50th Scientific Symposium of the UJNR Aquaculture Panel

PROGRAM

Theme: **Control and Management of Aquaculture Disease**

Dates and Time

US Eastern Time:	November 14th (Monday), 2022; 19:00 to 21:04 November 15th (Tuesday), 2022; 19:00 to 20:47
Japan Time:	November 15th (Tuesday), 2022; 9:00 to 11:04 November 16th (Wednesday), 2022; 9:00 to 10:47

Location: Online with the Microsoft Teams link for both days

Aim of the Symposium

The US-Japan Natural Resources (UJNR) Aquaculture Panel is a cooperative research exchange, between the US' National Oceanic and Atmospheric Administration (NOAA) and Japan's Fisheries Research and Education Agency (FRA), jointly addressing environmental and technical issues that affect the aquaculture industries of both nations.

The 2022, 50th UJNR Aquaculture Scientific Symposium is the second year of the current three-year theme of "Control and Management of Aquaculture Disease". Commercial aquaculture needs to manage pests and pathogens which can negatively impacts animal well-being and economical productivity. Investigation, identification, and development of effective controlling/mitigation measures against infectious diseases are therefore the top priority in the industries and governments in Japan and US.

Program

Note: All talks are scheduled for 17 minutes, which includes 14 minutes for presentation and 3 minutes for questions.

Monday, November 14th, 2022 (Eastern); Tuesday, November 15th, 2022 (Japan)

Welcome and Aim of the Symposium	19:00-19:05 (E)
Hideki Aono, Japan Panel Chair, FRA	09:00-09:05 (J)

Day 1, Session 1: Education and Disease Management

Moderator: Tomofumi Kurobe, Fisheries Technology Institute, FRA

Role of the Prefectural Fisheries Research Laboratories in Diagnosis of Aquatic Animals Satoshi Miwa, Fisheries Technology Institute, FRA	19:05-19:22 (E) 09:05-09:22 (J)
How Veterinarians Take Part in the Fish Diseases Control in Japan- A Perspective of Japanese Fisheries Veterinarians Shinpei Wada, Nippon Veterinary and Life Science University	19:22-19:39 (E) 09:22-09:39 (J)
Aquaculture Health Management in the United States Kathleen Hartman, Animal and Plant Health Inspection Service, USDA	19:39-19:56 (E) 09:39-09:56 (J)
Efforts to Strengthen Control and Management of Fish Diseases in the North Central and Great Lakes Region of the U.S. Myron Kebus, College of Veterinary Medicine, University of Minnesota	19:56-20:13 (E) 09:56-10:13 (J)

Day 1, Session 2: Disease Control

Moderator: Satoshi Miwa

Dealing With Unknowns- Investigation on Novel and Emerging Pathogens in Japan Tomofumi Kurobe, Fisheries Technology Institute, FRA	20:13-20:30 (E) 10:13-10:30 (J)
Discovering Pathogens, Developing Diagnostic Tools & Therapies against Infectious Diseases in Shrimp Arun Dhar, Aquaculture Pathology Lab, University of Arizona	20:30-20:47 (E) 10:30-10:47 (J)
Monitoring Dynamics of Red Sea Bream Iridovirus in Aquaculture Environment using Environmental DNA Yasuhiko Kawato, Fisheries Technology Institute, FRA	20:47-21:04 (E) 10:47-11:04 (J)

Tuesday, November 15th, 2022 (Eastern); Wednesday, November 16th, 2022 (Japan)

Day 2, Session 1: Fish and Shellfish Diseases

Moderator: Caird Rexroad, Agricultural Research Service, USDA

An Overview of the Current Status of OsHV-1 in the United States Colleen Burge, Bodega Marine Laboratory, California Department of Fish and Wildlife	19:00-19:17 (E) 09:00-09:17 (J)

Opportunity for Genetic Selection in Pacific Oyster to Increase Survival Against an OsHV-1 Microvariant from San Diego Bay (California, USA) 19:17-19:34 (E) 09:17-09:34 (J)
Neil Thompson, Agriculture Research Service, USDA

Studies on Erythrocytic Inclusion Body Syndrome (EIBS) in Farmed Coho Salmon Based on Genome Sequence of the Causative Agent Piscine Orthoreovirus 2 (PRV-2) 19:34-19:51 (E) 09:34-09:51 (J)
Tomokazu Takano, Fisheries Technology Institute, FRA

Spread and Transmission of Japanese Eel Endothelial Cells-Infecting Virus (JEECV) in Eel Aquaculture Farms 19:51-20:08 (E) 09:51-10:08 (J)
Kousuke Umeda, Fisheries Technology Institute, FRA

Day 2, Session 2: Advanced Feeding Strategy and Fish Immunology

Moderator: Luke Gardner, Moss Landing Marine Laboratory, California Sea Grant

ASC1-Deficiency Reduces Immune Responses during *Aeromonas hydrophila* Infection in Japanese Medaka, *Oryzias latipes* 20:08-20:25 (E) 10:08-10:25 (J)
Natsuki Morimoto, Fisheries Technology Institute, FRA

Functional Feeds and Ingredients to Target Fish Health and Optimize Performance 20:25-20:42 (E) 10:25-10:42 (J)
Brian Small, Aquaculture Research Institute, University of Idaho

Scientific Symposium Closing 20:42-20:47 (E)
Janet Whaley, US Panel Chair, NOAA Fisheries 10:42-10:47 (J)

Abstracts and Annotated Bibliographies

Abstract Index

Monday, November 14th, 2022 (Eastern); Tuesday, November 15th, 2022 (Japan)

Tuesday, November 15th, 2022 (Eastern); Wednesday, November 16th, 2022 (Japan)

Role of the prefectural fisheries research laboratories in diagnosis of aquatic animals

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Japan has 47 local prefectures. Each prefectural government has its own fisheries research laboratory, except for one prefecture. The government has issued an administrative directive “guideline for disease control of aquatic animals” which requires each prefectural government to place fish health experts who work on fish health issues at their fisheries research labs. These fish health experts directly deals with fish health in fish farms or public waters, including diagnosis of the diseases of aquatic animals.

The local fisheries labs give free diagnostic service to fish farmers. They also provide advice concerning aquaculture in general, and supervise drug usage and health management.

Followings are the major roles of the fisheries research laboratories or prefectural governments for the control of diseases, which are specified by the “guideline for disease control of aquatic animals”. Most of these are conducted by fish health experts.

- To visit fish farms periodically
- To gather information (species and numbers of animals cultured, location, movement of animals etc.) about the fish farmers in the prefecture as much as possible
- To gather information about occurrences of diseases
- To educate fish farmers and supervise hygiene management
- To diagnose diseases
- To report occurrences of specified, or emerging diseases to the MAFF and to ask for confirmatory diagnoses to the Fisheries Technology Institute.
- To take necessary measures to prevent diseases from spreading
- Observation of imported live animals from foreign countries for a specified period in fish farms.

For terrestrial animals, disease diagnosis is usually conducted by veterinarians in private practice, which requires national license. In addition, each prefectural government has its livestock hygiene service center with many animal health experts. The establishment of livestock hygiene center is directly required by a low, Livestock Hygiene Center Act, and the animal health experts are veterinarians. A livestock hygiene center works solely on the animal health issues.

On the other hand, prefectural fisheries research labs are usually based on the prefectural ordinance. They have wide range of duties concerning fisheries science, including surveys of fisheries resources, environmental monitoring, stock enhancement, etc. Fish health issues are just one of them, and usually only a small number of personnel is working on fish health. In addition, unlike a veterinarian, a fish health expert is not a qualification legally authorized by the government. Therefore, they cannot prescribe drugs for fish diseases which are not specified for the approval of the drug, whereas veterinarians can do it. Although there are cases in which a veterinarian is designated as a fish health expert, such cases are exceptions. This poses a substantial problem for the diagnostic system of aquatic animals in Japan. To solve this problem, the government is currently trying to involve veterinarians in the diagnostic system of aquatic animals.

How veterinarians take part in the fish diseases control in Japan

-A Perspective of Japanese Fisheries Veterinarians-

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In Japan, infectious disease-related issues in aquaculture have been mainly dealt by fish pathologists who do not have veterinary license, but received education in fisheries science and/or related disciplines. On the other hand, the topic, “Fish Diseases”, has been included in the national examination for veterinary license, since 1984. In addition, in 2011, Japanese Association of Establishments for Veterinary Education decided to include “Fish Diseases” as a compulsory subject of Applied Veterinary Medicine in Veterinary Education Model Core Curriculum. As a result, at least 70% of veterinarians in Japan have studied fish diseases in 17 schools of veterinary medicine. However, few veterinarians had been involved in the actual scenes of fisheries medicine for a long time.

In 2018, a list of veterinary practitioners correspondent for fish medicine at aquaculture scene (so-called “Fisheries Veterinarians = FVs”) was established and disclosed by the Food Safety and Consumer Affairs Bureau of Japanese Ministry of Agriculture, Forestry and Fisheries, based on their policy of prompt measures for fish diseases in domestic aquaculture. Currently, a total of 70 veterinarians are listed and some of them have already started their careers at actual aquaculture scenes. However, those FVs pointed out an issue that the latest information of aquaculture system and postgraduate training is not sufficient in the veterinary schools. In this presentation, I would like to introduce the recent situation of FVs in Japan, and present a perspective of Japanese FVs in the near future.

Aquaculture Health Management in the U.S.
Comprehensive Aquaculture Health Program Standards

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Introduction

Currently U.S. aquaculture industry sectors are operating without uniform, integrated and comprehensive standards for aquatic animal health. The commercial aquaculture industry sectors are burdened with varying health requirements for animal movement often resulting in expensive yet meaningless testing. Consistent program standards provide a science-based framework to verify aquatic animal health, allow for branding, provide leverage for negotiations with trade partners, both domestic and international, and facilitate safe animal movement.

Background

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 of farmed aquatic animals produced in U.S. private aquaculture industry sectors. The standards are 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.

CAHPS Overview

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. Participation in CAHPS, as well as the pathogens covered under the program is determined by the premises' aquatic animal health team.

Impact for U.S. Aquaculture Producers

APHIS recognizes that many U.S. aquaculture producers are moving live animals interstate and/or internationally and are burdened by duplicative and meaningless testing requirements for animal health. Adoption of CAHPS offers a uniform approach to verify farm-raised aquatic animal health as well as branding and leveraging opportunities.

Implementation

CAHPS provides standards to promote a meaningful and consistent approach to health management for aquatic animal movement, aiming to support safe trade while protecting aquatic animal health for U.S. commercial industry sectors and natural resources alike. CAHPS is a priority for the VS Aquaculture Program, but implementation is based on sufficient funding to support the oversight management of CAHPS.

Efforts to strengthen control and management of fish diseases in the North Central and Great Lakes region of the U.S.

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States within the North Central and Great Lakes region of the United States have regulations that guide control and management of fish diseases that may occur on fish farms hatcheries, and in wild populations of fish. The long-standing fish health regulatory infrastructure includes testing requirements for fish moved between states, between fish farms and hatcheries, and for fish released to the wild. Some states have training programs for fish farmers, fish farm veterinarians, and fish health professionals. These are described below:

- Fish Health Medicine Program – AAFV FishVets.org The intended audience for this online course is veterinarians. It contains modules on fish farm husbandry, water quality, biosecurity and risk management, fish health certificates, and a modified fish health assessment technique. Over 500 veterinarians throughout the world have completed this online course.

- Fish Health for Producers Fish Health Inspections - Northern Aquaculture Demonstration Facility | UWSP The intended audience for this online course is fish producers (i.e., fish farmers, fish hatchery managers, and fish health specialists). The aim is to help fish producers understand fish health testing, fish health certificates, and prepare them for fish health work that may be conducted at their facility on their fish. The six modules can be taken anytime and anywhere using narrated PowerPoint presentations and supplemental reading materials delivered using new educational technology software. This online course has attracted over 800 fish producers worldwide.

To improve the control and management of fish diseases, a newly funded project has been launched to incorporate consistent, science-based guidance to inform animal movement decisions in a viral hemorrhagic septicemia (VHS) outbreak in finfish. Still in its initial phases, the project activities thus far have been to convene a workgroup of federal, state, and tribal nation fish health regulators, fish farmers, hatchery producers, and other subject matter experts to develop science-based assessments of the risk of moving susceptible fish species from apparently uninfected premises to natural waters or other premises during a VHS outbreak in the Great Lakes (GL) region.

The risk assessments (RAs) will be translated into movement guidances, which provide regulators with a consistent risk-based framework to guide fish movement decisions in a VHS outbreak, and direct-action guides for fish producers, which facilitate the implementation of disease mitigation measures such as biosecurity on their premises. These materials will be widely distributed through a dedicated website, professional, trade, and academic conferences, and professional and lay publications. The workgroup will be the foundation for consensus among differing agencies, and the science-based RAs, movement guidances, and direct-action guides will facilitate the movement of fish in commerce during a large-scale fish disease outbreak, namely VHS in the GL, while concurrently controlling disease spread.

Annotated Key References

Laura Martinelli, Olivia Harris, Michael T. Collins, **Myron Kebus**, 2020, Efficacy of a Modified Health Assessment Utilized on Two Genetically Distinct Stocks of Rainbow Trout, Journal of Aquatic Animal Health, 32(2):pages 59-64.

Rainbow Trout *Oncorhynchus mykiss* are a salmonid fish maintained in aquaculture facilities and used as a source of protein around the world. Veterinarians need accurate, efficient tools and straightforward assessments to evaluate the health status of fish, like the rainbow trout, that are cultivated on farms. In this study, two genetically distinct groups of rainbow trout propagated at Rushing Waters Trout Farm in Palmyra, Wisconsin were evaluated. One group of rainbow trout originated from State A (n=20) and one group from State B (n=20), and they were both surveyed with a modified health assessment in a prospective, randomized, single-blind study. The adapted health assessment included external and internal gross evaluations, skin scrapes, gill clips, intestinal scrapes, and calculated indices including KTL and the hepatosomatic index. Generally, there were few significant differences in health assessment values between State A and State B fish. When this information was presented to the producer, it was discovered that perceived differences between groups were due to management errors in calculated growth rates. The results of this study add validity to the use of standardized health assessments for evaluating fish within aquaculture. This paper used the *Modified Fish Health Assessment* developed by Dr. Myron Kebus in 1994. He has trained over 300 veterinarians to conduct this procedure through online and hands-on training.

Grace Karreman, Kim Klotins, Julie Bebak, Lori Gustafson, Andrea Osborn, **Myron Kebus,** 2015, Aquatic Animal Biosecurity: A Case Study of Bioexclusion of Viral Hemorrhagic Septicemia Virus in an Atlantic Salmon Hatchery, Journal of Applied Aquaculture, 27(3):299-317.

A large North American salmon farm company requested assistance to prevent the introduction of Viral Hemorrhagic Septicemia Virus (VHSV) into one of its Atlantic salmon (*Salmo salar*) hatcheries. A systematic, disciplined approach was used based on risk assessment and the principles of infectious disease control principles. The hatchery's operations were analyzed for potential pathogen (VHSV) introduction using knowledge of the facility's physical layout and the operational process flow. The plausible routes of introduction were identified by tracing the movements of animals (fish), water, fomites, vectors and feed under everyday conditions. Mitigation measures for identified gaps were proposed using the known characteristics of the pathogen. Dr. Myron Kebus has used principles discussed in this article to train veterinarians and fish farmers on conducting biosecurity audits on fish farms.

VHSV Expert Panel and Working Group. 2010. Viral hemorrhagic septicemia virus (VHSV IVb) risk factors and association measures derived by expert panel. 2010. Preventive veterinary medicine. Vol 95:1-2. Pp 128-139.

Viral hemorrhagic septicemia virus (VHSV) is an OIE-listed pathogen of fish, recently expanding in known host and geographic range in North America. Through a group process designed for subjective probability assessment, an international panel of fish health experts identified and weighted risk factors perceived important to the emergence and spread of the viral genotype, VHSV IVb, within and from the Great Lakes region of the US and Canada. Identified factors included the presence of known VHSV-susceptible species, water temperatures conducive for disease, hydrologic connectivity and proximity to known VHSV-positive areas, untested shipments of live or frozen fish from known positive regions, insufficient regulatory infrastructure for fish health oversight, and uncontrolled exposure to fomites associated with boat and equipment or fish wastes from known VHSV-positive areas. Results provide qualitative insights for use in VHSV surveillance and risk-management planning, and quantitative estimates of contextual risk for use in a Bayesian model combining multiple evidence streams for joint probability assessment of disease freedom status. Consistency checks suggest that the compiled factors positively reflect expert judgment of watershed risk for acquiring VHSV IVb. External validation is recommended as the availability of empirical data permits. The panel included Dr. Myron Kebus. These risk factors are part of the current process to conduct a proactive risk assessment of VHSV IVb for fish farms in the Great Lakes Region of the U.S. by the University of Minnesota, Secure Food Systems team.

Dealing with Unknowns: Investigation on Novel and Emerging Pathogens in Japan

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In Japan, various fishes and shellfishes are cultured for production of meats or luxury items. These include freshwater and saltwater fishes and shellfishes (Japanese Eel, Tuna, Red Seabream, Yellowtail, Fugu, Oysters, Scallops, Sea Squirt) for meat production. Luxury items, such as peals (produced within Akoya Pearl Oyster) and Koi Carp, are also the major aquaculture products in Japan. Each fish or shellfish is susceptible to a unique range of pathogens, so that many different types of pathogens have been constantly identified. One of the main roles of the Diagnosis Group at the FRA (with support from other groups) is to identify the cause of mortalities due to infectious diseases. Our group consists of specialists with various expertise, such as virology, parasitology, bacteriology, histopathology, molecular biology, and anatomy and cell biology of fishes and shellfishes. Using the series of expertise with traditional and cutting-edge tools (e.g., histology, experimental challenge test, meta-transcriptome analysis), we perform diagnosis for specimens that researchers at local fisheries laboratories cannot identify the cause of death, which is called "unknown cases". We have identified many novel and emerging pathogens, such as viruses, bacteria, microsporidian, and myxosporean parasites. For some economically important diseases, we further developed diagnostic tests and controlling measures. Therefore, our group is functioning as the front line to fight against infectious diseases in Japan. In my presentation, I will explain some of our past cases and discuss challenges that we are facing now.

Discovering pathogens, developing diagnostic tools & therapies against infectious diseases in shrimp

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Over the past three decades, shrimp farming has been slowly transformed from a small family-based backyard farming activity to a global industry providing a major source of revenue and jobs for millions of people around the world, especially in Asia and Latin American countries with large coastal boundaries. Now as the industry is rising steadily, its growth and sustainability is jeopardized for periodic outbreak of infectious diseases caused by viruses, bacteria and fungi.

The Aquaculture Pathology Laboratory (APL) at the University of Arizona (UA) is a Reference Laboratory of the World Organization for Animal Health (OIE, Paris, France) for Crustacean Diseases. It is an United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) approved and ISO17025:2017 and ISO17025:2043 certified laboratory for crustacean disease diagnostics. Established in the mid-1970s, the laboratory attained global recognition through its research, services to shrimp industry, and training of aquaculture professionals worldwide. Today, the laboratory provides diagnostic testing to 30-35 countries/year. The laboratory also offers screening of therapeutics against microbial diseases, feed and feed additives, and quarantine services to shrimp industry worldwide[1]. The laboratory conducts "Proficiency Tests" to harmonize shrimp disease diagnostics across many laboratories throughout the world[2]. The laboratory offers a longstanding summer training course called "*Shrimp Pathology Short Course"* that is open to aquaculture professionals from around the world. The laboratory also provides in-country training worldwide upon request.

Over the years, researchers in APL discovered the etiology of many shrimp diseases, and developed diagnostic tools to prevent the spread of those diseases across the world. The discovery of an emerging lethal strains of infectious myonecrosis virus (IMNV) associated with recent outbreaks in Brazil is an example of such research endeavour[3]. A serendipitous discovery of a novel virus, *Penaeus vannamei* Solinivivirus associated with the IMNV outbreak in Brazil is a further testament of applied research legacy of the laboratory[4]. In order to expedite pathogen discovery and developing diagnostic tools, Dhar's team now combines genomic approaches with conventional pathology to discover pathogens and to develop disease diagnosis in shrimp. The concept was recently demonstrated to reconstruct the genome of a DNA virus, white spot syndrome virus, starting with thin sections (5 µm) of paraffin embedded tissue samples from archived histology blocks[5]. Currently, APL has a collection of over 100,000 histology tissue block dating back to mid-1970's. This collection of histology blocks is a treasure trove for pathogen discovery, studying the origin and evolution of viruses and their spread worldwide. A workflow was developed in the laboratory for genome reconstruction to enable molecular diagnostics. This will enable the industry to prevent the disease spread in near real time while shining a light on the epidemiology of diseases of shrimp aquaculture from the past. Researcher in Dhar's team are now using reverse genetic approach to develop an antiviral therapy that can be delivered via oral route to control viral diseases in shrimp.

Embedded References:

Aquaculture Pathology Laboratory-University of Arizona:

1. Schofield, P. J. Noble, B. L., Padilla, Tanner, and Dhar, Arun K. 2018. The history and future of the Aquaculture Pathology Laboratory. Global Aquaculture Advocate, Tuesday, 23 January 2018. https://www.globalseafood.org/advocate/history-future-aquaculture-pathology-laboratory/
   This article published on 23 January 2018 in the industry magazine, Responsible Seafood Advocate summarized the accomplishment of the laboratory under the leadership of the founding director, Professor Donald Lightner who retired in Fall 2015, and how the laboratory plans to continue his legacy and contribute further to address emerging global challenges in shrimp industry under the leadership of the new director, Professor Arun K. Dhar who joined the university in January 2017.
2. Aranguren Caro, F., Millabas, J., and Dhar, Arun K. 2018. A comprehensive look at the Proficiency Test for farmed shrimp. Global Aquaculture Advocate, Monday, 8 January 2018. https://www.globalseafood.org/advocate/proficiency-test-farmed-shrimp/
   This article summarized how the Proficiency Test conducted by the Aquaculture Pathology Laboratory-University of Arizona in harmonizing shrimp disease diagnosis by diagnostic laboratories around the world. Currently, the university conducts PT twice a year and about 50-55 countries participates on a yearly basis.

Discovery of emerging and novel viruses infecting marine shrimp:

3. Andrade, Thales P. D., Cruz-Flores, R., Mai, Hung N., and Dhar, Arun K. 2022. Novel Infectious Myonecrosis Virus (IMNV) variant is associated with recent disease outbreaks in *Penaeus vannamei* shrimp in Brazil. *Aquaculture, 554: 738159.* DOI: 10.1016/j.aquaculture.2022.738159

In recent years, infectious myonecrosis disease, caused by the Infectious Myonecrosis Virus (IMNV), has caused major losses to shrimp farmer in Brazil. Histopathological and genomic analysis revealed a novel IMNV genotype that might have been introduced to Brazil through the movement of animals from Asia.

4. Cruz-Flores, Roberto, Thales P.D. Andrade, Mai, Hung N., Alenton, Rod Russel R. and Dhar, Arun K. 2022. Identification of a novel solinivivirus with nuclear localization associated with mass mortalities in cultured Whiteleg shrimp (*Penaeus vannamei*). *Viruses,* 14, 2220. https://doi.org/10.3390/v14102220
   Further genomic analysis of the IMNV-infected samples (from Reference 3) revealed the presence of a new virus that has not been reported in shrimp, as of now. The new virus was found to be prevalent in many states in Brazil. It remains to be determined if the new virus has any synergistic effect along with IMNV in causing enhanced mortalities in shrimp farm in Brazil.

Expediting Pathogen Discovery by combining histopathology and genomic tools:

5. Cruz-Flores, Roberto Hung N. Mai, Siddhartha Kanrar, Aranguren Caro, L. Fernando and **Dhar, Arun K.** 2020. Genome reconstruction of White Spot Syndrome Virus (WSSV) from archival Davidson-fixed paraffin embedded shrimp (*Penaeus vannamei*) tissue. *Scientific Reports* 10: 13425. https://doi.org/10.1038/s41598-020-70435-x
   The authors for the first time demonstrated how the genome of a DNA containing virus, white spot syndrome virus can be reconstructed from archived histology tissue blocks. This opened a new horizon to study the origin and evolution of viruses in aquatic animals. The Aquaculture Pathology Laboratory has a collection of >100,000 histology blocks dating back to the beginning of shrimp industry in the mid-70'S. This repository serves a unique collection of biological materials to study the origin, evolution and spread of pathogens in shrimp aquaculture worldwide.

Monitoring red sea bream iridovirus in aquaculture environment using environmental DNA to understand disease outbreaks

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The appearance or density of pathogens in the aquatic environment surrounding fish farms is strongly influenced by disease outbreaks in the farms. Therefore, waterborne pathogens could be monitored by the use of environmental DNA (eDNA) collected from rearing water in fish farms. In this study, we developed and applied the eDNA method for monitoring red sea bream iridovirus (RSIV), which is listed as a notifiable disease by the World Organization for Animal Health due to its significant impact on the aquaculture industry. We first developed a RSIV concentration method from seawater using the iron-based flocculation, coupled with a large-pore-size filtration technique, to process a larger amount of seawater as viral load in environments seemed to be very low. Using the method, RSIV in 500 mL seawater was successfully concentrated into a 0.2-mL eDNA solution at a 2,500-concentration magnification. The recovery rate of the RSIV genome measured by a real-time PCR was higher than 80% in the RSIV-spiked artificial seawater. The limit of detection of this method was approximately $1x10^2$ RSIV copies/L of sample water, which is a more than 1000 times improvement compared to the conventional DNA extraction from water samples without concentration. Data from an experimental challenge test revealed that RSIV genome number in the rearing water increased more than 5 days before mortality started. This suggests the possibility of the prediction of a disease outbreak before the actual onset of mortality in net cages in seawater.

Subsequently, the eDNA of RSIV was monitored in a fish farm, which mainly rears juveniles and broodstock of red sea bream (*Pagrus major*) over 3 years (2016 to 2018) using the method mentioned above. A disease outbreak of RSIV occurred in juveniles in July 2017 in the farm. The data demonstrated indeed that the RSIV genome was detected from the eDNA at least 5 days before the RSIV outbreak. The amount of RSIV DNA in the seawater during the outbreak were highly associated with the numbers of daily mortality, and the copy number of the viral DNA reached a peak of 10^6 copies/L in late July in 2017, when daily mortality exceeded 20,000 fish. In contrast, neither clinical signs nor mortality was observed in the broodstock during the monitoring period, whereas the broodstock were confirmed to be RSIV positive by a diagnostic PCR test using tissue samples in October 2017. Interestingly, the viral load of eDNA in the broodstock net pens (10^5 copies/L seawater) was higher than that in the juvenile net pens (10^4 copies/L seawater) just before the RSIV outbreak in late June 2017. After elimination of all RSIV-infected surviving juveniles and 90% of the broodstock, the copy numbers of RSIV significantly decreased and few viral DNA copies were detected in the eDNA in the fish farm from April 2018 onward (fewer than 10^2 copies/L seawater). These results suggest that the virus shed from the asymptomatically RSIV-infected broodstock was transmitted horizontally to the juveniles and caused the RSIV outbreak in the fish farm.

Since the iron flocculation method is easy and reliable, many sampling points can be adopted to monitor RSIV in seawater. We are now trying to visualize horizontal dispersion of RSIV among net-pens via seawater.

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.
Environmental DNA (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 via eDNA monitoring, and it could be applied as a tool for application in aquaculture to control fish diseases.

An overview of the current status of OsHV-1 in the United States

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The spread, emergence, and adaptation of pathogens causing aquatic disease has been problematic for fisheries and aquaculture industries for the last several decades creating the need for strategic management and biosecurity practices. The Pacific oyster (*Crassostrea gigas*), a highly productive species globally, has been a target of disease and mortality caused by a viral pathogen, the Ostreid herpesvirus 1 (OsHV-1) and its microvariants (OsHV-1 µvars). OsHV-1 µvars have emerged globally with economically devasting mortalities occurring in some locations. OsHV-1 µvars have spread both within and between countries leading to concern among shellfish growers globally. To date, in the US, OsHV-1 detection and mortality have been isolated to two geographic areas in California based on both submitted diagnostic samples and sentinel monitoring programs. Sporadic mass mortalities of juvenile oysters (~60% mean losses) have occurred in Tomales Bay since 1992 where an OsHV-1 reference variant has been detected as early as 1995 through retrospective analysis. OsHV-1 was subsequently detected in two neighboring bays: Drakes Bay and Bodega Bay. A novel OsHV-1 microvariant, OsHV-1 µvar SD was first detected in San Diego Bay in 2018 at a shellfish nursery using active surveillance to establish a health history, followed by a mortality event of juvenile Pacific oysters. Following detection of OsHV-1 µvar SD, multiple research avenues have been explored to better understand potential impacts of OsHV-1 µvar SD on the shellfish aquaculture industry including sentinel monitoring and challenge experiments with multiple species and oyster family lines. Laboratory challenge trials have provided information on the relative susceptibility of commercially important species in the US to OsHV-1 µvar SD. Given the emergence and spread of OsHV-1 microvariants, a multi-state oyster sentinel program, with strong industry collaboration was initiated in 2020 to monitor prevalence/viral load and pathogenesis of OsHV-1 in juvenile Pacific oysters planted at commercial leases in California (Tomales Bay and San Diego Bay), Oregon (Tillamook Bay), and Washington (Totten Inlet and Willapa Bay). Data from 2020 and 2021, confirm OsHV-1 infection only in Tomales and San Diego Bays and enhance our understanding of OsHV-1 related losses in those bays.

Annotated bibliography

Fuhrmann, M., Castinel, A., Cheslett, D., Nozal, D.F., Whittington, R.J., 2019. The impacts of ostreid herpesvirus 1 microvariants on Pacific oyster aquaculture in the Northern and Southern Hemispheres since 2008. Revue Scientifique Et Technique- Office International Des Epizooties 38 (2), 491–509.

Fuhrmann and co-authors provide an analysis of challenges and responses of Pacific oyster aquaculture industries impacted by OsHV-1 microvariants. Individual farms within industries acted independently and lacked cohesion. Generally, OsHV-1 μvars lead to significant, widespread, and long-lasting disruptions to affected industries first with large economic losses followed by using compensatory production strategies. These strategies included increasing the number of spat placed on farms and higher market price for oysters. This review also indicated that biosecurity did not prevent large scale losses to these industries with both environmental and anthropogenic factors such as commercial or recreational shipping, uncooked seafood translocations, and rafting through carrier particles.

Burge CA**,** Shore-Maggio A., Rivlin ND. 2018. Ecology of emerging infectious diseases of invertebrates. *In* Ecology of Invertebrate Diseases Ed. Hajek, A. Shapiro, D. Wiley. http://onlinelibrary.wiley.com/doi/10.1002/9781119256106.ch16/summary

This book chapter provides a review of ecology of emerging infectious diseases of invertebrates including a section on "Molluscan Herpesvirus Infections of Bivalves." This review provides background information on emergence of OsHV-1 globally including emergence of specific variants and potential anthropogenic influences. Potential management responses and practices for control of OsHV-1 are also discussed. Additionally, the review includes other potential protective strategies for emerging diseases of invertebrates.

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.

The authors report the first detection of an OsHV-1 microvariant in the United States in a new shellfish nursery establishing health history in San Diego, California. Specific genome regions recommended by the OIE (World Health Organization) were amplified, sequenced, and compared to known global variants of OsHV-1. An initial infection trial using low virus concentrations showed this variant to be lethal to juvenile Pacific oysters.

Opportunity for genetic selection in Pacific oyster to increase survival against an OsHV-1 microvariant from San Diego Bay (California, USA)

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Ostreid herpesvirus 1 is a pathogen of global concern that has caused severe losses in Pacific oyster aquaculture on numerous continents. Two strains of OsHV-1 are known on the West coast of the United States, a 'reference' variant in Tomales Bay, California, and a recently discovered, more pathogenic microvariant from San Diego Bay, California. Genetic selection has proven to be effective at increasing survival to OsHV-1 infection using multiple breeding models in other regions of the world where the pathogen is broadly distributed in farming areas. However, less research has focused on increasing resistance to OsHV-1 preemptively using genomic selection methods, with the goal of reducing losses if OsHV-1 becomes endemic. The U.S. Pacific coast shellfish aquaculture industry desires a source of OsHV-1 'resilient' Pacific oysters to mitigate any losses that could occur if/when OsHV-1 pathogens appear in their local waters. Using juvenile (5-10 mm shell length) oysters produced in the USDA Pacific Shellfish Breeding Center, a laboratory challenge experiment was conducted on 15 families to identify the standing genetic variation for OsHV-1 survival, quantify heritability, and determine the utility of genomic selection methods compared to pedigree methods for OsHV-1 survival/tolerance. Mean mortality of all 15 families was 61% for the pedigree challenge, ranging from 29 to 90 percent mortality per family. The heritability of survival is estimated to be 0.66 using pedigree methods, and a broad range of estimated breeding values indicate adequate standing genetic variation for selective breeding. Genotype data was produced with the Axiom Oyster Genotyping Array and used to compare the results from the pedigree-model experiment with a common-garden genomic selection-model experiment. There is significant potential to increase survival to OsHV-1 from San Diego Bay using genetic selection given results from this study. Further development in genomic selection methodologies and refinements in laboratory challenge protocol may increase efficiency of selection and be fundamental in providing the U.S. Pacific coast shellfish industry with an OsHV-1 'resilient' stock of oysters to use for commercial production.

Annotated bibliography:

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.

Studies on erythrocytic inclusion body syndrome (EIBS) in farmed coho salmon based on genome sequence of the causative agent piscine orthoreovirus 2 (PRV-2)

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Erythrocytic inclusion body syndrome (EIBS) causes mass mortality in farmed coho salmon (*Onchorhynchus kisutchi*) in Japan. The causative agent of the disease is a virus with an icosahedral virion structure. However, the genome structure of the virus had not been characterized. We determined the entire genome sequence of this virus and found that it harbors 10 double-stranded RNA genome segments (L1, L2, L3, M1, M2, M3, S1, S2, S3, S4). Phylogenetic analyses based on S1 suggested that the virus is closely related to other piscine orthoreovirus (the causative agent of heart and skeletal muscle inflammation in farmed Atlantic salmon, PRV-1) and was accordingly named PRV-2.

Additional benefits of obtaining the PRV-2 genome sequence were the advancement of research to control EIBS, including the development of diagnostic methods and prototype vaccines. RT-PCR for detecting this virus serves as a tool for diagnosing and monitoring infected fish. In addition, the antiserum raised against the virus spike protein, which is encoded in the S4 segment, allowed detailed immunohistochemical observations of the EIBS-affected fish. Furthermore, a plasmid DNA constructed to express the spike protein in eukaryotic cells was found to be effective as a DNA vaccine by increasing antibody titers and inhibiting viral growth upon PRV-2 infection.

Annotated Bibliography of Key Works

Takano, T., *et al*. 2016. *Plos One*, Vol:11:10. e0165424

Genome of a virus purified from EIBS-affected coho salmon was determined. The phylogenetic analysis based on viral genes suggested that this novel virus is closely related to the piscine orthoreoviruses (PRVs) that infect salmonids, and it was named PRV-2. RT-qPCR was developed to detect the virus and then it revealed a significant increase in PRV-2 RNA in fish blood after the artificial infection of EIBS-naïve fish but not in that of fish that had recovered from EIBS. The degree of anemia in each fish increased as the PRV-2 RNA increased during an epizootic season of EIBS on an inland coho salmon farm. These observations supported PRV-2 as the causative virus of EIBS in farmed coho salmon in Japan.

Matsuyama, T., *et al*. 2021. *Aquaculture*, Vol:533. 736163

In this study, analysis of antiserum obtained from PRV-2 infection survivors identified σ1 as a PRV-2 antigenic protein. Since a DNA vaccine incorporating only σ1 was not effective against PRV-2 infection, the DNA vaccine was modified by linking various sequences to the σ1 gene. The modified DNA vaccines showed an improved efficacy such as increasing antibody levels and inhibiting viral growth. The modified DNA vaccines showed better efficacy in increasing the antibody titer and inhibiting viral growth.

Spread and transmission of Japanese eel endothelial cells-infecting virus (JEECV) in eel aquaculture farms.

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The Japanese eel *Anguilla japonica* has been prized as a healthy and luxurious food in Japan for hundreds of years. Viral endothelial cell necrosis of eel (VECNE) is a disease responsible for a significant economic impact on Japan's eel aquaculture since the 1980s. In 2020, the loss of production due to VECNE was approximately 42 tons, equivalent to 189 million JPY (1.3 million USD). Its typical symptoms are 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. The causative agent of VECNE is Japanese eel endothelial cell-infecting virus (JEECV), which is a DNA virus in the family *Adomaviridae*. A previous study showed that increased water temperature (35°C) reduces the mortality of eels intraperitoneally injected with JEECV. However, studies of the disease are still limited. In particular, information of the mode of virus transmission and the dynamic changes in the viral loads in eel culture ponds has been needed to control the disease in aquaculture farms.

In this study, we first aimed (1) to reproduce a more “natural” form of infection using a bath-challenge method and (2) to examine the effect of different temperatures on the transmission of JEECV via water. Naïve eels maintained at 30°C, 32.5°C, and 35°C were challenged with the water from another aquarium containing eels of 7 days after intraperitoneal injection of the homogenized gill of a diseased eel. The eels at 30°C and 32.5°C started to die on day 18 after the challenge, while no eels died at 35°C throughout the experiment. Cumulative mortality at day 60 was 30%, 15%, and 0% for 30°C, 32.5°C, and 35°C, respectively. The gills of dead and surviving eels were subjected to real-time PCR for quantifying viral DNA. At 30°C and 32.5°C, eels that died on day 18 to day 20 showed high viral loads (10^7 to 10^9 copies/mg tissue DNA) as well as typical symptoms of VECNE such as reddening of the fins, whereas those died after day 20 showed lower viral loads (10^3 to 10^5 copies/mg of tissue DNA). No viral DNA was detected at 35°C. The sera of surviving eels were tested by ELISA to detect IgM against the spike protein of JEECV. The antibody titers were lower at higher water temperatures, and those of eels at 35°C were as low as those of control naïve eels. These results (1) showed that JEECV was able to infect eels through water and (2) suggest that at high temperatures around 35°C, JEECV could not efficiently infect its host rather than it was eliminated by the antibody-mediated immunity.

In order to estimate the risk of JEECV infection, we also monitored environmental viral DNA in the pond water of eel farms that experienced mass mortality from VECNE. Environmental DNA in water samples was concentrated using iron flocculation and filtration and tested using real-time

PCR. The pond water contained a large amount of JEECV genome (10^6 copies/mL) during mass mortality. Afterwards, the viral DNA decreased but was still detectable even 9 months after the mass mortality, suggesting that eels that survived the disease shed JEECV for a long period of time. Together with the results from the bath-challenge experiment, the monitoring results strongly suggest that surviving eels and pond water can be infection sources of JEECV. Therefore, on the occurrences of the disease, it seems crucial to isolate the surviving eels and disinfect the contaminated ponds to prevent the spread 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.

ASC1-deficiency reduces immune responses during *Aeromonas hydrophila* infection in Japanese medaka, *Oryzias latipes*

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Apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC) is one of the components of inflammasome, which has crucial role in the inflammatory response. In mammals, ASC regulates caspase-1 activation, thereby inducing pyroptosis and producing activated inflammatory cytokines such as interleukin (IL)-1β and IL-18. In addition, ASC also interacts with receptor-interacting protein kinase 2 (RIPK2) and induces nuclear factor-κB (NF-κB) activation. However, the role of ASC is still poorly understood in fish. In this study, we focused on understanding the role of ASC in fish that were infected with *Aeromonas hydrophila* using Japanese medaka (*Oryzias latipes*) as fish model.

At first, we identified three *asc* genes (*asc1*, *asc2*, and *asc3*) from the Japanese medaka. These *asc* genes were tandem replicates on chromosome 16, and all three ASCs conserved the pyrin and caspase-recruitment domains, which are important for inflammasome formation. The *asc1* expression levels were significantly higher in several organs (i.e., skin, gill, intestine, kidney, and liver) than those of *asc2* and *asc3*, suggesting that *asc1* may act as a dominant *asc* in these organs. To investigate the functional role of ASC1 in medaka, we established ASC1-knockout (KO) medaka using CRISPR-Cas9 system, and a 7-bp deletion in the exon-3 of *asc1* gene was confirmed. Homozygous mutants, ASC1-KO and wild type (WT) medakas were infected with *A. hydrophila*, and their mortality rates were observed. ASC1-KO medaka showed higher mortality than WT in *A. hydrophila* infection. Following *A. hydrophila* infection, the kidney of ASC1-KO medaka exhibited significantly lower expression of NF-κB regulated genes (e.g., *il1b*, *il6*, *il8* and *tnfa*) and *ripk2* gene than in WT kidney. Moreover, after *A. hydrophila* infection, the bacterial burden, superoxide anion production, and lactate dehydrogenase release in the kidney cells of ASC1-KO medaka were significantly decreased compared to those in WT. These results suggest that the medaka ASC1 plays a critical role against *A. hydrophila* infection by inducing inflammatory responses and cell death for bacterial clearance.

Annotated Bibliography of Key Works

Morimoto N, Okamura Y, Kono T, Sakai M, Hikima J. 2021. Characterization and expression analysis of tandemly-replicated *asc* genes in the Japanese medaka, *Oryzias latipes*. Dev Comp Immunol., 115: 103894.
The authors identified the three types of *asc* genes in Japanese medaka, and suggested that *asc1* may act as a dominant *asc* in the Japanese medaka.

Morimoto N, Okamura Y, Maekawa S, Wang HC, Aoki T, Kono T, Sakai M, Hikima J. 2020. ASC-deficiency impairs host defense against *Aeromonas hydrophila* infection in Japanese medaka, *Oryzias latipes*. Fish Shellfish Immunol.,105: 427-437.
The authors established the ASC1-KO medaka using CRISPR-Cas9 system, and presented the mortality rate of ASC1-KO medaka that were infected with *A. hydrophila*. Moreover, the expression of inflammation-related genes after infection with *A. hydrophila* was analyzed by quantitative real-time PCR (qPCR) to elucidate the relationship between ASC1 and inflammation in medaka. To reveal the role of ASC in fish during *A. hydrophila* infection, we detected bacterial burden, superoxide anion production, and cell death in kidney cells using ASC1-KO medaka.

Sarkar A, Duncan M, Hart J, Hertlein E, Guttridge DC, Wewers MD. 2006. ASC directs NF-kappaB activation by regulating receptor interacting protein-2 (RIP2) caspase-1 interactions. J Immunol., 176 (8): 4979-86.
This paper suggested that ASC may interact with RIP2-mediated NF-kappaB activation, toward caspase-1-mediated processing of proIL-1beta by interfering with the RIP2 caspase-1 interaction.

Functional feeds and ingredients to target fish health and optimize performance

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ABSTRACT

Often in aquaculture, the control and management of fish disease begins with best management practices (BMPs) and quality feeds. Feeds not only must be nutritious but must be balanced with ingredients palatable and digestible to the fish. Historically, feeds high in fishmeal and fish oil have provided optimal nutrition, palatability, and digestibility. More recently, the trend away from the use of fishmeal in fish feeds has resulted in ingredient-induced pathologies, such as soybean meal-induced enteritis (SBMIE) of the distal intestine, which require creative management solutions. Through the characterization of SBMIE in rainbow trout, *Oncorhynchus mykiss*, our group at the University of Idaho has learned much about gut health and fish performance. These findings resulted in new research directions to characterize the gut microbiome and to incorporate functional feed ingredients and additives to boost gut health, immune function, and fish performance. Functional feed ingredients and additives (FFAs) can be used to extend beyond satisfying basic nutritional requirements for growth and feed utilization to support the health of the fish. Moreover, functional aquafeeds may result in enhanced growth, immunity, and stress resistance, thus enabling a shift away from chemotherapeutic and antibiotic treatments of fish. A large number of potential FFAs (prebiotics, probiotics, immunostimulants, nucleotides, vitamins, minerals, plant or algal extracts, and essential oils) are available for inclusion in aquafeeds; however, their efficacy is often circumstantial. Over the past several years, our group has evaluated numerous FFAs, but more recent work on additives to improve gut health and boost immune function, e.g. probiotics, prebiotics, and immune stimulants, have enlightened our view of functional ingredients and additives. Examples of the successful use of function additives include recent studies in our lab demonstrating enhanced growth with a novel, high omega-3 fatty acid oil, improved intestinal histopathology when replacing soybean meal with corn fermented protein, and marginally altered immune expression and microbiota of Atlantic salmon (*Salmo salar*) gut, gill, and skin mucosa when fed diets supplemented with mannan-oligosaccharides, coconut oil, or both. Integral to this research has been the training of international students and faculty through opportunities at the Hagerman Fish Culture Experiment Station. Through these opportunities, visiting scholars get experience in fish nutrition, gut and mucosal health, immune function, and genomic tools to further our global understanding of the interactions between fish genetics, diet & ingredients, and mucosal microbiomes as they relate to fish health and performance. By characterizing functional additives to create functional feeds with health promoting properties, we are supporting eco-friendly and sustainable aquaculture solutions.

Annotated Bibliography:

Encarnação, P., 2016. Functional feed additives in aquaculture feeds. In Aquafeed formulation (pp. 217-237). Academic Press.

Presents an early review of functional aquafeeds, including the diverse nature and characteristics of these feed additives, and their application into diet formulations targeting a specific purpose. The review covers additives, such as acidifiers, exogenous enzymes, that are used to improve the animals' performance by providing enhanced digestibility of the feed materials, or counteracting the negative effects of antinutrients, as well as other additives, such as probiotics, prebiotics, phytogenics, and immune-stimulants, that target the improvement of intestinal health, stress, and disease resistance.

Gatlin III, D.M. and Yamamoto, F.Y., 2022. Nutritional supplements and fish health. In *Fish Nutrition* (pp. 745-773). Academic Press.
Presents a more recent review of feed additives as functional modulators of the immune system with a focus on disease prevention. The authors discuss the concept of functional feeds and their ability to "extend beyond satisfying minimum nutritional requirements of the cultured organism to improving their health and resistance to commonly encountered stressors and disease-causing organisms." The review provides an overview of functional additives and the current and potential applications in aquaculture.

Hong, J., Bledsoe, J.W., Overturf, K.E., Lee, S., Iassonova, D. and Small, B.C., 2022. Latitude^{TM} Oil as a Sustainable Alternative to Dietary Fish Oil in Rainbow Trout (*Oncorhynchus mykiss*): Effects on Filet Fatty Acid Profiles, Intestinal Histology, and Plasma Biochemistry. Frontiers in Sustainable Food Systems, 6, p.837628.
This study demonstrates the successful replacement of fish oil with a novel, high omega-3 plant oil in rainbow trout feeds. Fish fed the novel oil outperformed fish fed the fish oil control diet. Furthermore, phagocytic respiratory burst in fish fed the complete replacement diet was significantly higher, while fish fed the 50% replacement diet had reduced inflammation of the distal intestine. Superoxide dismutase, catalase and lysozyme activities were also measured, but there was no difference between treatment groups. While the main objective of this study was to replace fish oil on a fatty acid basis, the results demonstrate that the novel plant oil has function properties, enhancing growth performance, stimulating innate immune function and reducing intestinal inflammation.

Bledsoe, J.W., Pietrak, M.R., Burr, G.S., Peterson, B.C. and Small, B.C., 2022. Functional feeds marginally alter immune expression and microbiota of Atlantic salmon (Salmo salar) gut, gill, and skin mucosa though evidence of tissue-specific signatures and host–microbe coadaptation remain. *Animal Microbiome*, *4*(1), pp.1-20.
Mucosal surfaces of fish provide cardinal defense against environmental pathogens and toxins, yet these external mucosae are also responsible for maintaining and regulating beneficial microbiota. To better their understanding of interactions between host, diet, and microbiota in finfish and how those interactions may vary across mucosal tissue, the researchers used an integrative approach to characterize and compare immune biomarkers and microbiota across three mucosal tissues (skin, gill, and gut) in Atlantic salmon receiving a control diet or diets supplemented with mannan-oligosaccharides, coconut oil, or both. Dietary impacts on mucosal immunity were further evaluated by experimental ectoparasitic sea lice (*Lepeophtheirus salmonis*) challenge.