Multilocus Sequence Typing of Clinical and Environmental Strains of *Vibrio parahaemolyticus* Isolated from the Pacific Northwest, U.S.A.
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Mentor: Mark Strom, Microbiology, Northwest Fisheries Science Center
Mentor: Jeff Turner, Oceanography

*Vibrio parahaemolyticus* (Vp) is a halophilic, Gram-negative bacterium common in the estuarine and marine environment. Some strains are responsible for causing severe gastroenteritis in humans, often as a result of consuming raw or undercooked seafood. In Japan and Taiwan, Vp is the leading cause of bacterial foodborne illness. In recent years, Vp outbreaks have become increasingly epidemic and spatially traceable to areas such as the Pacific Northwest, Gulf Coast, East Coast, India, Asia, and South America. Pathogenic strains are normally detected by screening environmental samples for the presence of virulence-associated genes, such as the thermostable direct hemolysin (tdh) and thermostable-related hemolysin (trh). To examine the hypothesis that pathogenicity could evolve and or increase due to the natural variation of allelic genes, we are conducting multilocus sequence typing (MLST) analysis of putative "virulence-associated" genes. These genes, which are shared between clinical and environmental strains, encode surface proteins and global virulence gene transcription regulators. Phylogenetic dendrograms of gbpA (a N-acetylglucosamine-binding protein) and toxRS (a global virulence gene transcription regulator) revealed multiple sequence types and a high degree of nucleotide diversity. Among these sequence types, clustering is indicative of evolutionary relationships among clinical and environmental strains, which is supported by the MLST analysis of Vp housekeeping genes. DNA polymorphism analysis of gbpA yielded a dN/dS (ratio of non-synonymous to synonymous mutations) of 0.22, indicating that this gene is under neutral or "purifying" selection. Current and future work is focused on the analysis of additional genes such as mshA (mannose-sensitive hemagglutinin and a type IV pilin), pilD (type IV prepilin peptidase) and tl (thermolabile hemolysin). Data for additional "virulence-associated" genes and the concatenation of these genes could yield insight into the emergence of a highly virulent Vp clonal complex in the Pacific Northwest.

Selective Distribution of Potentially Pathogenic *Vibrio parahaemolyticus* Strains in Oysters and Water from Shellfish Growing Areas in The Pacific Northwest
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Mentor: Rohinee Paranjpye, NOAA Fisheries
Mentor: Mark Strom, Microbiology, Northwest Fisheries Science Center

*Vibrio parahaemolyticus* is a Gram-negative bacterium that occurs naturally in the marine environment. Since its identification in 1950, awareness and interest has grown because it can cause gastroenteritis in humans from the consumption of raw or contaminated shellfish such as oysters. Currently, outbreaks due to *V. parahaemolyticus* associated gastroenteritis occur globally. Present risk assessment models in the U.S. are based on the presence and concentration of the gene for thermostable direct hemolysin (*tdh*) in oysters. However, in the Pacific Northwest (PNW) there has been little correlation between illnesses related to the consumption of raw oysters and the presence or concentration of *tdh*+ *V. parahaemolyticus* strains in oysters harvested from the implicated growing areas, suggesting that additional markers in *V. parahaemolyticus* strains may be responsible for these illnesses. The purpose of this project is to compare the distribution of total (*tl*) and potentially pathogenic (*tdh*) strains of *V. parahaemolyticus* strains in oysters and water from the select growing areas in the PNW. Sampling sites include Nahcotta (Willapa Bay) and Hoodsport (Hood Canal) and were sampled from May to August 2010. A second objective is to analyze and compare the genetic profiles of *tdh*+ environmental isolates with clinical strains from the PNW, using genetic analysis such as Repetitive-Extragenic Palindromic sequence analysis (REP-PCR). A comparison of environmental strains with the
clinical isolates from the same geographic areas will assist in understanding the population structure of PNW V. parahaemolyticus strains.

Distribution of Pacific Geoduck Larvae in Quartermaster Harbor in Relation to Oceanographic Properties
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Mentor: Bonnie Becker, Marine Ecology, UW Tacoma
Mentor: Yvonne Shevalier, Environmental Science, UW Tacoma

Marine invertebrate larvae are notoriously difficult to study in the field due to their microscopic size. Bivalve larvae are also particularly difficult to identify to species, as many bivalves look nearly identical during their pelagic larval stages. Bivalves are filter feeders, and therefore play a key role in the health of the bodies of water they settle in. They are also of economic importance, as they are often used as a food source in coastal areas and are commonly exported for this purpose. To gain a better understanding of the larval distribution of the Pacific geoduck within Quartermaster Harbor, WA, our team deployed and retrieved passive larval tube traps weekly from March through July of 2010 to take time-integrated plankton samples. Oceanographic data was concurrently collected via CTD to identify the status of the water column at the time of larval sample retrieval. We hypothesized that the larvae would be found in higher concentrations near the thermocline, and also where chlorophyll and dissolved oxygen were readily available. Filtered and preserved tube trap samples were sent to the University of California, Santa Barbara to be subjected to fluorescent in situ hybridization with cell sorting (FISH-CS) to determine the number of geoduck larvae present. CTD data and larval collections from different locations were analyzed to identify the potential relationship between larval distribution and oceanographic factors. Further study is aimed at understanding the mechanism of larval transport in the Pacific Northwest and how it influences geoduck recruitment and population dynamics.

Effects of the Red Sea Urchin on Benthic Invertebrate Communities: A Link to Spatial Subsidies
Ross Douglas (Ross) Whippo, Senior, Aquatic & Fishery Sciences
Mentor: Kevin Britton-Simmons, Friday Harbor Laboratories

Marine invertebrate larvae are notoriously difficult to study in the field due to their microscopic size. Bivalve larvae are also particularly difficult to identify to species, as many bivalves look nearly identical during their pelagic larval stages. Bivalves are filter feeders, and therefore play a key role in the health of the bodies of water they settle in. They are also of economic importance, as they are often used as a food source in coastal areas and are commonly exported for this purpose. To gain a better understanding of the larval distribution of the Pacific geoduck within Quartermaster Harbor, WA, our team deployed and retrieved passive larval tube traps weekly from March through July of 2010 to take time-integrated plankton samples. Oceanographic data was concurrently collected via CTD to identify the status of the water column at the time of larval sample retrieval. We hypothesized that the larvae would be found in higher concentrations near the thermocline, and also where chlorophyll and dissolved oxygen were readily available. Filtered and preserved tube trap samples were sent to the University of California, Santa Barbara to be subjected to fluorescent in situ hybridization with cell sorting (FISH-CS) to determine the number of geoduck larvae present. CTD data and larval collections from different locations were analyzed to identify the potential relationship between larval distribution and oceanographic factors. Further study is aimed at understanding the mechanism of larval transport in the Pacific Northwest and how it influences geoduck recruitment and population dynamics.

Examining the Tradeoff between Length, Age, and Reproductive Potential in Female Sockeye Salmon
Mira Gabrielle (Mira) Courage, Junior, Biology (General)
Howard Hughes Scholar, Mary Gates Scholar
Mentor: Thomas Quinn, Aquatic & Fishery Sciences

The ecological phenomenon of life-history tradeoffs has been studied across a diverse range of topics and organisms, from pathogen resistance tradeoffs in fruit flies, to photosynthetic tradeoffs in trees. Female sockeye salmon (Oncorhynchus nerka) face tradeoffs related to reproduction: What is the optimal age and size at which to mature and reproduce? Larger bodied, older females produce more eggs and so can achieve higher reproductive success, but they are also at a greater risk of being eaten by bears or stranding in shallow water. On the other hand, smaller, younger females have a higher chance of surviving to reproduce but produce fewer eggs. We studied these tradeoffs in female sockeye salmon from Hansen Creek, Alaska. From 1999 through 2010 female adult sockeye salmon (N = 58,760) were categorized by cause of death and measured for length. These data established the relationship between body size and likelihood that females would...
die of senescence, having completed spawning, or die prematurely from stranding or attacks by glaucous-winged gulls or brown bears. We then used data on individually-tagged fish (N = 881) to determine the probability of successful spawning by fish that died prematurely. These data indicated that 65.0% of the bear-killed females died prior to spawning, as did 45.8% of the gull-killed fish and 70.6% of those that stranded in shallow water. We then established the relationship between length and age at maturity and the production of eggs. Finally, for any given length, we will calculate the reproductive potential as a function of average egg production and likelihood of pre-spawning death. This will allow us to compare the calculated optimal length with measured average length, and therefore assess the tradeoff between fecundity and predation.

**Geomorphic Evolution and Salmon Adaptation in the Wood River Watershed of Southwest Alaska**

*Jeanelle Miller, Senior, Aquatic & Fishery Sciences*

*Mentor: Daniel Schindler, School of Aquatic & Fishery Sciences*

Recently glaciated landscapes are among the fast evolving landforms on Earth and represent moving targets to which biological systems adapt. Temporal reconstruction of the geomorphic evolution of landscapes allows for the assessment of the evolution of target species and can provide insights into the processes of colonization and microevolution in response to habitat and climate change. Glacial geomorphology of Alaska has traditionally focused on assessing the influence of ice on landscape evolution. However, glacial geomorphology has had important but unresolved effects on the distribution and evolution of Pacific salmon. The Wood River watershed of southwest Alaska consists of a network of five large lakes that were carved through successive glacial advances and retreats during the last glacial period and is one of the few areas with well-defined terminal moraines on land. The lake system supports high densities of sockeye salmon that are a major economic input to Bristol Bay’s fishery. This salmon stock is characterized by considerable biological diversity represented by dozens of populations showing local adaptations to the specific geomorphic characteristics of streams, rivers, and lakes throughout the watershed. The geomorphic evolution of the system was punctuated by a series of drastic, synchronized drops in lake levels. I used spatial data in a geographic information system to reconstruct previous lake conditions to assess the evolution of salmon habitat in this system. My analyses document an extensive dammed proglacial lake that greatly reduced stream habitat for sockeye salmon while still maintaining a high degree of separation between the proglacial lake and northern most lake in the system. These features of the evolution of this landscape may account for genetic divergence currently observed among the sockeye populations throughout the watershed.