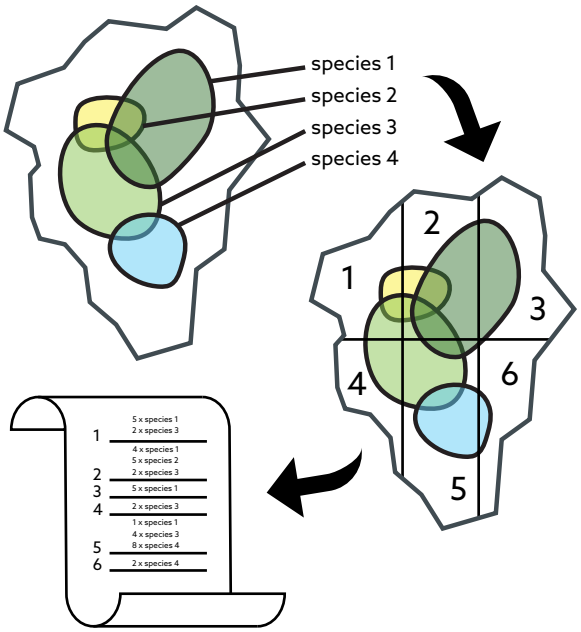
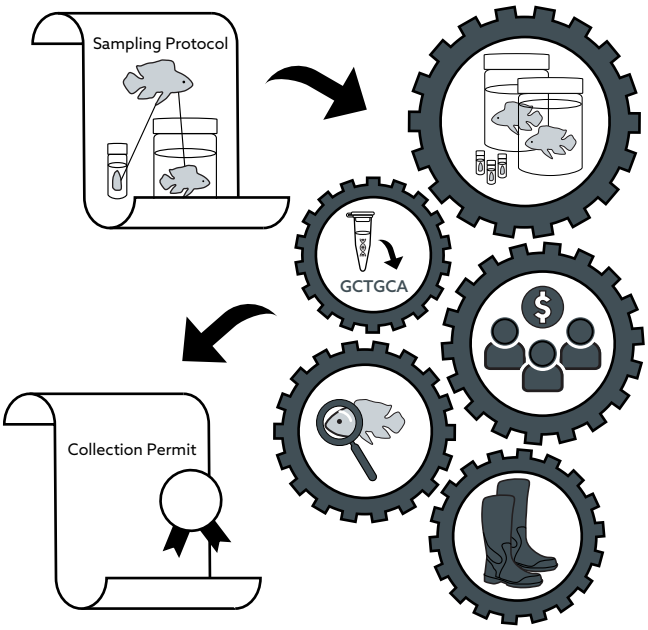


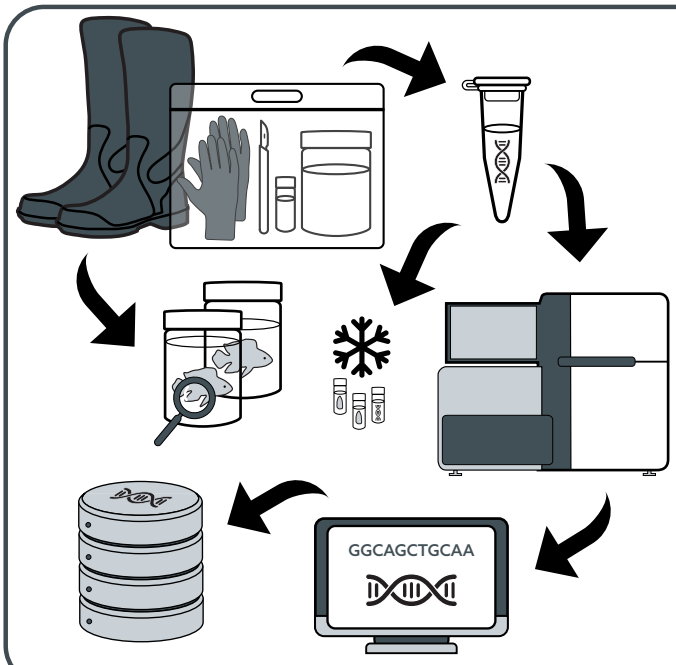
- Identify all target species present in your region of interest and determine their ranges.
- Divide your region into manageable subregions for sampling.
- Create a sampling plan to collect 3-10 individuals per species throughout its range.

- Within our study area, the state of Oregon, we identified 146 freshwater fish species and members of species complexes, both native and nonnative as resident target taxa.
- Because we were collecting freshwater species, it made sense to divided our study area into hydrologic units. Six parent regions—Coast, Lower Columbia, Willamette, Middle Columbia, Snake, and Closed Basins—were divided into 34 smaller basins.
- We set a goal of collecting 10 individuals of each species throughout its range.



- For your target taxa, research standard protocols for collecting and storing samples and, if taxonomic verification is needed, vouchers for downstream DNA analysis. Draft a sampling protocol and field datasheet for metadata.
- Gather your team, strategize collection, draft budget, seek funding. Identify where vouchers, if collected, samples and DNA extracts will be stored and taxonomically verified. Develop a wet-lab pipeline appropriate for your target taxa—adapted for HMW DNA if possible—locate a genetics lab and sequencing facilities.
- Apply for collection permits, if required.

- We developed a sampling protocol—included in SI—for the humane collection of fishes and stored tissues in 95% EtOH at -80°C to ensure proper preservation for DNA analysis. Our Field Notes Sheet is included in SI.
- Our core team included researchers from Oregon State University, the US Forest Service, and the Oregon Department of Fish and Wildlife (ODFW). We used the Cronn Lab for DNA extraction and library preparation, the Center for Qualitative Life Sciences for sequencing, and the Oregon State Ichthyology Collection (OSIC) to verify taxonomic assignments and store vouchers, tissues, and extracts.
- Our collection permits were managed by ODFW.



- Purchase supplies, assemble collection kits, and get boots on the ground to begin collection. Distribute samples to genetics lab and accession voucher specimens.
- Extract DNA, using HMW extraction protocols if possible. Accession samples—generally tissues or blood—and extracts and store at -80°C.
- Sequence samples once enough extracts are ready to fill a sequencing lane with sufficient coverage for your target taxa's mtDNA.
- Assemble mitogenome sequences using an appropriate bioinformatics pipeline.
- Store sequences on GenBank. Use a purpose-built client-server database, if desired.

- Details about our collection kit are included in SI. Tissues, extracts, and full-body vouchers are stored and taxonomically verified with OSIC.
- We extracted DNA from subsampled tissues using the Qiagen DNeasy Blood and Tissue Kit spin-column protocol for animal tissues. For more details, see Methods.
- We sequenced a mean of 67 samples per lane on an Illumina HiSeq 3000.
- SPAdes assembler successfully resolved most of our mitogenome sequences.
- Our data are available on GenBank and in SI, and obgpdb.org is reserved for a stand-alone client-server database.