Building a Comprehensive and Accessible DNA Barcode Database for Fish and Invertebrates in the San Francisco Estuary

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Background

- Environmental DNA (eDNA) metabarcoding methods rely on DNA barcode reference sequences for each species.
- Some SFE species already have DNA barcode reference sequences available in public databases, but some do not have have barcode data and many have only partial data.
- We are building a custom DNA barcode reference sequence database for SFE fish and invertebrates.
- This database is the first phase of a multiyear Prop 1 funded project to develop eDNA metabarcoding methods to complement existing SFE monitoring.
- The custom database will be made public as a resource to all scientists and will enable accurate taxonomic assignments from eDNA metabarcoding sequencing data. The data will also be useful for other genetic applications.

Approach

Collect SFE specimens → Extract DNA → PCR amplify ~5 barcode regions → DNA sequencing → Assemble database



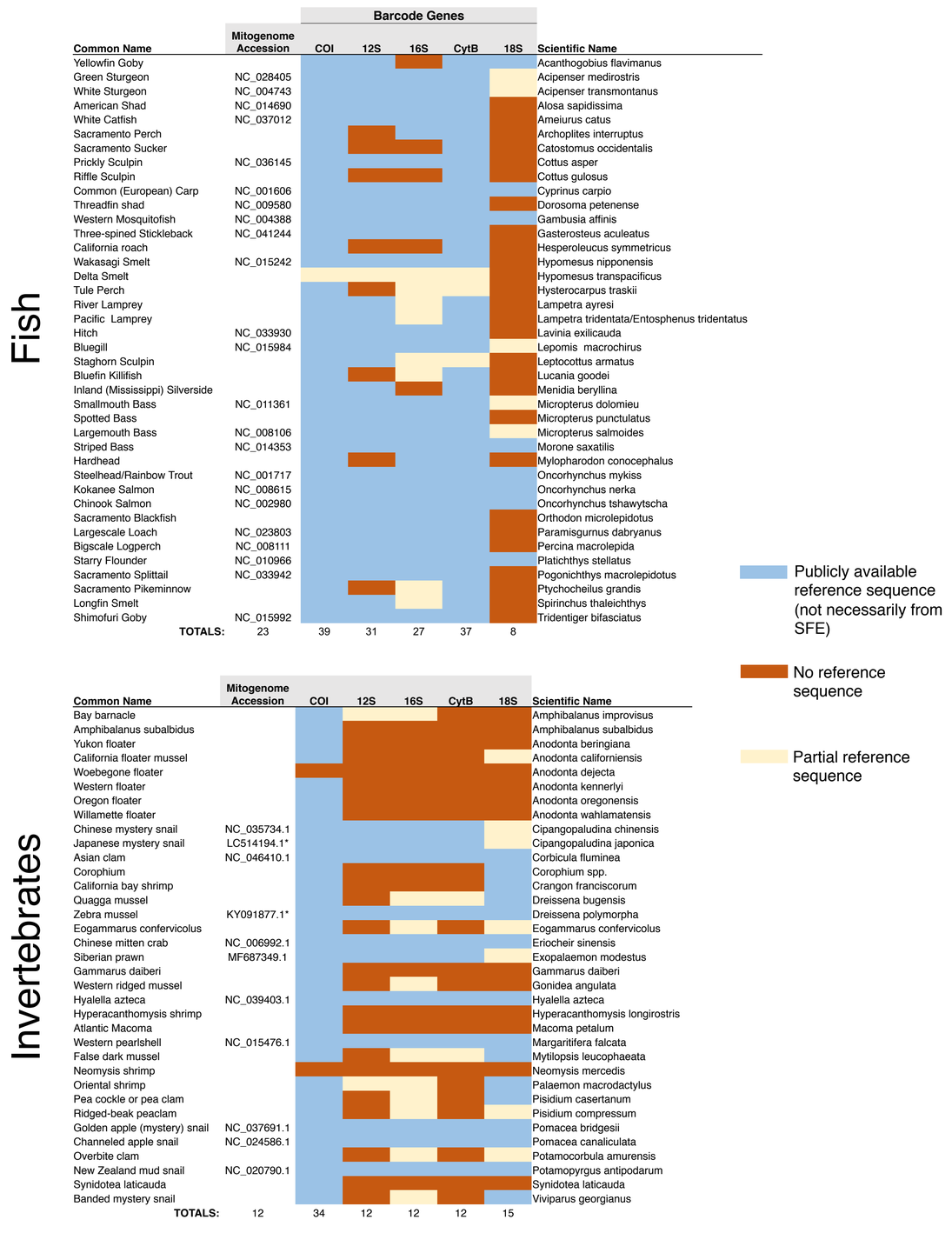
Collection of invasive zebra mussels (*Dreissena polymorpha*) from San Justo Reservoir, CA. Photos credit: Heather Perry, CDFW

- Specimen collection and DNA extractions ongoing
- PCR primer testing complete (see below)
- Goal for database is to sequence ~5 PCR products for each specimen
  - Some barcoding genes contain multiple barcodes
  - eDNA metabarcoding accuracy is improved by sequencing >1 barcode for each sample
  - For reference database sequencing, we are prioritizing PCR primers that will give us sequence information for multiple barcodes from one PCR/sequencing reaction
- DNA sequencing started July 2020 (delayed due to COVID-19 lab shutdown)
- Some extracted DNAs will also be used to create known DNA mixtures to test eDNA metabarcoding protocol prior to using with eDNA samples

PCR primers tested for Sanger DNA sequencing:

Gene	Type	Primer names (Forward/Reverse)	Reference
Cytochrome C Oxidase 1 (COI)	mitochondrial	jgLCO1490/jgHCO2198	Geller et al 2013
12S rRNA	mitochondrial	tRNA-Phe/teleo_R	Doble et al 2019
12S rRNA	mitochondrial	MiFish-U-F/MiFish-U-R	Miya et al. 2015
16s rRNA	mitochondrial	Ac16s-F/Ac16s-R	Evans et al. 2015
16s rRNA	mitochondrial	16s_Metazoa_fw/16s_Metazoa_rev	Shelton et al 2016
16s rRNA	mitochondrial	16S-V5-F/16S-V5-F	Riaz et al. 2011
Cytochrome B (CytB)	mitochondrial	L14912/H15149c	Burgener and Hubner 1998
18S rRNA	nuclear	SSU-F1/SSU-R568	Tanabe et al 2015
18S rRNA	nuclear	SSU-F1289/SSU-R1772	Tanabe et al 2015

Target Species



- Most SFE fish and invertebrate species have existing COI reference sequences
- References for other commonly used barcode genes (12S, 16S, Cytb, and 18S) are still needed for many SFE species
- Our objective is to fill in as many of these gaps as possible

Summary

- Database expected completion by **December 2020.**
- The database will improve the accuracy and utility of eDNA metabarcoding.
- Will enable biodiversity monitoring using eDNA metabarcoding, complementing and augmenting existing IEP monitoring.

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