Environmental and fish health assessment of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) from Walker River basin

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Lahontan Cutthroat Trout

- *Oncorhynchus clarkii henshawi*
- California and Nevada
- Extirpated from ~95% of their habitat
- Threatened species under the Federal Endangered Species Act
- State fish hatcheries have been increasing their production and stocking to expand fishing opportunities within their native drainage

https://www.wildlife.ca.gov/Conservation/Fishes/Lahontan-Cutthroat-Trout
Some pathogens of concern

- Parasites
  - *Ceratanova shasta*
  - *Myxobolus cerebralis*
  - *Tetracapsuloides bryosalmonae*

- Fungi
  - *Veronaea botryosa*

- Bacteria
  - *Flavobacterium columnare*
  - *Flavobacterium psychrophilum*
  - *Renibacterium salmoninarum*

- Viruses
  - IHN
  - IPN
  - VHS
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AFS-FHS Bluebook
http://www.afs-fhs.org/perch/resources/140832286331.3.1bkd2014.pdf
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- **Viruses**
  - Infectious hematopoietic necrosis (IHN)
  - Infectious pancreatic necrosis (IPN)
  - Viral hemorrhagic septicemia (VHS)
Some pathogens of concern

• Other aquatic animal pathogens
  – Ranavirus
  – *Batrachochytrium dendrobatidis*

Environmental DNA

- Powerful tool for:
  - Evaluating the presence of microorganisms
    - Direct observation is difficult or impossible
  - Assessing biodiversity in aquatic environments.
Objectives of Study

- To determine abundance of aquatic animal pathogens in resident LCT following conventional and molecular diagnostic methods.

- To determine abundance of fish and amphibian pathogens in sediment and water samples via eDNA analysis.

Preliminary step for the potential hatchery rearing of Walker basin strain LCT to be used for conservation and/or recreational purposes.
Sample collection

- July 2017
- N=60 LCT
  - Ovarian fluid
- N=6 water
- N=6 substrate
Environmental samples

- **Substrate**
  - Collection of 0.5 kg of substrate in individual containers at time of collection
    - 3 upstream and 3 downstream
  - A subsample (50-100g) saved at -80C for DNA extraction
    - MoBio PowerMax Soil DNA Isolation Kit
    - Concentration of DNA using MoBio protocol
      - Eluted with 200 µL water
Environmental samples

• Water
  • Collection of 1L of water in individual containers at time of collection
    • 3 upstream and 3 downstream
  • Vacuum filtration of water samples using 5 μm filter membrane
  • Acetone treatment (to dissolve filter paper), DNA extraction, and real-time PCR performed
Necropsy and sample collection

- Tissues from individual fish were pooled and subjected to DNA extraction MoBio Power Soil DNA Isolation Kit
  - Heart
  - Spleen
  - Kidney
  - Distal intestine
  - Biopsy of periocular tissue
Molecular diagnosis


• Quantitative PCR developed by UC Davis CORE PCR Facility.


• Nicole Strepparava, Thomas Wahli, Helmut Segner and Orlando Petrini. Detection and quantification of *Flavobacterium psychrophilum* in water and fish tissue samples by quantitative real time PCR. BMC Microbiology 2014, 14:105.


Molecular Diagnosis Results

• Environmental samples
  – Substrate
    • All negative
  – Water
    • One positive for *F. psychrophilum* (Ct of 35)

• Fish tissue samples
  – One positive for *F. columnare* (Ct of 38)
  – Internal control (Ct 23-27)
Molecular Diagnosis on Pooled samples

- N=12 pools (kidney/spleen)
  - 5 fish per pool
- *Tetracapsuloides bryosalmonae*
- *Nucleospora salmonis*
- All negative
Spleen/kidney subsamples were pooled (n=5 individuals)

Ovarian fluid

Grind the organs and suspend them in media with antimicrobials

Inoculate supernatants into EPC and CHSE-214 cell lines

Incubate at 15°C for 3 weeks

Look microscopically for cytopathic effect

No CPE was observed after 3 weeks of incubation
Conclusion and future work

• The sampled population appear to be free of the tested pathogens or have a lower load than the limit of detection of the assays.

• Samples from juvenile LCT and Brook trout (*Salvelinus fontinalis*) collected in September are pending.