UiT

THE ARCTIC UNIVERSITY OF NORWAY

FORBIO AND UIO/IBV COURSE: BIOINFORMATICS FOR ENVIRONMENTAL DNA SEQUENCING

Dietary DNA analyses

Galina Gusarova
The Arctic University
Museum of Norway, UiT

04.05.2021- on-line

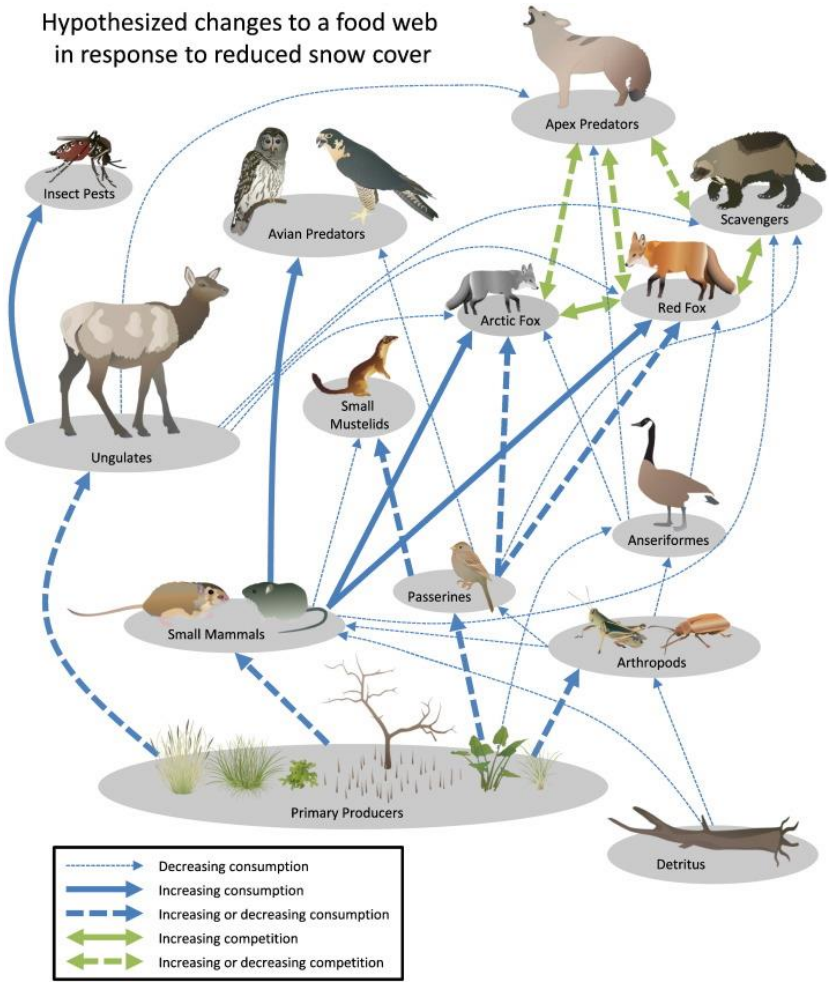
Dietary DNA analyses:
do not ignore that gut feeling

Why to study diet?

- important ecological traits, such as

dietary habits, food webs, and trophic niches;

- Ecosystem functionality;
- Feeding resources;
- Preditor-prey interactions;
- Biodiversity patterns;
- Tool for biodiversity assessments;
- Tool for nature management and conservation.



Penczykowski et al. 2017 Food Webs, Vol.13 80-91

Biodiversity patterns from biological samplers

- Prey DNA can be used to study biodiversity

frontiers in Marine Science

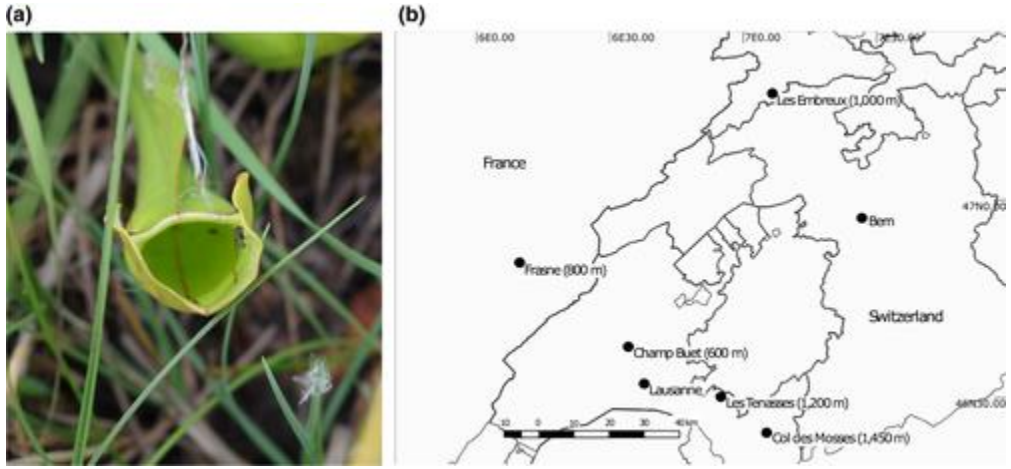
ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fmars.2020.605148

More Than Expected From Old Sponge Samples: A Natural Sampler DNA Metabarcoding Assessment of Marine Fish Diversity in Nha Trang Bay (Vietnam)

Marta Turon[1], Carlos Angulo-Preckler[1], Adrià Antich[2], Kim Præbel[1] and Owen S. Wangensteen[1]**

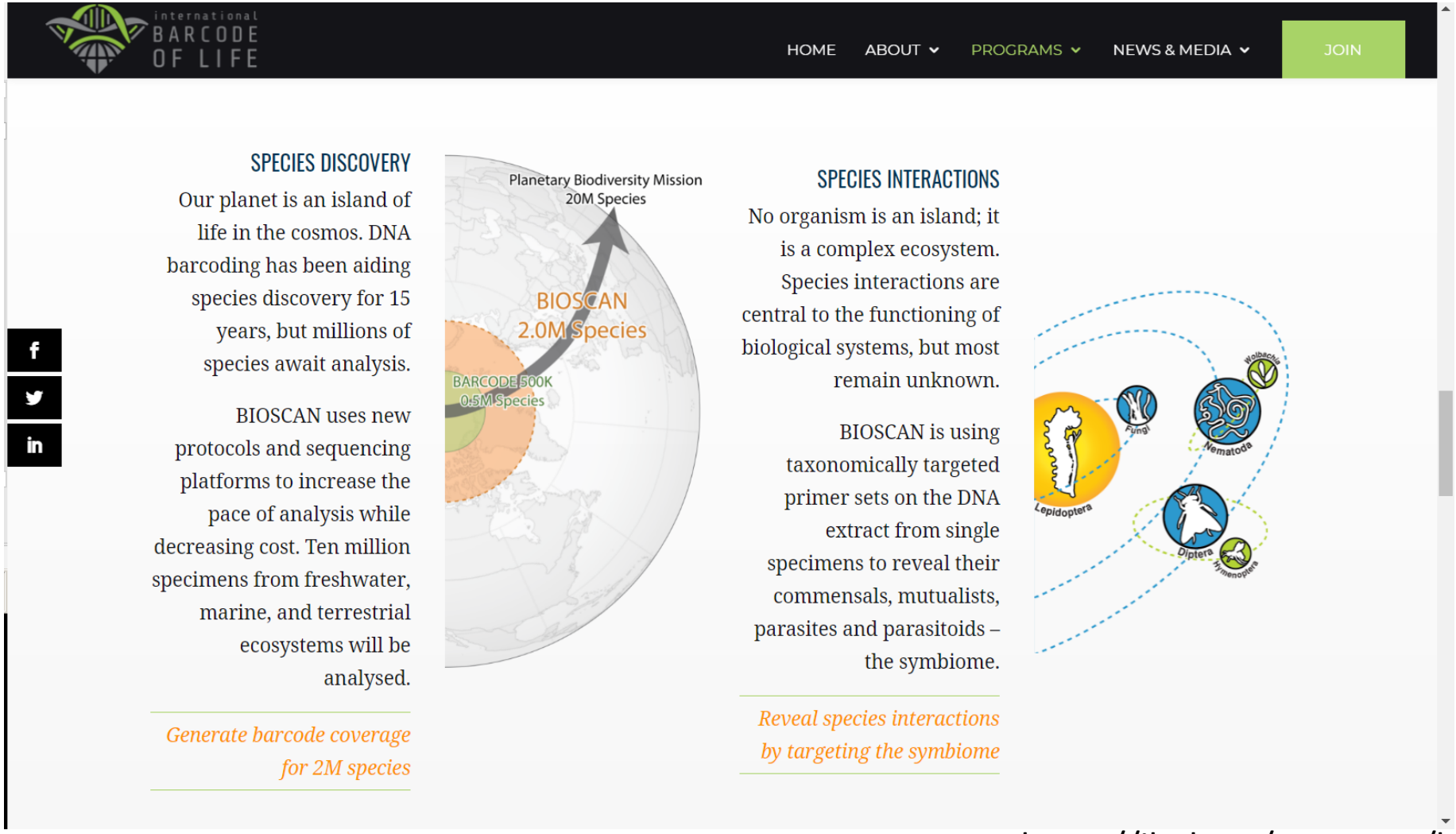
OPEN ACCESS

(a) *Sarracenia purpurea* with possible invertebrate prey at the lowest elevation (600 m) (photo credit: Axel Zander). (b) of the sampling sites in the Swiss Alps and France



Littlefair, JE, Zander, A, de Sena Costa, C, Clare, EL. DNA metabarcoding reveals changes in the contents of carnivorous plants along an elevation gradient. Mol Ecol. 2019; 28: 281–292. https://doi.org/10.1111/mec.14832

BIOSCAN's three research themes employ DNA barcodes to speed species discovery, to probe species interactions, and to track species dynamics



https://ibol.org/programs/bioscan/

Methods used to characterize diet

- Direct observations;
- Macro- or microhistology of stomach, gut or fecal content;
- Stable isotopes
- NIRS – near-infrared reflectance spectroscopy (nutritional content: total N, C etc)
- Dietary DNA


https://necsi.edu/predator-prey-relationships


Photo by J. Forbey


https://urbancoyoteresearch.com/research/stable-isotope-analysis-diet


https://www.mdpi.com/2073-4395/9/9/503/htm

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

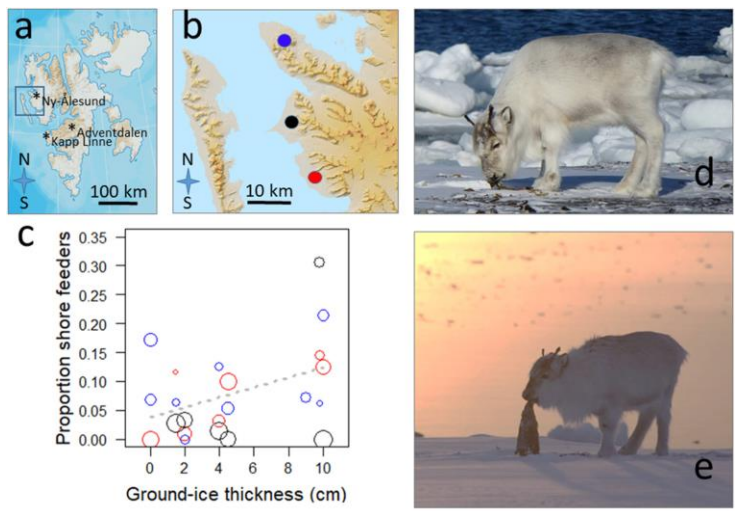
Article | Open Access

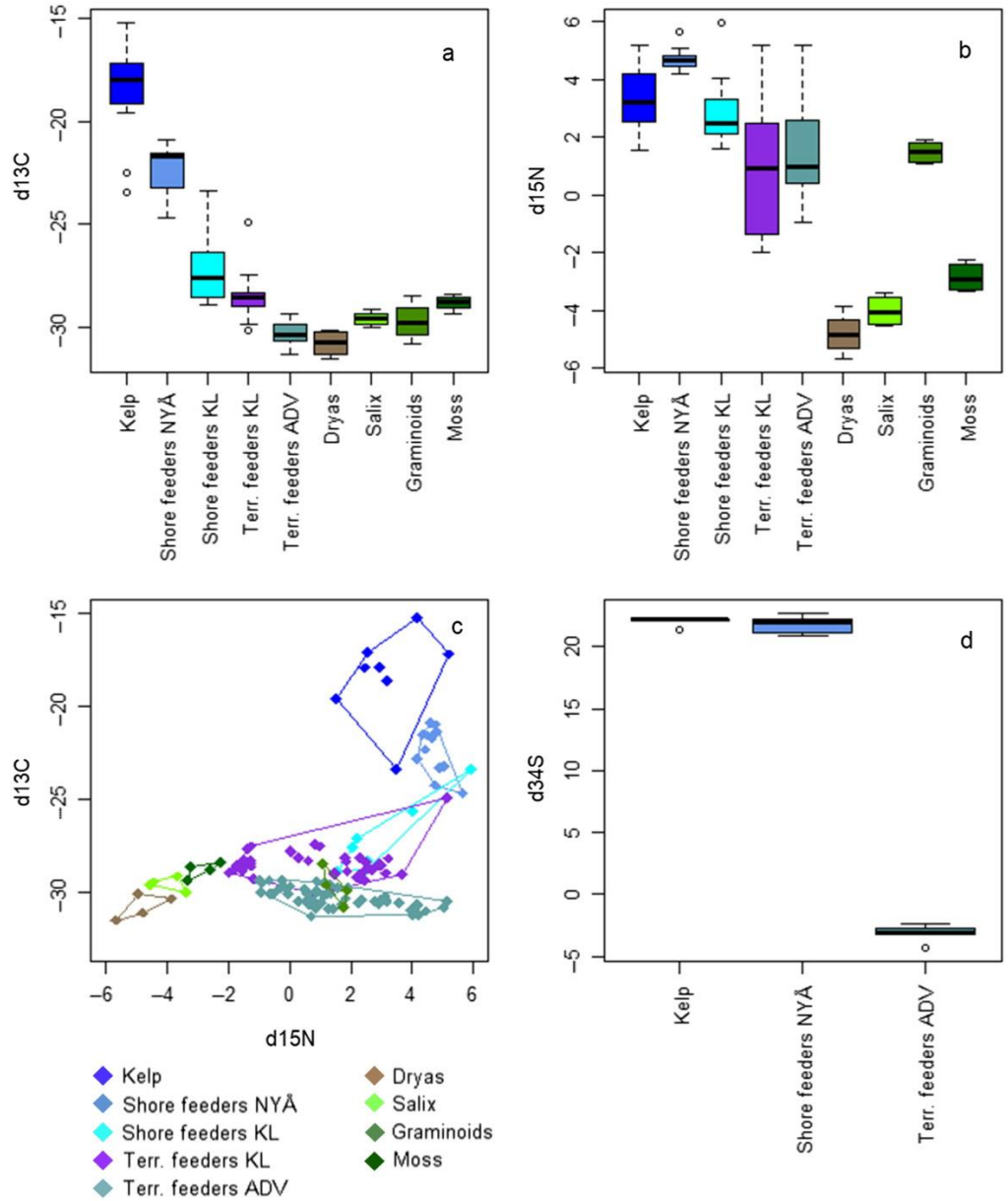
Reindeer turning maritime: Ice-locked tundra triggers changes in dietary niche utilization

Brage Bremset Hansen, Jon Runar Lorentzen, Jeffrey M. Welker, Øystein Varpe, Ronny Aanes, Larissa Teresa Beumer, Åshild Ønvik Pedersen

First published: 23 April 2019 | https://doi.org/10.1002/ecs2.2672 | Citations: 4

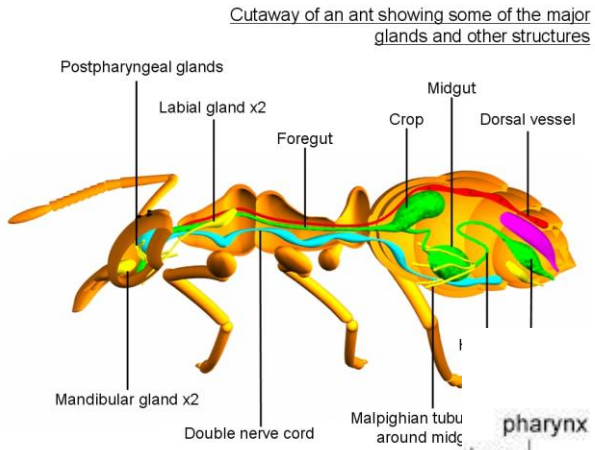
Corresponding Editor: James W. Cain.

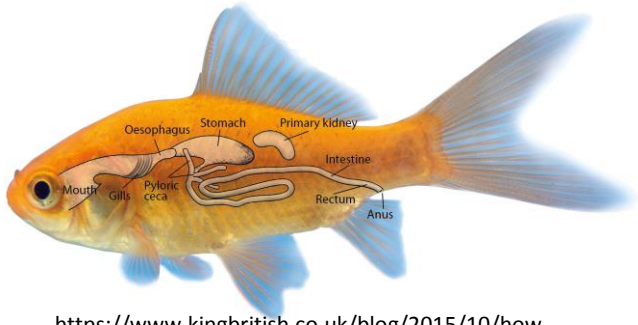




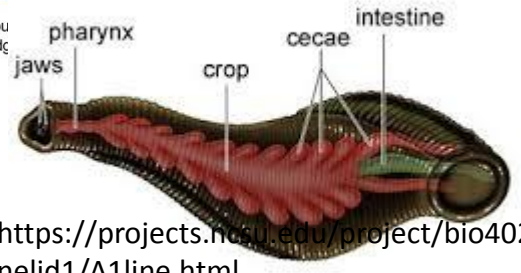
Dietary DNA

-environmental DNA approaches that use DNA extracted from gut, stomach, or fecal contents.

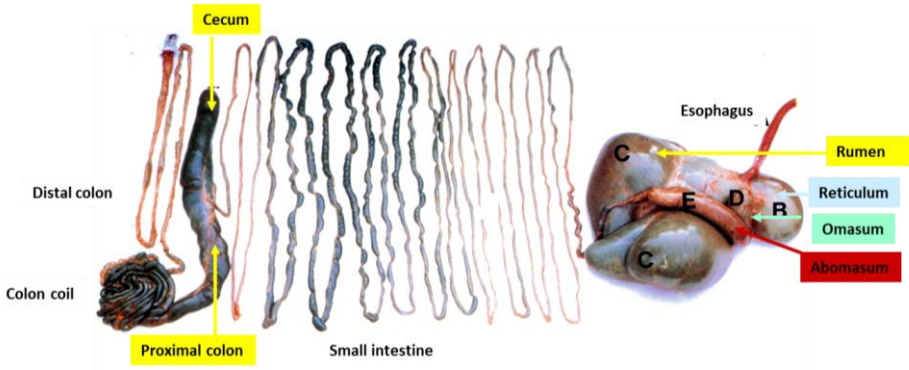




https://www.kingbritish.co.uk/blog/2015/10/how-does-a-fish-digestive-system-work



https://projects.ncsu.edu/project/bio402_315/Annelid1/A1line.html

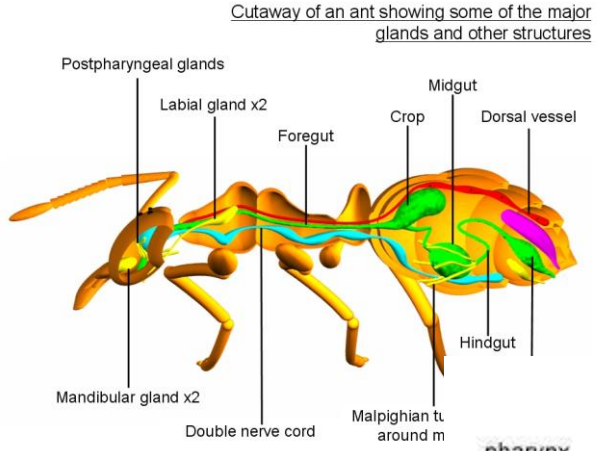


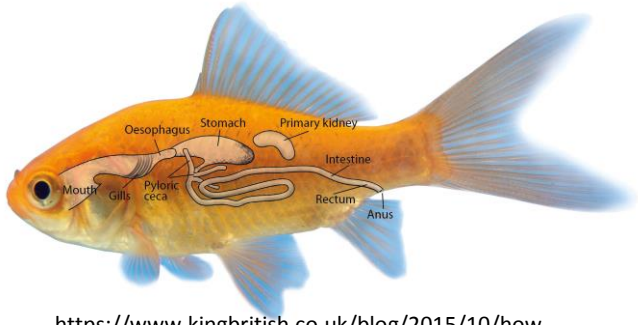
By Monica Sundset

Dietary DNA

-We sample from:

- Gastrointestinal tract (GI) - is a series of hollow organs joined in a long, twisting tube from the mouth to the anus;
- Animal by-products;
- The whole body.





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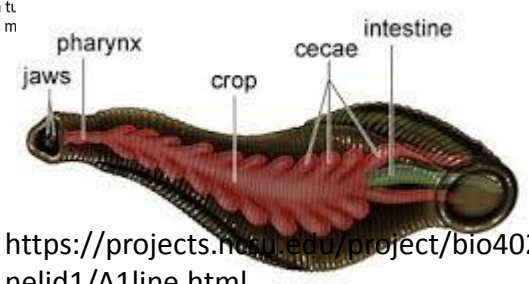

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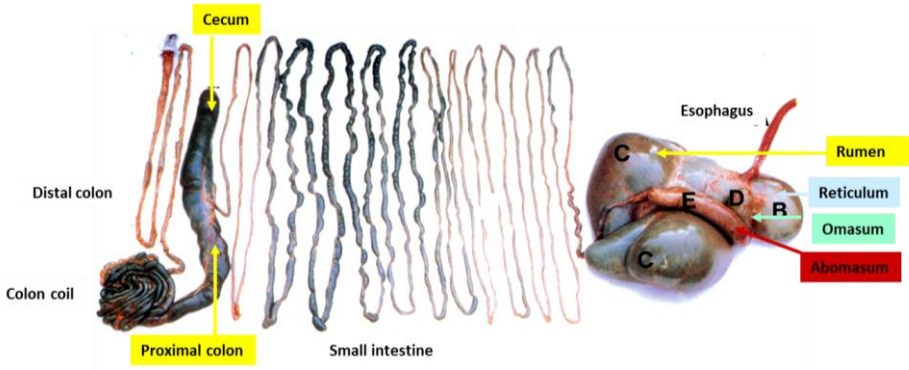
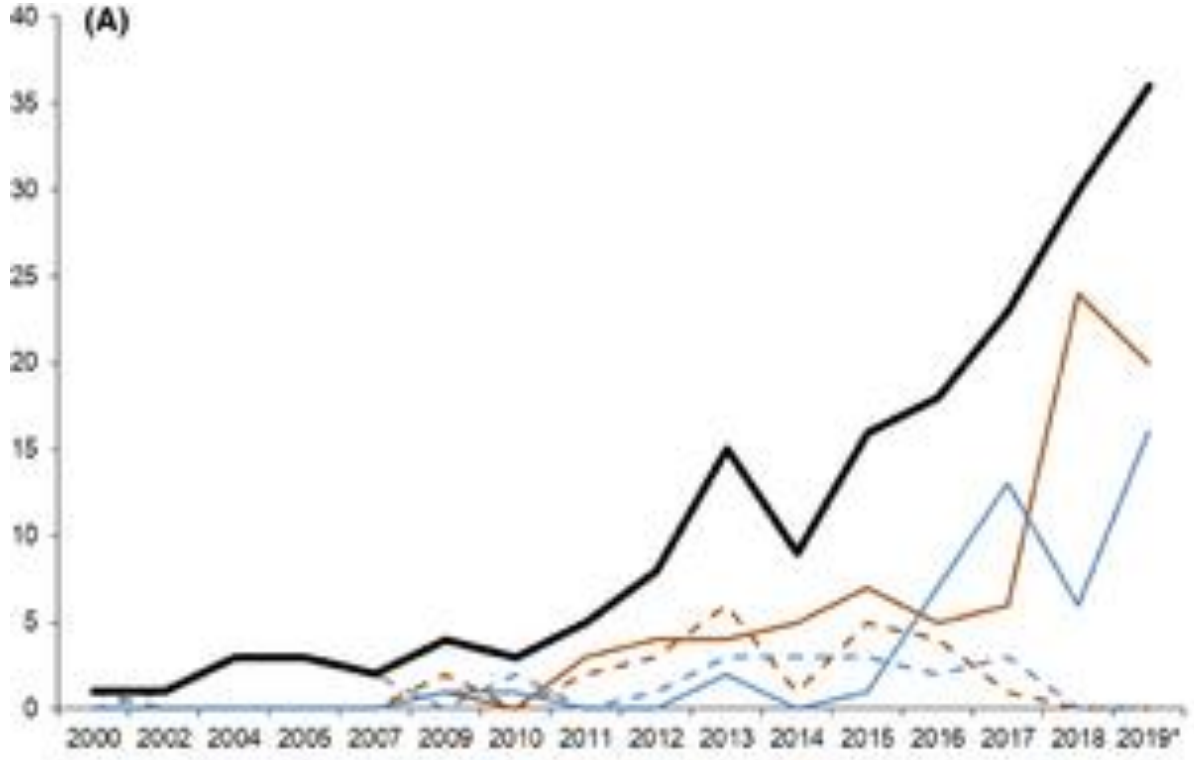
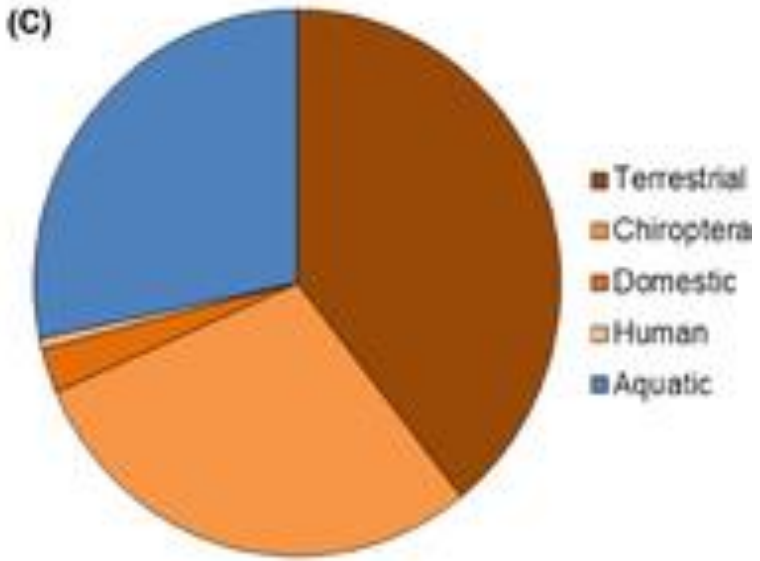


By Monica Sundset





Received: 5 June 2019 | Revised: 10 July 2019 | Accepted: 12 July 2019

DOI: 10.1002/edn3.27

REVIEWS AND SYNTHESIS

Environmental DNA

WILEY

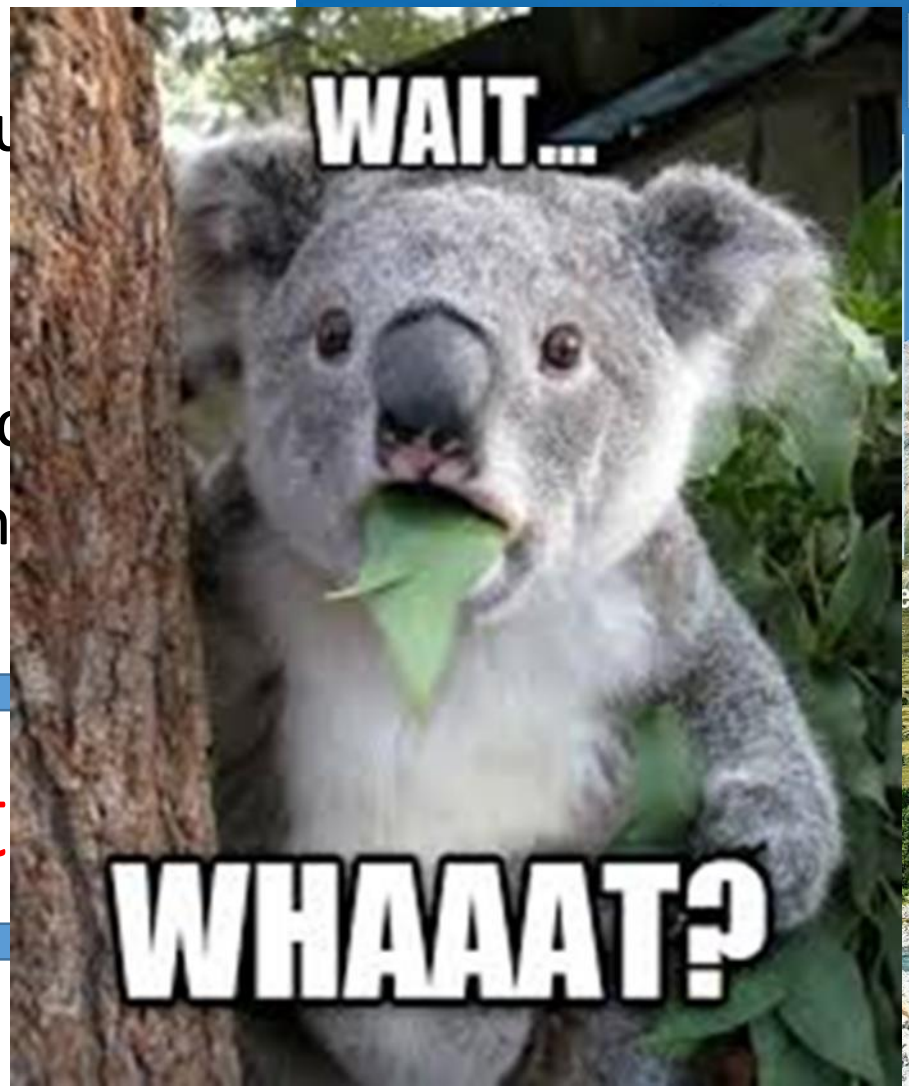
DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems

Lara Loureiro de Sousa[1] | Sofia Marques Silva[2] | Raquel Xavier[2]

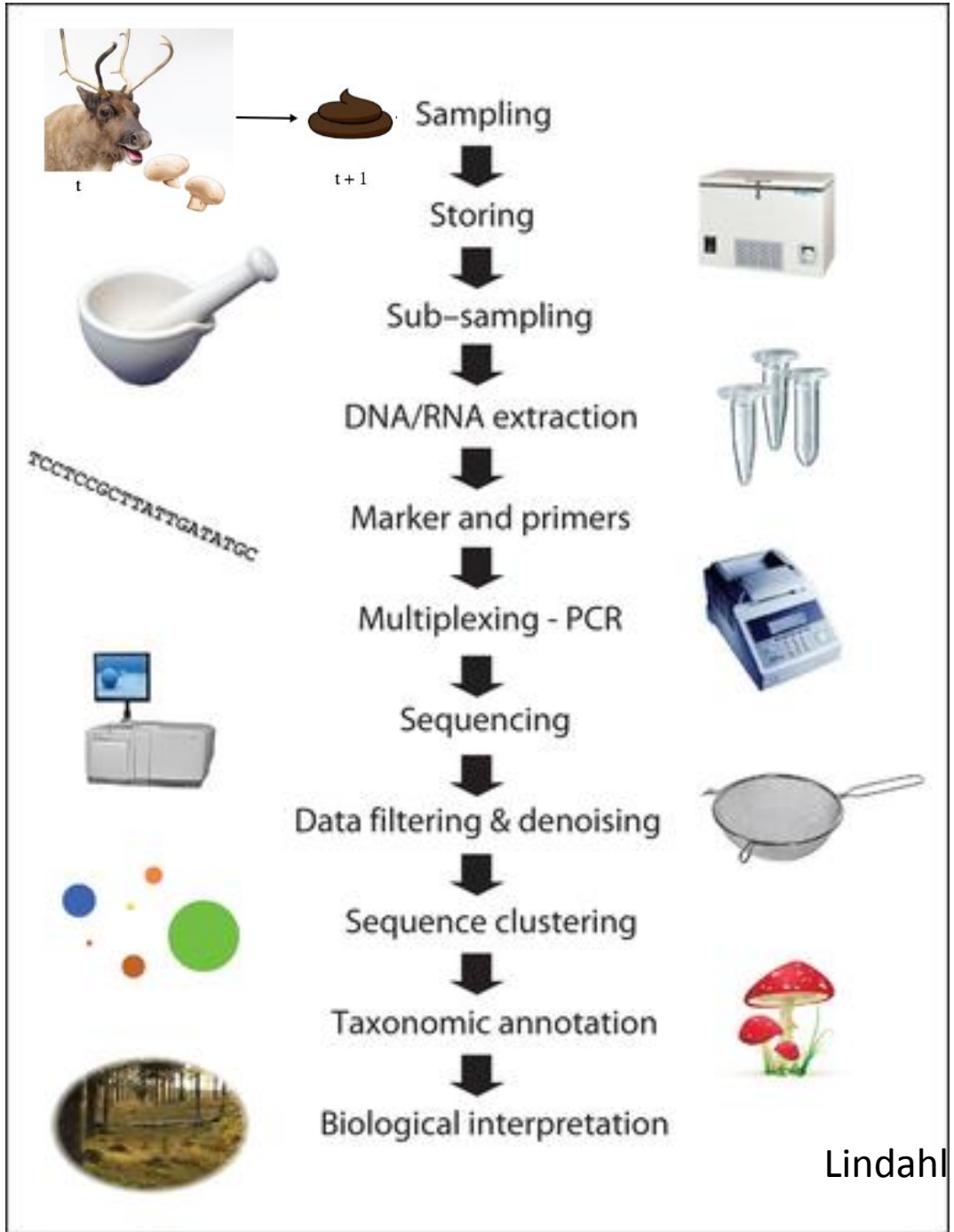
Dietary DNA analyses

- Appear among the first eDNA stu
- Sampling is straightforward
- dDNA is usually concentrated
- Easy to extract from stomach/fec
- Taxonomic diversity not too high

Easy and robust met



Rangifer tarandus,
Agaricus bisporus



Lindahl et al. 2013, modified

1. *Rangifer tarandus*,
2. *Agaricus bisporus*

18S

1. Fam. Hominidae
2. *Amanita muscaria,*
3. *Psilocybe semilanceata*

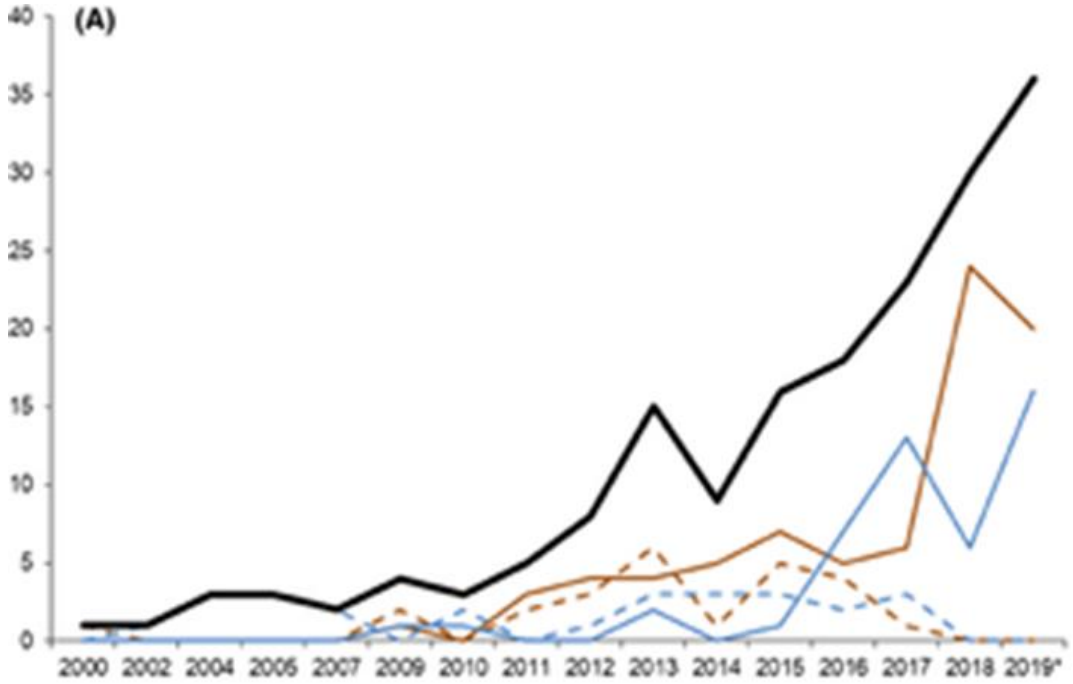
Lindahl et al. 2013, modified after
http://bardalgol.com/mushroom-troll-by-bard-algol/

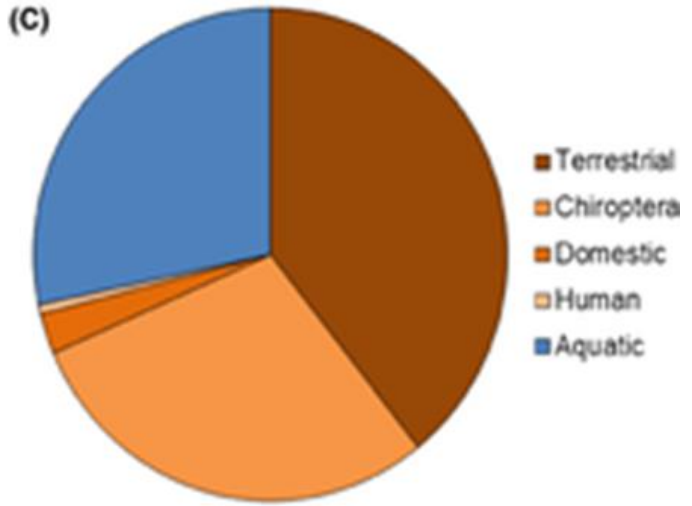
Dietary DNA: project set-up checklist

1. Type of environment for the target group: terrestrial, aquatic; contemporary or paleo- systems;
2. Sources of dDNA: whole body, GI tract, feces
3. Types of diet: herbivore, carnivore, omnivore
4. Relationship between the amount and type of dietary items and the inferred MOTUs an their sequence read numvers.

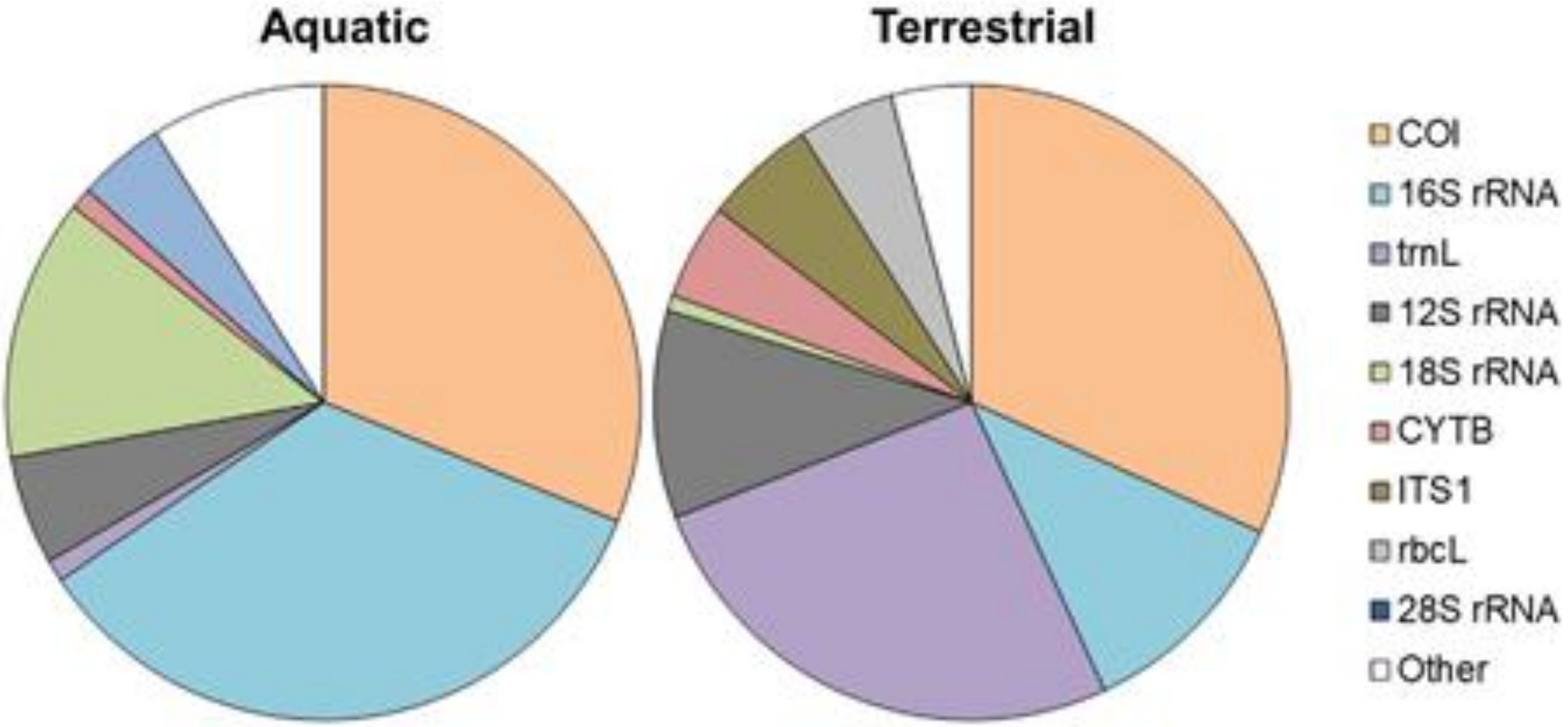
Aquatic environments – not always that simple…

- Impossible/not easy:
  - Collection pellets or feces
  - Direct observation of feeding interactions





Based on 150 studies analyzed, focusing on more than 250 vertebrate wild species, seven domesticated taxa, and humans.

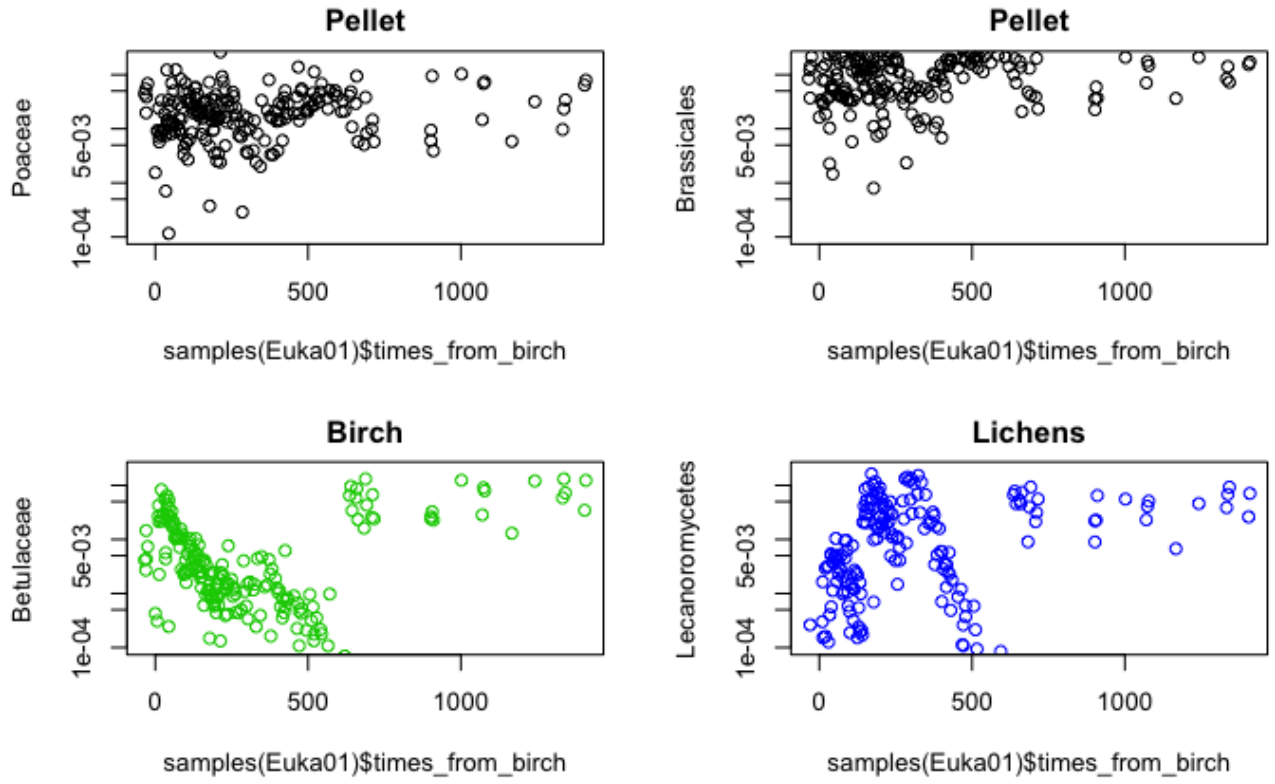


Environmental DNA, Volume: 1, Issue: 3, Pages: 199-214, First published: 08 August 2019, DOI: (10.1002/edn3.27)

Dietary DNA: project set-up checklist

1. Type of environment for the target group: terrestrial, aquatic
2. Sources of dDNA: GI tract, feces, whole body
3. Types of diet: herbivore, carnivore, omnivore

18S primers: DNA decay for lichens (14h) is two times more rapid compared to birch DNA (30h). 18S is a longer marker than GH and shorter than ITS



https://hungryhen.wordpress.com/2015/11/26/last-sunrise-in-tromso/

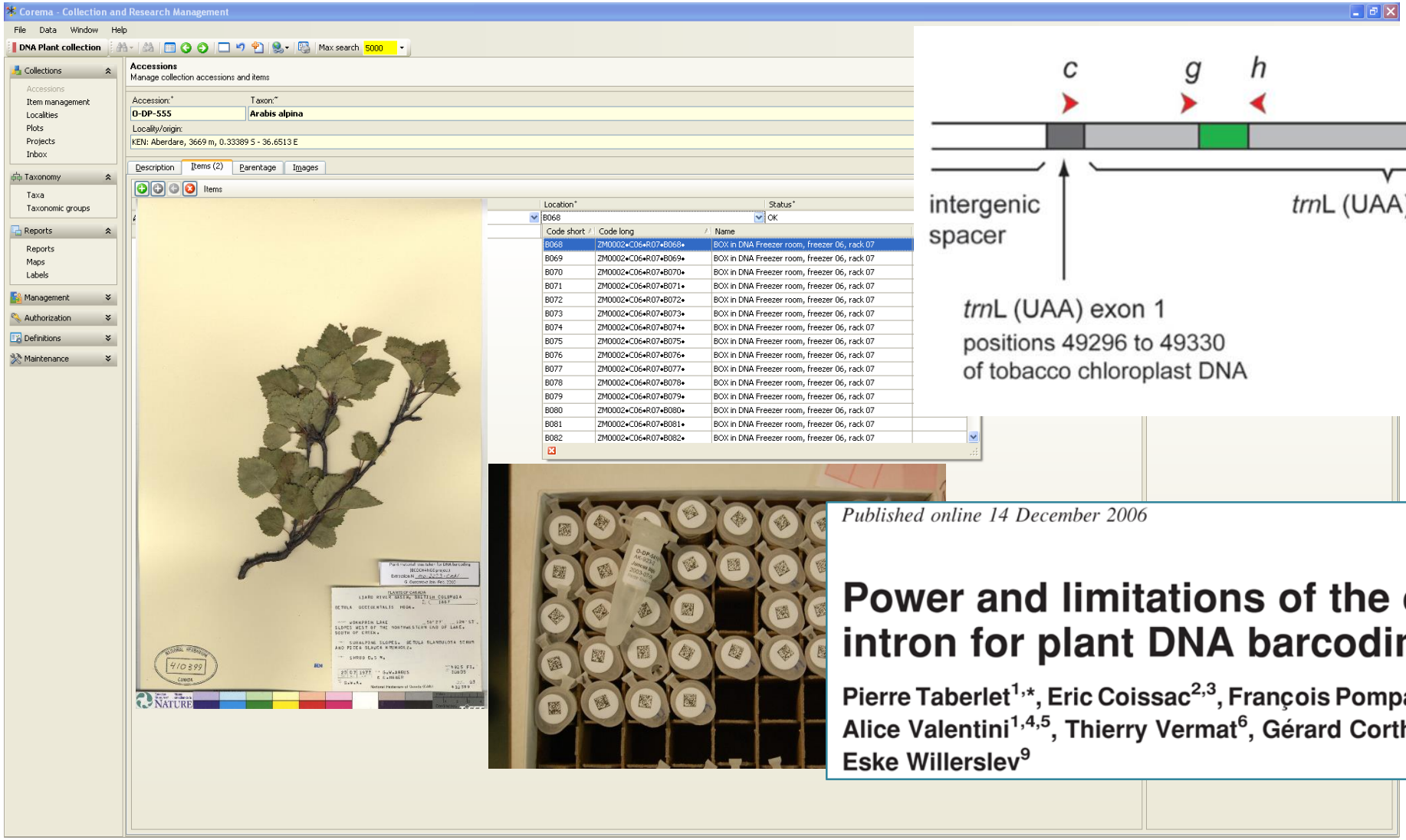
Dietary DNA: project set-up checklist

Read up DNA metabarcoding methodology & ecology literature:

- Target species ecology and diet;
- Trophic interactions;
- Seasonal or geographic fluctuations in diet due to resource availability.

Remember that potential biases can be introduced on every step of analyses from sampling to bioinformatics.

DNA Reference Library

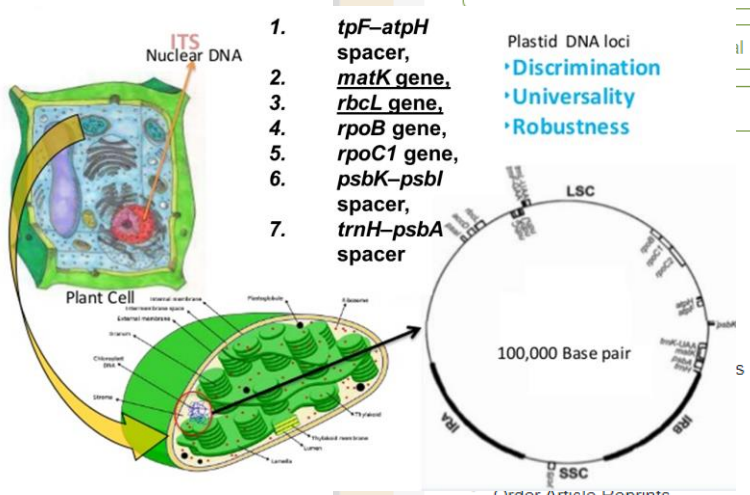


Published online 14 December 2006 *Nucleic Acids Research, 2007, Vol. 35, No. 3* ***e14***
doi:10.1093/nar/gkl938

Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding

Pierre Taberlet[1,*], Eric Coissac[2,3], François Pompanon[1], Ludovic Gielly[1], Christian Miquel[1], Alice Valentini[1,4,5], Thierry Vermat[6], Gérard Corthier[7], Christian Brochmann[8] and Eske Willerslev[9]

Genome skims of 2051 herbarium specimens from Norway/Polar regions compared with 4604 freshly collected, silica gel dried specimens mainly from the European Alps and the Carpathians



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The Treasure Vault Can be Opened: Large-Scale Genome Skimming Works Well Using Herbarium and Silica Gel Dried Material

by Inger Greve Alsos [1,*], Sebastien Lavergne [2], Marie Kristine Føreid Merkel [1], Marti Boleda [2], Youri Lammers [1], Adriana Alberti [3], Charles Pouchon [2], France Denoeud [3], Iva Pitelkova [1], Mihai Puşcaş [4], Cristina Roquet [2,5], Bogdan-Iuliu Hurdu [6], Wilfried Thuiller [2], Niklaus E. Zimmermann [7], Peter M. Hollingsworth [8] and Eric Coissac [2,*]

1 Tromsø Museum, UiT—The Arctic University of Norway, N-9037 Tromsø, Norway
2 LECA, Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, F-38000 Grenoble, France
3 Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France
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(This article belongs to the Special Issue Plant DNA Barcode)

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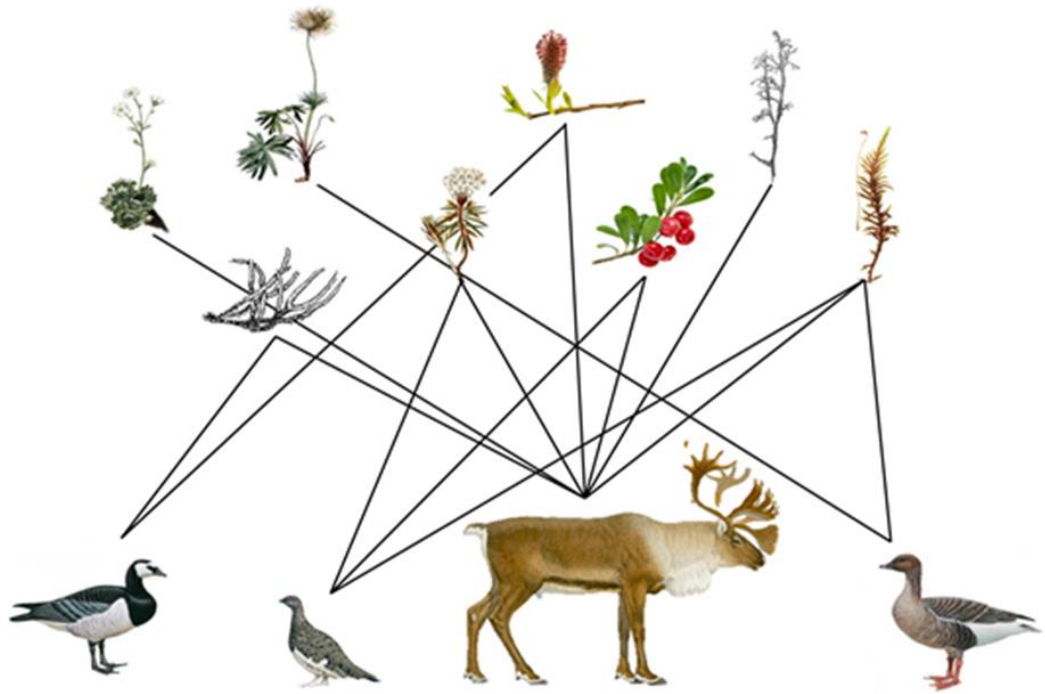
The Research Council of Norway

The REININ project

- Interspecific interactions
- Resource availability



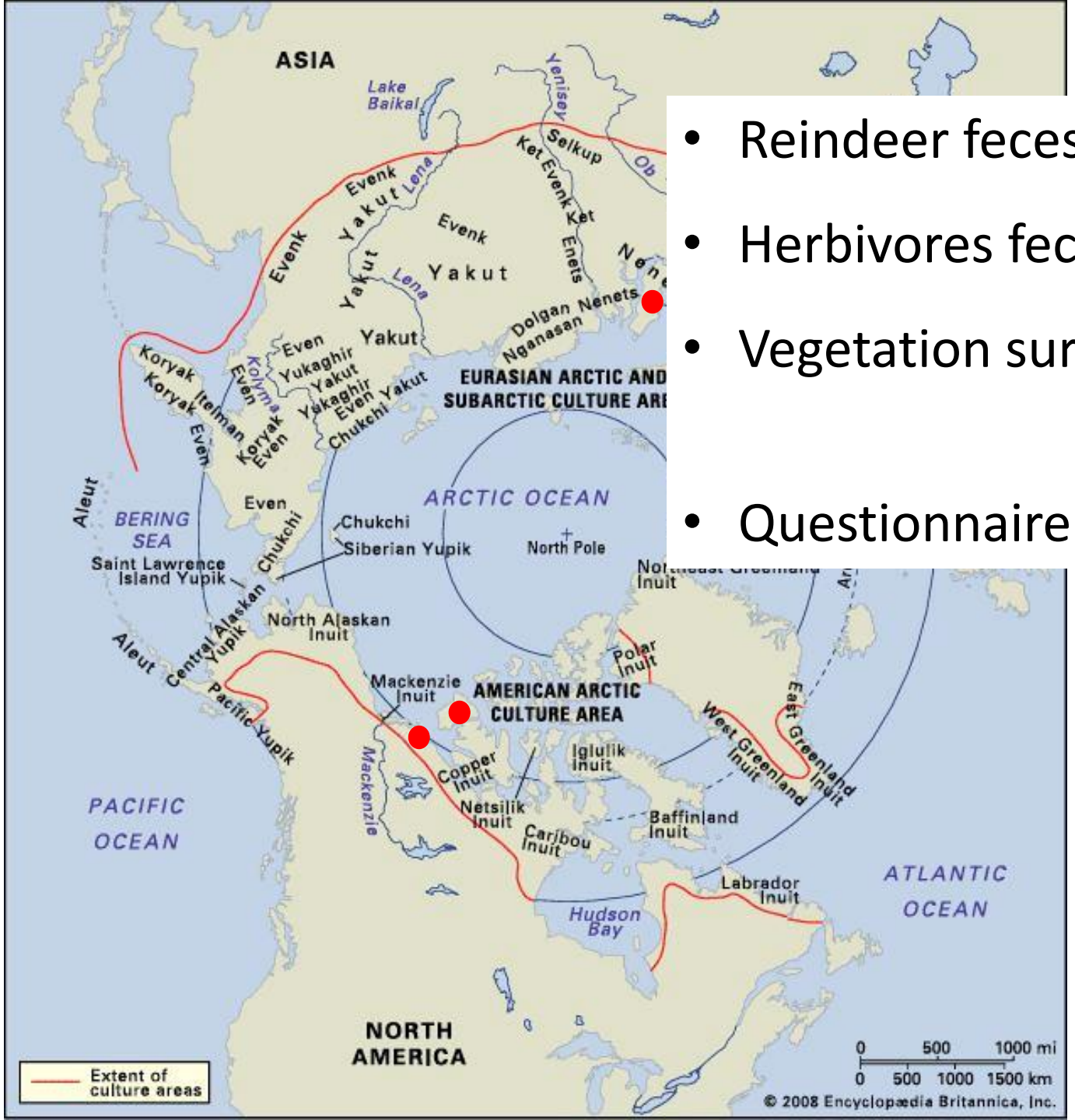
- Human exploitation
- Local knowledge

Yamal, Russia

Finnmark,
Norway

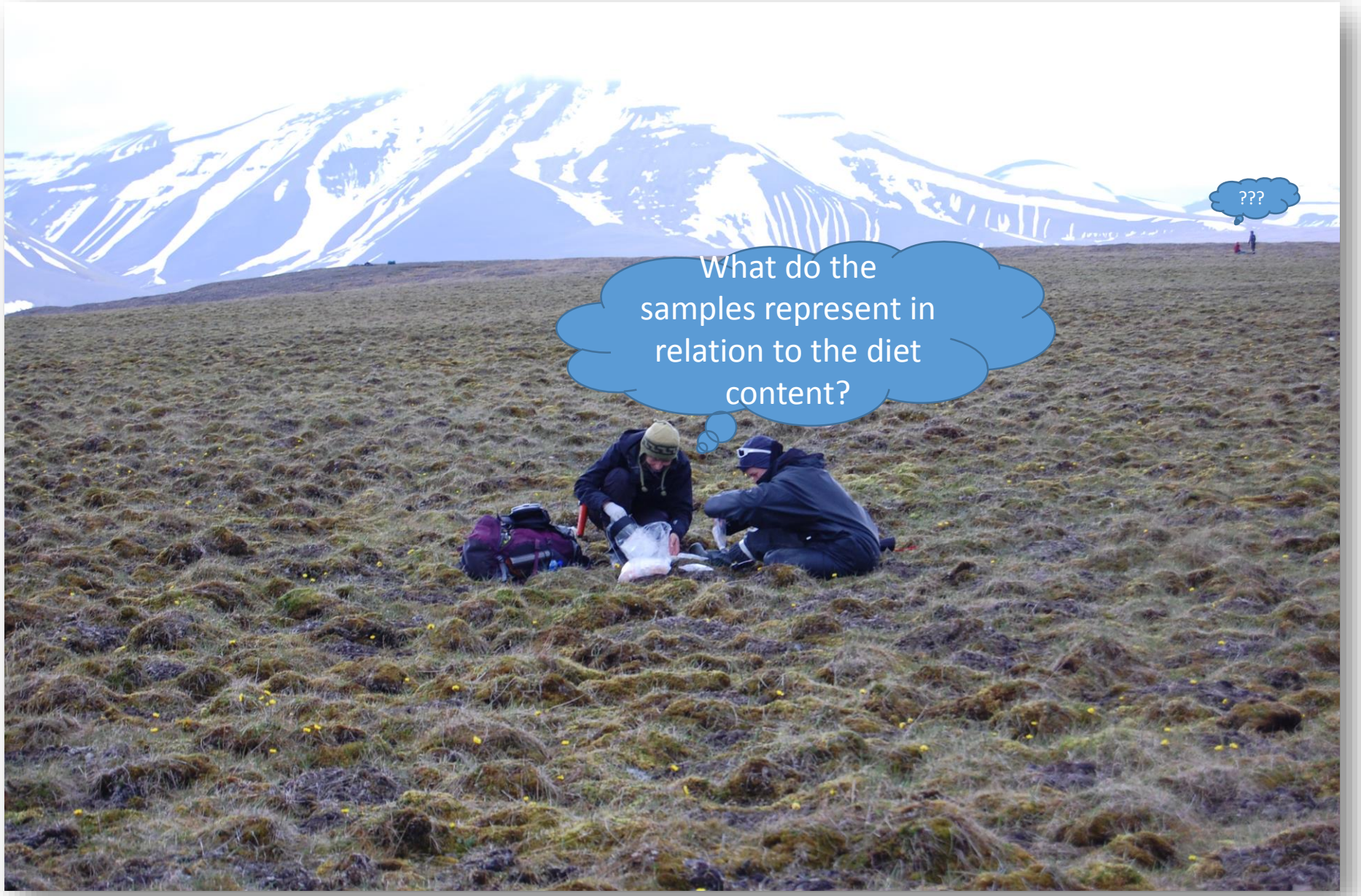
Svalbard

NWT, Canada



- Reindeer feces (monthly)
- Herbivores feces
- Vegetation surveys

- Questionnaire & focus groups


What do the samples represent in relation to the diet content?
???

dDNA detection in feeding experiments - Relationship between the amount and type of dietary items and the inferred MOTUs an their sequence read numbers.

- January-March 2018
- Method calibration
  - Gastrointestinal DNA transit time
  - Relative biomass
  - Species detection accuracy

Pernille Meyer

UNIVERSITETET I TROMSØ

UiT / NORGES ARKTISKE UNIVERSITET

DNA metabarcoding reveals long retention times and predictable decay dynamics of dietary DNA in reindeer

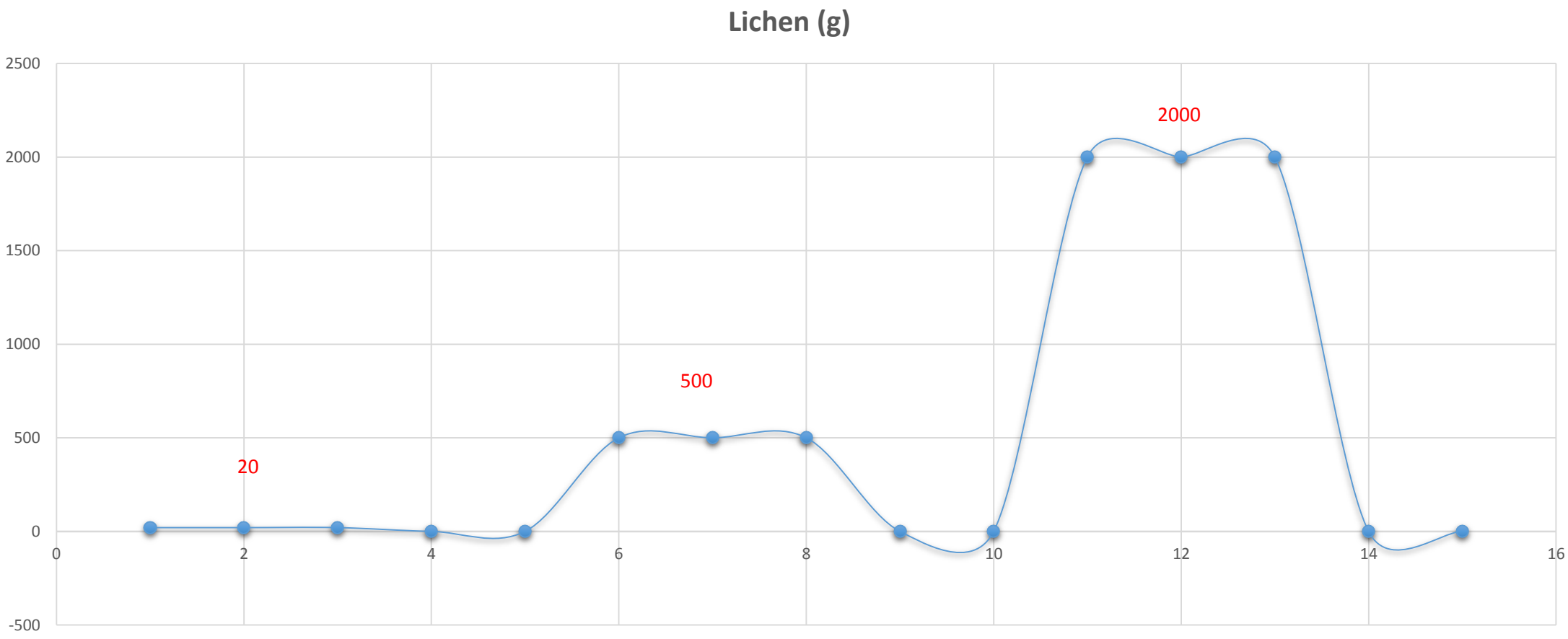
Running title: DNA metabarcoding diet analysis in reindeer

Pernille Meyer[1], Éric Coissac[2], Anne Krag Brysting[1], Oskar Rennstam Rubbmark[3], Leo Rescia[4], Lars Folkow[4], Monica Alterskjær Sundset[4], Galina Gusarova[1, 5], Stefaniya Kamenova[1]

- Increasing amount mixed lichen diet (mainly *Cladonia stellaris*)

How well is relative food biomass represented by output sequences?

Amounts of Lichen (g) indroduced during 14 days of feeding experiment



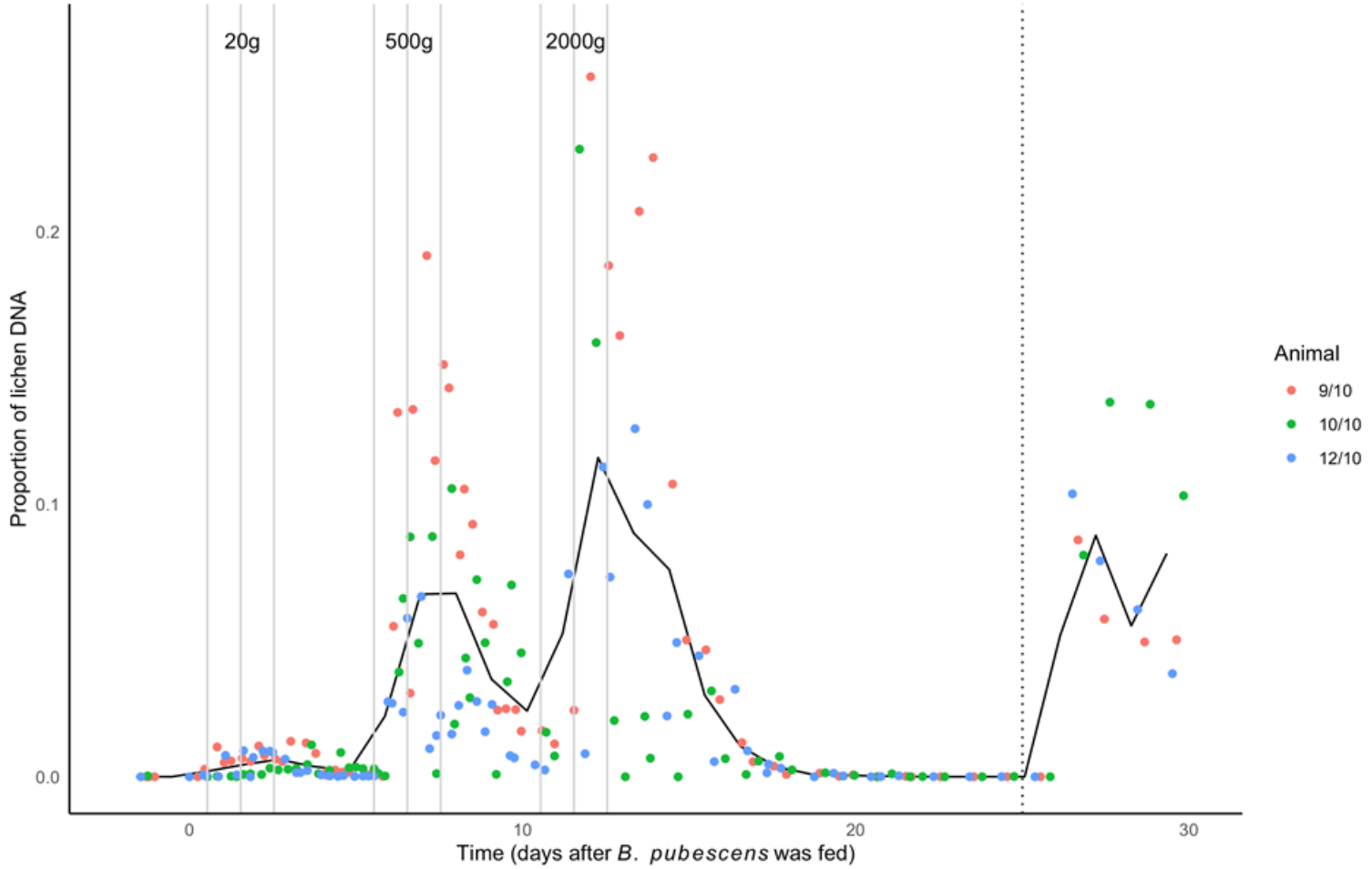
Laboratory set up

- AmpliTaq Gold 360 PCR Master Mix
- PCR reaction (with P6 loop trnL and 18S primers) was replicated three times
- with 1-2 negative controls per batch extraction & PCR
- For GH. Positive controls: the reads count is always proportional input amount of DNA (tested with 16 “artificial” plant species in a mix)
- sequencing libraries using a TruSeq PCR-free protocol (Illumina, USA) and completed 150bp pair-end sequencing with Illumina HiSeq 4000

Metabarcoding primers

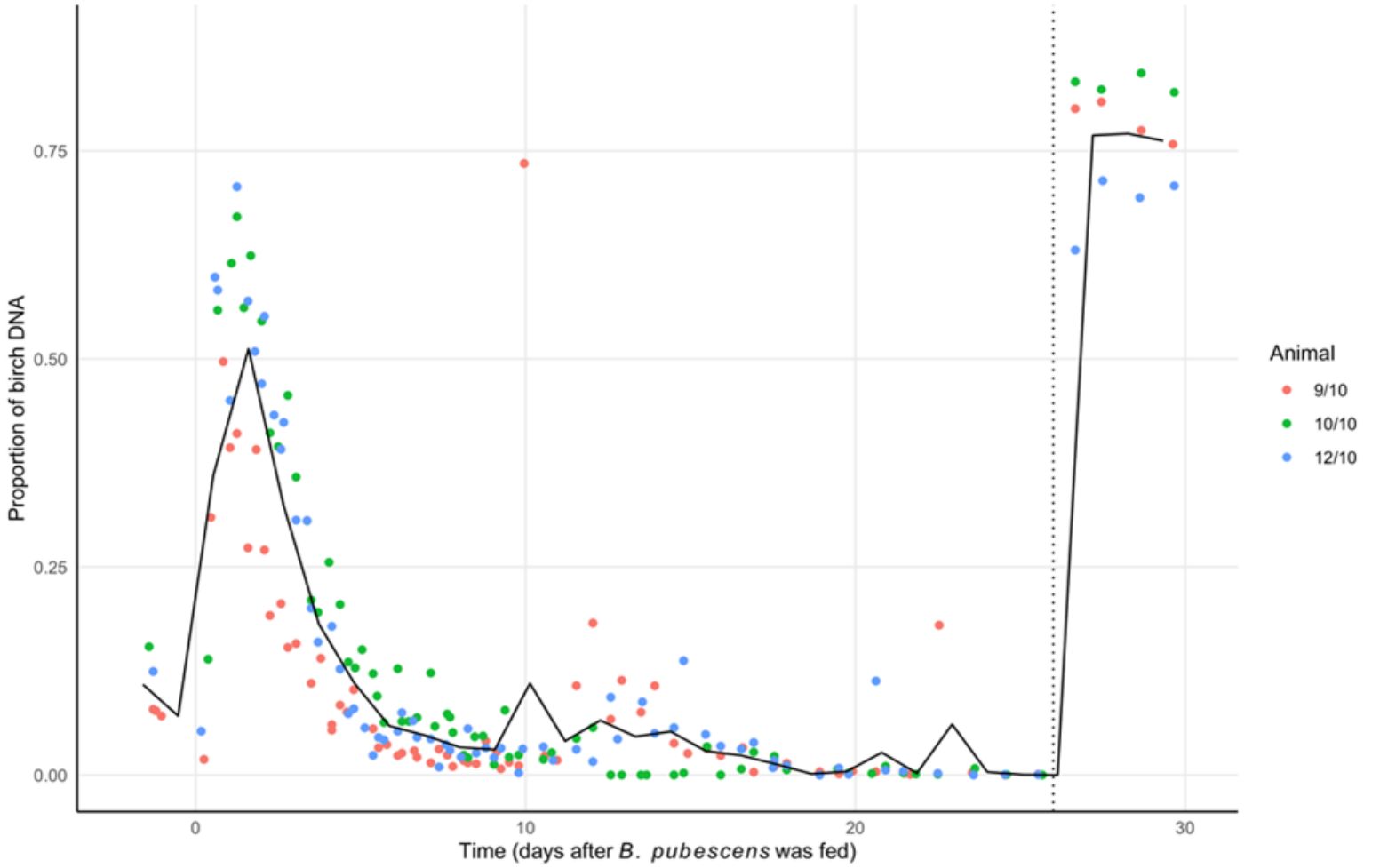
Amplifying	Primer name	Target	Forward primer	Reverse primer	Reference
Spermatophyta (seed plants)	Sper01	p6 loop of the trnL intron, cpDNA	GGGCAATCCTGAGCCAA	CCATTGAGTCTCTGCACCTATC	Taberlet et al., 2007
Eukaryotes	Euka02	18S, rDNA (V7 region)	TTTGTCTGSTTAATTSCG	CACAGACCTGTTATTGC	Guardiola et al., 2015
Fungi	Fung01	ITS1, nrDNA	GGAAGTAAAAGTCGTAACAAGG	CCAAGAGATCCGTTGYTGAAAGT	Epp et al., 2012
Bryophytes	Bryo01	Part of the trnL intron, cpDNA	GATTCAGGGAAACTTAGGTTG	CCATYGAGTCTCTGCACC	Epp et al., 2012; Taberlet et al., 2018

18S sequence read numbers correspond to proportional increase in food intake



Relative proportion of *Lecanoromycetidae* in diet from all sequences.
18S (Euka02 primers)

Detection of Birch (*Betula pubescens*)



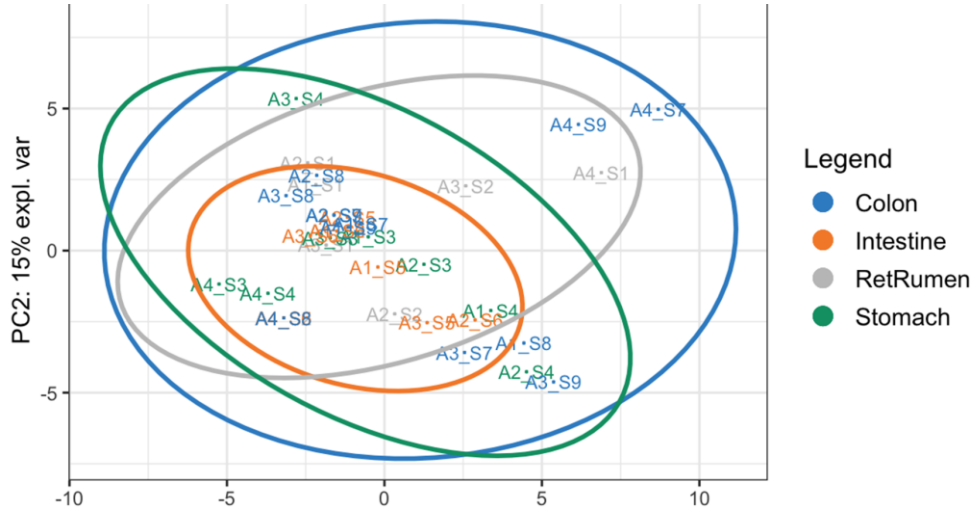
- Relative proportion of *Betulaceae* in diet, from all sequences. P6 loop trn*L* primers.

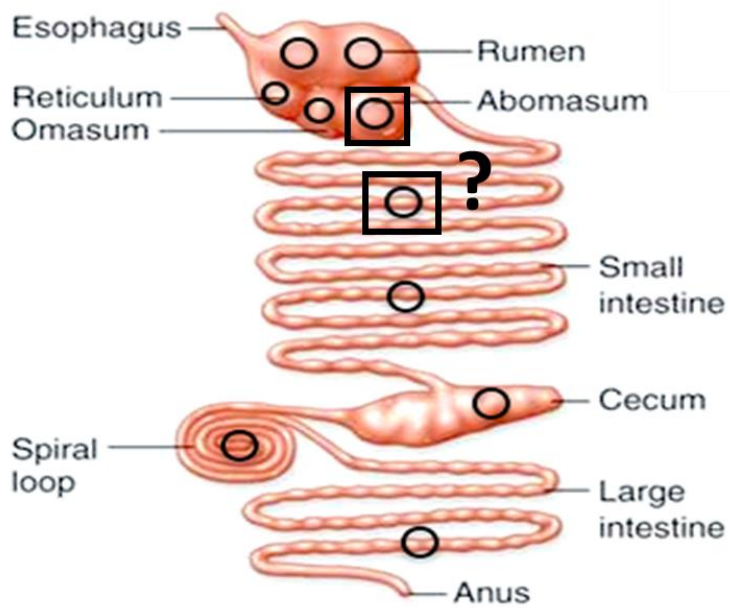
Conclusions from the feeding experiment

- great sensitivity in detection of low-abundant, recently ingested or highly-digested food material in herbivores with diverse diet and complex digestive system such as reindeer.
- DNA metabarcoding detects, in small proportions, food items ingested by reindeer over periods at least as long as 26 days -> integrative but qualitative assessments of diet over longer time periods
- the co-occurrence of fresh and earlier-ingested food items, detected within the same dietary sample, raises a note of caution about possible inferences of herbivores' diet selectivity based on estimates of in-field resource relative abundances

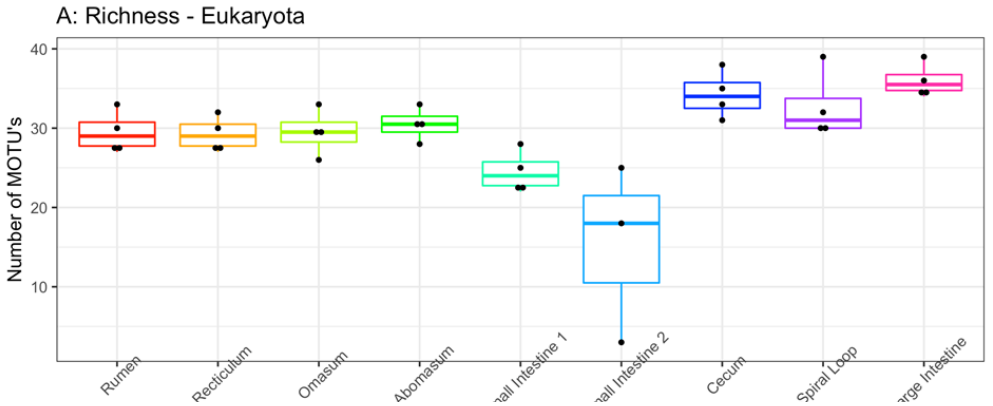
- How does detectability of DNA change through the gastrointestinal transit?

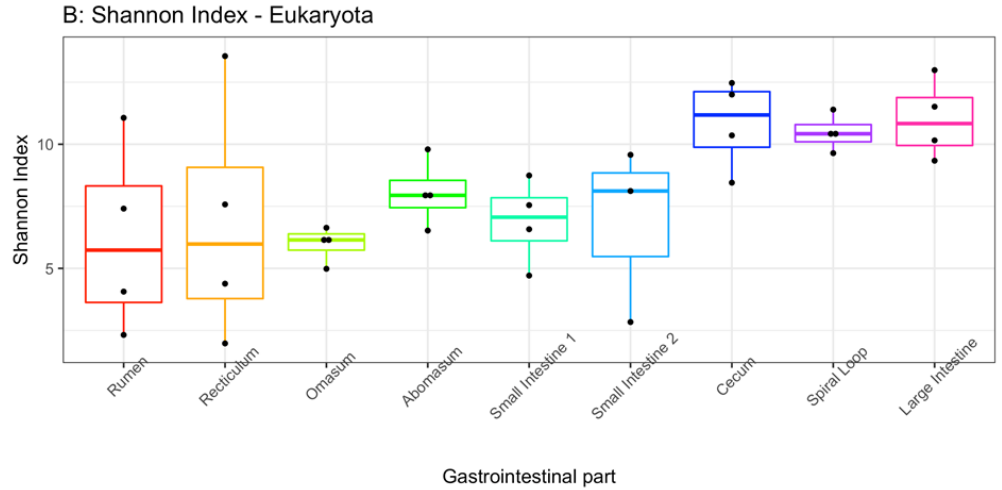
Tove Utsi, UiT

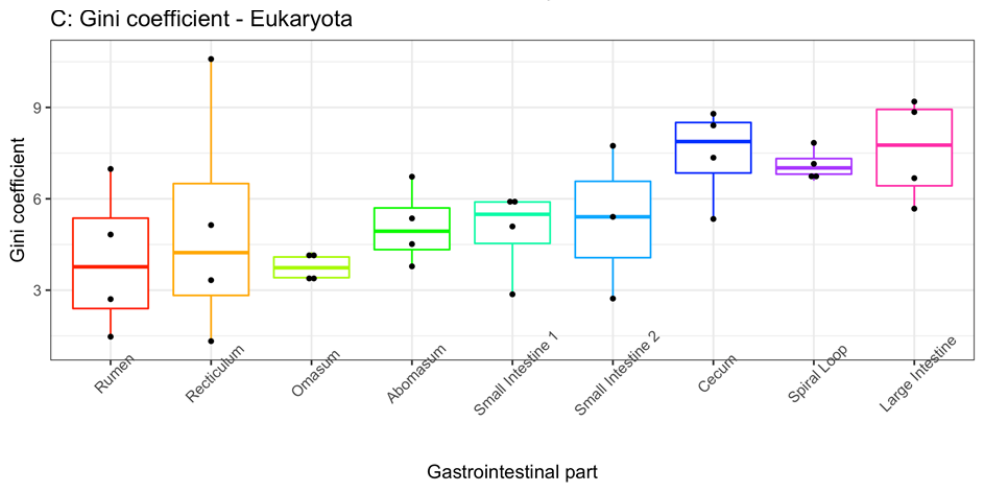


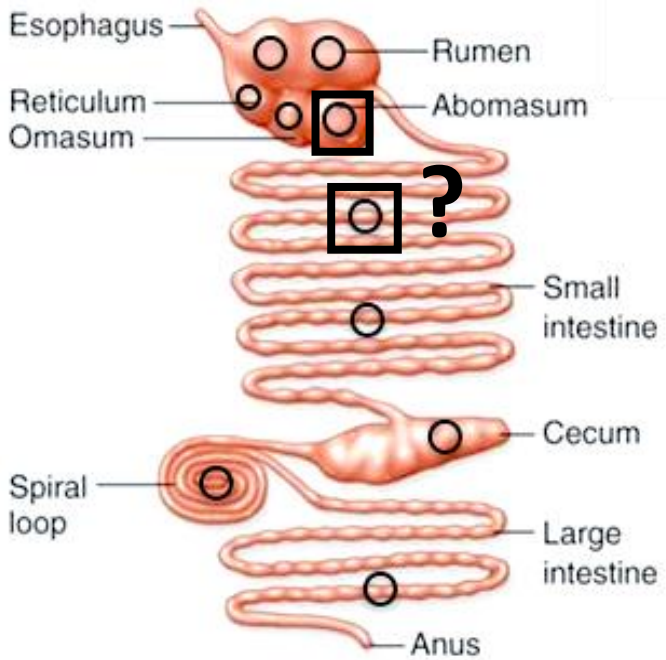


dDNA diversity varies through the different sections of the digestive tract in reindeer. In animals on natural pasture a higher diversity of dietary DNA was observed in the distal colon compared to the rumen, when using the seed plant primer and to a certain extent also with the eukaryote primer. (Meyer et al., unpublished data).


A: Richness - Eukaryota
Number of MOTU's
40
30
20
10
Rumen
Recticulum
Omasum
Abomasum
Cecum
Spiral Loop

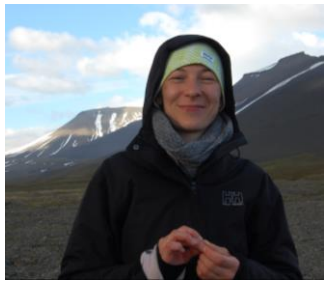

B: Shannon Index - Eukaryota
Shannon Index
10
5
Rumen
Recticulum
Omasum
Abomasum
Small Intestine 1
Small Intestine 2
Cecum
Spiral Loop
Large Intestine
Gastrointestinal part


C: Gini coefficient - Eukaryota
Gini coefficient
9
6
3
Rumen
Recticulum
Omasum
Abomasum
Small Intestine 1
Small Intestine 2
Cecum
Spiral Loop
Large Intestine
Gastrointestinal part

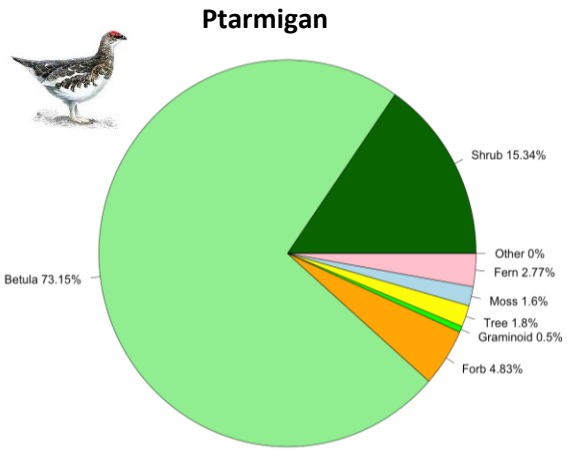

Esophagus
Rumen
Reticulum
Abomasum
Omasum
?
Small intestine
Cecum
Spiral loop
Large intestine
Anus

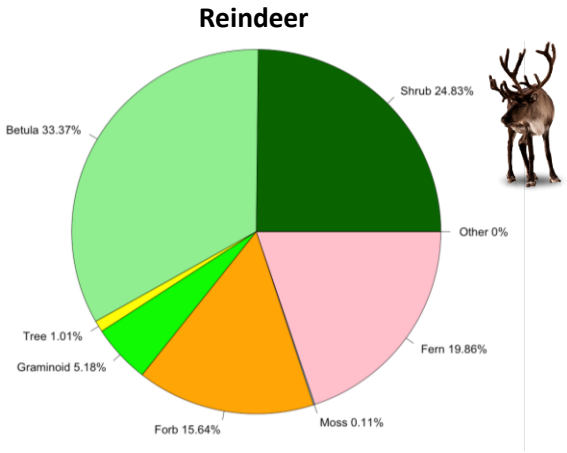
Proportions of MOTUs discovered in different herbivore species, Finnmark Norway – from species (MOTUs) lists to ecological conculsions

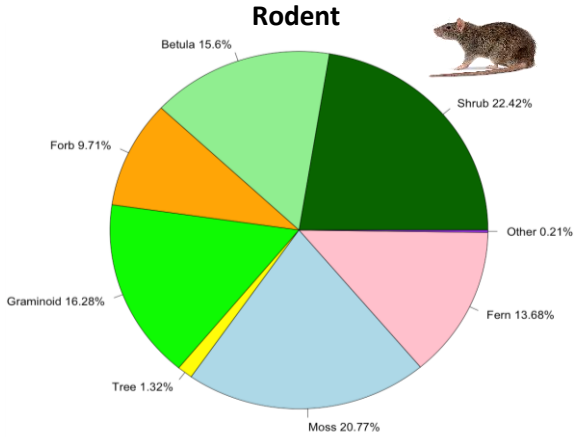
Lilja Steinthorsdottir, UiO

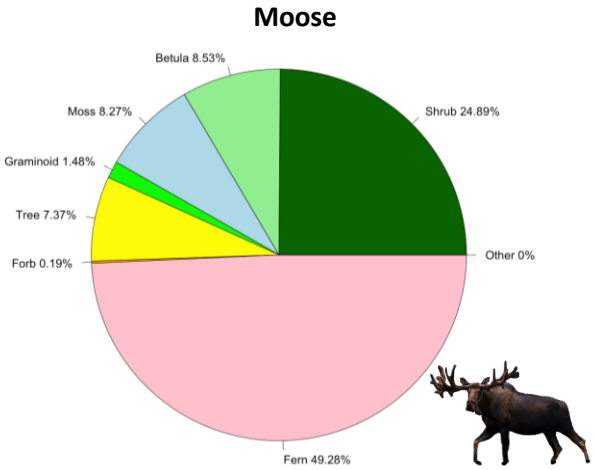


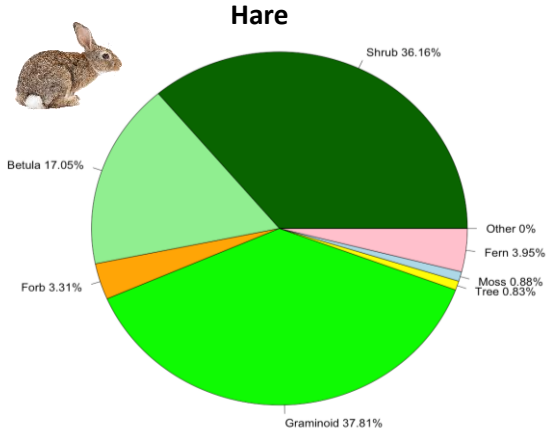
Stefaniya Kamenova, UiO

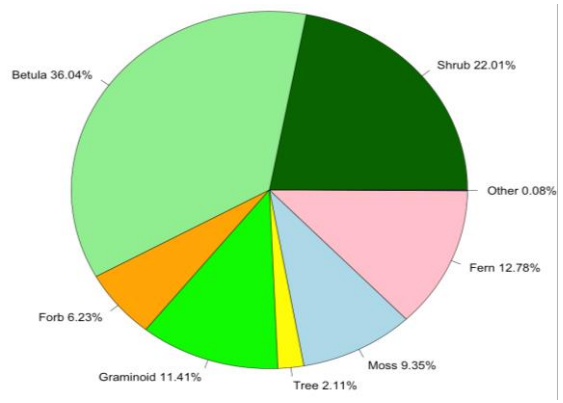












Article | Open Access | Published: 04 June 2019

Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content

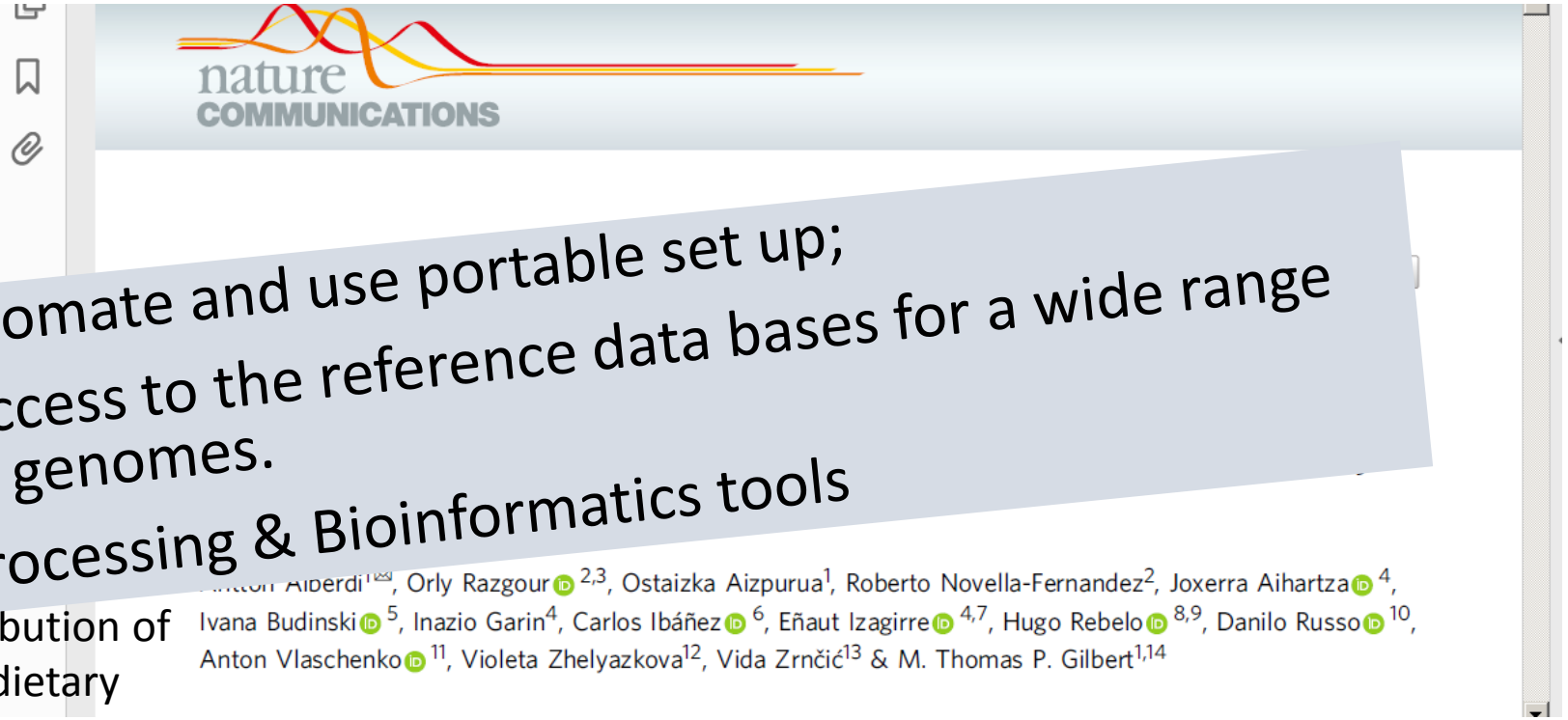
Francisco Javier Ancin Murguzur, Marjorie Bison, Adriaan Smis, Hanna Böhner, Eric Struyf, Patrick Meire & Kari Anne Bråthen

Scientific Reports **9**, Article number: 8259 (2019) | Cite this article

dDNA analyses in a broader ecological contex

Relationship between dietary niche breadth and spatial distribution features of European bats, by combining continent-wid
DNA metabarc
samples with s
modelling

- Possibility to automate and use portable set up;
- Development/access to the reference data bases for a wide range of taxa and loci, genomes.
- Efficient data processing & Bioinformatics tools



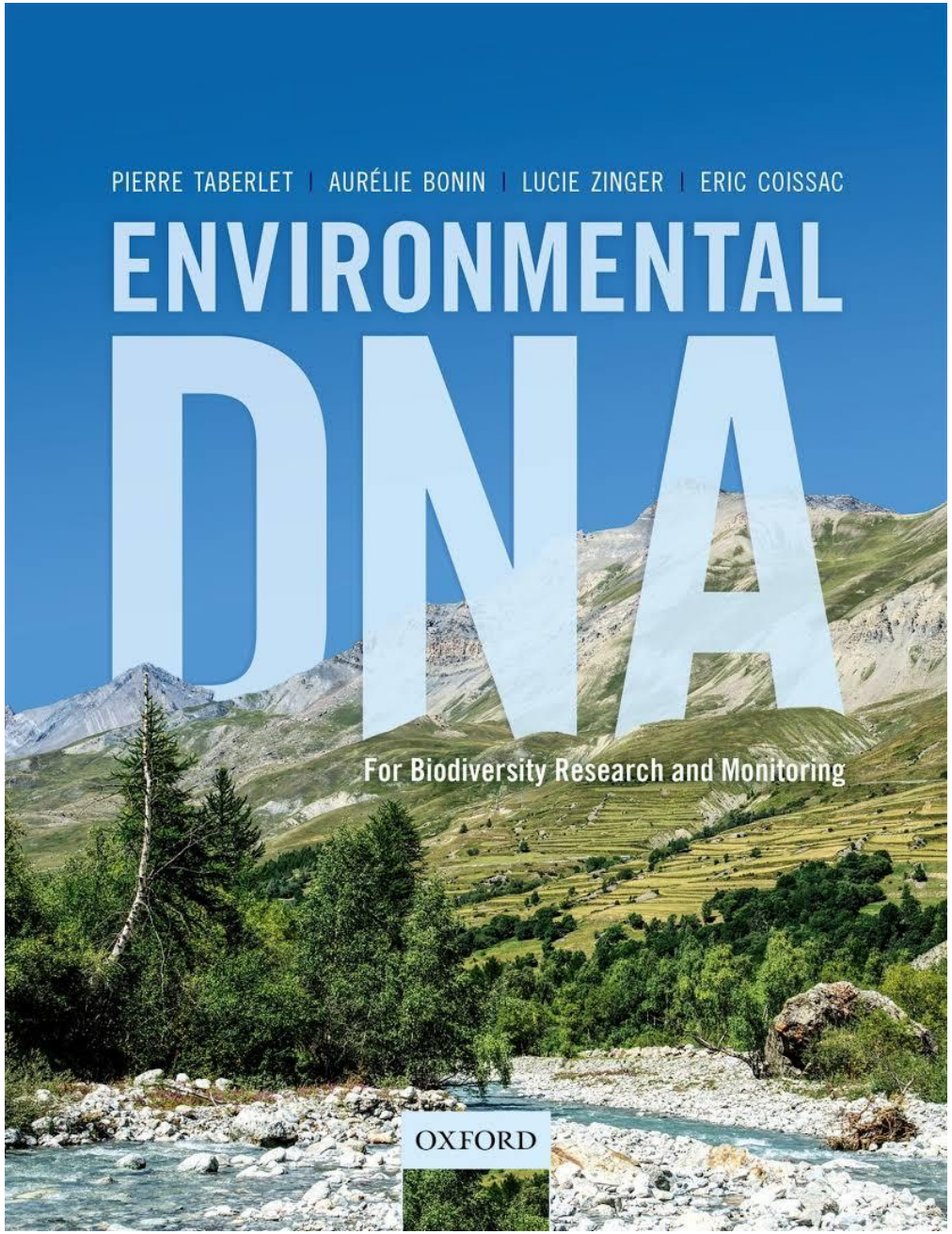
- range size is not
bats, the homog
species exhibits a strong correlation with dietary breadth.
- dietary breadth is correlated with bats' hunting flexibility.

These two patterns only stand when the phylogenetic relations between prey are accounted for when measuring dietary breadth.

Dietary DNA analyses

- Appear among the first eDNA studies
- Sampling is straightforward
- dDNA is usually concentrated
- Easy to extract from stomach/feces
- Taxonomic diversity not too high

Easy and robust method



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Canada

The University Centre in Svalbard

University of Manitoba

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