Hood Canal Coordinating Council
Jefferson, Kitsap & Mason Counties; Port Gamble S'Klallam & Skokomish Tribes
17791 Fjord Drive NE, Suite 118, Poulsbo, WA 98370

Hood Canal Regional PIC Program

eDNA Subgroup Meeting

Apr. 1, 2021, 10:00 am - 12:00 pm
Via Zoom

Meeting Objective:

- ID opportunities and coordinate resources to apply molecular methods for PIC investigations using environmental DNA (eDNA)

Attendees

Leslie Banigan, Kitsap Public Health District	Glenn Gately, Jefferson Conservation District
Anne Baxter, WA State Dept. of Ecology	Renee Johnson, Kitsap Public Health District
Seth Book, Skokomish Indian Tribe	Paul McCollum, Port Gamble S'Klallam Tribe
Hans Daubenberger, Port Gamble S'Klallam Tribe	Julian Sammons, Skokomish Indian Tribe
Mike Dawson, Jefferson Co. Public Health	Nate White, Hood Canal Coordinating Council

Welcome and Background

Any eDNA work is supplemental to the Regional PIC grant (it is not a deliverable and there is no dedicated money to pay for it)

Difference between eDNA and Microbial Source Tracking (MST):

- eDNA
  - Describes what DNA is in environment
    - Ex. Confirms if other animals beside humans are polluting the water
  - Useful if you don't know what you're looking for/don't know the pollution source
- MST
  - Useful molecular-based assays that identify specific genetic sequences in eDNA

Previous applications

- Jefferson County/Jefferson Conservation District/Skokomish Tribe
  - MST analysis in Chimacum Ck & Discovery Bay (worked with EPA Manchester Lab)
  - Sampling in Hood Canal and Duckabush watersheds
- Kitsap County
  - Collected eDNA samples in Lofall Creek (worked with EPA Manchester Lab)
- Port Gamble S'Klallam Tribe (PGST)
  - Experience doing eDNA analysis
    - Process: filter water sample, extract DNA, run through PCR to amplify DNA

sequences with high throughput method
        - Results compared to international gene datasets to determine the animals
        - Have to have a comprehensive library of DNA, otherwise may not know the animal causing the pollution (e.g. Sequim yak)
    - Useful on the reservation: insight into contamination sources that were unexplained, and outside the thinking at the time
        - e.g. river otters, raccoons, possums (these animals were confirmed with animal cams = useful technique to verify)
        - Also looked for human hits by looking for gut bacteria: none found
    - Most recently contracted with Smith-Root to process eDNA samples
        - Smith-Root has a relationship set up with a lab in Canada that has high throughput sequencing capabilities
- WA Dept. of Ecology
    - Used MST to look at bacterial communities in restored/unrestored streams
    - Used eDNA high throughput methods
        - $80/sample
        - Gives you a pie chart with bacteria (how often a read showed up in data):
        - Can be misleading if you compare environments with differing amounts of eDNA or if you have inhibitors present in a sample

Identify analytical needs and desired applications of eDNA

Analytical needs

- It is another tool in the toolbox to improve PIC investigations
- Situations where it is hard to ID pollution source
- Helps rule out other potential pollution sources

Desired applications

- Identify mysterious fecal pollution sources that may or may not be human
- Use eDNA investigations to clarify and explain the complicated science of bacterial water pollution issues so the public understands what the data means
- Pursue collection of a few preliminary eDNA samples to begin with, not a full project (a full project is more expensive and requires more staff capacity)

Brainstorm HCRPIC eDNA logistical/technical processes

HCRPIC partners decided on the following process to pursue eDNA:

<u>Form a technical workgroup to:</u>

- Determine the issues practitioners want solved
    - Will inform methodologies selected
    - Start with HCRPIC group, Anne's contacts
    - Ex. What warm-blooded animal species could be associated with (these indicator bacteria) at (this hot spot)?
- Determine sampling methodologies
    - Focus on warm blooded animals

    - Types of methodologies
        - High throughput sequencing
            - Gives you the best species it matched with
            - Need a DNA sequence library for different species to be effective
            - $80 quote, includes DNA extraction (Anne)
            - $250/sample (PGST)
        - qPCR
            - Gives you the relative abundance of a particular species
            - Works best if you know what to look for/have a question to answer
        - Microarray
            - Gives you a relative abundance of presence/absence of a species
            - Similar to qPCR
        - Open array
            - Best for when you know the variables you're looking for
            - Open array plates could be the best bang for the buck right now
            - Can also include salmon DNA, among other things
- Determine sample collection techniques
    - How will we process clean eDNA samples to prevent false negatives?
        - Want to avoid red herrings: need an accurate portrayal of water pollution that doesn't skew perception of problem toward animals, especially if there are human sources present, and vice versa
    - Best longevity storage practices
        - Don't want to store diluted DNA for long period (want it to be highest concentration possible)
        - Can put in -20, -80 freezer (shipping expensive for -80)
        - To be safe, filter in field, centrifuge to create pellet?
    - Develop easy procedure that can be replicated by anyone
        - Ex. Collect clean sample to avoid false positives
        - Ex. Field filtration
        - Ex. Centrifuge
        - Ex. Freezer storage

Find a (preferably local) lab willing to process eDNA results

- Local labs
    - Skokomish Tribe
        - Their water quality lab is unavailable for outside use (liability issues)
        - May still pursue eDNA sampling themselves just for Skokomish River, their reservation, mid-Skokomish Valley, and around their shellfish beds
    - PGST
        - Have some lab space they could set up for qPCR analysis to extract eDNA, but not amplification
            - Lab not currently set up: estimate $50,000-60,000 to set up
            - Prefer to partner with an existing lab over setting up their own lab

        - Can store samples for preprocessing (easier if there is a big batch) to save time if necessary, but main labs can do all the steps
        - They offered their auto sampler for use
    - USGS Marrowstone Lab
        - qPCR machine set up?
        - PGST has a good relationship with them
    - EPA Manchester?
        - Doesn't have much capacity?
        - More willing to help if there is an established eDNA/MST project?
            - Need a quality assurance plan in place for them to work with us?
            - Potential cost sharing if processing samples was part of a project?
    - University of Washington (UW)
        eDNA resources webpage
- Other labs
    - EPA (Cincinnati)/USGS (Portland):
        - Worked with Jefferson Co. to develop collection methods and process lake cyanobacteria samples
    - Source Molecular
        - May be able to process bulk samples?
        - Renee Johnson (Kitsap Co) has worked with them before
    - Smith-Root/Canada lab
        - Hans Daubenberger (PGST) has worked with them before

Find a way to pay for preliminary eDNA sample processing

- PGST offered to pay for three processing samples/month @ $80-100/sample

Long-term: set up a lab in Hood Canal?

- Skokomish Tribe may be willing to offer their lab if an official eDNA project is established
- Monitoring to Accelerate Recovery: potential funding source?

Next Steps

Individual subgroup members volunteered to pursue the following next steps:

Form a technical workgroup

- Leslie: Determine questions for practitioners
- Hans, Anne: Determine sampling methodologies
- Hans, Anne: Determine sample collection techniques

Find a (preferably local) lab willing to process eDNA results

- Paul M.: Reach out to USGS Marrowstone, EPA Manchester, UW eDNA resources
- Anne: Reach out to WDFW, DOH, other state labs

HCCC will check in with technical workgroup members at the June 2, 2021 HCRPIC Guidance Group meeting to determine progress made and whether/when to schedule a follow up

meeting.

In the meantime, technical workgroup/eDNA subgroup members can use this link to upload any relevant eDNA-related information to a new eDNA subgroup Box folder that everyone can access.