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Red fox carcass collection and dissection

Motivation

Red foxes are boreal generalist meso-predators expanding into and increasing in abundance in the low Arctic. They are a superior competitor to the endangered arctic fox, and they can exclude from productive denning areas as well as from resource subsidies such as carcasses in winter. Red foxes can even act as predator of arctic foxes. Increasing red fox populations have also a detrimental effect on many species of ground-nesting birds such as ptarmigan or waders. The most important driver of red fox expansion in the low arctic tundra are increased resource availability resulting from human activity. Ungulate carcasses, notably of semi-domestic reindeer, can play an important role in winter. Warmer winters, increased productivity resulting from warmer summers and the virtual absence of apex predators can also have a positive effect. As all predators in tundra ecosystems with cyclic small rodent populations, red foxes benefit from the small rodent resource pulses, which affect their reproduction, survival and migration. Red foxes are also hosts for various parasites. Sarcoptic mange (*Sarcoptes scabiei*) is a mite, which causes skin disease, and can be transferred to arctic foxes.

To mitigate the expansion of this meso-predator, red fox culling programs have been implemented in many places, but their success is variable. On Varanger peninsula, red fox culling has been carried out since 2005 as an experimental management action to conserve arctic foxes. Foxes are culled by the Norwegian Nature Surveillance (Statens Naturoppsyn, SNO) and local hunters are encouraged to hunt foxes and receive a payment for carcasses delivered to the project.

State variables: This protocol describes data collection for four state variables contributing to the monitoring target red fox (BT5) of the arctic fox module: red fox demography Varanger (V81), red fox diet Varanger (V82), red fox mange prevalence Varanger (V83) and red fox diseases and endoparasites (V84).

Reference to method: Devenish-Nelson et al. (2013) present a review of red fox demography data. Stomach content analyses have been used for instance by Killengreen et al. (2011) and prevalence of parasites and pathogens has been addressed by Mørk et al. (2019) and Tryland et al. (2018).

Spatial study design

All carcasses of red foxes shot on Varanger Peninsula are collected and examined. For this purpose, Varanger Peninsula is defined as the area delimited by the coast, Tana River in the west and the road E75 between Skiipagurra and Varangerbotn in the southwest.

Regular hunters hunt mostly close to the coast and close to the settlements. In addition, specific culling is carried out by the Norwegian Nature Surveillance (Statens Naturoppsyn SNO) in the core area for arctic foxes in the interior of the peninsula.

Temporal study design

Red foxes are culled by regular hunters during the hunting season between 15 July and 15 April every year. Most foxes are shot between November and April. The Norwegian nature surveillance culls red foxes mostly in late winter between February and April.

Procedure:

Culling and carcass registration

Hunters sign a contract with the project. Registered hunters send an sms within 6 hours after a fox is shot informing about the date, time and the location of the cull. Carcasses from hunters are collected and registered by the Norwegian nature surveillance at their offices in Vadsø and in Tana. For each fox, the following information is recorded:

- Date of culling
- Name of the hunter
- Municipality, where the fox was culled
- Location: Hunters are asked to report GPS coordinates of culling locations. Some report place names. For these coordinates are retrieved from www.norgeskart.no. If hunters only reported the municipality, a coordinate close to the coast in this municipality is chosen. The source of the coordinates is recorded as a separate variable.

After registration, the carcasses are stored frozen until further processing in the lab.

Until 2012, some foxes killed on the road were also received and recorded. For these, road_kill is recorded instead of the hunter.

Carcass examination and dissection in the lab

The red fox carcasses are examined according to two different protocols: a *simplified examination* and *full dissection*. Foxes culled by SNO, as well as a selection of foxes shot by following their tracks, independent of a bait, are subjected to full dissection.

Simplified examination

The examination should be carried out in a dissection room where the floor and the walls can be washed, and where there is good ventilation. The frozen carcasses are thawed over 2-3 days depending on the temperature in the room.

Each fox is attributed an ID number (these are continuous numbers which are unique in the project) and weighed to the nearest gram. The fox is sexed and the teeth are inspected to determine an approximate age. Sharp teeth and incisors, which still have mamelons (small bumps along the edge), indicate that the fox is a young of the year. With increasing age, the teeth wear off. Older foxes can lack teeth and have less sharp canines, premolars and molars. The fur of the fox is examined for mange. Mange often starts around the joints of the legs. If mange is suspected, but cannot be determined with certitude, a sample is taken for analysis by a veterinarian and stored in a small plastic box labelled with “red fox, mange” and the ID number of the fox.

For female foxes the reproductive status is determined by taking out the uterus and inspecting it. The uterus can be in four different stages: *thin* – meaning that the fox is not reproducing; *thickened and straight* – meaning either that the fox is in heat or has recently given birth; *thickened and curly* – corresponding to early pregnancy; pregnant with visible embryos. Some vixen have a thickened uterus also outside of the breeding season and there may be other reasons for the uterus to be thickened, such as disease. The uterus should be opened with scissors and inspected for placental scars. Count the number of scars. If the fox is pregnant count the number of embryos and measure the length of three embryo sacs. If the embryos are large enough to be taken out, weigh three of them. Length of the embryo sacs is recoded in mm and the weight in g

From each fox, the following samples are collected and stored in zip lock bags. The zip lock bags are labelled in advance.

- Fur from the base of the tail
- Four muscle samples of ca 3 x 3 x 1 cm are taken from the thigh muscle. One of them is packed in aluminum foil before storing it in the bag.
- The upper jaw should be sawed off well above the canines. The root of one canine will be used to age the fox based on counts of cementum annuli.

All samples are stored frozen.

Equipment needed:

- Scalpel
- Lab scissors
- Saw
- Zip lock bags for sample storage
- Aluminum foil for sample storage
- Scale to weigh the foxes
- Lab clothes including rubber boots and plastic apron
- Disposable gloves
- Pencil
- Preprinted registration form
- Small plastic boxes for sample storage
- Bin for discarding organic material

Information recorded in the lab:

- Estimated age
- Sex
- Weight (g)
- Mange. In case of manage record whether it is generalized or local, whether a sample has been taken and whether the fox has crusts, parts without fur or pigmented skin.
- State of the uterus: thin, thickened and straight, thickened and curly or pregnant.
- Number of placental scars (0 if none)
- Number of embryos
- Length of three of the embryos (total embryo sac) in mm
- Weight of three embryos (if the embryos are large enough) in g

Full dissection

In addition to the procedures described for *simplified examination* above, the following examinations and sample collection are carried out: The length of the fox is measured from the tip of the nose to the base of the tail. The fox needs to be straightened out and the measurement is taken three times with a thick thread. The thread is taken over the head down to the neck and from there back to the start of the tail.

After external examination, the skin of the fox is removed. Body condition is assessed by looking at the amount of fat present at the surface of the body. The fat score is estimated on a scale from 0 to 5 where 5 is fat, 4 is quite fat, 3 is normal, 2 is below normal, 1 is skinny and 0 is starving. Foxes with

score 4 or 5 should have a layer of fat on the lower back, in the middle of the back, which can be measured. The thickness of this layer is recorded in mm. For males, the testes are weighed.

Additional samples to take:

- Large muscle samples (one whole thigh muscle, which is used for determination of radioactive contamination)
- 5 – 9 ml of blood is sampled from the abdominal cavity with a plastic pipette.
- One rib bone
- Stomach
- Fecal sample of ca 3 g from the rectum or posterior colon
- One kidney (packed in aluminum foil in the plastic bag)
- Liver sample (ca 3 x 3 x 1 cm; packed in aluminum foil in the plastic bag)
- Brain sample (ca 2 x 2 x 2 cm; packed in aluminum foil in the plastic bag)
- The lower jaw in addition to the upper jaw.

All samples are stored frozen, except the blood samples.

Equipment needed (in addition to the equipment listed for *simplified examination*):

- Pipette to take blood
- Vial to store blood

Information recorded in the lab (in addition to the equipment listed for *simplified examination*):

- Body length (cm; three measurements)
- Fat score (0 to 5)
- Thickness of fat layer on the lower back (mm)
- Weight of testes

Stomach content analysis

Stomach dissection is carried out in a wet lab with good ventilations. The frozen stomachs are thawed for 1-2 hours. The plastic bags with stomachs can be put in cold water to thaw more rapidly.

The whole stomach is weighed to the nearest gram. Then the stomach is opened with scissors and the content is placed in a sieve. The skin is washed out (remains should be kept in the sieve) and weighed again. The content is sorted by dietary items. Small and well mixed items can be easier to sort when washing. The dietary items are identified optically and compared to a reference collection. If possible, bird remains are attributed to either ptarmigan, sea birds or other birds. Lemmings can be identified based on fur color. Otherwise, small rodents can be identified based on their teeth (under a microscope). Fur can also be examined under the microscope, notably to distinguish between arctic fox and hare. There are also useful internet sites with reference pictures of hair structure, for example http://www.microlabgallery.com/hair.aspx

Equipment needed:

- Sieve
- Sink
- Scissors
- Tweezers
- Plastic tray

- Scale weighing to a precision 0.1 g
- Registration form
- Petri dishes for storing samples to examine further
- Microscope
- Bin for discarding organic material

Information recorded in the lab:

- Weight of the full stomach
- Weight of the skin of the stomach
- Estimated volume percent of each dietary component
- Wet weight of each dietary component

Age determination based on cementum annuli

The age is determined for all foxes dissected according to the full dissection protocol (ie foxes shot by the Norwegian nature surveillance, as well as a selection of foxes shot by following their tracks, independent of a bait). To survey the age structure of the population, we aim at aging ca 60 foxes from Varanger peninsula and 40 foxes from Iesjavri. Thus, in addition to the fully dissected foxes, foxes shot between December and April are chosen at random from the remaining material. Because the age structure of females is particularly important to understand the demography of the population, these foxes are selected with a sex ratio of 2/3 females. Age determination is carried out at NINA by Frode Homstrøm.

Lab prodedure:

In order to extract a canine tooth to determine the age, the upper jaw is boiled for 1-2 hours. The tooth is taken out when it can be extracted smoothly, without damaging the root. The root is cut 15-20 mm above the tip. Subsequently, the cut off root tip is decalcified in 5% nitric acid. Depending on the thickness of the tooth, decalcification lasts for 1-3 days. After decalcification, the tooth is rinsed in water. Then the tooth is cut longitudinally into thin slices using a cryostat. Thickness varies between 1 and 50 my.

The tooth slices are dyed using Hematoxilin. The solution is prepared as follows using only deionized or distilled water. In 1 L of water, dissolve

1) 0.2 g sodium iodate (Merck.nr.: 1.06525.0100)
2) 50 g Aluminium potassium sulfate dodecahydrate (Merck.nr.: 1.01047.1000)
3) 1 g Hematoxylin cryst (Merck.nr.: 1.04302,0025)

Each component is dissolved in fully before adding the next one, in the order they are given. After everything is dissolved, the solution transferred to a bottle and should be stored in the dark.

The tooth slices are dyed by keeping them in the solution for 45 min at 20-25°C. After dying, the tooth slices are rinsed in water. The solutions can be used several times. If solid particles appear in it, it should however be discarded.

Finally the tooth slices are fixed between a slide and a cover slip using Kaiser's glycerol gelatin. Then the cementum annuli can be counted under a microscope. The quality of the age estimates is assured by comparison with animals of known age.

Assigning age based on cementum annuli:

Young foxes have an open pulpa. In this case, the tooth is not sliced and age is recorded as age 0, meaning young of the years. The pulpa usually closes at ca 9 month. Foxes with closed pulpa and no incremental lines shot in winter are also in their first year of live and thus of age 0. The age of the foxes with incremental lines corresponds to the number of lines + 1. To set a cutoff in the data, we assume that the line in the teeth appears on 1. November (Grue and Jensen 1979).

Fecal sample processing

The fecal samples are analysed individually at the Veterinary Institute for the presence of gastrointestinal parasites by counting helminth eggs. A modified McMaster technique is performed with a sensitivity of 40 eggs per gram of faeces, using a saturated NaCl and glucose flotation solution (specific gravity 1,23). Egg count per gram faeces is recorded as a measure of parasite abundance of three important parasite species in individual foxes: *Toxascaris leonina*, T*oxocara canis* and *Uncinaria stenocephala*.

Blood sample processing

Blood samples have been used previously for serological studies (e.g. Tryland et al. 2018) and for studies of environmental pollutants. For the moment, they are stored frozen for possible future analyses.

Data processing

In the lab, data are recorded in preprinted registration forms (Appendix)

Data from carcass examination and dissection are recorded in the file "template_redfox_carcass_examination.xlsx"

Data from stomach content analysis are recorded in the file "template_redfox_stomach_content.xlsx"

Data from fecal sample analysis are recorded in the file

"template_redfox_fecal_samples.xlsx"

Training requirements and specialized skills

People working in the lab need to be instructed by someone who knows the procedure. The aging based on counting cementum annuli and the parasite detection require specialized skills and are carried out in specialized labs at the Norwegian Institute for Nature Research (NINA) and the Veterinary Institute respectively.

References

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Appendix:

Registration forms for *simplified examination*, *full dissection* and *stomach content analysis*.

Attach the label of the fox here

Number in the project

Registration of simplified red fox carcass examination

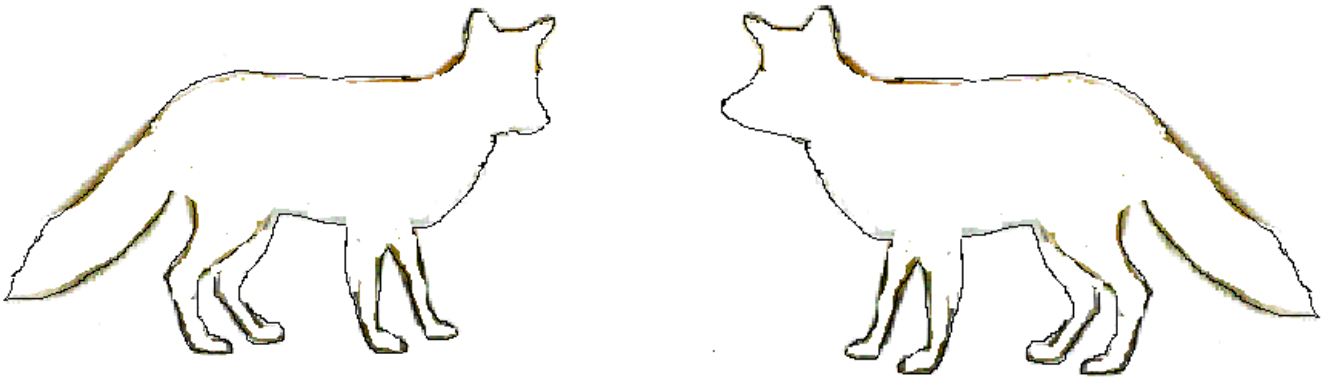
1. External examination

Estimated age (tooth wear):_____ Sex (M/F):____

Other information: _____

Mange: ____ No
____ Generalized ____ Local
Skin condition: _____ Crusts _____ Naked parts _____ Pigmentation
_____ Sample taken

External description and comments regarding ectoparasites (mange):
If mange is suspected, but not sure, take a skin sample for analysis by a veterinarian.



2. Samples

Upper jaw ☐

Muscle x 4 (from tigh, one of them packed in aluminum foil) ☐

Fur ☐

For females, the uterus is removed and opened to check for placental scars

_____ pregnant: _____ embryos. Length of the embryos: 1. _____2. _____ 3. _____

Weight of the embryos: 1. _____2. _____ 3. _____

_____ thin uterus _____ uterus thickened and straight _____ uterus thickened and curly

_____ number of placental scars (write 0 for none to record that it was checked)

Comments:

Date: Examined by:

Attach the label of the fox here

Number in the project

Registration of full red fox carcass dissection

2. External examination

Estimated age (tooth wear):_____ Sex (M/F):____

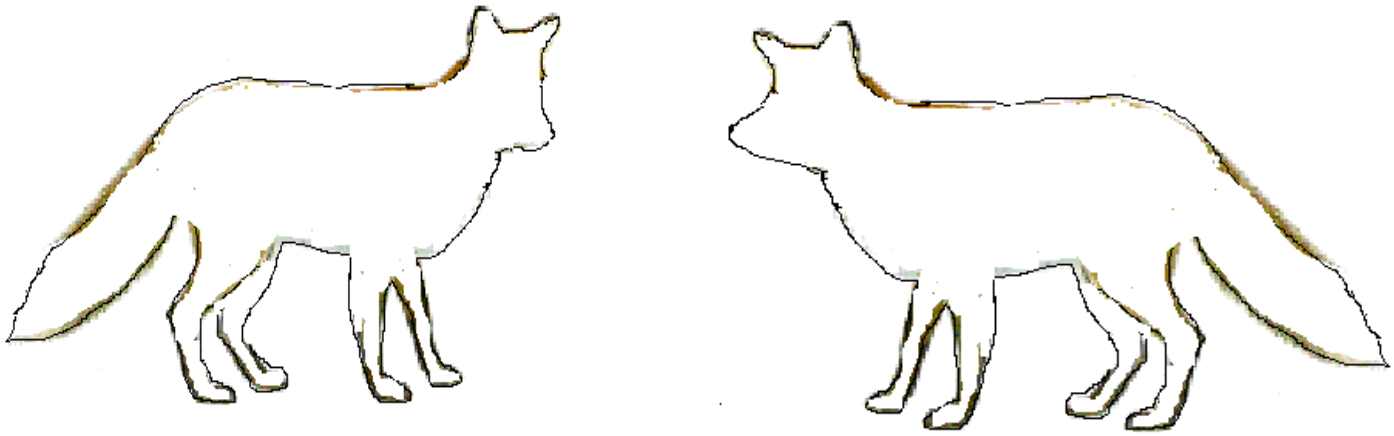
Other information: _____

Mange: ____ No
____ Generalized ____ Local
Skin condition: _____ Crusts _____ Naked parts _____ Pigmentation
_____ Sample taken

Body length from the tip of the nose to the base of the tail (3 measurements):

_____ _____ _____ (cm)

External description and comments regarding ectoparasites (mange):
If mange is suspected, but not sure, take a skin sample for analysis by a veterinarian.



3. Remove the fur of the fox

Fat index (0-5): _____ Thickness of the fat on the back (index 4-5): _____ (mm)

(5 = fat, 4 = quite fat, 3 = normal, 2 = below normal, 1 = skinny, 0 = starving)

Testicle weight: 1. _____ 2. _____ (g)

4. External samples

Muscle x 4, from thigh (3 in zip lock sampling bags, one additionally in aluminum foil) ☐

Large muscle sample, in zip lock sampling bag ☐

Fur, in zip lock sampling bag ☐

Rib bone, in zip lock sampling bag ☐

4. Dissection of abdominal cavity

For females, the uterus is removed and opened to check for placental scars

_____ pregnant: _____ embryos. Length of the embryos: 1. _____2. _____ 3. _____

Weight of the embryos: 1. _____2. _____ 3. _____

_____ thin uterus _____ uterus thickened and straight _____ uterus thickened and curly

_____ number of placental scars (write 0 for none to record that it was checked)

Blood sample from the abdominal cavity Torill ☐

Stomach with content, in zip lock sampling bag ☐ (*not for foxes from Iesjavri / SNO Alta*)

Feces, ca 3 g, in a small plastic box ☐

Liver, packed in aluminum foil and in a zip lock sampling bag ☐

Kidney, packed in aluminum foil and in a zip lock sampling bag ☐

5. Head

Brain sample, packed in aluminum foil and in a zip lock sampling bag ☐

Teeth x 2: saw off the snout of the fox ☐

How rotten was the fox: _____ fresh _____ moderately _____ considerably

Comments:

Date: Dissected by:

Fox ID _____Date: _____ Name: _____

	Percent	Weight	Comment
Whole stomach			
Stomach skin			

Empty stomach			
Reindeer			
Marine bird			
Ptarmigan			
Other/unknown bird			
Lemming			
Vole (unidentified)			
Tundra vole			
Grey-sided vole			
Unidentified small rodent			
Plants			
Human waste (describe)			
Fish			
Unidentified (describe)			