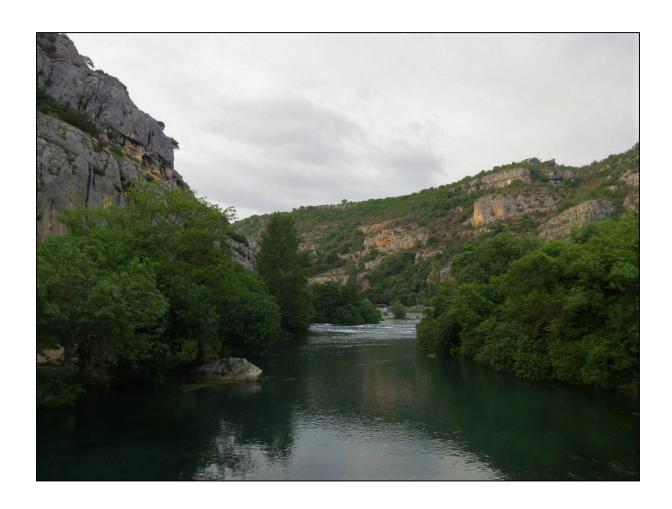
Operation Wallacea Croatia Terrestrial Research Program







2021 End of season report



Operation Wallacea Croatia Terrestrial Research Program - Krka National Park 2021

This end of season report is submitted as a summarization of methods and review of results from Operation Wallacea's 2021 research season, which ran in Krka National Park for five weeks between 29th June and 3rd August 2021. This report contains a summary of the methodologies and surveys employed, in addition to a summarization of the data collected during this time. The purpose is this report is:

- 1) To highlight the key findings of the 2021 research season
- 2) To codify survey methods and study site locations of the long term Opwall/Biota monitoring programme in Krka National Park

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1. Introduction

This report provides an overview of the results of the 2021 Operation Wallacea research programme in Krka National Park (KNP), Croatia. We present a detailed summary of the scientific methodologies used during the research program, a synopsis of core results from the field season, along with a detailed summary of the species detected by this survey work, and an appendices of all species in key study taxa detected by Operation Wallacea scientists since the project began (2017-2021).

The 2021 season represents the second year in which Operation Wallacea research teams have completed systematic surveys within KNP, following the 2019 season (the 2020 season was cancelled due to the impacts of the international COVID pandemic, while long-term monitoring in 2018 was disrupted by the change of location of the core survey area). These surveys focus on a select group of taxa (birds, butterflies, herpetofauna, bats) which are monitored in a standardised way to evaluate ecosystem community composition and habitat structure, and changes in this community and habitat structure. These methodologies also broadly match survey methods used for key monitoring groups in other Opwall terrestrial research sites, allowing for global-scale cross-site analysis of ecological patterns and trends.

Alongside these standardized monitoring surveys, a series of specialist research projects was also completed in 2021 (as in other years). Specialist projects this year included a preliminary study into the fungal diversity of Krka, the collection of bat ectoparasite and bat morphometrics data for a multi-site collaborative project led by the University of Ghent, and the development of novel methods for catching bats over lentic waterbodies.

2. Camps and transects

One camp on the edge of KNP was used by Opwall in the 2021 season (the Biota house at Puljane) with six transects being acceded from here. Transects (and survey sites located along each transect) are exactly the same as those used in the 2019 season.

The six transects are of variable length and shape and correspond to the principal habitat types occurring in KNP (Figure 1). As with other Opwall sites, permanent survey sites were established at regular intervals along each transect. These survey sites were located approximately 300m apart on each transect. Steep terrain, dense vegetation, and impassable drystone walls sometimes posed limitations on some survey site locations; in these cases survey sites were located at the next 50m interval along the transect. The transects are numbered (1-6) and on each of the routes the sites are numbered sequentially. Thus T3/3 is the third survey site along transect 3. GPS locations of all study site locations are provided in Appendix 1.



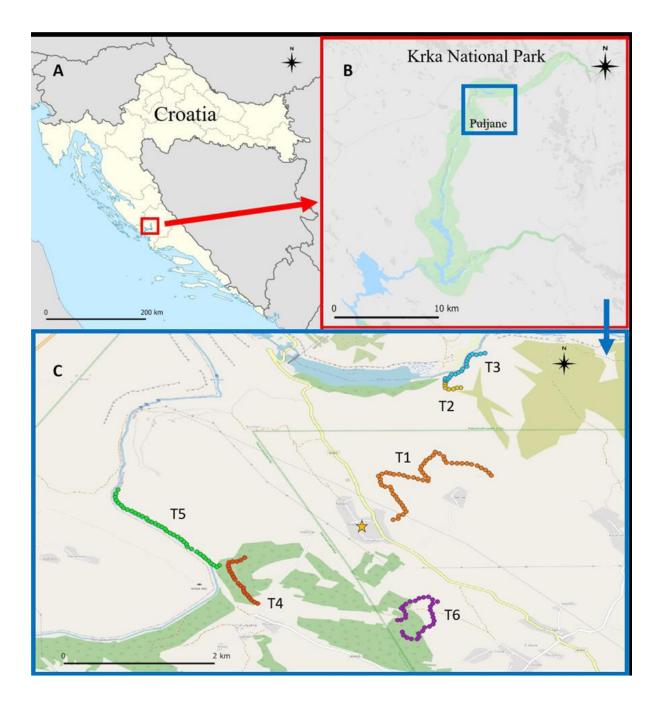


Figure 1: Map showing A) Location of KNP within Croatia; B) Location of the Operation Wallacea study area within KNP C) Location of transects within the KNP study area. A pale green line indicates the Park border. The yellow star represents the location of Puljane town (close to where the Operation Wallacea/Biota house is situated). Map sourced from Simões Nunes et al. (2020).



The six transects are representative of the major habitat types found in KNP and its immediate environs. These are summarized in Table 1 below, along with metadata regarding the location and length of each transect:

Table 1 – Details of habitat types, length, start and end points, and number of survey points on each of the six transects used by the Operation Wallacea project in Krka National Park. Adapted from Simões Nunes et al. (2020).

Transect number	Habitat represented	Length (m)	Coordinates	Survey sites per
1	Mediterranean scrub	2600	Start: 43.98832, 16.04937; End: 43.994172, 16.060951;	transect 7
2	Juniper-dominated valley slopes	350	Start: 44.00583, 16.05732; End: 44.00671, 16.05561;	2
3	Riverine forest	900	Start: 44.00676, 16.05548; End: 44.01041, 16.0602;	5
4	Dry scree slopes	1000	Start: 43.97719, 16.03329; End: 43.98328, 16.03175;	3
5	Riverine forest	1800	Start: 43.98254, 16.02773; End: 43.99021, 16.017157;	5
6	Mixed Mediterranean oak- juniper scrub	1540	Start: 43.973416, 16.050413; End: 43.974991, 16.049849;	5



3. Biodiversity monitoring

The main purpose of the Opwall monitoring program is to collect standardised data on key taxa to document changes in the ecosystems of KNP over time. A brief overview of survey methodologies is presented here.

3.1 Amphibians and reptile visual encounter surveys

Amphibian and reptile (herpetofauna) data are collected via visual encounter surveys (VES) which are completed three times on each transect during the course of the season. Each VES survey involves actively searching for herpetofauna along the entire length of each transect either in the morning (starting between 7:00-9:00h AM) or afternoon (starting between 15:00 – 17:00). For all observed animals' the distance along the transect is recorded as well as the species. Survey effort is quantified in time (marking start and end time for each survey), the number of participants and distance (length of the transect surveyed). Opportunistic surveys were also carried out continuously throughout the duration of the season by the herpetologist. Species, age, sex, date, time, and GPS location were collected for opportunistic sightings.

3.2 Birds

Bird communities in KNP were monitored using a combination of point counts and mistnetting of birds at constant effort mist netting stations. The combination of these two techniques provides a more complete overview of the bird communities present in KNP by capitalizing on the benefits of both methodologies. Mist netting has an element of inherent bias, by only providing a sample of the species present in the understory (e.g. it will not sample canopy and mid canopy species adequately) and captures are unlikely to reflect relative abundance of non-understory communities. However, the use of mist nets provides important quantitative information for understory species, including those that are inconspicuous or seldom vocal and thus often missed in point counts. The use of mist nets also minimises observer bias and produces results that are easily repeatable. Furthermore, the use of constant effort mist-netting protocol (as of 2012) provides important data on productivity, survivorship, phenology and longevity of several species.

Assessing bird diversity from point counts by recording all species detected requires a high level of observer skill. As such, variation between observers can be substantial in this type of survey, dependent upon experience and ability. The initial two days at camp were thus spent learning bird calls, where protocols for bird banding/mist netting and ageing/sexing local bird species in the hand were discussed and practised.



Overall, a total of six fixed mist-netting stations are present in the KNP study area, with each station being surveyed twice in the course of the season. Unfortunately, due to permitting restrictions in 2021, it was not possible to survey two of the mist-netting stations, and as such only four stations (representing eight survey days) were surveyed. Mist-netting was not conducted on successive days to remove observer effects of 'net shyness'. This allowed for relatively constant capture rates with birds experiencing less stress as a result (particularly regularly captured breeding individuals). On each banding day, eight nets were operated for six hours after opening time (dawn). In total, this constituted a total of 48 hours (384 net hours) during the course of the 2021 season.

3.2.1 - Point counts: Three 10-minute point counts were completed at each of the survey points on each transect at all camps throughout the season. Point counts were completed between 05:00am and 09:00am. In the rare event of heavy rains or strong winds that impede the accuracy of the survey, activities were cancelled. On all surveys, the weather conditions at the time of the point count were recorded. On arrival, a settle period of one minute was allowed prior to commencement of the survey. The count was subdivided in 2-5 minute intervals where all species detected were recorded. For the duration of the count (10mins), for each contact observed, the following details were recorded: species, audibly or visually detected, and approximate distance from the observer (to the nearest meter).

3.2.2 Mist-netting: Mist-netting was performed at the permanent constant effort mist-netting stations. Nets were checked at least once every 30 minutes, dependent on climatic conditions. Captured birds were extracted and placed in individual cotton bags while waiting to be processed. Birds were banded with uniquely-numbered aluminium rings (size according to species). The following morphometric, condition and breeding status data was taken:

- Maximum wing chord
- Maximum tarsus length
- Bill length (tip to feather)
- Tail length
- Weight
- Mass and Fat Scores
- Breeding Status
- Age and Sex

All information was noted on the provided bird banding data sheets. Furthermore, separate data was collected on net-effort hours. After a banding session, nets were furled or taken down. Nets were typically set-up on days prior to a survey morning at a given station and left furled overnight, easing early morning set-up times.



3.3 Bats

Bat communities in 2021 were surveyed via two methods: mist-netting at fixed netting stations (with an objective of sourcing standardized monitoring data) and targeted netting at fixed locations (with an objective of capturing as many bats as possible in order to obtain diversity and morphometrics data and sample as many bat parasites as possible).

3.3.1 – Standardized bat monitoring

Theoretically, standardized mist-netting surveys were planned to be completed on three nights at each of the six permanent netting stations (which were located at the same localities as the bird mist-netting stations – see section 3.2. above). However, in practise only three netting stations were surveyed, partly due to permitting restrictions, and partly due to extremely low capture rates which suggested standardized survey methods are not appropriate for mist-netting surveys in the habitats occurring in KNP.

For the standardized survey locations that were utilized in 2021, nets were placed in suitable areas (e.g., rides or open areas in the surrounding scrub) in which 45m of mist net (comprising three 12m x 2.5m and one 9m x 2.5m nets) were deployed. The GPS location of each mist net was recorded.

Mist-netting was conducted between 21:00pm and 03:00am. The nets were checked at least every 30 minutes throughout the survey period. All the bats were extracted from the nets following standardized protocols to minimize the stress and kept in capture bags for no longer than 30 mins. This time varied slightly depending on the size of the bat and the sex; pregnant females were released immediately with no measurements taken. Bats were weighed, sexed, and the length of the forearm and wing measured. In the case of cryptic species, additional measurements (e.g. tragus width, length of tibia) were taken as appropriate. Any ectoparasites observed were also collected and stored in ethanol. Each bat was marked by fur-clipping to ensure that any re-captured bats were immediately released with no further measurements being taken.

3.3.2 – Targeted bat surveys

Due to the low capture rates of standardized bat monitoring in KNP (see results below), targeted netting was also undertaken (based on existing records, anecdotal information or suitability of habitat) in locations which were predicted to be especially good for bat captures. While inherently biased (and thus unsuitable for standardized monitoring data), such targeted netting was important for obtaining morphometrics and bat ectoparasite data necessary for the successful completion of several of the specialist projects being completed in the Park (see below). Such targeted netting (including the use of static hand nets to capture roosting bats) was completed in roof spaces, abandoned houses, over still and running water bodies, and (where permitting allowed) at the front of cave entrances. Variable net lengths and netting strategies were used for such targeted studies, depending on the location in which netting was being conducted.



3.4 Butterflies

Formal butterfly surveys were completed in the KNP project following a modified Pollard count methodology (Pollard 1977) conducted along each of the projects transects. Entomologists walked each transect three times in the course of the season; either in the morning (typically starting between 07:00 and 08:00) or in the afternoon (typically starting between 16:00 and 17:00), these being considered peak activity times for butterflies in the region (Simões Nunes et al. 2020). Transects were walked at a standardised pace of 800m per hour. All butterflies observed visually or trapped in seine nets within 2.5m of either side of the transect line during the course of these standardised walks were recorded and identified wherever possible using an authoritative local guidebook (Tolman & Lewington 2009) as well as other sources. In addition to butterflies recorded on the standardised walks, entomologists also stopped at each of the survey stations on each transect to conduct 10 minute point counts, during which any butterflies seen within a 20m radius of the count station's central point were recorded. Butterflies which could not be identified in the field were captured and brought back to the Biota house for further observation, although no samples were exported in the course of the field season.

4. Habitat structure and Carbon surveys

Habitat structure surveys in the KNP project are conducted in 20m x 20m plots located at each survey station. The purpose of these habitat surveys is two-fold. First, general habitat structure data is sourced relating to ecosystem architecture, which in turn can be used to characterize differences between study sites and explain spatial and temporal patterns observed in other study taxa. Second, tree measurement data can be used to calculate carbon estimate data, which can be used to determine carbon stocks in KNP.

With the exception of a single canopy density measurement taken in the middle of the plot (see below), all general habitat measurements were taken in 5x5m subplots located in the corners of each 20m x 20m habitat plot. General habitat structure surveys obtain data on the following variables:

- 1: GPS co-ordinates of each plot corner
- 2: **Angle of slope** in the plot. Four measurements taken in each plot, pointing a clinometer directly towards the centre of the plot.
- 3: **Canopy density** per sub-plot. Determined by pointing a canopy scope with 25 equidistant dots at a 45° angle from each sub-plot corner into the centre of the plot, picking the biggest canopy gap within a 45° angle, and noting the number of dots that go onto sky, rather than



being blocked by vegetation. A fifth canopy density measurement is also taken at the centre point of the plot, looking directly up at a 90° angle.

- **4: Number of saplings** per sub-plot. Saplings are defined as trees >2m in height and <10cm CBH (Circumference at Breast Height).
- 5: **Understorey vegetation density** in each plot. Determined by using a 3m high touch-pole divided into six 0.5m sections. Relative understorey data for each section was determined by the number of pieces of live vegetation that touch each of the 0.5m sections of the pole. Four touch-test pole measurements were taken in each sub-plot (one in each corner), equating to 12 measurements for each plot as a whole.
- 6: **Grass height**. Determined by placing a 30cm plastic ruler vertically in the corner of each subplot (equating to four measurements per sub-plot and 12 measurements per plot overall) and identifying and measuring the longest blade of live grass touching the ruler. Once identified, the full height of each grass blade is determined by stretching it vertically next to the ruler.
- 7: **Vegetation cover.** Determined by visually assessing the percentage ground cover of shrubs, trees, and trees and shrubs in each sub-plot. As this measurement is subjective in nature, it is recommended that the same person (i.e. the habitat survey team leader) record this measurement throughout the course of the season, or at least as consistently as otherwise possible.

Tree measurements: Tree measurement and carbon stock estimate data is based around measurements of all trees within the entire 20x20m subplot (not just the subplots, as with most of the habitat structure variables). "Trees" are defined as possessing a CBH of >20cm. Dead trees were ignored. Shrubs (species that never obtain a height of >7m i.e. *Juniperus*) are never classified as trees regardless of the CBH size of their main stems. For each tree in the 20x20m plot the following variables were measured:

- 1) CBH value
- 2) Species (if known if not the Genus or Family was recorded)
- 3) Subplot each tree is present in (if applicable)

Additionally, at the end of each habitat survey, the tree survey team also recorded the height of the tallest tree in the plot. Tree height was calculated using a clinometer and a measuring tape to calculate the distance from the base of the tree and the angle from this point to the tree top, as well as the height of the recorder (measurement taken from the ground to eye-level). From this trigonometry can be applied to determine the height of the tree.



5. Reported results from 2021

5.1 – Herpetofauna

All transects were successfully surveyed three times in the course of the 2021 field season, and all opportunistic records were also noted. In total 19 individual reptiles and amphibians were encountered during formal survey work (constituting two amphibian species and five reptile species) and 62 opportunistic records (constituting five amphibian species and 16 reptile species). No new species records for the Park were recorded. Table 2a and 2b below summarized all species detected in the course of the field season overall.

Table 2a. Amphibian species detected by fieldwork in the Krka National Park study area in the 2021 field season.

Family	Common name	Latin name
Proteidae	Olm	Proteus anguinus
Bufonidae	Common toad	Bufo bufo
Ranidae	Agile frog Marsh frog	Rana dalmatina Pelophylax kurtmuelleri
Hylidae	European tree frog	Hyla arborea

Table 2b. Reptile species detected by fieldwork in the Krka National Park study area in the 2021 field season.

Family	Common name	Latin name
Testudinidae	Hermann's tortoise	Testudo hermanni
Emydidae	European pond turtle	Emys orbicularis
Lacertidae	Balkan wall lizard Green lizard Dalmatian wall lizard	Lacerta trilineata Lacerta viridis Podarcis melisellensis



Italian wall lizard Podarcis siculus

Dalmatian algyroides Algyroides nigropunctatus

Gekkonidae Mediterranean house gecko Hemidactylus turcicus

Anguidae European glass lizard Pseudopus apodus

Colubridae Four-lined snake Elaphe quatrolineata

Leopard snakeZamenis situlaDice snakeNatrix tessellataGrass snakeNatrix natrixDahl's whip snakePlatyceps najadum

European cat snake Telescopus fallax
Balkan whip snake Hierophis gemonensis

5.2 – **Birds**

Point count surveys in 2021 yielded a total of 503 individual bird records constituting 48 species, while mist-netting surveys caught 48 individual birds constituting 11 species. Point-counts, mist-netting, and opportunistic sightings combined detected a total of 151 species in Krka National Park in 2021. Two species (Goosander - *Mergus merganser* and Lesser grey shrike – *Lanius minor*) were new records for the Operation Wallacea terrestrial project, albeit not for the formal bird list maintained by the National Park authority Table 3 below summarizes all bird species detected in Krka National Park in 2021.

Table 3. Bird species detected by fieldwork in the Krka National Park study area in the 2021 field season.

Family	Common name	Latin name
Podicipedidae	Little grebe	Tachybaptus ruficollis
Phalacrocoracidae	Great cormorant Pygmy cormorant	Phalacrocorax carbo Microcarbo pygmaeus
Ardeidae	Grey heron Purple heron	Ardea cinerea Ardea purpurea
Anatidae	Mallard Goosander	Anas platyrhynchos Mergus merganser
Accipitridae	Common buzzard	Buteo buteo



Falconidae

Rallidae

Laridae

Caprimulgidae

European honey-buzzard

Short-toed eagle

European sparrowhawk

Pernis apivorus Circaetus gallicus Accipiter nisus

Common kestrel

Eurasian hobby

Falco tinnunculus Falco subbuteo

Common moorhen

Eurasian coot

Gallinula chloropus

Fulica atra

Scolopacidae Common sandpiper

Yellow-legged gull

Actitis hypoleucos

Larus michahellis

Columbidae European turtle-dove

Eurasian collared dove

Streptopelia turtur

Streptopelia decaocto

Caprimulgus europaeus

Cuculidae Common cuckoo

European nightjar

Cuculus canorus

Apodidae Common swift

Apus apus

Alcedinidae Common kingfisher Alcedo atthis

Meropidae European bee-eater Merops apiaster

Upupidae European hoopoe Upupa epops

Picidae Great spotted woodpecker

European green woodpecker

Dendrocopos major Picus viridis

Hirundinidae Barn swallow

House martin

Hirundo rustica Delichon urbicum

Alaudidae Crested lark

Wood lark

Galerida cristata Lullula arborea

Motacillidae Tawny pipit

> Tree pipit White wagtail Grey wagtail

Anthus campestris Anthus trivialis

Motacilla alba Motacilla cinerea



Oriolidae Eurasian golden oriole Oriolus oriolus

Laniidae Red-backed shrike Lanius collurio

Lesser grey shrike Lanius minor
Woodchat shrike Lanius senator

Corvidae Hooded crow *Corvus cornix*

Northern raven Corvus corax

Eurasian jay Garrulus glandarius

Sittidae Eurasian nuthatch Sitta europaea

Cinclidae White-throated dipper Cinclus cinclus

Paridae Sombre tit Poecile lugubris

Great tit Parus major

Eurasian blue tit Cyanistes caeruleus

Aegithalidae Long-tailed tit Aegithalos caudatus

Acrocephalidae Great reed warbler Acrocephalus arundinaceus

Eurasian reed warbler Acrocephalus scirpaceus

Eastern olivaceous warbler Iduna pallida

Cettidae Cetti's warbler Cettia cetti

Phylloscopidae Wood warbler Phylloscopus sibilatrix

Sylvidae Common whitethroat Sylvia communis

Garden warbler Sylvia borin

Eastern orphean warbler Sylvia crassirostris
Subalpine warbler Sylvia cantillans
Blackcap Sylvia atricapilla

Turdidae Common nightingale Luscinia megarhynchos

Eurasian blackbird Turdus merula

Muscicapidae Black-eared wheatear Oenanthe hispanica

European robin Erithacus rubecula

Fringillidae Hawfinch Coccothraustes coccothraustes

Chaffinch Fringilla coelebs
European goldfinch Carduelis carduelis



Passeridae House sparrow Passer domesticus

Emberizidae Cirl bunting Emberiza cirlus

5.3 – Bats

In 2021, the bat team completed nine nights of standardized mist-netting, and a further 19 nights of targeted mist-netting, supplemented by acoustic monitoring. Standardized netting captured a total of four individuals of two species, while targeted mist-netting captured 88 individuals of 13 species. Table 4 below summarizes all bat species detected in Krka National Park by all mist-netting efforts during the 2021 field season. This list includes visual observations and those records determined by acoustic analysis.

Table 4. Bat species detected by fieldwork in the Krka National Park study area in the 2021 field season.

Order	Family	Common name	Latin name
Chiroptera	Rhinolophidae	Greater horseshoe bat Lesser horseshoe bat Mediterranean horseshoe bat	Rhinolophus ferrumequinum Rhinolophus hipposideros Rhinolophus euryale
	Miniopteridae	Common bent-wing bat	Miniopterus schreibersii
	Vespertilionidae	Natterer's bat Long-fingered bat Greater mouse-eared bat Lesser mouse-eared bat Bechstein's bat Geoffroy's bat Kuhl's pipistrelle Savi's pipistrelle Common serotine	Myotis nattereri Myotis capaccinii Myotis myotis Myotis blythii Myotis bechsteinii Myotis emarginatus Pipistrellus kuhlii Hypsugo savii Eptesicus serotinus



5.4 – Butterflies

In 2021 the butterfly team completed the requisite three repetitions of each of the formal transects. Records from the formal surveys were supplemented further with opportunistic captures and observations. In total, the butterfly team detected 45 species during the course of the 2021 season. One of these (Purple hairstreak - *Neozephyrus quercus*) was a new record for the Operation Wallacea project in KNP. Table 5 below summarizes these species. Species marked * are new records for the 2021 season. Species marked † were not identified with 100% certainty.

Table 5. Butterfly species detected by mist-netting surveys in Cusuco National Park between June and August 2021.

Order	Family	Common name	Latin name
Lepidoptera	Papilionidae	Scarce swallowtail Southern swallowtail	Iphiclides podalirius Iphiclides feisthamelii
	Lycaenidae	Adonis blue† Chalk-hill blue† Small copper	Lysandra bellargus Lysandra coridon Lycaena phlaeas
		Holly blue Short-tailed blue Lang's short-tailed blue Silver-studded blue Anomalous blue Common blue Little tiger blue Brown argus Purple hairstreak*	Celastrina argiolus Cupido argiades Leptotes pirithous Plebejus argus Polyommatus admetus Polyommatus icarus Tarucus balkanicus Aricia agestis Neozephyrus quercus
	Pieridae	Eastern bath white Mountain small white Southern small white Eastern wood white Wood white Berger's clouded yellow Clouded yellow Cleopatra Brimstone	Pontia edusa Pieris ergane Pieris mannii Leptidea duponcheli Leptidea sinapis Colias alfacariensis Colias corceus Gonepteryx cleopatra



Nymphalidae Nettle-tree butterfly Libythea celtis

Marbled white

Speckled wood

Meadow brown

Wall brown

Dusky meadow brown

Melanargia galathea

Pararge aegeria

Maniola jurtina

Lasiommata megera

Hyponephele lycaon

Small heath Coenonympha pamphilus
Great banded grayling Brintesia circe

Woodland grayling

Grayling

Grayling

Tree grayling

The hermit

Silver-washed fritillary

Niobe fritillary

Red admiral

Hipparchia fagi

Hipparchia semele

Hipparchia statilinus

Chazara briseis

Argyniss phaphia

Fabriciana niobe

Vanessa atalanta

Southern comma
Spotted fritillary

Vanessa atalanta
Limenitis reducta
Polygonia egea
Melitaea didyma

Hesperiidae Dingy skipper Erynnis tages

Oberthür's Grizzled skipper Pyrgus armoricanus
Lulworth skipper Thymelicus acteon

Small skipper Thymelicus sylvestris

5.5 – Habitat structure

Habitat and forest structure data for KNP were collected each week during the 2021 field season. However, due to the reduced season length (Five weeks rather than the normal eight weeks), and reduced student numbers, not all habitat plots were surveyed in the course of the season. In total, 12 survey plots were surveyed, focusing on plots in T1 and T6. All T1 survey plots were completed with the exception of the point at 1350m, and all points on T6 were completed. Survey plots on T2, T3, T4 and T5 were not surveyed in 2021 and these represent the priority for the survey team at the start of the 2022 research season.



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