

# Analysis of The Microbial Content of An Invisible Fish Barrier

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## Abstract

- An Invisible fish barrier is an undefined barrier that is preventing the return of fish to spawning grounds
- Sites were chosen from previous year analysis and were characterized using the iNaturalist platform to identify species within the riparian zone
- 16SRNA sequencing was used for bacterial identification
- Sites that lack species diversity in their riparian zones, and were closer to urbanization, show diversity in potentially harmful microbes in correlation to low fish population.
- These sites also selected for gram-positive bacteria.
- Monitoring among these sites will be continued to identify find trends.

## Introduction

Stream microbiomes are composed of diverse collection of microbes that play an important role in keeping the surrounding riparian zones and life within the streams healthy and thriving. The Chehalis River watershed runs throughout southwestern Washington, emptying into Grays Harbor, an estuary of the Pacific Ocean. Native fish species include *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, *Gasterosteus aculeatus*, *Lampreta spp.*, and *Acipenser transmontanus* (1). The Chehalis River provides habitats in favor of fish spawning and juvenile development, but fish return populations upstream have decreased in recent years. Returns have been significantly lower than expected, falling below conservation objectives (2). Future spawning within the Chehalis watershed are not expected to meet spawning escapement goals. Understanding microbiome analysis of the Chehalis River in correlation to fish population, could help to statistically link microbes within the stream that are potentially causing an invisible fish barrier affecting fish return.

An invisible fish barrier is an undefined barrier that is blocking or preventing the return of fish. When water conditions are altered by human impacts or water quality becomes poor, it can result in microbial communities to shift. These communities can become harmful and potentially pathogenic to the surrounding hosts and environment (3). In this case, we believe the presence of certain microbes are influencing the fish return to spawning grounds, thus creating an invisible fish barrier.

Studies regarding stream microbiome analysis as correlated to fish returns have never been taken into consideration. In the past, studies have only looked at watershed aquatic life and surrounding environments as related to urbanization factors like culverts, pollution and dams (4). Although urbanization does influence the entire ecosystem, to analyze microbes specifically within the stream in relation to fish populations could help to find ways to improve overall water quality for fish health. This analysis may also provide a holistic indicator for necessary renovation and restoration of fish passages. Studies have shown that species of bacteria found in or on fish are also present within the stream in corresponding numbers, revealing that environmental parameters can be linked to altering fish microbiota structures (5).

In addition to providing habitat for fish and wildlife, the Chehalis River watershed is crucial to the culture and economy of the local residents. A developing challenge caused by the decrease in fish returns within the Chehalis River watershed is having to limit or cease commercial and recreational fishing, leading to consequences felt by the community at large. Streams that are fish bearing not only provide optimal health of the overall ecosystem, allowing other organisms within the stream to grow and improve the surrounding riparian zone, they support the human community nutritionally, economically, and culturally.

The aim of this study was to identify/characterize microbes within the Chehalis River at 7 sites, in correlation to fish populations, that are part of an invisible fish barrier preventing salmon from returning to their spawning grounds.

## Methods

With 50ml falcon tube, 2 water samples were taken from each of the 7 sites off the Chehalis River at 6-8 in below surface. Half of the falcon tubes were sent to Omega BioServices for 16SRNA sequencing for Bacterial ID. The other half were taken to the lab immediately for analysis on Mannitol and MacConkey plates.

Riparian zones were observed within a 10 ft radius from the water sampling sites with the iNaturalist platform to identify plants, fungi, and insects present. Comments were given regarding how many were noticed within the sites; few= 1-2, many= 3-10, abundant=>10. dissolved oxygen, pH and temperature were taken at each site to analyze water quality.

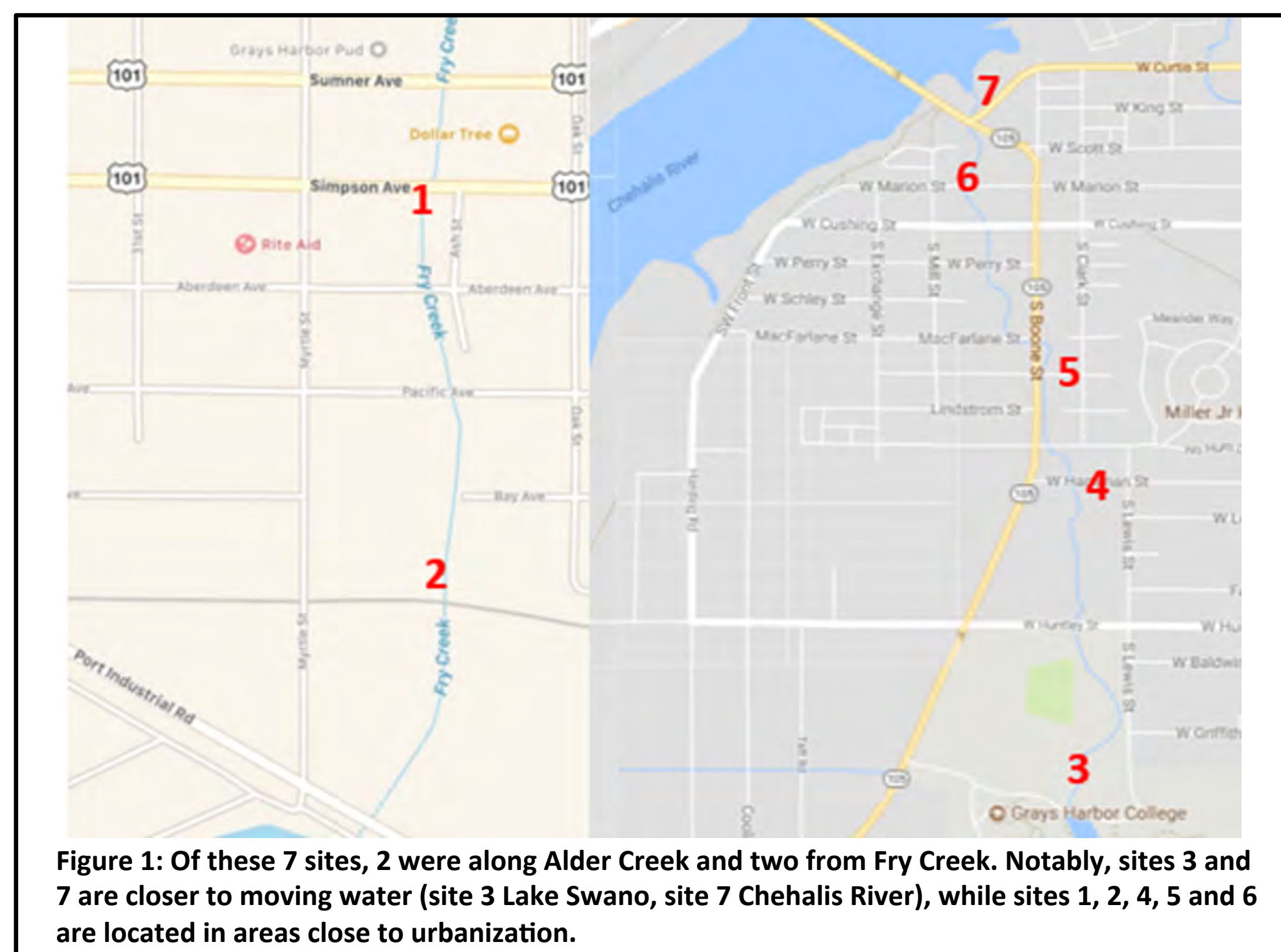


Figure 1: Of these 7 sites, 2 were along Alder Creek and two from Fry Creek. Notably, sites 3 and 7 are closer to moving water (site 3 Lake Swano, site 7 Chehalis River), while sites 1, 2, 4, 5 and 6 are located in areas close to urbanization.

## Results

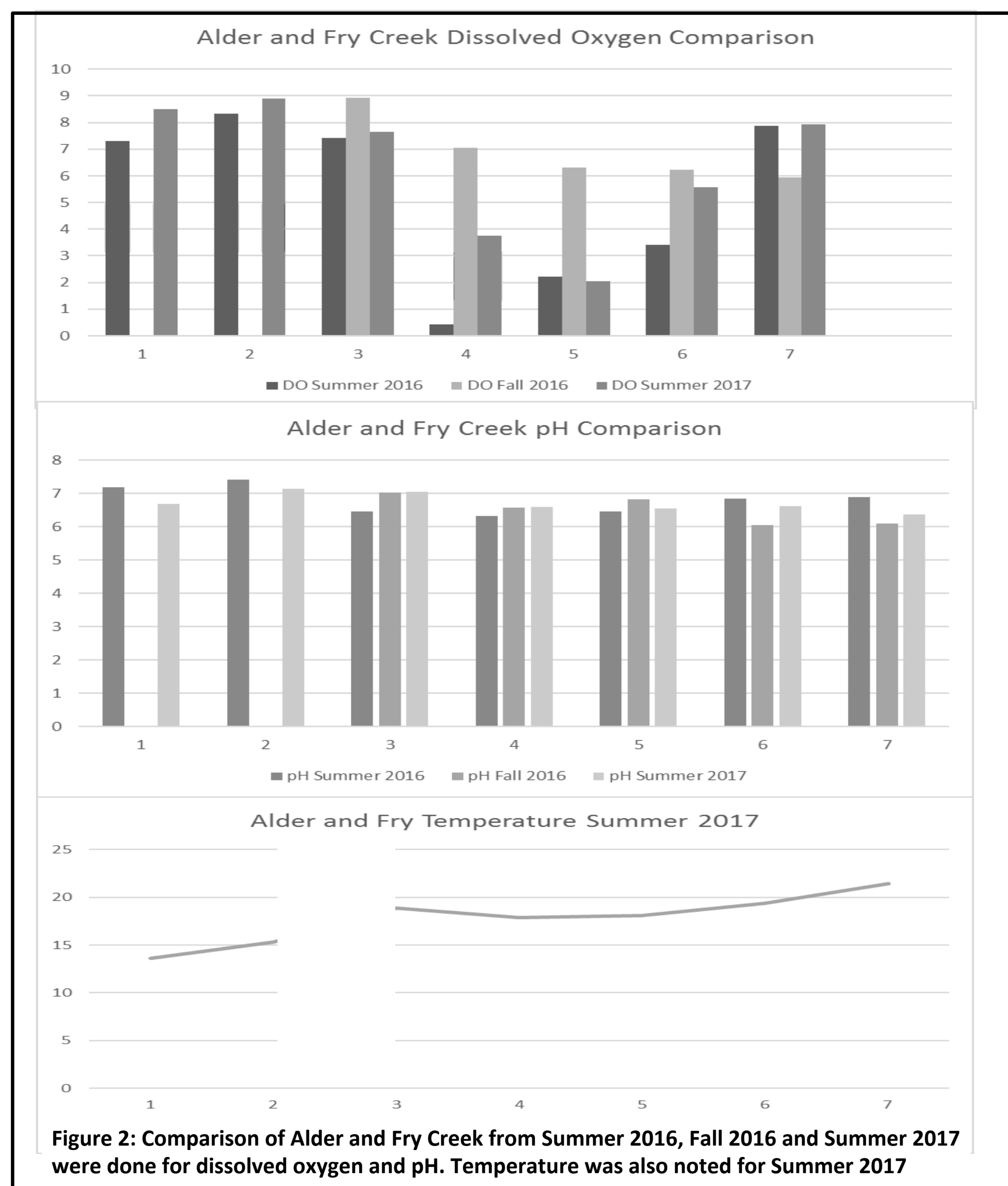


Figure 2: Comparison of Alder and Fry Creek from Summer 2016, Fall 2016 and Summer 2017 were done for dissolved oxygen and pH. Temperature was also noted for Summer 2017

| Genus          | Species      | Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Genus         | Species        | Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|----------------|--------------|------|---|---|---|---|---|---|---|---------------|----------------|------|---|---|---|---|---|---|---|
| Acer           | cinereum     |      |   |   |   |   |   |   |   | Azoreus       | disseminatus   |      |   |   |   |   |   |   |   |
| Alisma         | triviale     |      |   |   |   |   |   |   |   | Apis          | melifera       |      |   |   |   |   |   |   |   |
| Alnus          | rhombifolia  |      |   |   |   |   |   |   |   | Ariolimnax    | columbianus    |      |   |   |   |   |   |   |   |
| Alnus          | rubra        |      |   |   |   |   |   |   |   | Aquarius      | remigis        |      |   |   |   |   |   |   |   |
| Athyrium       | flix-femina  |      |   |   |   |   |   |   |   | Bombus        | melanopygus    |      |   |   |   |   |   |   |   |
| Cirsium        |              |      |   |   |   |   |   |   |   | Bombus        | voesnesensis   |      |   |   |   |   |   |   |   |
| Equisetum      | arvense      |      |   |   |   |   |   |   |   | Forficula     | auricularia    |      |   |   |   |   |   |   |   |
| Hedera         | helix        |      |   |   |   |   |   |   |   | Haploterena   | varicovirens   |      |   |   |   |   |   |   |   |
| Hypochaeris    | radicata     |      |   |   |   |   |   |   |   | Ichneura      | cerulea        |      |   |   |   |   |   |   |   |
| Impatiens      |              |      |   |   |   |   |   |   |   | Ichneura      | eragria        |      |   |   |   |   |   |   |   |
| Knautia        | pratensis    |      |   |   |   |   |   |   |   | Libellula     | quadrimaculata |      |   |   |   |   |   |   |   |
| Lemna          | minor        |      |   |   |   |   |   |   |   | Musca         | domestica      |      |   |   |   |   |   |   |   |
| Lysichiton     |              |      |   |   |   |   |   |   |   | Sympetrum     | illotum        |      |   |   |   |   |   |   |   |
| Malanthemum    | dilatatum    |      |   |   |   |   |   |   |   | Tyria         | jacobeae       |      |   |   |   |   |   |   |   |
| Oenanthe       | serotiflora  |      |   |   |   |   |   |   |   | Genus         | Species        | Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Oplismenus     | hirsutus     |      |   |   |   |   |   |   |   | Anas          | platyrhynchos  |      |   |   |   |   |   |   |   |
| Phalaris       | arundinacea  |      |   |   |   |   |   |   |   | Branta        | canadensis     |      |   |   |   |   |   |   |   |
| Picea          | sitchensis   |      |   |   |   |   |   |   |   | Cathartes     | aura           |      |   |   |   |   |   |   |   |
| Polystichum    | munifolium   |      |   |   |   |   |   |   |   | Corvus        | brachyrynchos  |      |   |   |   |   |   |   |   |
| Prunella       | vulgaris     |      |   |   |   |   |   |   |   | Cyanocitta    | stelleri       |      |   |   |   |   |   |   |   |
| Pteridium      | aquilinum    |      |   |   |   |   |   |   |   | Larus         |                |      |   |   |   |   |   |   |   |
| Rosa           |              |      |   |   |   |   |   |   |   | Passer        | domesticus     |      |   |   |   |   |   |   |   |
| Rubus          | spectabilis  |      |   |   |   |   |   |   |   | Phalacrocorax | auritus        |      |   |   |   |   |   |   |   |
| Rubus          | urinus       |      |   |   |   |   |   |   |   | Streptopelia  | desiaco        |      |   |   |   |   |   |   |   |
| Sambucus       | racemosa     |      |   |   |   |   |   |   |   | Turdus        | migratorius    |      |   |   |   |   |   |   |   |
| Schoenoplectus | acutus       |      |   |   |   |   |   |   |   | Genus         | Species        | Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Sparaganium    | emersum      |      |   |   |   |   |   |   |   | Canis         |                |      |   |   |   |   |   |   |   |
| Stachys        | chamissonis  |      |   |   |   |   |   |   |   | Castor        |                |      |   |   |   |   |   |   |   |
| Thuja          | plicata      |      |   |   |   |   |   |   |   | Felis         | catus          |      |   |   |   |   |   |   |   |
| Trifolium      | repens       |      |   |   |   |   |   |   |   | Gasterosteus  | aculeatus      |      |   |   |   |   |   |   |   |
| Tsuga          | heterophylla |      |   |   |   |   |   |   |   | Rana          | cascadae       |      |   |   |   |   |   |   |   |
| Typha          | angustifolia |      |   |   |   |   |   |   |   | Thamnophis    |                |      |   |   |   |   |   |   |   |
| Vaccinium      | parvifolium  |      |   |   |   |   |   |   |   | Oncorhynchus  | kisutch        |      |   |   |   |   |   |   |   |
|                |              |      |   |   |   |   |   |   |   | Ondatra       | zibethicus     |      |   |   |   |   |   |   |   |

Figure 3: Plant, insects and animals were documented at each site with the iNaturalist platform. Notes were given as seen; few 1-2, many 3-10, abundant >10.

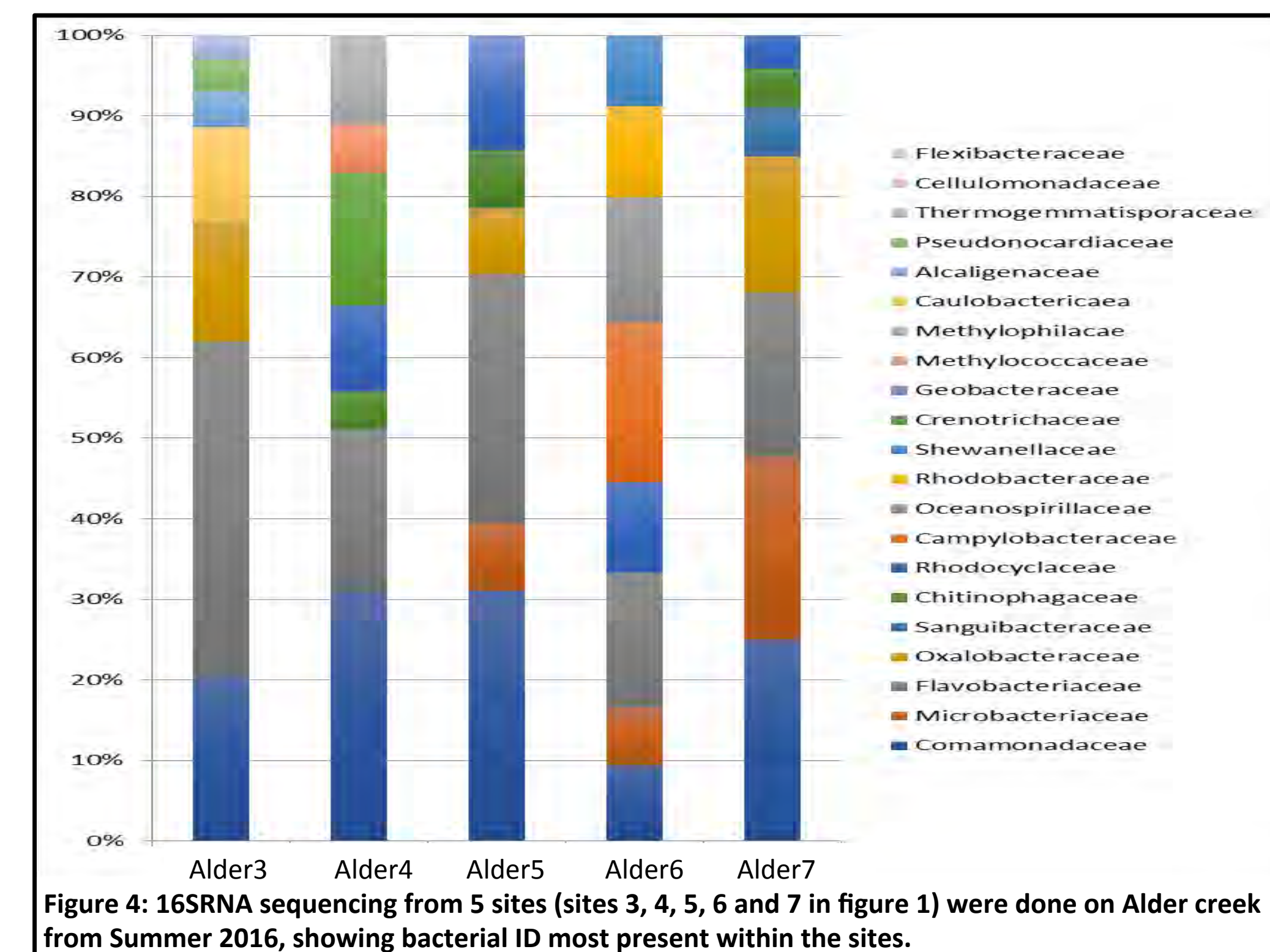


Figure 4: 16SRNA sequencing from 5 sites (sites 3, 4, 5, 6 and 7 in figure 1) were done on Alder creek from Summer 2016, showing bacterial ID most present within the sites.

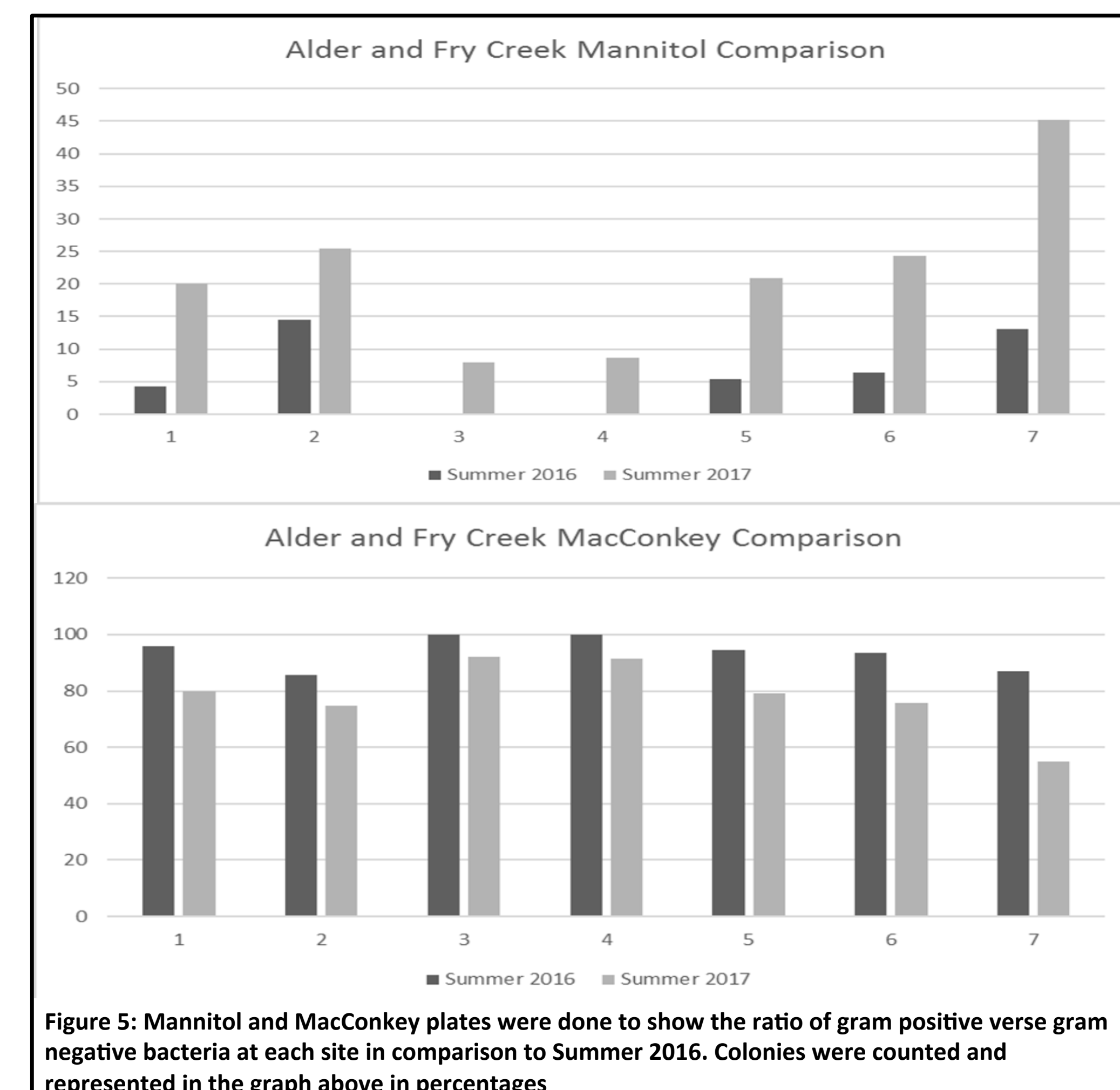


Figure 5: Mannitol and MacConkey plates were done to show the ratio of gram positive versus gram negative bacteria at each site in comparison to Summer 2016. Colonies were counted and represented in the graph above in percentages

## Discussion

Water quality throughout the 7 sites show roughly the same pH and dissolved oxygen when compared to Summer and Fall 2016, with slight deviation due to temperature changes in water affecting the streams ability to dissolve oxygen. At sites 4, 5 and 6 dissolved oxygen is lower as well as *Gasterosteus aculeatus* and *Oncorhynchus kisutch* population. Access to organic material like decomposition of plants or pollution run offs can affect streams water quality into becoming stagnant water(6). Aquatic life has a harder time surviving and provide an optimal growth for bacteria that utilize eutrophic conditions and anaerobes.

Healthy stream riparian zones contain diversity within plant, fungi, animal and insect species. Diversity among the ecosystems riparian zone provide the aquatic life in streams with food resources and habitat structure (7). Native plant species like *Athyrium flix-femina*, *Rubus ursinus*, and *Tsuga heterophylla* are most present in areas where *Gasterosteus aculeatus* and *Oncorhynchus kisutch* were documented. Riparian zones containing native species help water quality conditions by providing stabilized soil and the ability to filter time runoffs and abundance of harmful microbes. Areas where native species are high in diversity can be correlated to limiting influences on the invisible fish barrier.

Microbiome analysis of stream water is expected to show bacteria from the family *Flavobacteriaceae*, *Comamonadaceae* and *Pseudonocardiaceae*. Some species can be opportunistic pathogens and can be harmful to fish species but otherwise play a normal role in maintaining the streams health, although *Comamonadaceae* is associated with wastewater. In sites 5, 6, and 7 where there is high transient populations and pollutions, there is an abundance of the family *Campylobacteraceae*, *Sanguibacteraceae* and *Shewanellaceae*. *Campylobacteraceae* species can be commensal or pathogenic and can thrive as microaerophilic, anaerobic and/or aerobic in conditions where water quality is poor. Species of *Sanguibacteraceae* and *Shewanellaceae* are both opportunistic human and fish pathogens and can influence fish return.

The most prominent bacteria type present when ran on Mannitol and MacConkey plates appeared as being gram negative, which is consistent between summer 2016-2017, however there is an incremental increase in gram positive species that will be monitored closely to determine whether this is a trend. However, sites 6 and 7 show an increase in Gram-positive bacteria and can be linked to the 16SRNA sequencing. These sites showed an abundance of gram positive bacteria families which could potentially be contributing to the invisible fish barrier.

This is year two of an ongoing project. Fall 2016 and Summer 2017 water samples are in process for 16S sequencing. In addition to this analysis, aquatic species analysis will be added to this project Fall 2017, pending permit. Monitoring these sites will help correlate trends that are influencing riparian zones, water quality conditions and fish population.

## References

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