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Monitoring Methodology for European *Formica rufa* Species Group

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Abstract

The objects of the purposed methodology are the ants (*Hymenoptera: Formicidae*) from the European *Formica rufa* group (red wood ants). As protected species in the ecosystems in temperate forests, the trend of their populations' dynamic has to be observed periodically. The existing monitoring methods in the literature have been modified and optimized in the current paper. The purpose was to leave only the environmental parameters most related to the habitat preferences of red wood ants and which may be feasible in the field by a team of two experts per sampling site. The fieldwork is described in detail. The monitoring of the red wood ants is suggested to be done annually or in a maximum of five years.

Keywords: Fieldwork; Nest Density; Protected Species; Temperate Forests

Introduction

Formica rufa group (red wood ants) (Hymenoptera: Formicidae) contains mound-building species, which build perennial nests consisting of a mound of grass, litter, or conifer needles [1]. They play a keystone and ecologically dominant role in forest ecosystems [2]. They are the links of the food chain, successfully fight forest pest insects, participate in loosening, humification and enrichment of the soil, and contribute to the distribution of some plants.

They are spread in boreal and northern temperate forest ecosystems of North America, Europe and Asia. Ten species are present in Europe: *Formica* (*Formica*) *rufa* Linnaeus, 1761, *F.* (*F.*) *lugubris* Zetterstedt, 1838, *F.* (*F.*) *paralugubris* Seifert, 1996, *F.* (*F.*) *helvetica* Seifert, 2021, *F.* (*F.*) *polyctena* Foerster, 1850, *F.* (*F.*) *pratensis* Retzius, 1783, *F.* (*F.*) *aquilonia* Yarrow, 1955, *F.* (*F.*) *truncorum* Fabricius, 1804, *F.* (*F.*) *dusmeti* Emery, 1909 and *F.* (*F.*) *frontalis* Santschi, 1919 [3].

The following red wood ants are protected species in the IUCN red list [4] in Lower Risk/ near threatened category: *Formica lugubris, F. pratensis, F. rufa, F. polyctena* and *F. aquilonia*.

Because of their key role in the forest ecosystems, it is essential to monitor the favorable condition of their populations. In monitoring programs, nest density should be used as a dependent variable to assess the population dynamics by quantitative analyses [5]. Nest density estimation of RWA varies among different regions [6]. The reasons for decreasing nest densities may be: nest destruction, air and heavy metal pollution, collection of ant pupae for food for cage birds [7], the climate change, interspecies interactions, the presence of their competitors [8] and others. The differences in habitat characteristics in

each country, and the different methods of counting their density can make it difficult to compare results. Therefore, a common or close-in structure method may help interpret the results from separate regions. As the temperate forests have their landscape characteristics and specifics, a combination of sampling methods from other countries in the temperate climatic zone was used [7,9-16]. The methods were modified to take only the parameters which are most related to the habitat preferences of red wood ants and which may be feasible in the field by a team of two experts. Having the nest density and the changes in the environmental parameters in a given period, we may analyse the reasons for the changes in the population trends for conservation purposes. The method was checked and only partly given by [17].

Peculiarities in Conducting the Monitoring

To count red wood ant species density, sample transects were laid in previously selected suitable homogeneous biotopes. A homogeneous biotope is defined as a continuous polygon of the species' potential habitat falling within one monitoring territory characterized by similar environmental conditions. They are determined depending on the ecological preferences of each species, as for red wood ants they are quite similar (coniferous, mixed and deciduous forests, shrubs and ecotones). Therefore, the separate species could be searched in a common sampling transect (except *F. pratesis*, a species of mostly open habitats and ecotones).

Sample transects where nests were found can be used as stationary for future monitoring studies. The empty transects are not visited during the next monitoring, but additional ones are made.

The number of GPS points depends on the number of nests found. For each transect, one point is taken at the beginning, one at the end, and one point for each nest found inside the transect. In each selected territory for monitoring (maximum of 500 km2), at least 8 transects are made (10,000 m^2) [18].

Fieldwork

1. Visible nests are counted in sample transects 250 m long and 5 m wide (1250m^2) within a homogeneous biotope, by walking (Table 1, Figure 1). One field protocol is completed for each transect. An example of the protocol is given in the Supplementary data in [17]. The form data is needed for repeated observations of the red wood ants [10,19].

Figure 1: Homogeneous biotope for sampling transect (an example).

2. The GPS coordinates of each nest are taken and numbered individually (the number matches the GPS coordinate of the nest). During subsequent observations of the same sampling transects, the parameters of the previously numbered nests are monitored and the abandoned and newly discovered ones are noted.
3. Each nest is photographed with a digital camera along with some of the surrounding vegetation (Figure 2). A photo is also taken of the biotope at least up to a 30 m radius [10,20] (Figure 3).

Figure 2: Nest with the surrounding vegetation.

Figure 3: Nest with about 30 m radius biotope. Collecting ant specimens.

4. Nest parameters are measured (Table 2, Figure 4): base diameter [11] (for an elliptical base, minimum and maximum diameters are measured, which have to be averaged for convenience in statistical analyses and database entry), and height [9]. The volume later is calculated by half ellipsoid formula: $\mathbf{V = 2/3.\pi.(d/2)^2.h}$, where **d** is the diameter of the nest and **h** is the height. During the next monitoring of the same nests, a comparison between volumes is made to check the condition of the nest (decreasing, unchanged, growing, abandoned). The population vitality and the trend of the population dynamics are assessed based on the results.

Figure 4a: Measuring nest parameters - Diameter.

Figure 4b: Measuring nest parameters - Height.

5. Nests with active specimens are counted, as well as those that are abandoned [11].
6. About 10 specimens are taken from each nest for species determination in separate tubes [21] (Figure 3). When mixed colonies are encountered, the individual species are collected in the same tube. Tubes are pre-filled with 95-99% ethanol for DNA analysis if needed for identification.
7. The species of the specimens are identified, if possible, in the field with a magnifying glass with a minimum magnification of 20x. The final identification of species is done in the laboratory under binoculars according to determination keys.
8. Environmental parameters are identified (Table 3) and checked in the field protocol. The influences and threats may also be checked for future ecological analysis.

A weather condition which is NOT advisable to monitor for red wood ants are snowfall, presence of snow cover, heavy rain, fog, and average daily air temperatures below 10°C.

Monitoring Parameters

The monitoring parameters with their units of measurement, counting method and possible risks of errors are given in (Tables 1-3).

Unit of Measurement	Counting Method	Possible Risks of Reporting Errors
Number of nests within 1 sample transect (250 m x 5 m)	All nests within the reach of the transect are counted, including those partially located within it	Missing some of the small nests due to impenetrable terrain, difficult visibility due to dense undergrowth

Table 1: Number of Nests.

Nest Parameter	Unit of Measurement	Counting Method	Possible Risks of Reporting Errors
Diameter	Cm (data is recorded to the nearest 5 cm)**	Tape measure	Inaccurate reporting due to vegetation
Height	cm; For a nest located on terrain with a slope of more than 20°, the average height is taken	Tape measure	Inaccurate reporting due to vegetation
Active/ abandoned	+ / - indicate active or abandoned	Expert opinion after careful digging with a suitable stick, revealing part of the surface layer of the nest	The error may be due to late counting in the autumn season (after September) when ants move to winter nests in depth
Species	Latin name is indicated	With a magnifying glass with a minimum magnification of 20x; The final determination of the species is carried out in laboratory conditions under binoculars according to determination keys	Due to the high risk of error when determining the species in the field, the final determination takes place in a laboratory under binoculars *

Table 2: Nest Parameters.

*The taxonomy of some red wood ant species is unclear. For example, *Formica lugubris* and *F. paralugubris* are identified by genetic analysis [22]. Hybridization has been observed in some populations [3,17,23].

** Data taken in the field to the nearest 5 cm are intended to be compared with newly obtained data from the next visit to the same nests.

Environmental Parameters	Unit of Measurement	Counting Method	Possible Risks of Reporting Errors
Altitude*	Meters above sea level	GPS	Inaccuracy of the device; Lack of signal from satellites in dense forests or gorges (looking for the closest possible point with signal).
Exposure*	Indicate: N, NW, WE, E, SE, S, SW, W	Compass	Inaccuracy of the device
Slope	Degrees	Measuring into 4 classes by [10]: 1) 0-5o; 2) 5-15o; 3) 15-30o; 4) >30o	Inaccurate reporting by people without experience

Ecological groups of plants*	Defined by dominant plants species in respect of their adaptations to soil and air humidity in 5 categories (adapted by [24]): xerophytes (low humidity – succulents, *Euphorbia falcata, Dianthus petraeus, Poa bulbosa,* etc.); meso-xerophytes (drought-tolerant – *Hypericum perforatum, Adonis vernalis, Potentilla argentea, Quercus pubescens*, etc.); mesophytes (moderate humidity – Bellis *perennis, Festuca pratensis, Medicago arabica, Alcea rosea*, etc.); meso-hygrophytes (increased moisture affinity – *Ranunculus sceleratus, Carex distans, Juncus bufonis,* etc.); hygrophytes (high moisture – mosses, *Caltha palustris, Epilobium hirsutum, Oxalis acetosella*, etc.).	Expert opinion on the literature from the relevant region	Imprecise determination of the entire transect due to habitat heterogeneity. The evaluation is averaged.
Stone cover*	Percentage	Measuring into 5 classes by expert opinion: 1) < 5 %; 2) 5-25 %; 3) 25-50 %; 4) 50-75 %; 5) 75-100%.	Inaccurate determination of rock/ rock cover across the transect due to habitat heterogeneity. The evaluation is averaged.
Habitat	Determine if the habitat is a meadow, ecotone, coniferous forest, mixed forest, deciduous Forest or other	Expert opinion	Inaccurate determination of the habitat of the entire transect due to heterogeneity. The evaluation is averaged.
Grass cover*	Percentage	Expert opinion into 4 classes: 1) < 25%; 2) 25-50%; 3) 50-75%; 4) 75-100%.	Inaccurate determination of herbaceous vegetation cover of the entire transect due to heterogeneity of the habitat. The evaluation is averaged.
Canopy cover*	Percentage	Expert opinion in 5 classes: <20%, 20–40%, 41–60%, 61–80%, >80%.	Inaccurate determination of the assemblage of the entire transect due to habitat heterogeneity. The evaluation is averaged.
Undergrowth (shrub) density	Percentage	In 4 classes: <5%, 6–25%, 26–50%, 51–100%.	Inaccurate undergrowth density determination of the entire transect due to habitat heterogeneity. The evaluation is averaged.
Dominant tree species of the forest	Latin name	Expert opinion or available data from Forestry GIS database	Imprecise identification of the species due to habitat heterogeneity.
Forest mean age as years*	Years	Available data by Forestry GIS database in 4 classes: 1)0–50, 2)51–100, 3)101–150, 4)>150	Inaccurate age determination due to habitat heterogeneity. The evaluation is averaged.

Table 3: Environmental Parameters
*Environmental parameters of primary importance for the distribution of red wood ants according to [17].

Identifying Influences (current) and Threats (future)

1. Felling – Binary variable (presence/absence)
2. Fire - Binary variable (presence/absence)
3. Buildings - Binary variable (presence/absence)
4. Insecticide usage - Binary variable (presence/absence). Data would be taken by the relevant Forestry.

Monitoring Frequency in the Temperate Zone

Recommended Period of the Year During which Monitoring of each Species Should Take Place

Early summer is best for collecting ants in temperate zones [25]. Samplings in April are complicated due to the winter deformation of the mound (the nests are not well visible), and many of the young nests die in the winter. In late autumn, observations become difficult due to leaf litter – even large nests are difficult to see [19].

The recommended period according to the elevation is:

- Up to 1000m a.s.l.: May-September.
- Above 1000m a.s.l.: July-August.

Duration of a Single Observation

It takes an average of 1.5 hours per person to walk a transect 5 m wide and 250 m long. For a team of two people, the duration is 45 minutes to 1 hour (i.e. 6-8 hours per day). The duration of monitoring also depends on the number of nests found. A minimum of 10-15 minutes is required to retrieve the data from each nest. As 8 sample transects of 250 m per day are walked, an additional total of 2-3 hours or more is usually required to travel between individual transects.

Required Number of Field Visits within a Year

Each site/sample transect is visited once a year during daylight hours.

The Optimal Period for Repeating the Observations to Establish Trends in the State of the Populations

Annual monitoring is recommended. The maximal period is 5 years (Antonova, et al. in prep)

Equipment

Filed Equipment

1. Waterproof pants and jacket
2. Hiking boots, raincoat
3. Backpack minimum 65 liters
4. Compass
5. GPS/smartphone/tablet with digital topographic maps
6. Digital camera/smartphone
7. Field protocols/digital application for recording observations and photographs.

Consumables

1. Ethanol 95%
2. Polypropylene tubes 5-10 ml
3. Tweezers
4. Labels for labelling the samples
5. Pocket magnifier 20x
6. Pencil
7. Tape measure (10 m).

Fieldwork Safety Rules

1. Rubber gloves are used when collecting the samples against formic acid allergies,
2. It is mandatory to carry a first aid kit,
3. Avoid forest areas with fresh bear tracks.

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Conflict of interest

The author declares no conflict of interest.

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