ITEX herbivory protocol – trial version 2014

Herbivory is a main driver of tundra plant communities [1,2], and recent studies have shown that herbivores can modulate the responses of tundra plants to warming [3–7]. The International Tundra Experiment (ITEX; http://www.geog.ubc.ca/itex/) provides an experimental setting to test this idea across a large number of tundra sites.

The goal of this protocol is to provide guidelines for assessment of herbivory occurrence and intensity within ITEX plots (OTCs vs controls) and among study sites (controls at different sites). This information will allow a quantitative evaluation of herbivory, to address the following questions:

- ✓ If herbivory is similarly prevalent across tundra sites (by comparing control plots at different sites)
- ✓ If herbivory by vertebrates and invertebrates has a similar impact across tundra sites
- ✓ If herbivory occurs at different intensities within OTCs and in controls (due to exclosure of herbivores by the OTCs or due to the warming effect of OTCs), and whether this influences the responses of tundra plant communities to experimental warming

While the measurements proposed in this protocol will undoubtedly benefit the ongoing studies at each site, the data obtained would be also extremely valuable for collaborative research, e.g. comparisons across sites.

Because herbivores (both vertebrates and invertebrates) can affect plant communities directly, through plant biomass consumption, and indirectly, through trampling and nutrient deposition via faeces and urine[8], it is relevant to quantify both, the signs of herbivory and the signs of herbivore presence.

In this document, we will refer to "ITEX sites" as a group of pairs of OTCs and control plots occurring in places with broadly similar environmental conditions. For example, if you have plots on wet tundra but grouped at two different elevations, your groups will be regarded as two separate ITEX sites. Similarly, if your plots are at the same elevation but on three markedly contrasting habitats, for example in wet tundra, heath and dry tundra, your groups will be considered three separate ITEX sites.

This protocol consists of three levels of assessment: overall characteristics of the herbivore community, site-level assessment and plot-level assessment. Examples of the types of signs to be evaluated in the field, field data sheets and the data entry procedure are provided in the appendix.

1. Overall characteristics of the herbivore community

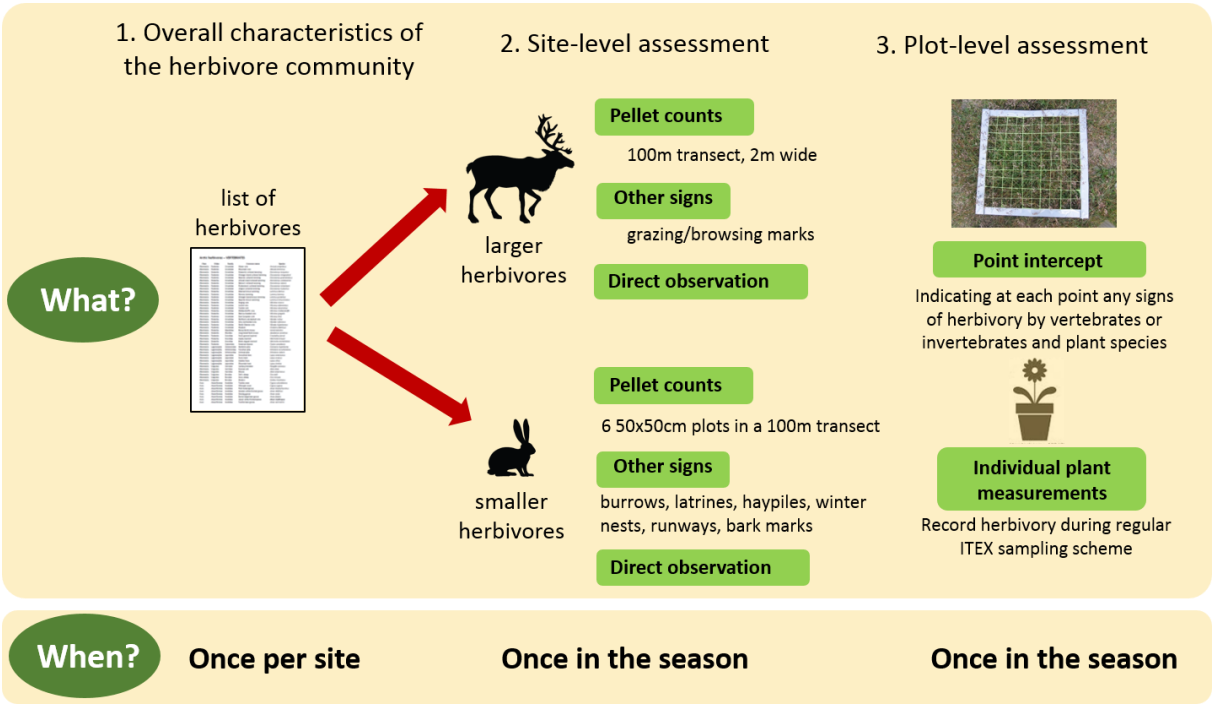
Overall description of the site, and relevant management practices that may affect herbivore populations

2. Site-level assessment

Local estimates of (vertebrate) herbivore presence and abundance in the area

3. Plot-level assessment

Fine-scale measures of herbivory and herbivore activity that can be related to plant measurements



Summary of proposed activities within this herbivory protocol. Ideally levels 2 and 3 will be conducted once in the season every year, but 'snapshot data' from different years will also be very useful.

1. Overall characteristics of the herbivore community

A brief description of the ITEX site will help in framing the specific monitoring protocols for the site and plot level assessments. General information on features of the site relevant to herbivore populations (e.g. if the area is under grazing management, hunting activities…) will be requested. Each ITEX site will be provided upon request with a preliminary list of potential herbivores, which will need to be updated using local information and consulting local experts, particularly regarding the presence of domestic herbivores. If available, data on densities of different herbivores, population fluctuations, status of populations (e.g. if migratory or resident) and accuracy of the observations would be highly desirable. Also, an indication needs to be made if the ITEX sites are within an exclosure fence that prevents access to any herbivore, either large or small mammals or birds. This site description will follow a basic template (Appendix 1) and needs to be completed once per ITEX site.

Background data on the potential occurrence and densities of herbivores and their distribution will help in defining overall herbivore activity in the area. This information will also help in defining the methods for herbivory assessment at the site- and plot-level (sections 2 and 3).

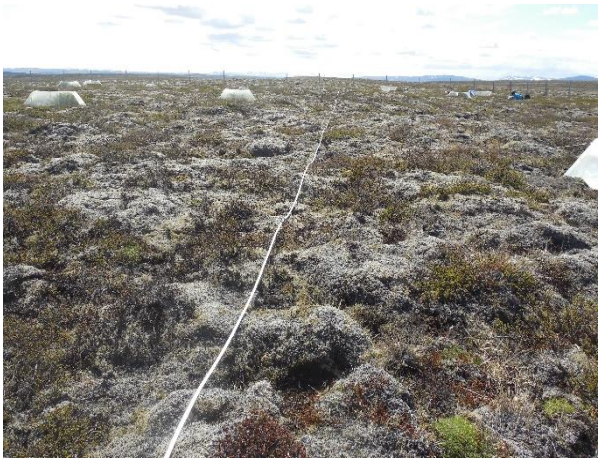
2. Site-level assessment of herbivory

For herbivores likely to have an impact at a scale larger than the ITEX plots (e.g., wide ranging animals such as reindeer/caribou or muskox, or for smaller mammals whose home range is larger than ITEX plots, e.g., lemmings and voles), recording herbivore presence at the site level is critical, because herbivory might be spatially variable and thus more difficult to detect in the small plant measurement plots. This assessment includes vertebrate herbivores only, as invertebrate herbivores tend to have a more localized effect and will be assessed at the plot scale. In some cases, signs of herbivore presence are not easily assigned to a certain

herbivore species, or they may only give an indication of relative abundance; nevertheless, this information is extremely valuable to approximate "herbivore pressure" at each site. Because we are interested in the effects of herbivores related to the ITEX sites, assessments at the site level will be conducted roughly in a 100m radius from the centroid of the group of ITEX plots at each site. Examples of field signs to be recorded and the field sheet to use for this part of the assessment are provided in Appendices 2 and 3.

Based on the list of potential herbivores in your site (section 1), you may need to use one or more of these methods:

Transects for pellet counts: a 100m line transect will be established within the site, trying to be representative of the plant community of the ITEX site. Ideally this transect will be permanently marked, pellets removed in each visit, and repeated in different years. Pellet removal ensures that in the next visit, only pellets deposited in the time between visits are counted and allows more accurate estimates of recent herbivore activity. In the first visit to the site (i.e. when establishing the transect for the first time), all pellets will be counted and removed; this first assessment, although not strictly comparable to subsequent ones because of pellets of unknown "ages", gives an indication of herbivore activity in the area. It is thus very important to note in your field data collection if the visit corresponds to a first survey of a transect or not. When species identification is not possible from the pellets, pellets will be assigned broadly to groups of herbivores (e.g., large mammals, small mammals, birds); whenever possible, take a picture of the 'unidentified' pellet.



Transect for pellet counts of large mammals (sheep) and birds (geese and ptarmigan) at an ITEX site in Auðkúluheiði (Iceland). The transect is set up between ITEX plots to capture herbivore activity at the ITEX site.

- For large mammals (caribou/reindeer, moose, sheep, muskox), some medium-sized mammals (marmots[9]) and birds (swans, geese, ptarmigan): the transect will be walked down slowly, recording the presence of pellets within a 2-m band (1 m to each side of the transect; using a 2m stick as a reference while walking down the transect is very helpful). Pellets frequently occur as groups; each group will be counted as one 'unit'. The distance along the transect at which each unit is found will be recorded.
- For some medium-sized mammals (hares) and small mammals (voles, lemmings, mice): because pellets of these animals are smaller, a squared sampling plot 50 x 50 cm will be used every 20 m along the transect (6 plots per transect). The number of pellets or the presence/absence of pellets will be recorded for each sampling plot. Pellets will be removed from the plots.

So, if you have herbivores from the first and second group at your site, you will need to do a combination of both methods, i.e. a 2 m band continuous transect, 100 m long, with square sampling plots every 20 m.

Other signs of herbivore activity: an area of 100 m by 100 m (1 ha) including the ITEX sites can be permanently marked for monitoring different signs of herbivore activity. The total number of signs will be recorded, and expressed as density (dividing the number of signs by the sampled surface). The time required for one person to search 1 ha thoroughly, by slowly walking parallel lines 10 m apart, is approximately 3-4 hours[10].

- Active burrows (marmots, ground squirrels, pikas, *Dicrostonyx* voles, mice): active burrows can be recognized by fresh digging and the presence of fresh faeces; old burrows often present spider webs. Count all burrows in the area, identifying species if possible, and noting if active or not.
- Latrines (marmots[9], ptarmigan, pikas)
- Hay piles (pikas)

- Winter nests (lemmings, voles): nests look like "a ball of cut grass, 12 cm in diameter"[10]. Distinguishing species is challenging and only fresh nests should be counted (old nests are usually flattened and grass is grey rather than tan-coloured[10]). Winter nests surveys are not very reliable in dense willow habitats, very rocky areas, or in tussock tundra where they are not very visible.
- Runways (*Lemmus* lemmings, *Microtus* voles): the number of active runways intersecting a transect line can be used to estimate abundances of lemmings and *Microtus* voles in wet habitats[10]. Active lemming runways have fresh pellets and/or fresh clippings of sedges or grasses. Transects can be done in exactly the same place year after year if permanent stakes are put out in wet habitats, and should be long enough to capture 10 or more runway intercepts[10].
- Grazing/browsing signs (caribou/reindeer, moose), grubbing (geese) and bark marks (hares, voles): notes on the presence of herbivory sign can be recorded while doing the pellet transect above

Because the signs of herbivore activity are likely to be long-lived, these assessments need to be conducted once in the season. Timing will depend on the type of sign being recorded; e.g. winter nests are best done shortly after snow melt (early in summer), while other signs can be assessed towards the end of the season (late July or August).

Direct observation of herbivores (all species, including invertebrates): numbers of herbivores seen within 100 m of ITEX sites should be recorded, together with a rough estimate of the time spent at the site each season (as a proxy for "survey effort"). These rates of encounter can then be used as an index of population size[11].

Information at the site level will help update the list of potential herbivores (section 1) and will give a more accurate estimation of actual herbivore activity in the area. This information will be valuable to evaluate the role of (vertebrate) herbivory across tundra sites.

3. Plot-level assessment of herbivory

The aim is to determine the intensity of herbivory in the ITEX plots (OTCs and controls), by collecting quantitative information (point frame of occurrence of herbivory and observations on individual plants) and qualitative (other observations of signs of herbivore activity). This will evaluate the local impact of herbivores at the plot level, including also invertebrate herbivores. We are mainly interested in broadly assessing the incidence of herbivory by vertebrates and invertebrates, without distinguishing species of herbivores because this might be more challenging; thus, damage on plants will be assigned only to either vertebrate or invertebrate herbivory. Because measurements of herbivory are typically cumulative, assessments of herbivory might be conducted only once in the season, preferably after the peak in biomass, and before plant senescence at the end of the season. This assessment can be done as part of your regular ITEX monitoring (see below), or as a standalone survey (point frame); if you are not planning on doing your ITEX monitoring in a given season but still want to assess herbivory, the point frame method should be used. Examples of damage caused by vertebrate and invertebrate herbivores and the field sheet to use for this part of the assessment are provided in Appendices 2 and 3.

This part of the assessment does not depend on the type of herbivores present at the site.

Point frame: the point intercept method will be used to assess the incidence of herbivory by vertebrates and invertebrates on the plant community. A quadrat, with 100 evenly distributed intercepts will be used, and *signs of herbivory* will be recorded at each intercept, indicating the plant species eaten and if damage is due to vertebrate or invertebrate herbivores. The size of the quadrat can be the same as used for vegetation analysis (usually 1x1 m or 75x75 cm) or any size down to 50x50 cm. Assessments at each intercept will include a 1 cm buffer, because herbivory (especially by invertebrates) might be very localized; by including the 1 cm buffer we maximize the chances of detecting herbivory in a standardized way. Record all distinct herbivory damages in

each intercept and distinguish between leaf and floral herbivory, and between vertebrate and invertebrate herbivory if possible. Herbivory (total, by vertebrates, by invertebrates) will be expressed as the proportion of point intercepts that showed obvious signs of herbivory. It is therefore important to note if there are any point intercepts on bare ground or on substrates where herbivory cannot be easily assessed (e.g. mosses and lichens), to subtract them from the total number of point intercepts (points without plants cannot have herbivory!). Using the point frame helps focus your attention to leaf herbivory that otherwise goes undetected; on average, each 100 intercept point frame will take around 5-8 minutes if only herbivory is recorded. This assessment can be combined with the regular ITEX vegetation monitoring.

Individual plants: when monitoring individual plants within the ITEX regular sampling schemes, herbivory can be recorded. This will provide an estimation of the incidence of herbivory on particular plant species. For each monitored plant, a visual estimation of the proportion of leaf herbivory using a scale from 0 to 4[12] (where 0 is no herbivory, 1: up to 30% of leaves eaten, 2: 30-60%, 3: 60-90%, and 4: 90-100%) will be used. For each plant, herbivory would be broadly classified as caused by vertebrates, invertebrates or both. Where possible, floral herbivory should be recorded too (as presence/absence).

Other signs of herbivore presence or activity in the plot: as in section 3, signs of herbivore activity and/or presence in the plots should be recorded. Here it is particularly important to pay attention to the presence of invertebrate herbivores (non-outbreaking), which might be overlooked when making the assessment at a larger scale. We could expect differences in herbivore use of plots with and without OTCs, for example by rodents, ptarmigans or invertebrates, due to an exclosure effect or due to the passive warming effect.

Plot-level assessment of herbivory will allow comparisons between plots (OTCs and controls) and across ITEX sites. Ultimately, this information will help in evaluating the role of herbivory as a driver of plant community responses to warming across a large number of tundra sites.

Timing and time commitment

Herbivore data could be collected at the beginning of the field season (some signs might be only detectable early in the season, e.g., lemming winter nests…), or at the end (cumulative signs of herbivory might be better assessed later in the season, before plant senescence). We expect sampling to take up to one day of work per ITEX site for two people over the whole season, preferably during the peak of the growing season. However, if you are able to do only part of the proposed activities, please do! And let us know dates. A rough estimate of time dedicated to each activity (needs to be adjusted to each site, depending on the herbivores present and the number of ITEX plots):

- Transect for pellet counts (if large and small mammals are present): 3 hr
- Point frame (for herbivory only): 10 min per plot; with 10 OTCs and control pairs (20 plots): 3.3 hr

Collected data

Data collected using the proposed (see appendix) or your own field sheets can be entered following the data entry procedure described in the appendix, or scanned copies of the field sheets can be sent to us (herbivory.network@gmail.com) at the end of the field season.

Materials

- 50 x 50 cm point frame – for pellet counts of smaller vertebrate herbivores (section 2) and for assessing herbivory at the plot level (section 3).
- 2 50 m tape measures – for establishing the transect (section 2).
- 2 m stick (or any other reference) to estimate the 2 m strip along the transect (section 2)
- wooden stakes (and marker) – to permanently mark the beginning and end of the 100 m transect (section 2).
- Plastic bag – for removing pellets from the transect and pellet plots (section 2). Pellets do not need to be kept, but have to be removed from the surroundings of the transect.

References

1. Mulder, C. P. H. Vertebrate herbivores and plants in the Arctic and subarctic: effects on individuals, populations, communities and ecosystems. *Perspect. Plant Ecol. Evol. Syst.* **2,** 29–55 (1999).
2. Jefferies, R. L., Klein, D. R. & Shaver, G. R. Vertebrate herbivores and northern plant communities: reciprocal influences and responses. *Oikos* **71,** 193–206 (1994).
3. Post, E. Erosion of community diversity and stability by herbivore removal under warming. *Proc. R. Soc. London B* **280,** 20122722 (2013).
4. Post, E. & Pedersen, C. Opposing plant community responses to warming with and without herbivores. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 12353–8 (2008).
5. Kaarlejärvi, E., Eskelinen, A. & Olofsson, J. Herbivory prevents positive responses of lowland plants to warmer and more fertile conditions at high altitudes. *Funct. Ecol.* **27,** 1244–1253 (2013).
6. Olofsson, J. *et al.* Herbivores inhibit climate-driven shrub expansion on the tundra. *Glob. Chang. Biol.* **15,** 2681–2693 (2009).
7. Speed, J. D. M., Austrheim, G., Hester, A. J. & Mysterud, A. Elevational advance of alpine plant communities is buffered by herbivory. *J. Veg. Sci.* **23,** 617–625 (2012).
8. Van der Wal, R., Bardgett, R. D., Harrison, K. A. & Stien, A. Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography (Cop.).* **27,** 242–252 (2004).
9. Karels, T. J., Koppel, L. & Hik, D. S. Fecal pellet counts as a technique for monitoring an alpine-dwelling social rodent, the hoary marmot (Marmota caligata). *Arctic, Antarct. Alp. Res.* **36,** 490–494 (2004).
10. Krebs, C. J., Reid, D. G., Kenney, A. J., Morris, D. W. & Gilbert, B. S. *Small mammal population monitoring. ArcticWOLVES sampling protocols*. (2008). at <http://www.cen.ulaval.ca/arcticwolves/files/protocols/small_mammal_abundance.pdf>
11. Hochachka, W. M., Martin, K., Doyle, F. & Krebs, C. J. Monitoring vertebrate populations using observational data. *Can. J. Zool.* **78,** 521–529 (2000).
12. MacLean, S. F. & Jensen, T. S. Food plant selection by insect herbivores in Alaskan arctic tundra: the role of plant life form. *Oikos* **44,** 211–221 (1985).

If you have any questions, please ask us! It is important that sampling is done in a consistent way accross sites. We will check email as regularly as possible, at herbivory.network@gmail.com

Appendices (not included in this draft; please contact us at herbivory.network@gmail.com)

- **Appendix 1.** Template for site description
- **Appendix 2.** Pictures of signs to be recorded in the field.
- **Appendix 3.** Example of data sheet