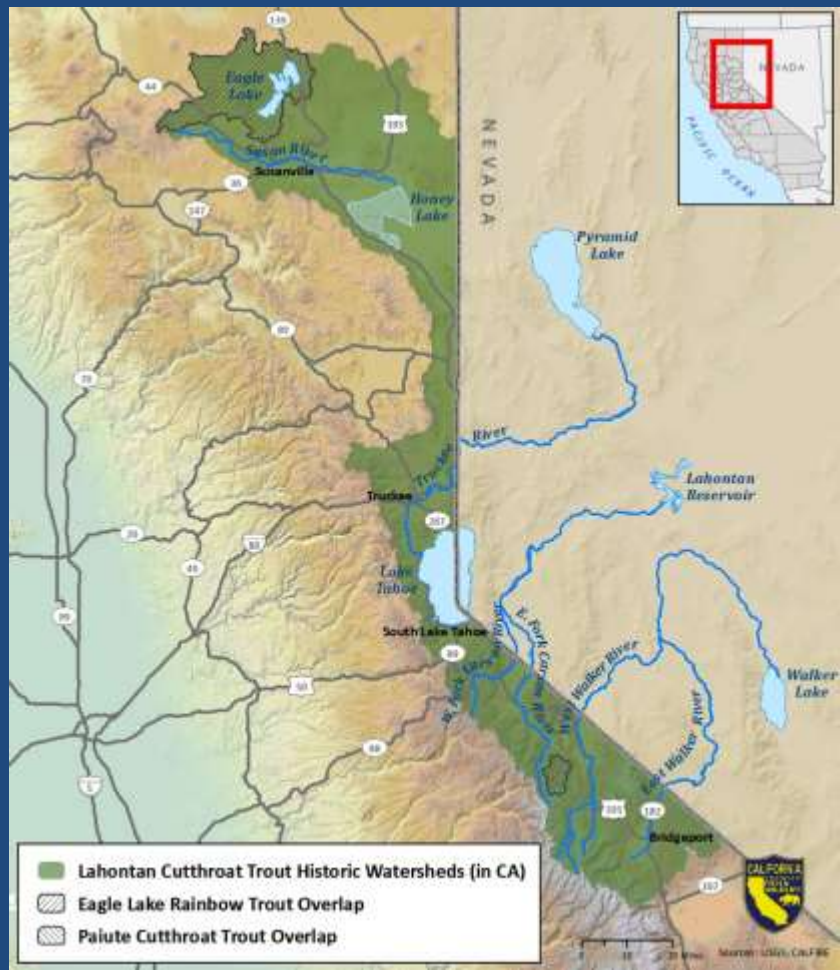
Environmental and fish health assessment of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) from Walker River basin

Esteban Soto[1,*], Joe Maret[2], Christine Richey[2], Kavery Mukkatira[2], Susan Yun[1], Kirsten Kenelty[1], Adrian Porras[1], Jeff Weaver[3], Dave Lentz[3]; Brett Anderson[4], Mark Adkison[2]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis
[2] Fish Pathology Laboratory, California Department of Fish and Wildlife
[3] Fisheries Branch, Heritage and Wild Trout Program, California Department of Fish and Wildlife
[4]Fisheries Branch, Inland Fisheries Assessment and Monitoring Program, California Department of Fish and Wildlife

Lahontan Cutthroat Trout

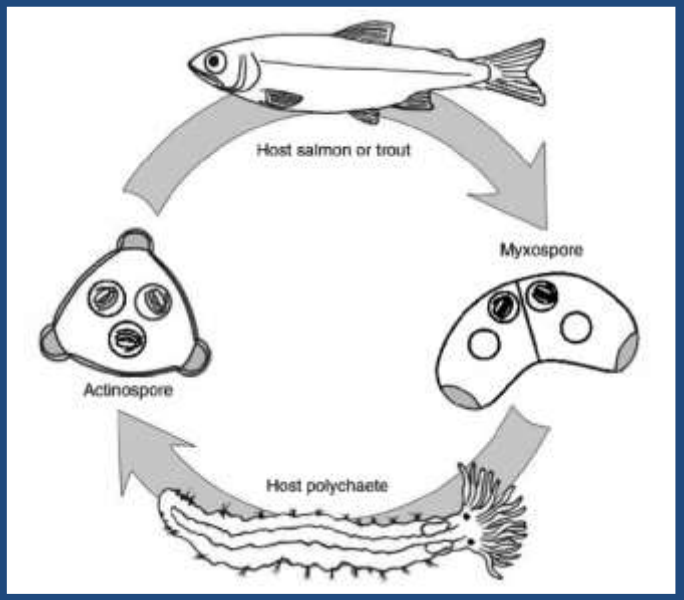
- *Oncorhynchus clarkii henshawi*
- California and Nevada
- Extirpated from ~95% of their habitat
- Threatened species under the Federal Endangered Species Act
- State fish hatcheries have been increasing their production and stocking to expand fishing opportunities within their native drainage



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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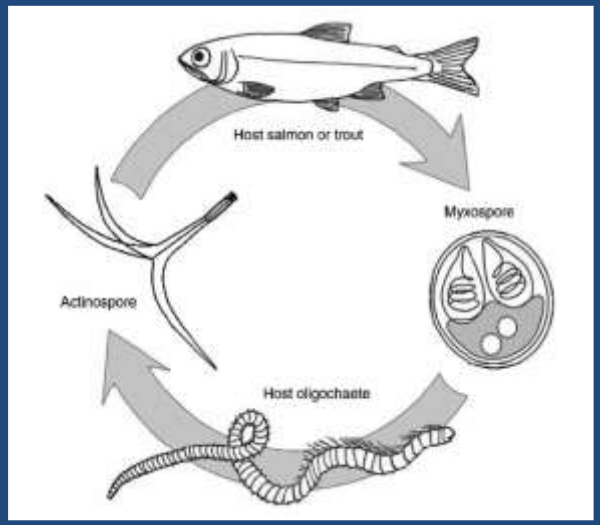

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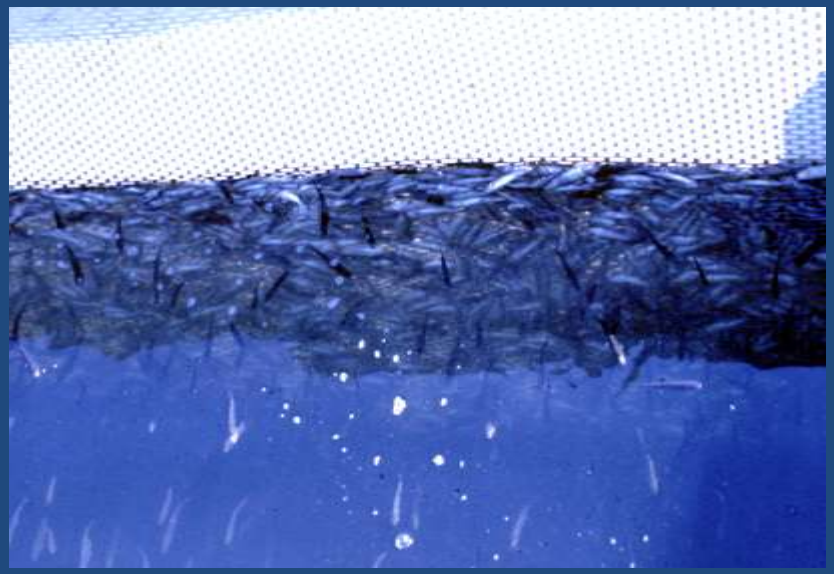

- Fungi
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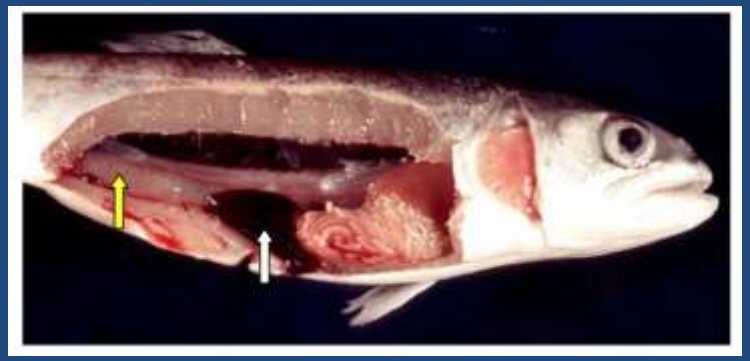

http://afs-fhs.org/perch/resources/14069248683.2.17nucleospora2014.pdf

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Figure 3. Abdominal distension associated with ascites in a juvenile Chinook salmon affected by BKD. Photo credit Ron Pascho.

Figure 1. Kidney lesions in a juvenile Chinook salmon *Oncorhynchus tshawytscha* affected by BKD. Photo courtesy of Dr. Caroline O'Farrell.

AFS-FHS Bluebook
http://www.afs-fhs.org/perch/resources/14069228631.3.1bkd2014.pdf

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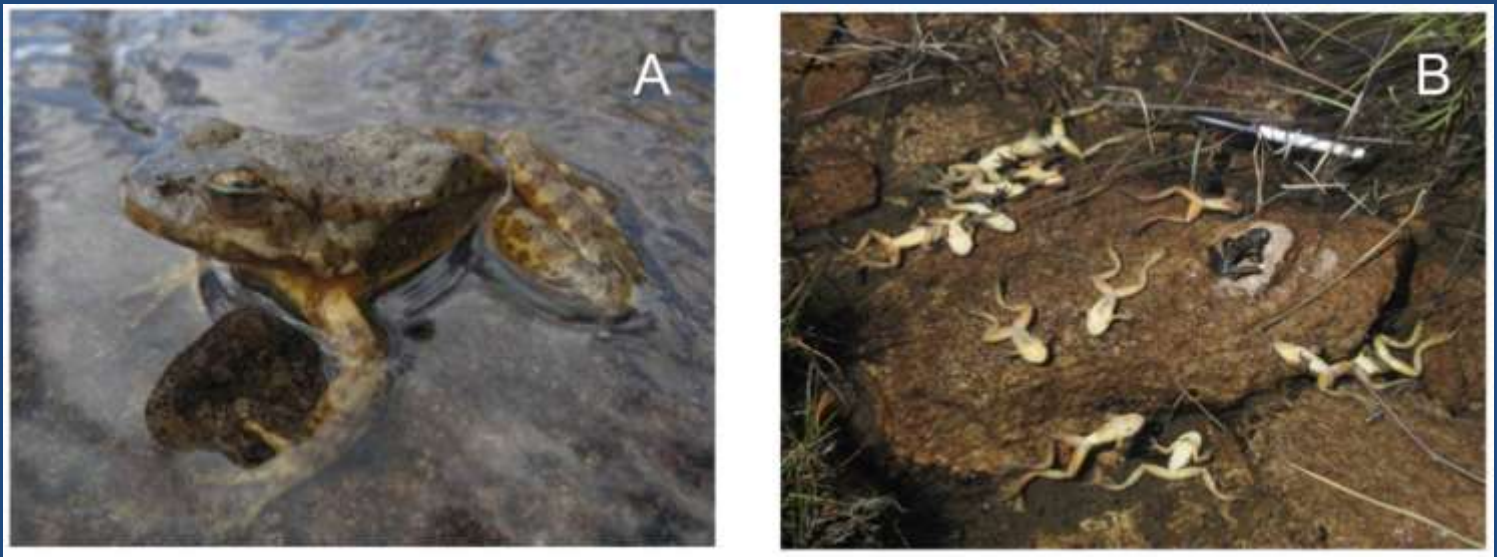
  - *Renibacterium salmoninarum*
- Viruses
  - Infectious hematopoietic necrosis (IHN)
  - Infectious pancreatic necrosis (IPN)
  - Viral hemorrhagic septicemia (VHS)

Some pathogens of concern

- Other aquatic animal pathogens
  - Ranavirus
  - *Batrachochytrium dendrobatidis*



PLoS One. 2012; 7(4): e35374.

Environmental DNA

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

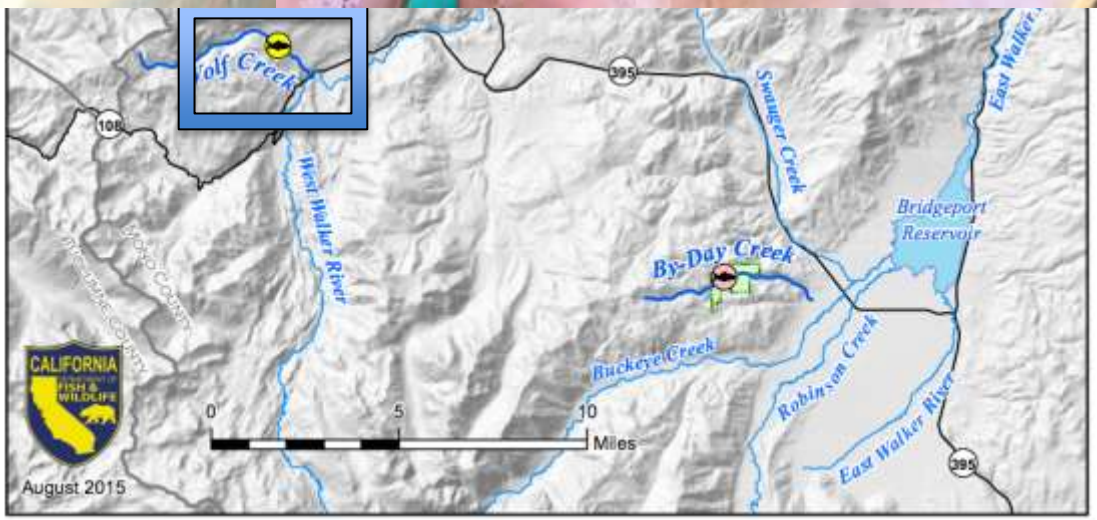
Objectives of Study

- 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

UCDAVIS VETERINARY MEDICINE

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

- Hallett SL, Bartholomew JL. (2006). Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Diseases of aquatic organisms 71(2):109-118

- Kelley GO, Zagmutt-Vergara FJ, Leutenegger CM, Myklebust KA, Adkison MA, McDowell TS, Marty GD, Kahler AL, Bush AL, Gardner IA, Hedrick RP. (2004). Evaluation of five diagnostic methods for the detection and quantification of *Myxobolus cerebralis.* Journal of Veterinary Diagnostic Investigation 16(3):202-211.

- Quantitative PCR developed by UC Davis CORE PCR Facility.

- Matthew C.Allender, DavidBunick, Mark A.Mitchell (2012). Development and validation of TaqMan quantitative PCR for detection of frog virus 3-like virus in eastern box turtles (*Terrapene carolina carolina*) Journal of Virological Methods. Volume 188, Issues 1–2, March 2013, Pages 121-125.

- Victor S. Panangala, Craig A. Shoemaker & Phillip H. Klesius. TaqMan real-time polymerase chain reaction assay for rapid detection of *Flavobacterium columnare*. Aquaculture Research, 2007, 38, 508-517.

- 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.

- Chase, D. M., D. G. Elliott, and R. J. Pascho. 2006. Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. Journal of Veterinary Diagnostic Investigation 18:375-380.

- Kent, M. L., J. Khattra, D. M. L. Hervio, and R. H. Devlin. 1998. Ribosomal DNA sequence analysis of isolates of the PKX myxosporean and their relationship to members of the genus *Sphaerospora*. Journal of Aquatic Animal Health 10:12-21.

- Protocol modified from: Barlough, J. E., T. S. McDowell, A. Milani, L. Bigornia, S. B. Slemenda, N. J. Pieniazek, and R. P. Hedrick. 1995. Nested polymerase chain reaction for detection of *Enterocytozoon salmonis* genomic DNA in Chinook salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms 23:17-23.

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

UCDAVIS VETERINARY MEDICINE

Virology

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