

Additional guidelines for the bull trout eDNA surveys

Foremost, thank you for helping with this project. Our goal is ambitious—to collect eDNA samples at regular intervals in nearly all cold-water habitats throughout the western U.S. that have a greater than 25% probability of occupancy by juvenile bull trout. Delineation of the cold-water habitats and predictions of occupancy are based on the NorWeST stream temperature model and Climate Shield habitat occupancy model (Isaak et al. 2015). To achieve that goal, we have developed a database and sampling framework that consists of a master set of geospatially referenced sampling locations at 1-km intervals within each cold-water habitat. We also identified sampling locations at this same interval based on the USFWS's designation of critical spawning and rearing habitat. Based on field tests of eDNA detection probabilities conducted by the National Genomics Center for Wildlife and Fish Conservation, this sampling approach will reliably determine the presence of populations of bull trout, as well as provide insights on non-spawning habitats used by adult and subadult fish. The result will be a rapid, robust, and repeatable range-wide assessment of natal habitats of this species.

Sampling

Sample collection and labeling. - Our eDNA collection protocol (Carim et al. 2016) addresses how to obtain a sample at a site, and identifies some issues we've encountered with the equipment or difficult circumstances. Although a protocol is included in the pump set you may have checked out from us, you can also contact us or see our website for the latest version of the protocol (<http://www.fs.fed.us/research/genomics-center/edna/>). There is one addition to that protocol: each sampling bag and its protective envelope should also be labeled with the site ID (see below), in addition to the stream name, geographic coordinates, and sampling date e.g.:

Boulder Creek
654-1 (**this is the site ID**)
11 652341 5231164 (**UTM coordinates, NAD83/WGS84**)
8/1/15

Spatial template and sampling approach. - We have mapped [eDNA sampling points](#) for each 4th-code hydrologic unit (8-digit HUC) throughout the western U.S. The spreadsheet that accompanies each map assigns a label (site_ID) to each eDNA sampling site, and groups sites within individual cold-water habitats (Patch_ID). The spreadsheet provides information on the probability of occupancy and on environmental covariates associated with each site or cold-water habitat. **Use that spreadsheet to electronically report your data to us, as well as sending us the labeled eDNA sample bags & envelopes.**

There are three requirements with regard to sampling logistics. First, it is important to **sample all the points in each cold-water habitat** (i.e., the set of eDNA points sharing a single Patch_ID). The Climate Shield model makes a prediction that juvenile bull trout will be present within a single cold-water habitat, but does not predict which part or how much of that habitat will be occupied. The eDNA sampling addresses that, but only if the entire cold-water habitat is sampled. Also, at some of those eDNA points, the stream may be dry or have insufficient flow to support a fish population (see Issues, below). We need your evaluation of those sites so we know they have been inventoried and not overlooked. Second, **the cold-water habitat should be sampled at one time** (that is, within a week or two). Bull trout are highly mobile, and that mobility can confuse our interpretation of which habitats are continuously occupied by juvenile life stages if sampling is prolonged. Third, to avoid the possibility that *you* are the source of bull trout eDNA in a sample, we recommend that you **sample a basin from downstream to upstream**. Sometimes, for reasons of safety or logistics, it is necessary to do otherwise. In those cases, if you are moving quickly, you tend to move faster than water is flowing and ameliorate this concern. If sampling requires more than one day, however, we recommend moving downstream to upstream.

Issues

Summer 2015 was the beta-testing phase of this project, and we found some bumps along the way as we shared our methods, maps, and priorities. Your suggestions for resolving any problems or identifying overlooked areas would be most appreciated as we begin the full project rollout from 2016 to 2018. Some issues we have already encountered and their solutions:

Streams at eDNA points are dry.	Record the date on the spreadsheet. Write "dry" in notes column.
Streams at eDNA points are < 0.5 m wetted width.	Record the date on the spreadsheet. Write "too small" in notes column. You may still collect the sample, which we will archive for studies of other species.
eDNA sampling points are on private land or dangerous to access.	If another location can be sampled within 0.5 km, sample that point and assign the same site ID. If no closer access is possible (or there is no access to any point on a stream), record the date on the spreadsheet and write "no access" in the notes column.
eDNA sampling points are assigned to locations known to be fishless (e.g., because of migration barriers).	These sites do not need to be sampled. The source of the information that bull trout are absent is requested. If there is uncertainty, we recommend collecting samples at the eDNA points.
eDNA sampling points are assigned to locations already known to host juvenile bull trout or spawning adult bull trout.	Because bull trout are already known to be present, these sites do not need to be sampled. The source of the information that bull trout juveniles or spawning adults is requested. Should you wish to sample to determine the upstream extent of bull trout, please sample at the designated eDNA points.

And if you have any questions, thoughts, or recommendations about the project, please contact us. Thanks!



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