78th Annual Shellfish Conference & Tradeshow

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TUESDAY 9/10

Ocean Conditions

Navigating Ocean Acidification in Shellfish Aquaculture: Stakeholder Perspectives and Implications Along the US Pacific Coast

Connor Lewis-Smith, Mackenzie Gavery, Karma Norman, Larken Root, Ryan Crim, Steven Roberts; NOAA, University of Washington, PSRF*

The shellfish aquaculture industry along the US Pacific Coast has been facing escalating ocean acidification and its associated challenges. This study delves into the economic and operational impacts of ocean acidification on shellfish aquaculture, focusing on stakeholder responses, adaptation strategies, and the broader implications for industry resilience and sustainability. Through an interview survey targeting shellfish growers and industry participants, which allowed for the simultaneous collection and pairing of quantitative and qualitative data, we gathered insights on the perceived threats of ocean acidification, adaptive measures currently employed, and the willingness to adopt innovative mitigation strategies, such as species diversification and environmental priming. Our findings reveal a nuanced understanding of the economic pressures and decision-making processes within the industry. The study emphasizes the differing perceptions of species portfolio expansion and the potential of parental priming techniques as proactive approaches to enhance resilience. Additionallangly, the economic considerations and barriers to implementing these strategies offer a unique perspective on the intersection of environmental challenges and sustainability in aquaculture. This research contributes to the broader discourse on governing aquatic food systems for resilience, providing empirical evidence to inform policy and regulatory frameworks to foster adaptive capacity and ensure the long-term sustainability of shellfish aquaculture in the face of environmental uncertainties.

Air priming as a management tool for transitioning hatchery produced oysters to the intertidal zone

Hollis Jones, Cassidy Cooper, Anne Todgham; University of California, Davis*

Aquaculture is the fastest growing food production sector globally and investing in research that has the potential to improve farmed species' resilience to climate change will be critical for industry growth. Stress priming, also called stress hardening or conditioning, can induce short or long-term stress ‘memory' that can enhance ability to cope with environmental challenges through an increase in protective cellular defense mechanisms. Priming has been well-studied in crop production and has growing recognition in aquaculture as the environments that aquacultured organisms are outplanted into are becoming increasingly unpredictable and extreme. One step in the aquaculture process that is particularly vulnerable is when oysters are shipped from a hatchery, where they are kept in a relatively homogeneous subtidal environment, to a grow-out farm, where they are exposed to air for several hours a day (low tide) and therefore unable to utilize aerobic respiration for energy acquisition. I hypothesize that a preliminary air exposure will result in a more successful transition from sub- to inter-tidal conditions by priming hatchery produced oysters for energy budget disruptions during emersion. In June 2023 I collaborated with Hog Island Oyster Co.'s hatchery team in Samoa, CA to expose hatchery produced oysters to a 6 hour air exposure 1, 2, and 3 days before exposure to intertidal conditions. I collected tissue samples from the primed and unprimed (control) oysters directly before they were exposed to their first intertidal cycle, directly after, and an hour after re-oxygenation. Tissue samples will be used to quantify glycogen and succinate content. Glycolysis plays a vital role in energy metabolism under both aerobic and anaerobic conditions, and measuring glycogen and succinate content,

an indicator of anaerobic respiration, will give us a more complete picture of energy homeostasis. The analysis of this data has not yet been completed but will be reported at the PCSGA & NSA conference in September 2024. If air priming has a positive influence on the oysters' ability to smoothly transition to the intertidal zone, it would be a reasonable management strategy that could be easily implemented during a normal work day.

Potential of seaweed to mitigate effects of ocean acidification on larval oysters in co-culture

Leah Wessler, Jennifer Clark, Andrea Frommel; Aquaculture and Climate Change Lab, Faculty of Land and Food Systems, University of British Columbia*

Ocean acidification (OA) conditions along the west coast of Canada and the US are corrosive and can negatively impact economically important marine calcifiers such as Pacific oysters (*Magallana gigas*). Currently, shellfish hatcheries combat ocean acidification by making artificial seawater, transporting larvae to other countries during vulnerable stages, or buffering water chemically. These methods can be unsustainable, expensive, or can affect the health of oysters later in life. Through photosynthesis, marine macroalgae uptake carbon from seawater, functioning as a potential tool for mitigating OA while providing a range of important products such as nutritious food, cosmetics, and biofuels. To quantify effects and fill knowledge gaps, our research examines the potential of tank cultivated Pacific dulse (*Devaleraea mollis*) to mitigate the harm caused by OA on oyster larvae in land-based co-culture. Preliminary data from this study shows that dulse can increase tank pH by over 0.4 units, depending on OA condition, and can maintain seawater buffering under continuous light for at least two weeks. We hypothesize that negative effects of OA on survival and calcification of wild and hatchery oyster larvae, and changes to the larval microbiome due to OA, will be mitigated by co-culturing the oysters with dulse. Carbonate chemistry will be modified to represent three OA conditions corresponding to ambient (400 µatm CO2), local upwelling (1000 µatm CO2), and upwelling + future projections (3000 µatm CO2). Two groups of larval oysters, one in co-culture with dulse and one in monoculture, will receive water from each OA level. Oyster samples taken at 14, 24, and 48 hours post fertilization will be analyzed for survival, growth, shell shape, and expression of shell formation and immune genes. Larvae from each experimental unit will be pooled for microbiome analysis, which has been linked to oyster immune function. Findings will provide data to model the CO2 uptake potential of macroalgae, applicable to industry and restoration efforts for both shellfish and macroalgae. Outcomes of this study will aid the shellfish industry in adapting to climate change while increasing sustainability and net income through increasing yield and providing crop diversification.

Rapid Response Plan for climate-related shellfish mass-mortality events in Washington State

Ashleigh Epps, Washington Sea Grant; Julieta Martinelli, Washington Department of Fish and Wildlife; and Rana Brown, Squaxin Island Tribe*

In June 2021, a heat dome event occurred during the lowest tide series of the year, leaving shellfish exposed to high air temperatures for 3 days. This event caused extensive mortalities of many shellfish species including manila clams (*Venerupis philippinarum*), native littleneck clams (*Leukoma staminea*), butter clams (*Saxidomus giganteus*), cockles (*Clinocardium nuttallii*), and razor clams (*Siliqua patula*). While the mortalities were evident, the fisheries management surveys used to gather information were conducted in sites subject to harvest and enhancements, leading to variability in the data, and limiting the statistical power of analysis. Given that extreme weather events are predicted to continue to happen, and even increase, locally and globally, there is a need to understand ecological variability in shellfish populations to be able to better detect the impact of severe events like the 2021 heat dome. The shellfish community of Washington State has joined forces to create a rapid response network with the purpose of creating a plan

to set best practices for sampling and monitoring shellfish throughout Washington. Specifically, the rapid response network aims to monitor beaches around Washington that have little to no harvest or enhancement twice per year to detect potential winter and summer mortalities. Many stakeholders including Tribes, shellfish growers, Washington Department of Fish Wildlife, Washington Sea Grant, and academic partners have joined the effort. In this talk, we will outline progress in the monitoring framework and lessons learned from the first two preliminary surveys conducted to test methods. We invite other partners in the Washington State shellfish community to provide feedback and join us in this effort to preserve and protect shellfish resources into the future.

Adaptive aquaculture: designing for shellfish production and coastal resilience

Matt Grosser; University of Washington

How can intentionally designed aquacultural installations make our coasts more resilient to climate impacts? This talk will explore the confluence of resilience infrastructure and nearshore mariculture from existing projects to speculative possibilities, specifically exploring the potential for this kind of work within the Puget Sound region.

Integrated Pest Management

Ghost shrimp busters: a review of efficacy, costs, and non-target effects of mechanical methods to control burrowing shrimp in oyster aquaculture

C. Haleh Mawson, Jennifer L Ruesink, Brian Allen, David Beugli, Steven Booth, Laura Butler, Bill Dewey, Cinde R Donoghue, Brett R Dumbauld, Kristine Feldman, Zachary Forster, S. Maria Garcia, Jackson A Gross, Bobbi Hudson, Wesley W Hull, Vikram Iyer, Aspen Katla, Laura Kraft, Blair Paul, Casey Pruitt, Aniruddh Vashisth, Kim D Patten; University of Washington*

Shellfish aquaculture benefits from non-chemical, operational-scale pest management tools to sustain or increase production. Since the 1920s, shellfish growers in Washington State (USA) have attempted to control the population of a native bioturbating pest, ghost shrimp (*Neotrypaea californiensis*). Even at low pest densities, burrowing ghost shrimp expel sediment that buries and smothers ground-cultured oysters on tidal flats. Successful mechanical control methods must be highly effective at reducing ghost shrimp density, but also relatively simple and cheap to implement with few or positive non-target effects on other tidal flat organisms, including eelgrass and oysters. Of 57 total trials from 1996 to 2023, 22 had quantitative data for treatment and reference plots suitable for effect size calculations. Methods included: 1) surface barriers, 2) shrimp removal, 3) sediment disruption, and 4) physical conditions intended to cause direct mortality (e.g. electricity, heat). Most surface barriers were not effective because they were penetrated by shrimp or swept away; a 5” thick gravel layer was an exception. Shrimp removal was effective with a water-jet technique developed to collect shrimp for bait at low tide, but scaling up proved unsuccessful. Sediment disruption through surface compaction was the most common farm-scale approach but did not reliably reduce shrimp densities, whereas consolidating sediment with vibration to 1 m depth had high efficacy but has been applied only in small plots. Effect sizes were not available for direct mortality methods, but high energy inputs were needed to kill shrimp in laboratory studies. Below nearly a meter of water-saturated sediment and tolerant of high pressure and anoxia, ghost shrimp are resilient against most mechanical control methods applied from the surface. Across all field methods, efficacy improved with effort (person-hours per area). Non-target effects on infauna became more pronounced after one month, including both positive and negative effect sizes, consistent with a community change following a reduction in bioturbation. In the six studies measuring epibenthic species, no overall positive response to shrimp control occurred, even though

the reason for control is to protect surface-dwelling species like oysters. This outcome illustrates the importance of pairing efficacy in terms of reduction of shrimp and improvement of farming. Although progress has been made in recent years, there remain few cost-effective mechanical control options that can be carried out without permits and that reduce shrimp to densities compatible with benthic shellfish aquaculture at a farm scale.

Describing Physiological Weak Points of Burrowing Shrimp to Ambient Ion and pH Stress as a Potential Target for their Control

Annika McCarty, Jennifer Ruesink, Andrea Durant; University of Washington*

Many shellfish growers in Washington state\'s Willapa Bay and Grays Harbor are facing significant negative impacts of increasing population densities of burrowing shrimp, *Neotrypaea californiensis*. These burrowing shrimp soften mudflats through bioturbation and cause shellfish, particularly Pacific oyster, to sink and suffocate. Previous methods used for the successful control of burrowing shrimp involved the use of insecticides (e.g. Carbaryl), and more recently proposed methods included the use of neonicotinoids (e.g. Imidacloprid). The use of pesticides for burrowing shrimp control has since been denied by the state, and our current project aims to identify chemical control methods of low environmental impact by characterizing weak points in the osmoregulatory physiology of *N. californiensis*. We hypothesized that changes in ambient seawater pH and potassium, K+, in burrows would negatively affect the ability of burrowing shrimp to excrete nitrogenous waste compounds and maintain systemic K+ levels in the hemolymph, leading to increased mortality. It is valuable that we first know how these animals \"work\", and here we present the beginnings of the first mechanistic model of ion transport at the gills of burrowing shrimp and the effects of system ion regulation and survival with altered ambient ion levels (mainly increased external potassium, K+, and changes to ambient pH, H+).

Rapid Rates of Change in Distribution of Burrowing Shrimp and Their Engineering Consequences for Tidal Flats

Brooke McIntyre, Joe Brockman, Brett Dumbauld, Wesley Hull, Nate Lewis, Haleh Mawson, Jennifer Ruesink; USDA ARS*

Burrowing shrimp (*Neotrypaea californiensis*) inhabit estuaries along the US Pacific coast and can dominate intertidal soft sediments and shape conditions through digging extensive burrows and deposit-feeding. Adult shrimp form beds where densities can be hundreds of m^{-2}. Here we report the rates at which shrimp bed boundaries can shift over time, and the consequent changes to ecosystem engineering. For six shrimp beds in Willapa Bay, WA, bed boundaries were mapped 1-3 times per year for 3 years to calculate rates of recession. Comparisons of sediment properties were made across shrimp bed boundaries and where shrimp densities changed over time. As shrimp transitioned from high to low density, sediment penetrability declined, and grain size and organic content increased. Eelgrass (*Zostera marina*) appeared as seedlings within one year of shrimp bed recession and began flowering after two years. Shrimp recruits were present on both sides of shrimp bed boundaries, including in areas of one- and two-years post shrimp bed recession. Overall, the consequences of shifting shrimp bed boundaries of this major ecosystem engineer spanned sediment and species interactions.

Burrowing shrimp density is a strong predictor of oyster performance via direct sediment burial

Wesley Hull, Jennifer Ruesink; University of Washington*

Because oyster performance can be affected by their sediment environment, both the underlying sediment properties of tide flats as well as the effects of bioturbating shrimp on sediments may affect oyster performance. Yet, while it is known that burrowing shrimp negatively impact oyster performance through burial, it is not known whether shrimp density alone is enough to accurately predict oyster performance, or whether effects of shrimp density mediated through their influence over sediment properties help explain variations in oyster performance. We examined how oyster survival and growth were affected by a) shrimp density, b) sediment properties of muddiness and penetrability, and c) shrimp density due to their effects on sediment properties (indirect effect). To do this we deployed seeded oyster cultch at 31 different sites varying in both shrimp density and sediment properties within Willapa Bay, WA. In our study, increasing shrimp density reduced oyster survival with complete mortality occurring between 50 – 100 shrimp m-2 and caused sediments to become less muddy and more penetrable. However, although shrimp made sediments less muddy and more penetrable, neither of those factors contributed to the negative impact of shrimp density on oyster survival. Likewise, decreases in growth were associated with increasing shrimp density, suggesting sediment burial from shrimp activity hampers oyster growth and that shrimp density is a strong predictor of oyster performance. Our study highlights the importance of examining interaction between biological and physical factors in shaping the survival of benthic species such as oysters and should be included in future research aimed at identifying causes and strategies for ensuring the resilience of important foundation species.

Trophic Level Responses to Different Longline Densities and Clustering

Maria Garcia, Jennifer Ruesink, Katie Houle, Andy Suhrbier; University of Washington*

This experiment examined how longline (oyster aquaculture type) density and aisle width could be altered to mediate negative effects on eelgrass. Longlines have the potential to be detrimental to eelgrass as they can shade eelgrass, or the eelgrass can get caught on the longlines and dry out on low tides. This experiment was used to ask two questions: 1. How does altering the density of longlines and the aisle width between them affect eelgrass presence? 2. Do these effects extend to higher trophic levels? Longline density and aisle width were altered at two sites in Willapa Bay, WA. Density was altered by having reference sites with no longlines, normal density, and half density. Another set of conditions kept the density of longlines but altered the spacing between them by having sets of longlines, clusters, close together with larger aisles. This set of conditions was designed with collaboration from aquaculture farms as a solution that would be economically viable for companies to implement. The response variables spanned four trophic levels and included eelgrass, epiphyte load, epifauna load and community structure, and nekton abundance and community structure. Lower longline densities allowed for more eelgrass. However, when the overall density of longlines remained the same, effects on eelgrass were reduced when longlines were clustered (increased aisle width). Despite changes in eelgrass, neither longline density nor aisle width extended to higher trophic levels.

Public Health

Occurrence of human pathogenic bacteria in seawater and oysters from Sally Cove in Rehoboth Bay, Delaware

Kelvin F. Ofori, Ali Parsaeimehr, and Gulnihal Ozbay; Delaware State University*

The Eastern oyster (*Crassostrea virginica*) is consumed raw by nearly 20 million Americans. Their filter-feeding nature causes the accumulation of various contaminants from seawater within their tissues. Bacterial contamination of seafood causes over 76% of all seafood-borne outbreaks. The Sally Cove in

Rehoboth Bay (latitude, longitude; 38˚38.932'' N, 075˚07.631'' W) is one of the main oyster aquaculture sites along the Atlantic coast in Delaware, producing oysters for consumption through surface and bottom oyster cultures. However, the proximity of Sally Cove to residential areas poses a substantial risk of potential cross-contamination of human pathogenic bacteria into the seawater and oyster cultures. This study aimed to detect the occurrence of eight human pathogenic bacteria in seawater and oysters from surface and bottom cultures in Sally Cove and how they are influenced by water quality parameters. Seawater and oysters were sampled from surface (0.10 m) and bottom (1.80 m) cultures once across four months, from July through October 2023. Also, physical seawater quality parameters were measured during sampling. For comparison, a site without oysters within Sally Cove was used as a control site. Seawater was centrifuged to obtain the sediment containing environmental DNA (eDNA) while the oysters were homogenized, pre-enriched, and cultured in Lysogeny broth or Tryptic soy broth. Bacteria were detected using PCR and rt-PCR. Results showed that indigenous marine bacteria such as *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, and *Clostridium* spp. were detected in seawater and oysters from the surface and bottom cultures across the entire four months. Similarly, human enteric bacteria such as Shiga-toxin *Escherichia coli* and *Salmonella enterica*, and *Staphylococcus aureus* were detected across the entire period. *Listeria* spp. was detected in samples from July through September but could not be detected in October. *Campylobacter* spp. could not be detected across the entire period. The temperature, salinity, pH, and dissolved oxygen of Sally Cove and control sites across the four months were in the range of 15.2 – 29.6°C, 29.33 - 31.87 g/kg, 7.23 – 7.95, and 3.72- 8.94 mg/L, respectively. Raw consumption of oysters from Sally Cove during summer and early fall poses risks of contamination of these pathogenic bacteria.

Keywords: Eastern oyster, Sally Cove, Rehoboth Bay, Pathogenic bacteria, Contamination

Using NOAA Observational and Modeling Tools to Address Vibrio Risk in the Pacific Northwest

Ava Ellett, Robert Daniels, Chris Schillaci, and John Jacobs; NOAA/National Ocean Service, National Centers for Coastal Ocean Science*

Vibrio parahaemolyticus has been a persistent issue for producers and consumers of raw oysters in the United States, and necessarily, much effort has been directed towards strategies for risk reduction. To date, most states use time and temperature controls to minimize post - harvest growth, and seasonal temperature averages to initiate and end control strategies. For the past decade, NOAA has been working with States and industry to apply NOAA forecast modeling and observational assets to better inform harvest and control strategies, and identify specific strains of clinical significance throughout the country. In this presentation, we will demonstrate: 1) the use of weather models to provide growing area scale projections of *Vp* growth under different control strategies in Washington, 2) how hydrodynamic models and satellite observations are being used to provide spatially explicit temperature guidance in Alaska, 3) the potential use of the new NOAA Salish Sea and Columbia River Ocean Forecast System for new guidance products and 4) whole genome sequencing efforts to identify strains of interest. These products are developed through partnerships and from stakeholder requests, and are distributed via the web.

Sustaining shellfish harvest amid the threat of harmful algal blooms: the SoundToxins partnership

Michelle Lepori-Bui, Michael J. Weir, Leif Anderson and Vera L. Trainer;* Washington Sea Grant

The SoundToxins partnership was established in 2006 as a cost-effective monitoring program to provide an early warning of harmful algal bloom (HAB) events to Washington State Department of Health (WDOH) through weekly phytoplankton monitoring. SoundToxins has 92 participants, including commercial shellfish growers, tribal managers, State agencies, environmental learning centers, and community volunteers, who monitor HABs with bottles, nets, and microscopes at more than 28 sites across Washington's inland waters.

A goal of SoundToxins is to avert human illnesses from toxic shellfish and the negative impacts on markets and harvester confidence. SoundToxins partners provide an early warning to WDOH, allowing them to prioritize analysis of shellfish samples from areas identified as having the greatest HAB risk (through HAB cell counts). In turn, WDOH can conduct more rapid assessments and earlier advisories when dangerous levels of HABs are detected. This can help shellfish growers and managers avoid costs associated with HAB events, such as product recall, and by allowing selective harvest, early harvest and depuration of toxic shellfish prior to harvest. More recently, SoundToxins has been informing growers about HABs that directly impact shellfish health, contributing to summer mortality events. During this session, we will provide an introduction to SoundToxins while describing some of its successes, then conclude with an opportunity for aquaculturists and harvesters to provide their opinions about SoundToxins via a short survey.

Occurrence of Polyfluoro Alkyl Substances (PFAS) in the marine environment

Schultz, Irvin; NOAA NWFSC

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemicals used in a wide range of industrial and consumer products to increase resistance to heat, stains, water and grease. A number of PFAS compounds such as PFOS (perfluorooctanesulfonic acid) and PFOA (perfluorooctanoic acid) are known to be global contaminants that are highly resistant to environmental degradation and can bioaccumulate to worrisome levels in aquatic animals. Laboratory studies with a variety of PFAS compounds have indicated they may pose significant human and environmental health risks. Many PFAS compounds are also water soluble and differ from other types of persistent contaminants such as polychlorinated biphenyls in that they tend to accumulate in blood rich tissues like the liver or kidney. Both PFOS and PFOA have been shown to undergo trophic transfer with potential to biomagnify at higher trophic levels. Thus, consumption of shellfish and finfish are considered a potential route of exposure of PFAS in both humans and higher trophic level marine animals. It is likely that edible seafood will receive increased scrutiny on PFAS levels. This presentation will provide an overview of PFAS and summarize recent reports of measured levels in marine shellfish and compare those with levels measured in other aquatic animals and other types of food.

PSP Alert: An Innovative, Rapid, Field-Deployable Kit for Measuring PSP Toxins in Shellfish

*Sepehr * Molazemhosseini, MD Daud Hossain Khan, Shivshankari Rajkumar, Roozbeh Safavieh; Sensoreal Inc.*

Rising ocean temperatures have increased the prevalence of toxic algal blooms globally, raising human exposure risks to paralytic shellfish poisoning (PSP) toxins. Although most commercial detection kits are lab-based, we introduce PSP Alert, a field-deployable and rapid solution. Our innovative sample preparation, combined with a digital analyzer and mobile app, provides user-friendly toxin detection at the point of harvest. It includes sample geo-tracking without cross-reactivity issues.

PSP Alert is a semi-quantitative PSP test kit that enables shellfish harvesters to immediately assess the presence and intensity of PSP toxins post-harvest, avoiding the costly delays associated with sample storage, transport, and centralized laboratory analysis. This product is particularly beneficial for remote and Indigenous communities by helping prevent the distribution and consumption of contaminated marine products.

The patent-pending core technology of PSP Alert features a unique and straightforward procedure to extract and detect all prevalent PSP toxins from shellfish tissue, converting them into a single detectable type—a capability not seen in other commercial kits. PSP Alert categorizes shellfish samples into low, medium, or

high toxin levels, significantly enhancing accuracy compared to kits that only provide positive or negative results, which can be inaccurate near the toxicity threshold.

PSP Alert's digital analyzer transmits data to electronic devices such as cellphones through a dedicated app. This method facilitates convenient data collection, storage, and logging, incorporating location and time tags.

Designed for use in resource-limited settings by untrained personnel, the kit features simple sample preparation and an automatic, portable digital analyzer suitable for use on ships or dockside.

Recently, our kit was benchmarked against the Canadian Food Inspection Agency (CFIA) gold standard toxin detection method and demonstrated a high level of agreement in test results across three different species.

The following link shows our kit in action: https://www.sensoreal.com/psp-alert

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Marine Pathogens and Diseases

Probiotic-induced protection of Pacific oyster (*Crassostrea gigas*) larvae from vibriosis in hatchery settings

Candice A. Thorstenson, Carla Schubiger, Spencer Lunda, Ryan S. Mueller, Chris Langdon; Oregon State University*

Vibrio coralliilyticus has been linked to mass mortality events of *Crassostrea gigas* larvae in hatcheries on the U.S. Pacific Coast, which can create a production bottleneck as oyster farmers rely on a steady supply of healthy oyster seed larvae from hatcheries. *Vibrio* are found ubiquitously in aquatic environments and, in addition to shellfish, *V. coralliilyticus* has been identified as a pathogen to fish and corals. There is a growing interest in developing non-antibiotic protocols to control pathogenic bacteria in hatcheries, such as supplementation with probiotics, to improve hatchery seed supplies. Probiotics are defined as live microorganisms that confer a health benefit to the host when administered in adequate amounts. Previously, at Oregon State University's Hatfield Marine Science Center in Newport, Oregon, we identified three marine bacteria that are highly beneficial to oyster larvae when supplemented exogenously. Applied in combination, the three bacterial strains significantly reduced acute mortalities of *C. gigas* larvae from exposure to *V. coralliilyticus* compared to each strain independently. Our current study further investigates the feasibility and efficacy of this probiotic combination under different formulations and protocols to prevent mortalities induced by *V. coralliilyticus*. In axenic larval culture, 80-90% of larvae treated with these probiotics within 24 hours of fertilization survived a lethal dose of *V. coralliilyticus* at 48 hours post fertilization. Further investigation into the timing of the probiotic application will result in development of a protocol for hatchery use as well as better understanding of the probiotic-larval oyster relationship.

Are Bivalve Shells Spreading Seagrass Wasting Disease?

Lindsay Alma, Gemma Field, Colleen Burge; University of California, Davis, Bodega Marine Laboratory*

Eelgrass beds play a key role in coastal environments by offering ecosystem services such as providing habitat for marine life, promoting biodiversity, sequestering carbon, and stabilizing shorelines. However, these vital ecosystems are threatened by environmental change and disease. Seagrass wasting disease, primarily caused by the pathogen *Labyrinthula zosterae*, is a factor contributing to the decline of eelgrass beds. Given that eelgrass beds often co-occur with wild oyster beds and bivalve aquaculture farms, understanding how these systems interact is critical for preserving eelgrass health. There is previous evidence that suggests that oysters may help reduce seagrass wasting disease through filtration, but they

may also transmit it via their shell. Our study investigates the role of oyster sanitization practices and live oyster presence in mitigating the impact of seagrass wasting disease. We inoculated live oysters and empty shells with an environmentally relevant level of *L. zosterae*. We applied four oyster sanitization treatments often used in the aquaculture industry: bleach, low salinity, depuration, and no treatment. The treated shells and live oysters were then introduced to eelgrass (*Zostera marina*). We assessed disease severity and presence of *L. zosterae* DNA on the shells after 10 days. Our results demonstrate that the sanitization treatments reduced the severity of seagrass wasting disease. Live oysters were effective in lowering the occurrence of *L. zosterae* DNA on shells compared to empty shells, likely due to filter feeding. This study emphasizes the importance of proper oyster sanitization before transplant and strategic placement of live oysters in an aquaculture setting to reduce disease, thus improving the health of eelgrass beds and the broader coastal ecosystem.

Identification of an Outbreak of Bivalve Transmissible Neoplasia in Soft Shell Clams (*Mya arenaria*) in the Puget Sound Using Hemolymph and eDNA Surveys

Zachary Child, Sydney Weinandt, Dorothy Lartey, Holden Maxfield, Angel Santos, Jay Dimond, Michael Metzger; Pacific Northwest Research Institute*

Bivalve Transmissible Neoplasia (BTN) is a transmissible cancer in which neoplastic cells are spread through the water column and taken up by new hosts (likely through filter feeding), after which they proliferate throughout the hemolymph and tissues of the newly-infected animal. In the soft-shell clam *Mya arenaria*, BTN had previously only been observed on the Northeastern Coast of the United States, as well as on Prince Edward Island, Canada. Here we observe previously undetected spread of *M. arenaria* BTN (MarBTN) on the Western Coast of the United States, in the Puget Sound, Washington. We first identified MarBTN in *M. arenaria* through testing of clam hemolymph with a qPCR assay. We found MarBTN in clams at Triangle Cove on Camano Island (81.5% in 2024), and the greater Stanwood area (77.3% in 2024), but not in other populations in Puget Sound. We then surveyed eDNA across multiple sites in Puget Sound to determine the spread of the cancer. MarBTN-specific mitochondrial eDNA was detected in seawater 10 miles northwest of Stanwood in Martha's Bay, with copy numbers remaining high through most of the Skagit River delta, as well as 8 miles west towards Saratoga Passage. Near Triangle Cove, elevated MarBTN copy numbers were found in Livingston Bay, with low positive samples as far south as Langley and Tulalip. eDNA samples from the Hood Canal, Admiralty Inlet, and Central and South Basins of the Puget Sound remain negative, and future monitoring remains needed to track the spread of this disease.

Selection for a single nucleotide polymorphism on chromosome 8 of the *Crassostrea gigas* genome improves resistance to *Vibrio coralliilyticus* and ostreid herpesvirus-1

Spencer L. Lunda[1], Konstantin Divilov[2], Timothy J. Green[3], Ben J. G. Sutherland[3], Jeremy C. Jennings[2], Mostafa Mandour[4], Xisheng Wang[5], Ling Jin[5], Ryan S. Mueller[1], and Chris Langdon; Oregon State University*

Selective breeding of the Pacific oyster *C. gigas* in the Western USA has improved the survival of specific breeding lines against pathogens. Selection for a SNP on chromosome 8 has led to additional increased survival of spat in Tomales Bay, California, where ostreid herpesvirus-1 (OsHV-1) is endemic. To evaluate whether this improved survival is due to increased resistance to OsHV-1, and to determine if selection for this SNP confers increased resistance to other pathogens, 12 biparental crosses of C. gigas were prepared to produce offspring that were either heterozygous for the putatively protective variant or homozygous for the reference allele (i.e., control families). Spat were reared for five months before undergoing a challenge with *Vibrio coralliilyticus* RE22 and for six months before performing challenges with a European microvariant of OsHV-1. At 3.5 months post-fertilization, spat were also planted in Tomales Bay, and growth, survival, and

prevalence of OsHV-1 infection in the field-planted oysters was assessed at 10 months post-fertilization. Spat from families with the heterozygous genotype showed a 28.1% increase in average survival compared to homozygous spat when challenged with RE22. Similarly, spat with the heterozygous genotype demonstrated 38.9% greater survival than the control families when challenged with OsHV-1 across two trials. This resistance phenotype extended to the field-planted oysters, where those with the heterozygous genotype had an average of 10.4% greater survival against endemic infections than control families. Oysters with this genotype were also found to have lower rates of infection with OsHV-1 in the field, and, of those infected with the virus, significantly lower viral loads were quantified. Survival in the field challenge was also found to be significantly correlated with survival in the laboratory challenges with both *V. coralliilyticus* (R^2 = 0.36, P = 0.041) and OsHV-1 (R^2 = 0.35, P = 0.043). Overall, our results confirm that the previously discovered chromosome 8 SNP confers resistance to OsHV-1, that this resistance extends to the common bacterial pathogen of oysters, *V. coralliilyticus* RE22, and indicate that that laboratory challenges with spat could serve as a surrogate for long-term field challenges.

Susceptibility of Eastern Oyster Families and Lines to OsHV-1 Variants

Colleen Burge, Ph.D.; University of California, Davis, Bodega Marine Laboratory, and California Department of Fish and Wildlife

There is concern over potential impacts to the shellfish industries in the US if OsHV-1, particularly the highly virulent OsHV-1 microvariants (µvars), were to spread beyond their current range. Laboratory trials were conducted exposing spat and juveniles of eastern oyster, *Crassostrea virginica*, to OsHV-1 variants. Through a bath challenge, spat from 30 eastern oyster families and two lines were exposed to a French µvar for seven days at 20 ppt and 22 ºC. Mortalities among the *C. virginica* families and lines ranged from 0-50% with a mean mortality of 15%. Juveniles from eight of the families whose spat had demonstrated survival ranging from low to high were selected for injection challenge studies with the French and San Diego variants for 10 days. Mortality ranged from 0-87% with a mean of ~23%. Survival of families and lines between bath challenges and injection challenges was highly correlated. Viral loads were higher in families with higher mortality and low in those with no or very low mortality. Viral DNA was also detected in tissues of surviving oysters; at higher levels in tissues of poorer performing families and at very low levels in families demonstrating little or no mortality. Results suggest that although some *C. virginica* lines and families are susceptible, many demonstrate tolerance and breeding for combating this virus is likely to be successful if necessary. A multi-trial genetic parameter analysis is in progress to determine the degree to which resistance to OsHV-1 in *C. virginica* is heritable.

Multiyear Monitoring of Ostreid Herpesvirus-1 (OsHV-1) Outbreaks in Juvenile Pacific Oysters (*Crassostrea gigas*) along the West Coast of the United States

Ford Evans, Marcela Prado-Zapata, Colleen Burge, Sukanya Dayal, Chris Langdon, Brett Dumbauld; Coastal Oregon Marine Experiment Station, Oregon State University*

Ostreid herpesvirus 1 (OsHV-1) has significantly impacted production of Pacific oyster (*Crassostrea gigas*) globally. Mortality events consistent with OsHV-1 were first observed in the United States as early as 1993 and, until recently, have been confined to Tomales Bay, California, and neighboring Drakes Bay and Bodega Bay. Detection of a novel OsHV-1 variant in San Diego Bay, California, in 2018 raised concern about the potential spread of this virulent pathogen to shellfish growing grounds further north along the U.S. West Coast.

Recognizing the risk of regional spread and the commercial impact of the OsHV-1 herpes virus, a multi-state oyster sentinel program, with strong industry collaboration, was initiated in 2020 to monitor the prevalence

and pathogenesis of this virus in juvenile Pacific oysters planted at commercial growing grounds in California, Oregon, and Washington. Industry partners counted spat every two weeks and collected samples for analyses, including OsHV-1 presence and viral load. Sentinel oyster deployment and monitoring has continued annually through 2024. Frequent sampling within each growing season allowed us to characterize spatial and temporal variation in survival and OsHV-1 load during C. gigas mortality events. When possible, two separate families (one selected for high survival when exposed to OsHV-1 in the field and the other an unselected control) were deployed at Tomales and San Diego Bays in California where oysters have historically tested positive for OsHV-1 to measure the effect of oyster genotype on the severity of OsHV-1 induced mortality in the field.

From 2020 to 2023, all samples collected from sites in Oregon (Tillamook Bay) and Washington (Willapa Bay and Totten Inlet) tested negative for OsHV-1. In contrast, OsHV-1 was detected in oyster tissue across all four years at the Tomales Bay site and during the 2020 and 2022 field seasons at the San Diego Bay site. Except for 2022, oyster survival at the end of the first growing season at the Tomales Bay site correlated with peak tissue viral load. Oyster genotype had a significant effect on survival and magnitude of peak viral loads in some, but not all, years. Spatial and temporal variation in both viral load and survival will be discussed in the context of sentinel monitoring for OsHV-1.

Powering American Aquaculture Workshop

Powering American Aquaculture: Opportunities for the co-location of marine energy and aquaculture

Mikaela Freeman, Jim Parson, Elizabeth Tobin, Lysel Garavelli, Laura Nelson, Nikki Sather, Hannah Hudson, and Andrea Mengual*

This will be an interactive workshop. We plan to do a short presentation that gives an overview of marine energy in general, how it can used in aquaculture operations, and discuss a few case studies from around the United States, including Puget Sound. Then we will have a discussion where we invite participants to ask questions, share concerns about marine energy, and suggest information they think would be beneficial to be communicated to the greater aquaculture community about this topic.

WEDNESDAY 9/11

Restoration

Analysis of New Size-Length Models for *Parastichopus californicus* & their Effectiveness in a Management Context

Zachary Vetak; Suquamish Tribe Shellfish Program

Due to historical overharvesting; populations of *Parastichopus californicus* have been depleted in the Puget Sound. As such new management tools that allow for more efficient and accurate population assessment are critical to better monitor the recovery of these populations.

This study explores the potential of new metrics being taken from specimens to better inform the relationship between size and weight of specimens; a challenging relationship to quantify due to their physiology. These metrics include circumference and the shape of body at time of measurement. To examine the efficacy of these metrics 812 cucumbers were sampled and measured from a specific region within the central Puget

Sound. These measurements were used to create a size index which was regressed against the whole wet weight of the specimens. The study also explores multiple regressions for this relationship, with some relying on the body shape of the specimen at time of processing while others do not. These models were tested via bootstrapped replication and analysis of the simulated data. The results suggest that all models were effective at estimating the weight of specimens with body shape having little to no influence on the result.

The temporal effect of the sampling procedure was also examined. While specimens were processed, they were stored in buckets of seawater on deck. Evaluation was conducted to see if specimens with the longest holding time showed any noteworthy difference in size or weight when compared to those sampled first. Neither size nor weight showed any trend upwards or downwards across sample processing.

Managing Shell-Boring Epibionts in Captive Abalone Stocks

Isaac Trevino, Colleen Burge, Blythe Marshman, Jim Moore, Jaclyn Plasterer; UC Davis- Bodega Marine Lab*

California's abalone populations have dramatically declined over the last 50 years due to overharvesting, disease and pests, and lack of habitat, leading to the white (*Haliotis sorenseni)* and black (*Haliotis cracherodii*) abalone being federally listed as endangered. Historically, there were 13 abalone aquaculture facilities in California with that number being reduced to two due to similar issues. Epibionts like the boring sponge (*Cliona celata*) and polydorid polychaetes *(Polydoridae: Polydora, Boccardia* and related genera) can significantly damage and weaken the shells of older abalone to the point of fracture. While captive abalone are not at risk of the predation that is exacerbated by epibiont infestation, the energy needed to produce new shell comes at the cost of muscle and gonad development which are vital for farming and breeding programs. California abalone farms have observed drastic losses in product due to intense polydora infestations. The California Department of Fish and Wildlife Shellfish Health Lab is responsible for maintaining shellfish health standards in commercial programs and to protect the state's endangered abalone species. The following studies are intended to combat a diversity of polydorid polychaetes at scale. Previously, the Shellfish Health Lab (SHL) developed a waxing treatment of epibiont infestations with a compound made from 66% beeswax and 34% coconut oil. The compound is applied to the dorsal surface of the shell and has been shown to be effective in reducing or eliminating infestations of polydorid polychaetes in captive abalone shells. The SHL is currently experimenting new methods to increase scale and efficiency of the slow and tedious waxing process to be applied in aquaculture facilities. However, this treatment is less effective against *Cliona* due to its weak bond causing the wax to fall off over time. As a result, the SHL is actively testing the use of underwater epoxy in place of waxing treatments. The SHL expects epoxy to provide supplementary shell structure and seal *Cliona* inside, effectively suffocating it. Trials have shown the epoxy to be safe for application to live abalone and preliminary data from epoxy applications lasting three, six, and nine weeks resulted in 64.2, 48.71, and 67.76 percent reduction of overall *Cliona* surface area coverage.

Oyster reef recovery: responses to shell replenishment on public fishing grounds

Alexandria Marquardt, Melissa Southworth, Andrew Scheld, Andrew Button, Roger Mann; Virginia Institute of Marine Science*

Oysters are a benthic dominant, critical ecosystem engineer, and important fishery species in temperate estuaries worldwide. Despite their importance, oyster populations have declined globally. The Rappahannock River in Virginia was essentially closed to harvest for several decades due to epizootics and low oyster abundance. In 2007, the Virginia Marine Resources Commission (VMRC) implemented rotational management in the Rappahannock River. VMRC created 6 management areas, where 2 areas are open per year and each area is open to harvest every 3 years. Oyster harvest rotation allowed for the reestablishment of an active fishery in the Rappahannock River. In addition, VMRC routinely performs shell replenishment,

the provisioning of additional shell material or hard substrates, when the underlying reef falls below management thresholds. Oyster shells have high turnover rates and are a limited, expensive resource; thus, optimizing repletion efforts to maximize restoration success is critical to maintain ecosystem services and support local economies. This project integrates long term data sets including annual oyster population surveys, shell replenishment records, and commercial harvest reports to understand both biological and economic responses to shell replenishment. We used generalized linear mixed effects models to explore how harvester efficiency, market sized oyster abundance, recruitment, and the underlying reef structure respond to shell inputs. Research findings will be used to create a decision tool for managers to inform management decisions and replenishment practices.

Effect of macroalgae diet on growth rate and nutrition of the pinto abalone, *Haliotis kamtschatkana*

James Crimp, Schery Umanzor, Maribel Montiel Vera, Lindsay Meyer; University of Alaska Fairbanks*

Cultivating the pinto abalone, *Haliotis kamtschatkana*, has gained attention over the past decade due to its potential in commercial aquaculture and its role in restoring depleted wild stocks. To enhance the viability of farming *H. kamtschatkana* for commercial and restoration purposes, more information is needed on the optimal macroalgal diet that maximizes its growth and nutrition. This study compares the suitability of two commonly cultivated species of macroalgae as feed: *Saccharina latissima*, a kelp with relatively low protein content, and *Devaleraea mollis*, a rhodophyte with higher protein content. Fifty *H. kamtschatkana* specimens, each measuring 50 ± 10 mm, were collected from the wild and fed either *S. latissima, D. mollis*, or an alternating diet of the two for 200 days. Feed consumption was measured weekly, while shell growth and weight change were recorded bi-monthly. Despite consuming significantly more *S. latissima* than *D. mollis*, there was no significant difference in specific or linear growth rates across the diets. Feed conversion efficiency was significantly higher for *D. mollis* than *S. latissima*, although there was no significant difference in protein conversion efficiency. Proximate analyses showed no significant difference in ash, protein, lipid, carbohydrate, or caloric content across the diets. Additionally, sexual dimorphism was observed, with females exhibiting significantly higher daily food consumption and growth rates than males. The results indicate that while both macroalgae species are suitable as feeds for *H. kamtschatkana* cultivation, the benefits of *D. mollis* are less pronounced compared to other commercially cultivated abalone species. This study provides actionable insights for those interested in cultivating *H. kamtschatkana* for commercial or stock enhancement purposes. It also adds valuable context to the physiology of an environmentally and culturally important species in the Northeast Pacific.

Genetics and Broodstock

Pacific oysters (*Crassostrea gigas*) dramatically recalibrate models for the upper limit of the eukaryotic mutation rate.

Rachel Hua, Nathan D. Churches, Peter L. Chang, Jordan Chancellor, Sergey V. Nuzhdin; USC*

Advanced terrestrial selection breeding programs for food crops serve as a template for future domestication of ocean-based crops. One consideration for marine organisms and their suitability for artificial selection is their mutation rate. Bivalves, one of aquaculture's most heavily produced crop, are thought to maintain among the highest observed mutation rates. Predicted rates of de novo mutations have been indirectly calculated from mutation loads or segregation distortions, frequently from allozyme or microsatellite data in the Pacific oyster (*Crassostrea gigas*). Based on solid theoretical framework, the observed levels of heterozygosity, given effective population size, cannot be easily explained unless the rate of de novo mutations far exceeds other eukaryotes. However, to date, no trios (two parents and their offspring) have

been sequenced at sufficient depth to directly evaluate this hypothesis. Using Pacific oysters, this study utilized three single parent pairings, and randomly selected juvenile individuals from each corresponding family, in order to empirically determine mutation rate. Our findings rank among the highest ever recorded for any organism (10-5 per nucleotide), and dramatically recalibrate the upper limit of the eukaryotic mutation rate from previous models. The authors realize the boldness of this claim, and encourage future work to confirm these results. If borne out, the data herein provide the first direct measurement of mutation rate in a broadcast spawning species with considerable commercial importance, thereby informing best breeding practices.

Improving hatchery efficiency: New insights into sex determination in Pacific oysters.

Bernarda Calla, Jingwei Song, Mark Yeats; USDA-ARS Pacific Shellfish Research Unit*

We are investigating the mechanisms of sex determination and differentiation in the Pacific oyster *Magallana gigas* (previously *Crassostrea gigas*), a sequential hermaphrodite. Despite many studies aimed to identify the factors that lead Pacific oysters to express male or female phenotypes, there is still a very incomplete understanding of the specific causes and molecular mechanisms driving sex expression in this species.

Achieving a better understanding of the sex determination process has many potential practical applications. For example, this information can be used to manipulate sex and fertility outcomes resulting in more efficient use of resources by balancing sex ratios in our broodstock. In addition, this knowledge will inform the development of tools aimed to preventing oysters from heavily investing metabolic and energy resources in reproduction, a known cause of summer mortalities. We have screened mantle tissues for signals of early sex determination using RNA and DNA sequencing. Results from these screenings will be presented.

Gender Reveal for Oysters? An Investigation of Early Sex Determination Mechanisms in Pacific Oysters (*Crassostrea gigas*)

Jingwei Song, Michael Banks, Bernarda Calla; Oregon State University*

Pacific oysters, the most widely cultivated oyster species globally, contribute to over $89 million in sales in the United States alone. Oysters are intriguing sequential hermaphrodites, undergoing sex changes at various points during their lifespan. Nevertheless, the absence of secondary sex characteristics and the lack of sex chromosomes in this species remain a challenge for studying basic mechanisms of sex determination and also for the non-lethal sexing of animals. We set out to answer two questions to address cryptic sex states among oysters: 1. Are there specific early and/or later gene expression patterns that reliably predict final sex state of oysters at spawning time? and 2. Can these gene expression signals be detected from sampling hemolymph, which serves as the invertebrate equivalent of blood? Hemolymph samples were repeatedly and non-lethally collected four times from the same group of marked individual oysters during the period prior to conditioning and throughout gonad maturation. Sex of each oyster were determined at the end of gonad maturation by gonadal biopsy. RNA-seq was used as a comprehensive discovery method to detect temporal differential expression patterns in male and female hemolymph samples. Low levels of expression of *DMRT*, *FOXL2*, *CgSOX*, and *SOX8* were found in the hemolymph, which are known to be involved in sex differentiation in oysters. Resolving markers and mechanisms of oyster sex determination will enhance the efficiency of future spawning and crossing practices, potentially enabling controlled production of specific sex ratios and/or sterile oysters.

Application of a Low-density Amplicon Panel to Support Breeding and Selection Programs in Pacific Oysters

Ben J. G. Sutherland, Konstantin Divilov, Neil F. Thompson, Thomas A. Delomas, Spencer L. Lunda, Christopher J. Langdon, Timothy J. Green; Vancouver Island University*

Selective breeding programs for Pacific oyster are necessary to generate lines that are resistant to specific stressors (e.g., viral infections), that are high yield or fast growing, or that are generally resilient to the multiple challenges oysters face in the ocean environment. Breeding programs may focus on family-based selection using pedigrees, marker-assisted selection, and/ or genomic selection. In Pacific oyster and other shellfish species, these breeding programs must also ensure the maintenance of genetic diversity in the breeding lines, due to the severe impacts of inbreeding that can occur from high genetic load. Fortunately, many genetic tools are available to support these approaches, but cost must be also considered given that available funding for programs may be limited relative to other agricultural species. For these reasons, we recently developed and made publicly available a 592-marker Pacific oyster amplicon panel to genotype at low resolution. In this talk, we will present the various ways that we are using this panel in the context of all three selective breeding approaches mentioned above. We will also present the ways we aim to make the panel adaptable to future developments as new genomic insights are discovered in the Pacific oyster, and the successes and challenges that we have faced to-date applying this panel to oysters from multiple breeding programs in the US and Canada.

The development of a new Kumamoto oyster breeding population based on individuals collected from the Ariake Sea, Japan, in 2006

Chris Langdon, Claudio de Melo, Konstantin Divilov, Noah Merz, Neil Thompson;* Coastal Oregon Marine Experiment Station, Oregon State University

Kumamoto oysters are highly regarded on the US West Coast due to their deep-cupped shells and firm summer meats. Previously, it had been reported that some commercial Kumamoto stocks were highly inbred and were contaminated by accidental hybridization with Pacific oysters. Consequently, in 2006, Kumamoto oysters were collected from the Ariake Sea, Japan, to supplement existing commercial West Coast Kumamoto stocks and to develop a new breeding population for the Molluscan Broodstock Program (MBP). The G_1 generation and early life stages of the G_2 generation of the new Japanese Kumamoto stock were maintained in quarantine and subjected to numerous histological exams and genetic tests to ensure the absence of detectable diseases of concern, before release and planting of G_2 spat at farm test sites.

Harvest traits (yield, survival, growth, shell dimensions and shape) of families of the G_2 and G_3 generations were determined at a sub-tidal farm site in Oregon and compared to those of established West Coast commercial stocks. A single MBP Kumamoto breeding population was produced in the G_5 generation by crossing individuals from Japanese families and West Coast stocks. To create each generation, individuals within families with desirable traits were selected and crossed, while minimizing inbreeding rates. Analysis of the ITS1/2 genomic regions of samples from the G_5 and previous generations indicated no genetic hybridization with Pacific oysters.

Mean individual weights and shell lengths of the G_3 Japanese families were significantly less (Tukey-Kramer; $p<0.05$) than those of families produced from established West Coast commercial stocks; however, other harvest traits (yield, survival, average individual weight, shell dimensions and shape) were not significantly different. Harvest traits of crosses between the Japanese and West Coast populations were intermediate in value compared with those of the two parental populations. Heritabilities for G_5 harvest traits were high, ranging from 0.83 for shell depth to 0.97 for survival, indicating a high potential for genetic improvement through selection. Genetic correlations were generally positive among harvest traits, except for shell shape

(depth/(width + length) which showed negative (but non-significant) correlations with individual weight and meat content, indicating that selection for a higher shell shape ratio may have a negative effect on these other traits.

Assessment of genetic diversity in Pacific oysters from the US West Coast using whole genome resequencing

Bernarda Calla, Neil Thompson; USDA-ARS Pacific Shellfish Research Unit*

The genetic diversity of a population defines its ability to adapt to episodic and fluctuating environmental changes. For species of agricultural value, available genetic diversity also determines their breeding potential. Under this view, comprehensive knowledge of the extant gene pool in Pacific oysters in the US West Coast (*Magallana gigas*, previously *Crassostrea gigas*) remains fundamental to the development of practices that maintain health and productivity in this species. This is the first study that uses whole-genome resequencing to evaluate the genetic diversity within and between naturalized and captively reared populations of Pacific oysters in the US West Coast. We identified over 20 million high-confidence SNPs and used those to evaluate population genetic parameters with the aim of cataloguing genetic diversity and to better understand the effects of hatchery rearing and adaptation in this species.

Harvesting microhaplotypes within an existing SNP panel substantially increases power for relationship reconstruction in POGS breeding populations.

Neil Thompson, Ben Sutherland, Tim Greene, Tom Delomas; USDA-ARS Pacific Shellfish Research Unit*

Amplicon sequence panels have become a staple of modern genetic analysis workflows. Numerous advantages to amplicon panels exist, including the ability to interrogate target SNPs and other variation within each amplicon. This type of data is not accessible using array genotyping methods. Yet, oftentimes amplicon panels are designed and optimized for SNPs and do not harness the extra statistical power that is available from using multi-nucleotide alleles. In this study we evaluated a recently designed SNP panel using three Pacific oyster populations from the USDA POGS breeding program, and evaluated if microhaplotypes provided higher statistical power for likelihood based relationship reconstruction than target SNP and highest Minor Allele Frequency SNPs. The microhaplotype data set used the smallest number of loci (n = 362), yet due to the high number of alleles per locus within each population (average 4.88 alleles per locus) resulted in the highest statistical power for relationship reconstruction. The main difference between target SNP, highest MAF SNP and microhaplotype analyses was a compression of the log likelihood distributions between relationship types in the SNP analyses compare to the microhaplotype analysis. Remarkedly little difference existed between MAF-SNP and target-SNP analyses suggesting that there is little to be gained from optimizing bi-allelic loci compared to microhaplotypes. Overall, these results are promising for the use of microhaplotypes in a number of aquaculture applications including simple parentage analysis to enable more complex breeding designs, as well as for pedigree imputation based genomic selection methodologies which depend on infallible relationship assignments.

Relative performance and mortality of diploid and triploid Pacific oysters throughout Puget Sound in relation to environmental conditions

Craig Norrie, Jacqueline Padilla Gamino, Paul McElhany, Shalin Busch, Jonathan Davis; University of Washington*

Triploid (individuals that possess an additional set of chromosomes) Pacific oysters, *Crassostrea gigas*, make up a significant portion of farmed oysters in Washington and worldwide. Their faster growth and higher

marketability provide significant benefits to oyster growers. Triploid oysters, however, experience higher mortality than diploids. Because these mortalities typically occur in summer when environmental conditions are more stressful, it is hypothesized that they are due to interactions between environmental conditions and chromosome number. The physiological mechanisms behind these differences in mortality, however, are unclear. Understanding drivers of triploid mortality is key to enhancing production and profitability of the aquaculture industry. Here, we examined how multiple interacting climate change stressors (temperature, pH, hypoxia) impact Pacific oyster performance throughout Puget Sound, Washington. We outplanted diploid and triploid (individuals with an additional set of chromosomes) oysters at four shellfish farms, monitored their performance (mortality, growth, respiration), and collected environmental information (temperature, oxygen, pH) in summer 2023 and 2024. We observed a pulse of high mortality at only one site, with the timing of this mortality coinciding with a drop in oxygen levels. No other differences in mortality were observed between sites. Spatial differences in oxygen consumption existed, however differences did not correlate with variation in environmental conditions. Results indicate oyster performance varies between sites with no clear impact of a single stressor on oyster performance or survival. This highlights the need to consider variation in multiple interacting stressors over small spatial scales to develop strategies to manage the impacts of climate change in coastal ecosystems.

Multitrophic Interactions

Recruitment and losses through the life cycle for intertidal clams in Willapa Bay, Washington

Jennifer Ruesink, Emily Grason, Lidia Garcia, Ella Ong; University of Washington*

When bivalve aquaculture production relies on natural recruitment, yields may decline due to density-independent constraints at multiple life stages. These life stage transitions include larval settlement, which is typically variable in space and time, rapid losses of newly-settled individuals, and additional mortality from predators or abiotic factors as the bivalves grow. Recruitment monitoring and outplants were used to evaluate the potential contributions of limiting factors at different life stages in two non-native clam species in Willapa Bay, Washington, USA (Manila clams, *Ruditapes philippinarum*; softshell clams, *Mya arenaria*). Recruits (250-500 µm) did not differ in cumulative abundance in 2023 relative to prior years of monitoring (2-3 times since 2011). Recruits surviving to the end of the summer represented 12% or less of those that had arrived but still showed spatial patterns consistent with rates of arrival. Manila clams outplanted across an elevation gradient suffered high mortality below mean lower low water regardless of size class (small: 8 mm; large: 20 mm) where native rock crabs were abundant. Clam growth declined at higher tidal elevations consistent with inundation time. European green crabs (*Carcinus maenas*) were relatively abundant at mid-tidal levels where commercial clam aquaculture typically occurs in the bay, and where small clams were particularly reduced without predator protection from mesh. While these data support that green crabs may be reducing survival of 1-year-old clams at mid-intertidal elevations, they also identify earlier bottlenecks to repopulating commercial clam beds, which point out why seeding clams is used to maintain consistent production. The relative roles of predators across size classes of clams in Willapa Bay, including those that could cause losses of ca. 1 mm clams within 1 mm mesh bags, need further exploration before green crabs can be singled out as a new limit to clam yields.

Landscape Scale Assessment of Eelgrass, Oyster Aquaculture, and Channel-fringing Habitat Provided to Managed Nekton Species in a US Pacific Coast Estuary.

Nathaniel Lewis, Brett R Dumbauld, Brooke McIntyre, and David Beugli*; *USDA-Agriculture Research Service*

Remote sensing tools have become a valuable tool for assessing interactions between shellfish aquaculture and submerged aquatic vegetation at the estuarine landscape scale. A geographic information system (GIS) with data layers for eelgrass and active aquaculture distributions was first developed in Willapa Bay, Washington in the mid-2000's. Data was collected using an extensive ground survey and ortho-imagery (4-band) captured from fixed-wing aircraft and information on active aquaculture boundaries, aquaculture bed type and bed use collected via interviews with shellfish growers. These historical data served as a baseline for comparisons with updated GIS layers of eelgrass and active aquaculture distribution based on new high resolution 2020 ortho-imagery. Imagery was captured during ideal low tide conditions and allowed for improved classification of eelgrass as well as visible evidence of culture, equipment, and physical use of the culture beds that was then cross-checked and verified with industry. Overall, eelgrass coverage estimates in Willapa Bay declined slightly from 5,938 ha in 2009 to 5,551 ha in 2020. While active oyster aquaculture increased from 1,764 ha to 3,137 ha, this can largely be attributed to better definition of "active" aquaculture, since "total" oyster aquaculture was also estimated to be 3,474 ha in 2005. We present an example of how these new estimates can be used alongside a targeted and comprehensive local scale survey of habitat use to evaluate estuary-wide provision of habitat for two managed species, Dungeness crab and English sole where we targeted channel fringing habitat (<25m from channels) based on previous assessments of habitat use by these species.

Evaluating Image Analysis for Measuring Dungeness Crab (*Metacarcinus magister*) Megalopae in a Monitoring Network

Claire Curran, P. Sean McDonald; University of Washington

The Pacific Northwest Crab Research Group (PCRG) is a monitoring network aiming to predict adult Dungeness crab biomass through counting and measuring Dungeness crabs during their megalopae larval stage. Currently, measurements are taken using a caliper in the field, which can be time consuming for researchers. ImageJ is a free-downloadable photo processing program, which allows users to measure objects within the photograph using a reference scale bar in the image (Ferreira & Rasband, 2011). The aim of this study is to (1) produce a method for the use of ImageJ to measure megalopae, (2) evaluate if ImageJ can generate measurements that are accurate to measurements in the field, and (3) use ImageJ to measure multiple megalopae in the same picture. Dungeness crab megalopae were captured using a light trap deployed at the Shilshole Bay Marina, in Seattle, Washington from April to August, 2024. Samples were taken on the Monday, Wednesday, Friday, and Saturday of the sampling period. During sampling, measurements of the total length, carapace height, and carapace width were taken using a caliper. In addition to these field measurements, individual pictures were taken of the megalopae and the same measurements were done using ImageJ. In addition to individually taking pictures of megalopae, photographs of multiple megalopae at once were taken for measurement using ImageJ. The field measurements were compared to the ImageJ individual and group photo measurements to assess accuracy. The average difference between field measurements and ImageJ measurements was less than 1 millimeter for all three measurements taken (total length, carapace height, and carapace width) in both group photos and individual photos. The ImageJ measurements for individual pictures differed from the field collected measurements by approximately +/-0.552mm for total height measurements, +/-0.568mm for carapace height measurements, and +/-0.476mm for carapace width measurements. ImageJ measurements for group pictures differed from field collected measurements by approximately +/- 0.519mm for total height measurements, +/- 0.765mm for carapace height measurements, and +/- 0.435mm for carapace width measurements. With the new methods and results of this study, a more efficient technique for measuring, not only larval Dungeness crabs, but also other larval marine species, could be adapted in future research. By using ImageJ, multiple megalopae can be measured at once, minimizing the handling of the crabs and decreasing the effort of researchers.

Public Education and Engagement

A trusted data oyster with a clam like formula and statistical mussel helping to meet information needs!

Diana Short, Katherine Marshall; National Animal Health Monitoring System (NAHMS) USDA*

This is a short overview talk to inform shellfish growers, hatcheries, and industry stakeholders on what the National Animal Health Monitoring System (NAHMS) does for animal agriculture. The USDA initiated NAHMS in 1983 to help collect, analyze, and disseminate data on animal health, management, and productivity across the United States. Since that time NAHMS has worked collaboratively with animal agriculture sectors to design and conduct studies to meet information needs. This presentation addresses: Who is NAHMS? What can NAHMS do for shellfish growers and stakeholders? What does it mean to be a Data Oyster with a Clam-like approach and Statistical Mussel? What information needs can NAHMS help with and what tools does NAHMS have available to meet its mission.

Lightning Round

Water Column Stratification of Mariculture Sites Across the Gulf of Alaska

Sierra Greene, Ginny Eckert; University of Alaska Fairbanks*

To better support the developing mariculture industry within Alaska, a localized focus on the oceanography of current farms is needed to enhance future site selection. In shallow, near shore regions within the Gulf of Alaska, stratification can occur in the summer months due to freshwater input and glacial runoff. Stratification in coastal zones can lead to decreased vertical nutrient supply, reduced primary production, and depleted oxygen levels throughout the water column; resulting in ideal conditions for harmful algal blooms and less suitable conditions for mariculture growth. This research will determine the variability of water column stability and stratification throughout the year at farm sites spread across the Gulf of Alaska. To do this, we partnered with eight kelp and oyster farmers in Kodiak, Kachemak Bay, and Prince William Sound, who will collect monthly CTD vertical profiles of salinity and temperature on their farms. From this farmer collected data, the density, simpson stratification index (SSI), and Turner angle of the water column will be calculated to determine shifts in stratification throughout the year and within each region. Characterizing the spatiotemporal variability of stratification at each site will provide insight on the effects of seasonal freshwater influences on site location, allowing potential farmers to have a more accurate perception on where to expect optimal growth.

Automating Oyster Aquaculture with the Sun

Johnny Shockley, Steve Pattison; Blue Oyster Environmental / Solar Oysters LLC*

Oyster aquaculture technology has been relatively static in an industry needing to grow to meet global protein demand and advance restoration. Oyster aquaculture is labor intensive, often growing less than a half million oysters per acre depending on gear types and site location. Floating and off-bottom gear is typically positioned in the near-shore environment or in the upper few feet of the water column, limiting overall growth and increasing conflicts with landowners and other stakeholders.

The Solar Oyster Production System (SOPS) has been developed to take advantage of offshore space that is typically not used by other oyster aquaculture systems. The SOPS supports a series of cages in up to twenty feet of water and utilizes solar power to mechanically rotate the cage array through the water column and above the water, providing desiccation/air exposure and the opportunity for mechanized washing, which minimizes overall labor inputs. The rotation sequence can be programmed to meet the needs of the grower. The mooring system and robust design of the platform allows location of the SOPS in higher-energy environments, with integrated anti-poaching technology that allows units to be placed further offshore, potentially minimizing stakeholder conflict. SOPS can grow spat-on-shell oysters for restoration or from seed for market oysters. Approximately 150,000 oysters from seed can be grown on one 40' x 25' SOPS prototype. In the Chesapeake Bay watershed, nitrogen and phosphorus credits for oysters harvested from aquaculture operations can be traded on the nutrient credit market. Use of the SOPS for 1 acre of high-density oyster aquaculture may remove nutrients equivalent to treating stormwater runoff from over 100 acres of impervious surface.

A SOPS prototype was launched in 2021 and loaded with spat-on-shell oysters in coordination with the Chesapeake Bay Foundation. For two years, growth was successful and approximately 400,000 diploid oysters were deposited on a reef located in the Baltimore Harbor. This was using one third of SOPS' capacity. Triploid seed oysters were grown to gauge the effectiveness of the technology. In 2024, SOPS operated on Hoopers Island Oyster Company's lease to gauge its effectiveness on an oyster aquaculture farm. Full commercialization of SOPS is targeted for 2025.

An Ulva Update: Farm to Farm Connections

Emily Buckner and Hannah Garfield; Puget Sound Restoration Fund

Puget Sound Restoration Fund's new 'Sea-to-Land' program has been exploring the removal of excess seaweed (e.g. *Ulva* spp.) from shellfish farms as a way to improve local water quality and help sustain healthy shellfish growing areas. This work has particularly focused on supporting growers in establishing sustainable seaweed removal practices and identifying potential upland markets for this new 'product'. In this talk, we'll touch on a few highlights from this past year, including testing passive drying systems for harvested seaweed, investigating the potential for *Ulva* as an animal feed additive, and partnering with Master gardeners and soil scientists to better understand seaweed's potential as a beneficial soil amendment.

Applications of Drone Technology for Sustainable Production and Management of Intertidal Shellfish Farm

Katie Houle; Pacific Shellfish Institute

Advances in remote sensing technologies for precision agriculture, including small aerial drones, have increased in recent years and become more cost effective and accessible for public consumers to operate. We investigated the use of a small, “off-the-shelf” aerial drone to produce high-resolution (< 1 cm/px) imagery of intertidal shellfish farms for inventory development, vegetation mapping and monitoring for nuisance species. Drone flights were completed during summer months in 2023 and 2024 on intertidal shellfish farms in Washington state. Imagery was collected with a DJI Mavic 3 multispectral drone with on board RGB, visible light camera (20 MP) and multispectral camera with Green, Red, Red Edge, Near Infrared sensors (5 MP). Images were post-processed and georeferenced into orthomosaics using the photogrammetry software Agisoft Metashape v.2.1.2. Raster orthomosaics were imported into ArcGIS Pro to identify benthic features of interest. Aquaculture gear (clam nets, off-bottom culture) were heads up digitized into vector polygon layers. Bottom grown Pacific oysters, eelgrass and macroalgae were classified using geoprocessing tools for Object-based Image Analysis (OBIA). The OBIA process segments a raster

image by grouping pixels together into vector objects for further land use classification. The classified features were converted from raster to vector polygon layers and total area (acres) of each feature were calculated. With known planting or stocking densities multiplied by total area cultured, estimates of shellfish biomass can be calculated. Future research will include training a deep learning model to identify both live and dead bottom grown Pacific oysters to efficiently map shellfish mortalities from aerial drone imagery.

Real Time Fecal Coliform "Sensor" Trials

Andy Suhrbier, Pacific Shellfish Institute

Remote shellfish farms are water quality data limited due to shipping costs and distance to certified laboratories. While a promising solution to understanding fecal contamination, derived near real time total and fecal coliform data from deployed sensors is still in its infancy. The Pacific Shellfish Institute evaluated the Proteus water quality unit, using Tryptophan and CDOM sensors to calculate fecal and total coliforms, in Alaska and Washington. New algorithms were created for these marine environments for total and fecal coliforms. These algorithms appeared to work sufficiently to derive trends in timing, depth and rainfall for fecal coliforms in and around shellfish farms, including remote locations. While this unit should not be used in any regulatory manner at its current state of evaluation, future trials are encouraged to gain insight on localized trends and algorithm/sensor suitability.