Symposium Schedule

51st Scientific Symposium of the UJNR Aquaculture Panel

"*Control and Management of Aquaculture Disease*"

Hilton Garden Inn
5 Park Street
Freeport, Maine
August 29th and 30th, 2023

51st Scientific Symposium of the UJNR Aquaculture Panel
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Hilton Garden Inn
5 Park Street, Freeport, Maine

Aim of the Symposium

The NOAA Office of Aquaculture, in cooperation with USDA's Agricultural Research Service, will host the 51st UJNR Aquaculture Panel Scientific Symposium in Freeport, Maine, United States. The UJNR Aquaculture Panel is a cooperative research exchange between the U.S. and Japan, jointly addressing environmental and technical issues that affect the aquaculture industries of both nations.

The 51st UJNR Aquaculture Scientific Symposium is the final year of the current three-year theme of Control and Management of Aquaculture Disease. With an increasing global demand for seafood and a rapidly growing aquaculture industry, the prevention and control of infectious disease is important for all sectors including fish, shellfish, and seaweeds. Disease problems often constitute large economic losses in aquaculture. Effective control of disease is a top industry and government priority. This year's presentations will focus on the status and understanding of disease occurrences, current systems for monitoring, treatment and prevention.

Program

Tuesday, August 29, 2023

Registration 12:00 - 13:00

Welcome and Aim of the Symposium
Janet Whaley, US Panel Chair, NOAA Fisheries Office of Aquaculture 13:00-13:15

Maine Shellfish Industry
(Moderator: Luke Gardner)

Maine's Scallop Farming Industry Emerges, as a Direct Outcome of Japan/US Technology Transfer
Hugh Cowperthwaite, Coastal Enterprises, Inc., Fisheries and Aquaculture
Dana Morse, Maine Sea Grant and University of Maine Cooperative Extension 13:15-13:45

Shellfish and Seaweed Epidemiology and Health Management
(Moderators: Luke Gardner and Kousuke Umeda)

Viral and bacterial diseases of marine shellfish in Japan
Tomomasa Matsuyama, Fisheries Technology Institute,
Fisheries Research and Education Agency 13:45-14:15

Finding a focus on summer mortality of aquacultured oysters
Ryan Carnegie, Virginia Institute of Marine Sciences,
College of William & Mary 14:15-14:45

Providing healthy outcomes for the critically endangered white abalone, *Haliotis sorenseni*
Colleen Burge, Univ. of California Davis Bodega Marine Laboratory,
California Department of Fish & Wildlife 14:45-15:15

Break 15:15-15:30

An overview of disease and pests on cultivated kelp, with a focus on Alaska
Jordan Hollarsmith, Alaska Fisheries Science Center, NOAA 15:30-16:00

Response of two Pacific oyster populations to OsHV-1 microvariant challenge
Neil Thompson, Agricultural Research Service,
United States Department of Agriculture 16:00-16:30

Closing remarks 16:30-16:45

Wednesday, August 30, 2023

Registration 08:30 - 08:50

Opening Remarks
Janet Whaley, US Panel Chair, NOAA Fisheries Office of Aquaculture 08:50-09:00

Maine Marine Aquaculture industry
(Moderator: Ken Riley)

Emerging aquaculture trends in the Gulf of Maine
Damian Brady, Aquaculture Research Institute, University of Maine 09:00-09:30

Finfish epidemiology and genomics
(Moderators: Ken Riley and Tomokazu Takano (first half), Yasuhiko Kawato (latter half))

Estimating transmission risk of red sea bream iridovirus between fish farms via seawater using environmental DNA
Yasuhiko Kawato, Fisheries Technology Institute,
Fisheries Research and Education Agency 09:30-10:00

Investigating routes of pathogen spreading in a saltwater fish farm
Tomofumi Kurobe, Fisheries Technology Institute,
Fisheries Research and Education Agency 10:00-10:30

Break 10:30-10:45

Development of a multiplex PCR to investigate genetic diversity of *Flavobacterium psychrophilum* infecting ayu (*Plecoglossus altivelis*)
Tomokazu Takano, Fisheries Technology Institute,
Fisheries Research and Education Agency 10:45-11:15

Phylogenomic Characterization of Nucleocytoplasmic Large DNA Viruses In Poikilothermic Vertebrates 11:15-11:45
Tom Waltzek, Washington Animal Disease Diagnostic Laboratory,
Washington State University

Analysis of Japanese flounder family resistance to two different bacterial diseases against *Edwardsiellosis*
Hiroyuki Okamoto, Fisheries Technology Institute,
Fisheries Research and Education Agency 11:45-12:15

Lunch Break 12:15-13:30

Finfish Health and Disease Management
(Moderators: Caird Rexroad and Tomofumi Kurobe)

Prevention of viral endothelial cell necrosis of eel (VECNE) in aquaculture farms
Kousuke Umeda, Fisheries Technology Institute,
Fisheries Research and Education Agency 13:30-14:00

Infectious salmon anemia virus investigations at the USDA National Coldwater Marine Aquaculture Center
Mark Polinski, Agricultural Research Service,
United States Department of Agriculture 14:00-14:30

Framework for National Aquaculture Health
Kathleen Hartman, Animal & Plant Health Inspection Service,
United States Department of Agriculture 14:30-15:00

The manufacture and use of autogenous vaccines to address emerging threats to aquaculture
Bill Keleher, Kennebec River Biosciences 15:00-15:30

Break 15:30-15:45

USDA efforts to improve management of sea lice, *Lepeophtheirus salmonis*, on domestic salmon farms
Mike Pietrak, Agricultural Research Service,
United States Department of Agriculture 15:45-16:15

Use of modern technologies to advance Atlantic salmon reproductive systems research
Erin Legacki, Agricultural Research Service,
United States Department of Agriculture 16:15-16:45

Scientific Symposium Closing
Janet Whaley, US Panel Chair, NOAA Fisheries Office of Aquaculture 16:45-17:00

Panel Members and Speakers

Japan:

Hideaki Aono (FTI)	Chair	FRA, Fisheries Technology Institute
Ryuji Kuwahara	Vice-Chair	FRA Headquarters, General Planning and Coordination Department
Masatsugu Takano	Committee Member	FRA Headquarters, General Planning and Coordination Department
Hirofumi Furuita	Assistant Secretary	FRA, FTI, Aquaculture Research Department
Hiroyuki Okamoto	Secretariat/Symposium Presenter	FRA, FTI, Aquaculture Research Department

Tomofumi Kurobe Aquaculture Research	Secretariat/Symposium Presenter	FRA, FTI,
Tomomasa Matsuyama Aquaculture Research	Symposium Presenter	FRA, FTI, Department
Tomokazu Takano Research Department	Symposium Presenter	FR, FTI, Aquaculture
Yasuhiko Kawato Aquaculture Research	Symposium Presenter	FRA, FTI, Department
Kousuke Umeda Aquaculture Research	Symposium Presenter	FRA, FTI, Department

United States:

Janet Whaley International Commerce	Chair	NOAA Fisheries, Office of Affairs, Trade, and
Luke Gardner	Vice-Chair	California Sea Grant
Caird Rexroad Research Service	Panel Member	USDA, Agricultural
Ken Riley Aquaculture	Panel Member	NOAA Fisheries, Office of
Kathleen Hartman Plant Health Inspection	Symposium Presenter	USDA, Animal & Service
Neil Thompson Research Service	Symposium Presenter	USDA, Agricultural
Mark Polonski Research Service	Symposium Presenter	USDA, Agricultural
Mike Pietrak Research Service	Symposium Presenter	USDA, Agricultural
Erin Legacki Research Service	Symposium Presenter	USDA, Agricultural
Jordan Hollarsmith Fisheries Science Center	Symposium Presenter	NOAA, Alaska
Thomas Waltzek University	Symposium Presenter	Washington State
Dana Morse University of Maine Extension	Symposium Presenter	Maine Sea Grant and Cooperative
Hugh Cowperthwaite Inc., Fisheries and	Symposium Presenter	Coastal Enterprises, Aquaculture

Damian Brady Aquaculture Research	Symposium Presenter	University of Maine, Institute
Ryan Carnegie Mary, Virginia Sciences	Symposium Presenter	College of William & Institute of Marine
Colleen Burge Department of Fish and Wildlife	Symposium Presenter	California
Bill Kelleher Biosciences	Symposium Presenter	Kennebec River

51st Scientific Symposium of the UJNR Aquaculture Panel

Control and Management of Aquaculture Disease

Symposium Abstracts

1. Maine's Scallop Farming Industry Emerges, as a Direct Outcome of Japan/US Technology Transfer

Hugh Cowperthwaite[1] and Dana L. Morse[2]

[1]Fisheries and Aquaculture. Coastal Enterprises, Inc. 30 Federal Street, Brunswick, ME. 04011, *Email:* Hugh.Cowperthwaite@ceimaine.org
[2]Maine Sea Grant College Program, and University of Maine Cooperative Extension. Darling Marine Center, 193 Clark's Cove Road, Walpole, Maine, 04573, *Email:* dana.morse@maine.edu

Abstract
Landings of farm-raised Atlantic scallops (*Placopecten magellanicus*) exceeded $100,000 in 2022 (Maine Dept. of Marine Resources, Landings Division); an encouraging indication of advancement towards enterprise profitability (Fig. 1) . Markets for whole/live and shucked products have been strong, commanding prices above wild-harvested product. Producers have attracted private and traditional capital - including Coastal Enterprises, Inc. - further indicating that financial reviews of scallop farms are positive enough for such investments. The present view is that scallop farming in Maine is emerging as a real sector of the industry, and one which can entertain future growth.

Figure 1. Maine landings of farmed and wild scallops, courtesy of Maine Dept. of Marine Resources.

2018-2022* Commercial Maine Landings					
Species	2018	2019	2020	2021	2022*
oysters (Tot)					
Pounds	2,790,213	3,276,796	3,528,426	6,038,941	3,383,684
Value	$7,286,107	$7,665,497	$4,843,465	$13,483,755	$8,393,344
oysters (AQ)					
Pounds				5,244,572	3,108,333
Value				$11,274,199	$7,831,181
periwinkle					
Pounds	664,847	573,794	264,302	496,199	482,323
Value	$638,593	$599,696	$297,226	$821,817	$800,086
sandworms					
Pounds	196,532	208,676	235,998	197,749	183,734
Value	$1,487,826	$1,585,795	$2,102,155	$1,974,171	$1,904,603
scallop (Tot)					
Live Pounds	5,032,595	3,613,589	5,619,483	4,811,691	4,349,866
Meat Pounds	604,153	433,804	674,608	577,634	522,193
Value	$6,359,662	$4,545,669	$6,975,003	$9,298,397	$8,768,193
scallop (AQ)					
Live Pounds				3,072	3,373
Meat Pounds				369	405
Value				$81,629	$103,220

Much of the work has focused on concrete operational issues: spat collection techniques and equipment, lantern and pearl net production, ear hanging techniques and machinery, biofouling management, etc. The authors have imported a variety of equipment from Mutsu-Kaden Tokki Co of Aomori City in Aomori Prefecture, Japan; one producer has purchased equipment from the HAMADE Company of Towa Denki Seisakusho, Inc, of Hakodate, Hokkaido, Japan. Pearl and lantern nets have come from the Jin Fishing Net Company, also of Aomori City, Japan.

Other positive signs of growth for scallop farming in Maine:

- A market analysis of farmed Atlantic sea scallops showing promise
- A Farmed Scallop Recipe Cookbook to help consumers, chefs and wholesaler dealers understand product formats and options
- Significant investment in shared equipment to give multiple growers opportunity to grow scallops
- Increasing numbers of shellfish dealers carrying whole scallops
- Increasing number of producers selling live product.
- Improved data describing PSP and ASP levels in scallop tissues, and at different sites; critical for risk management, farm siting, and proper regulation.
- A strong bioeconomic model for scallop production (Coleman et al 2021), already in use by industry.
- Decades of relationship building and collaboration between industry in Maine and Japan

Pests and Diseases:

P. magellanicus is susceptible to a suite of pests, pathogens and predators; these will be critical to understand as production grows. Concern exists regarding the bacterium *Williamsia maris*, polychaete *Polydora websterii*, sponge *Cliona celata*, the nematode *Sulcascaris sulcata,* and an undentified apicomplexan parasite (Inglis et al 2016)*;* all of which can have strongly negative impact on a shellfish crop. Other, unidentified organisms (Fig. 2) have been observed to strongly degrade scallop tissues; and even if mortality is not the result, marketability and growth are clearly at risk. Additionally, human health risks from phycotoxins are real and significant, given the capacity of P. magellanicus to collect and retain saxitoxin and domoic acid, in particular (Bricelj and Shumway, 1998).

Figure 2. Wild, unmarketable scallops from Maine, exhibiting unknown pathology.

Regulations and management protocols are in place to minimize the human health risks from phycotoxins, to gather relevant data, and to minimize the spread of pests, predators and pathogens during the entire scallop cultivation process.

References

Bricelj, M. and S. Shumway. 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. *Rev. Fish. Sci.* 6(4); 315-383.

Coleman, S., Morse, D., Brayden, W.C, and D. Brady. 2021. Developing a bioeconomic framework for scallop culture optimization and product development. *Aquaculture Economics & Management*, DOI: 10.1080/13657305.2021.2000517

Inglis, S., Kirstmundsson, A., Freeman, M., Levesque, M. and K. Stokesbury. 2016. Gray meat in the Atlantic sea scallop, *Placopecten magellanicus*, and the identification of a known pathogenic scallop apicomplexan. *J. Invert. Path*. v 141, p 66-75. https://doi.org/10.1016/j.jip.2016.10.008

Maine Dept. of Marine Resources, Landings Division. https://www.maine.gov/dmr/sites/maine.gov.dmr/files/inline-files/LandingsBySpecies.Table_.pdf

2. Viral and bacterial diseases of marine mollusks in Japan

Tomomasa Matsuyama

Pathology Division, Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Mie, Japan, *Email: matsuyama_tomomasa55@fra.go.jp*

Abstract

In Japan, over 30 mollusk species have been cultivated or produced for stock enhancement. Of these, the species produced on a large commercial scale are oysters (*Crassostrea gigas*), scallops (*Mizuhopecten yessoensis)*, pearl oysters (*Pinctada fucata)*, and abalones (*Haliotis* spp.). Unlike finfish aquaculture where feeding costs contribute to 60-70% of production expenses, bivalve shellfish aquaculture is highly cost-effective as it does not require feeding. However, certain operations within shellfish aquaculture, such as oyster peeling, are labor-intensive, often involving local fishery cooperatives for equipment maintenance and human resource management. The aquaculture of mollusks is more dependent on the natural environment compared to fish aquaculture, making it susceptible to infectious disease outbreaks and fluctuations in marine conditions.

Japan has often experienced farmed and wild mortality in mollusks, many of which had no identifiable cause. However, the application of Next-Generation Sequencing (NGS) in this field has led to the discovery of several new pathogens. Using NGS, our laboratory has identified a spirochete[1)] and a birnavirus[2)] as the pathogens of two important diseases of Akoya oyster (*P. fucata*), Akoya oyster disease and summer atrophy. These diseases have caused a significant decrease in pearl production in this country. We have also identified the virus belonging to the Asfarviridae as the pathogen of abalone amyotrophia[3)]. This disease causes mass mortality of juvenile *Haliotis* spp., but the causative agent had remained unknown for more than 20 years. Although none of the organisms fulfilled Koch's postulates, they are considered causative agents based on their characteristics and epidemiological evidence. In this presentation, I will introduce viral and bacterial diseases in Japanese mollusks, focusing on the three pathogens we have recently identified.

Annotated Bibliography of Key Works

Matsuyama, T., Yasuike, M., Fujiwara, A., Nakamura, Y., Takano, T., Takeuchi, T., ... & Nakayasu, C. (2017). A Spirochaete is suggested as the causative agent of Akoya oyster disease by metagenomic analysis. *PloS one*, *12*(8), e0182280.

Akoya oyster disease is an infectious disease that has been occurring since 1994 and causes mass mortality of adult oysters. In this study, the pathogen was presumed to be a spirochete closely related to the genus *Brachyspira*, named "*Candidatus* Maribrachyspira akoyae," through 16SrRNA metagenomic analysis and other methods. Subsequent epidemiological investigations have consistently linked this bacterium to Akoya oyster disease, providing an explanation for the occurrence of the disease.

Matsuyama, T., Miwa, S., Mekata, T., Matsuura, Y., Takano, T., & Nakayasu, C. (2021). Mass mortality of pearl oyster (Pinctada fucata (Gould)) in Japan in 2019 and 2020 is caused by an unidentified infectious agent. *PeerJ*, *9*, e12180.

Mass mortality of 0-year-old pearl oysters and anomalies in adults has been observed in Japan's major pearl farming areas in the summer since 2019. Although adult oyster mortality was low, both adult and juvenile oysters underwent atrophy of the soft body, detachment of the mantle from the nacre, and deposition of brownish material on the nacre. In this study, the pathogen was presumed to be a non-enveloped virus less than 100 nm by infection testing. A currently ongoing study has identified the causative agent as a virus closely related to the genus Entomobirnavirus through comparative metatranscriptome analysis and infection tests utilizing the purified virus.

Matsuyama, T., Kiryu, I., Mekata, T., Takano, T., Umeda, K., & Matsuura, Y. (2020). Pathogenicity, genomic analysis, and structure of abalone asfa-like virus: evidence for classification in the family Asfarviridae. Journal of General Virology, Accpet

In previous studies, we identified the pathogen of abalone amyotrophia and tentatively named it Abalone asfa-like virus (AbALV). In this paper, we show that AbALV is classified into the family Asfarviridae based on genomic analysis and electron microscopy of AbALV virions.

3. Finding a focus on summer mortality of aquacultured oysters

Ryan B. Carnegie

Virginia Institute of Marine Science, William & Mary, P.O. Box 1346, Gloucester Point, Virginia 23062, U.S.A., *E-mail: carnegie@vims.edu*

Abstract
As aquaculture grows, the challenges of maintaining biosecurity and understanding endemic and emerging disease threats under dynamically changing marine environments will continue to grow as well. Fundamental to this will be improved networking: working together across regions and among different stakeholder groups—industry, regulation, the scientific community, coastal societies—to reduce isolation and more effectively manage and find solutions to emerging problems. In the shellfish aquaculture realm in the eastern and southern U.S.A., the creation of a Regional Shellfish Seed Biosecurity Program (RSSBP) is exemplary of a new approach to networking. This program, begun in 2014, with sponsorship from NOAA and the USDA Animal and Plant Health Inspection Service Veterinary Services, brings together the shellfish pathology community, aquaculturists, and state and federal regulators to more effectively manage shellfish health and biosecurity in the context of interstate commerce in aquaculture mollusc products. This program has created a Hatchery Compliance Program to strengthen engagement between producers and the pathology community, to improve shellfish health through promotion of best practices, and to create streamlined commerce in the most biosecure shellfish products. It has created a Regional Shellfish Biosecurity Surveillance Database to consolidate disease surveillance data across all the coastal states and make it publicly available to facilitate decision-making on shellfish transfers by regulators. It has created a Regional Shellfish Health Advisory Council with representatives from the pathology community, industry, regulation, and extension to assist the states with difficult decisions concerning transfers. And it has created a Pathology Working Group to improve information sharing, coordinate development and application of diagnostic approaches, and promote dialogue that will improve understanding of, and responses to, emerging challenges.

The rise of "summer mortality" in aquacultured eastern oysters (*Crassostrea virginica*) is one of the great challenges of our time for both the oyster aquaculture industry as well as the scientific community. This syndrome has defied clear characterization. First noted in 2012, it is expressed in elevated mortality (typically 30%, sometimes over 80%) in 1-year-old oysters in intensive aquaculture, generally around the timing of peak reproductive development from late spring to early summer. Factors contributing to this mortality may include the stress of reproduction at a time of year when metabolic demands are high

because of high temperatures, but nutrition may be sub-optimal; the genetics of the aquacultured oysters; the farm-level husbandry practices being employed; and acute environmental stress. Farms along nearly the entire Atlantic and Gulf of Mexico coasts of the U.S.A. have been affected. Specific pathogens are not known to be involved. The networking established and promoted by the RSSBP has become the foundation of collective responses to this regional problem. This presentation will offer a more detailed perspective on the summer mortality problem, thoughts on underlying causes, and a vision of where research collaboration between the aquaculture industry and the scientific community needs to go for solutions to be established that will improve the sustainability of aquaculture production in the region.

Annotated Bibliography of Key Works

Carnegie, R.B., I. Arzul, and D. Bushek. 2016. Managing Marine Diseases in the Context of Regional and International Commerce: Policy Issues and Emerging Concerns. Philosophical Transactions of the Royal Society B 371: 20150215. http://doi:dx.doi.org/10.1098/rstb.2015.0215

This paper presents the paradox of the list, the paradox of advanced diagnostics, and the paradox of uncertainty noted in the abstract above. It is a key publication highlighting the "blind spots" in disease management created by use of notifiable lists and advanced diagnostics in particular, and presents a unique perspective on the troubled response to OsHV-1.

Guévélou, E., R.B. Carnegie, J.M. Small, K. Hudson, K.S. Reece, M.M. Rybovich, and S.K. Allen, Jr. 2019. Tracking triploid mortalities of eastern oysters *Crassostrea virginica* in the Virginia portion of the Chesapeake Bay. Journal of Shellfish Research 38: 101-113. http://doi.org/10.2983/035.038.0110

This paper describes the initial observations of summer mortality ("triploid mortality") on the U.S. East Coast.

ICES. 2019. Workshop on Emerging Mollusc Pathogens (WKEMOP). ICES Scientific Reports 1:57. 19 pp. http://doi.org/10.17895/ices.pub.5577

This paper highlights thirty-six priority areas for improvement in our detection and responses to emerging diseases. While mollusc-focused, the priorities are transcendent, equally relevant to fish and crustacean systems.

Gustafson, L.L., I. Arzul, C.A. Burge, R.B. Carnegie, J. Caceres-Martinez, L.H. Creekmore, B. Dewey, R. Elston, C.S. Friedman, P. Hick, K. Hudson, C. Lupo, B. Rheault, K.A. Spiegel, and R. Vásquez-Yeomans. 2021. Optimizing surveillance for early disease detection: Expert guidance for Ostreid herpesvirus surveillance design and system sensitivity calculation. Preventive Veterinary Medicine 194: 105419. http://doi.org/10.1016/j.prevetmed.2021.105419

This is an important new publication that highlights a key area of potential focus for detection of emerging pathogens, the passive surveillance represented by farm-level observations made every day by aquaculturists.

4. Providing healthy outcomes for the critically endangered white abalone, Haliotis sorenseni

Colleen A. Burge[*1], Blythe C. Marshman[1], Audrey A. Deutsch[2], Chelsey A. Souza[2], Kristin M. Aquilino[2], James D. Moore[2], & Alyssa R. Frederick[2]

Presenting author*

[1]California Department of Fish and Wildlife, Bodega Marine Laboratory, University of California Davis, PO Box 247, Bodega Bay, CA 94923, **Email: colleen.burge@wildlife.ca.gov*
[2]Bodega Marine Laboratory, University of California Davis, PO Box 247, Bodega Bay, CA 94923

Abstract
In 2001, white abalone (*Haliotis sorenseni*) was the first marine invertebrate species to be listed under the US Endangered Species Act. The recovery plan identified restoration aquaculture and outplanting as the primary actions to save the species. Monitoring health for any captively bred population both before and during outplants is an important aspect. A major impediment to initial attempts to culture the white abalone was the disease Withering Syndrome caused by *Candidatus* Xenohaliotis californiensis (*Ca*Xc)[1]. *Ca*Xc affects all species of native abalone in California that have been tested to date, and white abalone are highly susceptible with up to 100% mortality after exposure to *Ca*Xc[2].

The California Department of Fish and Wildlife, Shellfish Health Laboratory (SHL), located at the University of California, Davis Bodega Marine Laboratory (BML), has been instrumental in monitoring and improving the health of white abalone in the White Abalone Captive Breeding Program (WACBP). Careful observation and prevention of infectious disease and pests, as well as treatment of infected captive abalone, have propelled the success of captive production over the past decade, resulting in thousands of outplanted white abalone. The SHL conducts *Ca*Xc testing via quantitative PCR for all captive white abalone held among a dozen partner facilities. The successful treatment of *Ca*Xc-infected abalone using an antibiotic bath as a treatment is important for maintaining healthy broodstock. SHL also monitors pests such as sabellid polychaetes and *Cliona spp.* (boring sponge), which can compromise shell integrity. Shell abnormalities caused by these pests can make white abalone more vulnerable to infection and/or cause shells to break, sometimes resulting in mortality. SHL is exploring treatment options, including wax and epoxy applications, to asphyxiate pests. Managing the treatment and prevention of spread of pathogens new to the WACBP is a growing focus of the SHL. The SHL also conducts experimental research to support the WACBP. For example, work in the SHL showed that a phage hyperparasite of *Ca*Xc may protect red abalone at ambient and elevated temperatures but that white abalone were highly susceptible to *Ca*Xc associated losses at elevated temperatures[3]. A recent experiment focuses on the potential for heritable resistance in white abalone which will help the WACBP determine potential broodstock from populations that are more resistant for future abalone production. Routine monitoring and research into new treatments for pests and pathogens are essential for the survival of the species and has implications for restoration and commercial aquaculture for other abalone species.

Annotated Bibliography of Key Works

[1] Crosson, L.M., Wight, N., VanBlaricom, G.R., Kiryu, I., Moore, J.D. and Friedman, C.S., 2014. Abalone withering syndrome: distribution, impacts, current diagnostic methods and new findings. *Diseases of aquatic organisms*, *108*(3), pp.261-270.

The authors present a review on abalone Withering Syndrome (WS) of abalone including providing information on the distribution, impacts, and diagnostic tools. Importantly, it provides information on the current range of WS impacts including the northern eastern Pacific of North American in California, USA and Baja California, Mexico. The geographic range is expected to be broad as infected abalone have been transported to multiple countries. This review describes a phage hyperparasite that has potential to reduce losses associated with WS. Additionally, it provides key diagnostic tools used to detect CaXc such as qPCR, *in situ* hybridization, and histology.

[2] Crosson, L.M. and Friedman, C.S., 2018. Withering syndrome susceptibility of northeastern Pacific abalones: a complex relationship with phylogeny and thermal experience. *Journal of invertebrate pathology*, *151*, pp.91-101.

Crosson and Friedman describe differences in susceptibility of abalone species to WS in the north eastern Pacific. Importantly, this study focused on the temperature necessary to initate disease in red (*Haliotis rufscens*), pink (*H. corrugata),* and pinto (*H. kamtschatkana).* Mean percent WS-induced mortality of multiple abalone species (white, black red, pink, green, and pinto) are discussed in the context of phylogeny (distance from white abalone), infection intensity, temperature ranges, and optimum

growth. Species with cool water evolutionary histories are the most susceptible to *Ca*Xc (i.e. white and pinto abalone) with evolutionary distance from white abalone predicting relative susceptibility using the cytochrome oxidase *c* submit I).

[3]Vater and co-authors from the SHL describe the relative susceptibility of white and red abalone *Ca*Xc infected with the phage hyperparasite. Importantly, this study indicates that white abalone held at elevated water temperatures is highly susceptible to *Ca*Xc associated losses. This study indicates that temperature is an important consideration for outplant of white abalone.

5. An overview of disease and pests on cultivated kelp, with a focus on Alaska

Jordan Hollarsmith*[1], Tiffany Stephens[2], Schery Umanzor[2]

Presenting author*

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17109 Point Lena Loop Road, Juneau, AK 99801, **Email: jordan.hollarsmith@noaa.gov*
[2]University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, 17101 Point Lena Loop Road, Juneau, AK 99801

Abstract
Kelp (order Laminariales) disease and pest management are critical areas of concern, with far-reaching implications for established and nascent kelp aquaculture industries. Kelp farmers along the northwest coast of North America (British Columbia, Canada, and Alaska, US) have reported various infections and infestations on cultivated kelp that have had deleterious effects on their yields, often rendering the crop unsuitable for harvest. While progress has been made to avoid some infestations, such as efforts to deter Pacific herring (*Clupea pallasii*) from spawning on aquaculture infrastructure, many other diseases and pests remain unidentified and, therefore, uncontrollable. To the best of our knowledge, the kelp aquaculture industry along the North American west coast has encountered a spectrum of challenges, ranging from infestations of invertebrate organisms like caprellids, suctorians, and bryozoans, to vertebrate species such as spawning Pacific herring. Additionally, infections have led to the formation of galls, blisters, and malformed thalli, with the precise causative sources remaining elusive. While previous research points toward potential bacterial and parasitic origins, definitive conclusions have yet to be reached. Improving our understanding of these pests and pathogens and the conditions under which they thrive will be necessary as the kelp aquaculture industry grows in the number and size of farms in North America.

Annotated Bibliography of Key Works
Apt K. 1988. Galls and tumor-like growths on marine macroalgae. Diseases of Aquatic Organisms 4:211–217.

This older paper provides a helpful overview of some of the causative (or presumed causative) agents of gall formation on macroalgae. It defines galls as growths that are "characterized by host cell hyperplasia and hypertrophy producing an abnormal callus-like unorganized cell proliferation." Table 1 provides a helpful summary of research on drivers of gall formation on a variety of macroalgae species; drivers include bacteria, fungi, algae, animals, and other ("unknown" or "pollution"). Specific to kelp, Apt describes an endophytic algae (*Streblonema spp.*) that can form round wart-like tumors or convoluted elongated ridges on the stipes of *Nereocystis luetkeana* and other kelp species. While observations of galls on macroalgae have been made for over 100 years, at the time of this publication, few causative agents had been definitively identified.

Egan S, Fernandes ND, Kumar V, Gardiner M, Thomas T. 2014. Bacterial pathogens, virulence mechanism and host defense in marine macroalgae. Environmental Microbiology 16:925–938.

A review of bacterial interactions with marine macroalgae. Authors note that pathogenicity has not been clearly separated from saprophytic behavior or secondary colonization after disease initiation for most bacterial species found on macroalgae. They conclude that pathogenicity, and therefore the occurrence of macroalgae disease, is likely to increase with climate change as environmental stress can make macroalgae more susceptible to opportunistic pathogens. In order to determine pathogenicity, one must ideally fulfill Koch's postulate (i.e. pathogen present in infected tissue but not in healthy tissue; pathogen must be isolated in culture; the introduction of the culture to a healthy individual results in disease; the pathogen must be re-isolated from the newly disease individual), however this is rarely feasible in practice and virulence may depend on environmental conditions, not only the presence of a presumed pathogen. Macroalgae lack cell-based immune responses and therefore use other antibacterial strategies. Many species produce bioactive secondary metabolites that inhibit epibiotic growth on the thallus or select for certain 'beneficial' bacterial species (i.e. the probiotic hypothesis). Another strategy is the production of metabolites by the macroalgae or by other bacteria that interfere with bacterial communication networks and gene regulation (e.g. bacterial quorum sensing systems). Pathogen-induced defense mechanisms may also serve to alert adjacent conspecific macroalgae to proactively up-regulate a defense response. Table 1 contains a helpful list of reported bacterial pathogens in marine macroalgae.

Wang G, Lu B, Shuai L, Li D, Zhang R. 2014. Microbial diseases of nursery and field-cultivated *Saccharina japonica* (Phaeophyta) in China. Algological Studies 145–146:39–51.

Alginic acid-decomposing bacteria are the dominant bacterial communities in the seawater in cultivation areas and on healthy *Saccharina japonica* sporophytes, however in conditions of environmental stress (e.g. increased temperature or variation in light intensity) and high-density cultivation, these bacterial species can become pathogenic. Prevention strategies include selecting healthy mature sporophytes and discarding the decayed parts of blades, seeding sporelings at an appropriate density, maintaining clean and cold sporeling culture conditions, and adding erythromycin to inhibit bacterial growth. Defense responses of *S. japonica* to pathogenic bacteria included oxidative bursts and antioxidant enzyme activities. Confirming that a given bacteria causes disease (i.e. achieving Koch's postulate) has been difficult as the proposed causative bacteria lose their virulence after being cultured in the lab.

Ward GM, Faisan Jr JP, Cottier-Cook EJ, Gachon C, Hurtado AQ, Lim PE, Matoju I, Msuya FE, Bass D, Brodie J. 2020. A review of reported seaweed diseases and pests in aquaculture in Asia. Journal of the World Aquaculture Society 51:815–828.

This paper provides an excellent overview of seaweed diseases in Asian aquaculture (and since most of the research is in Asian aquaculture, it captures a lot of known diseases). Of the kelp species, it includes *Saccharina japonica* and *Undaria pinnatifida*. The tables of diseases, descriptions, host species, and identified causes are particularly useful.

6. Response of multiple Pacific oyster populations to OsHV-1 microvariant challenge

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Abstract

Ostreid herpesvirus 1 is a pathogen of significant concern to the Pacific Coast Shellfish industry as multiple California bays are known to harbor microvariant and reference viral strains. Genetic selection programs in Oceania, North America and Europe as well as substantial academic research have demonstrated the effectiveness of selection to increase field survival where OsHV-1 is endemic. The USDA ARS Pacific Shellfish Research Unit initiated the Pacific Oyster Genomic Selection project (a Pacific oyster breeding program) in 2023 and has access to two distinct populations of Pacific oyster currently maintained by Oregon State University (OSU). Differences in survival response to OsHV-1 challenge are known to occur between Pacific oyster strains, as well as between oyster species. However, it is unknown if the 2 populations curated at OSU display different survival rates when challenged with OsHV-1 microvariants. To assess the populations, the USDA produced 71 families. This group of Year Class 2023 contained 43 purebred Miyagi families, 11 purebred Midori families, 11 hybrid families (one Midori and one Miyagi parent), and 6 Willapa Bay families created using naturalized Pacific oysters (an unselected control). Spat were challenged against a European isolate of OsHV-1 microvariant in an AQC3 facility using a plate assay. Nested ANOVA analysis on survival data found that significant survival differences occurred based on oyster population. The Midori population exhibited significantly higher survival compared to the Miyagi population. A significant effect of family within population occurred too, demonstrating that within a population significant variation in survival occurred. The results from this study indicate that using the Midori population to found the POGS project breeding nucleus could more rapidly achieve high OsHV-1 survival, presuming the lab challenge results correlate with field survival performance where OsHV-1 occurs on the North American Pacific Coast. Field studies, using the same families in this experimental laboratory study are ongoing, but this result is a promising first step towards producing a Pacific oyster population that displays high survival against OsHV-1 pathogens for the United States Pacific oyster aquaculture industry.

Annotated Bibliography of Key Works

Degremont, L., Nourry, M. and Maurouard, E., 2015. Mass selection for survival and resistance to OsHV-1 infection in *Crassostrea gigas* spat in field conditions: response to selection after four generations. *Aquaculture*, *446*, pp.111-121.

The authors demonstrate that genetic selection to OsHV-1 infection is an effective method for improving survival. This research is the first to report such studies on the spat stage as well as realized heritabilities for OsHV-1 survival. A moderate heritability that ranges from 0.34 to 0.63 was found across 4 generations of selection, and gain in the trait ranged from approximately 20% to 60% compared to unselected controls. Using the simplest form of selection, mass selection (breeding from survivors), was shown to be an effective method for increasing survival and resistance to field exposure of OsHV-1. Interestingly an effect of animal size was highly correlated with field survival, this resulted in OsHV-1 selected populations having drastically higher yields than unselected controls. This study is foundational for breeding programs, as it demonstrates positive selection for OsHV-1 survival can rapidly accumulate.

Divilov, K., Schoolfield, B., Morga, B., Dégremont, L., Burge, C.A., Mancilla Cortez, D., Friedman, C.S., Fleener, G.B., Dumbauld, B.R. and Langdon, C., 2019. First evaluation of resistance to both a California OsHV-1 variant and a French OsHV-1 microvariant in Pacific oysters. *BMC genetics*, *20*(1), pp.1-9.

This study assessed the survival of families from the Oregon State University Molluscan Broodstock Program to multiple strains of OsHV-1 in multiple environments. Families were exposed to a less pathogenic OsHV-1 reference strain in Tomales Bay California, and siblings were exposed in laboratory challenge to a French OsHV-1 microvariant strain. Heritability in the field and laboratory trials was moderate to high, however the correlation in survival between the two trials was weak. This result suggested that OsHV-1 resistance mechanism may not be identical for microvariant and non-microvariant strains, or that laboratory based-assays may not be well suited to predict field performance. However, multiple factors could influence the lack of correlation between experiments, including most importantly that different OsHV-1 strains were used in each experiment, and that it's likely OsHV-1 was not the only pathogen present in Tomales Bay. Overall, this research demonstrates for the first time that a population

of Pacific oysters available to the U.S. West coast industry has the potential for genetic improvement in OsHV-1 survival. A broad range of survival existed for the families tested indicating that sufficient standing genetic variation for the trait exists. This research is important for establishing that Pacific oysters could be bred in the United States for increased OsHV-1 survival.

Burge, C.A., Friedman, C.S., Kachmar, M.L., Humphrey, K.L., Moore, J.D. and Elston, R.A., 2021. The first detection of a novel OsHV-1 microvariant in San Diego, California, USA. *Journal of Invertebrate Pathology*, *184*, p.107636.

This research reports the first detection of an OsHV-1 microvariant in North America. Using DNA sequencing methods, the authors demonstrate that the viral strain present in San Diego Bay California is most similar to other microvariants worldwide as compared to the OsHV-1 virus found in Tomales Bay California. A transmission experiment, using injection and bath exposure methods found that the San Diego microvariant produced lethal outcomes for multiple oyster populations. This research is important in identifying the first occurrence of a microvariant strain and demonstrating the pathogenicity of the virus found in San Diego Bay.

Gutierrez, A.P., Symonds, J., King, N., Steiner, K., Bean, T.P. and Houston, R.D., 2020. Potential of genomic selection for improvement of resistance to ostreid herpesvirus in Pacific oyster (*Crassostrea gigas*). *Animal Genetics*, *51*(2), pp.249-257.

Using a laboratory exposure, the authors tested a population of Pacific oysters from Cawthron Insitute's selective breeding program against OsHV-1 (strain not identified) in a common garden experiment. The juveniles originated from families that had divergent response to OsHV-1 exposure in field conditions. A down-selected set of families which demonstrated poor survival and a high performing set of families, were used to produce spat tested in this study. After genotyping with the Axiom Oyster Genotyping Array and animal model analyses it was determined that genomic selection methods (GBLUP) had higher prediction accuracy that pedigree methods (PBLUP). Genomic (GBLUP) prediction was 19% more accurate using all available SNP data compared to pedigree PBLUP prediction. Moderate heritability was reported in this study with a pedigree estimate of 0.25 and a 0.37 genomic estimate. No major effect loci were identified using a GWAS analysis, suggesting that genomic selection methods are best suited for improvement in this trait. This study is the first to demonstrate the potential gain in prediction accuracy for OsHV-1 survival using genomic selection methods in Pacific oyster.

Friedman, C.S., Reece, K.S., Wippel, B.J., Agnew, M.V., Dégremont, L., Dhar, A.K., Kirkland, P., MacIntyre, A., Morga, B., Robison, C. and Burge, C.A., 2020. Unraveling concordant and varying responses of oyster species to Ostreid Herpesvirus 1 variants. Science of The Total Environment, 739, p.139752

This study used a paired laboratory injection challenge and field exposure to OsHV-1 of oysters from three species, and three strains (or sources) of Pacific oyster. Two OsHV-1 microvariants (France and Australia) were injected into each group of oyster, and a field exposure to a non-microvariant OsHV-1 in Tomales Bay California occured. Differences in survival were present across species, and by strain within Pacific oyster. A second important finding was that surviving oysters regardless of species or strain, had lower viral load estimates than mortalities. This data suggests the physiological response of the animal that confers survival is one that limits replication of the OsHV-1 virus, or allows the animal to clear the viral infection. Interestingly, this study found a moderate to strong correlation between French microvariant survival and field survival to OsHV-1 in Tomales Bay, but did not find any significant correlation between the Australian microvariant survival and Tomales Bay field survival. These inconsistent correlation results highlight the need for paired laboratory and field studies to evaluate the performance of oyster families and strains against OsHV-1 variants.

Divilov, K., Schoolfield, B., Cortez, D.M., Wang, X., Fleener, G.B., Jin, L., Dumbauld, B.R. and Langdon, C., 2021. Genetic improvement of survival in Pacific oysters to the Tomales Bay strain of OsHV-1 over two cycles of selection. *Aquaculture, 543*, p.737020.

Using the Miyagi population of Pacific oysters in the Oregon State University breeding program, the authors demonstrate that selection for field survival in Tomales Bay California, where a non-microvariant of OsHV-1 is endemic and outbreaks seasonally, can be achieved using pedigree methods. Heritability of survival was moderate, and over two selection cycles achieved approximately a 10 percent increase in breeding values per selection cycle.

7. Emerging aquaculture trends in the Gulf of Maine

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Abstract

While aquaculture is often described as one of the fastest growing food production systems in the world, that progress is often non-linear and complex when we focus on any particular location. This type of place-based perspective on aquaculture can be helpful in understanding other complex social-ecological-economic systems. Maine is home to an incredibly diverse aquaculture industry with over 24 different species being cultivated across more than 150 production farms. While the dominant revenue-generating species is the Atlantic salmon, the industry is diversifying and experiencing a rapid expansion in the farming of shellfish and seaweed. Recently, there has been a surge of interest in establishing large land-based recirculating aquaculture systems (RAS). Currently, Maine hosts four such facilities in various stages of development, which propose to produce a range of species including salmon, trout, eel, and yellowtail fish. The Aquaculture Research Institute works with the seaweed, shellfish, and finfish industries in the state to create a more sustainable industry since each production system requires different tools. For example, seaweed processing recently passed the million pound threshold in Maine for the first time due to interactions between the seaweed industry and the American lobster fleet. One emerging shellfish aquaculture trend with promise is aquacultured sea scallops. Growers in Maine put the first aquacultured sea scallop on the market in 2019 but any new industry requires significant learning by doing and technology transfer. Finally, our group is working closely with Recirculating Aquaculture Systems, such as Whole Oceans, Kingfish Maine and Nordic AquaFarms to allow for sustainable use of intake water and a full characterization of the impact on receiving waters. A theme of our work is to take established oceanographic tools such as remote sensing satellite systems and oceanographic buoys to better characterize aquaculture environment interactions. For example, by combining multiple observing platforms, we are identifying new areas for shellfish aquaculture expansion, characterizing important feedbacks that alter carrying capacity, and incorporating climate related factors into future aquaculture growing area projections. In short, I will do my best to present a Maine travelogue through an aquaculture lens.

8. Estimating transmission risk of red sea bream iridovirus between fish farms via seawater using environmental DNA

Kawato Y*[1], Takada Y[1], Kurobe T[1], Nakagawa Y[2], Mizuno K[3], Harakawa S[3], Kawakami H[3], Yoshihara Y[4], Ito T[1]

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Abstract
Zone and zoning are important concepts to establish biosecurity measures in semi-open aquaculture systems such as net pens or cages among which the rearing water (i.e. environmental water) can move freely. Although the concept is defined by the World Organization for Animal Health (WOAH) for aquatic animals, it is usually difficult to determine the shortest distance between zones at which the transmission risk of a pathogen through environmental water can be ignorable. In this study, we assessed the actual transmission risk of red sea bream iridovirus (RSIV), which is listed as a notifiable disease by WOAH, via environmental water among net pens. The environmental DNA (eDNA) method using iron-based flocculation coupled with large-pore filtration (John et al. 2011; Kawato et al. 2016; Kawato et al. 2021) was used to monitor RSIV DNA copies in seawater from fish farms. In the surveillance for three years at 10 fixed points (n = 306) around net pens, there were only seven samples in which the viral load exceeded 10^4 copies/L in seawater in an aquaculture environment. RSIV dispersion in seawater from a net pen during the disease outbreak was visualized by the inverse distance weighting method using multiple-sampling datasets from another fish farm. The analysis demonstrated that the center of the net pen had a high viral load, and RSIV seemed to be quickly diluted by the tidal current. Finally, the transmission risk of RSIV-contained environmental water was estimated by an experimental challenge test using red sea bream *Pagrus major*. The fish (approximately 10 g) were exposed to seawater containing RSIV (10^3, 10^4, 10^5, 10^6, and 10^7 copies/L) continuously for 3 days, which mimics the field exposure of RSIV based on the monitoring data in the aquaculture environment. The rates of fish from which the virus was detected in the 10^5, 10^6, and 10^7 copies/L groups were 10%, 55%, and 100%, respectively, whereas no RSIV was detected in the 10^3 and 10^4 copies/L groups. A probit analysis of the challenge test indicated that the inferred infection rates of seawater containing $10^{5.9}$ copies/L and $10^{3.1}$ copies/L of RSIV were 50% and 0.0001%, respectively. These results suggest that the transmission of RSIV among fish farms via seawater is highly associated with the distance between the net pens, and the environmental water is not always an infection source for the transmission of RSIV between fish farms. Thus, we may even consider that the environmental water could be a potential wall reducing the transmission risk of RSIV between fish farms, although, in reality, it would not be that simple, as conditions change from case to case. Therefore, a farming zone established on the basis of geographical features such as a small bay or an inlet would be a reasonable unit for the prevention of RSIV. This study also suggests that appropriate hygiene management, such as disinfection measures, is very important even in a semi-open aquaculture system to reduce the risk of disease outbreaks caused by waterborne viruses.

This study was supported by the Regulatory Research Projects for Food Safety, Animal Health, and Plant Protection (JPJ00867.19190702) funded by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

Annotated Bibliography of Key Works
John SG, Mendez CB, Deng L, Poulos B, Kauffman AK, Kern S, Brum J, Polz MF, Boyle EA, Sullivan MB. 2011. A simple and efficient method for concentration of ocean viruses by chemical flocculation. *Environ Microbiol Rep*, 3:195–202.

This work demonstrated efficacy of iron-based flocculation and large-pore-size filtration to concentrate ocean viruses. The iron flocculation technique was superior to tangential flow filtration in terms of cost, time, and recovery rate. Bacteriophage infecting ocean bacteria can be successfully concentrated and recovered without losing infectivity.

Kawato Y, Ito T, Kamaishi T, Fujiwara A, Ototake M, Nakai T, Nakajima K. 2016. Development of red sea bream iridovirus concentration method in seawater by iron flocculation. *Aquaculture*, 450:308–312.

It is the first report that the iron flocculation technique was applied to concentrate a virus causing fish disease. Since eDNA was directly extracted from the iron flocculation-trapped filter without elution step, the procedure until real-time PCR improved simpler and time effective.

Kawato Y, Mekata T, Inada M, Ito T. 2021. Application of environmental DNA for monitoring red sea bream iridovirus at a fish farm. *Microbiol Spectr*, 9:e0079621.

eDNA could be applied in monitoring waterborne viruses of aquatic animals. However, there are few data for practical application of eDNA in fish farms for the control of disease outbreaks. The results of our field research over 3 years targeting eDNA in a red sea bream (*Pagrus major*) fish farm implied that RSIV outbreaks in juveniles originated from virus shedding from asymptomatically virus-infected broodstocks. Our work identifies an infection source of RSIV in a fish farm by eDNA monitoring, and it could be applied as a tool for application in aquaculture to control fish diseases.

9. Investigating routes of pathogen spreading in a saltwater fish farm

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Abstract
Infectious diseases spread among fish net pens in saltwater aquaculture settings and cause mass mortalities. It is thought that pathogens mainly spread via water current in the oceans, however an our recent study reveals that water current may not be the primary route of spread of a viral agent; in the study a viral agent, red sea bream iridovirus, was readily diluted by environmental water along with the distance from a fish net pen with diseased fish and the concentrations of the viral agent became below the threshold that infection can be established to naïve fish. This raised a question, “If viruses are not spreading via water current, then how do they spread in saltwater aquaculture settings?”. To address the question, we hypothesized that ‘personnel who involved in aquaculture unintentionally spread viruses via daily aquaculture activities by contaminating production sites (e.g., tools, net-pens, gloves, boats, pieces of equipment) with viruses originate from diseased or dead fish’. We assumed that use of contaminated tools was another route of spreading pathogens. To test the hypothesis, we performed a wipe test in a fish farm during active viral infections. In this study, we monitored emerging viral agents, adomavirus and parvovirus, which possibly cause mortalities in sea bream. On site, we collected samples from grounds, landing nets, containers where dead fish are temporarily stored, frames of fish net pens, gloves from a diver, ship’s wheel, environmental water, and more. Those samples were subjected to genomic DNA extraction, followed by PCR testing. The viral DNA was mainly detected from parts or areas where dead fish were handled, such as containers for temporarily storing dead fish, gloves and landing nets for picking up dead fish, suggesting fish farmers who treated dead fish and these tools can be a possible source of infectious diseases. The data from our study suggest that proper management of dead fish may be able to mitigate spreading viral infectious diseases in saltwater aquaculture settings.

Annotated Bibliography of Key Works

Kawato Y, Takada Y, Mizuno K, Harakawa S, Yoshihara Y, Nakagawa Y, Kurobe T, Kawakami H, Ito T. Assessing the transmission risk of red sea bream iridovirus (RSIV) in environmental water: Insights from fish farms and experimental settings. Microbiology Spectrum (*in revision*)

10. Development of a multiplex PCR to investigate genetic diversity of Flavobacterium psychrophilum infecting ayu (Plecoglossus altivelis)

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Abstract
Bacterial cold water disease (BCWD) causes serious problems in freshwater fish worldwide. In Japan, *Flavobacterium psychrophilum,* the causative agent of BCWD, was first isolated from cultured ayu, *Plecoglossus altivelis,* in 1987. More than 30 years have passed since then, and the disease is still inflicting serious problems in both wild and farmed Ayu. The fish is highly prized as a delicacy as well as a popular game fish in Japan. Therefore, effective vaccines to prevent BCWD and the breeding of fish resistant to the disease are desired. To evaluate the efficacy of these disease control techniques using *F. psychrophilum*, it is necessary to know the virulence of bacterial stains. However, information is limited on the diversity in the phenotypes or genotypes of *F. psychrophilum*.

To examine the genomic diversity of the bacterium, pan-genome analysis was conducted using the whole genome sequences of a total of 46 *F. psychrophilum* isolates from BCWD-affected ayu and salmonid fish, as well as environmental water. As a result, we identified 188 genes that were only found in the isolates from Ayu. Furthermore, we found that the presence and combination of these genes varied among the isolates. Sixteen genes that may represent genetic diversity were selected from the 188 genes and specific PCR primers were designed. All these primers were mixed, and multiplex PCR was performed using genomic DNA extracted from each *F. psychrophilum* isolate as template. Finally, genotyping was conducted based on the band patterns obtained by electrophoresis of the PCR products.

To date, we have analyzed more than 150 isolates and found that the isolates from ayu can be classified into at least 27 genotypes. Among them, several genotypes were isolated at high frequencies from BCWD-affected ayu collected from geographically different sites and were considered to be the major genotypes causing BCWD in ayu.

Annotated Bibliography of Key Works

Nagai, T. and Nakai T. 2020. *Fish Pathol*., 55, 71–79.

The authors demonstrated the susceptibility of ayu to infection by *F. psychrophilum* differs among hatchery stocks. They challenged two different Ayu stocks, DS stock and AS stock, with 18 *F. psychrophilum* isolates derived from diseased Ayu. As a result, six (6) isolates were more virulent to DS stock than to AS stock, whereas the other 12 isolates were more virulent to AS stock than to DS stock. The study shows that even *F. psychrophilum* infecting Ayu may have different characteristics (i.e., pathogenicity) depending on the isolates. This paper motivated us to embark on a study of the genetic diversity of *F. psychrophilum* isolates from Ayu.

Duchaud, E., *et al*. 2018. *Front. Microbiol.*, 9, 138.

The genomic diversity of the *F. psychrophilum* was analyzed using genome sequences of 41 isolates. The results revealed this bacterial species harboring a limited genomic diversity both in terms of nucleotide diversity, with ~0.3% nucleotide divergence inside CDSs in pairwise genome comparisons, and in terms of gene repertoire, with the core genome accounting for ~80% of the genes in each genome. The pan-genome showed nevertheless "open" according to the scaling exponent of a power-law fitted on the rate of new gene discovery when genomes are added one by one. This may suggest that genes encoded in regions other than the core genome (accessory genes) affect the phenotype of each isolate. Therefore, we attempted to develop a multiplex PCR genotyping method that considers the presence or absence of accessory genes as an indicator.

11. Phylogenomic Characterization of Nucleocytoplasmic Large DNA Viruses In Poikilothermic Vertebrates

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Abstract

Nucleocytoplasmic Large DNA Viruses (NCLDVs) are an important phylum (*Nucleocytoviricota*) of viruses infecting homeothermic and poikilothermic vertebrates. This phylum includes many well-known viral families including the *Poxviridae*, *Asfarviridae*, *Iridoviridae*, *Ascoviridae*, *Phycodnaviridae*, *Mimiviridae*, and *Marseilleviridae*. Unlike homeothermic vertebrates, NCLDVs of poikilothermic vertebrates have been underreported due to a lack of interest/resources and a technology gap. The advent of next-generation sequencing technologies have revolutionized the discovery and genomic characterization of NCLDVs from poikilotherms. In this presentation, we report the discovery of novel mimiviruses, poxviruses, and iridoviruses infecting fish and reptiles. We report a novel branch of the family *Mimiviridae* that infect and induce lethal cutaneous diseases in critically endangered sturgeon species (e.g., white sturgeon [*Acipenser transmontanus*] from the Kootenai River in Idaho, USA or shovelnose and pallid sturgeon [*Scaphirhynchus platorynchus* and *S. albus*, respectively] from the Missouri River Basin) (Hedrick et al., 1990, Kurobe et al., 2011). The white sturgeon mimivirus genome is 427,714 bp and predicted to encode 365 open reading frames within the unique region and inverted terminal repeats. The carp edema virus (CEV) is a poxvirus that has negatively impacted cultured and wild common carp (*Cyprinus carpio*) varieties in the USA (Lovy et al., 2018) and around the globe since its discovery in Japan in the 1970s (Oyamatsu et al., 1997). The CEV genome was recently reported to be 456,821 bp and predicted to encode 392 open reading frames (Mekata et al., 2021). CEV and the salmon gill poxvirus together form the deepest branch within the subfamily *Chordopoxvirinae*, family *Poxviridae*. Finally, erythrocytic necrosis virus (ENV) has been detected in a variety of reptile species as well as more

than 20 species of anadromous and marine fishes throughout the Atlantic and Pacific Oceans (Emmenegger et al., 2014). The complete genome of ENV from a Peninsula ribbon snake (*Thamnophis sauritus*) was determined to be 111,413 bp and predicted to encode 115 open reading frames. Phylogenetic analysis based on 19 conserved iridovirus genes revealed that ENV from ribbon snake groups within the family *Iridoviridae* as a unique branch separate from known iridovirus genera. The aforementioned studies suggest that much of what we think we know regarding NCLDV taxonomy will be challenged as viruses from a greater diversity of poikilothermic vertebrates are sequenced.

Annotated Bibliography of Key Works

Emmenegger EJ, Glenn JA, Winton JR, Batts WN, Gregg JL, Hershberger PK. 2014. Molecular identification of erythrocytic necrosis virus (ENV) from the blood of Pacific herring (*Clupea pallasii*). Veterinary Microbiology. 174: 1–2.

Viral erythrocytic necrosis (VEN) is a condition affecting the red blood cells of more than 20 species of marine and anadromous fishes in the North Atlantic and North Pacific Oceans, including Pacific herring (*Clupea pallasii*). The causative agent, erythrocytic necrosis virus (ENV), possesses virion features similar to members of the family *Iridoviridae.* However, comprehensive phylogenetic studies to confirm this hypothesis have been hampered by a lack of sequence data. The authors of the present study generated a 1448 bp fragment of the putative DNA polymerase gene that supported the inclusion of ENV in a novel genus in the family *Iridoviridae* that contains erythrocytic viruses from other poikilotherms.

Hedrick RP, Antonio DB, Munn RJ. 1997. Poxvirus like agent associated with epizootic mortality in juvenile koi (*Cyprinus carpio*). FHS Newsletter. 25:1-2.

Carp edema virus (CEV) is an unclassified poxvirus that infects skin and gill tissue to cause koi sleepy disease. Ronald Hedrick's research group at UC Davis, CA, USA provided the first description of CEV in a koi wholesaler in CA, USA in 1996. This was the first description of CEV outside of Japan since its discovery in the 1970s. Subsequently, CEV has frequently been detected in diseased cultured koi carp and wild stocks of common carp in the United States.

Hedrick RP, Groff JM, McDowell T, Wingfield WH. 1990. An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*. Dis Aquat Organ. 8:39-44.

Ronald Hedrick's research group provided the first description of an iridovirus-like agent from white sturgeon. The authors confirmed the lethal viral disease was restricted to the skin and provided the first description of the associated microscopic lesions. This study confirmed the virus, white sturgeon iridovirus (WSIV), could be transmitted via contaminated water to uninfected white sturgeon. WSIV was later determined to be a significant threat to the production of healthy white sturgeon juveniles in hatcheries across the Pacific Northwest of North America.

Kurobe T, MacConnell E, Hudson C, McDowell TS, Mardones FO, Hedrick RP. 2011. Iridovirus infections among Missouri River sturgeon: initial characterization, transmission, and evidence for establishment of a carrier state. J Aquat Anim Health. 23(1):9-18.

Ronald Hedrick's research group provided a second description of an iridovirus-like agent in cultured pallid and shovelnose sturgeon from the Missouri River Basin. Again, the authors confirmed the disease was restricted to the skin and the virus could be transmitted horizontally to naïve juveniles. The virus, the Missouri River Sturgeon Iridovirus (MRSIV), was argued to pose a significant impediment to the production of cultured pallid sturgeon juveniles destined for restocking and the replenishment of dwindling wild stocks in the Missouri River Basin of the United States.

Lovy J, Friend SE, Al-Hussinee L, Waltzek TB. 2018. First report of carp edema virus in the mortality of wild common carp *Cyprinus carpio* in North America. Dis. Aquat. Organ. 131:177–186.

In the USA, CEV was first detected in 1996 in a California koi wholesaler and has since been reported sporadically only within imported and domestic koi. In the present study, the authors confirmed

CEV for the first time in the United States in a wild stock of common carp. The authors confirmed the diseased carp displayed the expected microscopic lesions in the gills and visualized CEV virions by transmission electron microscopy within branchial epithelial cells. The CEV infection was further confirmed by conventional and quantitative PCR.

Mekata T, Kawato Y, Ito T. 2021. Complete Genome Sequence of Carp Edema Virus Isolated from Koi Carp. Microbiol Resour Announc. 10(16):e00239-21.

The authors of this study report the complete genome sequence of a carp edema strain from koi carp. The CEV genome was reported to be 456,821 bp and predicted to encode 392 open reading frames. To date, this is the largest viral genome reported from a vertebrate. The CEV genome sequence is expected to facilitate the future development of improved molecular diagnostics and vaccines.

Oyamatsu T, Hata N, Yamada K, Sano T, Fukuda H. 1997. An etiological study on mass mortality of cultured colorcarp juveniles showing edema. Fish Pathology. 32, 81–88.

The authors performed elegant experimental challenges using filtered gill tissue homogenates to confirm a poxvirus, carp edema virus (CEV), was the etiological agent responsible for koi sleepy disease (KSD). Although previous crude challenge studies had confirmed an infectious agent was the likely cause of KSD, the etiological agent had not been identified. Poxvirus-like particles had been detected in the branchial epithelium of diseased koi during natural epizootics; however, focused studies to understand the pathogenicity of this poxvirus had not been conducted. The authors of this study conducted a series of examinations employing infection trials and electron microscopy to establish CEV as the etiology of KSD.

12. Analysis of Japanese flounder family resistance to two different bacterial diseases against Edwardsiellosis

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Abstract
Japanese flounder (*Paralichthys olivaceus*) farming in Japan is conducted at high densities in land-based tanks, and infectious diseases such as Streptococcosis and Edwardsiellosis can cause significant damage. Vaccines have been developed against these diseases, but they still occur sporadically in aquaculture. Therefore, there is a need to develop strains that are resistant to bacterial infections. Although a family line of flatfish that exhibits resistance to Streptococcus has been developed, it has not yet been fully developed into a commercial strain, and has not yet been put into practical use. On the other hand, *Edwardsiella tarda* is currently causing more damage than *Streptococcus iniae*. Because *Edwardsiella tarda* is an intracellular parasitic bacterium, it is known to recur opportunistically due to environmental changes, even in aquaculture once the disease has subsided, causing significant cumulative damage. We have experimentally demonstrated that the previously developed Streptococcosis-resistant family also

exhibits a certain degree of resistance to Edwardsiellosis. The resistance of the same strain to these two different bacterial diseases may indicate the existence of common steps in both types of bacterial infections. In this report, we compare the results of infection tests against Streptococcosis and Edwardsiellosis and the genomic information of individuals that died in infection tests against *Edwardsiella tarda* and those that survived, and report the results of our search for DNA regions associated with resistance.

Annotated Bibliography of Key Works

Ozaki A, Okamoto H, Yamada T, Matsuyama T, Sakai T, Fuji K, Sakamoto T, Okamoto N, Yoshida K, Hattori K, Araki K, Okauchi M. 2010. Linkage analysis of resistance to Streptococcus iniae infection in Japanese flounder (*Paralichthys olivaceus*). *Acuaculture*, 308, Supplement 1:S62–S67.

This work demonstrated, 159 microsatellite markers selected from genetic linkage maps of Japanese flounder and F_1 progeny from crosses between Streptococcal disease-resistant and disease-susceptible parents were used for detection of QTL associated with resistance to this disease.

13. Prevention of Viral Endothelial Cell Necrosis of Eel (VECNE) in Aquaculture Farms

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Abstract

Viral endothelial cell necrosis of eel (VECNE) is one of the diseases responsible for a significant economic impact on Japan's eel aquaculture. In 2020, the loss of production due to VECNE was approximately 42 tons, equivalent to 189 million JPY (1.3 million USD). The causative agent of VECNE is Japanese eel endothelial cell-infecting virus (JEECV), which is a non-enveloped DNA virus in the family *Adomaviridae*. JEECV infects vascular endothelial cells and causes symptoms such as intense congestion in the central venous sinus (CVS) of the gill filaments, hemorrhage in the liver and kidney, and reddening of the fins and skin. Raising the water temperature is effective to reduce the mortality of infected fish. However, studies on how the virus spreads in an eel farm and how to prevent the transmission of the virus are still limited.

To identify the route of infection, we monitored JEECV in eels in a pond of an eel farm every month since April 2022, when glass eels were introduced. All eels tested were negative for JEECV before the introduction and remained negative until November. The virus DNA was first detected in December and thereafter the viral load gradually increased without mass mortality event. On the other hand, from October to December, mass mortality due to VECNE occurred among older eels (>1 year old) in other ponds. These suggest that JEECV transmitted from a contaminated pond to the monitoring pond in the same farm, not from the outside of the farm.

We next conducted a bath-challenge experiment to examine whether the virus transmission occurs via water. Eels intraperitoneally injected with diseased tissue homogenate were maintained in an aquarium and the rearing water was added to other aquaria containing naïve eels at different temperatures. The eels at 30°C and 32.5°C started to die on day 18, while no eels died at 35°C throughout the experiment. Eels that died earlier in the experiment showed high viral loads as well as typical symptoms of VECNE,

whereas no viral DNA was detected from the surviving eels at 35°C. These results showed that JEECV remains infectious outside of a host and suggest a high temperature (35°C) prevents its transmission. This could also explain the reason why VECNE occurs more often after summer when the water temperature decreases.

To prevent pond-to-pond transmission of JEECV, it is also important to keep hands, clothing, and equipment free from the virus. However, eel farmers in Japan often pay little attention to the hygiene management for their farms and the efficacy of disinfectants against JEECV has not been investigated. We first established a cell line of vascular endothelial cells from the bulbus arteriosus to grow the virus *in vitro*. JEECV produced an obvious cytopathic effect (CPE) in these cells. Ethanol and sodium hypochlorite (chlorine bleach) were tested for the efficacy to disinfect the virus. Both ethanol and sodium hypochlorite showed a clear virucidal effect after a short time of exposure. These results suggest that the appropriate use of disinfectants is a promising way to reduce the risk of pond-to-pond transmission of the virus.

Annotated Bibliography of Key Works

Ono, S, K. Wakabayashi, and A. Nagai. 2007. Isolation of the Virus Causing Viral Endothelial Cell Necrosis of Eel from Cultured Japanese Eel *Anguilla Japonica*. Fish Pathology 42(4): 191–200.

This is the first report that JEECV is the causative virus for VECNE. The authors established a cell line JEEC from vascular endothelial cells of Japanese eel. CPE with hypertrophied nuclei was found in JEEC inoculated with the filtrate of homogenized gills of diseased fish. After intraperitoneal injection of the virus, eels showed congestion in the CVS of gill lamella, with 60% cumulative mortality. The virus was recovered from the gills, liver, and kidney of the infected fish.

Mizutani, T., Y. Sayama, A. Nakanishi, H. Ochiai, K. Sakai, K. Wakabayashi, N. Tanaka, E. Miura, M. Oba, I. Kurane, M. Saijo, S. Morikawa, and S. Ono. 2011. Novel DNA Virus Isolated from Samples Showing Endothelial Cell Necrosis in the Japanese Eel, Anguilla Japonica. Virology 412(1): 179–87.

This is the first report of the full genome sequences of JEECV. The authors developed PCR assays specific for JEECV based on the genome sequence. JEECV was detected in both naturally and experimentally infected eels, suggesting that JEECV potentially causes VECNE.

Tanaka, M., T. Satoh, WJ. Ma, and S. Ono. 2008. Effectiveness of Increasing Temperature of Rearing Water and Non-Feeding against Viral Endothelial Cell Necrosis of Eel. Fish Pathology 43(2): 79–82.

The authors evaluated the effectiveness of increasing water temperature and non-feeding against VECNE. Eels intraperitoneally injected with JEECV showed increased cumulative mortality with elevating water temperature in the range between 20°C and 31°C, while mortality at 35°C was as low as that at 20°C. More than 3 days at 35°C were needed to reduce mortality, and non-feeding conditions enhanced the effect of treatment at 35°C. Eels that survived the primary challenge at 35°C showed high resistance to re-challenge with JEECV.

14. Infectious salmon anemia virus investigations at the USDA National Coldwater Marine Aquaculture Center

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Abstract

Infectious salmon anemia virus (ISAV) can cause a lethal anemic disease in salmon for which culture and movement of infected fish is regulated in most countries. Two phenotypically distinct variants of ISAV, however, complicate risk and associated regulation. One phenotype is the well characterized highly virulent ISAV-HPRΔ variant associated with anemia, commonly referred to as ISAV-HPR deleted. The second is the avirulent variant known as ISAV-HPR0 which is not associated with disease and has a non-supportive invitrome. Recent work at the USDA National Coldwater Marine Aquaculture Center has focused on improving diagnostic methods for rapidly and accurately identifying ISAV variants using a single molecular test as well as investigating the vertical (parent-to-offspring) transmission risk associated with ISAV-HPR0 in a broodstock culture system. Here we discuss the development of a new rapid multiplex RT-qPCR assay for detecting and differentiating ISAV phenotypes and its validation against more than 30 genetically diverse ISAV isolates collected from North America and Europe processed in 3

international laboratories. We have since used this new assay to screen more than 800 eggs from 16 family crossings of parents with active ISAV-HPR0 infections and conducted additional long-term monitoring of offspring for which derived likelihoods for egg-associated transmission risks will be discussed.

Annotated Bibliography of Key Works

Rimstad, E. and Markussen, T., 2020. Infectious salmon anaemia virus—molecular biology and pathogenesis of the infection. Journal of Applied Microbiology, 129(1), pp.85-97.

The authors provide a current review of scientific knowledge regarding what is known about the phenotypic differences associated with ISAV, the molecular biology and pathogenicity of this important aquatic orthomyxovirus that has a long history of infecting Marine fish in open net-pen farms in many parts of the world.

Marshall, S.H., Ramírez, R., Labra, A., Carmona, M. and Muñoz, C., 2014. Bona fide evidence for natural vertical transmission of infectious salmon anemia virus in freshwater brood stocks of farmed Atlantic salmon (Salmo salar) in Southern Chile. *Journal of virology*, *88*(11), pp.6012-6018.

The authors demonstrate for the first time that ISAV-delete virus can be passed from mother to progeny via visualizing virus particles by electron microscopy and immunofluorescence staining of ISAV particles within unfertilized eggs. Although this study does not demonstrate continued persistence of the virus following fertilization and subsequent hatch, it does demonstrate a potential for infectious particles to persist within eggs of infected females.

Christiansen, D.H., Petersen, P.E., Dahl, M.M., Vest, N., Aamelfot, M., Kristoffersen, A.B., Jansen, M.D., Matejusova, I., Gallagher, M.D., Jónsson, G. and Rodriguez, E., 2021. No Evidence of the Vertical Transmission of Non-Virulent Infectious Salmon Anaemia Virus (ISAV-HPR0) in Farmed Atlantic Salmon. *Viruses*, *13*(12), p.2428.

This study demonstrated that farmed progeny of ISAV-HPR0 infected broodstock did not develop infection with ISAV-HPR0 obtained from their parents. This study utilized infected broodstock to identify substrains of ISAV-HPR0 and demonstrated that although many of their progeny ultimately became infected with ISAV-HPR0 during net-pen culture, none of the new infections were of the same substrain found in their parents, indicating that the infection was acquired from an alternative source. This suggests that unlike ISAV-delete, ISAV-HPR0 may not be able to be vertically transmitted to the same effectiveness.

15. Framework for National Aquaculture Health Management in the U.S. National Aquaculture Health Plan & Standards and Comprehensive Aquaculture Health Program Standards

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Abstract

Currently the U.S. aquaculture industry is operating without uniform, integrated and comprehensive standards for aquatic animal health management, testing and verification. The commercial aquaculture farming sectors are burdened with varying health requirements for animal movement which often result in expensive yet meaningless testing, inferring no confidence in the health status of the tested population over time. The National Aquaculture Association (NAA) in collaboration with the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), have

developed the Comprehensive Aquaculture Health Program Standards (CAHPS) to establish a framework for the improvement and verification of the health status of farm-raised aquatic livestock produced in U.S. aquaculture farming communities. These standards provide a science and risk-based framework to establish and verify aquatic animal health, allow for branding, provide leverage for negotiations with trade partners, both domestic and international, and facilitate safe animal movement. The operationalization of CAHPS is supported by the National Aquaculture Health Plan and Standards: 2021-2023 as a health inspection option. CAHPS outlines a series of pillars that ensure animal health, individual farm biosecurity, and, most critically, provide a framework that allows for the verification and recognition of livestock health. The pillars that make up CAHPS are: 1) aquatic animal health team; 2) risk evaluation; 3) early detection system and surveillance; 4) disease investigation and reporting; and 5) response and recovery. There are 3 ways to participate in CAHPS – 1) CAHPS Farm, 2) CAHPS National and 3) CAHPS Global. However, CAHPS cannot exist without the infrastructure and framework of the national plan which provides guidance on uniform pathogen reporting practices, and standardization for laboratory quality management and test methodologies.

16. The manufacture and use of autogenous vaccines to address emerging threats to aquaculture

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Abstract
Vaccines have a long history of success within the aquaculture industry. The majority of these vaccines have been fully licensed products which have long development times and cannot be changed to address emerging threats easily. The use of autogenous vaccines, which can target farm specific pathogen risks, has grown dramatically in recent years and offers a more cost effective and flexible solution to address risk and animal loss on the farm. Vaccine programs can be tailored to meet a company's specific needs and can be updated as often as necessary providing an excellent return when compared to other approaches.

Annotated Bibliography of Key Works

de Ruyter, T. et al. 2023. Comparative Evaluation of Booster Vaccine Efficacy by Intracoelomic Injection and Immersion with a Whole-Cell Killed Vaccine against *Lactococcus petauri* Infection in Rainbow Trout (*Oncorhynchus mykiss*). Pathogens, 12(5): 632.

Lactococcus petauri is an important emergent bacterial pathogen of salmonids in the USA. The purpose of this study was to evaluate the protection conferred to rainbow trout (*Oncorhynchus mykiss*) against *L. petauri* by formalin-killed vaccines in immersion and injectable forms, as well as the enhanced protection afforded by booster vaccination. The study is illustrative of how autogenous vaccines can be manufactured and deployed to address a serious emerging threat.

Barnes, et al. 2021. Autogenous vaccination in aquaculture: A locally enabled solution towards reduction of the global antimicrobial resistance problem. Reviews in Aquaculture, 14(2): 907-918.

The authors identify technical, bureaucratic and infrastructural transitions that could facilitate implementation of autogenous vaccination in low- and middle-income countries (LMIC) aquaculture against challenging socio-economic and environmental backgrounds. The benefits of autogenous vaccination to animal welfare, transboundary biosecurity, local farmer and industry economics, and to public health, favor implementation in aquaculture as a locally enabled solution to the global problem of antimicrobial resistance.

Mondal, H. and J. Thomas. 2022. A review on the recent advances and application of vaccines against fish pathogens in aquaculture. Aquaculture International, 30: 1971-2000.

The present review emphasizes on the current advances in technology and future outlook with reference to different types of vaccines used in the aquaculture industries. Beginning with traditional killed/inactivated and live attenuated vaccines, this work culminates in the review of modern new generation ones including recombinant, synthetic peptides, mucosal and DNA, subunit, nanoparticle-based and plant-based edible vaccines, reverse vaccinology, and monovalent and polyvalent vaccines.

17. USDA efforts to improve management of sea lice, Lepeophtheirus salmonis, on domestic salmon farms

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Abstract
Sea lice are the most economically important pathogen in the commercial salmon farming industry. Globally management strategies have been shifting from drug-based options to nondrug-based strategies as lice populations have shown the ability to develop resistance to most currently available drug treatments. Many of the nondrug treatment strategies are not feasible in Maine due to the oceanographic and environmental characteristics of the region. Currently, the Maine industry is restricted to a single treatment method. The USDA is developing the use of lumpfish as cleanerfish to help as a potential short to long term solution in collaboration with industry and academic partners. The current focus of the USDA is the development of captive reared brood stock populations originating from local Gulf of Maine stocks. We are also incorporating selection for sea lice resistance in our selective breeding program as a long-term solution to the issue. Heritability estimates from our program of 0.18-0.20 reveal a moderate heritability to select for lice resistance. A retrospective analysis of our efforts since 2015 based on progeny testing revealed that pedigree-based PBLUP methods had a slightly higher accuracy of prediction (0.47) when compared to genomic predictions using ssGBLUP (0.42) or wssGBLUP (0.37). These accuracies may improve with increased training set size, but they demonstrate the ability for substantial genetic gains to be made towards sea lice resistance. The USDA will continue to focus on these two sea lice management strategies, while looking for other potential strategies that may prove effective for the US domestic industry.

Annotated Bibliography of Key Works

<u>Lumpfish</u>

Imsland, A. K. D., & Reynolds, P. (2022). In lumpfish we trust? The efficacy of lumpfish *Cyclopterus lumpus* to control *Lepeophtheirus salmonis* infestations on farmed Atlantic salmon: A review. Fishes, 7(5), 220.

Debate about the efficacy of lumpfish as a cleaner fish is common despite adoption by industry in most major North Atlantic producing countries. This manuscript reviews the existing controlled studies examining the efficacy of lumpfish. While sea lice reduction can vary across the studies, all studies showed a decrease in sea lice on salmon when lumpfish were present compared to controls.

Fairchild, E. A., Pietrak, M. R., & Burr, G. S. (2021). Lumpfish hatchery handbook. Northeast Regional Aquaculture Center publication #301-2021, pp. 49. Maryland: Northeast Regional Aquaculture Center https://www.nrac.org/latestpublications

The is non-peer reviewed handbook produced in collaboration between the University of New Hampshire and the USDA ARS. The handbook goes over lumpfish biology, spawning techniques, husbandry, feeding, disease and other important issues. It is a good guide to lumpfish hatchery operations as practiced in the US. This is a living document with updates and new chapters planned based on current research efforts.

Sea Lice/Selective Breeding

Vallejo, R. L., Pietrak, M. R., Milligan, M.M., Gao, G., Tsuruta, S., Fragomeni, B. O., Long, R. L., Peterson, B. C., & Palti, Y. (Submitted). Genetic architecture and accuracy of predicted genomic breeding values for sea lice resistance in the St John River aquaculture strain of North American Atlantic salmon. Aquaculture

The manuscript evaluates the potential for selective breeding for resistance to sea lice in the USDA's breeding program. It finds heritability estimates for sea lice density between 0.18-0.20 which are in line with other published results. Importantly this is the first known study to evaluate the potential for selective breeding using both a cross-validation analysis (CVA) and progeny testing of selection candidates (PTSC). Estimated accuracy values using the CVA approach are high and in-line with previous published studies while the PTSC approach yielded good accuracy values of 0.37-0.47.

18. Use of modern technologies to advance Atlantic salmon reproductive systems research

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Abstract

Control of reproductive function in captivity is essential for the sustainability of commercial aquaculture production. The study of reproduction in fish exists with the juxtaposition of an increased need of quality seedstock combined with the need for reproductive sterility. While seemingly opposite sides of reproductive research, these two problems can be improved with the use of new technology to dive deeper into the basic reproductive biology of fish.

In Maine, North American (NA) Atlantic salmon production has experienced a dramatic linear decrease in the eye-up rate of embryos. Along with temperature, and diet, the maternal endocrine environment is suggested to be an important factor in the development of fertilized eggs to the eye-up stage. Steroid hormone pathways which are essential for the development of oocytes differ depending on two distinct time periods: vitellogenesis and oocyte maturation. The successful completion of these two events most likely dictates time of spawning and therefore quality oocytes for fertilization. In addition to oocyte maturation, maternal hormones are necessary for oocyte viability after ovulation and can potentially dictate embryo survival. The study of fish steroidal pathways has traditionally been performed by focusing on one steroid hormone at a time using immunoassay technologies. As immunoassays are limited to one steroid hormone per assay, multiple assays and potentially multiple extractions are required to develop a full endocrine profile. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a

versatile analytical technique for steroid hormone measurement, providing direct detection methods which employ highly resolved unique masses and fragmentation patterns based on the atomic and molecular structure. The use of LC-MS/MS for the measurement of steroid hormones in North American Atlantic salmon has provided one of the most complete endocrine pathways measured in any fish species to date. These pathways included new insights into steroid metabolism through the 5α reductase pathway and the potential synergistic mechanisms between glucocorticoids and androgens required for improved embryo survivability through the eye-up stage.

On the flipside there is a need for the development of sterile Atlantic salmon broodstock which produce sterile offspring to be used in both net pen and land-based aquaculture systems to maximize domestic seafood production. The utilization of gene editing techniques such as CRISPR/cas9 to eliminate early maturation of production quality fish is a direct way to increase salmon production and reduce loss. Early maturity results in compromised growth, health, flesh quality and higher susceptibility to disease reducing the quantity of fish available for consumption. The development and implementation of sterile Atlantic salmon, which do not become reproductively mature, for use in commercial production will negate early maturity losses. CRISPR/cas9 technology allows for the rapid development of sterile fish, but current methodologies are not suitable for the upscaled use in commercial aquaculture nor do they solve the contradiction of a reproductively viable yet sterile broodstock. A new method combining gene editing (CRISPR/cas9) technology and germ cell (eggs and sperm) transplantation will support the mass production of sterile progeny suitable for use in commercial aquaculture facilities. The use of CRISPR/cas9 technology will both support the development of sterile fish but also provide a platform to study the biology behind early maturation or fish maturation in general.

Annotated Bibliography of Key Works

Legacki et al 2023 Using skin mucus for the identification of ovulation biomarkers in North American Atlantic salmon (*Salmo salar*) Aquaculture Vol 575:15 https://doi.org/10.1016/j.aquaculture.2023.739717

In Maine, North American Atlantic salmon production has experienced a 20% decrease in eye-up eggs, the point of embryo development where the black eye spot is visible. The maternal endocrine environment is suggested to be an important factor for ovulation and viable embryos but needs to be further investigated. Combining new sampling matrices (skin mucus) and advanced technologies (liquid chromatography tandem mass spectrometry (LC-MS/MS)) will advance the knowledge of the endocrine status in reproductively mature North American Atlantic salmon. Mucus and plasma were collected from sexually mature female North American Atlantic salmon then analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Fish collected post-ovulation were slotted into groups based on eye-up rate, fish with ≥ 70% eye-up rate and fish with ≤ 70% eye up rate. Mucosal concentrations of steroid hormones correlated with circulating concentrations of related steroids and reflect changes in reproductive physiology. The significant changes in mucosal and circulating steroid concentrations of the 5α reduced pregnane, allopregnanolone suggests a role of 5α-reduced pregnanes during ovulation in North American Atlantic salmon. To our knowledge this is the first time any 5α-reduced pregnane has been reported in teleosts. Mucosal concentrations of precursor glucocorticoids significantly elevated after ovulation combined with significant correlation with hydroxylated pregnanes suggests a dual mechanism needed for ovulation between glucocorticoids and pregnanes. Additionally, glucocorticoids and androgens appear to influence the viability of fertilized eggs highlighting the need for further research into the role's multiple hormones play in both oocyte maturation and embryo viability.

Zohar et al 2021 Fish reproductive biology – Reflecting on five decades of fundamental and translational research. General and Comparative Endocrinology Vol 300:1

Driven by the broad diversity of species and physiologies and by reproduction-related bottlenecks in aquaculture, the field of fish reproductive biology has rapidly grown over the last five decades. This review provides perspectives on fish reproductive biology integrating fundamental and applied developments and milestones. The understanding of the brain-pituitary–gonadal axis led to overcoming

the failure of farmed fish to ovulate and spawn in captivity, establishing a predictable, year-round production of eggs. Dissecting the molecular and hormonal mechanisms associated with sex determination and differentiation drove technologies for producing better performing mono-sex and reproductively-sterile fish. The growing contingent of passionate fish biologists, together with the availability of innovative platforms such as transgenesis and gene editing, as well as new models such as the zebrafish and medaka, have generated many discoveries, also leading to new insights of reproductive biology in higher vertebrates including humans. Consequently, fish have now been widely accepted as vertebrate reproductive models. The evolution of fish reproductive biology occurred due to the molecular and biotechnological revolutions in the life sciences, which enabled a higher resolution of fish reproductive and endocrine processes, answer more questions, and dive into deeper comprehension.

Houston, R.D., Bean, T.P., Macqueen, D.J., Gundappa, M.K., Jin, Y.H., Jenkins, T.L., Selly, S.L.C., Martin, S.A.M., Stevens, J.R., Santos, E.M., Davie, A., Robledo, D., 2020. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat Rev Genet 21(7), 389-409. https://doi.org/10.1038/s41576-020-0227-y

Aquaculture production is derived from numerous, exceptionally diverse species that are typically in the early stages of domestication. Genetic improvement of production traits via well-designed, managed breeding programmes has great potential to help meet the rising seafood demand driven by human population growth. Supported by continuous advances in sequencing and bioinformatics, genomics is increasingly being applied across the broad range of aquaculture species and at all stages of the domestication process to optimize selective breeding. In the future, combining genomic selection with biotechnological innovations, such as genome editing and surrogate broodstock technologies, may further expedite genetic improvement in aquaculture.

Okoli, A.S., Blix, T., Myhr, A.I., Xu, W.T., Xu, X.D., 2022. Sustainable use of CRISPR/Cas in fish aquaculture: the biosafety perspective. Transgenic Res 31(1), 1-21. https://doi.org/10.1007/s11248-021-00274-7

Aquaculture is becoming the primary source of seafood for human diets, and farmed fish aquaculture is one of its fastest growing sectors. The industry currently faces several challenges including infectious and parasitic diseases, reduced viability, fertility reduction, slow growth, escapee fish and environmental pollution. The commercialization of the growth-enhanced AquAdvantage salmon and the CRISPR/Cas9-developed tilapia (Oreochromis niloticus) proffers genetic engineering and genome editing tools, e.g. CRISPR/Cas, as potential solutions to these challenges. Future traits being developed in different fish species include disease resistance, sterility, and enhanced growth. Despite these notable advances, off-target effect and non-clarification of trait-related genes among other technical challenges hinder full realization of CRISPR/Cas potentials in fish breeding. In addition, current regulatory and risk assessment frameworks are not fit-for purpose regarding the challenges of CRISPR/Cas notwithstanding that public and regulatory acceptance are key to commercialization of products of the new technology. This study discusses how CRISPR/Cas can be used to overcome some of these limitations focusing on diseases and environmental release in farmed fish aquaculture. The authors further present technical limitations, regulatory and risk assessment challenges of the use of CRISPR/Cas, and proffer research strategies that will provide much-needed data for regulatory decisions, risk assessments, increased public awareness and sustainable applications of CRISPR/Cas in fish aquaculture with emphasis on Atlantic salmon (*Salmo salar*) breeding.