeijer and Blair 1997). Records of *H. albicilla* registered as living in nests and in territorial pairs were drawn on to maps. For remote areas, the number of individuals was estimated by abundance extrapolation based on an expert assessment of habitat suitability using topographic maps at the scale of 1:500,000 and 1:200,000 and using satellite Landsat images. At the first stage, all areas that were similar to areas in which *H. albicilla* was observed breeding, were identified using satellite images and topographic maps. After confirmation of the birds' residence in these areas, the number of breeding pairs was extrapolated by taking into account the available space suitable for establishing breeding territories as well as the average size of nesting sites and distances between them (defined based on field and published data).

In addition to observations recorded along the same survey routes as for *H. albicilla*, *Anser erythropus* was studied by satellite telemetry. Adult moulted birds (n=6) accompanying litters were equipped with plastic collars with fixed satellite "NORTH STAR" transmitters at nesting sites in the south-west of Putorana. The transmitters allowed the birds' locations to be traced for eight months. The telemetry data were processed using Argos-tools (http://gis-lab.info/programs/argos/index-rus.htm) and the Google Maps mapping service allowing the birds' movements to be traced in real-time (http://gis-lab.info/projects.piskulka.html) using scalable space Landsat images. Uncertainty in object position did not exceed 10 m. Descriptions of the habitats in the resting areas during migration were compiled using large-scale maps, space images and regional physico-geographical summaries (Gvozdetskiy and Mikhailov 1978, Dementiev 1979, Proshin 1979), as well as questionnaires (V.A. Arkhipov and E.A. Zhuravlev, pers. comm.). Faecal samples from *A. erythropus* were also analysed in the breeding area.

Results and discussion

The collected data allowed identification of the nesting area, estimation of the number of breeding birds and an assessment of the breeding habitat and migration. Figure 2

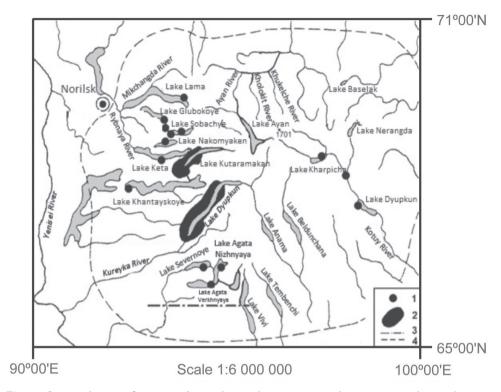


Figure 2. Distribution of *Anser erythropus* during the nesting period on Putorana Plateau. **I** meeting points of territorial pairs, litters and non-breeding individuals **2** long-term successful nesting areas **3** southern boundary of breeding range; 4: Putorana Plateau border.

shows stable and relatively large core areas for breeding of Anser erythropus which are consistently formed on the lakes in the western part of the Putorana Plateau: Kutaramakan (30-40 breeding pairs), Dyupkun (60-100 pairs) and Agata Nizhnyaya and Severnoye (10-15 pairs). At least 220 pairs nest each year in the surveyed 35,000km² territory. The largest nesting swarm on Lake Dyupkun belongs to one of the largest swarms on the Taimyr Peninsula, with an average occurrence of two pairs of geese per 10km of coastline (excluding the mountainous northeast segment, where there are 2.5 pairs/10km). A small group of 10-12 pairs was present in the eastern part of the Putorana Plateau in the Kotuy riverheads. Although a population of A. erythropus may nest on the Vivi, Tembenchi, Annama and Beldunchana lakes, these lakes have not been investigated. Long-term monitoring results allowed the southern line for the distribution of A. erythropus to be drawn 250 km further south of the Keta and Kutaramakan lake potholes than previously thought. According to our updated data, the nesting area of Anser erythropus completely covers the western part of the Putorana Plateau, extending southwards to 66°5. The southern border extends through the system of two lakes: Agata Verkhnyaya and Agata Nizhnyaya (Fig. 2). Putorana Plateau has therefore been conclusively shown to be the southernmost point for the distribution of A. erythropus on the Taimyr Peninsula which is a key region for the species' reproduction within the Taimyr sector in its range. The number of breeding birds in this region comprises 10–15% of the Taimyr population.

The critical nesting factor in Putorana is the presence of a wide flat coastal area of the lakes with sedge—mixed herbs and osier. These areas, extending for tens of kilometres along the lacustrine coast, provide the geese with both plentiful, easily accessible food and secure hiding places in case of danger. The average nesting density in the most favourable habitats is two pairs per 10 km of coastline. The average litter size (n=59) is four chicks.

Telemetry tracking has shown that the Putorana population hibernates in Syria and Iraq, migrating through Western Siberia, Kazakhstan, the Caspian Sea, Iran and Turkey. These migration routes are part of the global migration flows that are common to the population of *A. erythropus* breeding in the western half of its range, from Western Taimyr to Southern Yamal and Northern European (Fig. 3). Within the range of the full annual life cycle, the Putorana *A. erythropus* are most vulnerable at their migration stops, especially in Northern Kazakhstan, where people actively hunt waterfowl (Morozov and Syroechkovskiy Jr 2002, V.A. Arkhipov and E.A. Zhuravlev, pers. comm.).

The results of monitoring other protected species, i.e. the *white-tailed eagle (Haliaeetus albicilla)*, provided the basis for the assertion that the modern state of this species' nesting on Putorana Plateau is stable. There were no drastic changes in the number of the species in the past decade. An average of one territorial pair travels within about 1,176 km² and the forest landscapes in which *H. albicilla* nests comprise no more than 50% of the plateau area — about 500–580 km² (Romanov 2009). The relative abundance of the species per 10km of the route is shown in Table 1.

Table 1 shows that the average distance between adjacent nests varies from 11.7 to 39 km and the maximum concentration of nests is registered in the Ayan river valley. In comparison, nests were found every 10–20 km at the end of the 1950s and the beginning of the 1960s to the west of Keta lake and in the Rybnaya and Khantayka river valleys. The records of Dorogov (1988), who investigated extensive areas of Putorana Plateau in 1975–1986, showed that, on average, nesting pairs were found every 25–30 km in the lake or river valleys. The minimum distance between two adjacent nests was 6–7 km in the Ayan river valley (Dorogov 1988), 15 km at the Kotuy riverhead (Dorogov 1988) and 6 km at the Kholokit riverhead (survey data).

In early spring, the wellbeing of *H. albicilla* on Putorana Plateau is directly related to the abundance of carrion and the remains of prey left by terrestrial predators and the nesting areas of most pairs coincide with the areas of wild reindeer mass migration. The shift in the main reindeer migrations from western to eastern Putorana during 1970–1980 was probably one of the most significant factors that negatively affected *H. albicilla* population dynamics in some western areas of Putorana. However, the reduced number of nesting pairs on the western plateau did not mean an automatic reduction in the overall number of Putorana family groups. Following the reindeer migration routes indicated a smooth transition of breeding *H. albicilla* into the interior and eastern regions of the plateau.



Figure 3. Flight scheme of *Anser erythropus* based on the results of telemetry tracking. Note: **1,2,3** flight trajectories of three lesser white-fronted geese; **A** Azerbaijan; **B** Armenia; **C** Syria.

Overall estimates show that about 170 pairs nest in the Putorana Plateau territory, of which at least 70 pairs nest in potholes of western lakes (Romanov and Rupasov 2009). Based on data from the Red Book of the Russian Federation (Pavlov 2001) on the number of individuals in Russia (about 2,500 pairs), the Putorana population accounts for about 7% of the total number of *H. albicilla* in Russia or 17% of the total number nesting in Siberia (Romanov 2009). These calculations show that Putorana Plateau is a key area for the species' reproduction in the Asian part of Russia. Its population may be affected negatively by harvesting old-growth forests on floodplains,

Location	Route length (km)	Survey year	Number of breeding pairs	Average number of breeding pairs per 10 km of the route	neighbouring	Source
Middle reach of the Ayan River	70	1989	6	0.86	11.7	Romanov and Rupasov 2009
Pothole of Lake Ayan	70	1988	4	0.57	17.5	Romanov and Rupasov 2009
Pothole of Lake Kutaramakan	80	1990	4	0.5	20	Romanov and Rupasov 2009
Potholes of the lakes Nakomyaken, Sobachye and the eastern terminus of Lake Glubokoye	100	1999	4	0.4	25	Romanov and Rupasov 2009
Valley of the Mikchangda River	110	2004	3	0.27	36.7	Rupasov and Zhuravlev 2007
Basin of the Severnaya River	430	2003	11	0.26	39	Romanov and Rupasov 2007
Upstream of the Kotuy River	100	2007	3	0.3	33	Romanov and Rupasov 2009
Upstream of the Kotuy River	300	1983	4*	0.13*	75*	Dorogov 1988
Upstream of the Kotuy River	350	1984	5*	0.14*	70*	Volkov 1988

Table 1. Number of white-tailed eagles on Putorana Plateau.

Note: * as a result of non-specialised records (without a targeted search for nests), the obtained data may be underestimated.

direct interaction with man, local and seasonal decreases in the abundance and availability of fish (this effect was especially reflected in summer nutrition) and reduction in the availability of carrion in the spring due to wild reindeer migration route changes (Romanov and Rupasov 2009).

The biogeographical features of Putorana Plateau, in particular its location within the boundaries of the Yenisei zoogeographic border which is one of the largest meridional biogeographic borders of Eurasia, support abundant biological and landscape diversity in the region, a transitional nature of the fauna and many endemic and rare species. The annual seasonal migration of the world's largest population of wild Taimyr reindeer (*Rangifer tarandus*) attracts many predators and acts as a regulator for these predators' distribution, abundance and reproductive behaviour. Thus, research and monitoring of *Anser erythropus* and *Haliaeetus albicilla* are considered top priorities and represent major international environmental challenges, demonstrating the need for maintaining the Russian Subarctic nature protection status for Putorana Plateau and for continuing research on rare and protected species in its territory.

Case study 2: Kazakhstan

The problem of biodiversity protection is particularly acute in regions affected by global climate change. These regions include steppe landscapes with preserved relict plant

species and unique ecotopes, demonstrated by the forest outliers of the steppe region in Central Kazakhstan – the Pleistocene relicts of a single forest range that had contact with taiga forests of Western Siberia and with mountain and submontane forests of Altai in the cold and wet Pleistocene age. The presence of rare boreal and nemoral species surviving in these woods has led to the unique nature and high conservation value of these steppe landscapes. Amongst the total number of rare and endangered plants in Kazakhstan (about 600), 175 species reside in steppe landscapes.

The aim of this study was to assess the botanical diversity of Karkaraly National Park within the Karkaralinskie and Kent mountain ranges and the changes to this diversity during 2007–2014, in order to identify the most important ecotopes for rare and relict species in the studied region.

Natural conditions

Kazakhstan is a large country located in central Eurasia. It covers an area of 2,715,000 km², stretching nearly 3,000 km from west to east and 1,600 km from north to south. The landscape in Kazakhstan is diverse. The Kazakh Hummocks and Karkaralinskie and Kent mountain ranges are located in the central part of the country (Fig. 4).

The climate in the republic is sharply continental. The average January temperature ranges from -19°C in the north to -5°C in the south and the average July temperature ranges from +17°C in the north to +31°C in the south. Summer is hot and torrid everywhere in the country. The temperature can reach +50°C. Winter in the country is dry and cold and the temperature can reach -58°C (Thomas 2010).

The research area is located within the Kazakh Hummocks and limited to the Karkaralinskie and Kent mountain ranges. The coordinates of the area are 49°25'00'N and 75°25'00E. The area of Karkaraly National Park, where most of the research was undertaken, is 112,120 ha.

By botanical–geographical zoning, the research area belongs to the Bayanaulsko–Karkaralinsko–Kent district in the Eastern–Kazakhstan sub-province of Zavolzhsko–Kazakhstan province in the Prichernomorsko–Kazakhstan sub-region of the Eurasian steppe region (Karamysheva and Rachkovskaya 1973). For the landscape, the Bayanaulsko–Karkaralinskiy hills form part of the Western Siberian-Kazakhstan steppe region in Central Kazakhstan, forming the Karkaralinskaya province (Nikolayev 1999).

The area covered by Karkaralinskie and Kent mountains is an ancient Paleozoic shield that, during Neogene-Quaternary time, underwent powerful geomorphological transformations that led to the modern look of these mountains, with their peaked ridges, abundance of screes and narrow, difficult-to-access canyons. The soil in the area is mainly represented by dark chestnut and mountain chestnut soils. The small islets of meadow chernozem soil is associated with mountain ranges and confined to river valleys. Intermountain valleys feature salt-washed chernozems and there are widespread solonetzic and alkaline soils in degradations (Nikolaev 1999).

At the end of the 19th century, the first serious geobotanical studies were performed in the Kokshetau forests by a professor at Kazan University, Gordyagin (1897). This

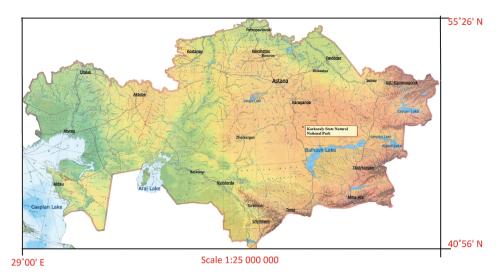


Figure 4. The research region in the Republic of Kazakhstan.

professor suggested that the forest outliers in the steppe region were a remnant of the single forest range which formerly connected the north to the taiga forests in Western Siberia and the east to the mountains and submontane forests in Altai. A similar idea was later expressed by Krasheninnikov (1939), who considered the steppe forest outliers to be the remains of a forest-steppe belt stretching from the Southern Urals to Altai during the cold and wet Pleistocene age.

The significant floristic unique nature of the East-Kazakhstan sub-province consists in the high percentage of species that have spread towards the east, such as the eastern Palearctic, eastern Kazakhstan, eastern Kazakhstan-Mongolic, eastern Kazakhstan and southern Siberian-Mongolic regions (Karamysheva and Rachkovskaya 1973). The presence of a wide range of humid boreal plant genera (e.g. *Chimaphila, Moneses, Pyrola, Orthilia*) is typical for this sub-province. All these genera grow in the low-mountain ranges of the Eastern Kazakhstan sub-province which is characterised by pine forests and open *Pinus sylvestris* forests and by the *Betula pubescens* birch gallery forest along streams and small rivers.

Pine forests mostly grow on mountain ranges with an understory of *Rosa spinosissima*, *Rosa majalis*, *Juniperus sabina*, *Lonicera tatarica*, *Padus avium* and *Crataegus sanguinea* which account for 71.3% of the total mountain forest area. Birch forests (*Betula pendula*, *B. pubescens*) are confined to the slopes with exposure to the north and northeast and to intermontane valleys along rivers and streams. Birch forests occupy 10% of the forested area. Aspen forests (*Populus tremula*) comprise about 2% of the forested country and are confined to relief depressions, valleys of rivers and streams and the base of round slopes.

Pine forest outliers are an amazing natural phenomenon of the western Siberian-Kazakhstan steppes. Conservation of these epibiotic complexes in the depths of the

steppe area favour specific edaphic conditions (loose, salt washed sands or granites). However, where the forests have been destroyed by people, natural recovery has become impossible. Many places have retained the names of forests that were lost long ago (Nikolaev 1999).

Materials and methods

The materials for this study were compiled during expeditions during the summer (June and July) in 2007–2014. The area of research occupies a territory of about 100,000ha in the Karkaralinskiy and Kent mountain ranges, the steppe river valleys and the intramontane bolted areas. Monitoring of rare and relict species was undertaken both in the interfluvial zones and in the mountains. Routine geobotanic descriptions have been made, floristic lists on each type of ecotopes have been compiled and a herbarium has been collected. The investigations were undertaken along routes (distance from 2 to 25 km) and permanent plots in ecotopes which are important for rare and relict species of flora. Overall, 360 leaves deposited in the herbarium were studied, with 216 geobotanic descriptions being mapped across 70 routes.

The research also includes analysis of the lists of rare and protected species of Kazakhstan plants (373 species) (Institute of Zoology and Scientific Society "Tethys" 1999), with the aim of defining biotopical preferences of the species in some families. A floristic diagram method, based on studies by Gnatyuk and Kryshen (2005), has been used to depict the relationships between different plant families. Statistical methods have been used to estimate the biodiversity of different biotopes. Alpha- and beta-diversity indices have been calculated using the indices of Whittaker and Shannon and assessed using cluster analysis (Dikareva and Leonova 2014).

Results and discussion

Figure 5 shows the relative contributions of different plant families to the 175 rare and relict plant species observed. *Compositae* (17%), *Poaceae* (10%) and *Ranunculaceae* (10%) predominate. These families are followed by *Ammaryllidaceae*, *Liliaceae*, *Crassulaceae* and *Boraginaceae* (7% each). This correlation is mostly similar to the overall family correlation with all flora in a steppe area.

The highest indices of alpha- and beta-diversity were located on stream banks, valleys of temporary streams, lake banks, floating bogs in the limnetic zones of lakes, sphagnum bogs, raised bogs and swamp-subor forests, crevices of stone chunks, vegetation at the basis of rocky mountain ridges, pine forests on the flanks and shelves of high mountains with an understory of moss or moss and grass and sticky alder forests.

We divided the studied ecotopes into ten groups according to their importance for rare and relict species and identified groups of specific and unique ecotopes.

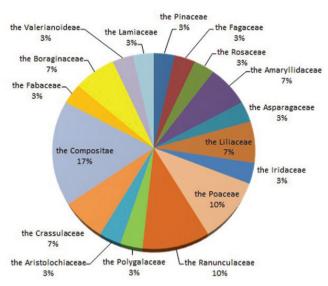


Figure 5. Floristic content of rare species in the Republic of Kazakhstan.

Group 1. Stream banks and shady canyons. Most important

This ecotype is the most important one for the conservation of rare and relict species. Species of the families Rosaceae (Padus avium, Crataegus alpinum, Sorbus sibirica, Rubus idaeus, Filipendula ulmaria), Grossulariaceae (Ribes nigrum, Ribes hispidum), Umbelliferae (Heracleum sibiricum, Angelica sylvestris, Pleurospermum uralense), Primulaceae (Lysimachia vulgaris, Naumburgia thyrsiflora), Compositae (Ligularia sibirica, Crepis sibirica), Equisetaceae (Equisetum sylvaticum, E. pratense) and Ericaceae (Pyrola rotundifolia, P. minor) dominate. Slightly less widespread are the species of the families Adoxaceae (Viburnum opulus), Onocleaceae (Matteucia struthiopteris), Athyriaceae (Athyrium filix-femina), Dennstaedtiaceae (Pteridium aquilinum), Cyperaceae (Scirpus sylvaticum), Rubiaceae (Galium boreale), Geraniaceae (Geranium sylvaticum) and Salicaceae (Salix caprea). Orchidaceae (Dactylorhiza maculata) was rarely seen.

Group 2. Important

This group includes sphagnum bogs, raised bogs and swamp-subor forests. They are characterised by families with species that are specific to the bogs of the northern taiga, as follows: Salicaceae (Salex Lapponum), Ericaceae (Oxycoccus palustris), Droseraceae (Drosera potundifolia, D. anglica), Cyperaceae (Rhyncospora alba, Eriophorum angustifolium, E. gracile, Carex vaginata, C. loliacea, C. buxbaumii, C. rostrata, C. magellanica), Scrophulariaceae (Pedicularis palustris, P. sceptrum-carolinum), Orchidaceae (Spiranthes sinensis) and Menyanthaceae (Menyanthes trifoliata). Some families grew on mounds. The representatives of these families, which usually grow in coniferous taiga, are Caprifoliaceae (Linnaea borealis), Orchidaceae (Goodyera repens) and Ericaceae (Vaccinium vitis-idaea, Pyrola rotundifolia, P. minor).

Group 3. Intermediate importance

Moss-grown and moss-grass-grown pine forests on the shoulders and tails of mountains are less favourable compared with the sphagnum bogs. The plant species, characterising these pine forests, belong primarily to the families Caprifoliaceae (*Linnaea borealis*), Orchidaceae (*Goodyera repens, Neottianthe cucullata*), Ericaceae (*Moneses uniflora, Chimaphila umbellata, Orthilia secunda, Pyrola chlorantha, P. rotundifolia, P. minor*) and Cystopteridaceae (*Gymnocarpium dryopteris, G. robertianum, G. tenuipes*).

Group 4. Less important

This group includes boil places, lakeshores and crevices of granite chunks in equal proportion. The following rare Orchidaceae were located near springs, with constant running and humifying water: *Cypripedium calceolus, Cyprepedium macranthon, Dactylorhiza fuchsia* and *Dactylorhiza maculata. Corallorhiza trifida* grew in the moss cover along the banks of streams that flow from springs. The following *Pyrola* species were observed: *Pyrola rotundifolia* and *Pyrola minor*. In addition, the fern *Gymnocarpium dryopteris* was found.

Group 5. Lacustrine ecotopes

Ecotopes of many rare boreal relicts, such as *Lycopodium clavatum* and *Diphasiastrum complanatum*, are located along the edges of lakes. On the edge of lakes in the pine forest, there are many individuals of the fern *Pteridium aquilinum*. A rare species, *Dryopteris carthusiana*, also grew here. On the lakeshores of Svetloe and Zerkalnoe in the Karkaralinskie mountains, the species *Trientalis europaea*, which is exceptionally rare for Kazakhstan, has been preserved. It grows in groups in the pine-birch forest on peaty soils, on pap at the base of birch trunks. *Equisetum hyemale* forms the entire tangle at the margin of Lake Borovoe in the mouth of the Imanayskiy well spring. *Vaccinium vitis-idaea* is located mainly close to lakes.

Group 6. Rocky ecotopes

Rocky inselbergs at the edges and on the sides of mountains and mountain uplifts are home to *Rubus idaeus* and the ferns *Asplenium septentrionale*, *Polypodium vulgare*, *Woodsia ilvensis* and *Cystopteris fragilis* which grow in crevices filled with fine grained soils. *Pentaphylloides fruticosa* and *Chamerion angustifolium* grow on the rocky edge of the Sinyukha mountain (southern side) and *Saxifraga sibirica* grows in shady moist crevices on northern side.

Group 7. Sticky alder forests

Black alder communities (*Alnus glutinosa*) are found in stream valleys and often in deep shady canyons that shelter many rare boreal relicts. Growth of boreal species in alder stands prefer abundant running humifying water, a wealth of soil, well-developed leaf-litter and a shadowing leaf canopy. *Circaea alpina*, *Circaea lutetiana*, *Delphinium elatum*, *Athyrium filix-femina*, *Matteucia struthiopteris* and others are found in this habitat type.

Group 8. Seasonal ecotopes

Two of the least important ecotopes for relict species are valleys of temporary streams and niches at the bottom of rocky edges of mountains in equal proportion. From the mountainsides in some places, streams flow down that are fully flowing after rains but dry up during dry summer periods. The boreal flora of the temporary stream valleys is less prevalent, including only *Ribes nigrum*, *Salix caprea*, *Solidago virgaurea*, *Rubus saxatilis*, *Galium boreale* and some others.

Group 9. Rocky shelters

On the northern sides of higher mountains and on mountain uplifts at the bottom of steep-sided rocky edges, snow usually accumulates in the winter and usually does not melt until the beginning or middle of June. There are shady places that offer shelter from the wind amongst large rocky inselbergs formed by a heavy layer of fine-grained soil. In these places, moisture is abundant as a result of melting snow and rain flowing down from the rocky edges as well as from the occurrence of condensation in crevices. Such shady niches serve as ecotopes for several rare relict plants. For example, *Juniperus communis* in the form of bunches and small trees (up to 3m), grow in the niches at the bottom of Sinyukha mountain in the Karkaralinskie mountains; however, in more open spaces, it takes the form of an elvin wood. *Rubus idaeus, Ribes nigrum, Athyrium filix-femina* and *Dryopteris filix-mas* were also observed.

Group 10. Lacustrine floating bogs

The least favourable ecotope for the conservation of rare species are the floating bogs in limnetic zones. The floating bogs on the lakeshores serve as distinctive ecotopes for the fern *Thelypteris palustris* which forms sporadic tangles. *Equisetum palustre* and *Equisetum fluviatile* were also observed.

The common feature of all these ecotopes is the presence of multiple rare and relict species that contribute to the high biodiversity and unique nature of the region (Fig. 6). This biodiversity is favoured by natural protection against fires, presence of a moisture reserve during dry periods, provision of ongoing stagnant semi-flow and flowing humification due to spring-well outlets, subsoil water proximity and water vapour condensation contained in the air and due to the damping impact of lakes.

The re-studies of vegetation of the evaluated areas have shown a gradual increase in species numbers and diversity, suggesting a favourable effect in the protection regime introduced in the national park and the decrease in grazing pressure (Dikareva and Leonova 2014). When composing recommendations to establish new strictly protected areas within the Karkaraly National Park, we took into account how much the ecotope favoured the existing high diversity of rare, relict and protected species (Fig. 7).

Thus, the annual floristic monitoring and biogeographical assessments of the ecotope's coverage of rare plant species allowed the identification of priority areas for Strictly Protected Natural Areas. These areas are primarily the stream banks, especially in deep shady crevices, sphagnum bogs, raised bogs and swamp-subor forests, as well as mossgrown and moss-grass-grown pine forests on the shoulders and tails of high mountains. Thus, for Karkaraly National Park, zoning is recommended based on the ecotopes with

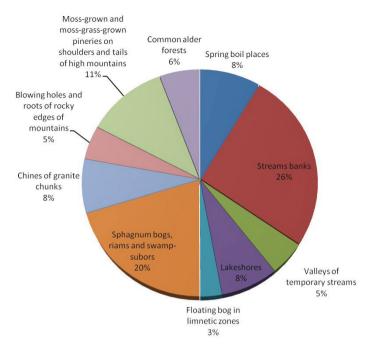


Figure 6. Percentage of rare species confined to a particular ecotope of the study area.

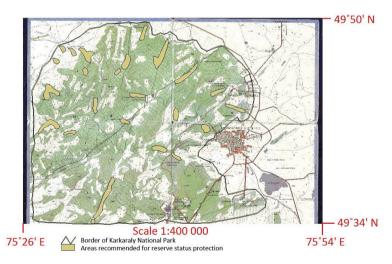


Figure 7. Existing protected territories and those that are recommended for protection in Karkaraly National Park.

the highest diversity of rare and relict plant species. In the shaded area on the map, protective measures, including prohibitions on visiting, should be imposed and regular monitoring should be undertaken. To improve the efficiency of the network of protected areas for the preservation of the unique plant biodiversity of the Karkaralinskie mountains, expansion of geobotanical research in this region must continue (Gerstner 2014).

Case study 3: Kaliningrad region

The Kaliningrad region is unique in Russia; it is Russia's western enclave, both geopolitically and naturally. The region belongs to an inhabited urbanised territory that surpasses the Baltic States, Belarus and the North-West Federal region of Russia in population density, degree of urbanisation, intensity of agriculture and density of the traffic network. The high degree of agricultural development and deep transformation of natural complexes, wide development of hydro-engineering, transport and forest-based and agro-industrial systems greatly affect attempts to preserve landscapes and ecosystems in a near natural state to prevent the numbers and ranges of rare and endangered species of plants and animals from decreasing. Despite the existing network of special protected natural areas in the Kaliningrad region, the area's status and activities do not fully comply with modern conservational concepts and international obligations which Russia has regarding conservation of biological and landscape diversity. The traditional specificity of this area requires special measures for the conservation and recovery of the most important natural complexes that are of common European importance. This specificity also calls particular attention to compromises between conservational and economic interests. One approach may be the development of conservation strategies for rare species based on a detailed examination of the regional pattern of biodiversity.

Natural Conditions

The Kaliningrad region is located on the western outskirt of the East European plain at the southeast coast of the Baltic Sea between 55°19'N and 54°19'N and 19°38'E and 22°52'E (Fig. 8). The region borders the Lithuanian republic to the north and east, Poland to the south and the Baltic Sea coastline to the west. The region's area, including waters, amounts to 15,100 km². The landscape is generally flat with predominantly vast low grounds in the central and northern parts (Pregolskaya, Polesskaya, Sheshupskaya, Nizhnenemanskaya) and in the uplands in the south (Varmiyskaya, Vishtynetskaya). These low grounds are characterised by a high diversity of origin and microrelief. Rare forms of relief are located in the territory's landscape, such as the deltaic plain of the Neman river, washed-over fens of the Curonian Lagoon coastline and sand spits (Curonian and Baltic) with wandering dunes which are unique to the Baltic region.

Based on natural and climatic conditions, the Kaliningrad region belongs to the south-taiga forest zone. The territory is characterised by abundant humifying, medium heat provision and a relatively steady temperature regime with a mild winter, cool summer and a long autumn period. The landscape of the Kaliningrad region has transitionary features between eastern and western Europe that are observed in the vegetation and soil layers of the territory. Zonal types of plants in the region's territory are represented by mixed broad-leafed fir forests (*Picea abies, Quercus robur, Carpinus betulus, Fagus sylvatica*) and nemoral forests with a grass layer that includes boreal floristic elements. Their differential characteristic is the high amount of broad-leafed



Figure 8. The region of study – Kaliningrad region.

species (up to 20%) and sticky alder (up to 15%), respectively. The region's territory is bordered with beech and fir forests. However, since the 17th century, the natural forest range has been cut down in the main part of the territory for grazing. Currently, the natural ecosystems of the Kaliningrad region are represented by forests, wetlands, meadows and dune complexes as well as the Baltic Sea water complexes. The biodiversity of the Kaliningrad region consists of 1,436 species of tall plants of which 26% are endangered and 338 terrestrial vertebrate species (mammals and birds), about half of which are rare and threatened (Dedkov and Grishanov 2010). Above the Kuronian Spit, about 20 million birds make their annual seasonal migration along the Belomor-Baltic route. The features of geographic location, historical development and natural conditions are the prerequisites and factors for high ecosystem diversity and biodiversity, the conservation of which is crucial for the whole European continent.

Materials and methods

This study involved the authors' own field materials on rare and protected species of plants and animals gathered in 2000–2013 in the Kaliningrad region, the contemporary records of I. Kant Kaliningrad University and national and regional Red Book materials (Pavlov 2001, Dedkov and Grishanov 2010). All this data was compiled into a common database and then analysed and conceptualised using cartographic methods. The data included 83 species of high plants and 53 terrestrial vertebrate species (136 species in total), which have been under federal and regional protection for 80 years, including the time when the studied territory was a part of East Prussia.

The cartographic modelling of species was performed via grid mapping (a method of square grids). This method supported spatial statistical analysis of species distribution with a large amount of chronological data. This method was used for the first time in

Great Britain (Perring and Walters 1962) and then realised successfully in the course of a long-term project (1972–2010) on creating the Atlas "Flora of Europe" (Humpries et al. 1999, AFE (accessed: 14.12.2015). Flora mapping in these investigations is performed on a universal basis using a grid of squares on a geographical map of the same area, the borders of which include registering the species using a binary (presence or absence) approach. The opportunities for successful implementation of the square grid method for spatial analysis of the plants and individual groups of animals were shown by different authors (Bukhar and Koroleva 1994, Uotila 1999, Seregin 2012, Kalyakin et al. 2014).

Although European projects used a single-square (50×50 km) grid, no single grid was used for the territory of Russia. Thus, while performing regional investigations, researchers should create square grid systems of the actual region. For the Kaliningrad region, a subdominant double grid of squares (large and small) has been prepared using GIS-technologies (Sokolov 1999, Koroleva and Neronov 2007, Koroleva et al. 2008, Koroleva 2014). In the large-square grid, the grade frame is marked horizontally every 4' starting from 54°16' and vertically every 8' from 19°36'. Thus, the Kaliningrad region's territory was divided into 258 relative squares with an area of 63.75 km² each. All the squares had a number reflecting the horizontal structure of the grid and a letter identifying the squares by their vertical placement (for example, L14). For more precise localisation of the species location, each square is then divided into four sectors (each with an area of 15.94 km²), marked by the letters a, b, c, d (Fig. 9). All the phases of mapping (create database, select series, draw maps) were undertaken using the programme MapInfo Professional, version 12.5.

The method of grid mapping was used for the Kaliningrad region, in addition to compiling traditional floristic and faunistic maps and this made it possible to perform a biogeographical assessment to detect the protected biota (flora and fauna), a benchmark assessment of historical floristic monitoring data and a current valuation of modern territorial conservation measures.

Results and discussion

Separate distribution maps of protected plant and animal species, certain groups and families are the initial stages and transition elements of biodiversity mapping (Koroleva 2013). These maps reveal valuable information related to the protected species' territories, show the priorities in wildlife conservation and allow development of a strategy for conservation of rare species and their ecotopes in the region. "Hot spots" of biodiversity are identified by overlaying protected plant and animal species maps.

As can be seen from Figures 10 and 11, the priority biodiversity conservation areas in the Kaliningrad region include the Curonian and Baltic Spits, Sambiyskaya and Varmiyskaya Uplands, Sheshupskaya and Polesye Lowlands, Neman and Pregolya river deltas and the Curonian Lagoon coastline. The highest priorities amongst them are the Curonian Spit (over seven species in a square) and the Vishtynetskaya Upland (over six species), where the largest number of protected flora and fauna species are registered.

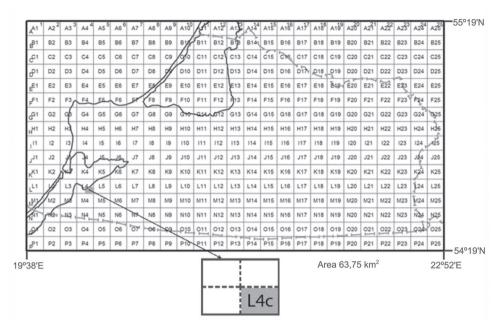


Figure 9. The square grid of the Kaliningrad region with an example of numeration of the "small square"

Dynamic trends in the distribution and/or disappearance of protected species using retrospective and modern mapping are shown in terms of the protected plants from the Red Book of the Russian Federation. The benchmark study of available archive materials from a historical perspective (until 1945) and with modern data allow the following conclusion to be drawn: The territories located east of Polesye and the south Gvardeyskiy districts showed fewer protected plant species in the last decade. The survey has also shown that, in the western and north-western regions, however, both the frequency of rare species and the amount of species in a single territory increased. This obviously testifies to the efficacy of protection measures in these regions. (Fig. 12).

Figure 12 shows the areas that require emergency protection measures due to the location of the species which are under international protection. These species are the orchid *Cypripedium calceolus* L. (lady's slipper), which is listed in the Red Book of IUCN and is found on the coast of Vistula Bay; *Epipogium aphyllum* Sw. (ghost orchid), which grows in the south Pravdinskiy district; and *Orchis morio* L. (greenwinged orchis), which is known to grow in the Curonian Spit. Two further species are listed in the Convention on International Trade in Endangered Species of Wild Flora and Fauna. Four species are threatened with extinction in this area: *Botrychium simplex* E. Hitchc. (grape fern), which is observed on the coast of Vistula Bay, in the outskirts of Krasnoznamensk and Svetlogorsk and in the Curonian Spit; *Orchis mascula* L. (purple orchis), which grows in the Neman and Chernykhovskiy districts; *Gladiolus palustris* Gaudin (cornflag helobius), which is found only in the Pravdinskiy district; and *Taxus baccata* L. (common yew), which grows in the Krasnoznamenskiy district and in

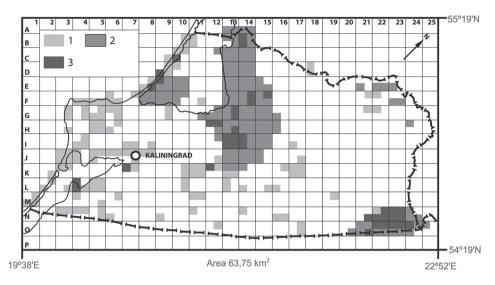


Figure 10. The protected plant and animal species spread in the territory of Kaliningrad region. **I** plants **2** animals **3** plants and animals

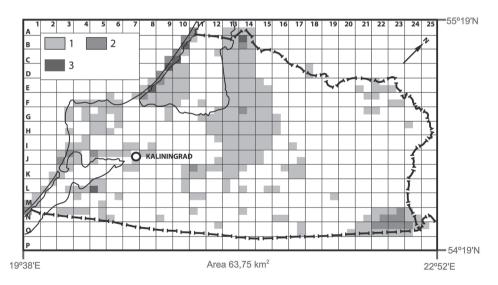


Figure 11. Species saturation (number of species in a square) of the protected plant and animal species in the territory of Kaliningrad region. **I** 1-3 species per square; **2** 4-6 species per square; **3** 7-9 species per square.

the outskirts of Kaliningrad. A large number of the protected species from the family *Orchidaceae* can be observed in the Kaliningrad region (Fig. 13).

Dimensional cartographical analysis of the protected biodiversity elements undertaken using GIS-technologies, databases and computer design reflects the historical and modern distribution of the protected flora and fauna species and shows how

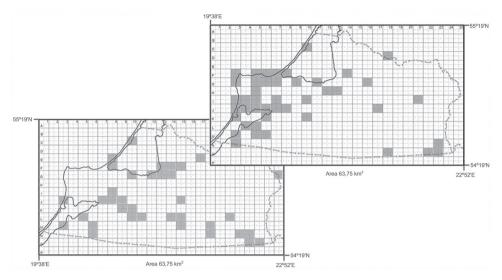


Figure 12. The protected plant species distribution in the territory of Kaliningrad region in the middle of XX century (bottom figure) and early XXI century (top figure).

close they are to extinction in the range and region. This analysis also identifies the areas of high species richness. The key areas in the Kaliningrad region include the Curonian and Baltic Spits, the Sambiyskaya Upland and the coastline of the Sambiyskiy peninsula, Neman river delta, the Vishtynetskaya and Varmiyskaya Uplands, the Pregolskaya and Polessye Lowlands and Sheshupskaya Plain. Currently, less than 15% of the prioritised biodiversity conservation areas including the national park "Curonian Spit" (which is on the List of World Cultural and Natural Heritage UNESCO) are under territorial protection. For the rest, including the unique dune complexes, large forest ranges, watersides and upland moors, state protection measures do not apply. For valuable natural complexes, the Kaliningrad region has developed a range of conservation projects and offers, amongst which the most valuable align with the biogeographical assessment results. These projects and offers can be regarded as previously developed schemes in the specially protected areas (Tsybin 2004), providing different levels of protection at a world, Baltic, border and regional level. The scheme system of the specially protected areas has been created by taking into account the natural structure of the region, adjacent territories of neighbouring states and peculiarities of land use. The system includes all already existing specially protected areas while improving the conservation status of the most valuable ones in terms of landscape and biological diversity (i.e. the Vistula Spit, natural reserves "Gromovskiy" and "Dunnyi"). Additional specially protected areas, in which threatened species and unique ecosystems are located, are also included in the system. Realisation of this system will help to conserve natural complexes which have Balticwide importance and which represent an important natural element in the ecological network of Europe.

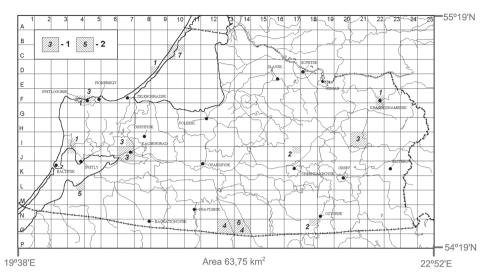


Figure 13. The plant species distribution for which there is a threat of extinction in the range. **A** species that are threatened with extinction in the range (under natural protection, category I) **B** species that are under international protection. The numbers designate the species: **I** *Botrychium simplex* E. Hitchc. **2** *Orchis mascula* L. **3** *Taxus baccata* L., **4** *Gladiolus palustris* Gaudin **5** *Cypripedium calceolus* L. (IUCN Red Book) **6** *Epipogium aphyllum* Sw. (Convention on International Trade in Endangered Species of Wild Fauna and Flora) **7** *Orchis morio* L. (Convention on International Trade in Endangered Species of Wild Fauna and Flora).

Conclusions

Wildlife, biological and landscape diversity conservation is currently considered to be a leading direction of sustainable development. To realise this conservation, strategic documents (concepts) must be developed that define the formation of the regional (national) network of specially protected natural areas, which include, along with all typical, rare and unique landscapes, the ecosystems, separate communities and ecotopes of rare and endangered species from the Red Books.

Biogeographical approaches may serve as a basis for the development of concepts and implementation plans for regional biodiversity conservation. These approaches require researchers to undertake regular monitoring and quantitative accounting of biota, to analyse and assess the conservation value and biogeographical specificity of the territories, to define the priority and efficiency of the species and ecosystems conservation and to plan conservation undertakings.

The assessment of the number of *Anser erythropus* and *Haliaetus albicilla* and their breeding population size in the Subarctic under national and international protection shows the importance of the Putorana Plateau as a key region for reproduction of these species in the Asian part of Russia. This increases the plateau's conservation importance. Relict pine forest outliers in the steppe zone of Central Kazakhstan conserve many rare and endangered plants in favourable ecotopes. For these forests, we recommend a strict reserve status of conservation within the boundaries of Karkaraly

National Park. In highly urbanised regions (e.g. in the Kaliningrad region), where almost all the territories preserved in a natural state should be protected, an efficient and effective conservation principle should be realised. In accordance with that principle, the proposed conservation approach, differentiated by its level and priority, will help to conserve the most valuable natural complexes and objects that merit being included into a common European ecological network.

The three examples shown in this research belong to different geographical districts of the Palearctic region, with various degrees of exploration, anthropogenic transformation of the landscapes and development of protected natural area systems. The conducted research also differs by scale, object and method. However, they demonstrate new opportunities, generalisation, visualisation and cross-spectrum analysis of biologic and geographical information of conservation biogeography for practical conservation aims.

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