Freshwater Sponges in Eastern Washington:
Environmental Constraints, Species Composition, and Associated Photobionts

Lucy Roussa, Camille McNeely, & Jessica Allen
Department of Biology, Eastern Washington University

Background & Sample Design

❖ Freshwater sponges are filter-feeding animals that harbor non-obligate algal symbionts in their tissues. In return for the sponge's protection, the algae provides its host with sugars produced from photosynthesis.

❖ Freshwater sponges in North America are poorly studied compared to other freshwater invertebrates.

❖ There are no published studies of freshwater sponge distribution in eastern Washington.

❖ The objective of this study is to explore habitat requirements, community composition, and photobiont preference of freshwater sponges.

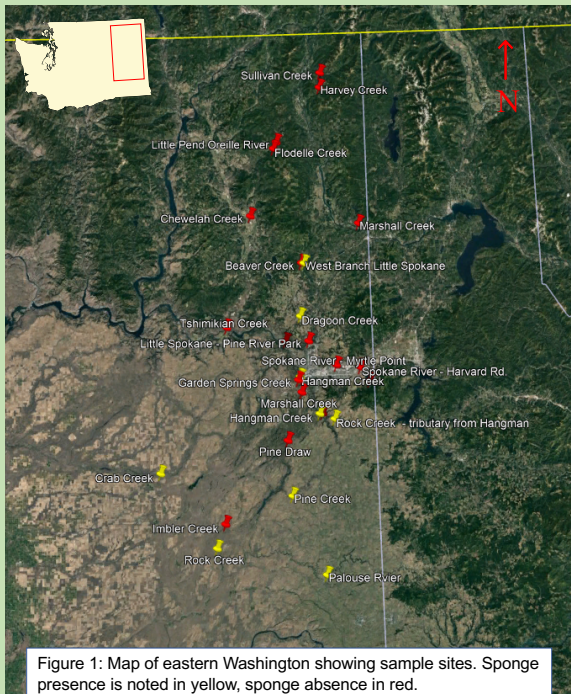

Figure 1: Map of eastern Washington showing sample sites. Sponge presence is noted in yellow, sponge absence in red.

❖ 27 streams in Eastern Washington were searched for 1 hour each, and at least 100 substrate particles were examined per site.

❖ We measured water quality parameters including temperature, dissolved oxygen, turbidity, pH, oxidative-reductive potential (ORP), and concentrations of nitrogen and phosphorus.

❖ Where sponges were present, we collected specimens to identify using DNA sequencing and microscopy. Flow was measured each time a sponge was collected.

❖ Laboratory work on sponge and algal DNA sequencing is still ongoing.
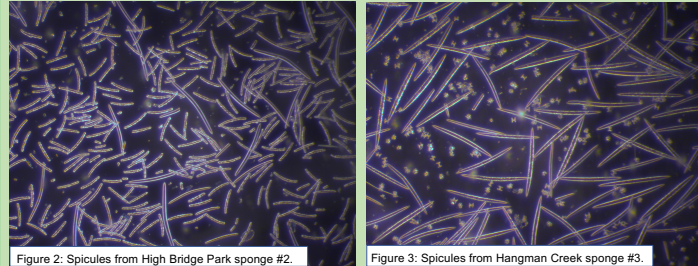
Microscopy: Morphology of Spicules

Figure 2: Spicules from High Bridge Park sponge #2.

Figure 3: Spicules from Hangman Creek sponge #3.

❖ Sponge tissue was digested in nitric acid for two hours to dissolve tissue. This leaves behind spicules, the silicate "backbone" of sponges.

❖ Morphology of spicules (megascleres, microscleres, and gemmuloscleres) is variable amongst genera, making them a vital tool for identification.
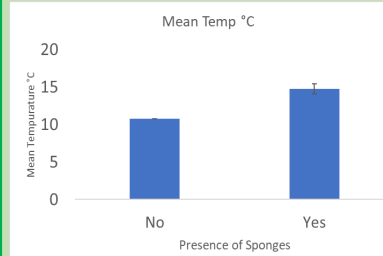
The Importance of Temperature

❖ Freshwater sponges have adapted to environments with harsh seasonality. Their dormant stage, known as gemmules, allows them to survive being completely frozen and complete desiccation.

Figure 5. Water was significantly warmer at sites where sponges were present compared to sites without sponges (t-test, t = 3.43, df = , P= 0.003).

No other water quality variables differed significantly between sites with and without sponges.
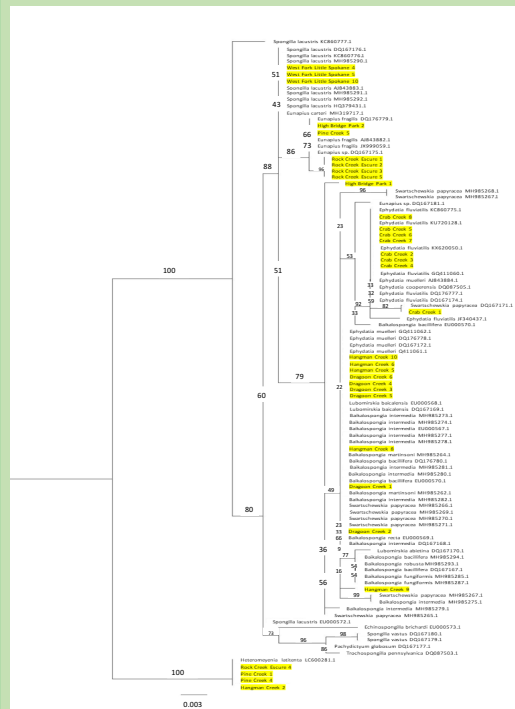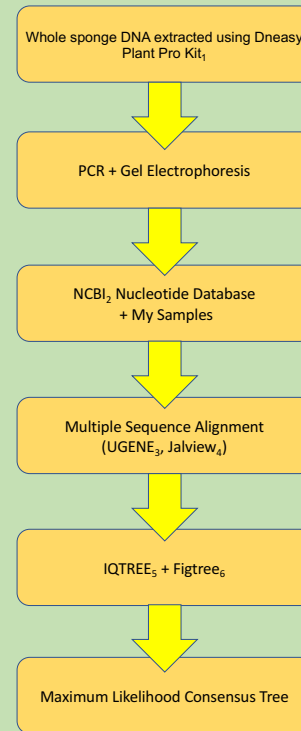
Phylogenetic Tree from COI Gene Region

Figure 4: Maximum-likelihood tree built using COI gene sequences in IQTree with 1,000 bootstrap replicates. Support values represent percentage of bootstrap replicates that recovered the same topology. Samples sequences for this study are highlighted in yellow.

DNA Pipeline

Whole sponge DNA extracted using Dneasy Plant Pro Kit[1]

⬇

PCR + Gel Electrophoresis

⬇

NCBI[2] Nucleotide Database + My Samples

⬇

Multiple Sequence Alignment (UGENE[3], Jalview[4])

⬇

IQTREE[5] + Figtree[6]

⬇

Maximum Likelihood Consensus Tree

Results Summary & Next Steps

❖ Sponges were found in sunny, shallow streams but not forested, narrow streams which suggests the sponges prefer streams with more photosynthetic activity. This preference may benefit their algal partners or be linked to availability of suspended food particles.

❖ We confirmed 4 common North American genera in Eastern Washington through a combination of spicule morphology and genetic differences in the COI gene region.

❖ At least 2 additional taxa within our samples are not clearly associated with North American taxa. We hope to clarify their taxonomy though sequencing an additional gene region.

❖ Algal DNA still needs to be sequenced. The rbcL and ITS regions will be used to identify algal species.

Acknowledgments

References: 1. Dneasy Quaigen, Inc., 2. Bethesda (1988) National Library of Medicine (US), National Center for Biotechnology Information. [cited 2022 Mar 13]. Available from: https://www.ncbi.nlm.nih.gov/ 3. Okonechnikov K, Golosova O, Fursov M, the UGENE team. Unipro UGENE: a unified bioinformatics toolkit . Bioinformatics 2012 28: 1166-1167. doi:10.1093/bioinformatics/bts091 4. Waterhouse AM, Procter JB, Martin DM, et al. (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25(9):1189-91. 5. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37(5):1530-4. 6. Andrew Rambaut Institute of Evolutionary Biology University of Edinburgh

Ashworth Laboratories Edinburgh EH9 3JT

TRiO McNair Scholar Program