

Diet segregation of Northwest Pacific pinniped communities; application of novel high-throughput DNA techniques to scat

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Fisheries and Oceans
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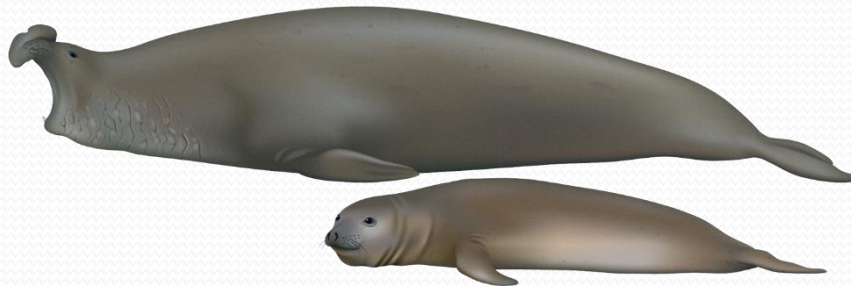
Pinnipeds in British Columbia

Family Phocidae – True seals (2 spp)

Harbour Seal *Phoca vitulina*



Northern Elephant Seal *Mirounga angustirostris*

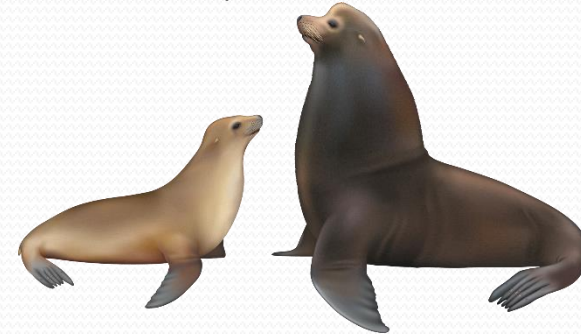


Family Otariidae – Eared seals (3 spp)

Steller Sea Lion *Eumetopias jubatus* ★



California Sea Lion *Zalophus californianus*

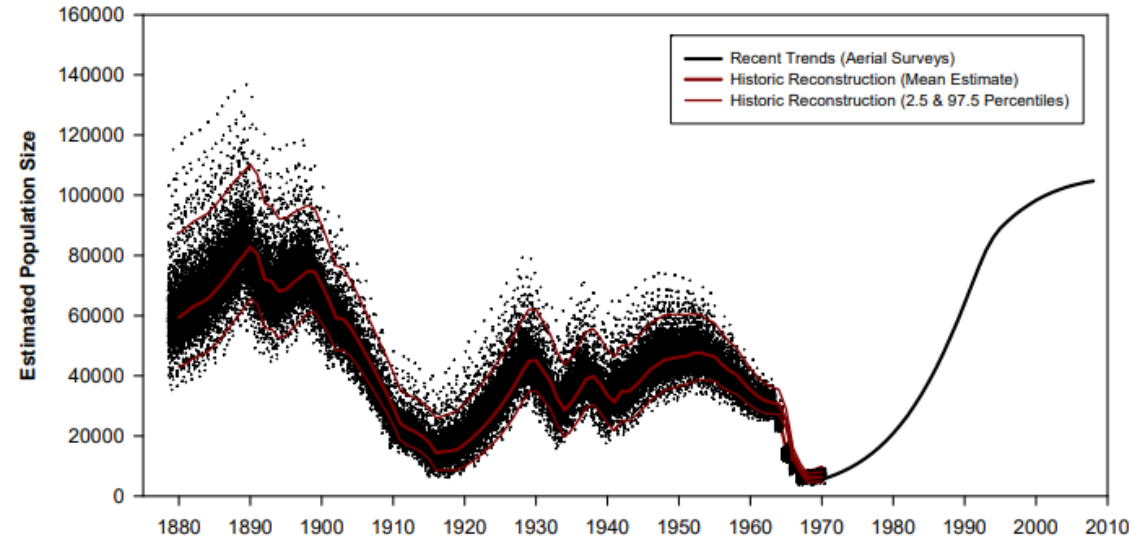
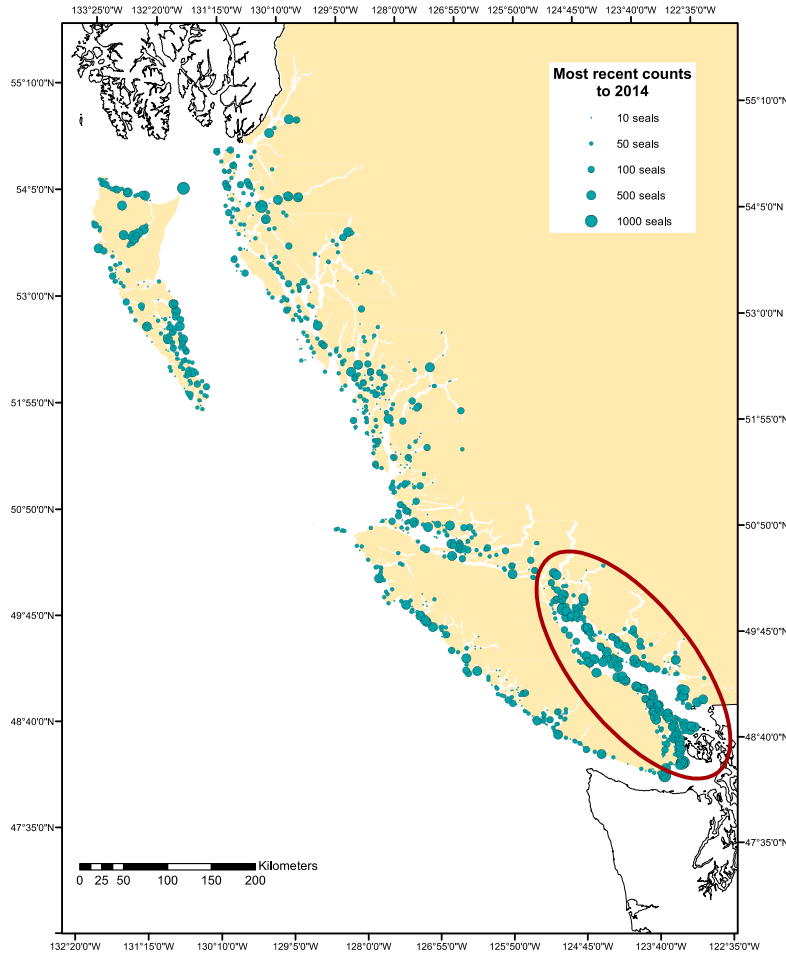


Northern Fur Seal *Callorhinus ursinus* ★★

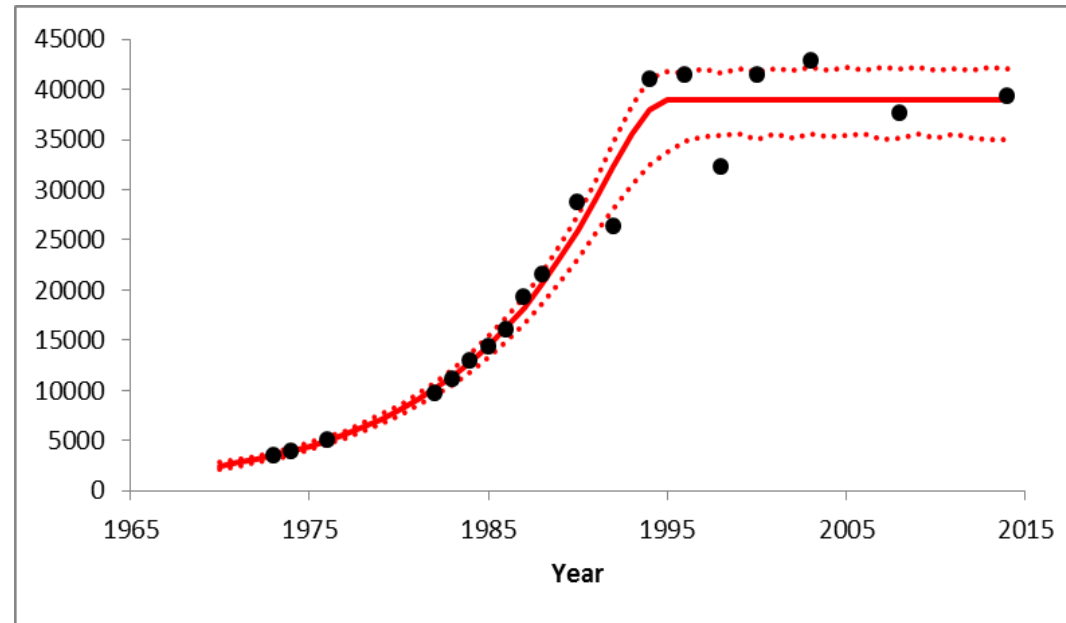


Harbour seal abundance: BC

coast wide

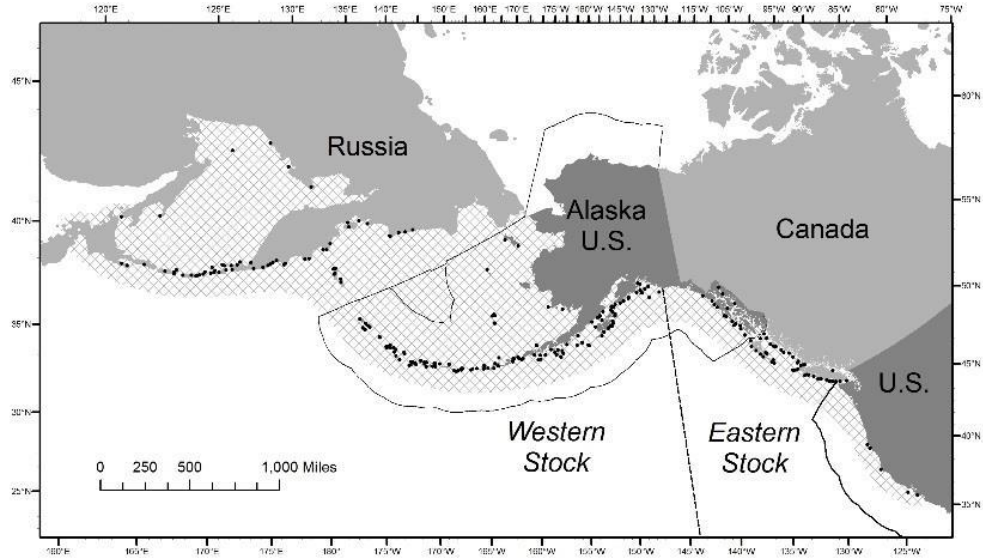


DFO. 2010. Population Assessment Pacific Harbour Seal (*Phoca vitulina richardsi*). DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2009/011.

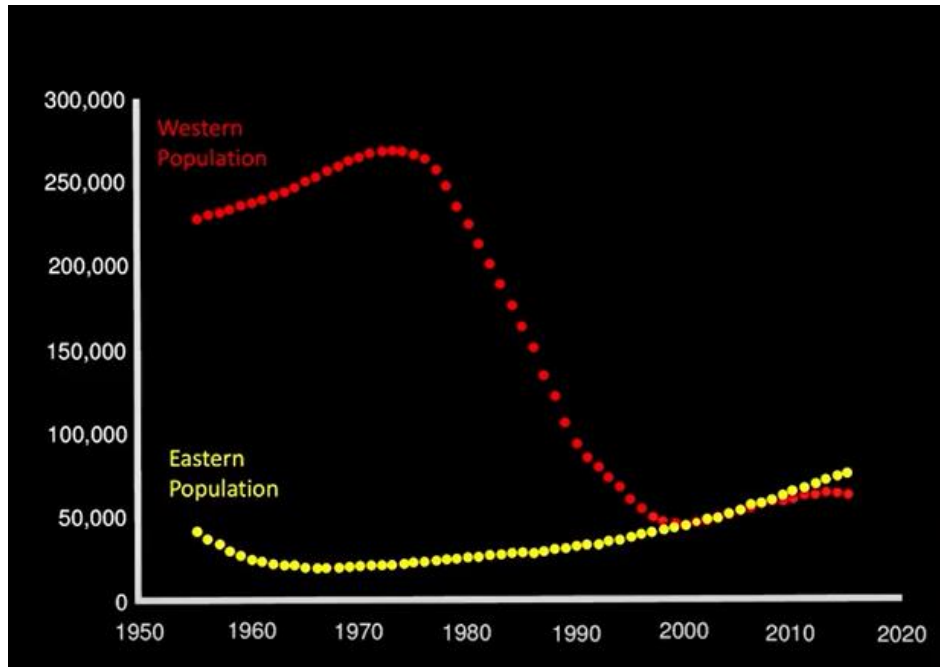
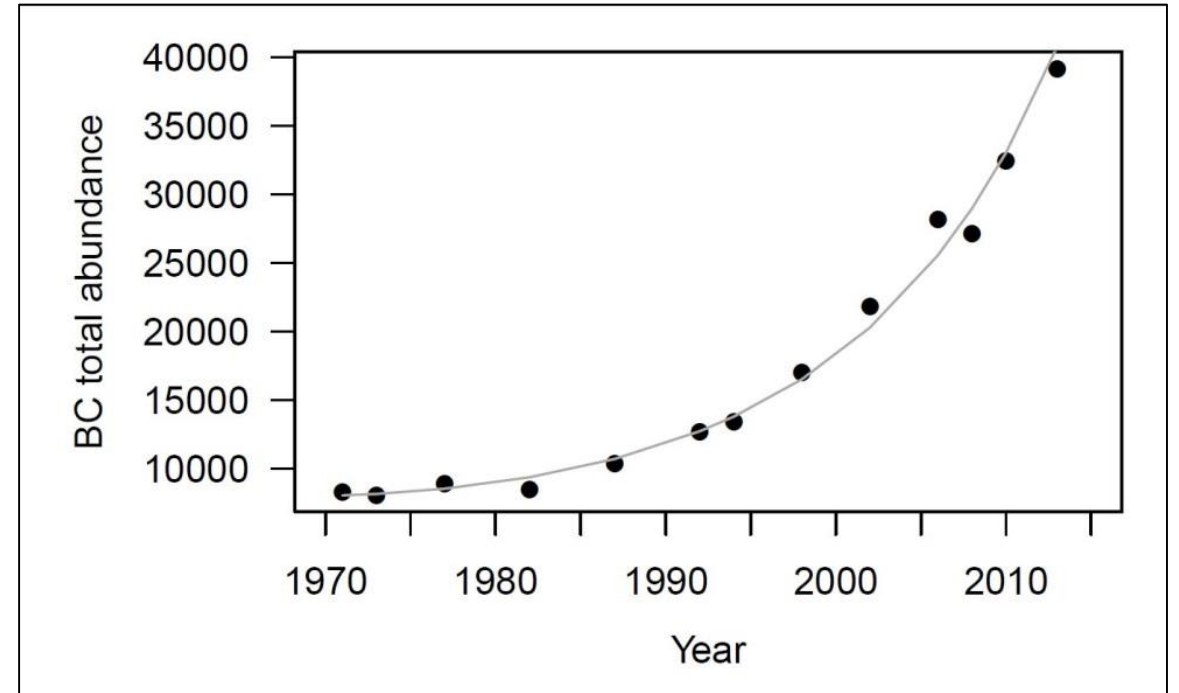


Strait of Georgia

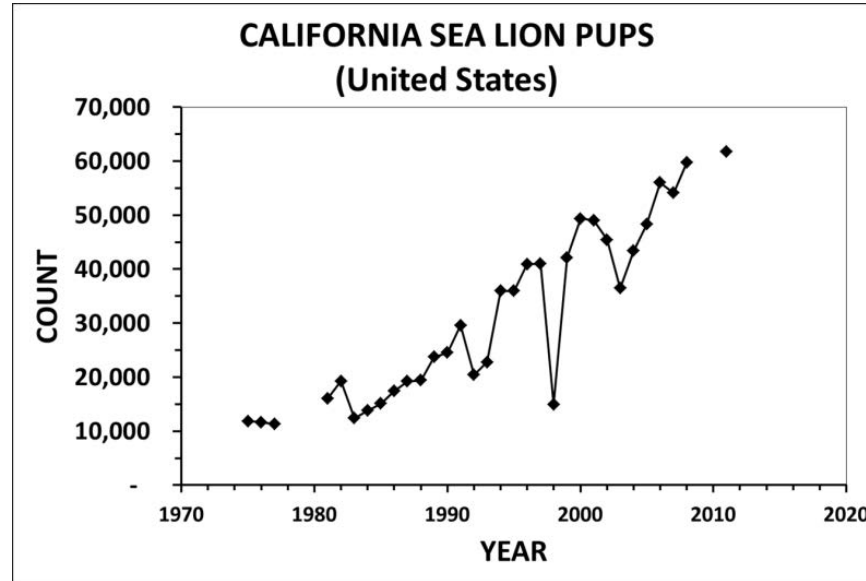
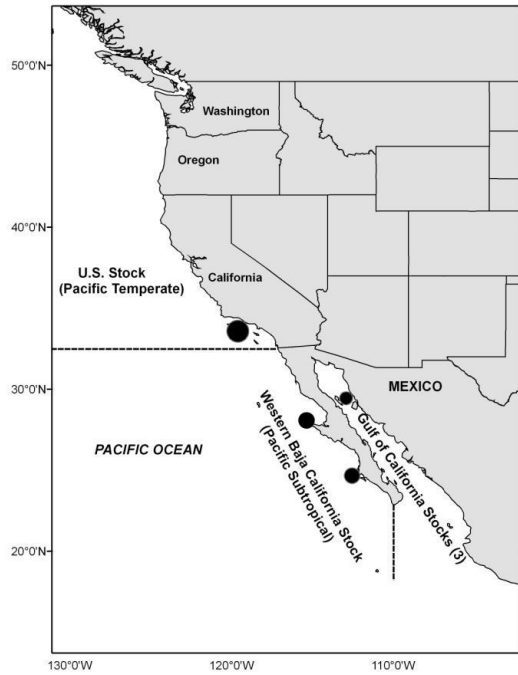
Steller Sea Lion



BC coast



California Sea Lions



- Breeding season surveys done outside of B.C.
- Relative overwintering counts updated opportunistically from fall/winter SSL surveys



Pinniped communities of Pacific Northwest

- To varying degrees, these species utilize both pelagic and benthic habitats from riverine estuaries to the oceanic zone beyond the continental shelf representing a broad range of foraging strategies
- Each of the species tend to be opportunistic in their diets, exploiting seasonally abundant prey, feeding on a mix of pelagic forage fish such as herring and benthic fish such as rockfish
- However, explicit diet data for these species in BC are sparse and have been taken opportunistically and irregularly
- Little is known about how key prey are partitioned both within and between species over varying spatial and temporal scales

Why Scoop Poop?



Diet assessment

Recovery of marine mammal populations is (re) raising concerns regarding their impact on fisheries.

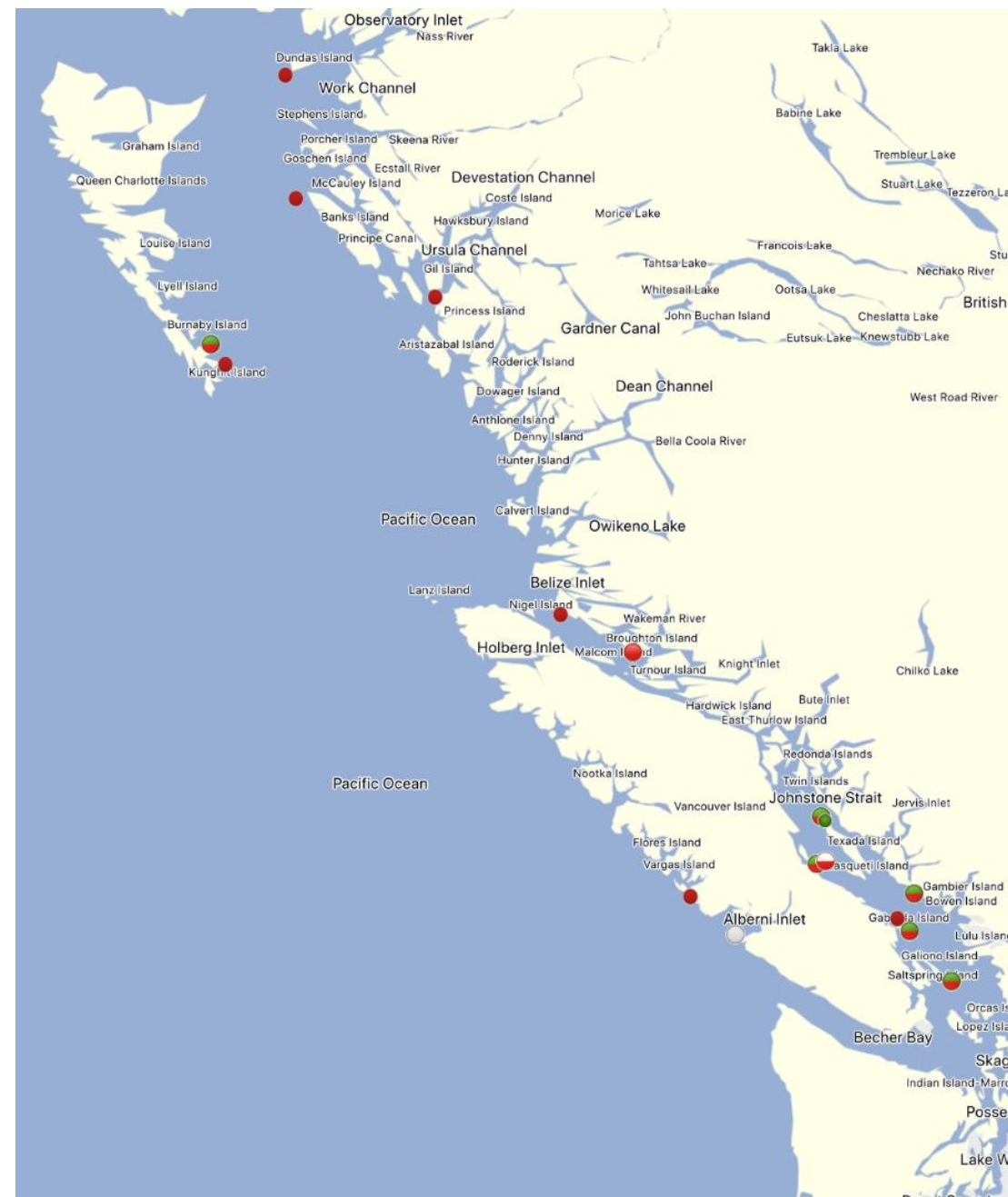
DFO has initiated new research to update knowledge of the temporal (monthly/annual) and spatial (river-estuary and non-estuary sites) variation in pinniped diets using:

scat-based methods

- Samples are readily obtained
- hard-part identification and DNA-metabarcoding provide accurate species composition of the diet and size-class of prey
- DNA from the animal itself can be extracted from scat providing value added information such as sex and population structure

2014-2017 scats analyzed to date through DNA metabarcoding

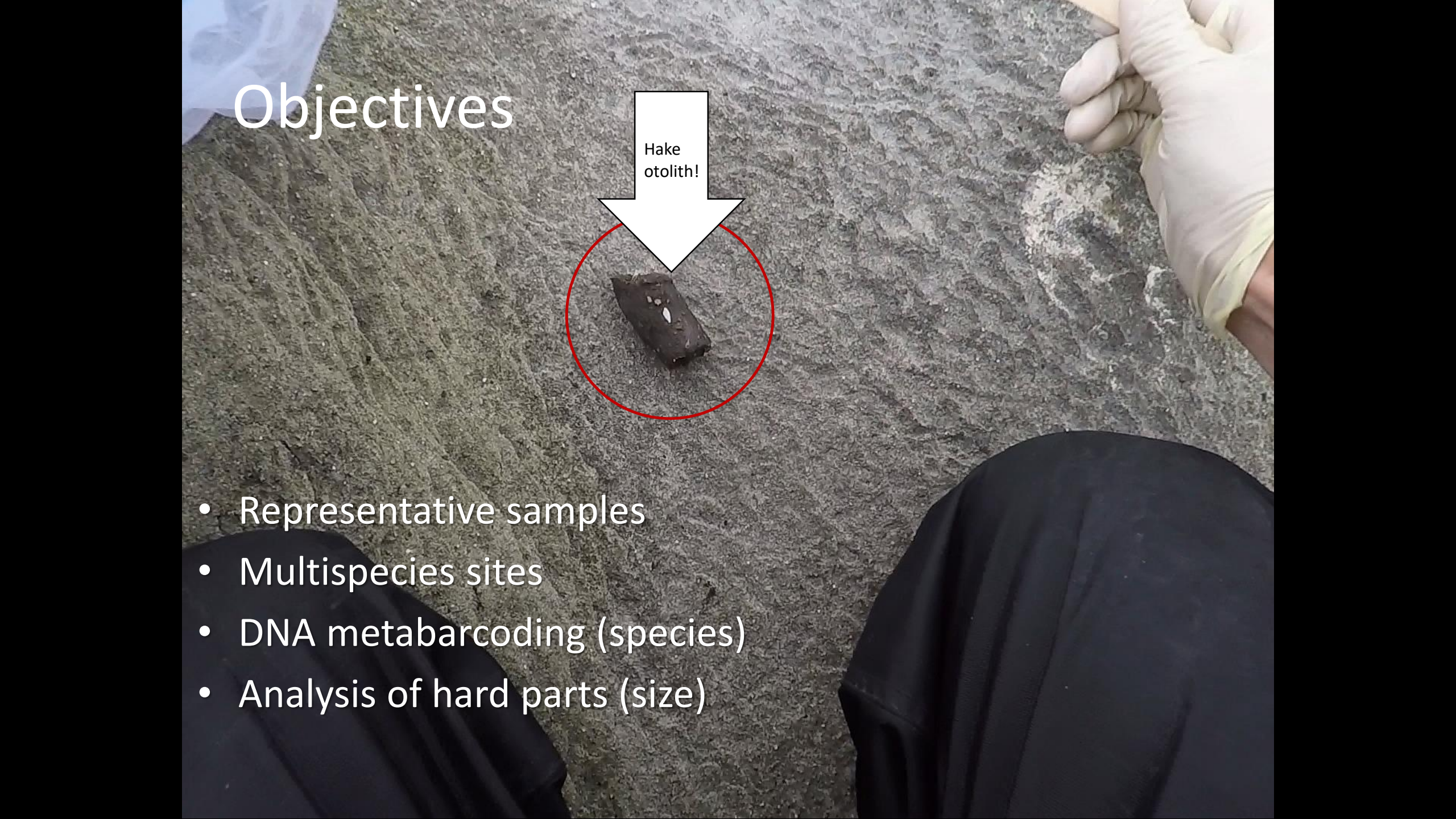
species	analyzed	TBA
Harbour	1248	-
Steller	331	229
California	151	35



Core sampling 2018:2020: monthly

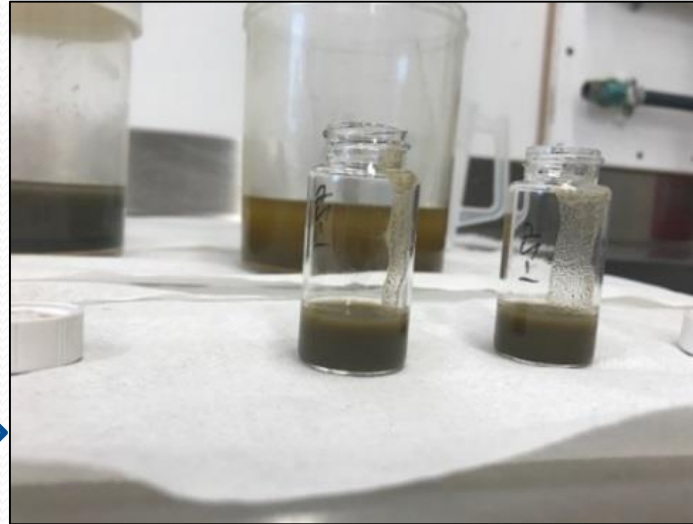


Objectives

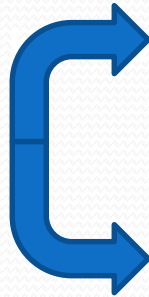


Hake
otolith!

- Representative samples
- Multispecies sites
- DNA metabarcoding (species)
- Analysis of hard parts (size)



Subsample for genetic analysis



Cleaning for hard parts analysis

Molecular Scatology: advantages and approach

- **Highly sensitive-high probability of prey species detection**
important for low proportion prey OR prey that are underrepresented by traditional hard part analysis
- **Can provide refined taxonomic assignment**
- **DNA metabarcoding** or “amplicon sequencing diet analysis” and “high-throughput sequencing diet analysis”
the ability to provide accurate “quantitative” estimates of diet rather than only being able to detect the presence of prey species in a sample

Approach: individual scat sample

1. A multiplex PCR reaction done with the extracted DNA using 16S primer sets designed to amplify both fish and invertebrate DNA.
2. Samples individually labeled with index sequences, pooled amplicons were sequenced on multiple Illumina MiSeq runs (v2-300 cycle SE).
3. DNA sequences compared with a custom BLAST reference database composed of 16S sequences of species known to occur in the geographic region.
4. To remove potential DNA contaminants, species sequences that comprised <1% of a single sample removed prior to calculating sample diet percentages.
5. Prey species taxonomic assignments were renormalized to generate proportional DNA summaries for each individual scat sample

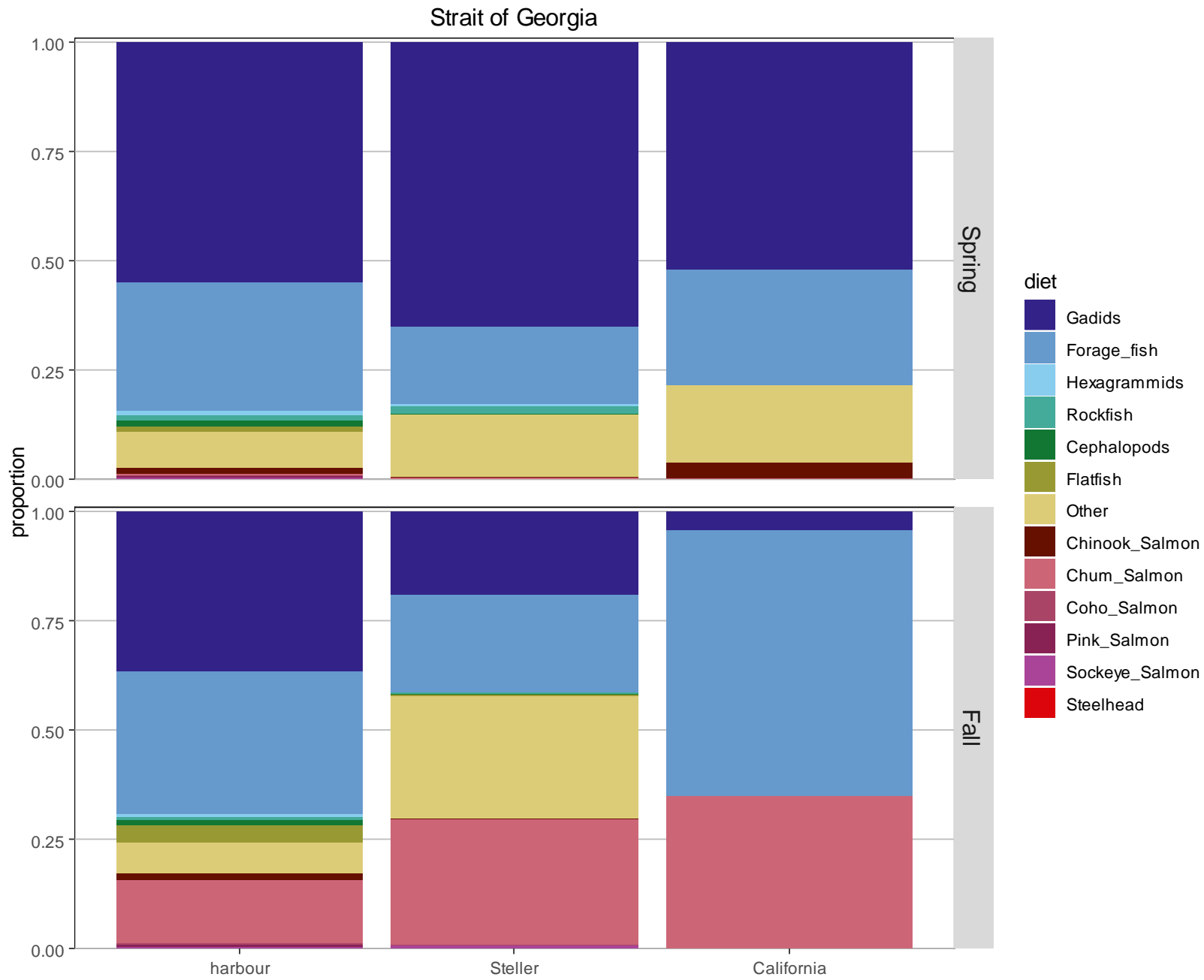
Molecular Scatology: biological biases

- 1. Tissue bias:** mass-specific differences in target gene copy number between food species
 - ~ species-specific methodological biases (e.g. DNA extraction bias, PCR bias, sequencing bias, quality-filtering bias, etc.)
- 2. Digestion bias:** differential digestion of food species DNA
 - ~ related to proximate composition

Temporal variation in Steller diets: West Coast Vancouver Island



Species variation in diets: harbour seals, Steller and California SL in the Strait of Georgia



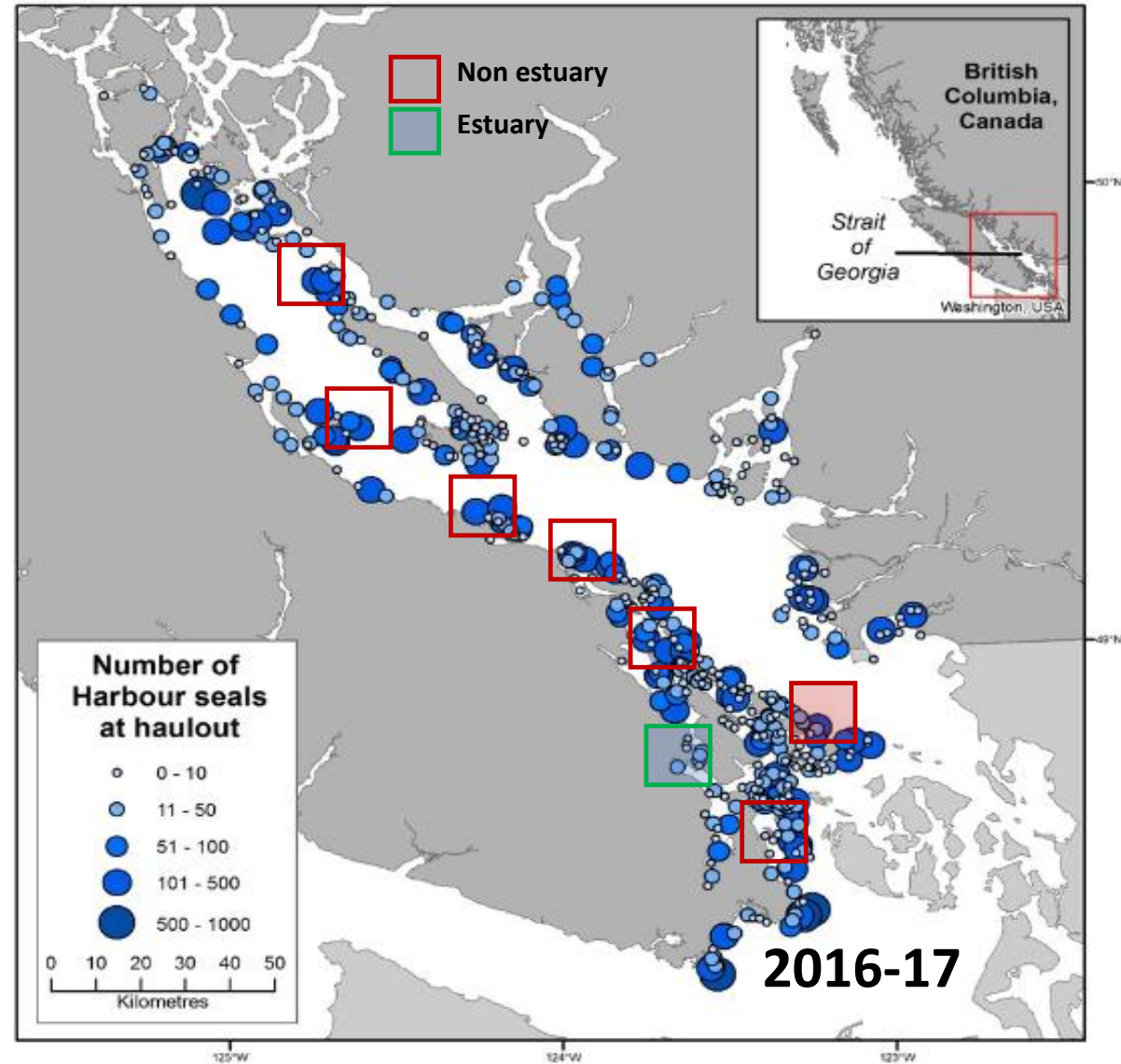
Harbour seal scat collections - Strait of Georgia

70 scats/month (April – November 2016 and April-May 2017)

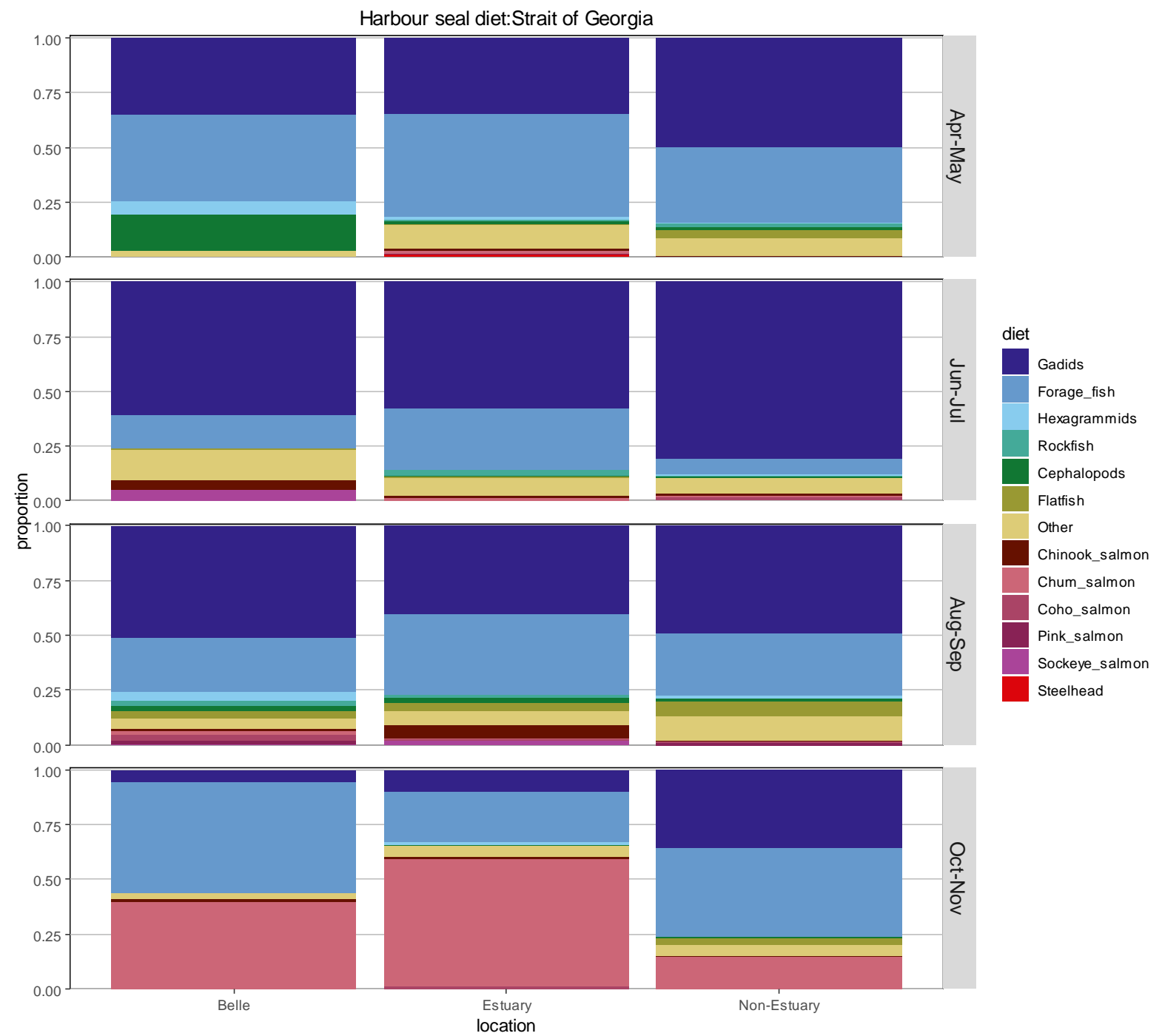
1 estuary site (Cowichan Bay)
~ 400 samples

7 primary non-estuary sites

+ *other non-estuary sites*
~1300 harbour seal and ~300 sea lion samples

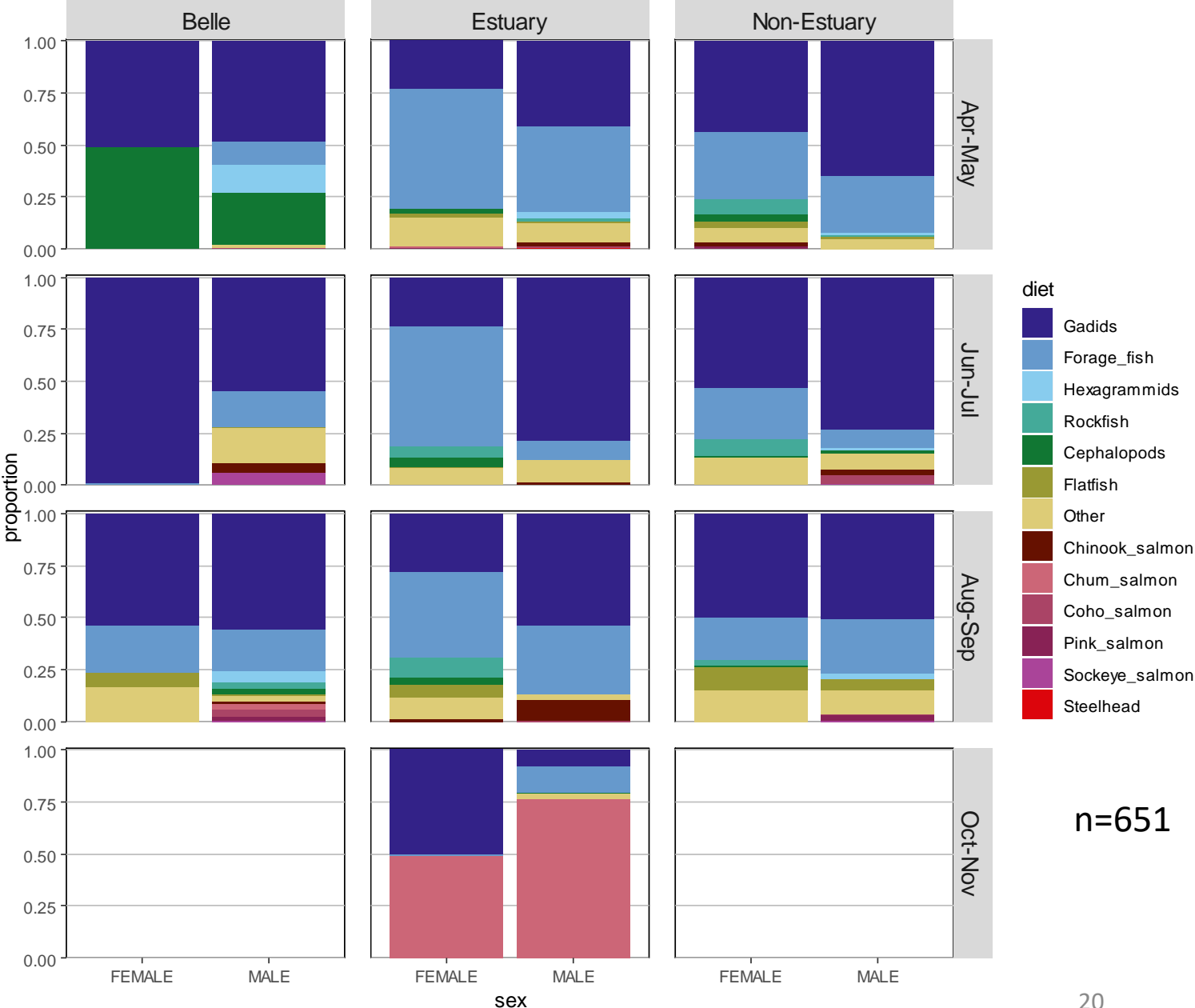


Spatial variation in diets: harbour seals



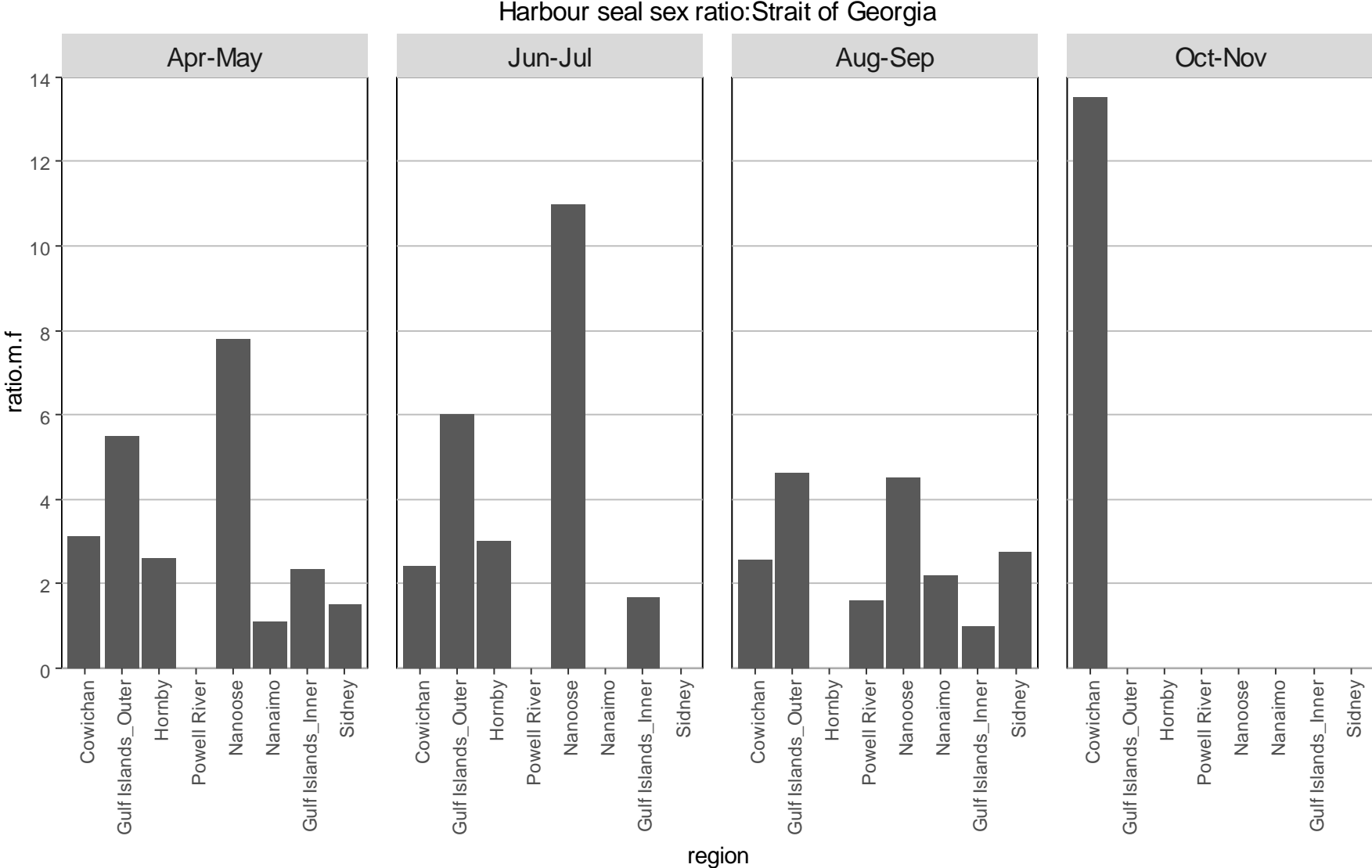
Sex variation in diets: harbour seals

Harbour seal diet: Strait of Georgia



Sex ratio: harbour seals, Strait of Georgia

male: female
mean = 3.5:1



Future work

1. captive feeding studies:

- Validating DNA metabarcoding to quantitatively describe pinniped diet (David Rosen, University of British Columbia/Vancouver Aquarium)

2. other eDNA applications from scat:

- *Infectious agents profiles*-the interaction between infectious agent carrier state and predation susceptibility
- Pinnipeds as samplers of *harmful algal blooms*

3. Other genetic applications

- Stock/individual
- Functional genomics

Thank-you!

