

Social media photo content for Sierra Nevada: a dataset to support the assessment of cultural ecosystem services in protected areas

Andrea Ros-Candeira^{1,2}, Ricardo Moreno-Llorca^{1,2}, Domingo Alcaraz-Segura^{1,3},
Francisco Javier Bonet-García⁴, Ana Sofia Vaz^{1,3,5}

1 *Laboratorio de Ecología (iEcolab), Instituto Interuniversitario de Investigación del Sistema Tierra en Andalucía (IISTA-CEAMA), Universidad de Granada, Avda. del Mediterráneo s/n, Granada 18006, Spain*
2 *Grupo de Ecología Terrestre, Departamento de Ecología, Universidad de Granada, Facultad de Ciencias, Campus Fuentenueva s/n, 18071, Granada, Spain*
3 *Departamento de Botánica, Universidad de Granada, Facultad de Ciencias, Campus Fuentenueva s/n, 18071, Granada, Spain*
4 *Departamento de Botánica, Ecología y Fisiología Vegetal, Área de Ecología, Universidad de Córdoba, Edificio Celestino Mutis (C-4), 14014 Córdoba, Spain*
5 *Research Network in Biodiversity and Evolutionary Biology, Research Centre in Biodiversity and Genetic Resources (InBIO-CIBIO), Campus Agrário de Vairão, Rua Padre Armando Quintas, PT4485-661 Vairão, Portugal*

Corresponding author: Andrea Ros-Candeira (a.roscondeira@gmail.com)

Academic editor: Yiannis Matsinos | Received 18 July 2019 | Accepted 10 December 2019 | Published 13 March 2020

<http://zoobank.org/6EFE874A-62B6-4FD0-86A2-CB139B9B1F01>

Citation: Ros-Candeira A, Moreno-Llorca R, Alcaraz-Segura D, Bonet-García FJ, Vaz AS (2020) Social media photo content for Sierra Nevada: a dataset to support the assessment of cultural ecosystem services in protected areas. *Nature Conservation* 38: 1–12. <https://doi.org/10.3897/natureconservation.38.38325>

Abstract

This dataset provides crowd-sourced and georeferenced information useful for the assessment of cultural ecosystem services in the Sierra Nevada Biosphere Reserve (southern Spain). Data were collected within the European project ECOPOTENTIAL focused on Earth observations of ecosystem services. The dataset comprises 778 records expressing the results of the content analysis of social media photos published in Flickr. Our dataset is illustrated in this data paper with density maps for different types of information.

Keywords

Biosphere reserve, cultural ecosystem service, nature-based experience, social media content, social-ecological research

Introduction

The modern human epoch is characterised by dynamic social-ecological changes, with local communities and individuals showing an important role in ecosystem integrity and health (Rands et al. 2010). In this context, nature conservation mechanisms in protected areas have been increasingly re-shaped to accommodate social aspects of ecosystems (Chan et al. 2006). The establishment of a global network of Biosphere Reserves (UNESCO) is an emblematic effort in this regard, being established to promote strategies that reconcile biodiversity conservation with the sustainable use of ecosystem services (Reed 2016).

Ecosystem services are generally known as the contributions that are obtained from nature (MEA 2005). They include raw material from ecosystems, recognised as provisioning services (e.g. timber and food) and the results from ecological functioning (e.g. hazard mitigation and pollination), i.e. regulating ecosystem services (MEA 2005). Ecosystems also offer non-material benefits, known as cultural ecosystem services, for example, through recreational and inspirational activities (Fish et al. 2016). Despite increasing focus on ecosystem services, assessment approaches have been particularly challenging for cultural ecosystem services (Blicharska et al. 2017).

Evaluations of cultural ecosystem services have been struggling with the inability to capture their subjectivity and utilitarian value (Fish et al. 2016). Conventional assessments include, for instance, the use of public polls which are often expensive and show limited spatio-temporal coverage, as well as biodiversity mapping that tends to merely capture the potential supply of cultural services (Blicharska et al. 2017). In the “information age”, the use of “big data” from social media has become a promising approach to monitor nature-based experiences associated with cultural services (Hausmann et al. 2018).

A plethora of social media information has been produced and shared at unprecedented rates, revolutionising traditional methods to address human culture (i.e. culturomics; Ladle et al. 2017), including in the light of conservation problems (see Ladle et al. 2016 for a review). Closely related to culturomics is the content analysis of digital photos posted and shared in social media platforms, such as Flickr (Richards and Friess 2015). These photos contain geographic and temporal information, allowing the mapping of cultural ecosystem services, at high spatial resolutions and for specific time periods in a straightforward and fast way (Vaz et al. 2018).

Despite increasing evaluations of social media information, there is a general deficiency of publicly available databases of photo content analysis. Analysing and mapping the cultural value of ecosystems allow the identification and location of places where nature contributes most to cultural identity and heritage, human health, environmental education and opportunities for nature enjoyment (Soga and Gaston 2016). Under appropriate management strategies, those places can provide great opportunities to promote social support for nature conservation alongside the sustainable use of Biosphere Reserves (Infield 2001).

Our expectation in describing and making available this dataset is to promote the sharing of other similar datasets in order to locate, describe and quantify potential cultural services in protected areas worldwide.

Project details

The dataset was compiled within the context of the H2020 project “ECOPOTENTIAL: improving future ecosystem benefits through earth observations” (<http://www.ecopotential-project.eu>), which has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 641762. ECOPOTENTIAL is focused on internationally recognised Protected Areas, blending Earth Observations, data analysis and modelling of current and future ecosystem conditions and services. ECOPOTENTIAL considers cross-scale geosphere-biosphere interactions at regional to continental scales, addressing long-term and large-scale environmental and ecological challenges.

Spatial coverage

General spatial coverage

The dataset covers a 1,722 km² area corresponding to the UNESCO Biosphere Reserve Sierra Nevada. Sierra Nevada is a mountainous region located in Andalusia (Granada and Almería provinces), in southern Spain. The altitude ranges from 860 m a.s.l. to the summits, where the highest peak reaches 3,479 m a.s.l. The climate is Mediterranean, presenting cold winters and hot summers, with pronounced summer drought (July–August). The annual average temperature decreases in altitude from 12–16°C below 1,500 m to 0°C above 3,000 m a.s.l. and the annual average precipitation is about 600 mm. Annual precipitation ranges from less than 250 mm in the lowest parts of the mountain range to more than 700 mm in the summit areas, where, above 2,000 m altitude, winter precipitation is mainly in the form of snow. Topographically, it is a heterogeneous area, with strong climatic contrasts between the sunny, dry south-facing slopes and the shaded, wetter north-facing slopes.

Sierra Nevada hosts more than 80 endemic plant species (Blanca 2001) and more than 2,300 taxa of vascular flora in total, representing the 33.2% of Spanish flora (Lorite 2016), being amongst the most important biodiversity hotspots in the Mediterranean region (Blanca et al. 1998). Overall, Sierra Nevada comprises 27 habitats types from the habitat directive, as well as 31 fauna species (20 birds, 5 mammals, 4 invertebrates, 2 amphibians and reptiles) and 20 plants species listed in Annex I and II of Habitats and Birds Directives. Besides being included in a biosphere reserve, Sierra Nevada has additional legal protections: Special Protection Area and Site of Community Importance (Natura 2000 network); and National and Natural Park.

Regarding its general socioeconomic characteristics, there were 61 municipalities with 90,048 inhabitants in 2017. The population average age is 48.3 years (ten years greater than the population of large urban areas closer to the national park). The main economic activity is services, mostly related to rural tourism (45% of people employed, 75% of registered businesses). Secondary economic activities are farming and construction sector (25% of people employed in each). Finally, the percentage of people

working in industrial sector stands around 5%. Registered unemployment in relation to total population is lower than the urban areas (9.3% versus 10.1%), but the net income per inhabitant is half that of urban areas (3,597€ versus 7,158€) (SIMA 2019).

Coordinates

36°55'04"N and 37°14'25"N Latitude; 3°36'26"W and 2°35'41"W Longitude

Temporal coverage

1972–2017

Methods

Sampling description

We focused on the screening of photos from a popular social media platform: Flickr (<https://www.flickr.com>). We used the application programming interface (API) in Flickr (<https://www.flickr.com/services/api/>) to collect publicly available information published by the users. To protect the users, the obtained information was kept anonymised through the study. Using this API, we collected geographically referenced social media data indicating a time window (between the start of Flickr in 2004 and 2017) and a bounding box with a pair of coordinates around our test area. After transforming the output JSON/XML files to .XLS format, we geoprocessed the data using a GIS in order to clip only those data points included within our case Biosphere Reserve boundaries and to prepare them for the content analysis of random stratified samples (see below). A total of 20,048 photos were downloaded and their information was stored as an excel file with the following attributes: date, latitude, longitude and picture Uniform Resource Locator (URL).

We stratified our sampling over four strata differing in their nature protection regime (National versus Natural Park) and tourist dynamics (rural versus recreational tourism). Specifically, we randomly selected a set of 210 photos across the limits of the National Park (corresponding mostly to the area with the highest elevation of the Biosphere) and another set of 210 photos within the remaining area, coincident with the Natural Park. A third set of 210 photos was considered across ski resorts, corresponding to areas with the highest movement of visitors in autumn and winter. The remaining photos ($n = 259$) were selected considering the rural areas of the reserve, which were expected to host more visitors during spring and summer. Our final dataset comprised 778 photos from 708 different Flickr users.

Method step description

We checked each individual photo ($n = 889$) to evaluate its suitability for the content analysis: unidentifiable photos (e.g. due to poor quality) or photos capturing non-natural and indoor elements (e.g. inside parking places or private and business properties) were not considered for the content analysis. Additionally, photos which were not available, for instance, since they were eliminated or protected by users' rights, were also not analysed. After applying the former exclusion criteria, we conducted a "directed content analysis" (following, for example, Hsieh and Shannon 2005; Martínez-Pastur et al. 2016; Oteros-Rozas et al. 2018). For this purpose, we manually classified each photo ($n = 778$), based on predefined categories (see Table 1). The photo content analysis was first conducted considering the main feature or topic dominating in each photo indicated in the "Main content" variable, whose categories express key cultural elements from ecosystems contributing to the use of cultural ecosystem services by people, in agreement with the new Common International Classification of Ecosystem Services (Haines-Young and Potschin 2018). These categories were associated with each photo, considering the main photographic focus on: fauna and flora or nature and landscape features, as well as on cultural, religious, rural, sports, gastronomy and recreation elements. In cases in which more than one feature or element could be recognised in the photo, we used more than one category to classify the photo. The order of the key elements which define the topic of the photo was based on the album/roll of the user. Specifically, the first topic considered was the element that was identified as the most frequently photographed by the user. The remaining topic was indicated as a secondary category.

In order to provide more detailed information about the photo's content, we further classified each photo considering: (1) The main nature and human features represented in the photo (e.g. lake, natural forest, mountain peak etc.). Again, more than one category per variable could be attributed in cases in which an individual photo showed the dominance of different nature and human features. (2) The type of prevailing sports activity (e.g. hiking, horse riding etc.), when one of the main topics of the photo was "Sports". (3) The represented faunal groups (e.g. ungulate, insect etc.), in those cases in which the main content of the photo was focused on fauna (e.g. categories "Fauna/Flora" and "Birdwatching"). Therefore, these last two variables (Sports activities and Faunal groups) depended on the classification attributed to the first variable "Main content".

Quality control description

The classification of photos into the above-mentioned categories was evaluated by two independent users. Before analysing the content of the whole dataset, a test set of 100 randomly chosen records was first considered and classified. After analysing this test set, the classification procedure was refined for a second round. For both classification

Table 1. Variables and categories considered for the classification of social media photo content, including their description and classification criteria.

Variable	Category	Description	
General information of the photo			
Coordinates	Latitude of the photo	Coordinate reference system: EPSG 4326-WGS 84	
	Longitude of the photo	Coordinate reference system: EPSG 4326-WGS 84	
Date	The date when the photo was taken	Format: day/month/year	
Main content of the photo			
Main content	Birdwatching	A bird or group of birds are the main content of photo	
	Fauna/Flora	Fauna and/or flora are the main content of the photo	
	Nature & Landscape/Seascape	The photo is mainly focused on nature or landscapes/seascapes in general	
	Cultural	Cultural elements are the main content, including harvest of pine nuts or traditional buildings	
	Religious	Religious elements are the main content, including processions, pilgrimage, churches, carriages or carts as part of pilgrimage	
	Rural	Elements associated with rural tourism are the main content, including lodges, rural activities, villages or other related infrastructures	
	Sports	Sports elements are the main content, such as those associated with biking, hiking or running	
	Gastronomy	Gastronomy is the main content of photo, including dining at restaurants or traditional products	
	Recreation	The main content of the photo is on recreational areas or similar public infrastructures, including barbecues or playgrounds	
	Other type	The photo is dominated by other elements that are not related with the former categories	
Detailed content of the photo			
Sports activities	Alpine skiing	Alpine skiing is the topic of photo	
	Cross country skiing	Cross country skiing the topic of photo	
	Climbing	Climbing is the topic of photo	
	Road cycling	Road cycling is the topic of photo	
	Mountain cycling	Mountain cycling is the topic of photo	
	Downhill cycling	Downhill cycling the topic of photo	
	Running	Running is the topic of photo	
	Hiking	Hiking is the topic of photo	
	Paragliding	Paragliding is the topic of photo	
	Horse riding	Horse riding is the topic of photo	
	Canoeing	Canoeing is the topic of photo	
	Other type	Other type of sports activity is the main topic of photo	
	Not applicable	The photo is not focused on any sports activity	
	Nature and human features	High mountain	High mountain is the topic of photo
		Mid-mountain	Mid-mountain is the topic of photo
Mountain peak		Mountain peak is the topic of photo	
Horizon		Horizon is the topic of photo	
Natural forest		Natural forest is the topic of photo	
Anthropic forest		Anthropic forest is the topic of photo	
Shrub		Shrub is the topic of photo	
Grassland		Grassland is the topic of photo	
Lake, pond		Lake is the topic of photo	
River		River is the topic of photo	
Sky		Sky is the topic of photo	
Urban/built environment		Urban/built environment is the topic of photo	
Non-urban/built environment, infrastructures		Non-urban/built environment, infrastructure, is the topic of photo (e.g. rural infrastructure, refuges and recreation infrastructure)	
Humans, selfies		People, including selfies, are the topic of photo	
Other type		Other type of feature is the main topic of photo	
Faunal groups	Not applicable	These categories are not applicable	
	Mammal	Mammal is the topic of photo	
	Ungulate	Wild ungulate is the topic of photo (e.g. Iberian ibex)	
	Waterbird	Waterbird is the topic of photo	
	Wader	Wader is the topic of photo	
	Raptor	Raptor is the topic of photo	
	Passerine	Passerine is the topic of photo	
	Reptile	Reptile is the topic of photo	
	Fish	Fish is the topic of photo	
	Insect	Insect is the topic of photo	
	Other type	Other type of fauna is the main topic of photo	
	Not applicable	The photo is not focused on any type of fauna	

rounds, the consistency between the two users was analysed through general agreement and kappa statistics. The statistics indicated an increase in classification consistency from the first to the second test set. Specifically, a good consistency between users was found, with agreement levels ranging between 65% (sports activities) and 88% (faunal groups) and kappa values between 0.58 (nature and human features) and 0.60 (sports activities).

Dataset overview

Figure 1 shows the spatial location of the photos considered in the dataset. The eastern part of Sierra Nevada is by far represented by a higher volume of photos, as indicated by warmer colours. This pattern seems to match the location of ski resorts and rural villages (the “Alpujarras”), which are characterised by a high touristic demand in the Biosphere Reserve. Several other photos are widely represented in westernmost regions of Sierra Nevada. In this case, the location of pictures seems to coincide with the prevalence of walking trails in the Biosphere Reserve.

This spatial pattern is also evident for the different categories assigned to the dataset (Figure 2). An exception is rather observed for the faunal groups variable, in which a relative density of photos is also represented in western regions of the Biosphere Reserve.

We are confident that our dataset (and derived maps) add detail on the potential location of different cultural contributions to people. Specifically, the spatial projections derived from this study can provide useful information for management decisions, for example, on prioritising land planning efforts and resources (Krishnaswamy et al. 2009). They can also be used to maximise synergies between biodiversity conservation and cultural values (Turnhout et al. 2013), identify conflicting areas (or disservices) emerging from recreational activities (such as skiing) tourism and strictly protected zones (with protected habitats/species; Van Cuong et al. 2017), support the monitoring of the natural and cultural capital through remote observations (i.e. “Digital conservation”; Arts et al. 2015) and assist on data collection and dissemination for scientific research and evidence-based conservation (Sherren et al. 2017).

Despite the usefulness of our dataset, some considerations must be recognised when using this and other similar datasets, in the cultural services’ arena. For instance, the spatial reference precision of social media photographs can bias the geolocation of collected data (Figueroa-Alfaro and Tang 2017). Still, this issue was likely insignificant in our study, due to the illustration of photographs through a Kernel function (i.e. as a heat-map). We are also aware that distinct social media platforms (such as Instagram, Panoramio) have different audiences, users and temporal/spatial characteristics, which affect the way they can be used (Van Zanten et al. 2016). In our study, we adopted the Flickr platform, due to its more nature-orientated users, ease of data analyses and broad spatial and temporal coverage. Nevertheless, we encourage the inclusion of different types and sources of social media information that can be

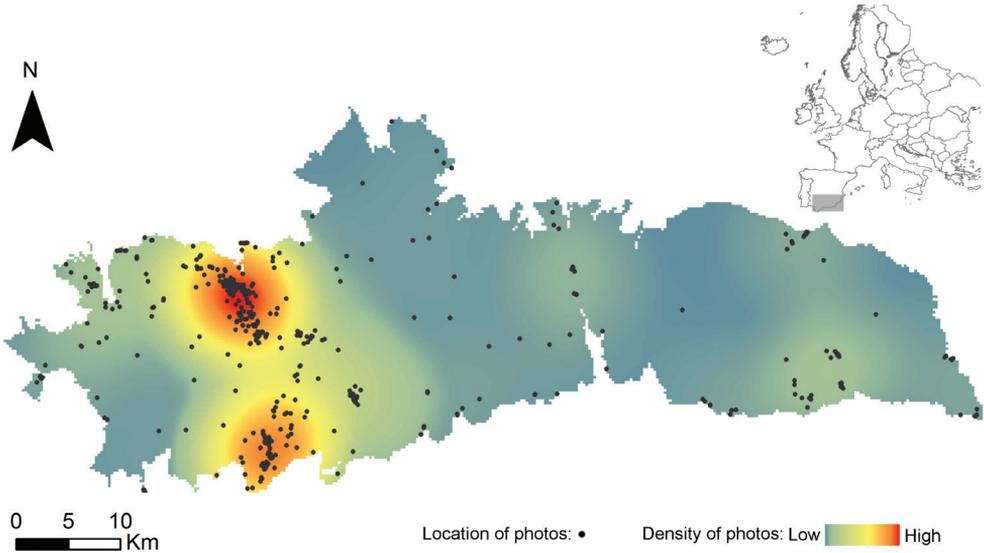


Figure 1. Location of Flickr photos considered in the dataset (n = 778). The location of each photo is represented by a dark circle. For visualisation purposes, the map also shows heatspots of photos (kernel density), highlighting the areas with the highest and lowest photo densities in Sierra Nevada Biosphere Reserve.

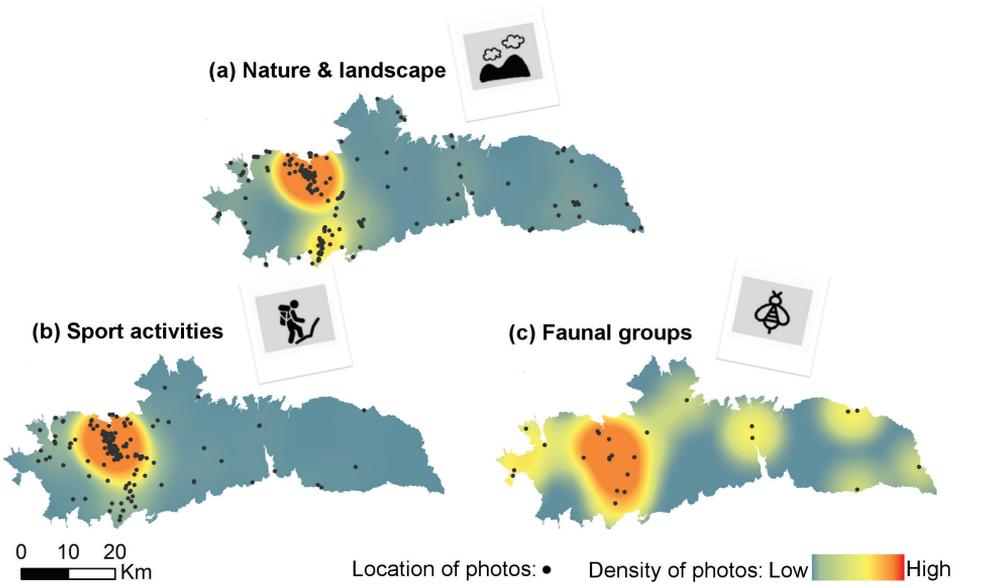


Figure 2. Illustration of Flickr photos showing: (a) nature & landscape as the main content, (b) sports activities and (c) faunal groups. The location of each photo is represented by a dark circle. The density of photos (kernel density) is also shown.

complementary to the dataset we propose (Oteros-Rozas et al. 2018). Furthermore, social media users make decisions on which photos they share in social networks, without necessarily meaning that the photos, shared online, express the most preferred and valued elements of the landscape (Malik et al. 2016). Therefore, any effort to further understand the cultural preferences of social media users, should examine the motivations underlying their choices and perceptions in relation to other (social) determinants (e.g. socio-demography, economy), for instance, through traditional state-preference methods or even through social media natural language processing of picture comments. However, attention should be warranted when interpreting and communicating social patterns into more detail (Van Berkel et al. 2018). This was the main reason why our dataset does not compile, nor analyse, data which were protected by users' privacy.

Dataset description

Object name: Georeferenced features of cultural ecosystem services in Sierra Nevada: a dataset based on social media photo content analysis.

Character encoding: UTF-8

Format name: CSV

Format version: v 2.0

Data published through: <https://doi.org/10.6084/m9.figshare.8943509.v2>

Publication date of data: 17-12-2019

Language: English

Licences of use: This dataset is licensed under the Creative Commons Attribution 4.0 International Licence (CC BY 4.0) <https://creativecommons.org/licenses/by/4.0/>

Acknowledgements

This work has been carried out within the H2020 project “ECOPOTENTIAL: Improving future ecosystem benefits through earth observations” (<http://www.ecopotential-project.eu/>), which has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 641762. This work was partially conducted under the agreement "Convenio de Colaboración entre la Consejería de Medio Ambiente y Ordenación del Territorio y la Universidad de Granada para el desarrollo de actividades vinculadas al Observatorio de Cambio Global de Sierra Nevada, en el marco de la Red de Observatorios de Cambio Global de Andalucía" and the eLTER H2020 project “European Long-Term Ecosystem and Socio-Ecological Research Infrastructure” funded by the European Union’s Horizon 2020 programme under grant agreement No 654359. Icons shown in Figures 1 and 2 were provided by Freepik from www.flaticon.com and adapted to our original content.

References

- Arts K, van der Wal R, Adams WM (2015) Digital technology and the conservation of nature. *Ambio* 44(S4): 661–673. <https://doi.org/10.1007/s13280-015-0705-1>
- Blanca G, Cueto M, Martínez-Lirola MJ, Molero-Mesa J (1998) Threatened vascular flora of sierra nevada (southern spain). *Biological Conservation* 85(3): 269–285. [https://doi.org/10.1016/S0006-3207\(97\)00169-9](https://doi.org/10.1016/S0006-3207(97)00169-9)
- Blanca G [Ed.] (2001) *Flora amenazada y endémica de Sierra Nevada*. 400 pp.
- Blicharska M, Smithers RJ, Hedblom M, Hedenäs H, Mikusiński G, Pedersen E, Sandström P, Svensson J (2017) Shades of grey challenge practical application of the cultural ecosystem services concept. *Ecosystem Services* 23: 55–70. <https://doi.org/10.1016/j.ecoser.2016.11.014>
- Chan KMA, Shaw MR, Cameron DR, Underwood EC, Daily GC (2006) Conservation planning for ecosystem services. *PLoS Biology* 4(11): 2138–2152. <https://doi.org/10.1371/journal.pbio.0040379>
- Figueroa-Alfaro RW, Tang Z (2017) Evaluating the aesthetic value of cultural ecosystem services by mapping geo-tagged photographs from social media data on Panoramio and Flickr. *Journal of Environmental Planning and Management* 60(2): 266–281. <https://doi.org/10.1080/09640568.2016.1151772>
- Fish R, Church A, Winter M (2016) Conceptualising cultural ecosystem services: A novel framework for research and critical engagement. *Ecosystem Services* 21: 208–217. <https://doi.org/10.1016/j.ecoser.2016.09.002>
- Haines-Young R, Potschin MB (2018) Common International Classification of Ecosystem Services (CICES) V5.1 and Guidance on the Application of the Revised Structure. European Environment Agency: 53. <https://doi.org/10.3897/oneeco.3.e27108>
- Hausmann A, Toivonen T, Slotow R, Tenkanen H, Moilanen A, Heikinheimo V, Di Minin E (2018) Social Media Data Can Be Used to Understand Tourists' Preferences for Nature-Based Experiences in Protected Areas. *Conservation Letters* 11(1): 1–10. <https://doi.org/10.1111/conl.12343>
- Hsieh H-F, Shannon SE (2005) Three approaches to qualitative content analysis. *Qualitative Health Research* 15(9): 1277–1288. <https://doi.org/10.1177/1049732305276687>
- Infield M (2001) Cultural values: A forgotten strategy for building community support for protected areas in Africa. *Conservation Biology* 15(3): 800–802. <https://doi.org/10.1046/j.1523-1739.2001.015003800.x>
- Krishnaswamy J, Bawa KS, Ganeshiah KN, Kiran MC (2009) Quantifying and mapping biodiversity and ecosystem services: Utility of a multi-season NDVI based Mahalanobis distance surrogate. *Remote Sensing of Environment* 113(4): 857–867. <https://doi.org/10.1016/j.rse.2008.12.011>
- Ladle RJ, Correia RA, Do Y, Joo GJ, Malhado ACM, Proulx R, Roberge JM, Jepson P (2016) Conservation culturomics. *Frontiers in Ecology and the Environment* 14(5): 269–275. <https://doi.org/10.1002/fee.1260>

- Ladle RJ, Jepson P, Correia RA, Malhado ACM (2017) The power and the promise of culturomics. *Frontiers in Ecology and the Environment* 15(6): 290–291. <https://doi.org/10.1002/fee.1506>
- Lorite J (2016) An updated checklist of the vascular flora of Sierra Nevada (SE Spain). *Phytotaxa* 261(1): 1–57. <https://doi.org/10.11646/phytotaxa.261.1.1>
- Malik A, Dhir A, Nieminen M (2016) Uses and Gratifications of digital photo sharing on Facebook. *Telematics and Informatics* 33(1): 129–138. <https://doi.org/10.1016/j.tele.2015.06.009>
- Martínez-Pastur G, Peri PL, Lencinas MV, García-Llorente M, Martín-López B (2016) Spatial patterns of cultural ecosystem services provision in Southern Patagonia. *Landscape Ecology* 31(2): 383–399. <https://doi.org/10.1007/s10980-015-0254-9>
- MEA [Millenium Ecosystem Assessment] (2005) *Ecosystems and Human Well Being*. Island Press, Washington, DC.
- Oteros-Rozas E, Martín-López B, Fagerholm N, Bieling C, Plieninger T (2018) Using social media photos to explore the relation between cultural ecosystem services and landscape features across five European sites. *Ecological Indicators* 94: 74–86. <https://doi.org/10.1016/j.ecolind.2017.02.009>
- Rands MRW, Adams WM, Bennun L, Butchart SHM, Clements A, Coomes D, Entwistle A, Hodge I, Kapos V, Scharlemann JPW, Sutherland WJ, Vira B (2010) Biodiversity Conservation: Challenges Beyond 2010. *Science* 329(5997): 1298–1303. <https://doi.org/10.1126/science.1189138>
- Reed MG (2016) Conservation (In)Action: Renewing the Relevance of UNESCO Biosphere Reserves. *Conservation Letters* 9(6): 448–456. <https://doi.org/10.1111/conl.12275>
- Richards DR, Friess DA (2015) A rapid indicator of cultural ecosystem service usage at a fine spatial scale: Content analysis of social media photographs. *Ecological Indicators* 53: 187–195. <https://doi.org/10.1016/j.ecolind.2015.01.034>
- Sherren K, Smit M, Holmlund M, Parkins JR, Chen Y (2017) Conservation culturomics should include images and a wider range of scholars. *Frontiers in Ecology and the Environment* 15(6): 289–290. <https://doi.org/10.1002/fee.1507>
- SIMA (2019) Multiterritorial Information System of Andalusia. <https://www.juntadeandalucia.es/institutodeestadisticaycartografia/sima>
- Soga M, Gaston KJ (2016) Extinction of experience: The loss of human-nature interactions. *Frontiers in Ecology and the Environment* 14(2): 94–101. <https://doi.org/10.1002/fee.1225>
- Turnhout E, Waterton C, Neves K, Buizer M (2013) Rethinking biodiversity: From goods and services to “living with”. *Conservation Letters* 6(3): 154–161. <https://doi.org/10.1111/j.1755-263X.2012.00307.x>
- Van Berkel DB, Tabrizian P, Dorning MA, Smart L, Newcomb D, Mehaffey M, Neale A, Meentemeyer RK (2018) Quantifying the visual-sensory landscape qualities that contribute to cultural ecosystem services using social media and LiDAR. *Ecosystem Services* 31: 326–335. <https://doi.org/10.1016/j.ecoser.2018.03.022>

- Van Cuong C, Dart P, Hockings M (2017) Biosphere reserves: Attributes for success. *Journal of Environmental Management* 188: 9–17. <https://doi.org/10.1016/j.jenvman.2016.11.069>
- van Zanten BT, Van Berkel DB, Meentemeyer RK, Smith JW, Tieskens KF, Verburg PH (2016) Continental-scale quantification of landscape values using social media data. *Proceedings of the National Academy of Sciences of the United States of America* 113(46): 12974–12979. <https://doi.org/10.1073/pnas.1614158113>
- Vaz AS, Castro-Díez P, Godoy O, Alonso Á, Vilà M, Saldaña A, Marchante H, Bayón Á, Silva JS, Vicente JR, Honrado JP (2018) An indicator-based approach to analyse the effects of non-native tree species on multiple cultural ecosystem services. *Ecological Indicators* 85: 48–56. <https://doi.org/10.1016/j.ecolind.2017.10.009>

Searching for snakes: ball python hunting in southern Togo, West Africa

Neil D’Cruze^{1,2}, Lauren A. Harrington², Délagnon Assou^{3,4}, Delphine Ronfot⁵,
David W. Macdonald², Gabriel H. Segniagbeto^{3,4}, Mark Auliya^{5,6}

1 World Animal Protection, 222 Grays Inn Rd., London WC1X 8HB, UK **2** Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Abingdon OX13 5QL, UK **3** Laboratory of Ecology and Ecotoxicology, Department of Zoology and Animal Biology, Faculty of Sciences, University of Lomé, BP 1515, Lomé, Togo **4** Togolese Society for Nature Conservation (AGBO-ZEGUE NGO). 06 BP: 6057, Lomé, Togo **5** Zoological Research Museum Alexander Koenig, Department Herpetology, Adenauerallee 160, 53113, Bonn, Germany **6** Department of Conservation Biology, Helmholtz Centre for Environmental Research GmbH – UFZ, 04318, Leipzig, Germany

Corresponding author: Neil D’Cruze (neil.dcruze@zoo.ox.ac.uk)

Academic editor: E. Di Minin | Received 31 October 2019 | Accepted 28 February 2020 | Published 13 March 2020

<http://zoobank.org/E239A308-5E74-4E8D-BF39-9690C1707A56>

Citation: D’Cruze N, Harrington LA, Assou D, Ronfot D, Macdonald DW, Hoinsoudé Segniagbeto G, Auliya M (2020) Searching for snakes: ball python hunting in southern Togo, West Africa. Nature Conservation 38: 13–36. <https://doi.org/10.3897/natureconservation.38.47864>

Abstract

The ball python (*Python regius*) is the single most exported live CITES-listed species from Africa, with a large proportion of snakes being sourced from Togo, West Africa, officially via a system reported nationally as “ranching”. This study represents the first in-depth review of ball python hunting being carried out by rural communities in Togo for nearly 15 years. Our approach, focused at the bottom of the trade chain, permitted extensive detailed data to be collected from hunters, and provides a unique insight into the practices, drivers and impacts associated with this type of large-scale commercial wildlife trade activity. We show that ball python hunting remains an economically valuable endeavour for these rural hunters. However, it also highlights a number of potential legal, conservation and animal welfare issues associated with the current hunting practices being carried out in Togo (and neighbouring range States) to supply the snake farms and ultimately the international exotic pet trade. Our findings suggest that the methods applied on the ground do not accurately reflect those being reported to national authorities and international regulatory mechanisms such as CITES. This irregular, if not illegal, trade may also be unsustainable, as suggested by hunters reporting that there are fewer ball pythons in the wild than there were five years

previously. We recommend that additional scientific investigation (focusing on the size and status of the wild population), better management, and enforcement of regulations, are required to ensure that ball python populations are managed in a sustainable, legal and traceable way.

Keywords

CITES, conservation, exotic pet, ranching, wildlife trade

Introduction

Snakes are hugely popular as exotic pets and consequently are traded alive globally in large numbers (e.g., Hoover 1998; Auliya 2003; Bush et al. 2014). Many are sourced from Africa and other tropical regions of the world (Bush et al. 2014; Jensen et al. 2018), where wildlife harvest and trade can play an important role in local livelihoods (e.g., Roe 2008). Increasingly, snakes, and other reptiles, are captive-bred for the pet trade both in source and destination countries (Brant 2001; Bush et al. 2014; Herrel and Meijden 2014). In some source countries, reptiles may also be produced via a process of “ranching”, one definition being the “*rearing in a controlled environment of animals taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood*” (Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES] definition; Lyons et al. 2017).

In contrast to captive breeding or “wildlife farming”, a topic that has received considerable attention in the scientific literature (see Tensen 2016 and references therein), ranching (as defined by CITES) is dependent on collection (hunting) of wild individuals. However, unlike wildlife farming, there have been relatively fewer studies, of which we are aware, of large-scale management operations that export live wild animals and their derivatives using the CITES source code “R”, and their links with (and impacts on) wild populations, with the exception of those addressing crocodylian ranching (e.g., Jenkins et al. 2004). A species of particular interest in this regard is the ball python (*Python regius*), which is the single most exported live CITES-listed species from Africa (CITES Trade Database, <https://trade.cites.org>), the vast majority of which are exported as “ranching” specimens (source code R) (CITES Trade Database, <https://trade.cites.org>; see also Robinson et al. 2015).

In 2010, the ball python was evaluated as Least Concern on the IUCN Red List of Threatened Species (<https://www.iucnredlist.org>), but with high offtake levels and exploitation for the international pet trade (e.g., D'Cruze et al. 2020) cited as a main potential threat (Auliya and Schmitz 2010). According to the CITES trade database (<https://trade.cites.org>), over three million individual ball pythons have been exported from West Africa since the first recorded exports in 1982, with total annual exports from Range States averaging c.100,000 since 2007 (also see Robinson et al. 2015). More than 98% of ball python exports from Range States originate from Togo, Benin and Ghana, destined predominantly for consumers in the United States of America, Europe and Asia (CITES Trade Database, <https://trade.cites.org>; Robinson et al. 2015; Harrington et al., in prep.).

The export of “ranchéd” specimens of ball pythons from West Africa can be traced back to a number of registered reptile “farms” operating in Benin, Ghana and Togo (de Buffrénil 1995). In Togo, most of these farms are based in, or less than, 10 km from the capital of Lomé (Ineich 2006) although some of the wildlife trade facilities have branches or “depots of animals” in villages elsewhere in the country. This type of “reptile production” was first introduced in West Africa in the 1960s, and became established in Togo in the early 1990s (de Buffrénil 1995). As of 2006, there were seven farms known to be operating in Togo (Ineich 2006; UNEP-WCMC 2014), with an agreed national annual export quota of 62,500 “ranchéd” specimens and 1,500 “wild” specimens in place since 2007 (UNEP 2019).

The most recent examination of the different python production systems in Togo was carried out by Ineich (2006), following concerns raised by the EU Scientific Review Group in 2005 in relation to an exceeded quota of wild-sourced specimens from Togo, between 1999–2003 (UNEP-WCMC 2005). Ineich (2006) described the UNELAT (Union Nationale des Eleveurs et Exportateurs d’Animaux du Togo – National Union of Animal Breeders and Exporters of Togo) exporting association which was put in place in 2000, in part, to help provide liaison and circulation of local CITES export-authority information. In Togo, all farms, of which four were affiliated with UNELAT, relied on the annual involvement of numerous villagers who hunted snakes and delivered them to these farms prior to export (Ineich 2006).

Ball python “ranching” in Togo (as outlined in UNEP-WCMC 2014) depends on the collection of gravid females and egg clutches, that are found by following tracks in farmland or forest, breaking open hollow palm trunks, and / or digging up abandoned rodent burrows (Harris 2002). According to Ineich (2006), exporters also trained Togolese “producer” villagers and provided them with the necessary equipment for temporarily keeping gravid females and hatching eggs, before selling the resulting juveniles to the farms. A key stage of this ranching system involved releasing the females after they had laid their eggs and also a proportion of the juvenile ball pythons produced annually (stated to be 20% at that time, Ineich 2006; UNEP-WCMC 2014). However, Ineich (2006) also reported that hunters collected non-gravid and male ball python specimens, together with non-CITES listed reptile species for direct export via the farms (Ineich 2006).

Ineich (2006) concluded that the practice of ball python ranching in Togo was being done in relatively healthy conditions. He suggested that (1) the technical knowledge of the exporters had improved between 2004 and 2006, (2) capabilities of the CITES authorities had likewise improved, and (3) the exporters / authorities exchanges were “excellent” and therefore constituted a solid basis for the future with exporters showing willingness to *perfect the management and monitoring of the ball python populations that they exploit* (Ineich 2006). However, Ineich’s (2006) review, which was broadly supportive of the different python production systems in Togo, was carried out over a decade ago. In the fast-moving arena of wildlife trade, subject to increasing consumer demand and often dependent on wild populations of unknown size and status, we considered it timely to provide an update on this situation in Togo, focusing specifically on the practices, and role of, local hunters.

With that aim, we present here data on the hunting activities of 58 rural hunters involved in the harvest of ball pythons in southern Togo. The specific objectives of our study were:

- (1) To describe how ball pythons are harvested and how this activity relates to trade regulations.
- (2) To gain insights into the potential impacts this activity might have on ball python conservation.
- (3) To gain insights into the extent to which this activity supports local livelihoods.

Methods

Study area

The Togolese Republic is a relatively small West African country located between Burkina Faso (in the North), Ghana (in the West), Benin (in the East) and the Atlantic Ocean (in the South). It covers a total land area of 56,600 km² (Dansi et al. 2013) with a population estimated at about eight million (World Bank 2019). The country is partitioned from North to South into five administrative regions (Savannah region, Kara region, the Central region, the Hills region and the Maritime region) inhabited by 21 principal ethnic groups (Adja, Akposso, Akébou, Ana, Anii, Atchè, Bassar, Ewé, Gam-Gam, Gourma, Ifè, Kabyè, Konkomba, Kotokoli, Lamba, Moba, Para, Tchokossi, Tém, Temmari and Yaka) (Dansi et al. 2013). However, ethnic groups predominantly in neighbouring countries, such as the Fon from Benin, are also present in adjacent parts of Togo (Heath 2010).

Togo has two climatic regions, the Southern and the Central regions characterised by a subequatorial climate with two rainy seasons and two dry seasons and the Northern region with Sudanian climate type, characterised by only two seasons (that is, a rainy season and a dry season) (Afidegnon 1999). As a consequence of its location, the Togolese landscape consists, from south to north, of a succession of ecosystems ranging from coastal grasslands to equatorial and wet tropical forests and ending in Sudan savannahs in the North (Segniabeto et al. 2011). These diverse ecosystems harbour a great number of animal species, as indicated by the presence of approximately 178 extant species of mammal (Amori et al. 2016), 43 species of lizard (Segniabeto et al. 2015) and 91 species of snake (Segniabeto et al. 2011).

Data collection

We used semi-structured interviews to gather information focused on ball python capturing and egg collecting practices and included questions related specifically to the numbers captured and released, where they were captured, income generated and

hunters' perceptions of their status in the wild (Suppl. material 1). We interviewed hunters that self-identified as hunting ball pythons, and that were willing to participate in the study, through a process of chain referral (Newing 2011), whereby participants recommended other potential participants or persuaded others to take part. This snowball sampling approach (Babbie 2004) is useful when researchers are interested in the opinions of a particular sector of the population (Potgieter et al. 2017) and, in this case, ensured that participants who could provide information pertinent to the study were selected as representatives of the ball python hunting community. Our primary goal was not to extrapolate to the wider community but to thoroughly understand the activities of the hunters we interviewed on the basis of a comprehensively built questionnaire (Suppl. material 1).

Interviews were conducted by four local field staff asking a set of predetermined questions that included open-ended, closed and multiple-choice questions (Suppl. material 1). Participants were initially asked some non-ball python related questions (including what domestic animals they kept and what type of meat they preferred to eat) to ensure that they were as comfortable and as relaxed as they could be before being asked more detailed questions about hunting ball pythons (Newing 2011). Between 27.08.18 and 24.08.19 interviews were conducted in 20 villages in Ewe and French and later translated into English (Fig. 1).

In accordance with the British Sociological Association Statement of Ethical Practice (BSA 2017), informed consent was obtained verbally from every survey participant prior to the interview, the objectives of the study were explained to participants and they were made aware of their rights to voluntarily participate or to decline, no identifying participant or household data were collected, and the database collated was entirely anonymous.

Data analysis

All statistical analyses were carried out in R (version 3.3.3; R Core Team 2017). All interviews were included in the analysis even if they contained missing data. Spearman's rank correlation coefficient was used to test for correlations among variables, and non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) to compare among groups. Monetary values were reported in West African CFA Francs (CFA) and converted to US dollars (USD) using 1 CFA = 0.0017 USD (conversion rate as of 26.04.19, xe.com). We used descriptive statistics, and frequency histograms to describe, and to illustrate, patterns and trends in the data. Estimated income from the ball python trade per hunter (I) was estimated as follows:

$$I = \frac{[(\text{number of snakes}^* \times \text{price per snake}) + (\text{number of eggs}^* \times \text{price per egg})]}{\text{size of hunting party}}$$

* This is the total number of snakes captured and purchased/total number of eggs collected and laid.

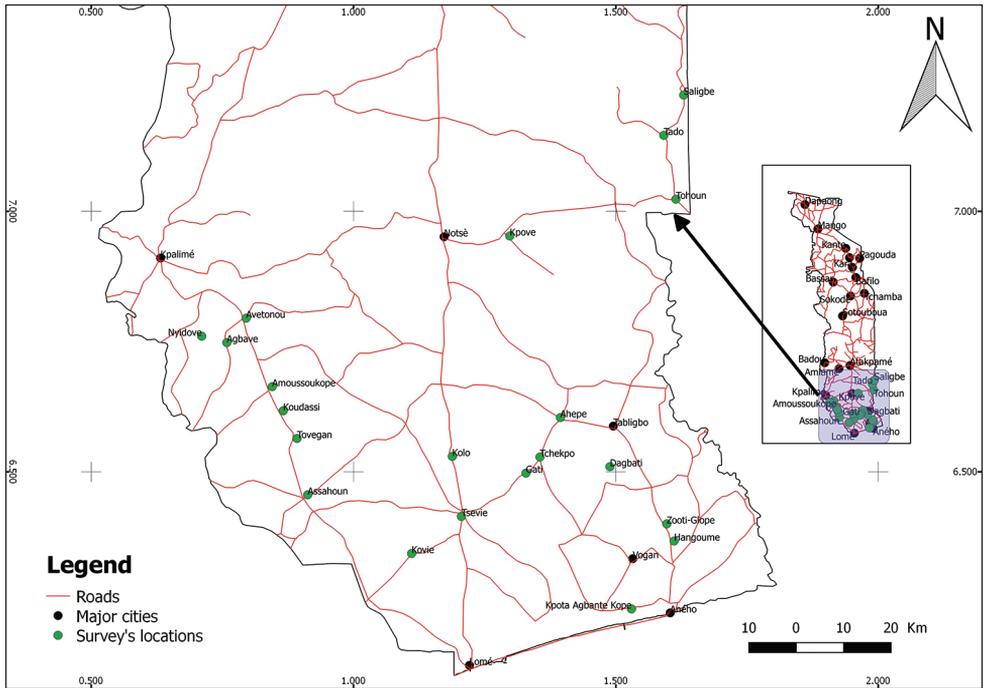


Figure 1. Location of the survey site districts in Togo, West Africa.

Results

Interviews lasted approximately one hour (between 37 minutes and 2 hours 2 minutes). All but three, of a total of 60 interviewees, hunted live ball pythons and collected their eggs (one individual did not hunt them personally but bought live snakes and eggs from other hunters). Hereafter we refer to 60 interviewees as follows; 57 hunters or 58 hunter / traders (noting that some hunters also “trade” by purchasing additional snakes and eggs from other hunters; we discuss this further below).

Hunter demographics

All but one of the interviewees (a 45-year-old widowed female) were males, 76% ($n = 45$) of which were in their 30s or 40s (age range = 16–61, mean = 39), and 95% ($n = 56$) of which were married. Most (77%; $n = 46$) owned between one and three houses (maximum = 10; two did not own a house), with between one and eight people in their household (mean = 6.9, maximum = 25), including between two and six children (mean = 4.2, maximum = 14; three did not have children).

Most (73%; $n = 44$) interviewees were Watchi / Ewe, 17% ($n = 10$) were Adja, others included Moba ($n = 3$), Fon, Kabye, and Lamba ($n = 1$ respectively). In terms of their religion, most (67%; $n = 40$) were Christian, although 32% ($n = 19$) were

animist, and one was Muslim. Education levels of interviewees were variable; although 38% ($n = 23$) were educated to secondary level, almost half (48%; $n = 29$) were educated only to primary level, and 13% ($n = 8$) reported that they had no formal education.

All but one interviewee (a “tailor/python reseller”) described themselves as hunters, farmers or both (over half, 62%; $n = 37$, said that they were hunters and farmers). Reported primary source of income was hunting (for 43% interviewees; $n = 26$) or farming (for 30% interviewees; $n = 18$), other primary sources of income given were “frog hunting” (8%; $n = 5$), “driving” (5%; $n = 3$), “retail” (3%; $n = 2$), “breeding” (3%; $n = 2$), “fishing” (2%; $n = 1$) and “ball python reselling” (2%; $n = 1$) (two interviewees stated that they were “studying”). Reported annual wages were variable: 10 interviewees reportedly earned > one million West African Francs (CFA) per year (equivalent to 1,700 USD; maximum 12 million or just over 20,000 USD) but most earned between approximately 100,000 and 600,000 CFA (median = 400,000 CFA per year or 679 USD; six interviewees earned < 100,000 CFA per year, minimum 40,000 CFA or 70 USD).

Ball python hunting

Ball pythons and their eggs were reportedly found by digging abandoned rodent burrows, breaking termite mounds, or by turning over dead oil palm trunks and piles of grass and leaves, and were most likely to be found in forest habitats (reported by $n = 49$ of 58 hunter/traders, 84.5%), farmland (70.1%; $n = 41$) or oil palm plantations (31.0%; $n = 18$). Other habitats where pythons could reportedly be found included fallow land ($n = 11$), rice fields owned by the interviewees ($n = 5$), teak plantations ($n = 2$), and Casava plantations ($n = 1$); note that hunters gave more than one answer. Pythons were found predominantly between January and March, with eggs collected most commonly in February and March (see Fig. 2). In total, 26% ($n = 15$) of hunters described locating ball pythons as being dependent on the presence of indirect signs such as tracks (some suggested that tracks could be seen after vegetation fires); similarly, 23% ($n = 13$) suggested that seeing a basking female ball python near a burrow (or “hole”), or signs that a snake had been basking there previously, indicated the presence of eggs inside the burrow.

Some hunters suggested that they hunted ball pythons as early as November, with peak hunting activity taking place in February and March, up until June (although one hunter said that he hunted ball pythons in August and September) (Fig. 2). Eggs were collected between January and May and as late as June (Fig. 2). All hunters reported hunting live snakes but tended to target either all snakes (male and female, adults and juveniles, 51%; $n = 29$) or females and juveniles (35%; $n = 20$); only seven hunters (12%) said that they hunted only females; one (2%) said that both males and females were hunted [i.e. over half of hunters (53%; $n = 30$) said that they hunted males, as well as females and juveniles]. All hunters also reported that they collected eggs. In addition to describing how they found the eggs, five hunters (9%) said that to obtain eggs they caught gravid females, and five (9%) explicitly referred to capturing and keeping

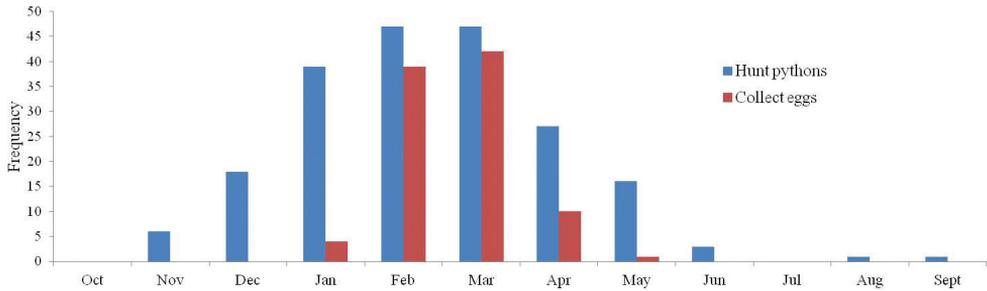


Figure 2. Reported peak months for harvesting ball pythons and their eggs; frequencies are the number of times the month was cited. Note that most hunters gave months as a range rather than a single optimal month so values sum up to more than the number of hunters ($n = 57$).

gravid females until they laid their eggs; two hunters said that they *collected the eggs and left the females*.

Hunters reported hunting parties averaging 5 hunters (range 1–10), that took part in between two and 150 hunting trips in the last 12 months (excluding one hunter who said he hunted on 300 separate days during this period) (mean = 58 hunting trips, median = 50; see Fig. 3a). They also reported capturing between 12 and 3,000 pythons (median = 120; Fig. 3b) and collecting between 12 and 5,000 eggs (median = 150; Fig. 3c) in total over the 12 months. Five hunters (9%) (all of whom said they captured female, male and juvenile snakes) reported capturing 1,000 or more pythons in the last year. However, most hunters (> 90% [91.2%]; $n = 52$) reported capturing a maximum of 500 pythons, and 17 (30%) capturing 50 or less. Similarly, one hunter reported collecting 5,000 eggs, whereas all others (98%; $n = 53$) reported that they did not collect more than 1,000 eggs. In addition, 17 (31%) individuals reported collecting 100 or less.

Dividing the reported total number of pythons captured in the last 12 months, and the total number of eggs collected, by the reported number of trips undertaken, for each hunter, suggested that hunters were capturing an average of between 0.3 (i.e. one every three or four trips) and 31 ball pythons (median = 2.5), and an average of between 0.3 (one every three or four trips) and 50 ball python eggs (median = 4.3) per trip. Approximately a fifth of hunters were capturing 10 or more ball pythons per trip (21%; $n = 12$) or collecting 10 or more eggs (20%; $n = 11$); most (ca. 70%) captured a maximum of five ball pythons per trip ($n = 40$), and fewer than six or seven eggs per trip ($n = 35$ and 40, respectively) (most likely a single clutch of eggs). Most ball python clutches comprise five to eight eggs (Aubret et al. 2003), and the eggs are strongly adherent so it is not possible to separate them without damaging the shell (Aubret et al. 2005).

In addition to direct ball python hunting activities, eight interviewees (including those who also captured snakes themselves, and one who only bought snakes and their eggs from hunters) reported purchasing between 2,000 and 3,000 additional ball pythons (mean = 1,900, minimum = 400, maximum = 2,700; excluding one interviewee who stated he bought 38,000) in the last 12 months. For the seven hunters, this additional purchasing increased their annual “collection” on average five-fold (range one to nine) to an average of 2,500 snakes in total (range 500 – 3,000). The one interviewee

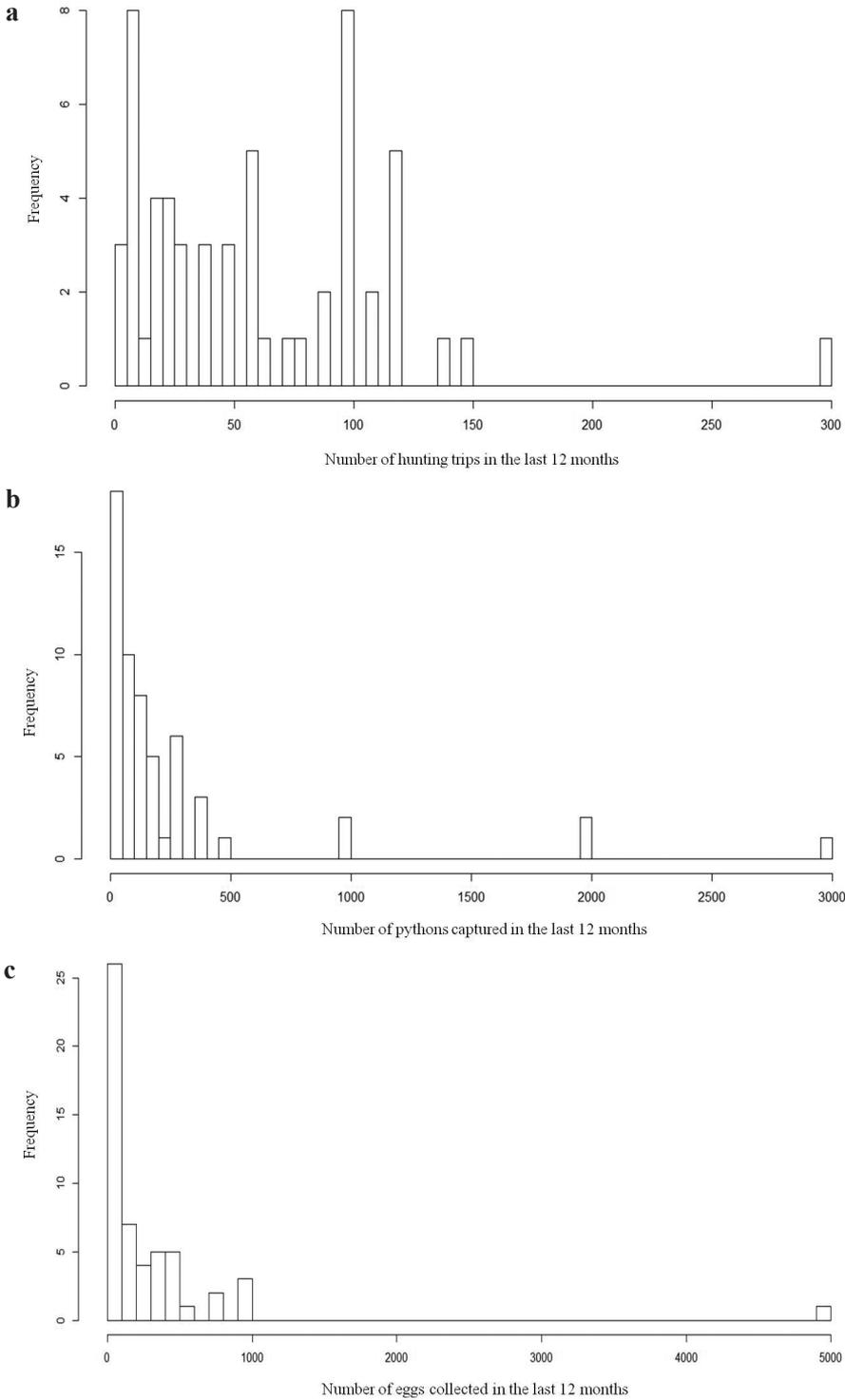


Figure 3. Frequency distribution of number of **a** ball python hunting trips **b** ball pythons captured and **c** ball python eggs collected, in the last 12 months as reported by 57 interviewed ball python hunters.

that only purchased ball pythons reported buying 1,500 individuals. Ten interviewees also reported that between 300 and 2,000 (mean = 1,320) eggs were laid by female ball pythons in captivity during one season prior to onward sale.

All hunters said that they sourced ball pythons in Togo, 60% ($n = 35$) stated that they sourced ball pythons only in Togo. However, almost a quarter (23%; $n = 13$) stated that they also hunted ball pythons in Ghana, 10% ($n = 6$) stated that they also hunted in Benin, and 7% ($n = 4$) stated that they hunted ball pythons in both neighbouring range states. All of the 58 hunter / traders that said they hunted ball pythons, collected their eggs, or bought ball pythons, reported that there were no quotas for either ball pythons or their eggs.

Most hunters / traders (86%; $n = 50$) stated that they released some ball pythons (range 5 – 1,000; median = 100, in the last 12 months; Fig. 4a) back into the wild. The numbers released were positively and significantly correlated with the numbers captured ($r=0.54$; $p<0.001$) but, on average, were fewer than the numbers captured (slope of regression = 0.68, $F_{1,49} = 24.91$, $p<0.001$) and there was considerable variation (R^2 adjusted = 0.32) such that, at an extreme the numbers released, per hunter, ranged between approximately one tenth and eight times their reported capture (median proportion released = 0.8; Fig. 4b). Over half of hunters that released pythons (58%; $n = 29$) released fewer snakes than they reported collecting. Nine (18%) hunters obtained ball pythons for release from the snake farms, 14 (28%) from the “middlemen”, and 27 (54%) did not say.

Most (64%; $n = 32$) of the 50 hunter / traders that released ball pythons back into the wild stated that they released ball pythons to allow them *to reproduce again*; others said, *so that they could be captured again next year* (12%; $n = 6$), or *to avoid impact on population size or make the hunting sustainable* (6%; $n = 3$). One hunter said explicitly that he released snakes *in [his] hunting area* in order to come back to capture them the following season. Most hunter / traders (66%; $n = 33$ of 50) stated that they released females, some also stated that they released females and juveniles (16%; $n = 8$), females and males (8%; $n = 4$), all sexes and age classes [males, females and juveniles, (6%; $n = 3$)], or just males (4%; $n = 2$).

With regards to release habitat, 18% ($n = 9$) stated that they released snakes into the forest. Others stated that they released snakes into farmland (6%; $n = 3$), fallow land (2%; $n = 1$), or, in most cases (68%; $n = 34$), a combination of forest, farmland, and / or fallow land; 6% ($n = 3$) said that they released ball pythons into either farmland or oil palm plantations.

Drivers of ball python harvest

Only two of the 60 interviewees (3%) said that they had collected ball pythons for personal consumption (both said that they liked to consume python meat occasionally and that they had slaughtered one and seven ball pythons in the last 12 months, respectively). The vast majority of interviewees (93%; $n = 56$), however, said that they did not eat either ball pythons specifically (65%; $n = 39$), or snakes more generally

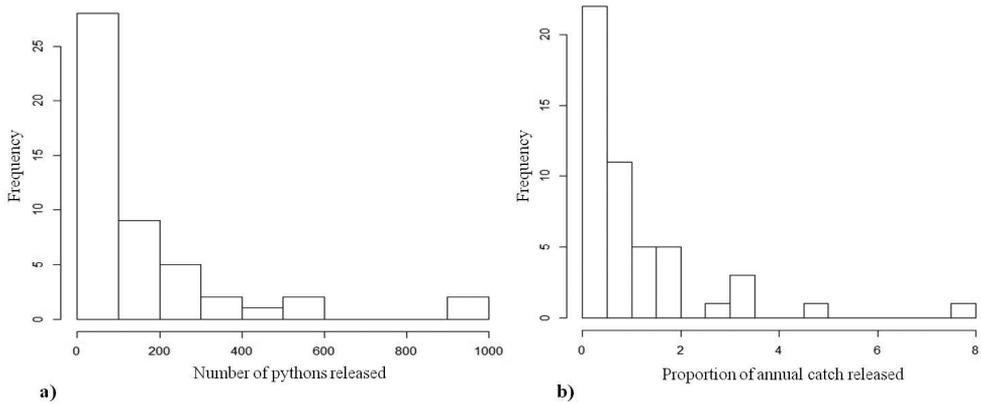


Figure 4. Numbers of ball pythons released back into the wild, shown as the frequency distribution of **a** reported numbers released, and **b** reported numbers released as a proportion of the reported numbers captured, in the last 12 months, based on answers given by 50 python hunters that released pythons at the end of the hunting season.

(28%; $n = 17$), primarily because ball pythons are considered as sacred (27%; $n = 15$ of 56 that stated that they did not eat ball pythons / snakes) and / or because it is not allowed in their society and culture (45%; $n = 25$).

None of the 58 hunter / traders said that they kept ball pythons as pets themselves, and none reported either using or selling ball python body parts (skins, bones or blood). Instead, all 58 hunters / traders said that they sold ball pythons and their eggs, either via “middlemen” outside (43%; $n = 25$) or inside (19%; $n = 11$) their village, or directly to snake farms (38%; $n = 22$) or both (2%; $n = 1$). Four hunter / traders (7%) sold ball pythons to snake farms in Benin ($n = 3$) or Ghana ($n = 1$). None sold ball python meat but five (9%) sold pythons and / or their eggs to Chinese restaurants in Lomé, the capital city of Togo.

Reported prices obtained for live ball pythons and their eggs varied greatly, for ball pythons between 1,200 and 10,000 CFA (median = 3,000, or between 2 and 17 USD, median = 5 USD), and for their eggs between 200 and 2,500 CFA (median = 500, < 0.5 to 4 USD, median = 0.9 USD). However, almost half of hunter / traders (44%; $n = 26$) reportedly sold snakes for 2,000 CFA (3.4 USD) or less (< 10%, $n = 5$, sold snakes for 10,000 CFA, or as much as 17 USD), and, the majority (93%; $n = 52$) sold eggs for 1,000 CFA (1.7 USD), or less (see Fig. 5).

Per hunting party, estimated annual income from ball python hunting (based on the reported number of snakes captured and the prices they were sold for, see Eqn. 1, Methods) ranged between 24,000 and 24 million CFA (median = 300,000 CFA; or between 41 and 41,000 USD, median = 510 USD), whilst that from egg collection (based on the reported number of eggs collected and the prices they were sold for) ranged between 6,000 and 7.5 million CFA (median = 90,000 CFA; between 10 and 12,750 USD; median = 153 USD), giving a total ball python harvest income of between 46,000 and 31.5 million CFA (median = 540,000 CFA; between 78 and 53,550 USD, median = 918 USD). The average estimated income of one hunter, ranged be-

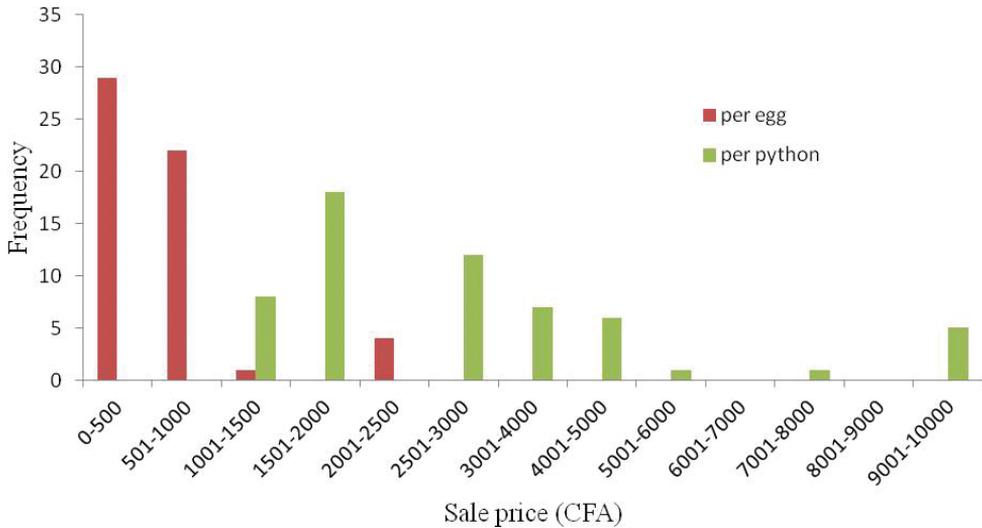


Figure 5. Sale price for ball pythons (green) ($n = 58$ hunter / traders), and ball python eggs (red) ($n = 56$ hunter / traders that gave prices for eggs), in West African CFA Francs (CFA) (1 CFA = 0.0017 USD, conversion rate as of 26.04.19, xe.com).

tween 12,250 and 5.25 million CFA (median = 102,250 CFA; between 20.8 and 8,925 USD, median = 173.8 USD), amounted to between 3 and 169% of a hunter's reported annual wages (median = 40%).

The highest reported prices (10,000 CFA for snakes and 2,500 CFA for eggs; 17 and 4.3 USD, respectively) were for sales to Chinese restaurants. Prices also tended to be higher when sold direct to farms or to middlemen outside the village than to middlemen inside the village (for ball pythons: median price obtained from sales to farms and middlemen outside the village = 3,000 CFA or 5.1 USD, middlemen inside the village = 2,000 CFA or 3.4 USD, Kruskal-Wallis $\chi^2 = 9.72$, $df=2$, $p=0.008$; for their eggs: median price obtained from sales to farms = 1,000 CFA or 1.7 USD, middlemen outside the village = 500 CFA or 0.9 USD, middlemen inside the village = 300 CFA or 0.5 USD, Kruskal-Wallis $\chi^2 = 19.03$, $df=2$, $p<0.001$).

Hunter / traders (45%; $n = 26$) further reported that the price of live snakes depended on the stage of the hunting season and the age class of the snake (57%; $n = 33$, with one hunter / trader reporting higher prices for gravid females), as well as “trends in the market” (14%; $n = 8$) and the effects of supply and demand (16%; $n = 9$). One hunter remarked, *if we have a lot of specimens and orders are scarce, the price per unit drops. But on the contrary, if we don't have python and the order is high (in terms of number), the price increases.*

Attitudes towards ball pythons and perceived population trends

When asked how they felt about ball pythons (on a scale of 1 to 5, with 1 being *strongly dislike* and 5 *strongly like*), there was a strong bias towards liking ball pythons, with

97% ($n = 58$) of all 60 interviewees stating that they either *quite liked* (42%; $n = 25$) or *strongly liked* (55%; $n = 33$) ball pythons (two interviewees stated that they *disliked* them). All 58 interviewees that said that they liked ball pythons suggested that the benefits provided by ball pythons were related to money or income; nine (16%) of these 58 specifically referred to money to *take care of their children*, and four (7%) referred to it as an important source of income (i.e. that *without [it], things would be very hard or difficult*). When asked why they felt this way (i.e. why they liked ball pythons), 45 (78%) of the 58 interviewees referred to ball pythons as not being dangerous or venomous, and three (5%) said they *could have fun with it*. The two interviewees who did not hunt or trade ball pythons said that they had no benefit. One hunter also said that ball pythons *regulate the population size of rodents that destroy our crops*.

Overall, most interviewees (75%; $n = 45$) stated that there were fewer ball pythons than there were five years ago: 67% ($n = 40$) stated that there were *quite a few less*, 8% ($n = 5$) stated that there were *a lot less*, while 7% ($n = 4$) that there were more, and 17% ($n = 10$) that there were the same number; one did not answer the question. Of the 45 interviewees that referred to an apparent decline in ball pythons in the last five years, 17 further commented on the possible cause of a decline (note that they were not asked this question directly). Eleven of 17 interviewees that commented on the cause of a decline referred directly to hunting, or to increasing numbers of hunters and farms (one stated that *As we are capturing them with their eggs, they don't have time to reproduce anymore*), four referred to tractors and cows (trampling), two to forest destruction, two to uncontrolled vegetation fires ($n = 2$), and one to climate change (prolonged dry seasons).

Discussion

This study represents the first review of ball python hunters and their experiences being carried out by local groups in Togo, West Africa for nearly 15 years (see Ineich 2006), and provides an assessment of a large-scale contemporary production system to supply live reptiles (exported as “ranchered” using CITES source code “R”) to the international pet trade. Our approach, focused at the bottom of the trade chain, permitted extensive detailed data to be collected from hunters, and provides a unique insight into the practices, drivers and impacts associated with this type of large-scale commercial wildlife trade (Fig. 6). We found that the practice of ball python hunting is an important source of income for those hunters involved. However, whilst our data do not include precise numbers of gravid females and adult males collected, or of juvenile production, there was little evidence of regulatory oversight at the hunter level with regard to (1) the release of females after they have laid their eggs, (2) release of 20% of the juveniles produced, or (3) collection of adult males within quotas for wild-caught specimens, as required under national management plans and CITES. Further, widespread suggestion among hunters that wild ball python populations have declined raises concern regarding the sustainability of this practice. Overall, we found little evidence of effective management, as outlined by Ineich (2006).



Figure 6. **A** hunter digging for a ball python (*Python regius*) **B** ball python uncovered from a rodent burrow **C** ball python eggs intended for commercial sale **D** Hunter with his catch of live ball pythons intended for commercial sale in Togo, West Africa. Images A-C Mark Auliya / World Animal Protection; image D, Delphine Ronfot / World Animal Protection.

On average, ball python hunting parties are typically composed of around five individuals that take part in around 50 hunts each year collecting 120 ball pythons and 150 eggs each. However, the roles of these individuals can extend beyond hunting (Fig. 7). We found that a proportion of hunters also act as middlemen [(14%; $n = 8$) buying between 2,000 and 3,000 ball pythons from other hunters over the last year] and (17%; $n = 10$) are also involved in the “ranching” of ball pythons (keeping gravid females at home until they have laid their eggs which are then sold to farms, as suggested by [Ineich 2006]).

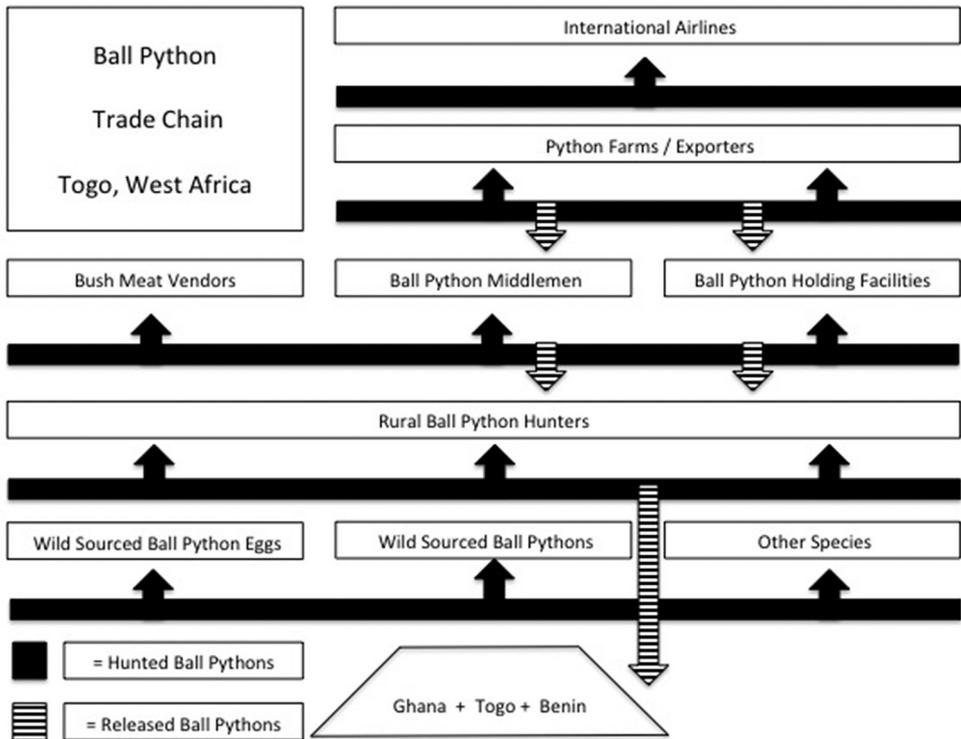


Figure 7. Ball python “chain of custody” demonstrating the various trade actors involved in commercial trade activity in Togo, West Africa. Ghana and Benin are included as source countries for wild caught ball pythons as per described by 23 hunters during interviews.

Why are hunters targeting ball pythons in Togo?

Our study demonstrates that ball python hunting remains an economically valuable endeavour for rural hunters in Togo, West Africa. Although ball python and egg hunting appear to be seasonal, taking place predominantly between the months of January and April, we estimate that, on average, a hunter receives 102,000 CFA (174 USD) from python and egg sales combined, representing approximately 40% of their annual income. Despite a degree of variability, collection rates reported by some hunters suggests that they can earn up to 5.3 million CFA (approximately 9,000 USD) from python and egg sales combined representing approximately 169% (almost double) their average annual income.

Overall, hunters described the sale of live ball pythons (maximum estimated annual income per hunting party of 24 million CFA) as a more financially lucrative endeavour than the sale of python eggs (maximum estimated annual income per hunting party of 7.5 million CFA). Although, on average, hunters collected approximately the same number of eggs as they captured snakes, the latter were reportedly worth approxi-

mately five times as much as a single egg. The lower value of eggs is presumably due to factors such as higher mortality rates in eggs and neonates, while gravid females are of presumably particularly high value because they will produce several offspring.

Is ball python hunting being conducted legally?

Our study reveals that some key aspects of python hunting taking place in Togo may run afoul of domestic and international legal requirements. For example, Article 78 of Law No. 2008-09 of the Carrying Forest Code strictly prohibits the hunting of gravid females, newborns, juveniles, eggs and the associated destruction of ball python burrows. Furthermore, Articles 79 and 80 of the Carrying Forest Code allow certain hunting activities, but the number of hunters that possess the required hunting permits is currently unknown. Another unknown is whether hunters possess the professional licence required under Article 81 of the Carrying Forest Code. In addition, Article 61 of the 005 Framework Law on the Environment participative manner to ensure its sustainability, which for ball pythons is questionable (also see below).

With regards to international regulations, our findings suggest that both exporter country and CITES source codes are in fact misrepresentative. Forty percent ($n = 23$) of the 58 hunter / traders interviewed during our study stated that they source ball pythons outside of Togo and 7% stated they sold them back to snake farms in Benin and Ghana meaning that reported exports from Togo, Benin and Ghana by CITES are in reality composed to at least some degree of snakes also sourced from neighbouring countries. In addition, 85% of the 58 hunters / traders stated that they hunt not only female ball pythons but also males and juveniles, which were then also sold to farms for export. This suggests that ball pythons exported from Togo are composed to at least some degree of snakes also sourced from the wild rather than “ranched” – although export of some wild-sourced specimens is permitted under CITES permit; the agreed quota is currently 1,500 (UNEP 2019). The number of adult snakes collected by hunters for direct export via farms is not known, but this “mixed” system creates considerable opportunity for mis-reporting (cf. Toudonou 2015), and once the snakes are in the farms it is difficult, if not impossible, to distinguish between wild-sourced and “ranched” specimens – a system that lacks transparency. At a regional level, this cross-border hunting activity, together with the misreporting of source codes, undermines domestic legal measures taken by neighbouring source countries: for example, Benin reduced their own quota for wild-sourced specimens in 2019 to 200 individuals (UNEP 2019) to regulate harvest in their country.

What are the impacts of hunting on ball pythons?

Although the global conservation status of the ball python has been assessed Least Concern according to the IUCN Red List of Threatened Species, population esti-

mates for ball pythons in West African ecosystems remain lacking (Auliya and Schmitz 2010). Despite their relatively wide distribution and adaptability to anthropogenically-altered habitat, regional concerns have already been raised about the impact of live python trade on the conservation of this species (e.g., Harris 2002; Toudonou 2015). Currently, there is no formal population monitoring initiative in place and as such it is not possible to assess whether or not this level of exploitation is sustainable. However, additional large-scale domestic use as bush meat and traditional medicine throughout its range (Harris 2002; Auliya and Schmitz 2010; Segniagbeto et al. 2013; D’Cruze et al., in prep.) and hunter perceptions of ball python population declines raise questions in this regard. We did not attempt to infer the cause of the perceived decline in ball python numbers and there are a number of additional factors that could cause such a decline, including habitat loss (i.e. expansion of agricultural areas into protected areas; see e.g., Harris 2002) and climate change (i.e. increasing frequency and duration of droughts; see e.g., Reading et al. 2010). However, these effects are not isolated, and habitat loss, for example, can increase a populations’ vulnerability to overutilization if the population becomes smaller or less resilient as a result and harvest levels remain the same (Regehr et al. 2017; see also Schai-Braun et al. 2019 for discussion of necessary adjustments to harvest quotas in response to population fluctuations caused by climatic events).

A key assumption of the current management process that underpins the export of ball pythons reported as “ranchered” from Togo is that wild release is carried out to balance this off take. However, our study highlights that in Togo this may not be carried out effectively. Although 86% of hunters stated that they released ball pythons back into the wild, the fact that more snakes were captured than were released suggests that the release of all females, in addition to the release of a proportion of offspring produced on the farms, is not being carried out as agreed national management regulations require. Furthermore, there are also questions regarding whether the re-release of ball pythons is being managed to avoid the introduction of disease (Auliya et al., in prep.).

In addition to questions regarding sustainability, there are a number of animal welfare issues associated with hunting practices currently being applied in Togo. Reptiles are recognised in the limited relevant research as being capable of a range of intellectual abilities and states including anxiety, distress, excitement, fear, frustration and pain (Lambert et al. 2019) suggesting that ball pythons, like other reptiles, have the capacity to experience suffering during capture, restraint, transport and subsequent captivity (Baker et al. 2013). We were not able to collect data on ball python morbidity and mortality rates during hunting and holding at farms. However, according to Harris (2002), the directors of two farms reported an estimated mortality rate between 1–2% at their facilities, and 1–5% during international transport; the latter information as the author notes, needs to be interpreted with caution as “*importers ... often falsify this data to increase profit*”. Further information on this matter would, in addition to quantifying efficiency of the system, provide insight into the extent and severity of animal welfare issues, but it is likely that snakes suffered stress during capture (in most cases physical removal from rodent burrows) and transport (live, in sacks filled with other

snakes) and possible that they sustained physical injuries – either or both may result in death or increased susceptibility to disease and / or infection (Baker et al. 2013). A proportion (17%) of hunters stated that they also kept gravid females themselves at home until their eggs were laid (which were then sold directly to farms). Whilst also not formally part of the study, post hoc visits to a number of hunters' "holding facilities" revealed sub-optimal captive conditions, and morbid animals with little to no veterinary, hygiene or disease transmission protocols in place.

What are the limitations of our study?

Caution is required in interpreting data derived from hunter interviews (e.g., Newton et al. 2008). For example, interviewees might have been reluctant to be honest about the magnitude of ball python hunting activities and there is a risk that the data underestimate overall hunting levels. Conversely, there is also a chance that some of the hunters may have overestimated their hunting success, or the value of their catch. In this study, it was puzzling that for some hunters the calculated proportion of their income provided by ball python hunting was greater than their reported annual income – this may in part be due to such overestimates (whether intentional or not) and / or to the cumulative effect of potential minor over-estimates for all variables included in the calculation. It is also possible that profits were not divided equally amongst all members of the hunting party, thus overestimating the total income generated for those receiving a smaller share. There are also further complicating factors such as the costs involved in purchasing additional snakes and eggs, and for this reason we did not include those hunters involved in further trading in our estimates of total income generated, but it is likely that this additional trading is potentially lucrative. Nevertheless, regardless of the precise proportion of total income provided by python hunting, our calculations suggest that it is currently substantial.

In addition, it was not possible to learn whether hunters only collected females with clear evidence of them being gravid, and / or whether they collected all females after the mating period, i.e. only assuming that they were gravid? Similarly, we do not know how many adult males were collected, how many adult females were released (post egg laying), or what proportion of juveniles produced in farms were in fact re-released. Also, we do not know whether wild captured specimens are intended for the purpose of consuming or supplying bush meat (markets) to other parts of the country or even across the country's border to Benin (Toudonou 2015), or even Nigeria (Eniang et al. 2006).

Our aim was not to assess the extent of impact or the total number of people involved, rather we sought to understand the practices undertaken by those who were willing to discuss that they hunted pythons and their reasons for doing so, and to assess whether there was cause for potential concern that might warrant further study. That 58 hunter / traders were willing to talk to us and that 40% ($n = 23$) openly admitted to an irregular if not illegal sourcing of ball pythons from neighbouring countries without

required CITES permissions suggests that they were being truthful. That 45 hunter / traders believed that ball python numbers were declining suggests further monitoring is required. According to Gorzula et al. (1997), there was “*sufficient hearsay evidence to suggest strongly that ball pythons are being smuggled from Ghana to Togo and Benin*”. Furthermore, Ineich (2006) stated that juvenile ball pythons originating from Benin are legally and illegally brought to Ghana. The overall similarity in the answers from hunters across 20 different villages from eight different districts, that might be located up to 100 km from each other, further suggests the validity of the data and the seriousness of our findings.

How can we protect ball pythons and people?

It is clear that hunting ball pythons to supplement the international exotic pet trade is a valuable source of income for the hunters currently engaged in this commercial trade. However, the long-term viability of this activity as a source of financial income is jeopardised by an apparent increase in the number of captive-bred ball pythons in key importing countries such as the USA (Barker and Barker 2006), and the increasing role of the USA (and some European countries) as a global exporter of captive-bred ball pythons (CITES Trade Database, <https://trade.cites.org>). A detailed analysis of international market forces is beyond the scope of our study. However, it appears that even if both demand for, and quotas for, ball pythons remained stable, the number of hunters required to meet current demand remains relatively small.

Currently, the ball python quota from Togo is set at 62,500 ranched individuals and 1,500 wild caught individuals (UNEP 2019). On average, the hunters interviewed during our study indicated that they harvested 120 live ball pythons to supplement the ranching process during a 12-month period. Assuming that each snake survived to produce one clutch of eight eggs (Aubret et al. 2003), all of which provided viable hatchlings, only approximately 61 hunters would be required to meet the 62,500 ranched individual quota currently put in place by Togo. Should consumer demand for ball pythons in key foreign markets shift away from ranched and / or wild captured ball pythons from West Africa, sustainable alternative livelihood initiatives (D’Cruze et al. 2010) may be useful in helping hunters to find new viable forms of income.

From a legal perspective, it is apparent that there are a number of complex and potentially conflicting pieces of legislation that relate to ball python hunting and subsequent commercial trade. As such, a streamlining exercise for the various existing pieces of domestic legislation would greatly benefit efforts to protect remaining wild ball python populations in Togo. The apparent intention of the Togolese authorities to update its capacity relating to the implementation of CITES would present an excellent opportunity to provide clarity and improved enforcement in this regard. Indeed, it is not clear that the source code R is appropriate for the “ranching” system described here, given the inclusion of the collection of gravid females, and other uncertainties such as how long offspring are held at farms for “rearing” prior to export.

In addition, clarification on the circumstantial requirements of ball python hunting licences and the penalties associated with violating relevant legislation is urgently required. Furthermore, the cross-border hunting activity reported during our study supports previous recommendations for increased control in the harvest and release of ball pythons (e.g., de Buffrénil 1995) and highlights the current need to define common legal tools in a tripartite agreement between the three main ball python range states in West Africa that are predominantly involved in their commercial export (Benin, Ghana and Togo).

Ultimately, confidence in the long-term sustainability of current ball python hunting practices, in terms of a source of financial income and wild population survival, is currently undermined by a lack of data on the status of wild ball python populations. Such data are essential to assign appropriate quotas (cf. Schai-Braun et al. 2019) that protect both people and ball pythons and are an urgent priority in this regard. However, such initiatives can be difficult to implement, time consuming and costly to fund (D'Cruze et al. 2009). In the interim, in light of the unregulated and potentially unsustainable nature of ball python hunting in Togo highlighted by this study, a revision of the Togo export quota should be considered as part of a more precautionary approach. Benin appears to have already taken such preventative action, recently reducing its export quota from 45,000 specimens that were exported as ranches in 2017 to 22,000 in 2018 and 2019 (UNEP 2019). Given the recognised current financial value of the international trade in Ball pythons to hunters in Togo, direct monetary compensation [e.g., payments for ecosystem services (Clements et al. 2010)] may prove useful to help minimize any unintended negative economic impacts on local communities.

Conclusion

Our study suggests that the ball python production methods applied on the ground in Togo do not accurately reflect (1) offtake levels reported in CITES trade data, (2) the methods being reported to national authorities or international regulatory mechanisms such as CITES, in terms of source country, (3) the extent to which current production methods are dependent on wild-sourced ball pythons, and (4) aspects of management practice (such as re-release of females and a proportion of juveniles). This irregular, if not illegal trade may also be unsustainable, for example as implied by hunters reporting that there are fewer ball pythons in the wild than there were five years ago. Although our study was focused only on Togolese hunters, it is clear that cross-border harvesting is taking place in Benin and Ghana. As such it is likely that these issues are not only restricted to Togo. In short, we highlight a number of uncertainties in the “ranching” process of ball pythons in Togo, and neighbouring countries, upon which legal, sustainable and humane trade depends, and that warrant further study. To create a trade that is transparent in all respects, we support previous conclusions that a solution cannot be sought at the national level but must also involve the neighbouring states Ghana and Benin (e.g., Jenkins 1998; Gorzula et al. 1997). A tri-state common strategy would need to be developed to reorder the trade with ball pythons for all stakeholders in terms of sustainability and traceability of animals (origin, source).

Acknowledgements

We wish to thank all of the hunters for their participation and patience during our study and all of the Masters students from the Ecology and Wildlife Management programme, from the Laboratory of Ecology and Ecotoxicology, Faculty of Sciences, University of Lomé (Kossivi Inès Akagankou, Afi Florence Konko, and Kafui Jeanne Dekawole) who helped us to conduct the survey. We also thank Komina Amevoïn and Guillaume Koffi K. Ketoh for their on-going support and collaboration. Mark Auliya and Lauren Harrington received a grant from World Animal Protection to carry out this research. Finally, we sincerely thank Agbo-Zegue NGO for providing necessary logistics for the study.

References

- Afidegnon D (1999) Les mangroves et les formations associées du Sud-Est du Togo: Analyse éco-floristique et cartographie par télédétection spatiale. PhD Thesis, Université Benin, Togo.
- Amori G, Segniagbeto GH, Decher J, Assou D, Gippoliti S, Luiselli L (2016) Non-marine mammals of Togo (West Africa): An annotated checklist. *Zoosystema* 38(2): 201–244. <https://doi.org/10.5252/z2016n2a3>
- Aubret F, Bonnet X, Shine R, Maumelat S (2003) Clutch Size manipulation, hatching success and offspring phenotype in the ball python (*Python regius*). *Biological Journal of the Linnean Society*. *Linnean Society of London* 78(2): 263–272. <https://doi.org/10.1046/j.1095-8312.2003.00169.x>
- Aubret F, Bonnet X, Shine R, Maumelat S (2005) Why do female ball pythons (*Python regius*) coil so tightly around their eggs? *Evolutionary Ecology Research* 7: 743–758.
- Auliya M (2003) Hot Trade in Cool Creatures: A Review of the Live Reptile Trade in the European Union in the 1990s with a Focus on Germany. TRAFFIC Europe, Brussels.
- Auliya M, Schmitz A (2010) *Python regius*. The IUCN Red List of Threatened Species 2010: e.T177562A7457411. <https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T177562A7457411.en>
- Babbie ER (2004) *The Practice of Social Research* (10th ed). Thomson/Wadsworth, Belmont, 493 pp.
- Baker SE, Cain R, van Kesteren F, Zommers ZA, D’Cruze N, Macdonald DW (2013) Rough Trade: Animal Welfare in the Global Wildlife Trade. *Bioscience* 63(12): 928–938. <https://doi.org/10.1525/bio.2013.63.12.6>
- Barker DG, Barker TM (2006) *Pythons of the World Volume II: Ball pythons*. VPI Library, Boerne, Texas.
- Brant WE (2001) Commercial production of reptiles for the US pet trade. IUCN SSC – Commercial captive propagation and wild species conservation. Selected background papers. White Oak Foundation, Jacksonville.
- BSA [British Sociological Association] (2017) Statement of Ethical Practice. BSA Publications. www.britisoc.co.uk/media/24310/bsa_statement_of_ethical_practice.pdf
- Bush ER, Baker SE, Macdonald DW (2014) Global Trade in Exotic Pets 2006–2012. *Conservation Biology* 28(3): 663–676. <https://doi.org/10.1111/cobi.12240>

- Clements T, John A, Nielsen K, An D, Tan S, Milner-Gulland EJ (2010) Payments for biodiversity conservation in the context of weak institutions: Comparison of three programs from Cambodia. *Ecological Economics* 69(6): 1283–1291. <https://doi.org/10.1016/j.ecolecon.2009.11.010>
- D'Cruze N, Henson D, Olsson A, Emmett D (2009) The Importance of herpetological survey work in conserving Malagasy biodiversity: Are we doing enough? *Herpetological Review* 40: 19–25.
- D'Cruze N, Kumar Sarma U, Mookerjee A, Singh B, Louis J, Prasanna Mahapatra R, Prasad Jaiswal V, Kumar Roy T, Kumari I, Menon V (2010) Dancing bears in India: A sloth bear status report. *Ursus* 22(2): 99–105. <https://doi.org/10.2192/URSUS-D-10-00033.1>
- D'Cruze N, Paterson S, Megson D, Warwick C, Coulthard E, Norrey J, Auliya M, Carder G (2020) Dropping the Ball? The welfare of Ball Pythons traded in the EU and North America. *Animals (Basel)* 10(3): 1–413. <https://doi.org/10.3390/ani10030413>
- Dansi A, Dantsey-Barry H, Dossou-Aminon I, N'Kpenu EK, Agré AP, Sunu YD, Kombaté K, Loko YL, Dansi M, Assogba P, Vodouhè R (2013) Varietal diversity and genetic erosion of cultivated yams (*Dioscorea cayenensis* Poir – *D. rotundata* Lam complex and *D. alata* L.) in Togo. *International Journal of Biodeversity and Conservation* 5: 223–239.
- de Buffrénil V (1995) Les élevages de Reptiles du Bénin, du Togo et du Ghana. Rapport d'étude réalisée pour le Secrétariat de la CITES, Juin 1995: 1–23.
- Eniang EA, Egwali E, Luiselli L, Ayodele I, Akani GC, Pacini N (2006) Snake bushmeat from the forest markets of south-eastern Nigeria. *Natura (Milan)* 95: 33–46.
- Gorzula S, Nsiah WO, Oduro W (1997) Survey of the Status and Management of the Royal Python (*Python regius*) in Ghana. Part 1. Report to the Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Geneva, 38 pp. http://ec.europa.eu/environment/cites/pdf/studies/royal_python_ghana.pdf
- Harris M (2002) Assessment of the status of seven Reptile species in Togo. Report to the Commission of the European Union, ref. EC 9810072: 1–58. http://jncc.defra.gov.uk/pdf/togo_sevenreptilespeciesvpt1.pdf
- Heath E (2010) Fon – Ethic group of the Republic of Benin whose ancestors built the powerful precolonial Kingdom of Dahomey; also called Agadja. In: Appiah KA, Gates HL (Eds) *Encyclopedia of Africa*. Oxford University Press, Oxford/New York, 1392 pp. 10.1093/acref/9780195337709.001.0001
- Herrel A, Meijden A (2014) An analysis of the live reptile and amphibian trade in the USA compared to the global trade in endangered species. *The Herpetological Journal* 24: 103–110.
- Hoover C (1998) The U.S. Role in the International live Reptile Trade: Amazon Tree Boas to Zululand Dwarf Chameleons. TRAFFIC North America, Washington.
- Ineich I (2006) Les élevages de reptiles et de scorpions au Bénin, Togo et Ghana, plus particulièrement la gestion des quotas d'exportation et la définition des codes 'source' des spécimens exportés. Rapport d'étude réalisée pour le Secrétariat de la CITES. Projet CITES A-251: 1–113.
- Jenkins RWG (1998) Management and use of *Python regius* in Benin and Togo (p. 11). Report prepared for Directorate General XI The Commission of the European Union.

- Jenkins RWG, Jelden D, Webb GJW, Manolis SC (2004) Review of Crocodile Ranching Programs. Conducted for CITES by the CROCODILE SPECIALIST GROUP of IUCN/SSC. January – April 2004. IUCN-SSC Crocodile Specialist Group. https://www.iucnscg.org/365_docs/attachments/protarea/CSG_-2b73a2ea.pdf
- Jensen TJ, Auliya M, Burgess ND, Aust PW, Strand J (2018) Exploring the international trade in African snakes not listed on CITES: Highlighting the role of the internet and social media. *Biodiversity and Conservation* 28(1): 1–19. <https://doi.org/10.1007/s10531-018-1632-9>
- Lambert H, Carder G, D’Cruze N (2019) Given the Cold Shoulder: A review of the scientific literature for evidence of reptile sentience and cognition. *Animals (Basel)* 9(10): 1–22. <https://doi.org/10.3390/ani9100821>
- Lyons JA, Natusch DJD, Jenkins RWG (2017) A Guide to the Application of CITES Source Codes. CITES, Gland. https://cites.org/sites/default/files/eng/prog/captive_breeding/E-Souce%20codes%20booklet%20-%20April%2017.pdf
- Newing H (2011) *Conducting Research in Conservation: a Social Science Perspective*. Routledge, Abingdon. <https://doi.org/10.4324/9780203846452>
- Newton P, Van Thai N, Robertson S, Bell D (2008) Pangolins in peril: Using local hunters’ knowledge to conserve elusive species in Vietnam. *Endangered Species Research* 6: 41–53. <https://doi.org/10.3354/esr00127>
- Potgieter GC, Weise FJ, Wachter B, Melzheimer J, Wiesel I, Stratford K (2017) Comment on Rust et al.: Human–Carnivore Conflict in Namibia Is Not Simply About Black and White. *Society & Natural Resources* 30(10): 1299–1303. <https://doi.org/10.1080/08941920.2017.1283077>
- R Core Team (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org>
- Reading CJ, Luiselli LM, Akani GC, Bonnet X, Amori G, Ballouard JM, Filippi E, Naulleau G, Pearson D, Rugiero L (2010) Are snake populations in widespread decline? *Biology Letters* 6(6): 777–780. <https://doi.org/10.1098/rsbl.2010.0373>
- Regehr EV, Wilson RR, Rode KD, Runge MC, Stern HL (2017) Harvesting wildlife affected by climate change: A modelling and management approach for polar bears. *Journal of Applied Ecology* 54(5): 1534–1543. <https://doi.org/10.1111/1365-2664.12864>
- Robinson JE, Griffiths RA, St John FAV, Roberts DL (2015) Dynamics of the global trade in live reptiles: shifting trends in production and consequences for sustainability. *Biological Conservation* 184: 42–50. <https://doi.org/10.1016/j.biocon.2014.12.019>
- Roe D (2008) *Trading Nature. A report, with case studies, on the contribution of wildlife trade management to sustainable livelihoods and the Millennium Development Goals*. TRAFFIC International and WWF International.
- Schai-Braun SC, Kowalczyk C, Klanssek E, Hackländer K (2019) Estimating Sustainable Harvest Rates for European Hare (*Lepus Europaeus*) Populations. *Sustainability* 11(10): 1–2837. <https://doi.org/10.3390/su11102837>
- Segniagbeto GH, Trape JF, David P, Glitho IA (2011) The snake fauna of Togo: Systematics, distribution and biogeography, with remarks on selected taxonomic problems. *Zoosystema* 33(3): 325–360. <https://doi.org/10.5252/z2011n3a4>

- Segniagbeto GH, Petrozzi F, Aidam A, Luiselli L (2013) Reptiles Traded in the Fetish Market of Lomé, Togo (West Africa). *Herpetological Conservation and Biology* 8: 400–408.
- Segniagbeto GH, Trape JF, Afiadmany KM, Rödel MO, Ohler A, Dubois A, David P, Meirte D, Glitho IA, Petrozzi F, Luiselli L (2015) Checklist of the lizards of Togo (West Africa), with comments on systematics, distribution, ecology, and conservation. *Zoosystema* 37(2): 381–402. <https://doi.org/10.5252/z2015n2a7>
- Tensen L (2016) Under what circumstances can wildlife farming benefit species conservation? *Global Ecology and Conservation* 6: 286–298. <https://doi.org/10.1016/j.gecco.2016.03.007>
- Toudonou CAS (2015) Ball python (*Python regius*). Species trade and conservation. Snake trade and conservation management (Serpentes spp.). An assessment of the impact of the pet trade on five CITES-Appendix II case studies PYTHON REGIUS. AC28 Inf. 4. <https://cites.org/sites/default/files/eng/com/ac/28/Inf/E-AC28-Inf-04.pdf>
- UNEP (2019) The Species+ Website. Nairobi, Kenya. Compiled by UNEP-WCMC, Cambridge. www.speciesplus.net. [Accessed: 07/01/2019]
- UNEP-WCMC (2005) Benin and Togo – A review of selected species in trade. SRG 35/4/2/1. Prepared for the European Commission Directorate General E – Environment ENV E.3 – Development and environment. United Nations Environment Programme World Conservation Monitoring Centre, Cambridge, 165 pp.
- UNEP-WCMC (2014) Review of *Python regius* from Togo (source R). UNEP-WCMC, Cambridge, 13 pp. http://ec.europa.eu/environment/cites/pdf/reports/SRG%2068_7%20Review%20of%20Python%20regius%20from%20Togo%20_public_.pdf
- World Bank (2019) Togo. <https://data.worldbank.org/country/togo>

Supplementary material I

Questionnaire

Authors: Neil D'Cruze, Lauren A. Harrington, Délagnon Assou, Delphine Ronfot, David W. Macdonald, Gabriel H. Segniagbeto, Mark Auliya

Data type: survey questionnaire form

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/natureconservation.38.47864.suppl1>

The first genetic assessment of wild and farmed ball pythons (Reptilia, Serpentes, Pythonidae) in southern Togo

Mark Auliya^{1,2*}, Sylvia Hofmann^{2*}, Gabriel H. Segniagbeto^{3,4}, Délagnon Assou^{3,4},
Delphine Ronfot¹, Jonas J. Astrin¹, Sophia Forat⁵, Guillaume Koffivi K. Ketoh³,
Neil D’Cruze^{6,7}

1 Zoological Research Museum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany **2** Department of Conservation Biology, Helmholtz Centre for Environmental Research GmbH – UFZ, Permoserstreet 15, 04318 Leipzig, Germany **3** Laboratory of Ecology and Ecotoxicology, Department of Zoology and Animal Biology, Faculty of Sciences, University of Lomé, BP 1515 Lomé, Togo **4** Togolese Society for Nature Conservation (AGBO-ZEGUE NGO). 06 BP: 6057 Lomé, Togo **5** L.f.A. Labor für Abstammungsbegutachtungen GmbH, Marie-Curie-Str. 1, 53359 Rheinbach, Germany **6** World Animal Protection, 222 Grays Inn Rd., London WC1X 8HB, UK **7** Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Abingdon OX13 5QL, UK

Corresponding author: Sylvia Hofman (sylvia.hofman@ufz.de); Mark Auliya (mark.auliya@ufz.de)

Academic editor: K. Henle | Received 17 December 2019 | Accepted 4 March 2020 | Published 13 March 2020

<http://zoobank.org/E8AE5C2E-41D6-470C-8C67-938C747617A4>

Citation: Auliya M, Hofmann S, Segniagbeto GH, Assou D, Ronfot D, Astrin JJ, Forat S, Ketoh GKK, D’Cruze N (2020) The first genetic assessment of wild and farmed ball pythons (Reptilia, Serpentes, Pythonidae) in southern Togo. Nature Conservation 38: 37–59. <https://doi.org/10.3897/natureconservation.38.49478>

Abstract

The ball python (*Python regius*) is the world’s most commonly traded python species for the “exotic” pet industry. The majority of these live snakes are produced via a number of python farms in West Africa that have been in operation since the 1960s and involved with “ranching” operations since the 1990s. However, to date no thorough taxonomic review or genetic studies have been conducted within its range, despite the fact that the evaluation of a species’ genetic variability is generally considered mandatory for effective management. We used mtDNA sequence data and eight polymorphic microsatellite markers to assess the underlying population genetic structure and to test the potential of the nuclear markers to assign farm individuals to wild reference populations in southern Togo. Despite the relatively large distances between sample locations, no significant genetic population structure was found, either in mtDNA sequence data or in the microsatellite data. Instead, our data indicate considerable gene flow among the locations. The

* These authors contributed equally to this work.

absence of a distinct population subdivision may have resulted from an anthropogenic driven admixture of populations associated with commercial wildlife trade activity in recent decades. Given the ongoing largely unregulated nature of the commercial ranching of ball pythons in West Africa, should a wild release component continue, as a first measure we recommend that the Management Authorities should develop an action plan with specific release protocols for python farms to minimise any potential negative conservation impacts resulting from admixture (genetic pollution) between farmed and wild individuals.

Keywords

COI gene, microsatellites, population assignment, *Python regius*, West Africa, wildlife trade management

Introduction

The ball python (*Python regius*) is native to open woodlands and savannahs of western Africa south of the Sahara extending east into north-western Uganda, and has been recorded from at least 18 countries (Barker and Barker 2006; Uetz et al. 2019). In 2009, the species was last assessed as “Least Concern” in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species with an “Unknown” population status (Auliya and Schmitz 2010). All Pythonidae species were included on CITES Appendix II in 1977 (except *Python molurus* that has been included on Appendix I since 1975) (UNEP 2019). The ball python is the world’s most common species of python traded internationally for the commercial exotic pet trade (Barker and Barker 2006; D’Cruze et al., in prep.).

Since 1997, several West African States within the range of ball python have established quotas for export, the majority of which relate to live specimens intended for commercial purposes (Fig. 1). In recent years the largest volumes were exported as “ranchered” specimens (CITES Source Code “R”). For example, between 1997 and 2018, 3,628,030 live specimens were reported from the main exporting countries combined (Togo, n = 1,252,500; Benin, n = 1,479,530; Ghana, n = 867,500) (CITES Trade Database, <https://trade.cites.org>). In the same period the total export quota for wild sourced live specimens was 206,101 (Togo, n = 33,501; Benin, n = 32,600; Ghana, n = 140,000) (UNEP 2019). Between 2008 and 2018, Ghana established an annual export quota of 200 captive-bred specimens, except 2013; export quotas for captive-bred specimens were not established in Togo and Benin during the study period 1997–2018. Export quotas set by other West African nations are relatively negligible in terms of volumes (UNEP 2019; Fig. 1).

In recent decades, there has been a shift away from sourcing wild caught ball pythons and towards sourcing them through “ranching” initiatives via python “farms” in the three main exporting countries (Robinson et al. 2015), to the extent that in the last 10 years 95% of live exports from Togo were recorded using CITES source code “R” with the majority destined for the USA (CITES Trade Database, <https://trade.cites.org>; D’Cruze et al., in prep.). According to CITES, ranching is defined as the *rearing in a controlled environment of animals taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood* (<https://cites.org/eng/res/11/11-16R15.php>). A common part of the ranching system is the release

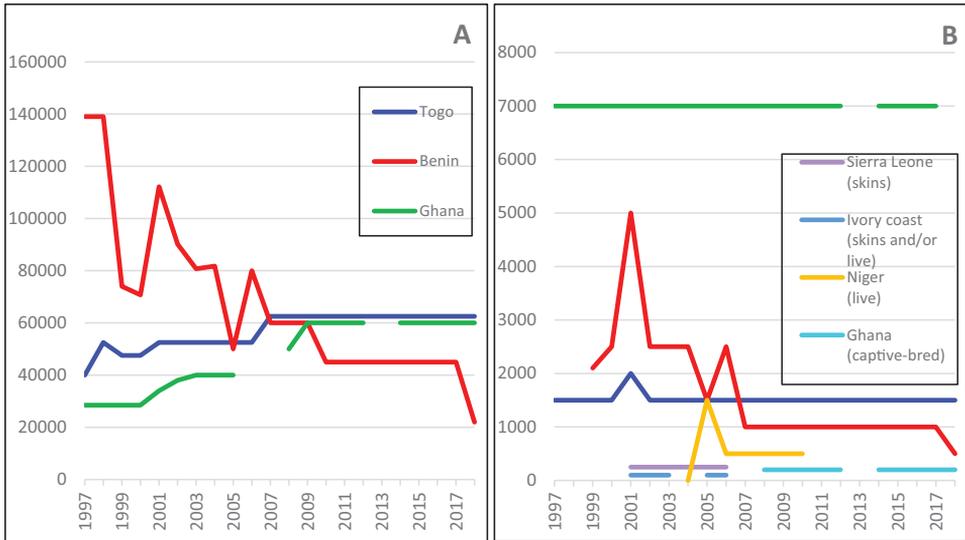


Figure 1. **A** Export quotas for ranched live ball python specimens from the three major exporting countries **B** export quotas for (1) wild live specimens from the three major exporting countries (for colours see **A**), for (2) wild specimens (live and skins), and for (3) captive-bred live specimens. Sources: <https://www.cites.org/eng/resources/quotas/index.php>, UNEP 2019.

of a percentage rate of neonates produced in captivity. In Togo for example, as recommended by Ineich (2006) this amount has been suggested to comprise 20% of the live snakes exported from Togo annually.

Between 1995 and 2016, numerous European Union (EU) and CITES-commissioned assessments and scientific studies were conducted in West Africa, including Togo (Segniagbeto et al. 2011). These initiatives aimed to evaluate management practices in the live reptile trade and in many cases had a particular focus on ball pythons (e.g., de Buffrénil 1995; Gorzula et al. 1997; Jenkins 1998; Affo 2001; Harris 2002; Harwood 2003; Ineich 2006; UNEP-WCMC 2010, 2014; Segniagbeto 2016). Although Ineich (2006) reported that trade was broadly being carried out under “relatively healthy conditions”, historically the results of these assessments have repeatedly reported a number of specific inconsistencies of the ranching strategies for Benin and raised questions for Togo that warrant additional scientific scrutiny (Ineich 2004, 2006; Toudonou 2003, 2004, 2007; Segniagbeto et al. 2011).

More recently, many of these inconsistencies have been confirmed as ongoing and have been summarised by D’Cruze et al. (in prep.) following a questionnaire-based study focused on ball python hunters in Togo. Specifically, this study found that: (1) the majority of surveyed hunters were not aware of national quotas relating to this CITES listed species; (2) that their harvest activity also involved illegal cross border collection and trade activities in other nearby range states (i.e., Benin, Ghana and Nigeria); and (3) that the release of gravid females collected in the wild and a proportion (20%, see above) of the resulting neonates (as part of the ranching program) was not monitored appropriately, with snakes being released without full care and con-

sideration given to key aspects, such as source location and the habitat of release sites (D’Cruze et al., in prep.).

Perceived and/or real inconsistencies relating to specific trade activities (as reported for ball python ranching – see above) can be forensically investigated by applying genetic methods that identify geographic origins and population structures of target species (Ogden 2012) (i.e., how is ball python ranching operated *per se*?). In addition, new information regarding the potential associated impacts of such trade activity on wild populations can also be elucidated (i.e., ball python conservation). However, to date no thorough taxonomic review or genetic studies have been conducted for the ball python within its range, despite the fact that the evaluation of a species’ genetic variability is considered mandatory for a fully effective and comprehensive management strategy (e.g., Sarre and Georges 2009; Frankham et al. 2014, and citations therein).

Here, we present the first molecular genetic analyses focused on the genetic structure and diversity of the natural population(s) of ball pythons in southern Togo [a region where the “vast majority of exploitation (in regard to ball pythons)” has been reported to take place (Harris 2002)] using eight polymorphic nuclear microsatellite loci and a fragment of the mitochondrial (mt) cytochrome c oxidase subunit 1 (COI) gene. COI is the most utilised marker for barcoding species (McGraw et al. 2012) and commonly used in phylogenetic studies on vertebrates (e.g., Saladin et al. 2019). Microsatellite markers are codominant, highly polymorphic and have high mutation rates and can therefore provide insight into small-scale genetic variation and ideally allow for the identification of specimens and their populations of origin (Schwartz et al. 2007; Palsbøll et al. 2013). Although microsatellites cannot directly impact a population negatively or positively, they have proven to be reliable indicators for the genetic diversity and allow for the comparison of genetic characteristics between populations, e.g. heterozygosity, allelic diversity, which has been noted to indicate levels of inbreeding or adaptive potential (Jump et al. 2009; Fraser et al. 2018; Lawrence et al. 2019). We used the microsatellite loci to test their predictive power for determining the origin of farmed (and traded) individuals and to assess the genetic differentiation between populations.

The main goal of this work is: (1) explore whether the genetic structure and divergence of wild ball python populations in Togo is consistent with their naturally expected low gene flow given their assumed low dispersal capacity (see below); (2) explore the role that commercial trade activities may have played to gain information on whether, and if so to what extent, ranching activities operating from facilities in python “farms” in Togo are impacting on wild populations; and (3) provide recommendations that can help inform existing and future initiatives focused on the conservation of this species.

Methods

Currently there are nine known farms that are registered to conduct commercial captive breeding and ranching of reptiles in Lomé. Seven of these farms were visited dur-

ing this study. These seven farms are thought to be responsible for exporting the majority (> 90%) of specimens globally (Segniagbeto 2016).

Sample collection

A non-invasive buccal swab method was applied to collect samples from 62 ball python specimens in Togo, including 21 samples from five of the nine known python farms currently located in Lomé and 41 samples from 12 wild populations located outside Lomé (Fig. 2). The number of samples differed between python farms and between locations of wild populations (ranging from 1–11) (Fig. 3, Suppl. material 1: Tables S1, S3).

DNA Extraction

Total genomic DNA was extracted from swab samples using the Blood and Tissue kit by Qiagen (Hilden, Germany) following the manufacturer's protocol. DNA extracts are available from the ZFMK Biobank, Bonn. The COI segment (674 bp) with primers HCO2198-JJ and LCO1490-JJ (Astrin and Stüben 2008) was amplified via the polymerase chain reaction (PCR) in reaction volumes of 20 µl, including 2.5 µl of undiluted DNA template, and using the Multiplex PCR Master Mix (Qiagen). Thermal cycling was performed on GeneAmp PCR System 2700 instruments (Life Technologies, Carlsbad, USA) as follows: hot start Taq activation: 15 min at 95 °C; first cycle set, touch down (15 repeats): 35 s denaturation at 94 °C, 90 s annealing at 55 °C (–1 °C/cycle) and 90 s extension at 72 °C. Second cycle set (25 repeats): 35 s denaturation at 94 °C, 90 s annealing at 40 °C and 90 s extension at 72 °C; final elongation 10 min at 72 °C. Amplicons were purified with the ExoSAP-IT Purification Kit (USB Corporation, Cleveland, Ohio) and sequenced in both directions using the PCR primers at MacroGen Europe's commercial Sanger sequencing service (Amsterdam, NL). All newly found haplotypes were deposited in GenBank (accession numbers: Suppl. material 1: Table S1).

Mitochondrial sequence data analysis

Molecular data were first phylogenetically analysed based on mtDNA to gain insights into spatial pattern of genetic variation and the level of genetic divergence among populations of the species in Togo.

The number of haplotypes was calculated using DnaSP 6 (Rozas et al. 2017). A median-joining (Bandelt et al. 1999) and a median (Bandelt et al. 1995) haplotype network was constructed in PopART v. 1.7 (Leigh and Bryant 2015) and SplitsTree v. 4.14.8 (Huson and Bryant 2006), respectively, to visualise relationships among haplotypes of the 12 localities (hereafter referred to as “populations”). Haplotype frequency distribution was mapped using ArcGIS 10.3.1 (ESRI, Redlands, CA, U.S.A.).

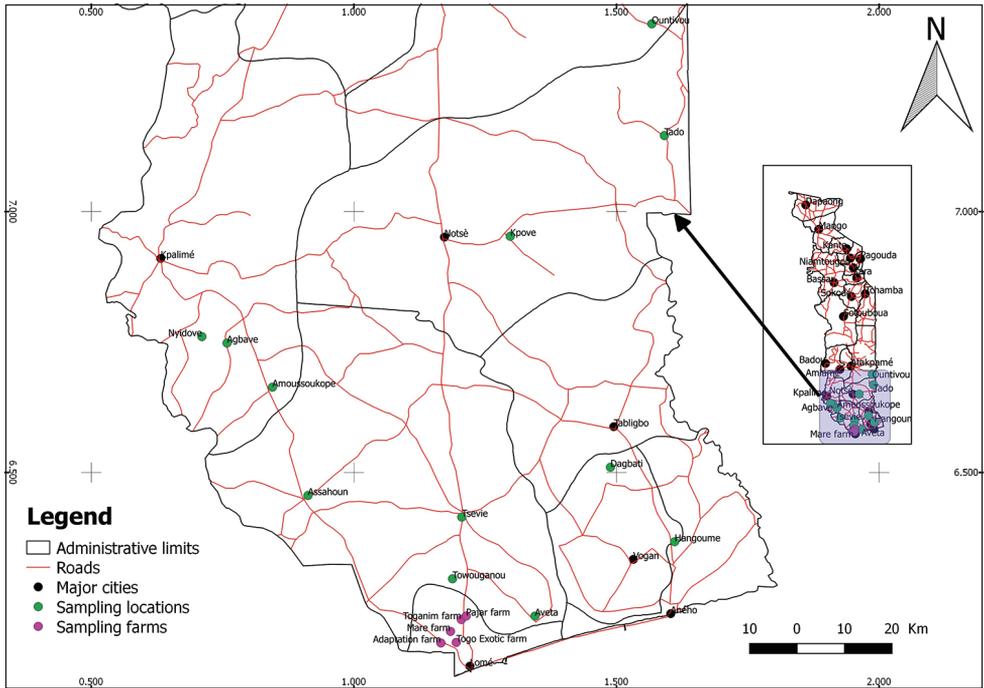


Figure 2. Southern Togo with sampling locations for wild *Python regius* (green) and housed in farms (red). Five specimens were sampled at each of three python farms, and at two farms each an additional three specimens were sampled. Number of wild specimens sampled per location: Hangoume, $n = 3$; Dagbati, $n = 2$, Kpové, $n = 3$, Assahoun, $n = 2$; Tado, $n = 3$; Tsevié, $n = 3$; Agbave, $n = 1$; Nyidove, $n = 1$, Amoussoukope, $n = 4$; Ouhangbé, $n = 6$; Towouganou (Zio), $n = 11$; Aveta, $n = 2$.

We also tested for significant differences in nucleotide and haplotype diversity between sample locations using permutation tests implemented in the R script genetic_diversity_diffs v1.0.6 (Alexander et al. 2016; R Core Team 2019) with 10,000 iterations. The script resamples from the combined haplotype frequencies over all populations, in order to test whether the observed differences in haplotype diversity between specific populations, are greater than expected by chance. For this analysis, we grouped all farms together due to their small geographic distances, as we did with the neighbouring localities Agbave and Nyidove from which only one sample was available each.

Phylogenies were reconstructed by Maximum Likelihood (ML) and Bayesian Inference (BI) methods, using RAxML v. 8.2.10 (Stamatakis 2014) as well as MrBayes v. 3.2.7a (Ronquist et al. 2012). We used *Xenopeltis unicolor* (NCBI accession no. LC075326) as outgroup and included additional COI-sequences of *P. regius* available from GenBank (AB177878; KX012740; KX012757; KX012789–KX012791; NC007399). Optimal partitioning schemes and substitution models for each partition were selected using the Bayesian Information Criterion in a greedy search algorithm and linked branch lengths in PartitionFinder v. 1.1.1 (Lanfear et al. 2012, 2014). BI analyses were then run using models and codon partitions as selected by PartitionFinder.

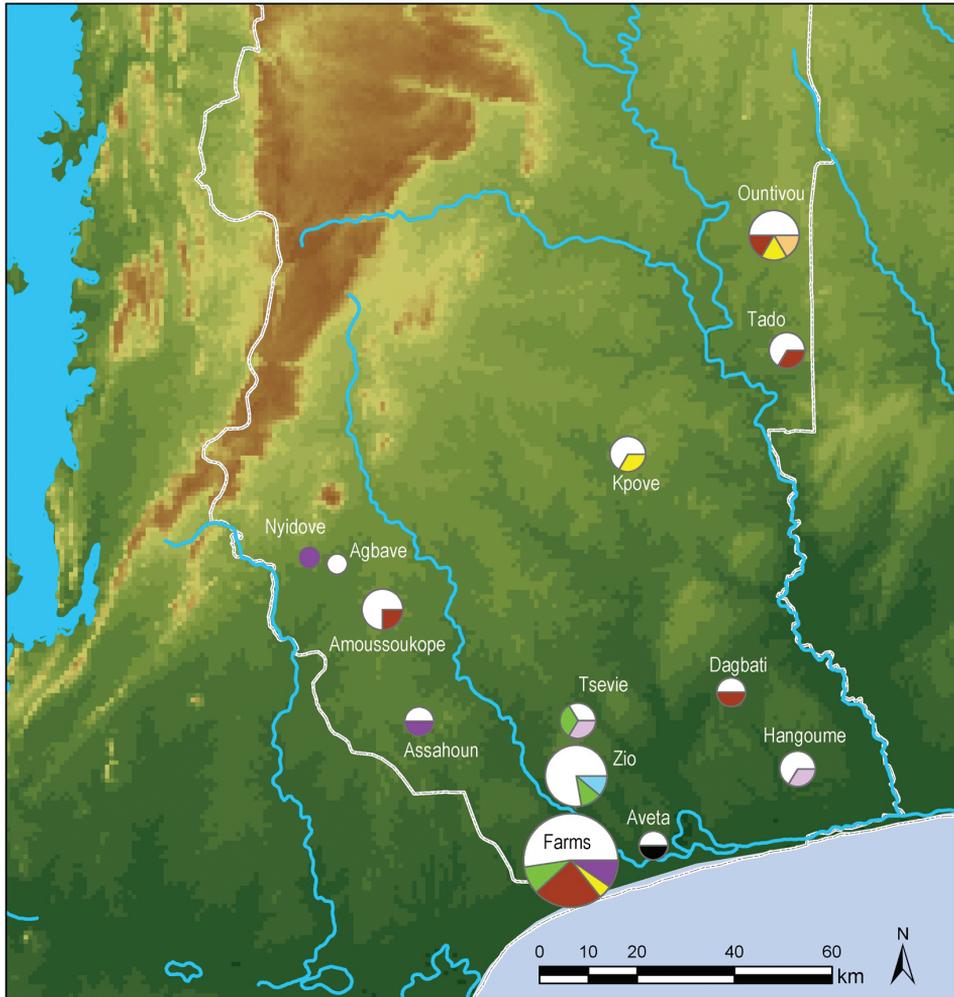


Figure 3. Map of the spatial distribution of haplotypes of *Python regius* in Togo. Circle sizes correspond to haplotype frequency. Source of the map: <https://www.esri.com>.

ML tree construction was performed under the GTR-GAMMA model applying the codon partition scheme preferred by PartitionFinder. Nodal support of the tree was assessed by rapid bootstrapping algorithm with 1,000 bootstrap replicates.

Microsatellite amplification, genotyping and analysis

We initially tested the cross-amplification of microsatellite loci previously described by Jordan et al. (2002) and Shaney et al. (2016). Out of these microsatellites, 11 loci cross-amplified in our samples and were genotyped for each sample using fluorescently labelled primers (for accession numbers see Table 1). Some of the primers were modified in their length to adjust the annealing temperature for a multiplex reaction. Before multiplexing, all primer pairs were tested in singleplex PCRs to check for correct amplification of the desired fragments. PCRs were performed in a 12,5 μ l reaction mix containing 5–40 ng DNA, 200 μ M dNTP's, 0,5 μ M each primer pair (reverse primer of each pair was labelled, see Table 1), 5 \times Q5 reaction buffer and 1 U Q5 High-Fidelity polymerase (New England Biolabs). Amplification conditions were as follows: 98 $^{\circ}$ C for 30 s followed by 33 cycles at 98 $^{\circ}$ C for 10 s, 64 $^{\circ}$ C annealing temperature for 30 s and 72 $^{\circ}$ C for 30 s, with a final extension at 72 $^{\circ}$ C for 2 min. Amplification products were separated on an ABI 3730 automatic sequencer. Raw data were analysed and evaluated using GeneMapper ID v3.2.1 (Applied Biosystems).

Microsatellite analysis

All markers were checked for scoring errors, large allele dropout and the possible presence of null alleles using Micro-checker v2.2.3 (van Oosterhout et al. 2004). Departure from Hardy–Weinberg equilibrium (HWE) was tested for each locus by Fisher's exact tests (Guo and Thompson 1992) using a Markov chain Monte Carlo (MCMC) approach with 10,000 steps and 1,000 iterations as implemented in GenePop v. 4.2 (Raymond and Rousset 1995). GenePop was also used to test linkage disequilibrium (LD) for all pairs of loci. P-values of LD were corrected using the FDR-method (Benjamini and Hochberg 1995). The number of alleles, polymorphic information content (PIC) (Botstein et al. 1980), expected (H_e) and observed (H_o) heterozygosity were calculated per locus using Cervus v. 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). FSTAT (Goudet 1995) was used to estimate and test (with 10,000 permutations) F_{ST} values between wild populations as well as between wild populations and farm individuals. GenALEx (Peakall and Smouse 2006, 2012) was used to compute the probability of identity (PI) with increasing locus combinations, i.e., the probability that two randomly chosen individuals in a population have identical genotypes.

Isolation by distance (IBD) was examined for the “wild” populations with reduced major axis (RMA) regression and Mantel test on matrices of genetic and geographic distances using IBD v.1.52 (Bohonak 2002) and 10,000 randomisations. Pairwise geographic distances between localities were calculated with der Geographic Distance Matrix Generator (Ersts 2019) and log-transformed. Genetic distance matrix ($F_{ST}/[1-F_{ST}]$; Rousset 1997) was obtained with GenePop, based on the eight polymorphic loci.

Individual assignment tests were performed to assign farm individuals to the population they have the highest probability of belonging to using a Bayesian approach

Table 1. Microsatellite primers used for multiplex reaction.

Sequence name	5' Modification	Primer Sequence (5'-3')	Genbank Accession No.
MS27-F	6-Fam	TTACACAACAACCGCCATAG	AF403219
MS27-R_mod*		TCCTTCTTATCCTGTTTACTCTGT	
KE959105.1-F	6-Fam	CACTGTTTTGGGCCATCTCC	KE959105
KE959105.1-R		GGGTTTAGGATGTGTTCTGATTCC	
KE955519.1-F_mod*	6-Fam	ATTTTAGCTGCAGGCTGTGG	KE955519
KE955519.1-R		TCTGCTAGGGCAAAACTGGG	
KE961431.1-F	6-Fam	GAAGGGAGGCCCAAATATCC	KE961431
KE961431.1-R		GAGAGACCTGGTGCAAACCC	
KE961083.1-F_mod*	6-Fam	GTCCCAAACATCCAGAGGG	KE961083
KE961083.1-R		GGATCAAACCTGGACAAGCC	
KE955203.1-F	Joe	TGCATTTTCTCTTCCACAGGG	KE955203
KE955203.1-R		ATCTTCTGGGAACCAACCC	
MS16-F	Joe	GAGTTCTGGTCTTGCTTTCG	AF403208
MS16-R		CAGGTACAACCTTCTCCAAC	
KE966557.1-F	Joe	GCCTCCTACTCAAAGGGTGG	KE966557
KE966557.1-R		CATGGGAGGCAAGGTAAGG	
MS9-F	Tamra	CAGTGGGCTTGAGATTGAC	AF403201
MS9-R_mod*		CCATTCTTAAAACTCTCACTC	
MS13-F	Tamra	AACAGAGAAGCACAAATCACC	AF403205
MS13-R_mod*		TGGCTCTCACTTGATATATTAGAAG	
MS5-F	Tamra	TAGGGTGTGAGTCATTGCTC	AF403197
MS5-R		TGGCATCCAGCAGTCATAG	

* modified primer length

according to Rannala and Mountain (1997) implemented in the program GeneClass2 (Piry et al. 2004). This method has been shown to outperform other assignment techniques, and has been specifically demonstrated to obtain high assignment accuracy for smaller sample sizes (Cornuet et al. 1999). Moreover, this assignment method has the advantage that it does not assume that the true population of origin is among the sampled populations (Manel et al. 2002). We included the eight polymorphic loci only, assumed a threshold of 0.05 and applied a Monte Carlo resampling of 100,000 simulated individuals (Paetkau et al. 2004). For broad comparison, we also performed self-assignment tests for the individuals from wild populations using the same parameter setting. We considered individuals with an assignment score > 0.7 to be assigned with confidence (Dellicour et al. 2011).

Results

MtDNA sequence information and phylogeny

The extraction of DNA from buccal swabs of 60 individual specimens from Togo proved successful; for two samples DNA extraction failed. The aligned sequence data set of these samples contained 674 bp with 13 variable characters of which 10 were parsimony informative. Translation of the gene segment revealed no frameshift muta-

tions or premature stop codons. Both BI and ML trees show that samples are split into three major clades, which, however, show no geographic structure and are only weakly differentiated (Suppl. material 1: Fig. S1). These clades are consistently recovered in the median network (Suppl. material 1: Fig. S2). Sequence comparison revealed a total of nine haplotypes, including a haplotype found by Dong and Kumazawa (2005) (AB177878); the nine haplotypes were deposited in GenBank (MN295674–82). One haplotype (hap1) was shared by all localities except Nyidove (close to the Ghanaian border); three haplotypes (hap7, hap8 and hap9) were unique to their geographic population (Ountivou, near the Benin border; Towouganou [Zio district] and Aveta), and not represented among the samples from any farm (Fig. 3). Remaining haplotypes (which differed from the most common haplotype by only 1–4 mutations) were found at moderate to low frequency (Figs 3, 4). Five among nine haplotypes (based on 21 individuals) were represented among farm animals.

Consistent with the phylogenetic trees, the haplotype network does not inform on a specific geographic structure and pairwise comparison of genetic diversity between the localities revealed no significant differences (Fig. 4, Suppl. material 1: Table S2).

Microsatellites – Genetic diversity, and individual assignment

Seven out of 60 individual DNA samples could not be amplified for any of the microsatellite loci and were excluded from subsequent analyses. At least eight microsatellites were polymorphic and four of them showed higher polymorphism with PIC values > 0.7 (Table 2). None of the loci showed indications for the presence of null alleles, large allele dropout or stuttering. The sample set from the “Adaptation farm” showed a higher number of homozygotes at locus 5-MS16. However, because these farm samples may originate from different populations, we considered this excess as uncritical for subsequent analyses. Tests for LD between all pairs of loci across populations yielded no significant genotypic disequilibrium; none of the loci deviated significantly from HWE. For polymorphic loci, the mean number of alleles was seven (2–25 alleles per locus), the observed heterozygosity ranged from 0.02 to 0.91, and the expected heterozygosity from 0.01 to 0.93. The probability of two random animals having identical genotypes was estimated at 2.0×10^{-8} (Table 2).

Information on population differentiation can ideally serve the management of genetic populations or, in our case, monitor national management regimes. The level of genetic differentiation (F_{ST}) between localities where wild individuals were sampled and specimens sampled from farms varied from 0 to 0.09 (Suppl. material 1: Table S3); none of the pairwise F_{ST} -values was significant, and there was no evidence of isolation by distance across populations ($Z = -11588.18$, $r = 0.24$, $p \leq 0.08$). Notably, F_{ST} -values among pairs that included a farm were remarkably low, indicating a potentially higher level of individuals from different populations among the farm samples that minimise the degree of genetic differentiation between farms and wild populations.

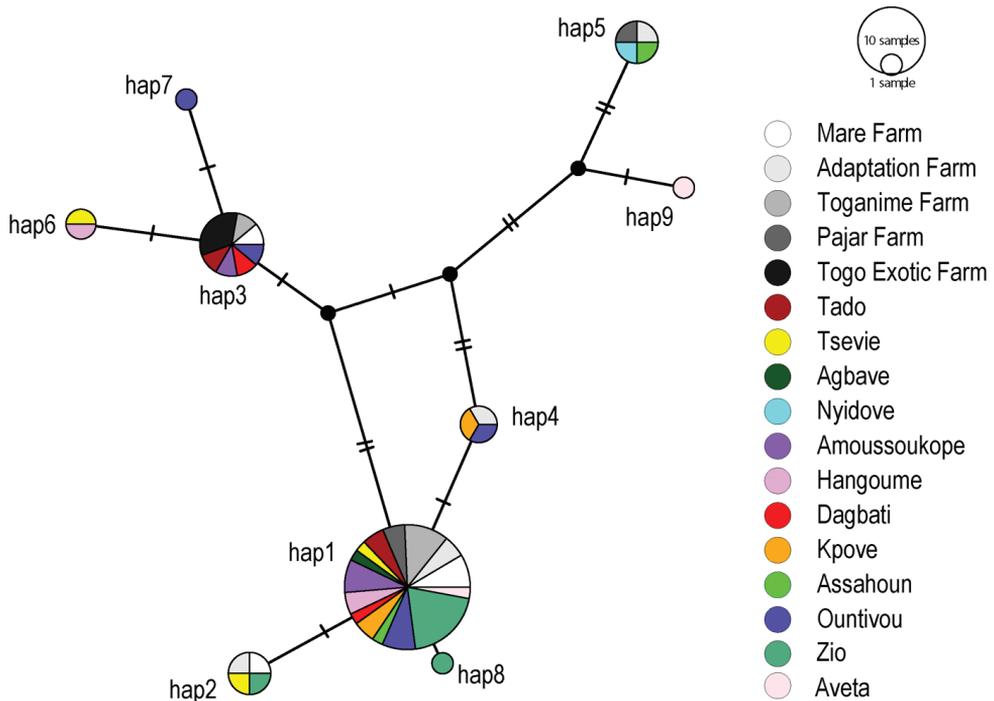


Figure 4. Haplotype network based on 674 bp of COI gene from 60 specimens of *Python regius* from Togo. Each circle represents a haplotype with its size proportional to the frequency of the haplotype. Ticks on branches connecting the haplotypes indicate nucleotide mutations. Localities are indicated by different colour.

Table 2. Genetic variability at 11 microsatellites applied in *Python regius*. Ho = observed heterozygosity; He = expected heterozygosity; PIC = polymorphism information content; PI = probability of identity for increasing locus combinations.

Locus	Repeat motif	Allelic range	No. of alleles	Mean no. of alleles	Ho	He	Missing data (%)	PIC	PI
12-MS27	(TTC)7	0	1	1	-	-	0	-	1.0
3-KE959105	(AATC)8	0	1	1	-	-	0	-	1.0
1-KE955519	(AC)90	14	8	3	0.72	0.72	0	0.68	1.2e ⁻¹
14-KE961431	(TC)10	0	1	1	-	-	0	-	1.2e ⁻¹
2-KE961083	(TTCC)13	22	12	5	0.85	0.87	3.64	0.85	3.8e ⁻³
7-KE955203	(TTC)35	35	2	1	0.02	0.02	0	0.02	3.6e ⁻³
5-MS16	(AAAG)12	30	10	4	0.75	0.82	5.56	0.78	2.3e ⁻⁴
6-KE966557	(AC)15	4	3	1	0.09	0.09	1.82	0.09	1.9e ⁻⁴
8-MS9	(AAAG)18	24	7	4	0.78	0.79	0	0.75	1.5e ⁻⁵
17-MS13	(TTTC)16	16	5	3	0.76	0.74	0	0.68	1.9e ⁻⁶
9-MS5	(TTTC)17	94	25	5	0.91	0.93	0	0.92	2.0e ⁻⁸
Mean			7	3		0.45		0.43	

Self-assignment of individuals from wild populations was successful and correct in 94% of the samples, with assignment probabilities ranging between 0.66 and 0.96 (mean 0.86); only two of these correctly allocated samples were assigned with lower confidence (0.66 – 0.70); (Suppl. material 1: Table S4). Sample MA26 (from Tsevié) was assigned with a slightly higher confidence to a different population than to the one where it was sampled from; sample MA50 (from Zio) couldn't be assigned to any of the wild population. In “real life” assignment, two of the farm individuals (MA15, MA19) could be assigned with high confidence to one of the wild reference populations, while a further five samples (MA3, MA11, MA12, MA15, MA20) were assigned with a probability > 0.6 (Suppl. material 1: Table S5).

Discussion

Severe inconsistencies in trade activities relating to this species have been previously reported from the main exporting countries in West Africa (Benin, Togo and Ghana) (see Ineich 2004, 2006; Toudonou 2003, 2004, 2007), yet despite the recognised importance of such data for sustainable management strategies, genetic variation among the species' populations has never been assessed, and molecular forensic methods for monitoring purposes have not been developed so far.

As such, our study is the first to report on the regional molecular phylogeny and genetic population differentiation of ball pythons from West Africa and to test the suitability of polymorphic microsatellites for tracking the origin of farmed individuals. Overall, these initial genetic findings from Togo indicate a relatively high mixing rate of ball pythons at the sampled localities, both within farms and wild populations, with no apparent bio-geographical trends, which may likely mirror the long-lasting anthropogenic use, and commercial trade of this species in Togo and other neighbouring range states in West Africa. However, further research to identify the degree of differentiation in non-harvested regions, and potential genetic homogenization at a larger spatial scale is required to verify this conclusively.

Ball python population genetics in Togo

There are no significant geographical or climatic barriers in the sample area and gene flow between populations of ball pythons has likely occurred over the last 10,000 years. However, unlike our study, genetic studies focussed on wild populations of other savannah inhabitant reptile species in West Africa have revealed phylogenetically distinct clades [e.g., the African helmeted turtle (*Pelomedusa subrufa*) (Vargas-Ramírez et al. 2010; Wong et al. 2010), the Puff adder (*Bitis arietans*) (Barlow et al. 2013), and the Egyptian cobra (*Naja haje*) (Trape et al. 2009)]. Likewise, phylogenetically distinct clades have also been previously reported for ball python prey species [e.g., Misonne's soft-furred mouse (*Praomys misonnei*)] (Nicolas et al. 2011).

Furthermore, stronger population subdivisions than those observed would be expected, at least across a wider geographic scale, given the low assumed dispersal capacity of ball pythons. Although an important research priority, to date field research provides some information on habitat use (Luiselli and Akani 1999), while studies on movements and home range sizes of ball pythons in West Africa are almost lacking. Little published information is currently available on daily and seasonal activities (Luiselli et al. 2007), however based on field experience and discussions with collectors in Benin, the species likely exhibits narrow home range (Toudonou, pers. comms.). Overall, it is suggested that the ball python represents a rather sedentary species (Luiselli, pers. comm.) that likely reflects other congeneric sedentary species, such as the Short-tail python (*Python brongersmai*), of which specimens have been shown to cover a mean distance of less than 200 metres in ca. 50 days (Erdelen et al. 1997, in: Auliya 2006).

There are other factors that can influence snake movement and dispersal, such as the seasonal flooding of python habitat during the wet season (Auliya 2006). Activity levels can also vary between savannah and forest populations (Luiselli and Angelici 1998), and males of congeners have reported higher activity levels when searching for females during the breeding season (Slip and Shine 1988). Although such aspects may have certainly influenced ball python population genetics in Togo, it is important to note that this species is reported to select specific microhabitats that provide numerous shelter opportunities and a constant prey source (Barker and Barker 2006).

Similarly, with regards to the ball python specimens sampled within python farms, haplotype diversity indicates that python farm ranching activity has historically targeted several populations but does not provide a clear spatial pattern or “trade chain” in this regard. The extent to which this observed lack of well-defined haplogroups is the result of regional trade activity or continuous historical gene flow/long-distance dispersal (or indeed *vice versa*) cannot be determined based on the mtDNA sequence data alone. However, it does raise a number of important questions regarding the impact of ongoing commercial trade activity on remaining wild populations.

In particular, it is currently unclear whether any of the haplotypes identified in Togo during this study actually originated and / or have current core distributions in neighbouring range States. This is a distinct possibility given that researchers have reported illegal cross-border hunting of ball pythons (de Buffrénil 1995) and other reptile species, such as the Müller’s sand boa (*Eryx muelleri*) (Vignoli et al. 2015).

Potential impacts of commercial trade on ball python populations

Our initial genetic findings line up with results obtained from a recent questionnaire-based study focused on python hunters in Togo, which reported that the collection and subsequent release of ball python ranched specimens was carried out in a relatively *ad hoc* and diffuse manner, without an effective monitoring process in place (D’Cruze et al., in prep.). Specifically, this study raised concerns that ball pythons may be released in insufficient numbers, in inappropriate habitats and geographic locations that may

be at least partly responsible for reported decreases in local wild ball python populations over the last five years (D’Cruze et al., in prep.).

It is beyond the scope of this study to assess the full impact that trade and associated ranching activity has had on the conservation status of wild ball python populations in Togo and other neighboring range states in West Africa. However, the challenges associated with implementing proper wild release protocols, and the multiple risks posed to focal species, their associated communities and ecosystem functions in both source and destination areas (including disease introduction and genetic pollution) are well known and should be mitigated (IUCN/SSC 2013).

In particular, ball pythons are known to harbour a number of infectious diseases (e.g., cryptosporidium, Yimming et al. 2016; nidoviruses, Uccellini et al. 2014) and parasites [including ticks (e.g., *Amblyomma latum*, Corn et al. 2011)]. For a recent review of the scientific literature (published between 2009 and 2019), which identified a list of nearly 150 underlying pathogens associated with this species, please see Green et al. (2020). The fact that ball pythons are being ranched under intensive commercial captive conditions prior to their release in large numbers (as has been reported in the CITES trade database), together with concerns regarding a lack of proper biosecurity measures at these facilities (D’Cruze et al., in prep.), arguably makes a higher level of monitoring and evaluation a key priority.

Moreover, the translocation of individuals of non-local origin may lead to introgression that disrupts spatial genetic structure, alters local genetic diversity, and ultimately threatens local adaptations (e.g., Söderquist et al. 2017 and references therein). Hybridisation between translocated farmed and wild individuals might also be a driver of biodiversity loss (Randi 2008; Sutherland et al. 2006; Söderquist et al. 2017).

Limitations of the genetic study

Our study provides only an initial insight into ball python genetic diversity in Togo. Yet, regardless of the low sample size per population, more than 90% of the samples from wild captures of ball python were correctly assigned to their population of origin, while 10% of farm samples could be assigned with high probability to one of the wild populations (and ca. 1/3 with a probability > 0.6). This could indicate that farms harvest populations, which have not been sampled in our study. However, the relatively low percentage of individuals assigned is similar to those reported in other studies and the limited assignment accuracy may result mainly from population pairs with a $F_{ST} < 0.05$ (Manel et al. 2002).

We also acknowledge that identifying the full population status and or exact geographic region of origin of ranched ball pythons in the wild requires a much denser sampling from a wider geographical area (including populations that have been subject to harvest in Ghana and Benin, in addition to reference samples of non-harvested populations across the species’ range), and most importantly, a higher sample size per (sub-) population (i.e., 25–30 samples; Kalinowski 2005; Hale et al. 2012). This would enable

us to identify geographic regions where substantial genetic admixture of farmed genotypes with wild populations may or may not have occurred. Moreover, when a larger sample size is applied, polymorphism may be revealed at the three additional loci (12-MS27, 3-KE959105, 14-KE961431) that were monomorphic in the samples used in this present study, which consequently may contribute “decisively” to forensic assignment.

It is important to note that larger than expected dispersal rates for this species may be partly responsible for the genetic admixtures reported in this study and more ecological studies are needed in this regard. However, based on current knowledge (cf. above), the ball python is likely a relatively sedentary species, assumed low dispersal capacity.

Recommendations

The long-term sustainability of the large-scale ranching and subsequent export of ball pythons from the main exporters in West Africa, such as Togo, is undermined by a lack of data on the status of wild ball python populations including their distribution, population trends and genetic structure. Such data is essential to effectively manage, monitor and evaluate the impact that this type of commercial trade activity may have on the conservation of this species and is an urgent priority in this regard. We recommend that future studies looking to build on our findings should aim to reduce the geographic sampling gaps to provide a denser coverage of samples over a larger area (including non-harvested regions), include samples from neighbouring range states (especially Benin and Ghana), include a higher number of samples per population/locality (25–30), and an increased number of relevant nuclear polymorphic markers (e.g., SNPs, microsatellites or SNP-STRs), to help better assign individual ball pythons to specific populations. Thus, systematic monitoring across a larger scale is needed to gain more insight of the spatial genetic population structure and the processes that are potentially associated with the uncoordinated translocation of farmed and wild individuals.

However, such research initiatives can be difficult to implement, time consuming and costly to fund (D’Cruze et al. 2009). In the interim, we recommend that Management Authorities should urgently develop a national action plan and specific release protocols for python farms to minimise any negative conservation impacts resulting from genetic pollution (whilst also minimising any risk of disease introduction). Should such efforts prove unsuccessful or unviable, additional measures such as a reduction in the number of ball pythons that are both captured and released may be required as part of a more precautionary approach. This type of action is not unprecedented; a quota reduction has already been adopted by the neighbouring range state and third largest exporter Benin, which recently reduced its ball python export quota from 45,000 ranches specimens in 2017 to 22,000 in 2019 (UNEP 2019). Whatever steps are taken, it is important to draw attention to the fact that given the cross-border harvest activity that underpins the ball python trade in West Africa, any future initiatives should not be restricted to Togo only but also extend to Benin and Ghana too (Gorzula et al. 1997; Jenkins 1998).

Conclusions

This study represents the first molecular genetic characterisation of ball pythons in Togo, one of the world's most traded snake species. Despite relatively large distances between sampled locations covering more than 12,773 km² (an estimate based on relevant district area sizes) no significant genetic population structure was identified, potentially implying a long-lasting human influence through domestic and international trade activities, or higher (long-distance) dispersal rates in ball pythons than the species' natural history would suggest. Although the ball python is not yet considered to be threatened by extinction, a modified genetic structure and a potentially associated loss of local adaptations should be nevertheless of concern from a conservation perspective. Self-assignments were correct for more than 90% of the samples from wild populations, and almost 1/3 of samples from farmed individuals could be allocated with higher probability to their potential population of origin. Although preliminary in nature, this study is the first of its kind for the ball python in West Africa. It has clearly demonstrated potential for the genetic assignment of ranched individuals that can assist management authorities with the ability to better monitor aspects of the ranching system and to trace trading activities in future.

Acknowledgements

We wish to thank the CITES Management Authorities of Togo (especially Mr Apla Yao Mawouéna and Mr. Okoumassou Kotchikpa) who facilitated access to reptile breeding farms. Thanks especially to Kinam Kombiagnou (Directeur de l'élevage, Ministère de l'agriculture, de l'élevage et de la pêche) for issuing the relevant permit. We further thank all farm owners (especially Mr. Koudeha, Emmanuel and Mrs Sambo) who accepted the examination of the specimens and the sampling of buccal swabs. Assistance from local guides was also indispensable during field work. We also thank Claudia Ertzbauer (lab manager of the ZFMK). Mark Auliya received a grant from World Animal Protection to carry out this research. Finally, we sincerely thank Agbo-Zegue NGO for providing logistics.

References

- Affo AAB (2001) Commerce international des Reptiles élevés en captivité au Togo: cas des pythons, tortues et caméléons. Unpublished training report, School for the training of wildlife specialist, Garoua, Cameroon, 1–41.
- Alexander A, Steel D, Hoekzema K, Mesnick SL, Engelhaupt D, Kerr I, Payne R, Baker CS (2016) What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)? *Molecular Ecology* 25(12): 2754–2772. <https://doi.org/10.1111/mec.13638>
- Astrin JJ, Stüben PE (2008) Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics* 22(5): 503–522. <https://doi.org/10.1071/IS07057>

- Auliya M (2006) Taxonomy, Life History and Conservation of Giant Reptiles in West Kalimantan (Indonesian Borneo). ntv-Publ., Münster, 432 pp.
- Auliya M, Schmitz A (2010) *Python regius*. The IUCN Red List of Threatened Species 2010: e.T177562A7457411. <https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T177562A7457411.en>
- Bandelt HJ, Forster P, Sykes BC, Richards MB (1995) Mitochondrial portraits of human population using median networks. *Genetics* 141: 743–753.
- Bandelt H, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16(1): 37–48. <https://doi.org/10.1093/oxford-journals.molbev.a026036>
- Barker DG, Barker TM (2006) *Pythons of the World. Volume II: Ball pythons*. VPI Library, Boerne, Texas, USA, 1–320.
- Barlow A, Baker K, Hendry CR, Pepplin L, Phelps T, Tolley KA, Wüster CE, Wüster W (2013) Phylogeography of the widespread African puff adder (*Bitis arietans*) reveals multiple Pleistocene refugia in southern Africa. *Molecular Ecology* 22(4): 1134–1157. <https://doi.org/10.1111/mec.12157>
- Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B. Methodological* 57(1): 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bohonak AJ (2002) IBD (Isolation By Distance): A program for analyses of isolation by distance. *The Journal of Heredity* 93(2): 153–154. <https://doi.org/10.1093/jhered/93.2.153>
- Botstein D, White RL, Skolnick M, Davies RW (1980) Construction of a genetic linkage map in man using restriction fragment polymorphisms. *American Journal of Human Genetics* 32: 314–331.
- Corn JL, Mertins JW, Hanson B, Snow S (2011) First reports of ectoparasites collected from wild-caught exotic reptiles in Florida. *Journal of Medical Entomology* 48(1): 94–100. <https://doi.org/10.1603/ME10065>
- Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153: 1989–2000.
- D’Cruze NC, Henson D, Olsson A, Emmett DA (2009) The importance of herpetological survey work in conserving Malagasy biodiversity: Are we doing enough? *Herpetological Review* 40: 19–25.
- de Buffrénil V (1995) Les élevages de Reptiles du Bénin, du Togo et du Ghana. Rapport d’étude réalisée pour le Secrétariat de la CITES, 1–23.
- Dellicour S, Frantz AC, Colyn M, Bertouille S, Chaumont F, Flamand MC (2011) Population structure and genetic diversity of red deer (*Cervus elaphus*) in forest fragments in north-western France. *Conservation Genetics* 12(5): 1287–1297. <https://doi.org/10.1007/s10592-011-0230-0>
- Dong S, Kumazawa Y (2005) Complete mitochondrial DNA sequences of six snakes: Phylogenetic relationships and molecular evolution of genomic features. *Journal of Molecular Evolution* 61(1): 12–22. <https://doi.org/10.1007/s00239-004-0190-9>

- Ersts PJ (2019) Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg [accessed on 2019-8-1]
- Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* 170: 56–63. <https://doi.org/10.1016/j.biocon.2013.12.036>
- Fraser DJ, Walker L, Yates MC, Marin K, Wood JLA, Bernos TA, Zastavniouk C (2018) Population correlates of rapid captive-induced maladaptation in a stream fish. *Evolutionary Applications* 12(7): 1305–1317. <https://doi.org/10.1111/eva.12649>
- Gorzula S, Nsiah WO, Oduro W (1997) Survey of the Status and Management of the Royal Python (*Python regius*) in Ghana. Part 1. Report to the Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Geneva, Switzerland, 1–38.
- Goudet J (1995) FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *The Journal of Heredity* 86(6): 485–486. <https://doi.org/10.1093/oxfordjournals.jhered.a111627>
- Green J, Coulthard E, Megson D, Norrey J, Norrey L, Rowntree JK, Bates J, Dharmapaul B, Auliya M, D’Cruze N (2020) Blind trading: A literature review of research addressing the welfare of ball pythons in the exotic pet trade. *Animals (Basel)* 10(2): 193. <https://doi.org/10.3390/ani10020193>
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48(2): 361–372. <https://doi.org/10.2307/2532296>
- Hale ML, Burg TM, Steeves TE (2012) Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS One* 7(9): e45170. <https://doi.org/10.1371/journal.pone.0045170>
- Harris M (2002) Assessment of the status of seven Reptile species in Togo. Report to the Commission of the European Union, réf. EC 9810072: 1–58. http://jncc.defra.gov.uk/pdf/togo_sevenreptilespeciesvpt1.pdf
- Harwood J (2003) West African Reptiles: species status and management guidelines for Reptiles in international trade from Benin and Togo. Report to the European Commission prepared for the European Commission, Directorate General E – Environment, ENV E.3 – Development and Environment, January 2003, UNEP-WCMC, i–v+1–51.
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23(2): 254–267. <https://doi.org/10.1093/molbev/msj030>
- Ineich I (2004) Les élevages de reptiles et de scorpions au Bénin et au Togo, plus particulièrement la gestion des quotas d’exportation et la définition des codes “source” des spécimens exportés. Rapport d’étude réalisée pour le Secrétariat de la CITES, report to UNEP-CITES Secretary, 1–81.
- Ineich I (2006) Les élevages de reptiles et de scorpions au Bénin, Togo et Ghana, plus particulièrement la gestion des quotas d’exportation et la définition des codes ‘source’ des spécimens exportés. Rapport d’étude réalisée pour le Secrétariat de la CITES. Projet CITES A-251: 1–113.
- IUCN/SSC (2013) Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii+57 pp.

- Jenkins RWG (1998) Management and use of *Python regius* in Benin & Togo. Report prepared for Directorate General XI, The Commission of the European Union (doc. SRG 8/5/3), 1–11.
- Jordan PW, Goodman AE, Donnellan S (2002) Microsatellite primers for Australian and New Guinean pythons isolated with an efficient marker development method for related species. *Molecular Ecology Notes* 2(1): 78–82. <https://doi.org/10.1046/j.1471-8286.2002.00135.x>
- Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. *Trends in Plant Science* 14(1): 51–58. <https://doi.org/10.1016/j.tplants.2008.10.002>
- Kalinowski ST (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? *Heredity* 94(1): 33–36. <https://doi.org/10.1038/sj.hdy.6800548>
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16(5): 1099–1106. <https://doi.org/10.1111/j.1365-294X.2007.03089.x>
- Lanfear R, Calcott B, Ho SY, Guindon S (2012) Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29(6): 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Lanfear R, Calcott B, Kainer D, Mayer C, Stamatakis A (2014) Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* 14(1): 1–14. <https://doi.org/10.1186/1471-2148-14-82>
- Lawrence ER, Benavente JN, Matte JM, Marin K, Wells ZRR, Bernos TA, Krasteva N, Habrich A, Nessel GA, Koumrouyan RA, Fraser DJ (2019) Geo-referenced population-specific microsatellite data across American continents, the MacroPopGen Database. *Scientific Data* 6(14). <https://doi.org/10.1038/s41597-019-0024-7>
- Leigh JW, Bryant D (2015) Popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6(9): 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Luiselli L, Akani GC (1999) Habitats of snakes in the rainforests of Eket (Akwa-Ibom State, south-eastern Nigeria) (Squamata: Serpentes). *Herpetozoa (Wien)* 11: 99–107.
- Luiselli L, Angelici FM (1998) Sexual size dimorphism and natural history traits are correlated with intersexual dietary divergence in royal pythons (*Python regius*) from the rainforests of southeastern Nigeria. *The Italian Journal of Zoology* 65(2): 183–185. <https://doi.org/10.1080/11250009809386744>
- Luiselli L, Akani GC, Eniang EA, Politano E (2007) Comparative ecology and ecological modeling of sympatric pythons, *Python regius* and *Python sebae*. In: Henderson RW, Powell R (Eds) *Biology of the Boas and Pythons*. Eagle Mountain Publ., LC, Eagle Mountain, 89–100.
- Manel S, Berthier P, Luikart G (2002) Detecting Wildlife Poaching: Identifying the Origin of Individuals with Bayesian Assignment Tests and Multilocus Genotypes. *Conservation Biology* 16(3): 650–659. <https://doi.org/10.1046/j.1523-1739.2002.00576.x>
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7(5): 639–655. <https://doi.org/10.1046/j.1365-294x.1998.00374.x>

- McGraw SN, Keeler SP, Huffman JE (2012) Forensic DNA Analysis of Wildlife Evidence. In: Huffman JE, Wallace JR (Eds) *Wildlife Forensics – Methods and Applications*, John Wiley & Sons, Ltd, Sussex, 253–269. <https://doi.org/10.1002/9781119953142.ch13>
- Nicolas V, Missouf AD, Denys C, Peterhans JK, Katuala P, Couloux A, Colyn M (2011) The roles of rivers and Pleistocene refugia in shaping genetic diversity in *Praomys misonnei* in tropical Africa. *Journal of Biogeography* 38(1): 191–207. <https://doi.org/10.1111/j.1365-2699.2010.02399.x>
- Ogden R (2012) DNA Applications and Implementation. In: Huffman JE, Wallace JR (Eds) *Wildlife Forensics – Methods and Applications*. John Wiley & Sons, Ltd, Sussex, 271–289. <https://doi.org/10.1002/9781119953142.ch14>
- Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: A simulation-based exploration of accuracy and power. *Molecular Ecology* 13(1): 55–65. <https://doi.org/10.1046/j.1365-294X.2004.02008.x>
- Palsbøll PJ, Zachariah Peery M, Olsen MT, Beissinger SR, Bérubé M (2013) Inferring recent historic abundance from current genetic diversity. *Molecular Ecology* 22(1): 22–40. <https://doi.org/10.1111/mec.12094>
- Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6(1): 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall R, Smouse PE (2012) GenALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics (Oxford, England)* 28(19): 2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. *The Journal of Heredity* 95(6): 536–539. <https://doi.org/10.1093/jhered/esh074>
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- Randi E (2008) Detecting hybridization between wild species and their domesticated relatives. *Molecular Ecology* 17: 285–293. <https://doi.org/10.1111/j.1365-294X.2007.03417.x>
- Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* 94(17): 9197–9221. <https://doi.org/10.1073/pnas.94.17.9197>
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and Ecumenicism. *The Journal of Heredity* 86(3): 248–249. <https://doi.org/10.1093/oxfordjournals.jhered.a111573>
- Robinson JE, Griffiths RA, St. John FAV, Roberts DL (2015) Dynamics of the global trade in live reptiles: shifting trends in production and consequences for sustainability. *Biological Conservation* 184: 42–50. <https://doi.org/10.1016/j.biocon.2014.12.019>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219–1228.

- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution* 34(12): 3299–3302. <https://doi.org/10.1093/molbev/msx248>
- Saladin B, Thuiller W, Graham CH, Lavergne S, Maiorano L, Salamin N, Zimmermann NE (2019) Environment and evolutionary history shape phylogenetic turnover in European tetrapods. *Nature Communications* 10(1): 249. <https://doi.org/10.1038/s41467-018-08232-4>
- Sarre SD, Georges A (2009) Genetics in conservation and wildlife management: A revolution since Caughley. *Wildlife Research* 36(1): 70–80. <https://doi.org/10.1071/WR08066>
- Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution* 22(1): 25–33. <https://doi.org/10.1016/j.tree.2006.08.009>
- Segniagbeto GH (2016) Study on four species of fauna subject to international trade in Togo 1–51. <https://cites.org/sites/default/files/eng/com/sc/67/E-SC67-15-A3.pdf>
- Segniagbeto GH, Trape JF, David P, Ohler AM, Dubois A, Glitho IA (2011) The snake fauna of Togo: Systematics, distribution, and biogeography, with remarks on selected taxonomic problems. *Zoosystema* 33(3): 325–360. <https://doi.org/10.5252/z2011n3a4>
- Shaney KJ, Adams RH, Kurniawan N, Hamidy A, Smith EN, Castoe TA (2016) A suite of potentially amplifiable micro-satellite loci for reptiles of conservation concern from Africa and Asia. *Conservation Genetics Resources* 8(3): 307–311. <https://doi.org/10.1007/s12686-016-0534-y>
- Slip DJ, Shine R (1988) Habitat Use, Movements and Activity Patterns of Free-Ranging Diamond Pythons, *Morelia spilota spilota* (Serpentes: Boidae): A Radiotelemetric Study. *Australian Wildlife Research* 15(5): 515–531. <https://doi.org/10.1071/WR9880515>
- Söderquist P, Elmberg J, Gunnarsson G, Thulin CG, Champagnon J, Guillemain M, Kreisinger J, Prins HHT, Crooijmans RPMA, Kraus RHS (2017) Admixture between released and wild game birds: A changing genetic landscape in European mallards (*Anas platyrhynchos*). *European Journal of Wildlife Research* 63(98). <https://doi.org/10.1007/s10344-017-1156-8>
- Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Sutherland WJ, Armstrong-Brown S, Armsworth PR, Tom B, Brickland J, Campbell CD, Chamberlain DE, Cooke AI, Dulvy NK, Dusic NR, Fitton M, Freckleton RP, Godfray HCJ, Grout N, Harvey HJ, Hedley C, Hopkins JJ, Kift NB, Kirby J, Kunin WE, Macdonald DW, Marker B, Naura M, Neale AR, Oliver T, Osborn D, Pullin AS, Shardlow MEA, Showler DA, Smith PL, Smithers RJ, Solandt JL, Spencer J, Spray CJ, Thomas CD, Thompson J, Webb SE, Yalden DW, Watkinson AR (2006) The identification of 100 ecological questions of high policy relevance in the UK. *Journal of Applied Ecology* 43(4): 617–627. <https://doi.org/10.1111/j.1365-2664.2006.01188.x>
- Toudonou ASC (2003) Diversité des serpents dans les agrosystèmes et leur importance socio-culturelle au sud et centre Bénin. Mémoire pour l'obtention du diplôme d'Ingénieur Agro-

- nome option: Aménagement et Gestion des Ressources Naturelles, Faculté des Sciences Agronomiques (FSA), Université d'Abomey Calavi, 1–85.
- Toudonou ASC (2004) Ball Python *Python regius*. <https://cites.org/sites/default/files/eng/com/ac/28/Inf/E-AC28-Inf-04.pdf>
- Toudonou ASC (2007) Exploitation and conservation of ball python (*Python regius*) in Benin. Thesis for obtaining Master of Science degree, major: Management & Planning of Natural resources- Speciality: Herpetology, Faculty of Agronomic Sciences (FSA), University of Abomey Calavi, 1–53.
- Trape JF, Chirio L, Broadley DG, Wüster W (2009) Phylogeography and systematic revision of the Egyptian cobra (Serpentes: Elapidae: *Naja haje*) species complex, with the description of a new species from West Africa. *Zootaxa* 2236(1): 1–25. <https://doi.org/10.11646/zootaxa.2236.1.1>
- Uccellini L, Ossiboff RJ, de Matos RE, Morrisey JK, Petrosov A, Navarrete-Macias I, Jain K, Hicks AL, Buckles EL, Tokarz R, McAloose D, Lipkin WI (2014) Identification of a novel nidovirus in an outbreak of fatal respiratory disease in ball pythons (*Python regius*). *Virology Journal* 11(1): 1–6. <https://doi.org/10.1186/1743-422X-11-144>
- Uetz P, Freed P, Hošek J (2019) The Reptile Database. <http://www.reptile-database.org>
- UNEP (2019) The Species+ Website. Nairobi, Kenya. Compiled by UNEP-WCMC, Cambridge, UK. Available at: www.speciesplus.net [accessed 15/07/2019]
- UNEP-WCMC (2010) Review of species/country combinations subject to long-standing import suspensions: reptile species from Africa. UNEP-WCMC, Cambridge.
- UNEP-WCMC (2014) Review of *Python regius* from Togo (source R). UNEP-WCMC, Cambridge. http://ec.europa.eu/environment/cites/pdf/reports/SRG%2068_7%20Review%20of%20Python%20regius%20from%20Togo%20_public_.pdf
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. *Molecular Ecology Notes* 4(3): 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Vargas-Ramírez M, Vences M, Branch WR, Daniels SR, Glaw F, Hofmeyr MD, Kuchling G, Maran J, Papenfuss TJ, Široký P, Vieites DR, Fritz U (2010) Deep genealogical lineages in the widely distributed African helmeted terrapin: evidence from mitochondrial and nuclear DNA (Testudines: Pelomedusidae: *Pelomedusa subrufa*). *Molecular Phylogenetics and Evolution* 56(1): 428–440. <https://doi.org/10.1016/j.ympev.2010.03.019>
- Vignoli L, Segniagbeto GH, Eniang EA, Hema E, Petrozzi F, Akani GC, Luiselli L (2015) Aspects of natural history in a sand boa, *Eryx muelleri* (Erycidae) from arid savannahs in Burkina Faso, Togo, and Nigeria (West Africa). *Journal of Natural History* 50(11–12): 749–758. <https://doi.org/10.1080/00222933.2015.1082659>
- Wong RA, Fong JJ, Papenfuss TJ (2010) Phylogeography of the African Helmeted Terrapin, *Pelomedusa subrufa*: Genetic Structure, Dispersal, and Human Introduction. *Proceedings of the California Academy of Sciences* 61: 575–585.
- Yimming B, Pattanatanang K, Sanyathitiseree P, Inpankaew T, Kamyngkird K, Pinyopanuwat N, Chimnoi W, Phasuk J (2016) Molecular Identification of *Cryptosporidium* Species from Pet Snakes in Thailand. *Korean Journal of Parasitology* 54(4): 423–429. <https://doi.org/10.3347/kjp.2016.54.4.423>

Supplementary material I

Supplementary tables and figures

Authors: Mark Auliya, Sylvia Hofmann, Gabriel H. Segniagbeto, Délagnon Assou, Delphine Ronfot, Jonas J. Astrin¹, Sophia Forat, Guillaume Koffivi K. Ketoh, Neil D'Cruze

Data type: molecular data

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/natureconservation.38.49478.suppl1>

Do both habitat and species diversity provide cultural ecosystem services? A trial using geo-tagged photos

Takeshi Osawa¹, Yusuke Ueno^{2,4}, Takaaki Nishida^{3,5}, Jun Nishihiro^{4,6}

1 Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, Minami-Osawa 1-1, Hachioji, Tokyo, 192-0397, Japan **2** Ishikawa Prefectural University, 1-308, Suematsu, Nonoichi-shi, Ishikawa-ken, 921-8836, Japan **3** Mitsubishi UFJ Research and Consulting Co., 5-11-2, Toranomom, Minato-ku, Tokyo 105-8501, Japan **4** Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan **5** Kyoto Sangyo University, Motoyama, Kamigamo, Kita-ku, Kyoto, 603-8555, Japan **6** Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, 305-8506, Japan

Corresponding author: Takeshi Osawa (arosawa@gmail.com)

Academic editor: Yu-Pin Lin | Received 14 May 2019 | Accepted 9 March 2020 | Published 18 March 2020

<http://zoobank.org/9A91F516-1C39-4E4B-B645-EA4C9313B069>

Citation: Osawa T, Ueno Y, Nishida T, Nishihiro J (2020) Do both habitat and species diversity provide cultural ecosystem services? A trial using geo-tagged photos. *Nature Conservation* 38: 61–77. <https://doi.org/10.3897/natureconservation.38.36166>

Abstract

Conservation for both biodiversity and ecosystem services are an important issue worldwide. However, knowledge of their relationship remains limited. As habitat structure is strongly related to regional biodiversity, we studied cultural ecosystem services by using habitat structure as a proxy for biodiversity. Specifically, we used human preference, assessed by using photos with location information (i.e. geo-tagged digital pictures) as an index of a cultural ecosystem service. We conducted nature walks in semi-natural environments for cognitively-impaired students from a local special school and studied the photos they took during the walks. We analysed the habitat preferences inferred from the photo locations and the composition of the photos—whether they were close-up, scenic or landscape views. The results showed that levels of human preference and biodiversity, indicated by habitat structure, had a positive relationship. During spring to autumn, when levels of biological activity are higher, people tended to show more preference in close-up views (i.e. the subject of the photo was the species itself). In winter, they tended to be interested in scenic views that were not strongly influenced by species diversity. Additionally, photos taken in areas with threatened species almost always included close-up views, although not of the threatened species themselves. Areas with high species diversity therefore appeared to be more appealing to the participants. These results suggest that habitat diversity could not only contribute to biodiversity, but also provide cultural ecosystem services. Habitat conservation for semi-natural environments could be synergised for both biodiversity conservation and general human well-being.

Keywords

Cultural services, habitat type, human interest, human-biodiversity relationships, non-material benefits, phenology

Introduction

Ecosystem services which provide benefits to people from ecosystems are important to human societies and to economies (Millennium Ecosystem Assessment 2005; Mace et al. 2012). Fostering a broad range of ecosystem services has become a dominant environmental paradigm that has opened up important conservation opportunities around the world (de Groot et al. 2010; Plieninger et al. 2013; Castro et al. 2014). However, as is the case with biodiversity, ecosystem services are declining worldwide, spurring scientists and policy-makers to act together to identify effective policy solutions (Mace et al. 2012; Balvanera et al. 2014; Boerema et al. 2017). Identifying the impacts of biodiversity and detecting ecosystem services on human well-being and making appropriate conservation efforts are crucial to addressing social requirements.

Motivations for the conservation of the natural environment differ (Mace et al. 2012; Mori 2017). For example, biologists and bird watchers may be primarily interested in biodiversity itself, whereas others may be more concerned with beautiful scenery or clean air (i.e. non-biological components) (Cumming and Maciejewski 2017). Realistically, setting conservation goals amongst such varied stakeholders is difficult because of their diverse motivations and/or preferences (Martin-López et al. 2012; Van Berkel and Verburg 2014; Garrido et al. 2017). This difficulty acts as a barrier to promoting biodiversity conservation activities by society as a whole, that is, activities that internalise the social–ecological systems. Approaches that could integrate such diverse motivations should be a central theme in practical conservation planning. In this context, illustrating the key role of biodiversity as a provider of general human well-being through ecosystem services is a good solution to mainstream the concept of biodiversity conservation. Studies evaluating biodiversity, including functional diversity and ecosystem services, have been extensively conducted and discussed in the past decade (Tilman et al. 2006; Mace et al. 2012; Cadotte 2013; Doi et al. 2013; Balvanera et al. 2014; Mori 2017; Schwarz et al. 2017; Thompson et al. 2018). However, an understanding of the relationships between biodiversity and ecosystem services remains limited (Cardinale et al. 2012; Balvanera et al. 2014; Boerema et al. 2017; Schwarz et al. 2017).

Physical structures (i.e. habitat structures) are strongly related to regional biodiversity and/or ecosystem functions and are relatively easy to observe; thus, they are often used as a proxy to evaluate biodiversity (Osawa et al. 2010a, b; Marull et al. 2015). If we could also detect ecosystem services derived from habitat structures that are likely to produce high biodiversity and/or ecosystem functions, it would be useful in integrating motivations for conservation. In this study, we tried to identify human well-being that is derived from habitats and their diversity and that relate to regional biodiversity and/

or ecosystem functions, focusing on cultural services, defined as non-material benefits derived from human–ecosystem relationships (Chan et al. 2011, 2012a). Non-material services, including cultural services, do not exclude other functions or services; thus, identifying a cultural service (i.e. an additional value) could contribute to options for the future development of a target ecosystem (Chan et al. 2012b; Van Berkel and Verburg 2014). However, these types of ecosystem services are difficult to evaluate because their value is non-economic (Chan et al. 2012a; Boerema et al. 2017; Schwarz et al. 2017; Gomes et al. 2018). To identify these services, we used human-interest photos tagged with location information (i.e. geo-tagged pictures), which have been previously suggested as potential indices of cultural services (Martínez Pastur et al. 2016; Yoshimura and Hiura 2017). Generally, people take a photo for objects in which they have a positive interest i.e. preferences. The Millennium Ecosystem Assessment (2005) categorised cultural services as mainly seven: *spiritual and religious, recreation and ecotourism, aesthetic, inspirational, sense of place, cultural heritage* and *educational* (Millennium Ecosystem Assessment 2005; Hernández-Morcillo et al. 2013) that almost of all categories would relate to human interests. Especially, *recreation and ecotourism* and *sense of place* are likely to relate directly with human interests i.e. preferences. Actually, previous studies indicated that revealed preference techniques have been useful in estimating the ecosystem service that is difficult to evaluate directly (Sherrouse et al. 2011; Casado-Arzuaga et al. 2014; Van Berkel and Verburg 2014).

In this study, we tried to assess the relationship between habitat structure and human interests i.e. their preferences using geo-tagged photos, with a focus on detecting ecosystem services derived from biodiversity-related components. A previous study indicated that people preferences are useful for assessing the demand side of the ecosystem services (Casado-Arzuaga et al. 2014). People demand is important for assessing a cultural service because cultural services are not solely a function or a one-way flow from natural ecosystems to people, but they are co-generated through the interaction of people and the environment (Casado-Arzuaga et al. 2014). We predicted that there would be positive relationships between the variety of habitat structures (as an index of biodiversity) and human interest i.e. preferences. Specifically, people do not prefer areas with a very simple habitat structure (i.e. those with low species diversity) because inherently human interests should be variety. Dallimer et al. (2012) indicated that the natural environment has generally been treated as uniform, such as green space, when testing non-material human well-being; thus, incorporating habitat structure could provide a new insight for this field. We also predicted that phenology would influence human interest, that is, there might be differences between seasons with high biological activity and those with low activity. Phenology also could cause habitat differences with time which could influence human interests. Thus, we predicted habitat diversity could contribute to both biodiversity and human well-being simultaneously with different mechanisms. This idea could expand our understanding of relationships between natural phenomena and non-material human well-being, so we tested these hypotheses in a semi-natural area.

Methods

Study area

The study was conducted in the Asabata Yusuichi retarding pond, Shizuoka Prefecture, Japan (35.02N, 138.40E, 55 ha; Fig. 1). This area is about a 30-minute drive from Shizuoka Station, a central station in the Prefecture. The terrain of this area is flat; thus, it has a relatively high risk of flooding. In fact, the area experienced heavy rains in 1974 known as “Tanabata gouu” and heavy flooding occurred. After this disaster, the Asabata Yusuichi retarding pond was constructed to reduce the risk of future flooding.

A nature restoration project managed by local governments has been continuing in the area since 2008 because it contains not only the pond but also several other types of habitats, such as wetland, grassland and secondary forest, containing several threatened species (Shizuoka Prefecture, Shizuoka City Council on Asabata Yusuichi 2008). After the project was established, resident groups were created to discuss the conservation, sustainable use and applicable management of natural resources, in collaboration with a wide range of stakeholders (<http://asabata.org/>, in Japanese, accessed on 20 Jan 2020). One of the resident groups aims to collaborate with a special school to support student studies and their independent living (<http://asabata.org/shien/%E3%83%99%E3%83%BC%E3%83%86%E3%83%AB%E9%BA%BB%E6%A9%9F%E9%83%A8%E4%BC%9A%E3%81%AE%E5%8F%96%E3%82%8A%E7%B5%84%E3%81%BF/>, in Japanese, accessed on 20 Jan 2020).

Data collection

We conducted nature walks in a semi-natural environment for participants and cognitively-impaired students from a local special school. We used photos taken by the participants during the walk events to evaluate human interest. Before conducting the analysis, we obtained permission and agreements with teachers and legal guardians for using these photos. We collected photos taken by the cognitively-impaired students who were unlikely to have any bias for ecosystem services. Thus, we predicted that the interests of non-impaired people would probably be influenced by lectures at nature walk events. For example, if an event lecturer is familiar with birds, then participants may tend to focus on birds. Alternatively, non-impaired participants might read brochures or reports published by the nature restoration project before the event and may focus on the threatened species in this area. Cognitively-impaired students would be unlikely to be biased. The special school is located near Asabata Yusuichi (Fig. 1) and collaborates with the nature restoration project, as described above, so the students often visit the area. Students are from class grades ranging from primary to high school (with ages about 7–18 years). We conducted a number of nature walks with students from 2015 to 2017 and collected photos from seven of these walks. During

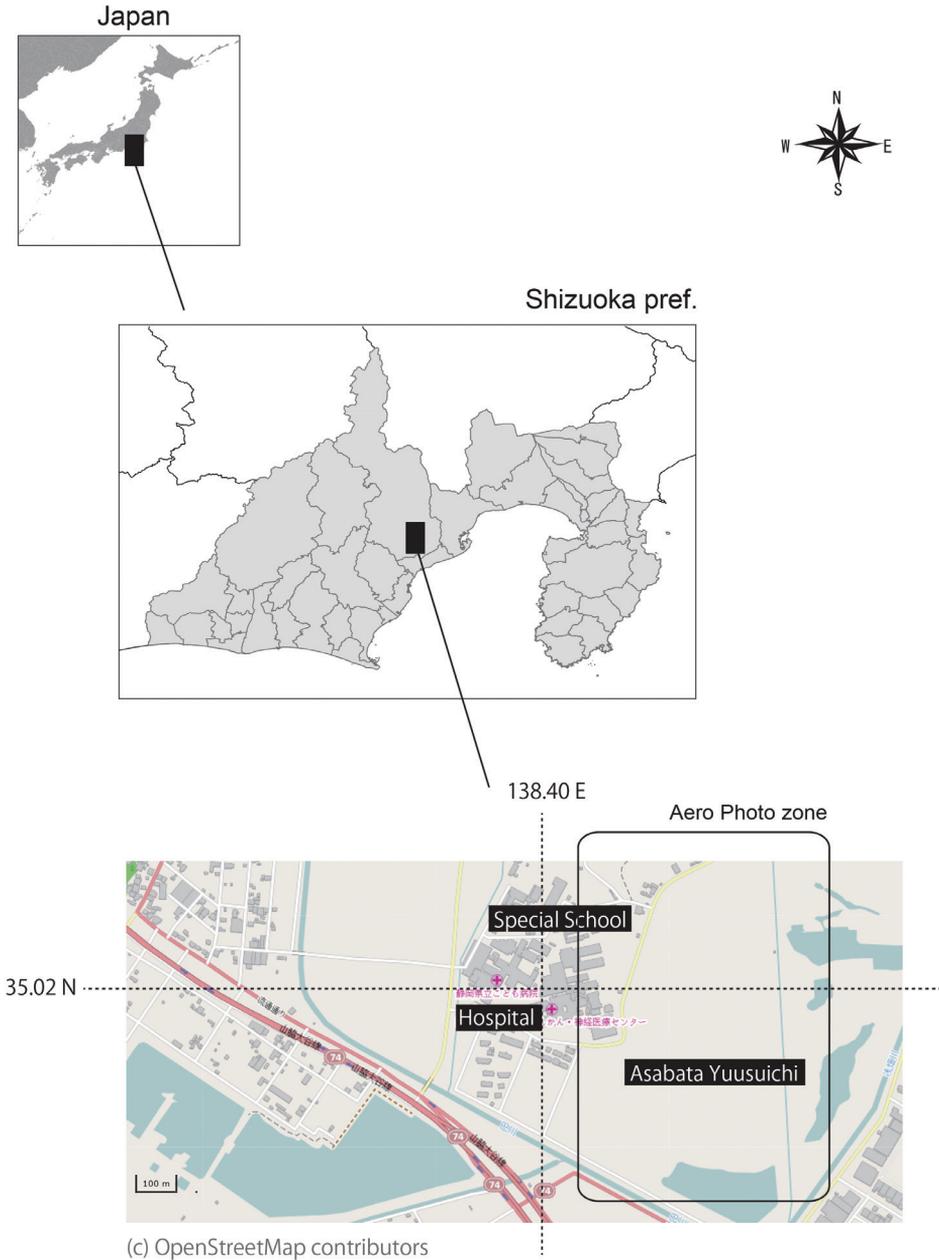


Figure 1. Study area and some land marks in this study. We conducted the study in the part of Asabana Yuuichi.

the walks, students took photos of areas or things that interested them using digital cameras with geo-tagging capability (Ricoh WG-4). We did not use photos without attached geo-tags for this analysis.

Definition of picture composition

We defined three types of picture compositions: close-up, scenery and landscape. If the subject in a photo was a single species or more than one species close enough to touch another, it was classified as a close-up view. If the subject was scenery that could be reached easily on foot, such as a grassland or a full view of a tree or stand, it was classified as a scenic view. If the subject was a landscape that could not be reached easily on foot, such as a mountain, it was classified as a landscape view. Typical pictures from each view are shown in Suppl. material 1: Fig. S1. Close-up views might be the most influenced by biological activity because people can define the target species, colour and shapes, while both scenic and landscape views would be less so, since wide-angle compositions tend to include non-biological objects. Before categorising the photos, we excluded some pictures that obviously overlapped with respect to objects and temporally adjacent time stamps.

Land cover, habitat classification and field survey

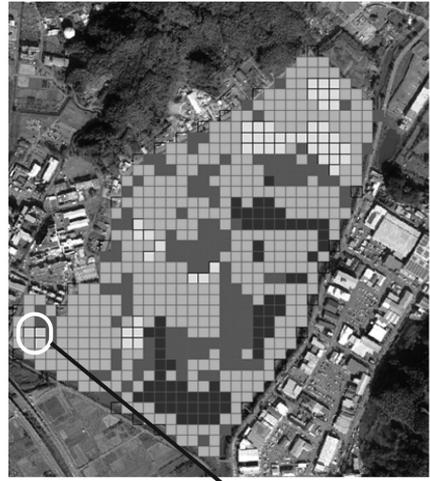
We used an aerial photograph taken in December 2016 to map the current land cover (Fig. 2a), which we classified as bare ground, forest, grassland, open water or paddy field according to our interpretation of the aerial photographs and ground-truth fieldwork. We used the land-cover types as an index of habitat and diversity. To create the land-cover map, we used GIS polygon data based on the photographs and conducted ground-truth fieldwork to evaluate the accuracy of the classifications. In this area, people can access all land-cover types, even the forest, because impenetrable vegetation is rarely present. We also developed GIS line data for the walking path. We divided Asabata Yusuichi into 30-m-square cells and assigned each grid to a category according to its dominant land cover class (Fig. 2b). We also categorised the cells according to whether they did or did not contain a walking path (Fig. 2c). We used these cells as analysis units to accommodate errors in the latitude/longitude coordinate values in the photograph metadata. We overlaid the photo location information and the land-cover map, assigned a dominant land-cover type to each photo location and noted whether or not the grid contained a walking path.

We also conducted a field survey from 2015 to 2017 to find and identify threatened plant species in the national Red List (<http://www.env.go.jp/press/103881.html>, in Japanese, accessed on 20 Jan 2020) and the prefectural Red Data Book (http://www.pref.shizuoka.jp/kankyoku/ka-070/wild/red_replace.html, in Japanese, accessed on 20 Jan 2020). If we found a threatened species, we photographed it with the same camera type as used by the study participants to obtain location information. We defined each cell that contained one or more threatened species as an important habitat area (Fig. 2d). To avoid any potential intrusion by plant collectors, we do not show detailed location information for the threatened plants.

(a) Aerial photograph in 2016



(b) 30-m resolution land cover map



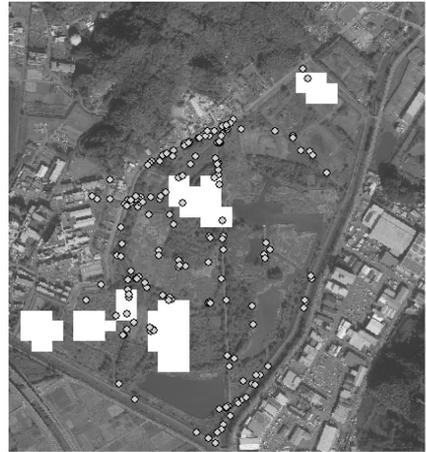
- Open water
 - Forest
 - Grassland
 - Bare ground
 - Paddy area
- Paddy area (3 grids)

(c) Map with points of shooting picture



Walking path

(d) 30-m grids with threaten plants



○ Points of shooting picture

Figure 2. Aerial photograph, land cover mesh map and location of shooting photos in the study area. Mesh size is 30 m square.

Species diversity

We conducted a field survey, a literature search and a public hearing to evaluate species diversity in each land-cover type. We focused on plants, birds, butterflies and dragonflies, which are generally well-known taxa in Japan. We collected data on fauna and flora and their habitat requirements of each species from published sources (Takagawa et al. 2011; Ozono et al. 2012) and web resources (<http://www.insects.jp>, in Japanese, accessed on 20 Jan 2020). If a species had two or more major habitats, we recorded them all. Therefore, the total number of habitats and the total number of species are not equal in some cases. All the species and their required habitats are shown in Suppl. material 3: Tables S1–S4.

Analysis

We tested each photographer's preference for land-cover type (i.e. land cover) with Fisher's test in the following manner. The ratio of the number of photos of a particular land cover to the total number of photos taken was calculated for each land cover. This ratio was compared with the ratio of the total number of cells containing the land cover to the total number of all cells. A land cover was deemed to be "preferred" if the ratio of the number of photos was significantly larger than that of all cells; conversely, it was considered "not preferred" if the ratio of the number of photos was significantly smaller than that of all cells. We conducted this analysis for the entire area and for the walking-path cells only to account for the accessibility in the cells. We also compared differences in photo compositions from spring to autumn (March to October), which has high levels of biological activity, with those from winter (November to February), when biological activity is low. Additionally, we counted the preferred picture composition in cells that contained threatened plant species. All analyses were conducted using R software v. 3.1.2 (<https://www.r-project.org/>, accessed on 20 Jan 2020).

Results

In total, 630 land cover cells were classified, of which 308 contained a walking path (Table 1). The ratio of cells containing a walking path did not bias any land-cover type ($p > 0.05$, Fisher's test on the ratio of the land cover types between all cells and those with a walking path), excluding open water ($p < 0.001$, Fisher's test). This result was expected because there were no bridges over the water. Thus, the walking-path effects did not matter with respect to the land-cover type in each photo. Eighty cells (27 with a walking path) contained at least one threatened plant species. We analysed 232 photos (153 grassland, 58 forest, 15 bare ground and 6 open water): 126 were close-ups, 100 were scenic views and 6 were landscapes (Table 1). In terms of land cover preference, the student photographers tended not to prefer open water ($p < 0.001$), but there were no land cover preference trends for the other land-cover types (Table 1).

Table 1. Number of 30 m land cover (LC) cells for each LC type and number of photos and type of composition. Numbers in parentheses indicate cells with a walking path or the number of photos taken in a cell with a walking path.

Land cover	LC mesh number	Picture number	Close-up	Scenery	Landscape
Grassland	374(202)	153(125)	77(63)	72(59)	4(3)
Forest	138(77)	58(55)	32(31)	24(22)	2(2)
Bare ground	47(19)	15(15)	13(13)	2(2)	0(0)
Open water	68(9)	6 ^{***} (0 [*])	4 [*] (0)	2 ^{**} (0)	0(0)
Paddy area	3(1)	0(0)	0(0)	0(0)	0(0)
Total	630(308)	232(195)	126(107)	100(83)	6(5)
Threatened plants	80(27)	17(10)	11(6)	6(4)	0(0)

Fisher's test *: $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Number of species and their primary habitats. Numbers in bold indicate dominant habitat for the species group. Bird habitat was divided into breeding and overwintering seasons.

Main habitat	Plant	Bird (breeding)	Bird (overwinter)	Butterfly	Dragonfly
Grassland	344	51	74	15	1
Forest	25	80	61	10	24
Wetland (paddy field)	110	73	118	0	24
Pond, bog (open water)	24	52	102	0	38
Other	10	62	129	11	24
Total number of species	513	219	219	23	49

Species number were derived from field survey, literature search, and public hearing. Habitat information were derived from published sources (Ozono et al. 2012; Takagawa et al. 2011) and web resources (<http://www.insects.jp>, in Japanese)

Table 3. Timing of the nature walks and number of total photos and each type of composition.

Year	Month	Season	Total	Close-up	Scenery	Landscape
2015	May	Spring	66	49	16	1
	June	Summer	44	35	8	1
	September	Autumn	36	28	8	0
	October	Autumn	1	1	0	0
2016	December	Winter	40	8	29	3
2017	January	Winter	37	4	32	1
	April	Spring	8	1	7	0
	Total		232	126	100	6

The numbers of plant, bird, butterfly and dragonfly species in each main habitat, which were derived from a field survey, a literature search and a public hearing, are shown in Table 2. The grassland was dominant for plants and butterflies. A pond/bog (open water) was the main habitat for dragonflies. The forest was the primary habitat for birds during the breeding season (spring–autumn), whereas a wetland (paddy field) was their main winter habitat.

Most of the photos taken from spring to autumn were close-ups, whereas almost every photo taken in winter was of scenery (Fig. 3, Table 3). Landscape views were rarely taken in either period.

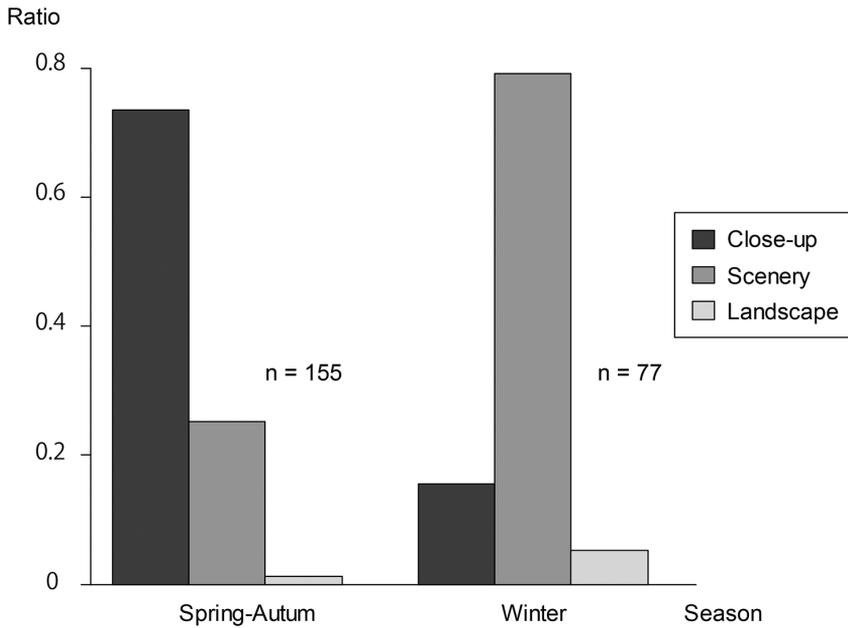


Figure 3. Barplot of the ratio of photo composition between spring to autumn and winter seasons.

Table 4. Composition of photos taken in cells that contained identified threatened plant species. Numbers in parentheses indicate cells with a walking path.

Seasons	Total number	Close-up	Scenery	Landscape
Spring, Summer and Autumn	12 (7)	11 (6)	1 (1)	0 (0)
Winter	5 (3)	0 (0)	5 (3)	0 (0)

We found 14 threatened plants in the study area (Suppl. material 3: Table S5). The composition of pictures taken in the cells containing threatened plants showed a clear trend. In spring to autumn, 11 of 12 photos were close-ups, whereas in winter, all five pictures were of scenery (Table 4). The subjects of the close-up photos were different in each picture (plants: *Pueraria lobata*, *Mallotus japonicus*, *Calystegia japonica*, *Solidago altissima*, *Triadica sebifera* and *Dioscorea japonica*; insects: *Orthetrum albistylum* and *Acrida cinere*; amphibian: *Rana rugosa*). There were, however, no close-up photos of threatened plants (Suppl. material 3: Table S6).

Discussion

We analysed the preferences of students who participated in taking photos of land-cover types using geo-tagged photos as an index of a cultural ecosystem service. Results showed that land cover (i.e. habitat) structure, which could contribute to biodiversity,

could also attract more people preferences i.e. indicator of cultural services. Additionally, our results suggested that natural or semi-natural land-cover types could provide cultural ecosystem services that cannot be provided by artificial land-cover types. These results suggest that the conservation of habitat diversity could contribute not only to regional biodiversity itself but also to human well-being as an additional value.

We classified five land cover types (grassland, forest, bare ground, open water and paddy field) to use as indices of habitat type. Each habitat type, except for bare ground, was used as the primary habitat for at least some of the flora and fauna surveyed in the area. These results indicate that habitat diversity could directly contribute to regional species diversity in the study area. In other words, if the number of habitat types decreased or the habitats degraded, species diversity could also decline. Our study area contains several habitat types as well as a mosaic structure of semi-natural habitats. Although we did not evaluate it in this study, this type of mosaic habitat structure could contribute to biodiversity and ecosystem functions (Tscharntke et al. 2005; Yoshioka et al. 2017). Thus, the land cover of Asabata Yusuichi has currently contributed to regional biodiversity and ecosystem functions, at least from a biological perspective.

People's preferences varied amongst the habitat types such that no single habitat, excluding open water, was notably more preferred over another. This result suggests that each habitat holds its own appeal. Thus, diverse habitat types, each having unique species components, may fulfil people's diverse subjective preferences. Our results suggest that each habitat could contribute its own species groups, so human interests and species diversity could be matched. Kasada et al. (2017) indicated that places with both high human interest and high biodiversity are valuable in maintaining human–biodiversity relationships (Kasada et al. 2017). An area that has diverse habitats and easy accessibility, such as Asabata Yusuichi, could contribute to both regional biodiversity and maintaining human–biodiversity relationships. If the overall habitat composition became simpler (i.e. the number of habitat types was reduced), the preferences in such habitats would be restricted to only people with preferences for those types. Therefore, we conclude that diverse habitats are an important resource for not only species diversity but also cultural ecosystem services.

We found clear seasonal differences in picture composition. During spring to autumn – a period with high biological activity – people tended to take close-up photos (i.e. of a particular species). Conversely, in winter, they tended to be preferred in scenic views, which were not strongly influenced by the species present. This trend was most notable in photos taken in the cells containing threatened plants. Of course, scenic and landscape views are also an important provider of cultural ecosystem service (Martínez Pastur et al. 2016; Yoshimura and Hiura 2017). However, this type of service could, to some degree, also be provided by an area with artificial components and non-biological materials. Conversely, artificial components and non-biological materials may not have the appeal of species diversity, as reflected by people's preference in close-up views. Natural or semi-natural habitat diversity is compatible with close-up and scenic types of cultural services. The most important point is phenology, which is an ecological phenomenon providing an additional ecosystem service. A previous study has also suggested that

natural and semi-natural mosaic landscapes could provide more services than an artificial landscape (Felipe-lucia and Comín 2015). This study revealed the importance of not only habitat diversity but also ecological phenomena in providing ecosystem services.

Biologists tend to concentrate on species diversity, with a particular emphasis on threatened species as an index of habitat value (Balvanera et al. 2014). In this context, habitats containing threatened species are considered to be of higher value than those without (Camaclang et al. 2014). In contrast, Dallimer et al. (2012) pointed out that the discrepancy between reality and perception of biodiversity was influenced by the biodiversity-identification skills of people and could produce a gap between biodiversity conservation and human well-being (Dallimer et al. 2012). In this study, we used photos taken by cognitively-impaired students who were unlikely to have any biases, such as focusing on species that have been assigned as threatened. Interestingly, people in our study took a higher proportion of close-up photos in areas containing threatened plants during spring–autumn, but did not photograph the threatened species themselves. In addition, objects were not biased towards highly visible species, such as those with large colourful flowers (Suppl. material 2: Fig. S2). This may indicate that the concrete preferences of biologists and/or society and those of our participants were different; however, the habitats that contained the threatened species did seem to appeal to the study participants even if their preferences were focused elsewhere. Although we were unable to identify any specific reason for this behaviour, future studies should test whether and why people have a greater preference for species in unique habitats, even if the species are not threatened.

Conclusions

The non-material value of ecosystems, including cultural services, should be evaluated as part of the total ecosystem value for humans (Chan et al. 2012a; Doi et al. 2013; Van Berkel and Verburg 2014). Thus, surveys that incorporate social and ecological perspectives provide novel evidence of the relationships between ecosystems and human society (Chan et al. 2012a; Doi et al. 2013; Garrido et al. 2017). However, cultural services do not represent purely ecological phenomena, but are rather the outcome of complex and dynamic relationships between ecosystems and humans (Fagerholm et al. 2012; Garrido et al. 2017). In this study, we used people preference as an index of cultural ecosystem service and found that the habitat diversity of a semi-natural environment with species diversity provides a cultural service that appears to fulfil diverse subjective interests. Cultural ecosystem services are co-generated with humans and the environment (Casado-Arzuaga et al. 2014); thus, interaction between people preference and habitat type is according to that idea. Additionally, we found one link between ecological phenomena and cultural services, namely, contact with the socio–ecological system. This contact could contribute to maintaining human–biodiversity relationships (Kasada et al. 2017). Based on these results, the habitat conservation of semi-natural environments that can be utilised by humans, such as natural parks, could synergise with both biodiversity conservation and general human well-being.

Acknowledgements

We thank Mr. A. Ono and Ms. Y. Kuriyama for their assistance with several activities. We also thank the students and teachers participating in the study. Two anonymous reviewers gave us several useful comments. This study was supported by the Environment Research and Technology Development Fund (4-1504, 4-1705) of the Ministry of the Environment, Japan.

References

- Balvanera P, Siddique I, Dee L, Paquette A, Isbell F, Gonzalez A, Byrnes J, O'Connor MI, Hungate BA, Griffin JN (2014) Linking biodiversity and ecosystem services: Current uncertainties and the necessary next steps. *Bioscience* 64(1): 49–57. <https://doi.org/10.1093/biosci/bit003>
- Boerema A, Rebelo AJ, Bodi MB, Esler KJ, Meire P (2017) Are ecosystem services adequately quantified? *Journal of Applied Ecology* 54(2): 358–370. <https://doi.org/10.1111/1365-2664.12696>
- Cadotte MW (2013) Experimental evidence that evolutionarily diverse assemblages result in higher productivity. *Proceedings of the National Academy of Sciences of the United States of America* 110(22): 8996–9000. <https://doi.org/10.1073/pnas.1301685110>
- Camaclang AE, Maron M, Martin TG, Possingham HP (2014) Current practices in the identification of critical habitat for threatened species. *Conservation Biology* 29(2): 482–492. <https://doi.org/10.1111/cobi.12428>
- Cardinale BJ, Duffy JE, Gonzalez A, et al. (2012) Corrigendum: Biodiversity loss and its impact on humanity. *Nature* 489: 326–326. <https://doi.org/10.1038/nature11373>
- Casado-Arzuaga I, Onaindia M, Madariaga I, Verburg PH (2014) Mapping recreation and aesthetic value of ecosystems in the Bilbao Metropolitan Greenbelt (northern Spain) to support landscape planning. *Landscape Ecology* 29(8): 1393–1405. <https://doi.org/10.1007/s10980-013-9945-2>
- Castro AJ, Verburg PH, Martín-López B, Garcia-Llorente M, Cabello J, Vaughn CC, López E (2014) Ecosystem service trade-offs from supply to social demand: A landscape-scale spatial analysis. *Landscape and Urban Planning* 132: 102–110. <https://doi.org/10.1016/j.landurbplan.2014.08.009>
- Chan KMA, Goldstein J, Satterfield T, et al. (2011) Cultural services and non-use values. *Theory Pract Ecosyst Serv Valuat Conserv*, 206–228. <https://doi.org/10.1093/acprof:oso/9780199588992.003.0012>
- Chan KMA, Guerry AD, Balvanera P, Klain S, Satterfield T, Basurto X, Bostrom A, Chuenpagdee R, Gould R, Halpern BS, Hannahs N, Levine J, Norton B, Ruckelshaus M, Russell R, Tam J, Woodside U (2012a) Where are Cultural and Social in Ecosystem Services? A Framework for Constructive Engagement. *Bioscience* 62(8): 744–756. <https://doi.org/10.1525/bio.2012.62.8.7>

- Chan KMA, Satterfield T, Goldstein J (2012b) Rethinking ecosystem services to better address and navigate cultural values. *Ecological Economics* 74: 8–18. <https://doi.org/10.1016/j.ecolecon.2011.11.011>
- Cumming GS, Maciejewski K (2017) Reconciling community ecology and ecosystem services: Cultural services and benefits from birds in South African National Parks. *Ecosystem Services* 28: 219–227. <https://doi.org/10.1016/j.ecoser.2017.02.018>
- Dallimer M, Irvine KN, Skinner AMJ, Davies ZG, Rouquette JR, Maltby LL, Warren PH, Armsworth PR, Gaston KJ (2012) Biodiversity and the Feel-Good Factor: Understanding Associations between Self-Reported Human Well-being and Species Richness. *Bioscience* 62(1): 47–55. <https://doi.org/10.1525/bio.2012.62.1.9>
- de Groot RS, Alkemade R, Braat L, Hein L, Willemsen L (2010) Challenges in integrating the concept of ecosystem services and values in landscape planning, management and decision making. *Ecological Complexity* 7(3): 260–272. <https://doi.org/10.1016/j.ecocom.2009.10.006>
- Doi H, Katano I, Negishi JN, Sanada S, Kayaba Y (2013) Effects of biodiversity, habitat structure, and water quality on recreational use of rivers. *Ecosphere* 4(8): art102. <https://doi.org/10.1890/ES12-00305.1>
- Fagerholm N, Käyhkö N, Ndumbo F, Khamis M (2012) Community stakeholders' knowledge in landscape assessments – Mapping indicators for landscape services. *Ecological Indicators* 18: 421–433. <https://doi.org/10.1016/j.ecolind.2011.12.004>
- Felipe-lucia MR, Comín FA (2015) Land Use Policy Ecosystem services – biodiversity relationships depend on land use type in floodplain agroecosystems. *Land Use Policy* 46: 201–210. <https://doi.org/10.1016/j.landusepol.2015.02.003>
- Garrido P, Elbakidze M, Angelstam P, Plieninger T, Pulido F, Moreno G (2017) Stakeholder perspectives of wood-pasture ecosystem services: A case study from Iberian dehesas. *Land Use Policy* 60: 324–333. <https://doi.org/10.1016/j.landusepol.2016.10.022>
- Gomes VHF, Ijff SD, Raes N, Amaral IL, Salomão RP, de Souza Coelho L, de Almeida Matos FD, Castilho CV, de Andrade Lima Filho D, López DC, Guevara JE, Magnusson WE, Phillips OL, Wittmann F, de Jesus Veiga Carim M, Martins MP, Irumé MV, Sabatier D, Molino J-F, Bánki OS, da Silva Guimarães JR, Pitman NCA, Piedade MTF, Mendoza AM, Luizé BG, Venticinque EM, de Leão Novo EMM, Vargas PN, Silva TSF, Manzatto AG, Terborgh J, Reis NFC, Montero JC, Casula KR, Marimon BS, Marimon B-H, Coronado ENH, Feldpausch TR, Duque A, Zartman CE, Arboleda NC, Killeen TJ, Mostacedo B, Vasquez R, Schöngart J, Assis RL, Medeiros MB, Simon MF, Andrade A, Laurance WF, Camargo JL, Demarchi LO, Laurance SGW, de Sousa Farias E, Nascimento HEM, Revilla JDC, Quaresma A, Costa FRC, Vieira ICG, Cintra BBL, Castellanos H, Brienen R, Stevenson PR, Feitosa Y, Duivenvoorden JF, Aymard CGA, Mogollón HF, Targhetta N, Comiskey JA, Vicentini A, Lopes A, Damasco G, Dávila N, García-Villacorta R, Levis C, Schiatti J, Souza P, Emilio T, Alonso A, Neill D, Dallmeier F, Ferreira LV, Araujo-Murakami A, Praia D, do Amaral DD, Carvalho FA, de Souza FC, Feeley K, Arroyo L, Pansonato MP, Gribel R, Villa B, Licona JC, Fine PVA, Cerón C, Baraloto C, Jimenez EM, Stropp J, Engel J, Silveira M, Mora MCP, Petronelli P, Maas P, Thomas-Caesar R, Henkel TW, Daly D, Paredes MR, Baker TR, Fuentes A, Peres CA, Chave J, Pena JLM, Dexter KG, Silman

- MR, Jørgensen PM, Pennington T, Di Fiore A, Valverde FC, Phillips JF, Rivas-Torres G, von Hildebrand P, van Andel TR, Ruschel AR, Prieto A, Rudas A, Hoffman B, Vela CIA, Barbosa EM, Zent EL, Gonzales GPG, Doza HPD, de Andrade Miranda IP, Guillaumet J-L, Pinto LFM, de Matos Bonates LC, Silva N, Gómez RZ, Zent S, Gonzales T, Vos VA, Malhi Y, Oliveira AA, Cano A, Albuquerque BW, Vriesendorp C, Correa DF, Torre EV, van der Heijden G, Ramirez-Angulo H, Ramos JF, Young KR, Rocha M, Nascimento MT, Medina MNU, Tirado M, Wang O, Sierra R, Torres-Lezama A, Mendoza C, Ferreira C, Baidier C, Villarroel D, Balslev H, Mesones I, Giraldo LEU, Casas LF, Reategui MAA, Linares-Palomino R, Zagt R, Cárdenas S, Farfan-Rios W, Sampaio AF, Pauletto D, Sandoval EH, Arevalo FR, Huamantupa-Chuquimaco I, Garcia-Cabrera K, Hernandez L, Gamarra LV, Alexiades MN, Pansini S, Cuenca WP, Milliken W, Ricardo J, Lopez-Gonzalez G, Pos E, ter Steege H (2018) Species Distribution Modelling: Contrasting presence-only models with plot abundance data. *Scientific Reports* 8(1): 1003. <https://doi.org/10.1038/s41598-017-18927-1>
- Hernández-Morcillo M, Plieninger T, Bieling C (2013) An empirical review of cultural ecosystem service indicators. *Ecological Indicators* 29: 434–444. <https://doi.org/10.1016/j.ecolind.2013.01.013>
- Kasada M, Matsuba M, Miyashita T (2017) Human interest meets biodiversity hotspots: A new systematic approach for urban ecosystem conservation. *PLoS One* 12(2): e0172670. <https://doi.org/10.1371/journal.pone.0172670>
- Mace GM, Norris K, Fitter AH (2012) Biodiversity and ecosystem services: A multilayered relationship. *Trends in Ecology & Evolution* 27(1): 19–25. <https://doi.org/10.1016/j.tree.2011.08.006>
- Millennium Ecosystem Assessment (2005) *Ecosystems and human well-being*. Island Press Washington, DC.
- Martin-López B, Iniesta-Arandia I, Garcia-Llorente M, Palomo I, Casado-Arzuaga I, Amo DGD, Gómez-Baggethun E, Oteros-Rozas E, Palacios-Agundez I, Willaarts B, González JA, Santos-Martín F, Onaindia M, López-Santiago C, Montes C (2012) Uncovering ecosystem service bundles through social preferences. *PLoS One* 7(6): e38970. <https://doi.org/10.1371/journal.pone.0038970>
- Martínez Pastur G, Peri PL, Lencinas MV, García-Llorente M, Martín-López B (2016) Spatial patterns of cultural ecosystem services provision in Southern Patagonia. *Landscape Ecology* 31(2): 383–399. <https://doi.org/10.1007/s10980-015-0254-9>
- Marull J, Tello E, Fullana N, Murray I, Jover G, Font C, Coll F, Domene E, Leoni V, Decolli T (2015) Long-term bio-cultural heritage: Exploring the intermediate disturbance hypothesis in agro-ecological landscapes (Mallorca, ca. 1850–2012). *Biodiversity and Conservation* 24(13): 3217–3251. <https://doi.org/10.1007/s10531-015-0955-z>
- Mori AS (2017) Biodiversity and ecosystem services in forests: Management and restoration founded on ecological theory. *Journal of Applied Ecology* 54: 7–11. <https://doi.org/10.1111/1365-2664.12854>
- Osawa T, Mitsunashi H, Niwa H, Ushimaru A (2010a) Enhanced diversity at network nodes: River confluences increase vegetation-patch diversity. *The Open Ecology Journal* 3(1): 48–58. <https://doi.org/10.2174/1874213001003010048>

- Osawa T, Mitsuhashi H, Niwa H, Ushimaru A (2010b) Differences in distribution patterns around river confluences among hydrophilic vegetation groups. *Ecological Research* 25(6): 1161–1169. <https://doi.org/10.1007/s11284-010-0741-z>
- Ozono A, Kawashima I, Futahashi R (2012) *Dragonflies of Japan*. Bun-Ichi syuppan, Tokyo. [in Japanese]
- Plieninger T, Dijks S, Oteros-Rozas E, Bieling C (2013) Assessing, mapping, and quantifying cultural ecosystem services at community level. *Land Use Policy* 33: 118–129. <https://doi.org/10.1016/j.landusepol.2012.12.013>
- Schwarz N, Moretti M, Bugalho MN, Davies ZG, Haase D, Hack J, Hof A, Melero Y, Pett TJ, Knapp S (2017) Understanding biodiversity-ecosystem service relationships in urban areas: A comprehensive literature review. *Ecosystem Services* 27: 161–171. <https://doi.org/10.1016/j.ecoser.2017.08.014>
- Sherrouse BC, Clement JM, Semmens DJ (2011) A GIS application for assessing, mapping, and quantifying the social values of ecosystem services. *Applied Geography (Sevenoaks, England)* 31(2): 748–760. <https://doi.org/10.1016/j.apgeog.2010.08.002>
- Shizuoka Prefecture, Shizuoka City Council on Asabata Yusuichi (2008) *Restoration plan for Asabata Yusuichi*. [in Japanese]
- Takagawa S, Ueta M, Amano T, et al. (2011) JAVIAN Database: A species-level database of life history, ecology and morphology of bird species in Japan [JAVIAN Database]. *Bird Res* 7: R9–R12.
- Thompson PL, Isbell F, Loreau M, O'Connor MI, Gonzalez A (2018) The strength of the biodiversity – ecosystem function relationship depends on spatial scale. *Proceedings. Biological Sciences* 285(1880): 20180038. <https://doi.org/10.1098/rspb.2018.0038>
- Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441(7093): 629–632. <https://doi.org/10.1038/nature04742>
- Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity – Ecosystem service management. *Ecology Letters* 8(8): 857–874. <https://doi.org/10.1111/j.1461-0248.2005.00782.x>
- Van Berkel DB, Verburg PH (2014) Spatial quantification and valuation of cultural ecosystem services in an agricultural landscape. *Ecological Indicators* 37: 163–174. <https://doi.org/10.1016/j.ecolind.2012.06.025>
- Yoshimura N, Hiura T (2017) Demand and supply of cultural ecosystem services: Use of geotagged photos to map the aesthetic value of landscapes in Hokkaido. *Ecosystem Services* 24: 68–78. <https://doi.org/10.1016/j.ecoser.2017.02.009>
- Yoshioka A, Fukasawa K, Mishima Y, Sasaki K, Kadoya T (2017) Ecological dissimilarity among land-use/land-cover types improves a heterogeneity index for predicting biodiversity in agricultural landscapes. *Ambio* 46(8): 894–906. <https://doi.org/10.1007/s13280-017-0925-7>

Supplementary material 1

Figure S1. Typical pictures in each composition

Authors: Takeshi Osawa, Yusuke Ueno, Takaaki Nishida, Jun Nishihiro

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/natureconservation.38.36166.suppl1>

Supplementary material 2

Figure S2. Pictures took in mesh which have any threaten species

Authors: Takeshi Osawa, Yusuke Ueno, Takaaki Nishida, Jun Nishihiro

Explanation note: All objects were not the threaten species itself.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/natureconservation.38.36166.suppl2>

Supplementary material 3

Tables S1–S6

Authors: Takeshi Osawa, Yusuke Ueno, Takaaki Nishida, Jun Nishihiro

Explanation note: **Table S1.** All plants and main habitats in the study area. **Table S2.** All birds and main habitats in the study area. **Table S3.** All butterflies and main habitats in the study area. **Table S4.** All dragonflies and main habitats in the study area. **Table S5.** List of threatened plants in the study area. **Table S6.** Picture objects list which took in area with threatened species from spring to autumn.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/natureconservation.38.36166.suppl3>

Environmental stress in *Parnassius apollo* reflected through wing geometric morphometrics in a historical collection with a possible connection to habitat degradation

Martin Štefánik¹, Peter Fedor¹

¹ Department of Environmental Ecology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Corresponding author: Martin Štefánik (stefanik16@uniba.sk)

Academic editor: R. Julliard | Received 25 November 2019 | Accepted 12 February 2020 | Published 18 March 2020

<http://zoobank.org/FBA7B6E8-AC78-4E83-9362-53F4490BC606>

Citation: Štefánik M, Fedor P (2020) Environmental stress in *Parnassius apollo* reflected through wing geometric morphometrics in a historical collection with a possible connection to habitat degradation. Nature Conservation 38: 79–99. <https://doi.org/10.3897/natureconservation.38.48682>

Abstract

Monitoring climate changes and habitat degradation in threatened species without negative impact to the populations can pose a considerable challenge. A rare chance to test the morphological response of wing shape and size to environmental factors on the mountain Apollo (*Parnassius apollo*) collected from 1938 to 1968 at a single location – Strečno mountain pass, N Slovakia presented itself in a historical collection. The canonical variate analysis showed a significant shift from a narrower to broader forewing, with more extremes in either extra broad or narrow forewings in the post- 1960 population. Analysis of existing data was conducted to determine the possible factors affecting this change. Generally, the comparative statistics of temperature and precipitation to morphology of individuals and their fluctuating asymmetry showed no, or weak, correlations. Two extreme weather events (ECEs), identified using the historical weather data, show no correlation of wing morphology to these events. Although no strong correlations can be drawn in case of the available weather data and morphology, the results of this study can be connected to strong anthropogenic effects of a large-scale road development project taking place in the vicinity of the collection site starting in November 1959 causing changes in the available habitat and therefore a shift in the wing morphology.

Keywords

Anthropic impact, butterfly, geometric morphometry, historical collection, *Parnassius Apollo*, wing

Introduction

Sudden or continual alterations to the environment caused by natural or anthropogenic processes have been of interest to the scientific community for some time (Stern et al. 1992). Monitoring these environmental changes has become an essential component of applied ecology, using suitable species (bioindicators) and their assemblages to analyze environmental stress and disturbance (Dubovský et al. 2010; Zvaríková et al. 2016). There is a large body of work concerning the negative effects in populations of arthropod fauna, including butterflies, to various environmental changes (Van Swaay and Martin 1999; Descimon et al. 2005; Wenzel et al. 2006; Nakonieczny et al. 2007; Van Dyck et al. 2009; da Rocha et al. 2010; Gibbs et al. 2011; Wallner et al. 2013). Due to the more extreme nature of these changes in mountainous regions the native populations face even higher risk of population reduction and potential extinction (Nogués-Bravo et al. 2007). The same risks must be expected for their host and nectar plants to which their life cycle is bound (Engler et al. 2011).

Among bioindicators, detectors may provide an important material to detect response based on their body morphology (Blake et al. 1994; Findlay and Houlihan 1997; Clarke et al. 2000; Spellerberg 2005; Zvaríková et al. 2016). Generally, all organisms exhibit a certain degree of morphological (phenotypic) plasticity (Ananthakrishnan and Whitman 2005; Hoffmann et al. 2005; Sukhodolskaya and Saveliev 2014), which describes the capacity of a genotype to display a range of phenotypes in response to changes in the environment (Garland and Kelly 2006; Whitman and Agrawal 2009) allowing organisms to maintain high fitness in the face of environmental heterogeneity (Pigliucci et al. 2006). The monitoring of these changes in morphology is heavily focused on the symmetries and asymmetries of individuals in attempts to describe the underlining factors of these changes (Ananthakrishnan and Whitman 2005; Zvaríková et al. 2016).

Asymmetry in individuals' morphology is often used to illustrate isolation effects, reduction in genetic diversity, and lower fitness of populations or environmental stress (Valen 1962; Parsons 1990; Frankham 2005; Allendorf et al. 2013; Beasley et al. 2013; Woods et al. 1999). Within previous research, population genetic diversity was negatively correlated to the level of asymmetry on multiple occasions (Vrijenhoek and Lerman 1982; Blanco et al. 1990; Hoelzel et al. 2002). Higher levels of morphological asymmetry in birds corresponded with higher mortality during extreme climatic events (Brown and Brown 1998). In contrast to these claims are examples of naturally highly inbred populations where the benefits of outbreeding are not always maintained (Fountain et al. 2015). Fluctuating asymmetry (FA) of individuals' morphology is often used as an indicator for population fitness, although the complex nature of the origin and expression of fluctuating asymmetry (FA) or lack thereof shows that a unification of these results may be impossible, and the problem therefore requires a case-by-case approach (Kaeppler 2012; Windig et al. 2000).

A reliable evaluation of insect morphology vs. environment interaction requires a long-term monitoring of a suitable model species with sufficient information on its

autecology as well as environmental disturbance details (Webster and Sheets 2010). Using historical collections, we offer an analysis of the mountain Apollo, *Parnassius apollo* (Linnaeus, 1758), a xeromontainous papilionid butterfly with diminishing distribution across Europe. The species has been in long-term decline since the 1900s due to lack of land management and global climate change (Todisco et al. 2010). Current populations are now usually present in small, often isolated patches, unable to colonize new habitats (Fred et al. 2006). Although its physiological predispositions would allow the Apollo butterfly to migrate longer distances, its successful reproduction remains restricted to the distribution of its larval host plant (*Sedum sp.*) located on open rock formations (Brommer and Fred 1999). The survival of these small populations was hypothesized to be due to the ability to maintain a low long-term effective population, which may result from a strong historic bottleneck or a founder event (Habel et al. 2009). Due to the continuous decline in population this species has been listed in the IUCN Red List of Threatened Species, the appendix II in CITES, as well as the annex IV of Habitats Directive.

The isolation effect on the phenotype of *Parnassius apollo* was previously tested on western European populations, showing similar levels of asymmetry throughout the region (Habel et al. 2012), however higher levels of asymmetry were presented by Adamski and Witkowski (2002) in a post-bottleneck population when compared to the pre-bottleneck individuals. The populations of the Carpathian region (Eastern/Central European) were determined as genetically homogeneous (Todisco et al. 2010), although multiple subspecies based on morphology are considered (Hrubý 1964; Capdeville 1978; Kizek 1997).

Although the pressures on selection in butterfly wing morphology remain largely unknown, the strongest factors seem to be habitat, predators and sex-specific behavior (Le Roy et al. 2019). Contrasting habitats (Jugovic et al. 2018), restoration attempts (Sivakoff et al. 2016) or landscape structure (Merckx and Van Dyck 2006) show impact on the resulting wing morphology in butterflies. The material of mounted *Parnassius apollo* specimens by a single collector at a location in the vicinity of an important north Slovakian trade and travel route presents an ideal dataset for the analysis of ecological (temperature and precipitation) and environmental (stress and disturbance) factors along a 30-year long period in the mid-20th century. A thorough analysis of the correlation strength of weather and extreme climatic events to the resulting changes in morphology was conducted. We hypothesize that the anthropic impact at the collection sites could induce a change in the morphology of the *P. apollo* wing observed through the geometric morphometry.

Material and methods

All 506 *Parnassius apollo* specimens used for this analysis belong to the historical collection (1938–1968) of the Slovak National Museum (Bratislava, Slovakia) and were mounted by Ján Zelný. Since the whole collection has been the work of a single col-

lector it presents an ideal study opportunity, reducing the variable of multiple collectors or preparation techniques used. The material refers to a single location at Strečno mountain pass, N Slovakia (49°10'07.6"N, 18°52'51.6"E) (Fig. 1), at the altitude of 450 m a. s. l.

Digitization and morphometric analyses

Each individual was photographed from the dorsal side using a digital camera (Canon 60D, 50 mm lens) under standardized light conditions, with the added size standard (5cm with 1mm increments) fixed at the height of the wing. Geometric morphometric analysis was based on 17 landmarks (10 landmarks on forewing and 7 on hindwing), situated terminally or on vena intersection (Fig. 2) to assure the repeatability of the landmarks on the highest numbers of individuals (Habel et al. 2012).

A database (.TPS file) of all digitized specimens was created using tpsUtil64 (version 1.70) software (<http://life.bio.sunysb.edu/morph/soft-utility.html>, accessed 23.11.2018) and imported to tpsDIG2x32 (version 2.26) software (<http://life.bio.sunysb.edu/morph/soft-dataacq.html>, accessed 23.11.2018) where all landmarks were set. TPS file containing the landmarks for each specimen was imported to MorphoJ software (version 1.06d) (Klingenberg 2008, 2011).

To address the potential influence of digitization or setting of the landmarks on our results, individuals were tested for digitization and measurement error. To test for digitization error, 50 specimens were randomly selected, photographed twice, measured and the results compared. The measurement error was addressed by measuring the whole dataset twice by the same person and compared (Palmer and Strobeck 1986; Klingenberg and McIntyre 1998).

The weather data was obtained from the archives of the Slovak Hydrometeorological Institute (SHMI) for the locations closest to the collection site. The precipitation data was recorded at location Vrútky, Slovakia (49°06'42.7"N, 18°55'26.2"E), 7 km south from the collection site and Varín, Slovakia (49°12'05.4"N, 18°52'12.4"E) 3 km north of the collection site. The closest site collecting temperature data was maintained by the Slovak Army at the Žilina Airport, Slovakia (49°13'59.1"N, 18°36'47.4"E) located 20 km west of the collection site. All data on temperature prior to 1944 was probably lost, therefore comparative statistics between morphometric data and temperature was conducted only for the dataset 1944–1968. The data of monthly precipitation and temperature, and yearly precipitation and temperature, were correlated to symmetric and asymmetric components, centroid size and Procrustes FA scores of Procrustes coordinates exported from MorphoJ and analyzed using “corr” package in R (RStudio Team 2015). The extreme climatic events (ECEs) were selected according to the ability of the collected weather data to display these events. In the case of extreme temperatures, the highest monthly and yearly temperature did not exceed the averages for the location and we therefore did not consider high temperatures from 1944 – 1968 to display any ECEs. In the case of low temperatures, February 1956, ac-

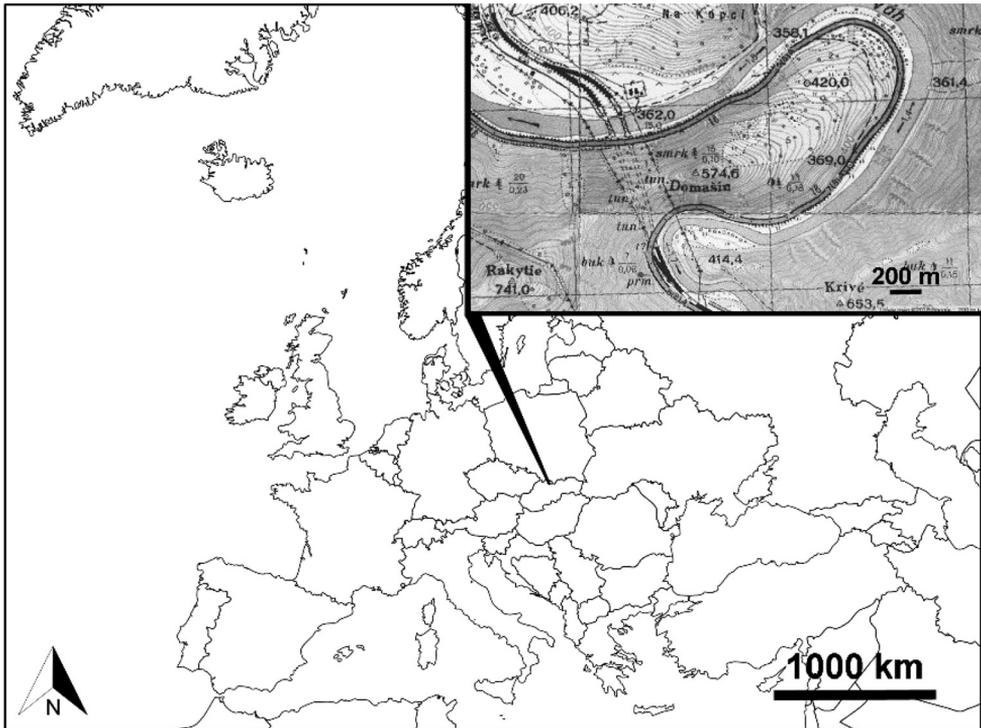


Figure 1. Location of Strečno mountain pass in European context and a close-up of the collection site on a historic map from 1955.

According to the SHMI, saw the lowest monthly temperature ever recorded for the location during the measured period 1944–2019. The heavy rainfall and flooding in 1958 can also be supported by the collected data. Based on this information we selected the low monthly February 1956 temperatures and 1958 heavy rainfalls and flooding as the two most important ECEs during our research period.

Data on the anthropic impact and road construction timeline was pieced together from articles and archives by The News Agency of the Slovak Republic (TASR), using mostly contemporary photographs with descriptions.

Statistics

Statistics were conducted separately for the fore and hindwing to rule out the possible misalignment of wings during preparation (Bookstein 1997; Alibert and Auffray 2002). Using the MorphoJ software (Klingenberg 2008) a Procrustes superimposition was conducted following 3 steps for fitting the landmarks: (1) scaling the landmarks to the same centroid size (hereinafter “size”); (2) shifting the landmarks to the same position and (3) rotating them to fit the orientation as closely as possible minimizing the sum of squared

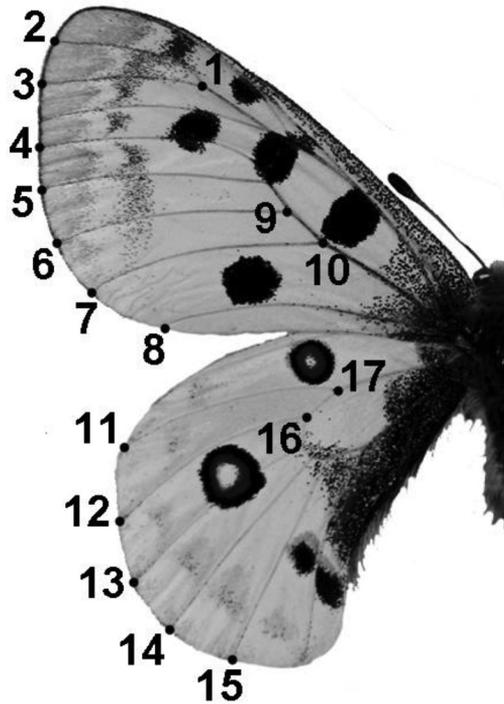


Figure 2. Left fore and hindwing of *P. apollo* used for the morphometric analysis, including the morphometric landmarks Forewing: 1 – R4 and R5 intersection, 2 – R4 terminally, 3 –R5 terminally, 4 –M2 terminally, 5 –M3 terminally, 6 –Cu1 terminally, 7 –Cu2 terminally, 8 –A2 terminally, 9 – Discal cell and Cu1 intersection, 10 – Discal cell and Cu2 intersection. Hindwing: 11 –Rs terminally, 12 – M1 terminally, 13 – M2 terminally, 14 – M3 terminally, 15 – Cu1 terminally, 16 – Discal cell and M1 intersection, 17 – Discal cell and Rs intersection.

distances of landmarks (Klingenberg 2015). Procrustes ANOVA in MorphoJ was used to determine the morphological variance found among individuals when comparing the left and right wing. The same method was used to compare the left and right wing within each individual to test if there is a significant deviation from bilateral symmetry of our selected landmarks (Klingenberg and McIntyre 1998). The deviation to bilateral symmetry across the temporal variable is further referenced as FA (fluctuating asymmetry). Procrustes residuals were used for canonical variate analysis (CVA) to test for differences between years using MorphoJ and PAST software (version 3.20) (Hammer et al. 2001). A scatterplot produced within the MorphoJ environment was created to display the comparison of the morphological changes. Boxplots of the FA, CV1 and CV2 scores were generated in the R software (version 3.4.4), using RStudio (version 1.1.456) (RStudio Team 2015). To compare the variance of morphology between years and between artificially created pre- and post- 1960 datasets a factorial analysis was conducted using the “aov” function in R. Also, a breakpoint factorial analysis using “segmented” package was conducted to test the observed change on the morphology (RStudio Team 2015).

Results

All 506 *Parnassius apollo* specimens (Tab. 1), including 342 males and 164 females, used for this analysis were checked for strength of measurement and digitization error, with negligible results when compared to the morphological changes. In accordance with no significant size, shape or fluctuating asymmetry differences of hind or forewings between sexes (Tab. 2), the specimens were pooled together for further analyses.

Table 1. A survey of the collected specimens.

Collection year	Collection period	Female	Male	Total
1938	15.6.–20.7.	10	16	26
1939	15.6.–20.7.	0	18	18
1940	15.6.–20.7.	9	10	19
1941	15.6.–20.7.	8	17	25
1942	15.6.–20.7.	9	16	25
1943	22.6.–20.7.	7	21	28
1946	22.6.–20.7.	8	15	23
1948	22.6.–20.7.	8	11	19
1950	22.6.–20.7.	9	11	20
1951	22.6.–20.7.	7	10	17
1953	25.6.–24.7.	10	15	25
1954	25.6.–24.7.	0	15	15
1956	25.6.–24.7.	0	13	13
1957	25.6.–24.7.	5	16	21
1958	25.6.–24.7.	3	10	13
1960	25.6.–24.7.	5	14	19
1961	25.6.–24.7.	4	4	8
1963	25.6.–24.7.	7	6	13
1965	25.6.–24.7.	52	86	138
1966	25.6.–24.7.	1	9	10
1968	25.6.–24.7.	2	9	11
Total		164	342	506

Table 2. ANOVA of shape, centroid size and fluctuating asymmetry scores between males and females.

Df	Sum sq	Forewing		
		Mean sq	F value	<i>p</i>
Shape				
1	0.000142379	0.000142379	1.238	0.2664
centroid size				
1	0.00129255	0.00129255	0.6782	0.4106
fluctuating asymmetry (FA)				
1	0.0000686794	0.0000686794	1.446	0.2297
hindwing	–	–	–	–
Shape				
1	0.000221659	0.000221659	2.233	0.1357
centroid size				
1	0.0000453208	0.0000453208	1.439	0.2308
fluctuating asymmetry				
1	0.0000592967	0.0000592967	0.8129	0.3677

Testing for digitization error by photographing 50 individuals under the same light conditions and digitization setup, no significant difference in landmark placement was detected (ANOVA, digitization error of forewing $p = 0.76$, hindwing $p = 0.56$). The same result of non-significant difference was recorded for the measurement error, remeasuring all 506 individuals twice (ANOVA, measurement error of forewing $p = 0.59$, hindwing $p = 0.45$).

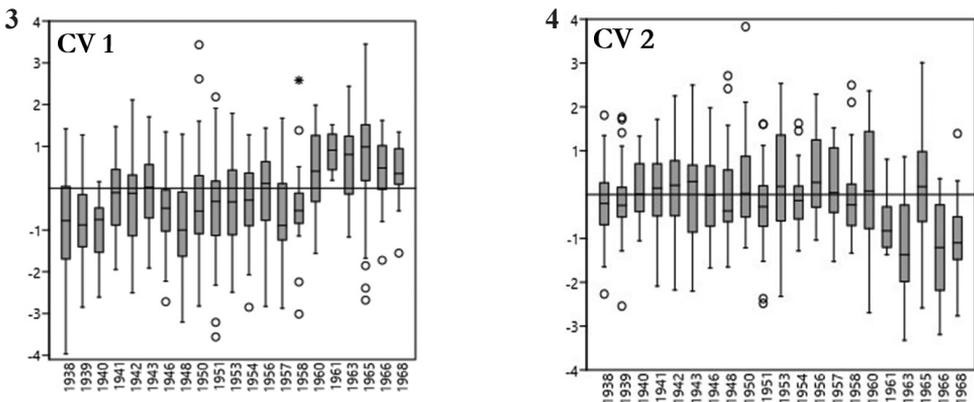
Fluctuating asymmetry (FA) throughout time

Rejecting the null hypothesis using Procrustes ANOVA ($p < 0.0001$) for the fore and hindwing corresponds with statistically significant levels in wing asymmetry within each year measured. As there was no significant variance in asymmetry throughout the years for the forewing (ANOVA, $p = 0.417$) as well as the hindwing (ANOVA, $p = 0.0564$), the asymmetry is statistically significant within each year, although with no fluctuation when comparing the time series.

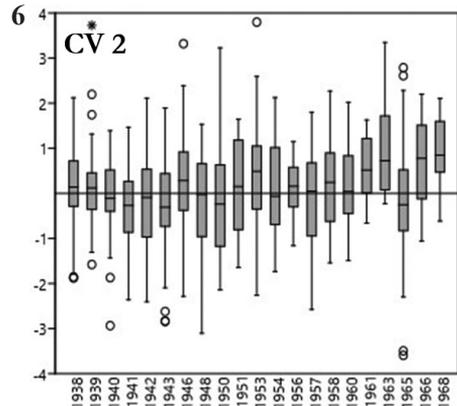
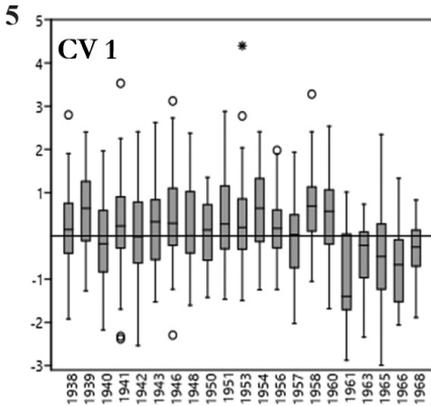
Wing shape change patterns

The Mann-Whitney's pairwise comparison, to test the significance of the differences in wing shape between each year of the time series (Appendix 1), and the Canonical variate analysis (CVA) show a significant change in the wing morphology starting with 1960 for the forewing and 1961 for the hindwing, continuing until the end of our dataset. Canonical variate 1 (CV1) and 2 (CV2) (Figs 3–6) describes 81% (for the forewing) and 76% (for the hindwing) of the total variance in wing morphology.

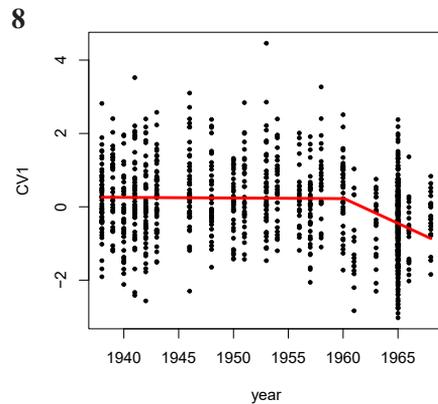
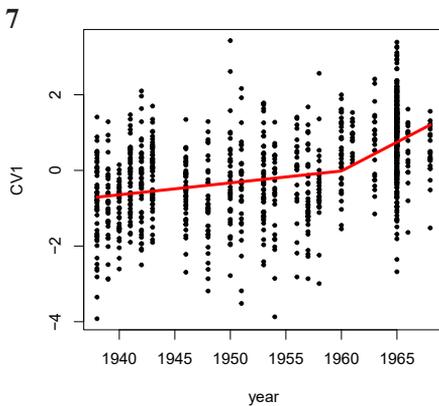
The shift observed starting in 1960 was tested using 2 factor analysis of variance where we tested the artificial pre/post 1960 datasets and the natural variation



Figures 3, 4. Mountain Apollo forewing shape changes displayed on the canonical variate 1 and 2. The boxplots cover the upper and lower 25%, separated by a line showing median inside the box, whiskers cover remaining upper and lower 25%, the white circles represent outliers and black stars are extreme values.



Figures 5, 6. Mountain Apollo hindwing shape changes displayed on the canonical variate 1 and 2. The boxplots cover the upper and lower 25%, separated by a line showing median inside the box, whiskers covering remaining upper and lower 25%, the white circles represent outliers and black stars are extreme values.



Figures 7, 8. Breakpoint regression analysis of the CV1 for the fore- and hind wing displaying the estimated breakpoint of the forewing CV1 in 1960 ($se = 0.0058$, $Pr(>F) = 6.42 \times 10^{-8}$) and for the hindwing in 1960 ($se = 0.0051$, $Pr(>F) = 0.00511$).

between years using CV1 (Tab. 3). To measure the shift in morphology observed in Figs 3–6 a breakpoint regression analysis was tested on CV 1 of the fore- and hindwing (Figs 7, 8).

Wing shape dynamics pre/post 1960

Due to the results of the Mann-Whitney's pairwise comparison (Appendix 1) and the CVA, observing a significant change in the wing morphology after 1960 (1961) we pooled the dataset into two groups (1938 – 1958 with 307 individuals and 1960 – 1968 with 199 individuals) to mitigate the possibility of a bias due to low numbers of individuals collected in some of the years. The scatter plots (Figs 9, 10) display the

Table 3. Canonical variate 1 (CV1) used in the analysis of variance for comparison of natural variance in wing morphology between years and the shift observed around 1960.

		Df	Sum sq	Mean sq	F value	Pr(>F)	
CV1 forewing	pre vs post 1960	1	381.9	381.9	381.922	$< 2 \times 10^{-16}$	***
	Years	19	60.7	3.2	3.194	4.81×10^{-6}	***
	Residuals	989	989	1			
CV1 hindwing	pre vs post 1960	1	117.6	117.6	117.601	$< 2 \times 10^{-16}$	***
	Years	19	64.8	3.41	3.412	1.14×10^{-6}	***
	Residuals	993	993	1			

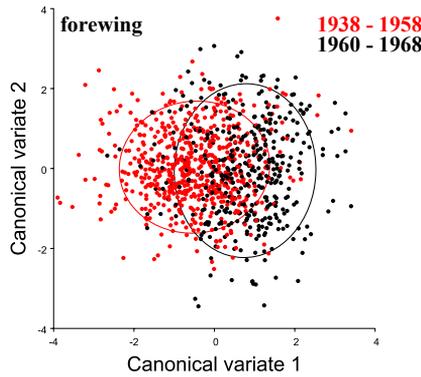


Figure 9. Scatter plot of the *P. apollo* forewing shape change along the first two canonical variates as axes. CV1 and CV2 cumulatively displaying 81.12% of the variability with 80% confidence ellipses.

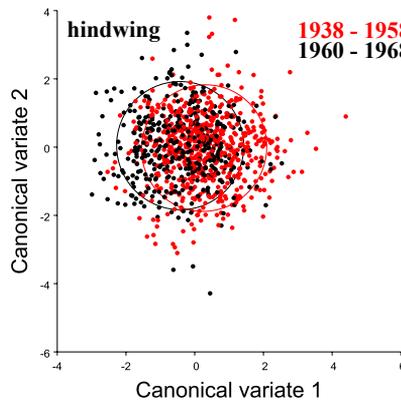


Figure 10. Scatter plot of the *P. apollo* hindwing shape change along the first two canonical variates as axes. CV1 and CV2 cumulatively displaying 76.39% of the variability with 80% confidence ellipses.

same pattern of statistically significant (<0.0001 ; $P = 0.05$) changes in morphology after being pooled.

The scatter plots of CV1 and CV2 are displaying a shift from a narrower to broader forewing, with more extremes in either extra broad or narrow forewings in individuals

from the 1960 – 1968 group (Fig. 9). The apex of the forewing is getting narrower and the landmarks M2 (4) and Cu2 (7) are moving closer along the time series.

For the hindwing (Fig. 10) the CV2 refers to a shift of the wing from broad to narrow along the Y axis. There is no general direction of hindwing landmark dynamics for CV1 and the movement on the hindwing is strongest at the M3 (14) and Cu1 (15), with an inwards landmark movement along the time series.

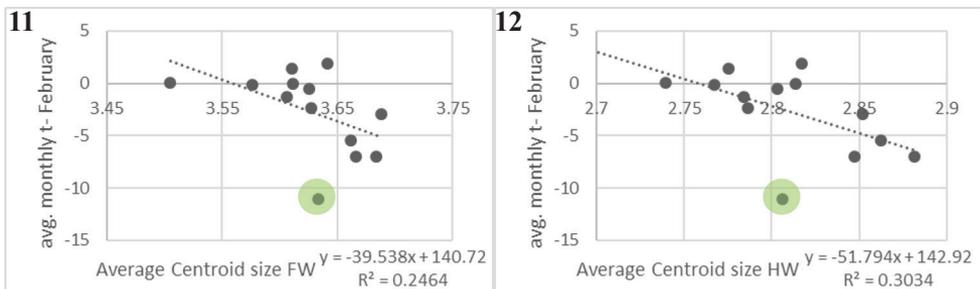
Weather and wing morphology correlation

Generally, the comparative statistics of temperature, precipitation and morphology of individuals and their FA showed no distinct correlation ($r^2 < 0.04$). The strongest correlation $r^2 = 0.25$ (forewing) and $r^2 = 0.30$ (hindwing) of standard deviation of the centroid wing size to the average monthly temperature was observed during February. The other positive correlation refers to January temperature vs. wing size ($r^2 = 0.21$ for forewing, $r^2 = 0.175$ for hindwing) and March temperature vs. wing size ($r^2 = 0.18$ for forewing, $r^2 = 0.16$ for hindwing).

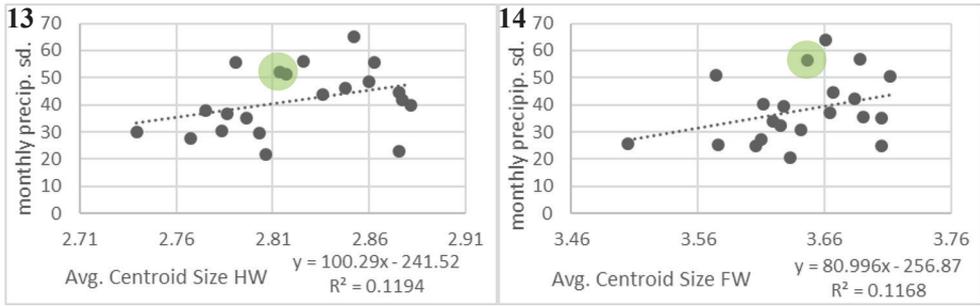
Extreme climatic events

Using historical weather data (temperature and precipitation) and photographic documentation of ECEs, we identified two extreme weather events (ECE) and compared them with the morphometric data (symmetric and asymmetric components, centroid size and Procrustes FA scores). The first ECE (a deep drop in monthly temperature during February 1956: -11.5°C) corresponds with no deviation from the average wing size despite the extremely cold weather, as the wing size parameter lies within the range of the dataset (Figs 11, 12).

Similarly, the extreme weather event, in this case heavy rainfall in June 1958, does not show any correlation to wing morphology ($R^2 < 0.02$), when comparing the daily, monthly, or annual precipitation total sum, average, min or max values at the study site. A weak positive correlation ($R^2 = 0.12$) was found for standard deviation



Figures 11, 12. The correlation of the Centroid size standard deviation of the *P. apollo* fore and hindwing to the average temperature in February (the extreme weather event highlighted with a green circle).



Figures 13, 14. The correlation of monthly sd of precipitation to Centroid size of the *P. apollo* fore and hindwing (the extreme weather event highlighted with a green circle).

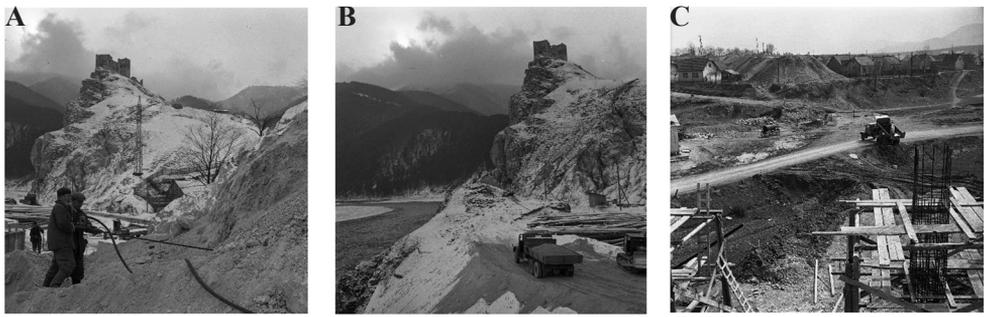


Figure 15A–C. Extensive construction works at the collection site, November 1959 (Kocián, 1959a, b, c).

of monthly precipitation to the wing size, when the wings grew larger with increasing variation of monthly precipitation. (Figs 13, 14).

Environmental impact

Since the changes in wing morphology show only weak correlations to temperature or precipitation, there is a strong suggestion of high anthropic effect. A large-scale road development project in November 1959 with heavy modification or complete destruction of some of the cliff faces was conducted in the immediate proximity of the collection site when constructing a new concrete road at Strečno castle, the Domašín meander and Žilina (Fig. 15A–C). The construction works were underway until at least 1965 and in 1964 heavy traffic was reported for the location.

Discussion

The Mann-Whitney’s pairwise comparison and the Canonical variate analysis (CVA) revealed a significant change in wing morphology starting between 1958 and 1960,

continuing to the end of the dataset. This is supported by the analysis of variance and the breakpoint regression analysis. The fluctuating asymmetry was statistically significant in all tested years, however with no significant changes over the period of 30 years, proving previous results and the hypothesis that *P. apollo* populations are able to maintain low long-term effective populations despite the high but constant amounts of asymmetry (Habel et al. 2009, 2012).

In principle, any external factor can produce plastic responses in organisms with the most prominent factors being temperature, photoperiod and humidity, where a single population can display annual or even seasonal variation (Blanckenhorn 2009). However, the analysis of the interaction of temperature and precipitation with morphology showed only weak or no distinct correlations. The first ECE (a deep drop in monthly temperature during February 1956) corresponds with no deviation from the average wing size despite the extremely cold weather, as the wing size parameter lies within the range of the dataset. February may be of high importance in the ontogeny of *P. apollo* as the eggs usually hatch at the end of February and the beginning of March (Žltková and Havranová 2017). Lower temperatures generally show positive effects on the overwintering eggs, although the overwintering larvae may be threatened by sudden temperature increases (Radchuk et al. 2013). Interestingly, Yu et al. (2012) found higher winter temperatures, especially in February, to have the highest negative correlation coefficient for the numbers of collected mountain Apollo specimens. Although our data does not show such a relationship, the wing size does correlate with the average monthly temperatures in February.

The second ECE with high short-term precipitation had little to no impact on the mountain Apollo populations at the study site; however we hypothesize that the abnormal rainfall may have had a negative impact on adults, pupas as well as host plants (McDermott Long et al. 2017). Although we do not reject the possibility of the ECEs' impact on wing morphology, the available meteorological data suggest only weak or no correlations to changes in pre/post 1960 datasets, therefore a different effect had to be considered when evaluating impact on wing morphology.

A road development in a complicated terrain of an incised meander of the Strečno mountain pass was carried out from 1959 to 1965. The populations of mountain Apollo were present along the cliff faces and the surrounding meadows, creating ideal conditions with all the essential properties present for this species. We hypothesize that the removal of large parts of cliff faces which were used for the leveling of the road and the use of the open meadows for storage of material or by movement of the construction machinery changed the habitat and reduced the number of habitable patches that the population can occupy and limited the resources at the location (Blake et al. 1994; Brommer and Fred 1999; Fred et al. 2006). If we consider that even positive attempts to restore the habitats can change the morphology of butterflies by indirectly altering the host plant presence or quality (Sivakoff et al. 2016), the road work must be considered a major factor. Direct and indirect alteration or complete destruction of the habitats during the construction phase had most likely an impact on the morphology of the mountain Apollo butterflies, although the complex and often subtle morpho-

logical changes do not allow us to hypothesize further regarding the possible outcomes to the foraging behavior or overall fitness of the affected populations.

A more subtle, but lasting effect of the newly built road was a reported increase in the numbers of vehicles at the location. Since the morphological changes of the wings were observable until the end of our dataset in 1968 we here hypothesize that the newly built road could have continually affected the mountain Apollo and their host and nectar plants (Bengtsson et al. 1989; Trombulak and Frissell 2000; Muñoz et al. 2015), until the butterfly completely disappeared from this location in the 1990s.

Due to its conservation status and the vulnerability of current populations of mountain Apollos, the historic collections often provide the only and possibly the last opportunity to analyze large numbers of these individuals. The populations collected by Ján Zelný at Strečno mountain pass were gathered in such numbers due to the attempts of discovering a new subspecies based on the wing color patterns, where the method of collection was to gather the largest number of individuals during each visit. In most cases the selective nature of the amateur collectors could include a bias by selecting the largest or subjectively most beautiful specimens. Due to the nonselective nature of the collection method used by Zelný and the fact that he was the only person collecting and mounting the specimens we concluded that even if he had a specific preference in collection of butterflies the bias is the same in all collected populations. The statistical analysis of environmental effects on the wing morphology of the 506 individuals of *P. apollo* from a single location, collected periodically almost at the same time in June and July, over 30 years creates a unique look into a historical population of *Parnassius apollo* no longer present at the location.

Conclusion

The asymmetry of wings did not significantly change over time, nor could it be correlated to the analyzed environmental factors. Only weak or no statistical correlation with the meteorological data within the analyzed timeseries was detected. There is a strong suggestion of anthropogenic impact due to road construction on the changes in wing morphology at the studied site.

Acknowledgements

We would like to extend our gratitude to the staff of the Slovak National Museum in Bratislava – Natural Science Museum and the archives of the Slovak Hydrometeorological Institute for providing the data and invaluable help during our research. We are also grateful to Ivana Cocherová, Júlia Haruštiaková, Vladimír Janský, Lubomír Rajter, and Michal Vlačiha for their help during the digitization of the samples. The research was funded by VEGA 1/0286/20.

References

- Adamski P, Witkowski Z (2002) Increase in fluctuating asymmetry during a population extinction: The case of the apollo butterfly *Parnassius apollo frankenbergeri* in the Pieniny Mts. *Biologia* 57: 597–560.
- Alibert P, Auffray JC (2002) Genomic coadaptation, outbreeding depression and developmental instability. In: Polak M (Eds) *Developmental Instability: Causes and Consequences*. Oxford University Press, Oxford, 116–134.
- Allendorf F, Luikart G, Aitken S (2013) *Conservation and the Genetics of Populations*. Wiley-Blackwell, London, 620 pp.
- Ananthakrishnan TN, Whitman D (2005) *Insect Phenotypic Plasticity: Diversity of Responses*. CRC Publishers, Boca Raton, 222 pp. <https://doi.org/10.1201/9781482294408>
- Beasley E, De A, Bonisoli-Alquati A, Mousseau TA (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. *Ecological Indicators* 30: 218–226. <https://doi.org/10.1016/j.ecolind.2013.02.024>
- Bengtsson BE, Elmquist H, Nyholm E (1989) On the Swedish apollo butterfly with an attempt to explain its decline. *Entomologisk Tidskrift* 110: 31–37.
- Blake S, Foster GN, Eyre MD, Luff ML (1994) Effects of habitat type and grassland management practices on the body size distribution of carabid beetles. *Pedobiologia* 38: 502–512.
- Blanckenhorn WU (2009) Causes and consequences of phenotypic plasticity in body size: The case of the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). In: Whitman, DW, Ananthakrishnan TN (Eds) *Phenotypic Plasticity of Insects: Mechanism and Consequences*. Science Publishers, Enfield, 369–422. <https://doi.org/10.1201/b10201-11>
- Blanco G, Sanchez JA, Vazquez E, Garcia E, Rubio J (1990) Superior developmental stability of heterozygotes at enzyme loci in *Salmo salar* L. *Aquaculture (Amsterdam, Netherlands)* 84(3–4): 199–209. [https://doi.org/10.1016/0044-8486\(90\)90086-3](https://doi.org/10.1016/0044-8486(90)90086-3)
- Bookstein FL (1997) *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge, 456 pp.
- Brommer JE, Fred MS (1999) Movement of the Apollo butterfly *Parnassius apollo* related to host plant and nectar plant patches. *Ecological Entomology* 24(2): 125–131. <https://doi.org/10.1046/j.1365-2311.1999.00190.x>
- Brown CR, Brown MB (1998) Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. *Evolution* 52: 1461–1475. <https://doi.org/10.1046/j.1365-2311.1999.00190.x>
- Capdeville P (1978) Les Races Géographiques de / Die Geographischen Rassen von *Parnassius apollo*. *Sciences Nat, Venette*, 193 pp.
- Clarke GM, Yen JL, McKenzie JA (2000) Wings and bristles: Character specificity of the asymmetry phenotype in insecticide-resistant strains of *Lucilia cuprina*. *Proceedings. Biological Sciences* 267(1455): 1815–1818. <https://doi.org/10.1098/rspb.2000.1215>
- da Rocha JR, De Almeida JR, Lins GA, Durval A (2010) Insects as indicators of environmental changing and pollution: a review of appropriate species and their monitoring. *Holos environment* 10(2): 250–262. <https://doi.org/10.14295/holos.v10i2.2996>

- Descimon H, Bachelard P, Boitier E, Pierrat V (2005) Decline and extinction of *Parnassius apollo* populations in France-continued. Studies on the ecology and conservation of butterflies in Europe. In: Kühn E, Feldmann R, Thomas JA, Settel J (Eds) Concepts and Case Studies. Pensoft Publishers, Sofia, 114–115.
- Dubovský M, Fedor PJ, Kucharyczyk H, Masarovič R, Balkovič J (2010) Zgrupowania wciornastków (Thysanoptera) pni drzew w różnowiekowych lasach dębowych Słowacji. / Assemblages of bark-dwelling thrips (Thysanoptera) of uneven-aged oak forests in Slovakia. Sylwan 154: 659–668.
- Engler R, Randin CF, Thuiller W, Dullinger S, Zimmermann NE, Araújo MB, Pearman PB, Le Lay G, Piedallu C, Albert CH, Choler P, Coldea G, De Lamo Xavier, Dirnböck T, Gégout J-C, Gómez-García D, Grytnes J-A, Heegaard E, Høistad F, Nogués-Bravo D, Normand S, Puşcaş M, Sebastià M-T, Stanisci A, Theurillat J-P, Trivedi MR, Vittoz P, Guisan A (2011) 21st century climate change threatens mountain flora unequally across Europe. Global Change Biology 17(7): 2330–2341. <https://doi.org/10.1111/j.1365-2486.2010.02393.x>
- Findlay CS, Houlahan J (1997) Anthropogenic correlates of species richness in southeastern Ontario wetlands. Conservation Biology 11(4): 1000–1009. <https://doi.org/10.1046/j.1523-1739.1997.96144.x>
- Fountain T, Butlin RK, Reinhardt K, Otti O (2015) Outbreeding effects in an inbreeding insect, *Cimex lectularius*. Ecology and Evolution 5(2): 409–418. <https://doi.org/10.1002/ece3.1373>
- Frankham R (2005) Stress and adaptation in conservation genetics. Journal of Evolutionary Biology 18(4): 750–755. <https://doi.org/10.1111/j.1420-9101.2005.00885.x>
- Fred MS, O'Hara RB, Brommer JE (2006) Consequences of the spatial configuration of resources for the distribution and dynamics of the endangered *Parnassius apollo* butterfly. Biological Conservation 130(2): 183–192. <https://doi.org/10.1016/j.biocon.2005.12.012>
- Garland T, Kelly SA (2006) Phenotypic plasticity and experimental evolution. The Journal of Experimental Biology 209(12): 2344–2361. <https://doi.org/10.1242/jeb.02244>
- Gibbs M, Wiklund C, Van Dyck H (2011) Temperature, rainfall and butterfly morphology: Does life history theory match the observed pattern? Ecography 34(2): 336–344. <https://doi.org/10.1111/j.1600-0587.2010.06573.x>
- Habel JC, Zachos FE, Finger A, Meyer M, Louy D, Assmann T, Schmitt T (2009) Unprecedented long-term genetic monomorphism in an endangered relict butterfly species. Conservation Genetics 10(6): 1659–1665. <https://doi.org/10.1007/s10592-008-9744-5>
- Habel JC, Reuter M, Drees C, Pfaender J (2012) Does isolation affect phenotypic variability and fluctuating asymmetry in the endangered Red Apollo? Journal of Insect Conservation 16(4): 571–579. <https://doi.org/10.1007/s10841-011-9442-3>
- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9. <https://doi.org/10.1016/j.bcp.2008.05.025>
- Hoelzel AR, Fleischer RC, Campagna C, Le Boeuf BJ, Alvord G (2002) Impact of a population bottleneck on symmetry and genetic diversity in the northern elephant seal. Journal of Evolutionary Biology 15(4): 567–575. <https://doi.org/10.1046/j.1420-9101.2002.00419.x>
- Hoffmann AA, Woods RE, Collins E, Wallin K, White A, McKenzie JA (2005) Wing shape versus asymmetry as an indicator of changing environmental conditions in insects.

- Australian Journal of Entomology 44(3): 233–243. <https://doi.org/10.1111/j.1440-6055.2005.00469.x>
- Hrubý K (1964) *Prodromus Lepidopter Slovenska*. Slovak Academy of Sciences, Bratislava, 962 pp.
- Jugovic J, Zupan S, Bužan E, Čelik T (2018) Variation in the morphology of the wings of the endangered grass-feeding butterfly *Coenonympha oedippus* (Lepidoptera: Nymphalidae) in response to contrasting habitats. *European Journal of Entomology* 115: 339–353. <https://doi.org/10.14411/eje.2018.034>
- Kaeppeler S (2012) Heterosis: Many genes, many mechanisms – End the search for an undiscovered unifying theory. *International Scholarly Research Notices* 12: 1–12. <https://doi.org/10.5402/2012/682824>
- Kizek T (1997) Ochrana jasoňa červenookého na Slovensku – Projekt „APOLLO“. Slovenská Agentúra Životného Prostredia, Banská Bystrica, 23 pp.
- Klingenberg CP (2008) MorphoJ. University of Manchester. http://www.flywings.org.uk/MorphoJ_page.htm
- Klingenberg CP (2011) MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11(2): 353–357. <https://doi.org/10.1111/j.1755-0998.2010.02924.x>
- Klingenberg CP (2015) Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. *Symmetry* 7(2): 843–934. <https://doi.org/10.3390/sym7020843>
- Klingenberg CP, McIntyre GS (1998) Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with procrustes methods. *Evolution; International Journal of Organic Evolution* 52(5): 1363–1375. <https://doi.org/10.1111/j.1558-5646.1998.tb02018.x>
- Kocián F (1959a) From the construction of concrete road Žilina – Strečno/Zo stavby betónovej cesty Žilina – Strečno. <https://vtedy.tasr.sk/zoom/428143/view?page=1&p=separate&tool=info&view=0,0,1148,1238>
- Kocián F (1959b) From the construction of concrete road Žilina – Strečno/Zo stavby betónovej cesty Žilina – Strečno. <https://vtedy.tasr.sk/zoom/428512/view?page=1&p=separate&tool=info&view=0,0,1249,1238>
- Kocián F (1959c) A second view on the construction of the room near Žilina/2 Pohľad na stavbu štátnej cesty pri Žiline. <https://vtedy.tasr.sk/zoom/449819/view?page=1&p=separate&tool=info&view=0,0,1331,1305>
- Le Roy C, Debat V, Llaurens V (2019) Adaptive evolution of butterfly wing shape: From morphology to behaviour. *Biological Reviews of the Cambridge Philosophical Society* 94. <https://doi.org/10.1111/brv.12500>
- Linnaeus C (1758) *Systema Naturæ per Regna Tria Naturæ, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*. Tomus I. Editio decima, reformata. Holmiæ, 824 pp. <https://doi.org/10.5962/bhl.title.542>
- McDermott Long O, Warren R, Price J, Brereton TM, Botham MS, Franco AM (2017) Sensitivity of UK butterflies to local climatic extremes: which life stages are most at risk? *Journal of Animal Ecology* 86: 108–116. <https://doi.org/10.1111/1365-2656.12594>
- Merckx T, Van Dyck H (2006) Landscape structure and phenotypic plasticity in flight morphology in the butterfly *Pararge aegeria*. *Oikos* 113(2): 226–232. <https://doi.org/10.1111/j.2006.0030-1299.14501.x>

- Ministry of Environment of the Slovak Republic (2019) Overview of historic meteorological extremes in Slovakia / Prehľad historických extrémov (extrémnych hodnôt) vybraných meteorologických prvkov na území Slovenskej republiky. <http://www.shmu.sk/sk/?page=1384>
- Muñoz PT, Torres FP, Megías AG (2015) Effects of roads on insects: A review. *Biodiversity and Conservation* 24(3): 659–682. <https://doi.org/10.1007/s10531-014-0831-2>
- Nakonieczny M, Kedzioriski A, Michalczyk K (2007) Apollo butterfly (*Parnassius apollo* L.) in Europe: Its history, decline and perspectives of conservation. *Functional Ecosystems and Communities* 1: 56–79.
- Nogués-Bravo D, Araújo MB, Errea MP, Martínez-Rica JP (2007) Exposure of global mountain systems to climate warming during the 21st Century. *Global Environmental Change* 17(3–4): 420–428. <https://doi.org/10.1016/j.gloenvcha.2006.11.007>
- Palmer AR, Strobeck C (1986) Fluctuating asymmetry: Measurement, analysis, patterns. *Annual Review of Ecology Evolution and Systematics* 17(1): 391–421. <https://doi.org/10.1146/annurev.es.17.110186.002135>
- Parsons PA (1990) Fluctuating asymmetry: An epigenetic measure of stress. *Biological Reviews of the Cambridge Philosophical Society* 65(2): 131–145. <https://doi.org/10.1111/j.1469-185X.1990.tb01186.x>
- Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *The Journal of Experimental Biology* 209(12): 2362–2367. <https://doi.org/10.1242/jeb.02070>
- Radchuk V, Turlure C, Schtickzelle N (2013) Each life stage matters: The importance of assessing the response to climate change over the complete life cycle in butterflies. *Journal of Animal Ecology* 82(1): 275–285. <https://doi.org/10.1111/j.1365-2656.2012.02029.x>
- RStudio Team (2015) RStudio: Integrated Development for R. <http://www.rstudio.com>
- Sivakoff FS, Morris WF, Aschehoug ET, Hudgens BR, Haddad NM (2016) Habitat restoration alters adult butterfly morphology and potential fecundity through effects on host plant quality. *Ecosphere* 7(11): e01522. <https://doi.org/10.1002/ecs2.1522>
- Spellerberg IF (2005) *Monitoring Ecological Change*. Cambridge University Press, Cambridge, 412 pp. <https://doi.org/10.1017/CBO9780511614699>
- Stern PC, Young OR, Druckman DE (1992) *Global Environmental Change: Understanding the Human Dimensions*. National Academy Press, 308 pp.
- Sukhodolskaya RA, Saveliev AA (2014) Effects of ecological factors on size-related traits in the ground beetle *Carabus granulatus* L. (Coleoptera, Carabidae). *Russian Journal of Ecology* 45(5): 414–420. <https://doi.org/10.1134/S1067413614050142>
- Todisco V, Gratton P, Cesaroni D, Sbordoni V (2010) Phylogeography of *Parnassius apollo*: Hints on taxonomy and conservation of a vulnerable glacial butterfly invader. *Biological Journal of the Linnean Society. Linnean Society of London* 101(1): 169–183. <https://doi.org/10.1111/j.1095-8312.2010.01476.x>
- Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation Biology* 14(1): 18–30. <https://doi.org/10.1046/j.1523-1739.2000.99084.x>
- Valen LV (1962) A study of fluctuating asymmetry. *Evolution; International Journal of Organic Evolution* 16(2): 125–142. <https://doi.org/10.1111/j.1558-5646.1962.tb03206.x>

- Van Dyck H, Van Strien AJ, Maes D, Van Swaay CA (2009) Declines in common, widespread butterflies in a landscape under intense human use. *Conservation Biology* 23(4): 957–965. <https://doi.org/10.1111/j.1523-1739.2009.01175.x>
- Van Swaay C, Martin W (1999) Red Data Book of European Butterflies (Rhopalocera). Council of Europe Publishing, Strasbourg, 260 pp.
- Vrijenhoek RC, Lerman S (1982) Heterozygosity and developmental stability under sexual and asexual breeding systems. *Evolution; International Journal of Organic Evolution* 36(4): 768–776. <https://doi.org/10.1111/j.1558-5646.1982.tb05443.x>
- Wallner AM, Molano-Flores B, Dietrich CH (2013) Using *Auchenorrhyncha* (Insecta: Hemiptera) to develop a new insect index in measuring North American tallgrass prairie quality. *Ecological Indicators* 25: 58–64. <https://doi.org/10.1016/j.ecolind.2012.09.001>
- Webster MARK, Sheets D (2010) A practical introduction to landmark-based geometric morphometrics. *The Paleontological Society Papers* 16: 163–188. <https://doi.org/10.1017/S1089332600001868>
- Wenzel M, Schmitt T, Weitzel M, Seitz A (2006) The severe decline of butterflies on western German calcareous grasslands during the last 30 years: A conservation problem. *Biological Conservation* 128(4): 542–552. <https://doi.org/10.1016/j.biocon.2005.10.022>
- Whitman DW, Agrawal AA (2009) What is phenotypic plasticity and why is it important. *Phenotypic plasticity of insects: Mechanisms and consequences* 1: 1–63. <https://doi.org/10.1201/b10201>
- Windig JJ, Rintamäki PT, Cassel A, Nylin S (2000) How useful is fluctuating asymmetry in conservation biology: Asymmetry in rare and abundant *Coenonympha* butterflies. *Journal of Insect Conservation* 4(4): 253–261. <https://doi.org/10.1023/A:1011332401156>
- Woods RE, Sgrò CM, Hercus MJ, Hoffmann AA (1999) The association between fluctuating asymmetry, trait variability, trait heritability, and stress : A multiply replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution; International Journal of Organic Evolution* 53(2): 493–505. <https://doi.org/10.1111/j.1558-5646.1999.tb03784.x>
- Yu F, Wang H, Wang S, Zhang Q, Ji R (2012) Response of *Parnassius apollo* population and vertical distribution to climate warming. *Acta Ecologica Sinica* 32(19): 6203–6209. <https://doi.org/10.5846/stxb201107281107>
- Žitková K, Havranová I (2017) (*Parnassius apollo* Linnaeus, 1758) na roky 2017 – ŠOP SR. Štátna ochrana prírody Slovenskej republiky, Slovakia. [http://www.sopsr.sk/files/jason-cervenooky-\(Parnassius%20apollo\).docx](http://www.sopsr.sk/files/jason-cervenooky-(Parnassius%20apollo).docx)
- Zvaríková M, Kiseliák J, Zvarík M, Masarovič R, Prokop P, Fedor P (2016) Ecological gradients and environmental impact in the forest dwelling *Haplothrips subtilissimus* (Thysanoptera: Phlaeothripidae) phenotypic variability. *Ecological Indicators* 66: 313–320. <https://doi.org/10.1016/j.ecolind.2016.01.038>

Rocky pine forests in the High Coast Region in Sweden: structure, dynamics and history

Jennie Sandström¹, Mattias Edman¹, Bengt Gunnar Jonsson¹

¹ Department of Natural Sciences, Mid Sweden University, SE-851 70 Sundsvall, Sweden

Corresponding author: Jennie Sandström (jennie.sandstrom@miun.se)

Academic editor: Klaus Henle | Received 28 March 2019 | Accepted 18 December 2019 | Published 27 March 2020

<http://zoobank.org/46A93E31-820B-4B9D-A75B-13FB9DD4BE1A>

Citation: Sandström J, Edman M, Jonsson BG (2020) Rocky pine forests in the High Coast Region in Sweden: structure, dynamics and history. Nature Conservation 38: 101–130. <https://doi.org/10.3897/natureconservation.38.34870>

Abstract

Almost all forests in Sweden are managed and only a small fraction are considered natural. One exception is low productive forests where, due to their limited economical value, natural dynamics still dominate. One example is the Scots pine (*Pinus sylvestris* L.) forests occurring on rocky and nutrient-poor hilltops. Although these forests represent a regionally common forest type with a high degree of naturalness, their dynamics, structure and history are poorly known. We investigated the structure, human impact and fire history in eight rocky pine forests in the High Coast Area in eastern Sweden, initially identified as good representatives of this forest type. This was done by sampling and measuring tree sizes, -ages, fire-scarred trees, as well as dead wood volumes and quality along three transects at each site. The structure was diverse with a sparse layer of trees (basal area 9 m² and 640 trees larger than 10 cm ha⁻¹) in various sizes and ages; 13 trees ha⁻¹ were more than 300 years old. Dead wood (DW), snags and logs in all stages of decay, was present and although the actual DW (pine) volume (4.4 m³ ha⁻¹) and number of units (53 ha⁻¹) was low, the DW share of total wood volume was 18% on average. Dead wood can be present for several centuries after death; we found examples of both snags and logs that had been dead more than 300 years. Frequent fires have occurred, with an average cycle of 40 years between fires. Most fires occurred between 1500–1900 and many of them (13) during the 1600s. However, fires were probably small since most fire years were only represented at one site and often only in one or a few samples. The rocky pine forests in the High Coast Area are representative of undisturbed forests with low human impact, exhibiting old-growth characteristics and are valuable habitats for organisms connected to sun-exposed DW. Management of protected rocky pine forests may well include small-scale restoration fires and the limited DW volumes should be protected.

Keywords

boreal, coarse woody debris, dendrochronology, fire history, natural forest, pine heath forest, shingle field pine forest

Introduction

Human influence on boreal forests has varied considerably over time and has dramatically transformed forests in Fennoscandia during the last centuries (Östlund et al. 1997). The multi-aged, structurally diverse and old-growth forests that once dominated the Swedish forest landscape have today been replaced by young, even-sized and single-aged managed forests (Östlund et al. 1997; Axelsson and Östlund 2001). In forests with low levels of human impact, there are structures and processes, such as various kinds of disturbances, (e.g. wind, fire, insects and browsing) that create a structurally diverse and heterogeneous forest (Bradshaw et al. 2011; Brumelis et al. 2011). However, studies of natural forest structure and dynamics are hampered by the limited availability of unmanaged reference forests (Kuuluvainen et al. 2017).

In Sweden, nutrient poor forests with low productivity ($< 1 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$) have been exempted from regular forestry by the Forest Act since the 1970s (Anonymous 2017). Approximately 4.6 M ha of forest land are left out from management because of low productivity (Fridman and Wulff 2018). Some of these forests occur on nutrient-poor, rocky areas on hilltops, where Scots pine (*Pinus sylvestris* L.) is the major tree species. Because of the nutrient-poor conditions, the distribution of trees is sparse and the forests are characterized by openness and sun-exposed conditions. There is no exact estimate on how much forest land consists of rocky pine forests; however, they constitute a significant share of the 14% of the Swedish forest land that is covered by unproductive forest (Forestry 2015).

Rocky pine forests can frequently be found in the High Coast Area in the eastern parts of Northern Sweden (Fig. 1). This area is characterized by a steep terrain and rugged topography and in combination with the highest isostatic up-lift in the world after the latest glaciation resulted in recognition as a World Heritage Area. The current land up-lift is 8 mm year^{-1} and total recovery is almost 300 m in altitude (Berglund 2012). The area that formerly was below sea level has been exposed to wave action and coastal erosion and, in particular, convex land surfaces often consist of bare bedrock and very shallow soil.

The low productivity in rocky pine forests in the High Coast Area, together with their inaccessibility, partly explains why many of these forests have escaped extensive human use. These forests mostly have continuity in old-growth characteristics with diverse canopy structure, old trees and dead wood. Hence, these types of forests could function as small refuges for organisms dependent on old-growth conditions. For example, many threatened insects are dependent on dead wood (Stokland et al. 2012), including sun-exposed dead wood (Wikars 2015). An example is *Chalcophora mariana* L., a beetle dependent on sun-exposed dead wood, and currently found at only two sites in Sweden, of which the Skule National Park in the High Coast Area is one of them (Ehnström and Bader 2013).

To date, very few studies about the structure and history of nutrient-poor, rocky pine forests have been made. This is true not only for Northern Europe but also on a more general, global scale. Some studies have been made on rocky black pine (*Pinus nigra* Arn.) forests in Spain and even though these forests resemble Scots pine forests in

northern rocky areas, they constitute a different forest type with a contrasting historical and landscape context (e.g. Fule et al. 2003; Rubiales et al. 2007; Hernandez et al. 2011; Camarero et al. 2013; Ehnström and Bader 2013).

Due to lack of knowledge of the special habitat that rocky, nutrient-poor pine forests constitute and the importance of baseline information on forests with low human impact, we have examined the structure, history of human use and fire history in rocky pine forests in the High Coast Region. We have used a combination of several methods; investigation of the current forest structure, the use of historical records and biological archives (dendrochronology), which allowed us to address the following questions: 1) What characterizes the forest structure and dynamic in rocky pine forests? 2) What is the fire history in the rocky pine forests? 3) To what extent has human use in the past influenced the rocky pine forests?

Methods

Study area

The study was conducted within a 15 × 75 km area (approximately at 62.5°–63.1°N, 17.9°–18.7°E, DD) in the High Coast Region situated in Västernorrland County located in the southern boreal zone of Sweden (Fig. 1). The High Coast Region on the east coast of Sweden along the Baltic Sea is characterized by a rugged and steep terrain. The area was covered with ice during the latest ice age and after the ice had melted the area was under sea level for millennia; only the highest hill tops > 285 m.a.s.l. could be seen as small islands (Lundkvist 1986). Most of the plots are situated on hilltops and the altitude ranges from approximately 50 to 230 m, i.e. below the highest coastline. Mean annual temperature in the region is around 3 °C, mean annual precipitation is 800 mm, the length of the growing season (number of days with temperature > 5° C) is approximately 150 days. The average maximum snow depth is 70 cm and the snow covers the ground 150–175 days every year (SMHI 2016). Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris*) are the two dominant tree species in the study area with Scots pine dominating on dry and nutrient poor sites. A general inventory of 26 sites with rocky pine forests was conducted in 2011 in the study area by the County Administration in Västernorrland with the aim to map core areas with high conservation values, e.g. presence of big and old trees, diverse forest structure and abundant dead wood (Salomonsson and Bader 2015). Eight of these 26 study sites with the highest ranking (high conservation values) were selected for this study.

Sampling for stand structure

In August 2014 we established three band transects at each site, resulting in total in 24 transects. We used band transects since they sample spatial heterogeneity better than circular plots. The studied forests are highly heterogeneous and exposed bedrock oc-

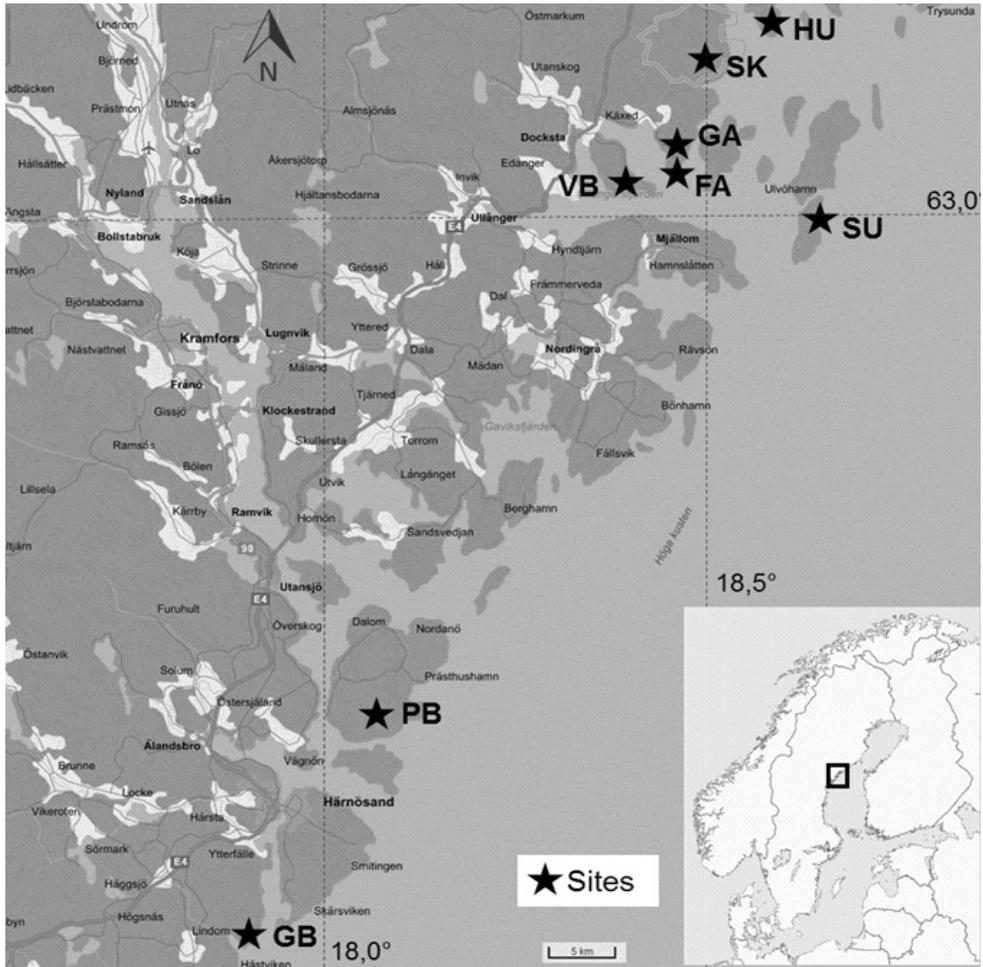


Figure 1. Study area. Location of the study area in the High Coast Area, Västernorrland County, in the southern boreal zone of central Sweden. Forest structure was investigated at eight sites with rocky pine forest (shown by stars, **GB** Gropberget, **PB** Porsmyrberget, **VB** Vårdkallberget, **FA** Fanön, **GA** Gårdberget, **SU** Southern Ulvön, **SK** Skuleskogen National Park, **HU** Hummelvik Nature Reserve)

curs mixed with parts of more closed forest. Transects were 10 × 100 m for living trees, but for dead wood sampling we expanded plots to 20 × 100 m to ensure a sufficient number of sampled dead wood units.

To avoid subjectivity in the placement of transects we randomized their placement by using a numbered grid that was placed over a map of the core area at each site and starting points as well as the direction (N, NE, E, SE, S, SW, W or NW) were randomly assigned, but with a minimum distance of 100 m. Within each transect, we recorded x and Y coordinates, species and diameter at breast height (DBH) for all living (stems ≥ 1.3 m high) trees. To describe the spatial distribution pattern, we calculated

the distance from each tree to the nearest tree in the \times direction and then computed the variance to mean ratio of these distance, i.e. a one-dimensional spatial analysis (Horne and Schneider 1995). We then used a χ^2 -test to test whether the distribution of trees where random or significantly aggregated. We extracted two increment cores at 30 cm height from each living tree (DBH \geq 10 cm) using a 6 mm increment borer (Haglöfs, Mora, Sweden). We measured the height of the tallest tree along the transect with a Laser Height Meter (Nikon Forestry 550) at 0 m, 50 m and 100 m.

All snags and logs with a maximum diameter of \geq 10cm and with their base inside a transect were included. We recorded both base- and top diameters, DBH, length and decay class. We used a four class system for decaying wood: 1 Hard dead wood – The volume of the stem consisting of $>$ 90% hard wood, hard surface, very little impact from wood-decaying organisms; 2 Partly decayed wood – 10–25% of the stems' volume consists of soft wood, a sheath knife goes through the surface but not through the whole sapwood; 3 Decayed wood – 26–75% of the stems volume consists of soft to very soft wood; 4 Substantially decayed wood – 75–100% of the stems volume consists of soft-very soft wood, a sheath knife can penetrate the whole stem, but a hard core can occur (Esseen et al. 2003). We collected samples for dead wood for dating with a chainsaw, taking only small samples (cookies) rather than whole cross-sections when possible. The dead wood samples were used for age determination and calculating time since death. Naturally created high stumps were recorded as snags. We used the conic-paraboloid formula (Fraver et al. 2007) to calculate the volume of dead wood, a formula that has a greater precision and lower bias than more commonly used formula. However, due to the shape of the trees with many branches and bent, crooked stems, the volume calculations are rough estimates rather than exact values. Cut stumps were not included in dead wood volume calculations but we noted diameter and species and took samples for dating to quantify past harvesting.

Sampling for fire history

In autumn (September–November) 2015 we carried out a comprehensive search of each site for fire scars in stumps, living and dead trees. Two persons visited each site one whole day, resulting in approximately 10 search hours at each site covering the core area and the adjacent forest. Every tree with a fire scar was mapped and samples were later collected with a chainsaw. We only took partial sections whenever possible to avoid unnecessary damage. A total of 52 fire-scarred wood samples were collected and dated from eight sites. An additional six samples from dead wood sampling in 2014 and 3 samples from Skuleskogen, sampled in 2010, also had fire scars and were included, resulting in a total of 61 dated samples. More than half of the samples (33) contained scars from repeated fires and the maximum number of fires in one sample was four.

Dendrochronology and cross-dating

We mounted cores from living trees and cross-sections from dead trees and sanded them with increasingly fine grain size until a fine polish was achieved (down to grain size 400 for all samples and to 600 for some samples) using standard methods (Speer 2010). Ring widths and number of rings (age) were measured using a scanner and the image analyzing software WINDENDRO (version 2014). We used Applequist (1958) pith locator in samples that had missing rings in the core. Age was determined by counting every year ring to the pith. A few samples had a rotten core and we then used the mean growth rate for each site and the DBH to estimate the age. As a measure of growth at each site, we used the average ring width from each tree and its variability for all trees at each site. Samples from living trees with high age and several samples from dead wood that were visually dated with high accuracy were selected to create a master chronology. We used the software program COFECHA (Holmes 1983; Grissino-Mayer 2001) to cross-date our samples and suggested years were always visually double-checked to make sure that distinct marker years (with typical late-wood features) were dated correctly. The program ARSTAN was then used to create a standardized master chronology and tree ring series with questionable dating were eliminated before standardization (Cook and Krusic 2005). The master chronology from living and some dead wood samples resulted in 130 samples covering 818 years (1197–2014) with a mean sensitivity of 0.30 and a relatively strong inter-correlation of 0.515. This master chronology was used to date samples from dead wood and as dead wood samples were dated, they were added to the master chronology. We were able to date 70% of all dead wood samples; 30% of the logs were too decomposed to allow sampling or difficult to date. The dated samples belonged to all decay classes but only a few samples in decay class four were possible to sample and date. The master chronology was also used to date fire scars and because the growth is related to precipitation during the main fire season in June, we noted the average ring width for all the fire years. The final chronology (see Appendix 1) with living as well as dead wood samples with and without fire scars resulted in 248 samples covering 819 years (1197–2015) with a mean sensitivity of 0.29 and an inter-correlation of 0.505 and with a minimum of ten samples, beginning with the year 1431. We also calculated time since death for the dead wood; however, we cannot rule out the possibility that we have overestimated this value as in some cases the outermost parts might have eroded. The computer program FHAES (Fire History Analysis and Exploration System, version 2.0) was used for structuring and analyzing fire data. FHAES was also used to produce fire history graphs.

Results

Site history

Very little written information is available that is relevant for the specific historical use of the rocky pine forests. The general history of the region is, however, relatively well documented (e.g. Lundkvist 1986; Baudou 1995). Based on findings of old

building structures in stone and grave cairns, it is clear that some places in the area have been populated by humans for at least 3,000 years (Baudou 1995). The few early settlers based their livelihood mostly on fishing and hunting (e.g. seal along the coastline) and fishing has been the main income in the region for a long time (Lundkvist 1986). The possibility for mining was explored during the 1700's at several places along the High Coast Region, e.g. at southern Ulvön and Hemsön (sites SU and PB), and, although it never became an industrial use, the attempts might have impacted the nearby forests to some extent (Andersson 1975; Lindh 1991). When the first large-scale wave of industrial usage of the forests started during the 1800's, it is unlikely that the nutrient-poor pine forests with rather small trees on the hill-tops along the High Coast also were exploited. In a map of Vårdkallberget from 1851 it is possible to read (in Swedish) that the area close to the hill-top where the rocky pine forests occur is named "Näs by skogsmark" and "Myre by skogsmark" (Renström and Hedström 1985). This means that this area was classified as a common forest resource, free to use for the village members, but to what extent it was actually used is not known. Close to one (VB) of the eight sites, remnants of two old tar pits have been found (Fig. 2). The pits are located just along the coastline and it is unclear whether the wood for the tar was collected nearby or from the pine forest in the hill-top (the hill-top is located approximately 500 m from the tar pit remnants). The area around VB was not populated before 1780 but nearby settlers used the area mainly for fishing even before the settlements (Renström and Hedström 1985; Lundkvist 1986). We only found a few harvested stumps on our plots, at one plot at GA and in two neighboring plots at the SU site, indicating very limited forestry activities. We found nine stumps at GA and ten stumps at SU, most of them heavily eroded, and we were only able to date six stumps. The outermost year ring from the stumps at SU was dated to 1736, 1827 and 1886 and for GA 1752, 1813 and 1828, corresponding to 200 years since harvest on average.

Stand structure

Scots pine was the dominating tree species in rocky pine forests and stands were very scattered and open; they had very little mineral soil and the ground was interspersed with bare rock and with a vegetation dominated by lichens and dwarf shrubs (Fig. 3). A general pattern was that the forest became even less dense and increasingly rocky higher up on the mountain. Trees had different sizes and they grow upright but also strongly leaning, some even grow horizontally despite being healthy and alive.

The general stand structure at the eight sites was characterized by sparsely distributed trees with a mean basal area of approximately $9 \text{ m}^2 \text{ ha}^{-1}$ and a density of $640 \text{ trees ha}^{-1}$ on average (Table 1). Variance to mean ratio showed that trees both had an aggregated and a random distribution pattern, depending on site (Table 1). The forests mainly consisted of Scots pine, which constituted approximately 90% of all the trees. The second most common tree species was Norway spruce, which constituted 7% on average. Deciduous trees were usually present but in very low density (Table 1). Less

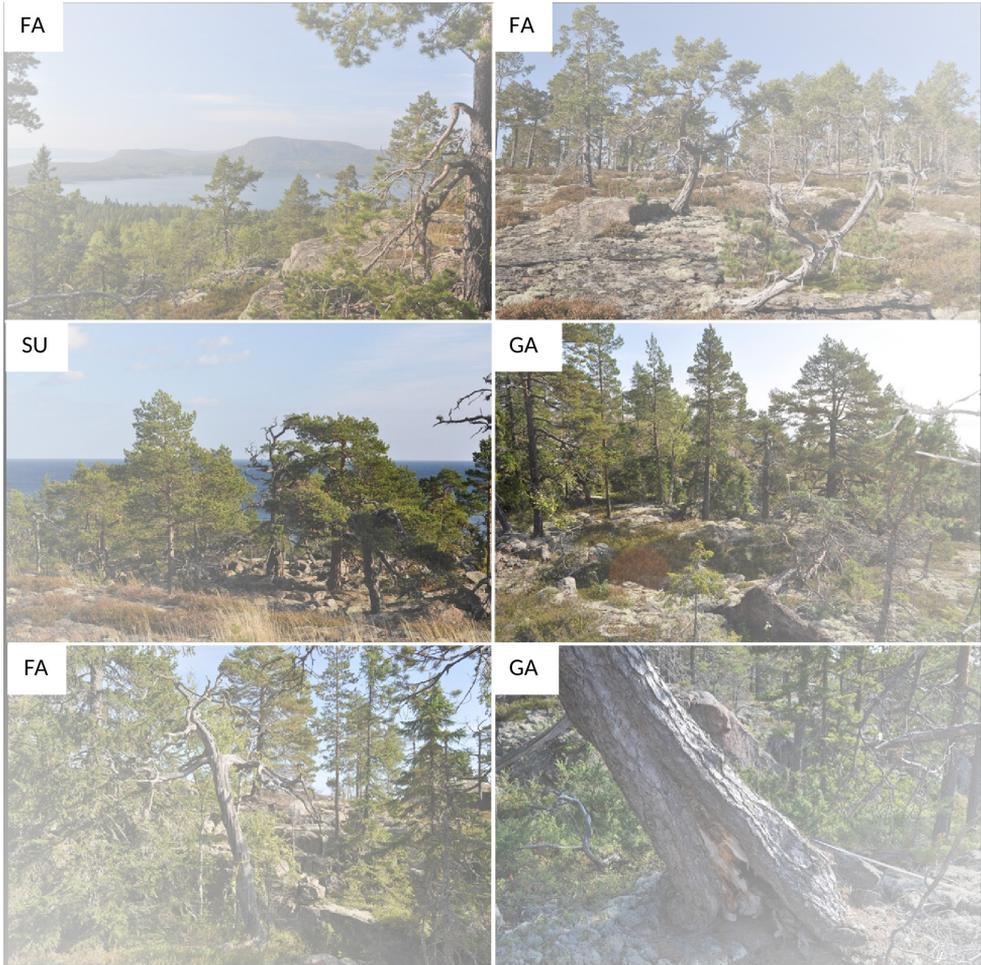


Figure 3. General description of rocky forests in the High Coast Area. Photos from Fanön (FA), Gårdberget (GA) and S. Ulvön (SU), which show the topography, typical characteristics in rocky pine forests with dispersed trees and dead wood in various sizes in rocky terrain, spruce interspersed at concave surfaces and a fire-scarred pine.

All ages are present for pine but really old spruces were lacking. The oldest spruces were a maximum of 275 years old whereas pines were older; approximately 1 tree ha⁻¹ was above 400 years old and 13 trees ha⁻¹ were more than 300 years old. The correlation between age and DBH was generally low ($R^2 = 0.31$ for pine and $R^2 = 0.11$ for spruce) but due to the large sample size significant for both tree species ($p < 0.001$ and $p < 0.05$ respectively; Fig 5). The oldest pine (418 years) was also the largest (48 cm DBH), while for spruce the oldest tree (259 years) was only 11 cm at breast height (Fig 5). The age span was wide across all diameter classes; for example, the age span of trees with a DBH of 10 cm varied from 28 to 282 years and from 57 to 216 years for pine and spruce, respectively.

Table 1. General stand structure. Stand structure data for eight rocky *Pinus sylvestris* dominated forests in the High Coast Region in Northern Sweden. Values are means with SE in parenthesis and based on three band transects per site. Site acronyms as in Figure 1.

Study site	GB	PB	VB	FA	GA	SU	SK	HU
Dbh (cm), Trees ≥ 1.3 m height	11.4 (0.6)	9.8 (1.2)	11.2 (0.8)	11.3 (2.8)	11.1 (1.0)	12.2 (0.7)	12.6 (0.3)	9.3 (0.6)
Basal area ($m^2 ha^{-1}$), Trees ≥ 1.3 m height	11.0 (1.9)	8.8 (0.9)	10.2 (1.1)	8.1 (2.4)	10.2 (1.9)	6.3 (0.8)	10.1 (2.1)	8.8 (0.9)
Maximum height, Pine (m), average	10.1 (0.67)	7.0 (0.27)	6.6 (0.23)	8.4 (0.42)	8.4 (0.45)	6.2 (0.05)	8.0 (0.67)	5.5 (0.25)
Age, Pine (yr), average ≥ 10 cm DBH	194 (16)	157 (10)	182 (10)	147 (4)	157 (15)	189 (21)	156 (30)	158 (24)
Age, Pine (yr), maximum	334	299	372	276	418	403	442	376
No. of living trees $ha^{-1} \geq 1.3$ m height	647 (94)	713 (91)	636 (76)	563 (39)	753 (234)	363 (19)	593 (118)	783 (72)
No. of living trees $ha^{-1} \geq 10$ cm DBH	280 (70)	300 (15)	297 (32)	300 (60)	323 (33)	220 (6)	303 (61)	363 (35)
Spatial distribution (variance to mean ratio, VMR)	1.9*	1.1	1.2	3.3*	1.2	1.7*	1.7*	1.0
Pine, share (%)	77 (15)	95 (3)	95 (1.4)	78 (6)	88 (11)	92.5 (4)	94 (1.3)	98 (2.3)
Spruce, share (%)	20 (13)	4 (2.2)	5 (1.4)	4 (0.2)	9 (7)	6 (4)	4 (0.8)	0 (0)
Deciduous, share (%)	3 (1.9)	1 (1.1)	0 (0)	18 (5.8)	3 (3.4)	1.5 (1.5)	2 (1.1)	2 (2.2)
Dead wood volume ($m^3 ha^{-1}$)	4.8 (1.09)	1.0 (0.49)	1.7 (0.31)	1.9 (0.52)	2.6 (1.30)	1.9 (0.89)	1.5 (0.76)	0.6 (0.21)
Standing								
Downed	3.3 (1.56)	1.7 (0.29)	1.6 (0.30)	3.3 (1.14)	2.0 (0.15)	2.0 (0.99)	4.0 (1.44)	1.2 (0.52)
Proportion dead wood, basal area (%)	18.9 (1.91)	13.5 (1.69)	16.7 (2.88)	21.7 (5.64)	16.7 (2.64)	26.1 (6.00)	20.3 (8.47)	9.9 (2.61)

*Significantly aggregated

Table 2. Tree-ring width based growth. The growth of living Scots pines measured as average annual tree-ring width in eight rocky *Pinus sylvestris* dominated forests in the High Coast Region in Northern Sweden. Values are means and SD from all trees > 10 cm DBH at the sites. Site acronyms as in Figure 1.

Study site	GB	PB	VB	FA	GA	SU	SK	HU
Growth (mm yr ⁻¹)	0.53	0.53	0.58	0.68	0.56	0.48	0.55	0.52
SD	0.21	0.20	0.29	0.38	0.26	0.25	0.24	0.19
n	82	85	84	70	83	59	49	89

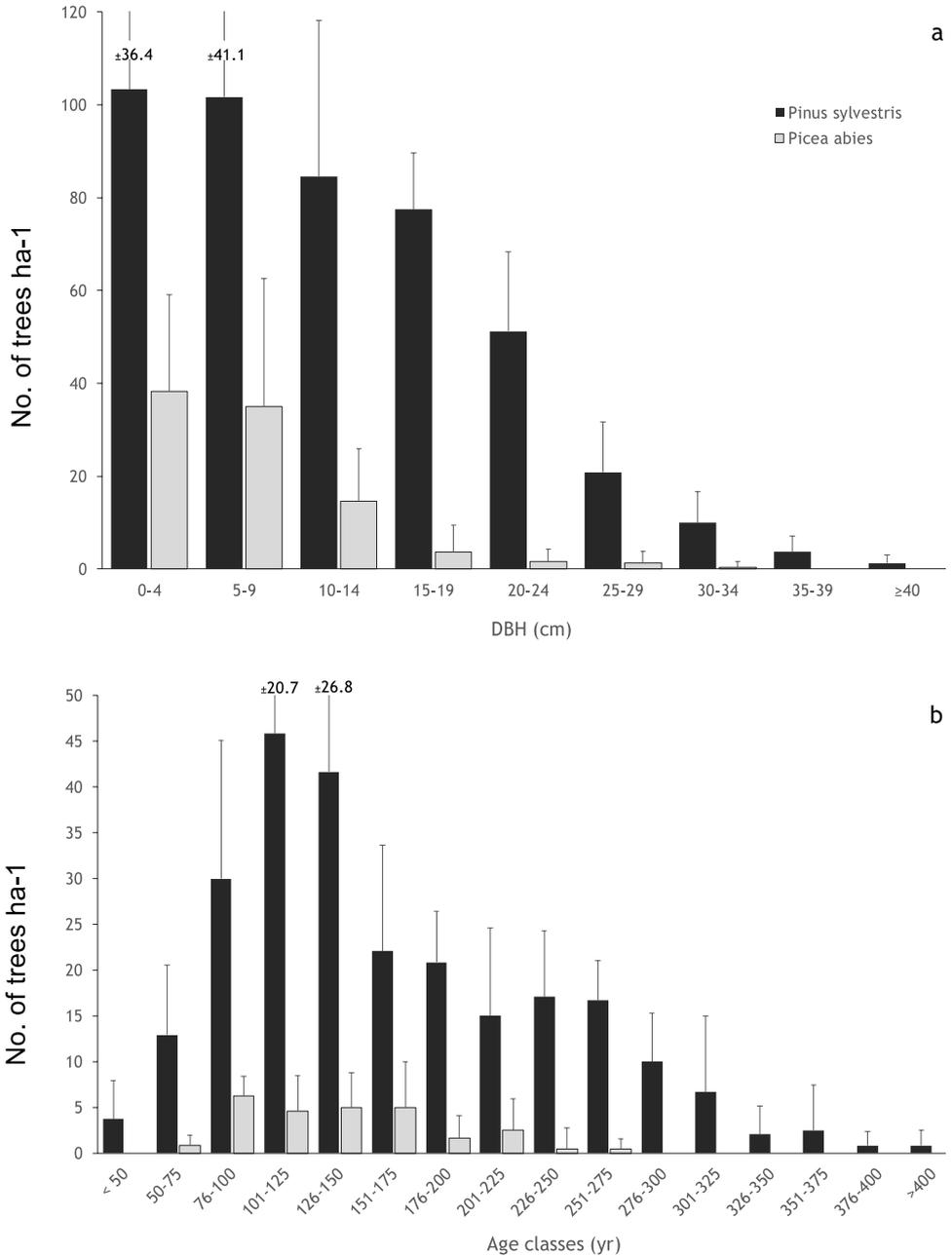


Figure 4. Categories of tree size and tree age. Summary of the distributions of diameter at breast height (DBH) (a) and age (b) (> 10 cm DBH) for living trees of pine and spruce at the eight sites combined (with mean and SD, n = 8).

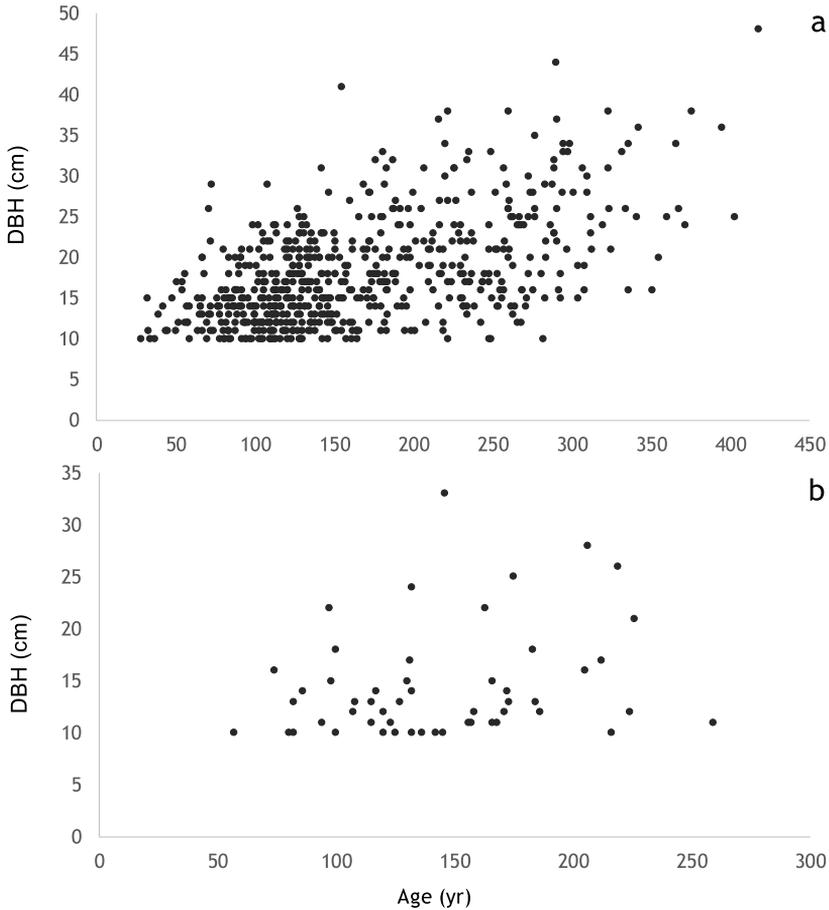


Figure 5. Relationship between size and diameter at breast height (DBH) for pine (**a**, $n = 624$) and spruce (**b**, $n = 52$). Only trees with a DBH larger than 10 cm are included.

Dead wood

The amount of dead pine wood was low (average of $4.5 \text{ m}^3 \text{ ha}^{-1}$, range: 2–8, $\text{SD} = 2.0$, $n=8$) and varied between sites, but both logs and snags were present at all sites (Table 1, Fig. 6a). On average, there were $53 \text{ pine DW units ha}^{-1}$ ($\text{SD} = 12.8$, $n=8$). DW in all decay stages were present and with decay stage 2 as the most common. However, at three sites dead trees in decay stage 1 were more common (FA, GA, HU; Fig. 6b). The average decay class for all dead wood was 2.45 and for dated dead wood samples 1.92. The difference stems from lack of datable samples from dead wood in decay class four. The basal area of DW in relation to total basal area, including living trees, varied between 10–26% at the different sites with an average of 18%. Most dead wood had died fairly recently; almost half of the dated dead wood samples had died during the last 50 years (Fig. 7).

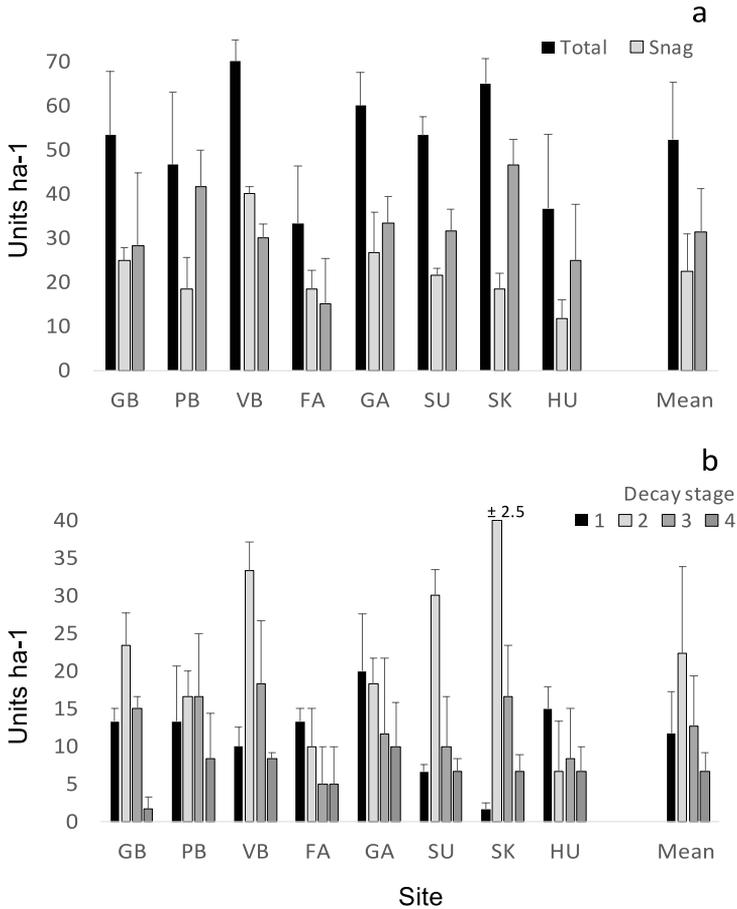


Figure 6. Number of logs and snags from pine in rocky pine forests at the eight different sites (SE, $n = 3$) as well as the total mean (SD, $n = 8$). The abundance of DW is classified as standing (Snag) or horizontal (Log) (a) and also classified in decay stages (b).

A majority of the DW, 88%, had died during the last 200 years, which corresponds to a DW addition of approximately 2 trees per ha⁻¹ and decade since the beginning of the 1800s. However, there were clear signs that dead wood can remain for several hundred years. The DW did not totally decay even in cases when the logs had been dead more than 300 years and we even found DW that had been dead more than 500 years (Fig. 7). Snags constituted a higher proportion of newly dead wood whereas logs were the most common DW type for units older than 350 years, but important to note is that there were also really old snags present. For DW that had been dead between 100–300 years the two types are equally common (Fig. 7). The average time since death for all dated dead wood units was 106 years (SD = 129, $n=220$), for snags 92 years (SD = 151, $n=109$) and for logs 120 years (SD = 129, $n=111$). The average age for the dated DW was 192 (SD = 82, $n=220$).

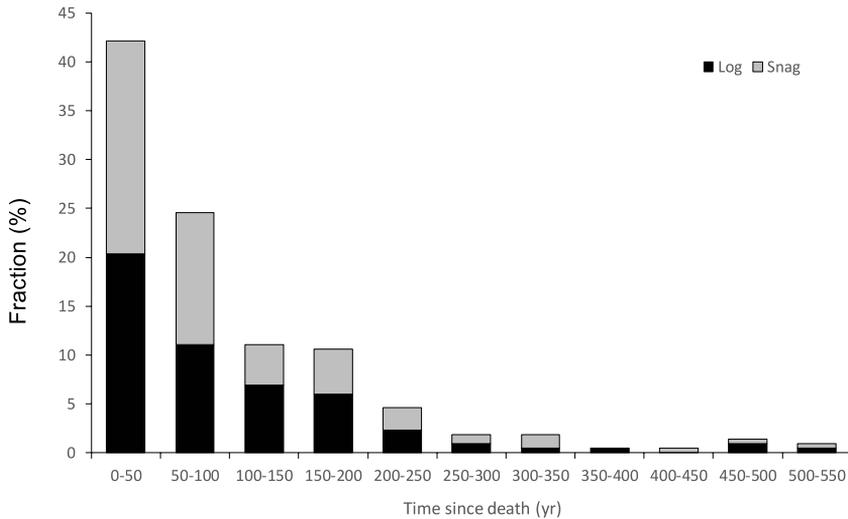


Figure 7. Time since death for dead wood for snags and logs.

Fire history

Most signs of fire were found in FA where 18 fire years were detected from 12 collected fire-scarred samples (Fig. 8a). FA was also the only site with individual trees that had experienced at least four fires. On average, 1.8 fires per sample from all sites was detected but on FA where most fires occurred, we detected 2.4 fires per sample. We did not find any fire-scarred trees at GB and only 3 fires could be detected at PB. On average, eight fire events per site were detected covering a time period from 1235 to 1923 but the number of detected fires varied between sites (Fig. 8a and Table 2). The period with most fires was during the 1600s and the 1800s when 13 fires occurred across all eight sites (Fig. 8b). We found signs of 47 fires between 1500 and 1900 and we detected very few fires earlier than 1500 and later than 1900. The earliest detected fire occurred in 1235 but as with most dated fires, this fire was only detected at one site (Fig. 8c). However, one of the fire years, 1693, was found at five sites. The fire in 1693 was found in PB, FA, SU, SK and HU but not in GA and VA which are located close to FA, SK and HU (Table 2). GA and FA are situated very close together but the only fire year that is common for the two sites is 1563. Approximately half of the fire-scarred trees (45%) had only one fire scar and trees with most scars (repeated fires) had four scars and was found at FA (see Appendix 2 for detailed information on samples with fire scars). In addition, most of the dated fire years was only detected in one sample (64%). The average fire interval varied between sites (shortest: FA 20.5 years and longest: VB: 65.5 years), but with an average fire interval of 42 years (excluding GB, where no signs of fire were detected) (Appendix 3). We also noted that many fire years had smaller year rings (indicating a dry fire season) than the average sizes in the master chronology. Twenty-eight fires had significantly smaller year rings than average, 25 had average size while only four had unusually large rings (Appendix 4).

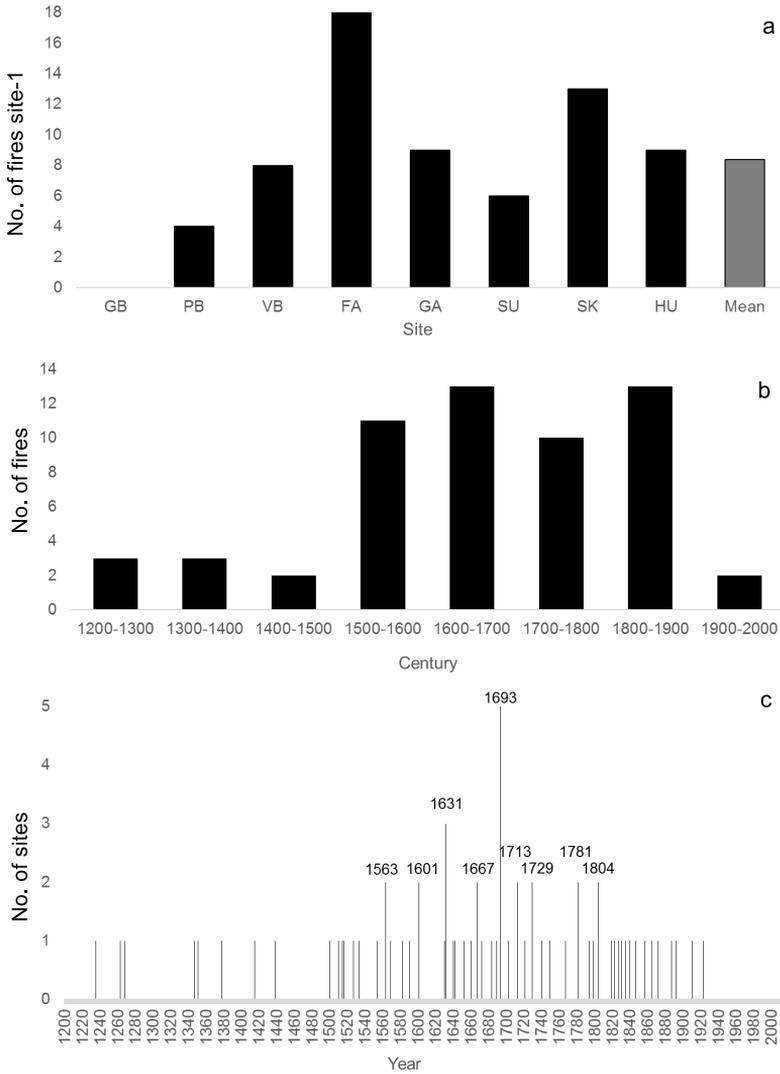


Figure 8. Fires in rocky pine forests. The total number of fires at the eight sites and the average from the sites (a). All detected fires are included, regardless of the number of samples the fire was detected in. Total number of fires are also separated into centuries (b) and individual fire years and their occurrence in the number of areas, with fire years occurring at more than one site highlighted (c).

Discussion

Forest structure

The studied rocky pine forests are characterized by a very open structure. The low density of trees can partly be explained by the scarcity of water and nutrients, as low productivity is generally connected with low basal area and openness (Liira et al. 2007). The rocky pine forests also have an uneven distribution of tree species, size and age. This creates a heterogenic and diverse environment and a wide age distribution typical

for old pine forests (Kuuluvainen et al. 2002). Attributes that usually are associated with old-growth forests are present, such as several canopy layers, high variation in tree sizes and ages and high spatial heterogeneity of tree distribution (irregular distribution of gaps) (Bauhus et al. 2009).

Although the diameter distribution pattern in our study is descending with size, the pattern is not a typical reversed J-shaped curve, common in many undisturbed forest stands (e.g. Parker and Peet 1984; Linder et al. 1997; Kuuluvainen et al. 2002; Rouvinen and Kuuluvainen 2005). In a study by Lilja and Kuuluvainen (2005), three different semi-natural (old-growth) pine stands had all a descending pattern, one that showed a reversed J-shaped pattern and two stands that were more similar to our findings. Rouvinen and Kuuluvainen (2005) found a bimodal pattern in a managed pine stand but a descending pattern, but no sharp reversed J-curve, in a natural pine stand. In a sub-xeric old-growth stand, dominated by pine, Uotila et al. (2001) found a pattern with most trees in intermediate diameter classes and not a clear descending pattern. Since we have no data on regeneration (trees < 1.3 m), we have no clear explanation to why we did not find a clear reversed J-shape on the diameter distribution in our study. It could be that the small trees are less frequent because there are only a few places/spots where trees can establish or it could be influenced by moose (*Alces alces*), which browses on small trees in winter. Neither did we find a bimodal diameter distribution pattern, often seen in forests with repeated disturbances, such as fires (e.g. Zenner 2005). This could indicate that the fires in this type of forests have been low-intensity fires of small sizes, as discussed below. In addition, the lack of bimodal diameter distribution pattern also could be an effect of lack of fires during the last 150 years.

As well as being a sign of naturalness, the heterogeneity and patchiness of tree distribution may also be influenced by an uneven water and nutrient availability for the trees. A tree that grows where the ground consists of bare rock has a very different potential for growth than trees in a concave patch covered with mineral soil, which probably can explain why the trees grew spatially irregularly at four of the sites. The trees generally have a low growth and high variability between trees at all of the sites, indicating also heterogeneity in growth. The high variability in growth between trees clearly contributes to the limited correlation between age and DBH. Kuuluvainen et al. (2002) found a stronger correlation between age and size; their R^2 was 0.58 for pine. However, they had a wider range of stand types included in their study and not only nutrient poor pine forest, which could explain the higher predictability. Nevertheless, they also found a lower correlation for spruce ($R^2=0.36$) than for pine, similar to our findings.

The average maximum tree height is generally low in rocky pine forests. The maximum tree height is related to height growth when the tree is young (Ryan and Yoder 1997), which in turn is influenced by genetic and environmental factors (Junttila 1986; Kaya et al. 1999; Pensa et al. 2005). Tree height seems to depend on a combination of temperature, precipitation and nutrient availability (Jansons et al. 2015) and the environment in the rocky pine forests is characterized by low water and nutrient availability and can probably partly explain the low mean maximum tree height. Other

factors that might influence tree height in the rocky pine forests are wind-exposure and openness (Tomczak et al. 2014).

The presence of old trees is one of the key features of natural forests (Östlund et al. 1997; Andersson and Östlund 2004). Old trees are relatively common in the studied rocky pine forests and we found approximately 13 pine trees ha⁻¹ older than 300 years and as many as 70 pines ha⁻¹ older than 200 years. As a comparison, Edwards and Mason (2006) found approximately 20 and 60 trees ha⁻¹ older than 200 years in two different old-growth pine stands in Scotland. In another Scottish old (and open) forest, only about 6 trees ha⁻¹ older than 250 years were found (Summers et al. 2008). This is in line with the findings of Kuuluvainen et al. (2002); they found approximately 5–10 trees ha⁻¹ older than 250 years in an old-growth pine forest. The oldest living pines in our plots were 418 years but we know that living trees older than 600 years (data not shown) existed in the studied area. In line with our findings, in old-growth forests in Finland, ages of more than 500 years for pine and almost 300 years for spruce were found (Kuuluvainen et al. 2002). In a study from a similar area, Hornslandet, approximately 200 km south from our study area, the authors actively searched for very old pine trees and found the oldest recorded pine in Sweden with an age of more than 750 years (Andersson and Niklasson 2004).

There are not many deciduous trees in the rocky pine forests and although birches (the most common deciduous species in the area) can tolerate rather dry conditions (Sutinen et al. 2002), regular drought may well limit the abundance of deciduous trees. In addition, it is likely that the density of deciduous trees is limited by the presence of moose (*Alces alces* L.), which can reduce the abundance of several deciduous species (e.g. rowan and aspen) when present (Edenius et al. 2002). The abundance of moose has since the 1970s massively increased in northern Sweden (Cederlund and Bergström 1996) and moose is today abundant throughout the whole of Sweden with the highest densities in the world (Skogforsk 2016). Moose is highly abundant also in the rocky pine forests in the High Coast Region and likely have a negative impact on the abundance of deciduous trees.

Dead wood and human impact

The CWD volume is very low and corresponding to levels in managed forest (Jonsson et al. 2016; Ylisirniö et al. 2012) and at a much lower level than what usually is common in natural forests with no or little human impact (e.g. Rouvinen and Kuuluvainen 2001; Karjalainen and Kuuluvainen 2002; Koster et al. 2005; Shorohova and Kapitsa 2015). Given the low tree density it is, however, more relevant to compare the proportion of dead trees to living trees. In the rocky pine forest the CWD share of the total basal area (living and all dead wood combined) is 18% on average, which is in line with several other pine-dominated forests with old-growth characteristics (e.g. Sippola et al. 1998; Siitonen et al. 2000; Rouvinen and Kuuluvainen 2001). By contrast, Karjalainen and Kuuluvainen (2002) found a higher proportion (32%) of the volume of

CWD in forest stands on dry soils. The variation in CWD:live wood volumes is greater in pine dominated forests than in spruce forests (Shorohova and Kapitsa 2015). We found a slightly lower proportion of dead wood in this study, but it should be noted that the ratio between dead and living trees is driven primarily by tree life span (mortality rate) and decay rates. Even though decay rates are slow in the studied rocky pine forests, it is to be noted that tree life span is likewise very long.

Charcoal and tar production has been common in the area a long time and the CWD quality in rocky pine forests is particularly suitable for tar production. Situated < 1 km from our sampling sites, two old remnants of tar pits have been found at one site, but there were no reported signs of old tar pits at the other seven sites. Hence, we cannot totally rule out the possibility that some CWD has been used for char and/or tar production in the studied area because these activities tend not to leave any visible traces behind.

The most common decay stage in this study is stage 2, which is in line with other pine forests with a high degree of naturalness (Karjalainen and Kuuluvainen 2002). The late decay stages were not as common in the study area as the most decayed wood (decay stage 4) constituted approximately only 12% of the total amount of CWD. In other studies of pine forests with high degree of naturalness the distribution of CWD in decay classes was more even (Rouvinen and Kuuluvainen 2001) or later decay classes were also more common (Sippola et al. 1998). One explanation for the relatively low presence of CWD in late decay stage found in this study could be reoccurring small fires because fire consumes logs in later decay stages to a higher extent than logs in earlier stages (Eriksson et al. 2013). It seems that CWD is accumulating slowly and can remain for very long periods in dry conditions. This makes every CWD unit very valuable and even a small outtake can have a large impact on the CWD-dependent organisms. The slow decay rate also explains why there is really old CWD; some has been dead for 300–500 years, in the rocky pine forest. In addition, we were not able to sample and date the most decayed dead wood, so the result is probably an underestimation of the amount of very old CWD.

Only two of the sites had signs of past cutting. A low frequency of manmade stumps indicates a high degree of naturalness (Uotila et al. 2001; Rouvinen et al. 2005). The outermost year rings on the six dated stumps varied between 1736 and 1886. This suggests that harvesting took place a very long time ago, although not necessarily giving the exact year of harvest since all stumps lacked bark and some erosion of the outer parts cannot be excluded. Consequently, the years should be regarded as the earliest possible cutting year but the actual cutting could have happened later. Nevertheless, there is evidence that the limited cuttings that did occur happened during the 1800s, or even earlier, and with no recent harvesting during the last 100 years. It is unlikely that rocky pine forests were targeted by industrial forestry since trees with large diameter are rare and the accessibility is very low. However, it is possible that settlers used the pine forests at small-scale for certain construction details, e.g. window frames, for which dense high quality wood was preferred. Such limited harvest/extraction is unlikely to leave any long-lasting visible traces.

Fire history

Surprisingly, many fires were detected in the rocky pine forests. There is not much fuel on the ground in the studied rocky pine forests, bare rock is common and trees are scattered. On the other hand, the ground surface easily becomes very dry and vegetated areas are mainly composed of reindeer lichens and dry mosses, which together with scattered dwarf shrubs potentially could carry a ground fire during dry years. Not many fires were detected before 1500. This could partly be a sampling artefact due to the limited number of fire scarred old trees and snags, but different climate and lower human population size cannot be ruled out as explanations for lower fire frequency in the beginning of our time series. Many fires happened during the 1600s, a pattern also observed by others (Zackrisson 1977; Niklasson and Granström 2000; Wallenius et al. 2004). The year 1693 stands out as a major fire year; a fire year that has also been documented by others (Drobyshev et al. 2014).

There was a population increase after the Black Death plague during the 1600s and several "slash and burn" immigrants from Finland also settled down in northern Sweden during this period, which can be an explanation for more frequent fires. However, most of the "slash and burn" farmers did not settle down along the coast area where fishing was the main livelihood but rather in the inland areas where the land was more suitable for the "slash and burn" cultivation technique (Lundkvist 1971). One fire happened in 1721 in Hummelvik nature reserve, a year when Russians made raids where they burned hundreds of farms along the coastline in the studied area (Lundkvist 1971; Lindh 1991). A village just outside Hummelvik nature reserve is mentioned as being burned (Lundkvist 1971), which could potentially explain the fire 1721 since the year ring has an average size. Unusually small year rings have been shown to be closely related to low precipitation in June in the Rocky pine forests in the High Coast Region and fire years are influenced by summer temperature and precipitation to a large extent in the northern region (Drobyshev et al. 2012; Drobyshev et al. 2014).

Most fire years were detected only in a few samples, which indicates that the fires in this area and forest type might have been small in size, but not necessarily rare events. A plausible explanation is that there are plenty of small-scale dispersal barriers for fires in this heterogenic landscape. Both bare rock and wet hollows often occur. Moist depressions, swamps and *Picea abies* patches often do not burn even when nearby dry patches do (Wallenius et al. 2004). The mean fire interval varied between 20.5 and 65.5 years at the different sites (except of one site with no fires found at all). These intervals included fire years that only were detected in one sample, and hence do not necessarily indicate that the whole forest area (site) burned. It is more likely that the fires were small in this heterogenic environment which creates a small-scale spatial variation influencing fire frequency and size (Wallenius et al. 2004). Many trees also survived the fires and many age classes were present. Taken together, this suggests that the fires were not stand-replacing, had low intensity but happened on a regular basis.

Conclusions

The rocky pine forests in the High Coast Region show a high degree of naturalness and possess many old-growth characteristics, e.g. presence of old trees, diverse structure and although the volume of dead wood is low, it constitutes approximately 18% of the total basal area. The dead wood is diverse with a variety of both snag and log sizes and can be present for a long time due to the slow decay rate. The diverse presence of dead wood, e.g. all decay stages represented, indicates that there is a constant supply of dead wood and that these types of forests have natural features. The high degree of naturalness is also supported by the lack of signs of human use; only a few man-made stumps have been found. All sites but one have clear signs of fires and it seems that the fires have happened quite frequently, but have been small in size. It is likely that these type of forests host a specific biota, evolved and adapted to the specific conditions that rocky pine forests constitute.

Acknowledgements

We are thankful to all landowners for allowing access to the study sites. We would like to thank for their much appreciated help in the field: Julia Hjalmarsson, Miriam Matheis, Jonas Orelund and Håkan Norberg. Thanks also for the valuable help from the County Administration in Västernorrland, especially to Pekka Bader, Jonas Salmonsson and Johan Uebel. We would also like to thank Prof. Lars Östlund for advice regarding signs of human use and for comments that improved the manuscript. Thanks to Prof. Evan Larsson for help with the master chronology and for comments that improved the manuscript. Many thanks also to Prof. Timo Kuuluvainen for comments that improved the manuscript.

References

- Andersson A (1975) Vegetationskartering av södra Ulvön. Härnösand.
- Andersson M, Niklasson M (2004) Rekordgammal tall på Hornslandet i Hälsingland. *Svensk Botanisk Tidskrift* 98: 333.
- Andersson R, Östlund L (2004) Spatial patterns, density changes and implications on biodiversity for old trees in the boreal landscape of northern Sweden. *Biological Conservation* 118(4): 443–453. <https://doi.org/10.1016/j.biocon.2003.09.020>
- Anonymous (2017) Skogsvårdslagen, Skogsvårdsstyrelsen.
- Applequist MB (1958) A simple pith locator for using with off-center cores. *Journal of Forestry* 56(2): 141.
- Axelsson AL, Östlund L (2001) Retrospective gap analysis in a Swedish boreal forest landscape using historical data. *Forest Ecology and Management* 147(2–3): 109–122. [https://doi.org/10.1016/S0378-1127\(00\)00470-9](https://doi.org/10.1016/S0378-1127(00)00470-9)

- Baudou E (1995) Norrlands forntid: ett historiskt perspektiv. Skytteanska samf., Bjästa.
- Bauhus J, Puettmann K, Messier C (2009) Silviculture for old-growth attributes. *Forest Ecology and Management* 258(4): 525–537. <https://doi.org/10.1016/j.foreco.2009.01.053>
- Berglund M (2012) The highest postglacial shore levels and glacio-isostatic uplift pattern in northern Sweden. *Geografiska Annaler Series a- Physical Geography* 94A: 321–337. <https://doi.org/10.1111/j.1468-0459.2011.00443.x>
- Bradshaw RHW, Josefsson T, Clear JL, Peterken GF (2011) The structure and reproduction of the virgin forest: A review of Eustace Jones (1945). *Scandinavian Journal of Forest Research* 26(S10): 45–53. <https://doi.org/10.1080/02827581.2011.517943>
- Brumelis G, Jonsson BG, Kouki J, Kuuluvainen T, Shorohova E (2011) Forest Naturalness in Northern Europe: Perspectives on Processes, Structures and Species Diversity. *Silva Fennica* 45(5): 807–821. <https://doi.org/10.14214/sf.446>
- Camarero JJ, Manzanedo RD, Sanchez-Salguero R, Navarro-Cerrillo RM (2013) Growth response to climate and drought change along an aridity gradient in the southernmost *Pinus nigra* relict forests. *Annals of Forest Science* 70(8): 769–780. <https://doi.org/10.1007/s13595-013-0321-9>
- Cederlund G, Bergström R (1996) Trends in the moose—forest system in Fennoscandia, with special reference to Sweden. *Conservation of Faunal Diversity in Forested Landscapes*. Springer, 265–281. https://doi.org/10.1007/978-94-009-1521-3_10
- Cook E, Krusic P (2005) Program ARSTAN: a tree-ring standardization program based on detrending and autoregressive time series modeling, with interactive graphics. Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY.
- Drobyshev I, Niklasson M, Linderholm HW (2012) Forest fire activity in Sweden: Climatic controls and geographical patterns in 20th century. *Agricultural and Forest Meteorology* 154–155: 174–186. <https://doi.org/10.1016/j.agrformet.2011.11.002>
- Drobyshev I, Granström A, Linderholm HW, Hellberg E, Bergeron Y, Niklasson M (2014) Multi-century reconstruction of fire activity in Northern European boreal forest suggests differences in regional fire regimes and their sensitivity to climate. *Journal of Ecology* 102(3): 738–748. <https://doi.org/10.1111/1365-2745.12235>
- Edenius L, Bergman M, Ericsson G, Danell K (2002) The role of moose as a disturbance factor in managed boreal forests. *Silva Fennica* 36(1): 57–67. <https://doi.org/10.14214/sf.550>
- Edwards C, Mason WL (2006) Stand structure and dynamics of four native Scots pine (*Pinus sylvestris* L.) woodlands in northern Scotland. *Forestry* 79(3): 261–277. <https://doi.org/10.1093/forestry/cpl014>
- Ehnström B, Bader P (2013) Åtgärdsprogram för jättepraktbagge. Naturvårdsverket Rapport 6584.
- Eriksson AM, Olsson J, Jonsson BG, Toivanen S, Edman M (2013) Effects of restoration fire on dead wood heterogeneity and availability in three *Pinus sylvestris* forests in Sweden. *Silva Fennica* 47(2): 954. <https://doi.org/10.14214/sf.954>
- Esseen P-AGA, Ståhl G, Sundquist S (2003) Fältinstruktion för nationell inventering av landskapet i Sverige, NILS. SLU, Institutionen för skoglig resurshållning och geomatik, Umeå.
- Forestry (2015) Forests and Forestry in Sweden. The Royal Swedish Academy of Agriculture and Forestry. <https://www.ksla.se/publikationer/ovriga-publikationer/forests-and-forestry-in-sweden>

- Fraver S, Ringvall A, Jonsson BG (2007) Refining volume estimates of down woody debris. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 37(3): 627–633. <https://doi.org/10.1139/X06-269>
- Fridman J, Wulff S (2018) Skogsdata 2018. https://www.slu.se/globalassets/ew/org/centrb/rt/dokument/skogsdata/skogsdata_2018_webb.pdf
- Fule PZ, Heinlein TA, Covington WW, Moore MM (2003) Assessing fire regimes on Grand Canyon landscapes with fire-scar and fire-record data. *International Journal of Wildland Fire* 12(2): 129–145. <https://doi.org/10.1071/WF02060>
- Grissino-Mayer HD (2001) Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-ring research*. <https://www.semanticscholar.org/paper/Evaluating-Crossdating-Accuracy%3A-A-Manual-and-for-Grissino-Mayer/20ae845b0294aba8ab98bb6375cc43e49d93dae8>
- Hernandez L, Rubiales JM, Morales-Molino C, Romero F, Sanz C, Gomez Manzaneque F (2011) Reconstructing forest history from archaeological data: A case study in the Duero basin assessing the origin of controversial forests and the loss of tree populations of great biogeographical interest. *Forest Ecology and Management* 261(7): 1178–1187. <https://doi.org/10.1016/j.foreco.2010.12.033>
- Holmes RL (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-ring bulletin*. <https://www.semanticscholar.org/paper/Computer-Assisted-Quality-Control-in-Tree-Ring-and-Holmes/b704f137a9f2a4c87efd7b16da018c62561cc2f>
- Horne JK, Schneider DC (1995) Spatial variance in ecology. *Oikos* 74(1): 18–26. <https://doi.org/10.2307/3545670>
- Jansons A, Matisons R, Zadina M, Sisenis L, Jansons J (2015) The effect of climatic factors on height increment of Scots pine in sites differing by continentality in Latvia. *Silva Fennica* 49(3): 1262. <https://doi.org/10.14214/sf.1262>
- Jonsson BG, Ekström M, Esseen P-A, Grafström A, Ståhl G, Westerlund B (2016) Dead wood availability in managed Swedish forests – Policy outcomes and implications for biodiversity. *Forest Ecology and Management* 376: 174–182. <https://doi.org/10.1016/j.foreco.2016.06.017>
- Junttila O (1986) Effects of temperature on shoot growth in northern provenances of *Pinus sylvestris* L. *Tree Physiology* 1(2): 185–192. <https://doi.org/10.1093/treephys/1.2.185>
- Karjalainen L, Kuuluvainen T (2002) Amount and diversity of coarse woody debris within a boreal forest landscape dominated by *Pinus sylvestris* in Vienansalo wilderness, eastern Fennoscandia. *Silva Fennica* 36(1): 147–167. <https://doi.org/10.14214/sf.555>
- Kaya Z, Sewell M, Neale D (1999) Identification of quantitative trait loci influencing annual height-and diameter-increment growth in loblolly pine (*Pinus taeda* L.). *Theoretical and Applied Genetics* 98(3–4): 586–592. <https://doi.org/10.1007/s001220051108>
- Koster K, Jogiste K, Tükia H, Niklasson M, Mols T (2005) Variation and ecological characteristics of coarse woody debris in Lahemaa and Karula National Parks, Estonia. *Scandinavian Journal of Forest Research* 20(sup6): 102–111. <https://doi.org/10.1080/14004080510042137>
- Kuuluvainen T, Mäki J, Karjalainen L, Lehtonen H (2002) Tree age distributions in old-growth forest sites in Vienansalo wilderness, eastern Fennoscandia. *Silva Fennica* 36(1): 169–184. <https://doi.org/10.14214/sf.556>

- Kuuluvainen T, Aakala T, Varkonyi G (2017) Dead standing pine trees in a boreal forest landscape in the Kalevala National Park, northern Fennoscandia: Amount, population characteristics and spatial pattern. *Forest Ecosystems* 4(1): 12. <https://doi.org/10.1186/s40663-017-0098-7>
- Liira J, Sepp T, Parrest O (2007) The forest structure and ecosystem quality in conditions of anthropogenic disturbance along productivity gradient. *Forest Ecology and Management* 250(1–2): 34–46. <https://doi.org/10.1016/j.foreco.2007.03.007>
- Lilja S, Kuuluvainen T (2005) Structure of old *Pinus sylvestris* dominated forest stands along a geographic and human impact gradient in mid-boreal Fennoscandia. *Silva Fennica* 39(3): 407–428. <https://doi.org/10.14214/sf.377>
- Linder P, Elfving B, Zackrisson O (1997) Stand structure and successional trends in virgin boreal forest reserves in Sweden. *Forest Ecology and Management* 98(1): 17–33. [https://doi.org/10.1016/S0378-1127\(97\)00076-5](https://doi.org/10.1016/S0378-1127(97)00076-5)
- Lindh T (1991) Hemsöns historia, D.1. Hemsö idrottsförening, Ålandsbro.
- Lundkvist T (1971) Boken om Säbrå: kommunens och socknarnas historia: Säbrå, Häggdånger, Stigsjö, Viksjö, Hemsö. Härnösand: Ångermannia, Härnösand.
- Lundkvist T (1986) Boken om Säbrå: Kommunens och socknarnas historia: Säbrå. Häggdånger, Stigsjö, Viksjö, Hemsö, Ångermannia, Härnösand.
- Niklasson M, Granström A (2000) Numbers and sizes of fires: Long-term spatially explicit fire history in a Swedish boreal landscape. *Ecology* 81(6): 1484–1499. [https://doi.org/10.1890/0012-9658\(2000\)081\[1484:NASOFL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1484:NASOFL]2.0.CO;2)
- Östlund L, Zackrisson O, Axelsson AL (1997) The history and transformation of a Scandinavian boreal forest landscape since the 19th century. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 27(8): 1198–1206. <https://doi.org/10.1139/x97-070>
- Parker AJ, Peet RK (1984) Size and age structure of conifers forests. *Ecology* 65(5): 1685–1689. <https://doi.org/10.2307/1939148>
- Pensa M, Salminen H, Jalkanen R (2005) A 250-year-long height-increment chronology for *Pinus sylvestris* at the northern coniferous timberline: A novel tool for reconstructing past summer temperatures? *Dendrochronologia* 22(2): 75–81. <https://doi.org/10.1016/j.dendro.2005.02.005>
- Renström A, Hedström B (1985) 15 byar i Kramfors kommun. Värns, Vibygerå socken: historisk bakgrund, byggnadsinventering, förslag till förnyelse och bevarande.
- Rouvinen S, Kuuluvainen T (2001) Amount and Spatial Distribution of Standing and Downed Dead Trees in Two Areas of Different Fire History in a Boreal Scots Pine Forest. *Ecological Bulletins* 2001: 115–127.
- Rouvinen S, Kuuluvainen T (2005) Tree diameter distributions in natural and managed old *Pinus sylvestris*-dominated forests. *Forest Ecology and Management* 208(1–3): 45–61. <https://doi.org/10.1016/j.foreco.2004.11.021>
- Rouvinen S, Rautiainen A, Kouki J (2005) A relation between historical forest use and current dead woody material in a boreal protected old-growth forest in Finland. *Silva Fennica* 39(1): 21–36. <https://doi.org/10.14214/sf.393>
- Rubiales JM, Garcia-Amorena I, Genova M, Manzanque FG, Morla C (2007) The Holocene history of highland pine forests in a submediterranean mountain: The case of Gredos

- mountain range (Iberian Central range, Spain). *Quaternary Science Reviews* 26(13–14): 1759–1770. <https://doi.org/10.1016/j.quascirev.2007.04.013>
- Ryan MG, Yoder BJ (1997) Hydraulic limits to tree height and tree growth. *Bioscience* 47(4): 235–242. <https://doi.org/10.2307/1313077>
- Salomonsson J, Bader P (2015) Skoglig naturvårdsinventering – av ett urval hållmarker längs Västernorrlands kust. The County Administrative Board of Västernorrland, Härnösand Rapport 2015: 2.
- Shorohova E, Kapitsa E (2015) Stand and landscape scale variability in the amount and diversity of coarse woody debris in primeval European boreal forests. *Forest Ecology and Management* 356: 273–284. <https://doi.org/10.1016/j.foreco.2015.07.005>
- Siitonen J, Martikainen P, Punttila P, Rauh J (2000) Coarse woody debris and stand characteristics in mature managed and old-growth boreal mesic forests in southern Finland. *Forest Ecology and Management* 128(3): 211–225. [https://doi.org/10.1016/S0378-1127\(99\)00148-6](https://doi.org/10.1016/S0378-1127(99)00148-6)
- Sippola AL, Siitonen J, Kallio R (1998) Amount and quality of coarse woody debris in natural and managed coniferous forests near the timberline in Finnish Lapland. *Scandinavian Journal of Forest Research* 13(1–4): 204–214. <https://doi.org/10.1080/02827589809382978>
- Skogforsk (2016) Sverige har världens tätaste älgstam. <https://www.skogforsk.se/kunskap/kunskapsbanken/2016/varldens-tataste-algstam/2018-08-01>
- SMHI (2016) SMHI. <http://www.smhi.se/klimatdata> [2016-02-18]
- Speer JH (2010) Fundamentals of tree-ring research. The University of Arizona press, Arizona.
- Stokland JN, Siitonen J, Jonsson BG (2012) Biodiversity in dead wood. Cambridge University Press. <https://doi.org/10.1017/CBO9781139025843>
- Summers RW, Wilkinson NI, Wilson ER (2008) Age structure and history of stand types of *Pinus sylvestris* in Abernethy Forest, Scotland. *Scandinavian Journal of Forest Research* 23(1): 28–37. <https://doi.org/10.1080/02827580701646513>
- Sutinen R, Teirilä A, Päänttjä M, Sutinen M-L (2002) Distribution and diversity of tree species with respect to soil electrical characteristics in Finnish Lapland. *Canadian Journal of Forest Research* 32(7): 1158–1170. <https://doi.org/10.1139/x02-076>
- Tomczak A, Jelonek T, Pazdrowski W (2014) Characteristics of selected morphological traits of trees in mature Scots pine stands exposed to wind. *Sylvan* 158: 183–191.
- Uotila A, Maltamo M, Uutera J, Isomäki A (2001) Stand Structure in Semi-Natural and Managed Forests in Eastern Finland and Russian Karelia. *Ecological Bulletins* 49: 149–158.
- Wallenius TH, Kuuluvainen T, Vanha-Majamaa I (2004) Fire history in relation to site type and vegetation in Vienansalo wilderness in eastern Fennoscandia, Russia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* 34(7): 1400–1409. <https://doi.org/10.1139/x04-023>
- Wikars L (2015) Åtgärdsprogram för skalbaggar på äldre död tallved. Naturvårdsverket Rapport 6629.
- Volkov A, Gromtsev A, Sakovets V (1997) Climax forests in the north-western taiga zone of Russia: natural characteristics, present state and conservation problems. In: Preprint of report for the meeting of the Learned Council Forest Research Institute, Karelian Research Centre, Russian Academy of Sciences.

- Ylisirniö AL, Penttilä R, Berglund H, Hallikainen V, Isaeva L, Kauhanen H, Koivula M, Mikola K (2012) Dead wood and polypore diversity in natural post-fire succession forests and managed stands – Lessons for biodiversity management in boreal forests. *Forest Ecology and Management* 286: 16–27. <https://doi.org/10.1016/j.foreco.2012.08.018>
- Zackrisson O (1977) Influence of Forest Fires on the North Swedish Boreal Forest. *Oikos* 29(1): 22–32. <https://doi.org/10.2307/3543289>
- Zenner EK (2005) Development of tree size distributions in Douglas-fir forests under differing disturbance regimes. *Ecological Applications* 15(2): 701–714. <https://doi.org/10.1890/04-0150>

Appendix I

Master chronology, marker years and ring widths

Abbreviations: ++ much bigger than normal; + bigger than normal; - Smaller than normal; -- much smaller than normal. All other years with ring widths are of average size. Bold years means that they are valuable marker years (unusually thin latewood = TH, unusually thick and dark latewood = DL, unusually thick latewood = B).

Year	Ring width	Year	Ring width						
1204	+	1329	+	1464	-	1647	+	1874	--
1207	-	1331	+	1466	--	1648	+	1878	+
1209	-	1332	+	1468	+	1656	+	1881	--
1210	--	1333	-	1473	--	1659	--	1886	+
1212	+	1335	+	1476	+	1665	+	1888	--
1213	--	1339	-	1484	++	1666	-	1890	++
1214	+	1347	-	1490	-	1667	-	1891	-
1216	+	1348	-	1491	+	1673	+	1896	+
1218	-	1351	-	1494	-	1686	+	1901	B
1219	-	1353	+	1498	+	1687	+	1902	--
1223	+	1357	+	1503	-	1693	--	1907	+
1228	+	1358	+	1505	+	1696	-	1911	-
1229	++	1362	-	1506	-	1698	-	1913	+ B
1230	+	1363	-	1508	-	1713	-	1917	-
1233	--	1364	+	1509	--	1723	+	1918	-
1234	--	1366	--	1511	-	1726	-	1922	+
1235	-	1368	+	1512	+	1736	-	1923	+
1239	-	1370	-	1513	+	1742	-	1924	++
1243	+	1372	+	1514	-	1747	-	1925	+
1244	++	1373	+	1525	++	1749	-	1928	+
1247	+	1376	++	1528	+	1752	+	1929	+ DL
1252	++	1381	+	1531	-	1771	-	1933	--
1256	--	1382	++	1533	-	1776	+	1934	-
1259	-	1384	-	1535	+	1777	++	1936	--
1261	+	1385	-	1538	--	1778	+	1939	B
1262	+	1386	-	1541	+	1781	-	1940	--
1263	-	1388	--	1543	-	1786	-	1943	-
1265	--	1389	-	1547	+	1792	+	1945	+
1267	++	1391	-	1550	--	1793	+	1946	+

Year	Ring width								
1268	+	1394	+	1551	+	1794	B	1947	+
1269	-	1396	++	1554	--	1795	--	1953	+
1274	--	1398	-	1561	++	1805	++	1954	+
1275	--	1401	+	1562	+	1806	+	1955	-
1278	++	1403	+	1568	--	1810	-	1957	++ DL
1279	+	1404	++	1572	--	1816	-	1959	--
1283	--	1406	++	1578	+	1821	B	1960	--
1285	++	1411	--	1583	+	1822	-	1961	-
1287	-	1418	--	1585	+	1827	+	1962	+
1291	-	1420	--	1588	--	1828	+	1969	--
1293	-	1422	-	1590	--	1831	-	1972	+
1296	+	1423	+	1594	+	1832	--	1974	+
1297	-	1425	+	1601	--	1835	-	1976	-- TH
1298	+	1426	+	1603	-	1840	+	1984	+
1299	-	1427	+	1606	-	1844	+	1987	+
1302	--	1428	+	1611	+	1847	--	1991	+
1303	+	1430	-	1615	-	1850	+	1992	--
1304	--	1432	--	1623	+	1851	+	1997	--
1305	+	1434	+	1625	+	1853	--	2000	+
1307	+	1442	-	1626	+	1859	TH	2002	-
1309	+	1446	--	1628	+	1860	B	2004	+
1312	--	1451	+	1630	-	1861	--	2008	--
1319	+	1454	+	1631	--	1862	-	2009	--
1321	+	1455	-	1633	-	1866	+	2011	+
1324	-	1459	-	1638	+	1868	+	2012	+
1325	--	1462	+	1642	-	1869	+	2014	+

10 or more samples from 1431

Total no. of samples	248
Age span	1197–2015
Total no. of years	819
Total no of rings	56329
Mean age	227
Sensitivity	0.29
Series intercorrelation	0.505

Appendix 2

Dated samples with fire scars and fire years

Site and Sample ID	Correlation with master	Pith year	Outermost ring year	Age	Year of fire1	Year of fire2	Year of fire3	Year of fire4	Sample type	Scar direction
Gropberget										
No fire scars detected										
Porsmyrberget										
BrandPO8	0,206	1467	1783	316	1702				Dead	
BrandPO12	0,248	1500	1990	490	1660	1693	1804		Living	
BrandPO14	0,614	1773	2015	242	1804					
Vårdkällberget										
VA1d20	0,275	1230	1505	275	1268	1347			Dead	
VA1d29	0,338	1671	1846	175	1740					
BrandVA1	0,454	1783	2013	230	1804				Living	E
BrandVA3	0,562	1580	1838	126	1835				Dead	N
BrandVA4	0,468	1661	2015	354	1804 or 1805				Living	SE
BrandVA5	0,56	1561	1854	293	1601	1631	1729		Dead	W
BrandVA7	0,606	1604	1896	292	1601				Stump	W
BrandVA10	0,323	1574	1819	269	1631				Living	E
BrandVA12	0,273	1731	2015	284	1749	1923				
Fanön										
BrandFA1	0,333	1518	2015	497	1781				Living	S
BrandFA4	0,342	1494	1647	153	1533				Stump	
BrandFA5	0,163	1526	1990	463	1582	1640	1713	1822	Dead	
BrandFA6	0,58	1712	2015	303	1822				Living	E
BrandFA7	0,338	1435	1863	428	1527	1563	1693	1819	Dead	E
BrandFA8B	0,492	1518	2004	486	1693	1798	1846	1910	Dead	N
BrandFA11	0,587	1484	1912	428	1590	1693	1827 (1828)		Dead	S, N
BrandFA12	0,57	1702	2015	313	1693	1857			Living	Fire 1 SE, Fire 2 N
BrandFA16	0,585	1599	1855	256	1689	1781	1822		Dead	SW
BrandFA19	0,296	1407	1546	136	1500				Dead	S
BrandFA20	0,382	1640	1972	332	1640	1693	1781	1846	Living	S
BrandFA22	0,343	1642	1837	195	1693	1781				
Gårdberget										
Garmlingen	0,397	1419	2014	595	1563	1601	1631	1713	Living	
GA1dStump1A	0,596	1409	1736	327	1438	1601			Dead	

Site and Sample ID	Correlation with master	Pith year	Outermost ring year	Age	Year of fire1	Year of fire2	Year of fire3	Year of fire4	Sample type	Scar direction
GA1dStump1B	0,663	1465	1713	248	1601	1713			Living	NW
BrandGA4	0,456	1698	2013	315	1713	1826–1827			Living	Fire 1 NW, Fire2 SW
BrandGA6	0,585	1436	2000	564	1516	1830			Living	NW
BrandGA10	0,306	1587	1905	318	1667	1830			Living	SE
BrandGAlst14	0,146	1773	1996	223	1830				Dead	
BrandGAX1	0,391	1338	1517	179	1378				Dead	
BrandGAX2	0,53	1582	1715	133	1667				Stump	N
BrandGAX3	0,3	1447	1723	276	1516	1601 or 1602	1667		Dead	W
S. Ulvön										
BrandULV1	0,3	1683	1868	185	1729	1864			Living	E
BrandULV2	0,237	1745	2015	270	1767				Living	E
BrandULV3	0,484	1760	1959	199	1767				Dead	S
BrandULV4	0,28	1641	1991	350	1693				Living	E
BrandULV5	0,37	1725	2015	290	1767				Living	NE
BrandULV6	0,543	1735	1817	82	1794				Living	W
BrandULV7	0,561	1749	1925	176	1892				Stump	S
BrandULV8	0,423	1587	2015	428	1794				Living	E
Skule										
BrandSKU2	0,305	1428	2004	576	1781–1782	1840			Living	W + E
BrandSKU6	0,196	1662	2014	352	1693	1840			Living	W
BrandSKU9	0,396	1657	2015	358		1840			Living	NE
BrandSKU1	0,225	1565	1826	261	1631				Dead	
BrandSKU2	0,212	1295	1626	331	1351	1415	1510		Dead	W
BrandSKUStump	0,476	1796	161	1796	1652	1693 or 1694			Stump	
BrandSKUStump8	0,451	1559	1853	294	1554	1631	1672		Stump	W
T4.126	0,617	1598	1808	210	1642				Dead	
T5.95	0,338	1203	1546	343	1235	1263	1351	1415	Dead	
T6.05	0,484	1421	1753	332	1510	1631			Dead	
Hummelvik										
HU1d02A	0,408	1864	2006	142	1872				Dead	
BrandHU5	0,365	1546	1983	437	1568 or 1569	1668 or 1678	1887		Dead	SE
BrandHU8	0,451	1411	1759	348	1458	1514	1569	1693	Dead	E
BrandHU9	0,201	1648	1918	270	1683	1872			Dead	E
BrandHU1st13	0,31	1617	1937	320	1721				Dead	NW
BrandHUSrump1	0,472	1446	1725	279	1514	1568 (+– 1 yr)	1630		Dead	
BrandHUSrump2	0,503	1469	1578	109	1514	1630			Stump	Stump

Appendix 3

Fire years and interval

Fire years. The individual fire years at the different sites and the number of samples where fires were detected and the average fire interval at each site (calculated as: number of fires/(last fire year minus first fire year)). GB = Gropberget, PB = Porsmyrberget, VB = Vårdkallberget, FA = Fanön, GA = Gårdberget, SU = Southern Ulvön, SK = Skuleskogen National Park, HU = Hummelvik Nature Reserve. Gropberget (GR) did not have any signs of fire.

No signs of fires	GB		PB		VB		FA		GA		SU		SK		HU	
	Fire year	n	Fire year	n	Fire year	n	Fire year	n	Fire year	n	Fire year	n	Fire year	n	Fire year	n
	1660 ±2 yr	1	1268	1	1500	1	1378 ±2 yr	1	1693	1	1235	1	1514	3		
	1693	1	1347	1	1527	1	1438	1	1729	1	1263	1	1569	3		
	1702	1	1601	2	1533	1	1516	2	1767	3	1351	2	1630	2		
	1804	2	1631	2	1563	1	1563	1	1794	2	1415	2	1668	1		
			1729	1	1582	1	1601	4	1864	1	1510	2	1683	1		
			1740	1	1590	1	1631	1	1892	1	1554	1	1693	1		
			1749	1	1640	2	1667	3			1631	3	1721	1		
			1804	3	1689	1	1713	4			1642	1	1872	2		
			1835	1	1693	6	1830	4			1652	1	1887	1		
			1923	1	1713	1					1672	1				
					1781	4					1693	1				
					1798	1					1781	1				
					1819	1					1840	3				
					1822	3										
					1827	1										
					1846	2										
					1857	1										
Average fire interval (yr)	36		65.5		20.5		50		33		46.5		41.5			

Appendix 4

Fire years and ring widths

Abbreviations: ++, much bigger than normal; +, bigger than normal; aver, average size; -, smaller than normal; --, much smaller than normal. GB = Gropberget, PB = Porsmyrberget, VB = Vårdkallberget, FA = Fanön, GA = Gårdberget, SU = Southern Ulvön, SK = Skuleskogen National Park, HU = Hummelvik Nature Reserve.

Fire years	Site (and number of samples)	Ring width in master
1235	SK (1)	-
1263	SK (1)	-
1268	VB (1)	+
1347	VB (1)	-
1351	SK (1+1)	-
1378+- 2 yr	GA (1)	aver
1415	SK (2)	aver
1438	GA (1)	-
1500	FA (1)	aver
1510	SK (2)	-

Fire years	Site (and number of samples)	Ring width in master
1514	HU (3)	-
1516	GA (2)	aver
1527	FA (1)	aver
1533	FA (1)	-
1554	SK (1)	--
1563	FA (1), GA (1)	aver
1569	HU (3)	aver
1582	FA (1)	aver
1590	FA (1)	--
1601	VB (2), GA (4)	--
1630	HU (2)	-
1631	VB (2), GA (1), SK (3)	--
1640	FA (2)	-
1642	SK (1)	-
1652	SK (1)	-
1660 ± 2 yr	PB (1)	aver (1659 --)
1667	GA (3)	-
1668/1669	HU (1)	aver
1672	SK (1)	aver
1683	HU (1)	aver
1689	FA (1)	aver
1693	FA (6), SU (1), SK (2), HU (1), PB (1)	--
1702	PB (1)	aver
1713	GA (4), FA (1)	-
1721	HU (1)	aver
1729	VB (1), SU (1)	-
1740	VB (1)	-
1749	VB (1)	-
1767	SU (3)	aver
1781	FA (4), SK (1)	-
1794	SU (2)	aver
1798	FA (1)	-
1804	VB (3), PB (2)	aver
1819	FA (1)	aver
1822	FA (3)	-
1827/1828	FA (1)	+
1830	GA (4)	-
1835	VB (1)	-
1840	SK (3)	+
1846	FA (2)	aver
1857	FA (1)	aver
1864	SU (1)	aver
1872	HU (2)	aver
1887	HU (1)	aver
1892	SU (1)	aver
1910	FA (1)	aver
1923	VB (1)	+