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Title: Moth larval abundance surveys Varanger

Motivation

Population outbreaks by geometrid moths is a key driver of vegetation dynamics and forest health in the ecotone forest bordering on the shrub tundra. Moth larval abundance is a target in the forest-tundra ecotone module of COAT.

State variables:

Moth larval abundance surveys are used for calculating the following state variables:

Moth abundance lowland transects Varanger (V24).

Reference to method:

This survey method has been in use for monitoring moth abundances in Troms since 1999 both in lowland transects similar to the ones currently used in Varanger, and in elevational gradients. The method is described in Ims et al. (2004) and Vindstad et al. (2019).

Spatial study design

Moth larval abundance surveys are conducted in the COAT Varanger regional design at four localities: Tana, Austertana, Bugøyfjord and Vestre Jakobselv. Each locality consists of 10 replicated sites placed in mature birch forest. All coordinates can be found in the coordinate file included in the dataset V_insect_defoliators_density_varanger on the COAT data portal.

Table 1. List of site IDs for the moth larvae abundance surveys.

locality	section	site_id
tana	NA	t_lar_1, t_lar_2, t_lar_3, t_lar_4, t_lar_5, t_lar_6, t_lar_7, t_lar_18, t_lar_9, t_lar_10
austertana	NA	a_lar_1, a_lar_2, a_lar_3, a_lar_4, a_lar_5, a_lar_6, a_lar_7, a_lar_8, a_lar_9, a_lar_10 (In 2015 only: a_lar_11, a_lar_12, a_lar_13)
bugoyfjord	NA	b_lar_1 , b_lar_2, b_lar_3, b_lar_4, b_lar_5, b_lar_6, b_lar_7, b_lar_8, b_lar_9, b_lar_10
vestre_jakobselv	NA	vj_w_lar_1, vj_w_lar_2, vj_w_lar_3, vj_w_lar_4, vj_w_lar_5, vj_w_lar_6, vj_w_lar_7, vj_w_lar_8, vj_w_lar_9, vj_w_lar_10

Design within site: The sampling sites are not marked in the field, and locating the sites using a handheld GPS to an accuracy of a few meters is sufficient. No permanent plots or other sampling units exist.

Temporal study design

Moth larval abundances are collected once a year in late June – early July depending on the phenological progress of the season. The sampling is timed to ensure that most larvae are in the late instars (4th and 5th instar). Of the two dominating species *Epirrita autumnata* tends to be approx. one instar ahead of *Operophtera brumata*, and sampling should hence preferentially take place when the majority of *E. autumnata* larvae are in late 4th or early 5th instar.

Procedure

At each site, moth larval densities are estimated by collecting 10 branches of about 80 cm length from 10 haphazardly chosen birch trees within an approximately 20-m radius around each site. Branches are preferably chosen from mature trees at a height of 1-2 meters above ground. Each branch is thoroughly shaken in a large plastic box with vertical sides (see image below), until all moth larvae have detached and fallen into the box. Be careful when carrying the branches to the box, so that larvae do not detach prematurely. At low-intermediate densities all 10 branches may be shaken into the box before counting, but at high densities is it recommended to count a few branches at a time, or to bring two boxes. The larvae in the box are subsequently sorted to species and counted. Larvae of *O. brumata* are melanistic (can vary in color from yellowish green – almost black). They are hence scored into three color groups (light, intermediate, dark) and the number of larvae reported for each group. After all larvae have been counted, 10 specimen of each species are collected in individual Eppendorf tubes, placed in one ziplock bag per species and brought back to the lab for genetic analysis. The bags should be labelled by site number, year and species. An identification guide for the species and guidelines for scoring to color groups can be found in the appendix to this protocol.

Left: Armlength branches are shaken into a solid white plastic box. Middle: Larvae are sorted into species and color groups and counted. Right: At high densities the number of larvae and the amount of debris might be high, and branches should be counted a few at a time.

Equipment needed

- 1-2 sturdy white plastic boxes with vertical sides. The size (width*length*height) should be approximately 40*60 *30 cm to ensure that arm length branches can be shaken into the box without losing larvae over the edges.
- Strong garden pruning shears (one per person) for cutting branches
- A handheld GPS with all site IDs loaded
- Eppendorf tubes for collecting larvae (~30 tubes per site)
- Small ziplock bags for collecting Eppendorf tubes (3 per site)
- Pencil and notebook

Information recorded in the field

Field notes are done in waterproof notebooks. For each site record the following:

- Date
- Name of observer
- Location name and site number
- Total number of larvae of the species *Epirrita autumnata* (EA, autumnal moth), *Operophtera brumata* (OB, winter moth), *Agriopis aurantiaria* (AA, scarce umber moth).
- Total number of winter moth larvae in each color category (OB_light – OB_intermediate – OB_dark)

Any geometrid species, beyond the three species listed above, which occur in frequent number (more than a few per site) and which are *unknown or new to the observers*, should be counted and a few specimen brought back to the lab for identification. This is done to ensure that any new species expanding into the area are recorded as early as possible. A notebook sheet can for instance be structured accordingly:

Location:			Date:		Observer:	
Site	EA	OB_light	OB_interm	OB_dark	AA	Note
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Sample processing after field work

Larvae collected for genetic analysis should preferably be placed in a freezer at -80°C the same day as they have been collected. If a -80°C freezer is not immediately available, place them in a regular freezer, and move them to -80°C as soon as this is possible.

Data processing

Each field worker is responsible for typing his/her own data unless otherwise agreed upon with the project leader. Field data on moth abundances should be typed the same day as they have been collected. A notebook containing un-typed data should never be brought back in the field the

following day, due to the risk of losing it. If it is practically impossible to type the data the same day, photocopy or photograph the un-typed sheets, and store the photocopy/photographs in a safe place.

Training requirements and specialized skills

Field workers must be able to reliably distinguish the outbreak species autumnal moth, winter moth and scarce umber moth. They must be able to distinguish any geometrid larvae from non-geometrids independent of species. Field workers must further be trained and inter-calibrated with each other on grouping the winter moth larvae into color categories.

References

Ims, R. A., Yoccoz, N. G., & Hagen, S. B. (2004). Do sub-Arctic winter moth populations in coastal birch forest exhibit spatially synchronous dynamics? *Journal of Animal Ecology*, *73*, 1129–1136. https://doi.org/10.1111/j.0021-8790.2004.00882.x

Vindstad, O.P.L., Jepsen, J.U.; Yoccoz, N.G., Bjørnstad, O., Mesquita, M. & Ims, R.A. (2019). Spatial synchrony in sub-arctic geometrid moth outbreaks reflects dispersal in larval and adult lifecycle stages. Journal of Animal Ecology 88: 1134-1145, https://doi.org/10.1111/1365-2656.12959

Appendices

Identification guide for geometrid larvae

Currently the winter moth (*O. brumata*) and the autumnal moth (*E. autumnata*) are responsible for almost all birch forest defoliation in Finnmark. In Troms, the scarce umber moth (*A. aurantiaria*) is locally equally abundant as the other two species. The scarce umber has so far not been recorded in Finnmark, but may be present, and is likely to occur there in the future as a northwards spread has been documented. Currently therefore the identification guide only include these three species. All three species hatch from eggs around the time of budburst of the host tree. First instar larvae are tiny (2-3 mm) and can be difficult to distinguish. Later instars however, are quite distinct.

Winter moth: Winter moth larvae may grow to a length of approx. 20 mm in the later instars. The are usually a greyish-green color with distinct dorsal-lateral light lines (Plate 1). They are melanistic however, and both body and head capsule may vary widely in color from a light yellowish green to almost black (Plate 2). Phenologically it tends to be 1 instar behind the autumnal moth.

Autumnal moth: Autumnal moth is the largest of the tree species, and may grow to approx. 30 mm in later instars. It is bright green with very little color variation and has a light lateral line. Phenologically it tends to be the most advanced of the three species.

Scarce umber moth: Scarce umber moth larvae may grow to 25-30 mm in length in later instars, but is much thinner than any of the other two. It is brown to black in color, resembling a thin twig. It is agile and moves about quicker that any of the other two species. Phenologically it is the latest of the three species, and may be 1 instar behind winter moth and 1-2 behind autumnal moth.

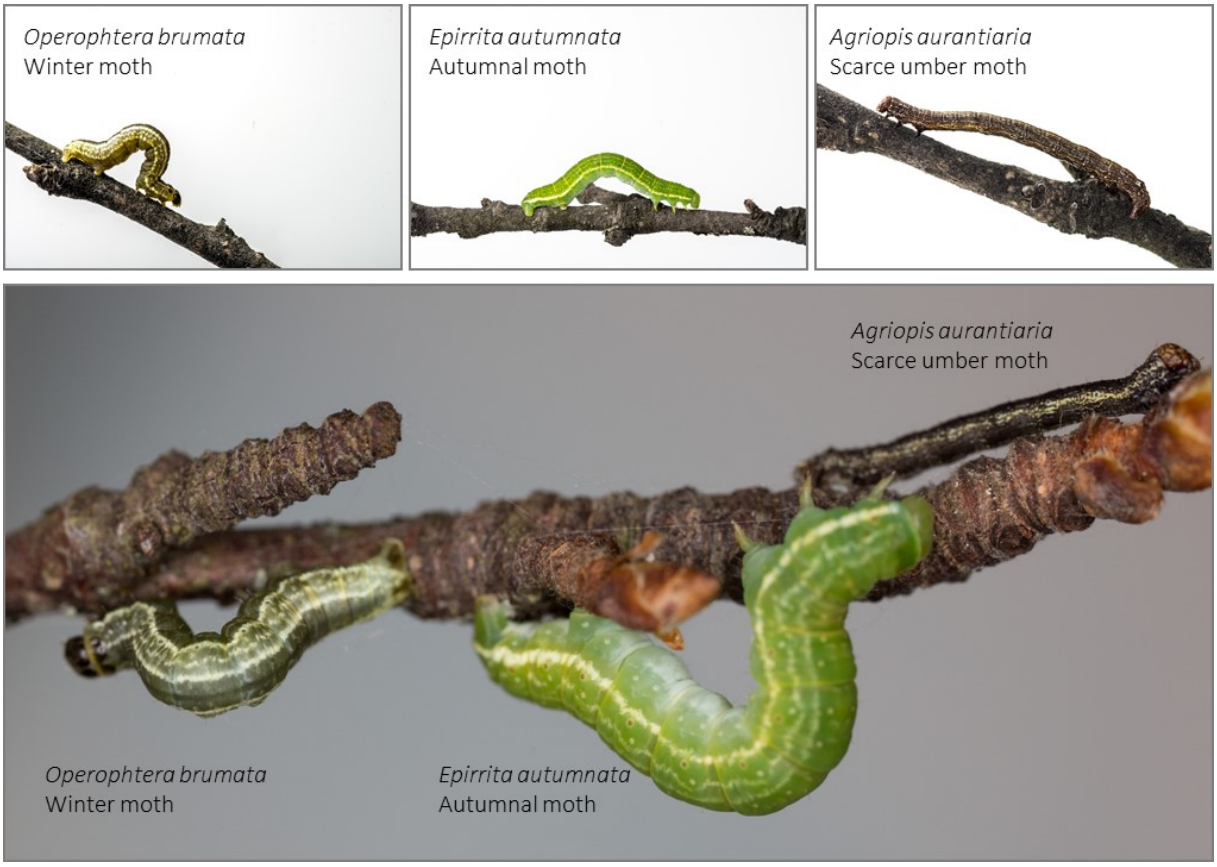

Plate 1. Late instar larvae of winter moth, autumnal moth and scarce umber moth. Photo: Moritz Klinghardt (top panel), Jon Aars (botton panel).

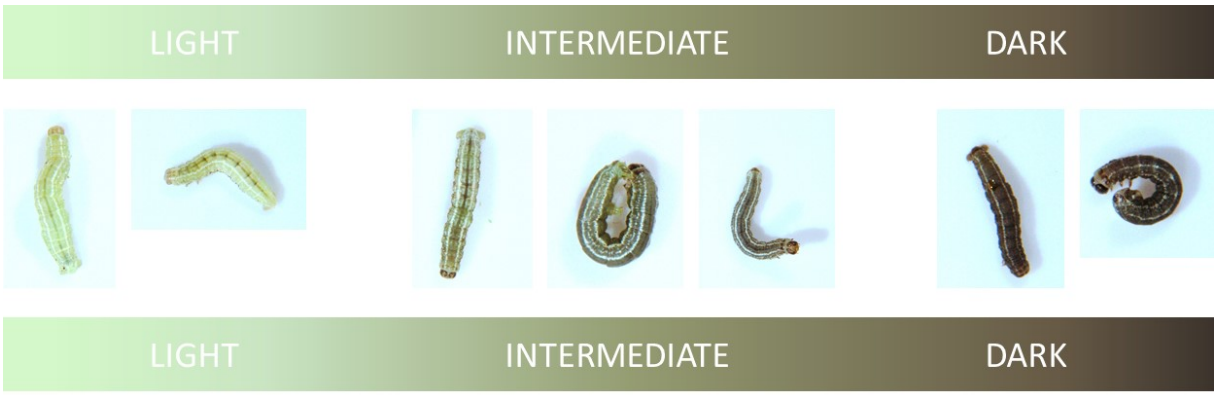

Plate 2. Color variation in the larvae of winter moth. The color of the larvae varies from very light yellowish green to almost black. They need to be divided into three groups; light, intermediate and dark. This division is not always straightforward and it is recommended to begin with the light and dark category, and place any uncertain borderline specimen in the intermediate category. Frequent calibration between field workers is further recommended to avoid consistent bias between observers. The color of the larvae is influenced by the current-year density of larvae, with a higher frequency of dark larvae at higher densities. It is therefore very important to keep in mind that the division should be made with reference to the whole potential color scale, not just the range of colors available in a given sample, or the range experienced by the observers in a given year. Photo: Mathieu Laparie.