Microbial Biomarkers for Pre-Spawning Mortality in Chinook Salmon

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Pre-spawning mortality (PSM) presents a major problem for population recovery of spring Chinook salmon in the Willamette River basin. In certain reaches and years, PSM can exceed 90%, despite efforts to improve habitat and passage operations. PSM rates are highly variable between populations and years. At the population level, PSM associates with temperature and the density of hatchery origin fish, but drivers of PSM risk for individuals within a given population and year are poorly understood, as is the timing of PSM initiation. In this study, our goal was to identify nonlethal biomarkers of senescence (a precursor to PSM) in individual Chinook salmon. Ultimately, this tool could help us predict PSM risk for individual fish at various points in their freshwater migration and maturation. This information could inform rapid mitigation efforts for populations with many at-risk individuals and could be used to identify the causes of individual-level PSM risk. To achieve our goal of developing biomarkers for PSM, we pursued two specific objectives:

Objective 1. Generate a laboratory model of senescence using hatchery-reared juvenile Chinook salmon.

1. Treat juveniles with slow-release immunosuppressive stress hormones (cortisol and dexamethasone).
2. Evaluate senescence in this model by comparing stress hormones, morbidity, and pathology with control fish.

Objective 2. Identify gut microbiota that serve as biomarkers of senescence and morbidity.

1. Compare gut microbiome communities from senescent and morbid juveniles with control and surviving juveniles and identify specific gut microbes that associate with senescence and morbidity.
2. Compare results from our model system with a small pilot dataset from healthy and senescent adult fish.

Objective 1.

Juvenile Chinook salmon were obtained from South Santiam Hatchery and were intraperitoneally injected with slow-release vegetable shortening implants of stress hormones (cortisol or dexamethasone), or implant-only controls. Nonlethal gut swabs were collected from each fish after three weeks and seven weeks. Half the fish were euthanized three weeks post treatment to assess pathology and plasma cortisol levels, and the remaining fish were euthanized seven weeks post treatment. Mortality was 28% in cortisol-treated fish, 44% in dexamethasone-treated fish, and 11% in control fish. Histopathological analysis identified gill lesions with opportunistic pathogens (e.g., surface bacteria and *Ichthyobodo necatrix*) as the immediate cause of mortality, indicating that stress hormones caused reduced immune response in treated fish.

Cortisol assays suggested that stress hormone implants were effective up to three weeks post treatment but diminished by seven weeks, therefore future studies using this model system will seek to extend treatments to create an improved model of senescence.

Objective 2.

Nonlethal gut swabs were sequenced to identify gut microbiota from 83 experimental juvenile fish and 7 adult fish (3 healthy and 4 senescent). We identified gut microbiome communities that were significantly associated with treatment and morbidity (Figure 1), and the specific bacterial groups driving these associations. Microbiome composition also differed between healthy and senescent adults collected from the Willamette Hatchery, and the differences observed between these groups reflected the patterns observed in our experimental data (Figure 2). Overall, our results support the potential of gut microbiota as individual-level predictors of senescence and pre-spawning mortality. Future efforts will focus on collecting nonlethal gut microbiome data from adult fish at various points in their freshwater migration and evaluating the accuracy of the gut microbiome as a predictor of PSM on different time scales.

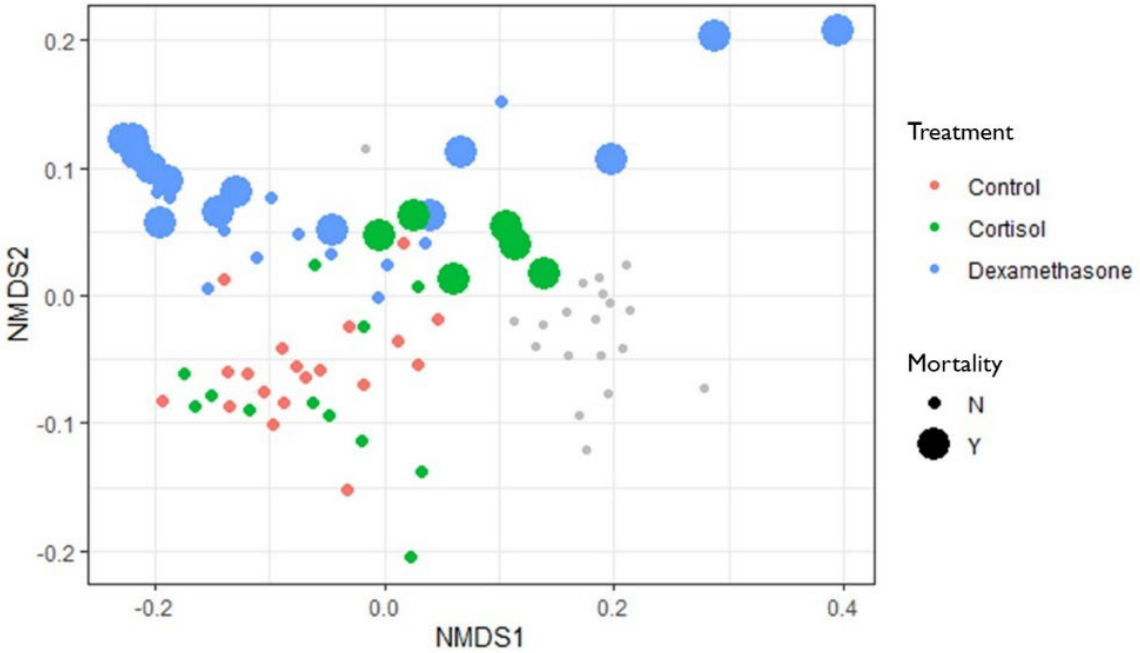

Figure 1: Microbiome community variation associated significantly with stress hormone treatment and morbidity among experimental juveniles. Distance between points represents microbiome community similarity. Treatment is indicated by color, and size indicates whether or not fish became morbid after sampling. Light gray points indicate fish that were collected at the last time point after treatment effects had waned.

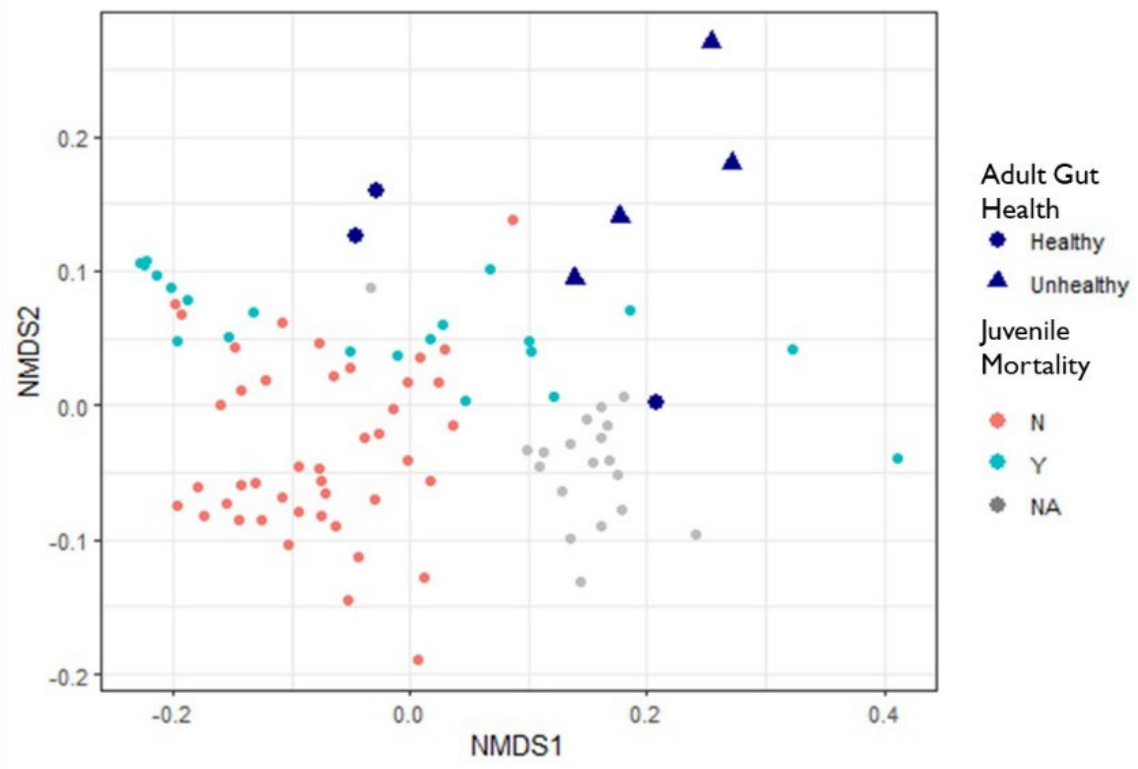

Figure 2: Adult fish had similar microbiome communities to those of morbid juveniles. The microbiomes of unhealthy adults (senescent based on histology) showed the most differences from surviving juveniles. Distance between points represents similarity between gut microbial communities. Smaller points indicate juvenile samples from the experimental model, with color indicating morbidity status. Larger, dark blue samples indicate adult samples, and within these, circles indicate healthy fish and triangles represent unhealthy (senescent) fish. Light gray points indicate juvenile fish that were collected at the last time point after treatment effects had waned.