DNA sequencing of Hoary marmot (*Marmota caligata*) stomach contents using metabarcoding

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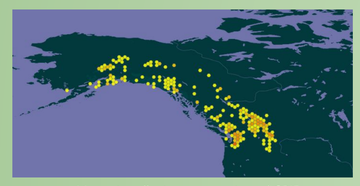
Problem

- Hoary marmots (*M. caligata*) are alpine-dwelling herbivores
  - research gap on diet
  - alpine habitat is expected to change
- We used DNA analysis of *M. caligata* stomach contents to compare their diets in alpine and coastal habitats

Background



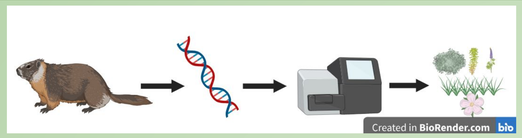
Marmota caligata range courtesy of GBIF

- *M. caligata* primarily occupy alpine tundra, rocky talus
- Southeast Alaska populations have been observed in coastal regions
- Thought to feed on grasses, flowering plants, mosses, roots, lichen[3]
- DNA amplification and metabarcoding allow for simultaneous identification of multiple taxa in one sample[1,2], providing insight into the *M. caligata* diet.

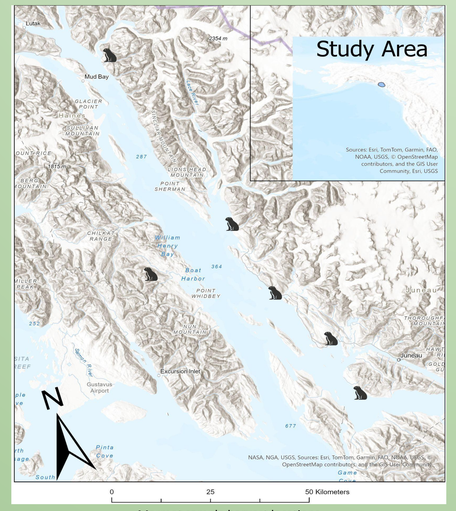
Goals

- Test methods to determine *M. caligata* diet and microbiome using DNA sequencing of stomach contents
- Understand current hoary marmot diet in Southeast Alaska
- Determine advantageous, alternative food sources which draw *M. caligata* from their usual alpine habitat
- Provide researchers with information on potential *M. caligata* environmental resiliency

Methods

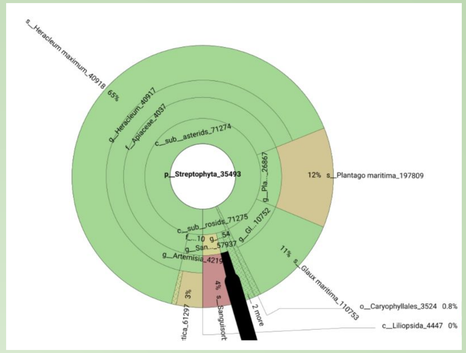


- Marmot stomachs (n=5), 5 samples per stomach
- Homogenize stomach contents via blending
- Extract DNA using Gentra Purgene Tissue Kit
- Quantify DNA using Nanodrop One[c]
- PCR1
  - iTru RBCLA-F, MRBCL-163-RI, UNIPLANT-R, UNIPLANT-F plant primers
- PCR2
  - add dual indices
- Send to IAB Core lab for sequencing on Illumina miSeq[4]
- Analyze using BLAST to identify consensus sequences using GenBANK DNA database[5]
- RDP classifier to estimate confidence in matches

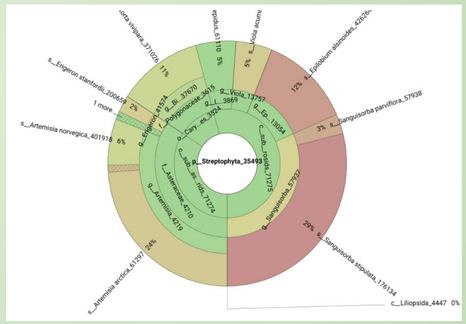


Marmot sample harvest locations

Results



These Krona plots show our results from two samples. Confidence in taxon identification is indicated through color.



Alpine Marmots (n=3)	Coastal Marmots (n=2)
Small-flowered woodrush	Cow parsnip
Luzula parviflora	*Heracleum maximum*
Boreal sagebrush	Boreal sagebrush
Artemisia arctica	*Artemisia arctica*
Alpine sagewort	Sea milkwort
Artemisia norvegica	*Glaux maritima*
Alpine bistort	Goose tongue
Bistorta vivipara	*Plantago maritima*
Garden heliotrope	Common dandelion
Valeriana officinalis	*Tarraxacum officianale*
Mountain sorrel	Sea sandwort
Oxyria digyna	*Honckenya peploides*
Purple sweet-cicely	Dandelions
Osmorhiza purpurea	*Tarraxacum* sp

Our mountain diet analysis also found *Viola* sp., *Lupinus* sp., *Geranium* sp., and *Erigeron* sp.

Citations

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Takeaways

- Samples from hoary marmots collected in either alpine or coastal habitats suggest some overlap in diet, with coastal samples including plant species that only grow at sea level (*P. maritima*, *H. peploides*).

Future Research

- In future research, we will be able to use fewer replicates per stomach as our results were consistent across samples
- More samples for research are recommended
- Creating a comprehensive list of coastal and alpine plant species is necessary for thorough cataloguing and comparison of diets

Plantago maritima, photo by M. Hogan, 2020, CC BY-NC-SA

Hoary marmot, photo by JD Anderson, 2016.

Artemisia norvegica, photo by T AbeLlyod, 2016 CC-BY-NC

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Hoary marmot, photo courtesy NPS (n.d.)