Multispecies/Metabarcoding with eDNA

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Multispecies/Metabarcoding with eDNA

- Potential uses
  - Detection of invasive species
  - Detection of pathogens
  - Community structure
  - Species range
  - Diet analysis

- eDNA sources
  - Water
  - Soil
  - Sediment
  - Scat
  - Air
  - Long term storage

Multispecies/Metabarcoding with eDNA

- Why multispecies/metabarcoding with eDNA?
  - Non-invasive
  - Cost effective (sampling)
  - Once developed, system is plug and chug
  - Multiple species-level differentiations
  - Redundancy (multiple loci/traditional survey)
  - Added value/Collaboration

- Problems
  1. Lack of sequence data
     - Primer design
     - Reference database development
  2. Analysis methods
  3. Bioinformatics pipelines
  4. Cost of initial development

Metabarcoding

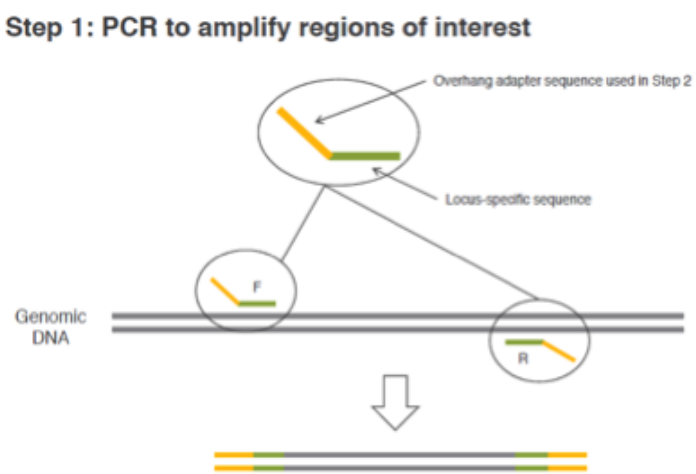
- PCR Amplicon based Next-Generation Sequencing

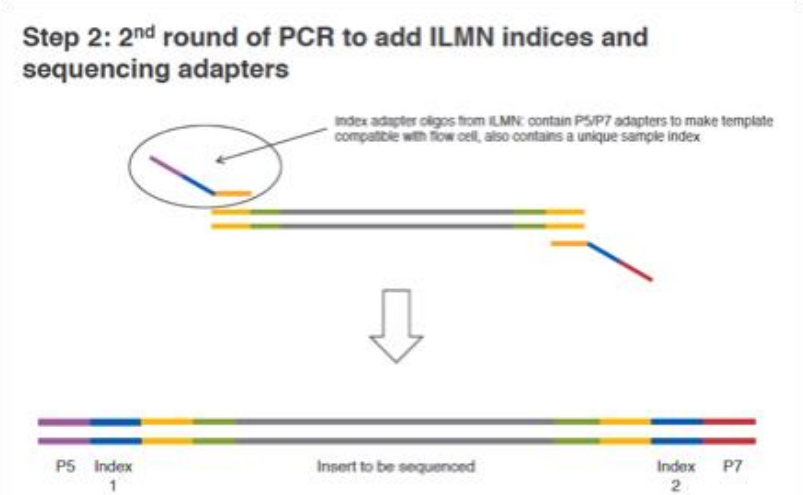
Step 1

PCR eDNA by sample by locus

Step 2

Pool Step 1 PCR products by sample





https://web.uri.edu/gsc/next-generation-sequencing/

- Illumina MiSeq = 384 samples
  - ~15 million reads Passing Filter
  - # loci – depends on desired coverage
  - Acceptable number of hits = 0.01% of Total Reads Passing Filter per sample (Bokulich et al., 2013)

Primer design and database development

- Steps:
  1. Get species list
  2. Check NCBI for sequence data, loci/species pairs
  3. Download all sequence data for loci/species pairs
  4. Align (MEGA)
  5. Look for potential primer sites
     - Fail? Go to 3
     - Worst case (Classical)
  6. Check potential primers against NCBI
     - Fail? Go to 3
  7. Download data from NCBI
  8. Repeat as needed for 2nd+ locus or missed species

Multispecies

- Alaska fish – 12S (Menning et al., 2018)

Acipenser medirostris
Acipenser transmontanus
Alosa sapidissima
Arctogadus glacialis
Boreogadus saida
Catostomus catostomus
Catostomus commersonii
Clupea pallasii
Coregonus autumnalis/clupeaformis
Coregonus clupeaformis
Coregonus laurettae
Coregonus nasus
Coregonus sardinella
Coregonus sp.3
Cottus aleuticus/bairdii/cognatus
Cottus asper
Couesius plumbeus
Dallia pectoralis
Esox lucius
Gadus macrocephalus
Gadus ogac
Gasterosteus aculeatus
Hypomesus olidus
Lampetra aepyptera
Lampetra fluviatilis
Leptocottus armatus
Lethenteron camtschaticum
Lethenteron reissneri
Lota lota
Oncorhynchus clarkii
Oncorhynchus gorbuscha
Oncorhynchus keta
Oncorhynchus kisutch
Oncorhynchus mykiss
Oncorhynchus nerka
Oncorhynchus tshawytscha
Osmerus mordax
Percopsis omiscomaycus
Prosopium coulterii
Prosopium cylindraceum
Prosopium williamsoni
Pungitius pungitius
Salmo salar
Salmo trutta
Salvelinus alpinus
Salvelinus alpinus/malma
Salvelinus confluentus
Salvelinus fontinalis
Salvelinus malma
Salvelinus namaycush
Stenodus leucichthys
Thymallus arcticus
Thymallus thymallus

Multispecies

- Alaska fish – 12S (Menning et al., 2018)
    - Added value (14 Feb., 2019)
        - 1662 unique Genera
        - 3764 unique species
        - 4829 unique sequences

https://www.adfg.alaska.gov/index.cfm?adfg=animals.main

Alaska mammals

- *Myotis keenii* – Keen's Bat
- *Myotis lucifugus* – Little Brown Bat
- *Cervus elaphus* – Roosevelt Elk
- *Oreamnos americanus* – Mountain Goat
- *Alces alces* – Moose
- *Ovibos moschatus* – Muskox
- *Ovis dalli* – Dall Sheep

Alaska amphibians

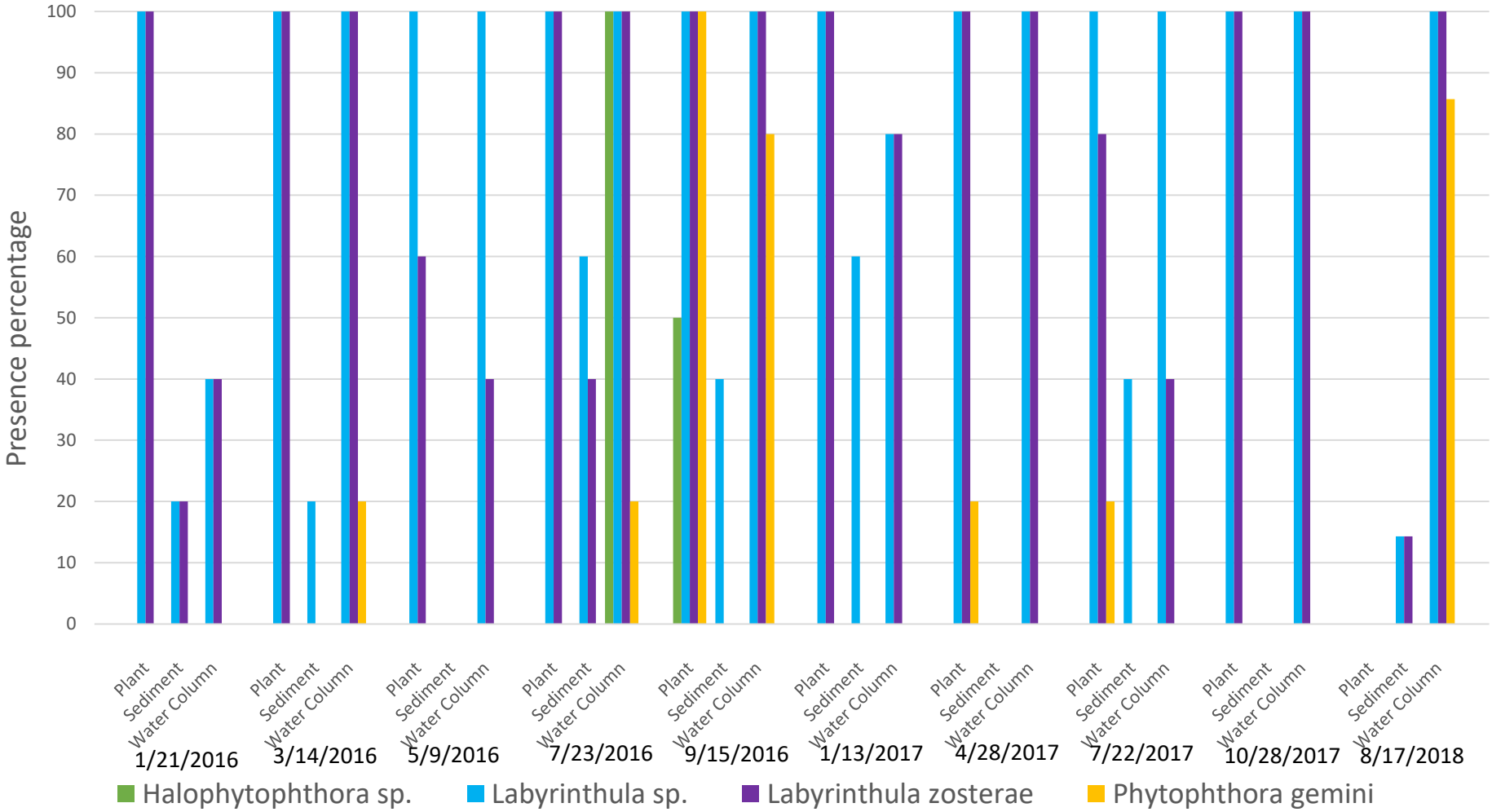
- *Taricha granulosa* – Roughskin newt

Multiplex samples

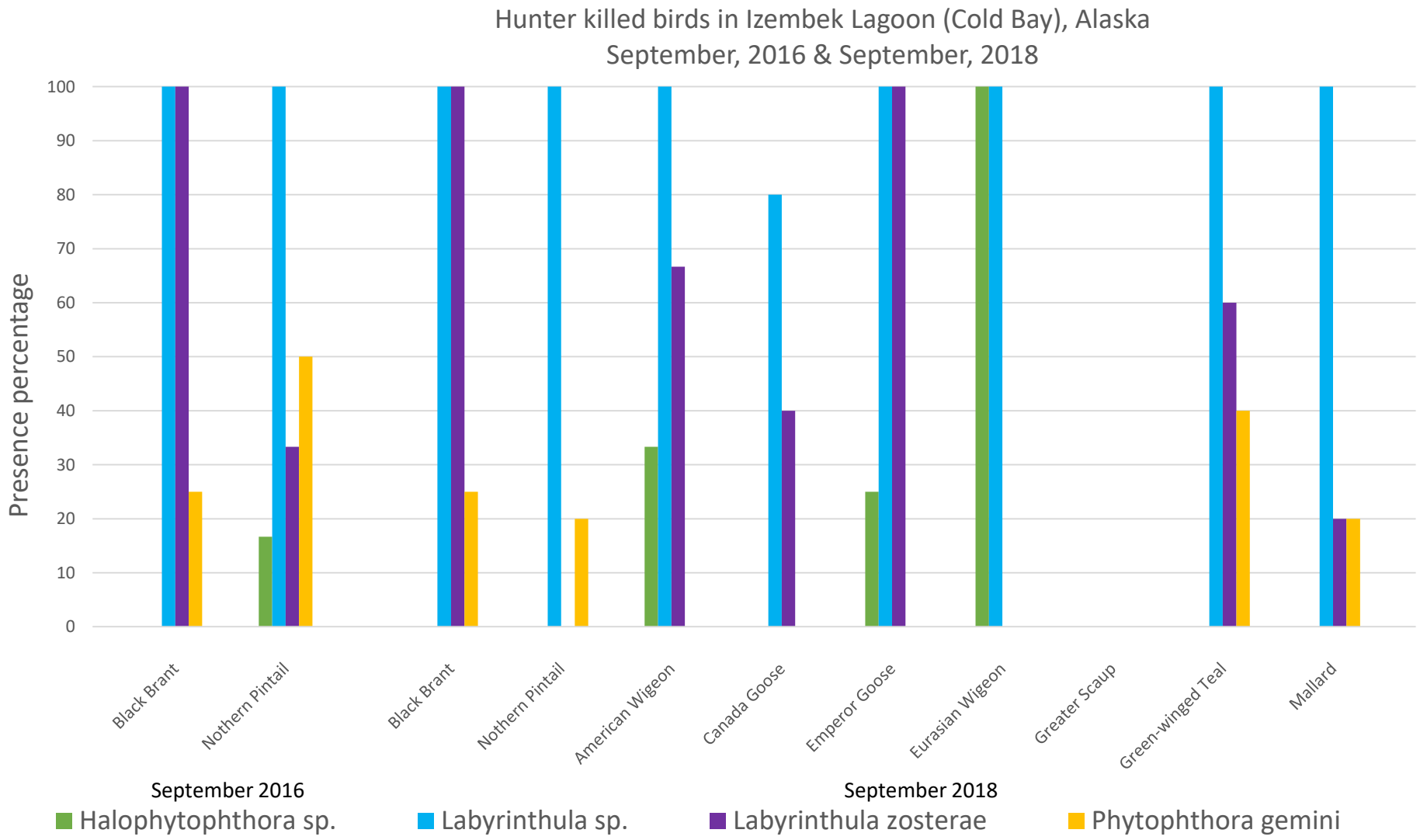
- Alaska fish – 12S (Menning et al., 2018)
- Alaska fish – *Coregonus* sp. (Cytb1, Cytb2) (writing)
- Alaska fish – *Cottus* sp. (Cytb) (writing)
- Alaska fish – *Oncorhynchus* sp. (Cytb) (writing)
- Alaska fish – *Osmerus* sp. (Cytb) (writing)
- Alaska fish – *Salvelinus* sp. (COI) (writing)
- *Elodea* sp. (5.8S, atpB-rbcL, trnL, trnL-F) (in prep)
- *Gavia* sp. (Cytb, NADH) (writing)
- Plants (trnL) (Taberlet et al., 1991; Taberlet et al., 2007)
- Seagrass (*Zostera marina*) pathogens (writing)
    - *Labyrinthula* sp. (5.8S, 18S)
    - *Halophytophthora* sp. (ITS)
    - *Phytophthora* sp. (COI, ITS)
- Cestodes (12S, 16S, 28S) (Greiman et al., 2018)
- Bacteria (16S) (Zeglin et al., 2016)

Multiplex samples

Izembek Lagoon (Cold Bay), Alaska
January 21, 2016 - August 17, 2018



Multiplex samples



Multispecies/Metabarcoding with eDNA

- Solutions
  1. Lack of sequence data – Experimental design
     - Initially conduct traditional survey in conjunction with eDNA development
  2. Analysis methods
     - Keep up with changes in the field
  3. Bioinformatics pipelines
     - Keep up with changes in the field
  4. Cost of initial development
     - Cheaper once system is developed
     - Plan ahead/Collaborate
     - Long term sample storage (EtOH, Longmire buffer (Longmire et al., 1997))

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Questions?

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