Introduction: Recent advances in genetics and genomics have facilitated the exploration of a myriad of scientific questions concerning the ecology and management of commercially and recreationally important marine, freshwater, and anadromous species (migrating from the ocean to spawn in freshwater). These methods can be used to characterize and monitor biodiversity, delineate stock structure in highly migratory fishes, and guide management of the lifeblood of the Pacific Northwest: salmon and steelhead. Culturing these species in hatcheries has been used to mitigate the impacts of hydropower development, habitat alteration, and overharvest since the early 19th century. Oregon Department of Fish and Wildlife (ODFW) operates more than 30 hatcheries throughout the state, and in 2017, released over 40 million fish[1]. It is well established, however, that hatchery-origin fish (HOR) have lower reproductive success (fitness) compared to fish of natural-origin (NOR) when both are spawning in the wild[2], yet the mechanisms remain unclear. My research will focus on investigating a potential genetic mechanism to provide hatchery managers with the knowledge necessary to minimize fitness differences and negative impacts on wild populations.

Research Plan: To investigate genetic mechanisms of fitness differences, I will measure telomere length in two species of Pacific salmonids. Analogous to a cap on the end of a shoelace, telomeres are long repetitive nucleotide sequences located at the terminus of chromosomes in most eukaryotic cells. These repeats keep the core genetic material protected and prevent fusion between neighboring chromosomes. However, these repeat sequences are truncated with each cell division and at a critical threshold, the cell is signaled for destruction. Thus, telomeres have been implicated in aging and due to their responses to oxidative stress, have been used as biomarkers in the overall physiological state of an organism[3]. Previous work examining the physiological effects of telomere length in birds[4] and Atlantic salmon (*Salmo salar)*[5] demonstrates their ability to predict individual longevity and their importance in overall success of the organism, but this has not been performed in Pacific salmonids to date.

Objectives & Hypotheses: My hypothesis is that telomere length is a genetic mechanism contributing to the observed fitness differences in HOR and NOR salmonids. I will use a combination of experimental manipulation in a hatchery to test for the effects of hatchery rearing on telomere length, and a multigenerational genetic pedigree to analyze telomere length relative to the known total lifetime fitness between and among HOR and NOR salmon.

Rearing density and steelhead genetics: I will collaborate with staff at the Oregon Hatchery Research Center (OHRC), ODFW, and the State Fisheries Genetics Lab to collect 12 male and 12 female winter steelhead (*Oncorhynchus mykiss*, the anadromous rainbow trout) from the Siletz River, OR. All male and female steelhead will be spawned in 1x1 crosses to produce 12 families, the offspring of which will be distributed between nine tanks into three treatments: 1 high density treatment (HD) and 2 low density treatments (LD1, and LD2). The HD treatment will consist of 3 tanks, each holding 200 fish per family, totaling 2,400 fish per tank. The 2 LD treatments will consist of 3 tanks each holding 20 fish per family, totaling 240 fish per tank. After offspring have been reared at the OHRC for 6 months, I will collect a sample of muscle tissue from 240 individuals within each of the nine tanks, extract DNA from these sampled tissues and perform genetic parentage analysis to assign offspring back to the parents using a panel consisting of 192 single nucleotide polymorphisms (SNPs). Using these assignments, we will subsample 10 offspring per family from each of the tanks for telomere analysis using quantitative polymerase chain reaction (qPCR) and the Applied Biosystems 7500 Real-Time PCR System. This method utilizes probes to produce a fluorescent signal as the reaction progresses and calculates the starting concentration of nucleic acid, or copy number, based on the cycle at which the sample fluorescent signal crosses a baseline threshold (C_t) relative to reference standards[6]. To acquire a telomere length approximation, I will previously described methodologies[7] using a single-copy reference gene developed

using the *O. mykiss* genome and comparing the relative telomere copy number to this single copy reference. To test for meaningful differences between treatments and families of steelhead, I will conduct a Welch's t-test and use linear mixed effect models using R statistical computing package.

Telomere length and total lifetime fitness in chinook salmon: Using the same genetic methods described above, I will investigate the effects of telomere length on total lifetime fitness using tissue samples previously collected from HOR and NOR spring Chinook salmon (*O. tshawytscha*) from the South Fork McKenzie River, OR[8]. Total lifetime fitness is estimated as the number of offspring from adults released above the dam returning as spawners 3-5 years later, assigned through genetic parentage analysis. The genetic analyses and knowledge of adult fish through the genetic pedigree will allow for the testing of differences between HOR and NOR between and within each group with known high fitness (e.g. > 10 offspring per parent) or low fitness (e.g. < 10 offspring per parent). The results of this research could demonstrate conservation of telomere function across taxa and in this case, could also be used to improve hatchery rearing practices for Chinook to benefit commercial harvest opportunities and aid in conservation efforts.

Intellectual Merit: Given the wide use and dependence of anadromous salmonid hatcheries in the western United States and Canada, these projects have the potential to generate novel findings aid in production for fishing opportunities and conservation efforts. Pre-1850 estimates of salmon and steelhead in the Columbia River alone range from 10-15 million fish annually[9]. Today, according to ODFW, all runs of salmon and steelhead in the Columbia River are threatened or endangered under the federal Endangered Species Act. Thus, improvements to current practices are necessary to ensure sustainable harvest while minimizing the impact on wild fish.

Broader Impact: This research has the potential to significantly improve hatchery practices throughout Oregon and western North America, enhancing sustainable harvest opportunities for salmon and steelhead and aiding conservation efforts. While conducting this research, I will have the opportunity to interact with a variety of stakeholders and interest groups, including the general public through educational efforts at the OHRC and the Tillamook Estuaries Partnership. I will participate in Linn-Benton County's Salmon Watch program, teaching local youth about salmon biology and the importance of watershed health. Additionally, I will engage with stakeholders like Oregon Salmon Commission and recreational fishing associations to understand different social and economic perspectives, along with others in the scientific community in state and federal agencies.

References:: [1] ODFW Fish Propagation Annual Report for 2017 (2018), **[2]** Araki et al. 2008. *Evol. Appl* **(1):** 342-355, **[3]** Reichert & Stier, 2017. *Biol. Lett.* **(13)**, 20170463, **[4]** Heidinger et al. 2012. *PNAS* **(109)**: 1743-1748, **[5]** McLennan et al. 2018. *Molecular Ecology* **(27)**: 804-814, **[6]** Cawthon RM (2002). *Nucleic Acids Res.* **(30)** e47, **[7]** Naslund et al. 2017. *Oecologia* **(177)**: 1221-1230, **[8]** Sard et al. 2016. *Anim. Conserv.* **(19)**: 570-577, **[9]** Lackey et al. (2006). *Salmon 2100: The Future of Wild Pacific Salmon*