# 49th Scientific Symposium of the UJNR Aquaculture Panel

## PROGRAM and ABSTRACTS

Theme: Control and Management of Aquaculture Disease
Dates/Times Eastern Time: November 2nd and 3rd, 2021; 7:00 to 9:00 p.m.
Dates/Times Japan Time: November 3rd and 4th, 2021; 8:00 to 10:00 a.m.
Location: Online with the Microsoft Teams link for both days **HERE**

## 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 2021, 49th UJNR Aquaculture Scientific Symposium is the first year of the current three-year theme of "Control and Management of Aquaculture Disease". Commercial aquaculture must manage pests and pathogens that can cause disease and negatively impact animal well-being and economic productivity. Investigation and identification of effective controls, mitigation measures, and treatments of disease is therefore a top industry and government priority in Japan and the US.

## Program

*Note: All talks are scheduled for 20 minutes, which includes 15 minutes for presentation and 5 minutes for questions.*

### Tuesday, November 2, 2021 (Eastern); Wednesday, November 3, 2021 (Japan)

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| **Welcome and Aim of the Symposium** | 19:00-19:05 (E) |
| Michael Rust, US Panel Chair, NOAA Fisheries Office of Aquaculture | 08:00-08:05 (J) |

### Session 1: Disease Control

(Moderators: Satoshi Miwa, Caird Rexroad)

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| **Isolation and Characterization of Hirame Aquareovirus (HAqRV):** | 19:05-19:25 (E) |
| **A New Aquareovirus Isolated From Diseased Hirame *Paralichthys olivaceus*** | 08:05-08:25 (J) |

Yasuhiko Kawato, Fisheries Technology Institute, FRA

**Weissellosis: An Emerging Disease of Farmed Rainbow Trout** 19:25-19:45 (E)
Tim Welch, National Center for Cool and Cold Water Aquaculture, 08:25-08:45 (J)
USDA/ARS

**Development of a Method to Quantify Endogenous IFNγ Protein in** 19:45-20:05 (E)
**Amberjack Species** 08:45-09:05 (J)
Yuta Matsuura, Fisheries Technology Institute, FRA

**Use of a *Flavobacterium columnare* DnaK Recombinant Protein Vaccine** 20:05-20:25 (E)
**to Guard Against Columnaris Disease in Channel Catfish** 09:05-09:25 (J)
Miles Lange, Aquatic Animal Health Research Unit, USDA/ARS

**α-glucosidase Activity of *Nocardia seriolae* Isolated From Seawater Fish** 20:25-20:45 (E)
**in Japan** 09:25-09:45 (J)
Yoshiko Shimahara, Fisheries Technology Institute, FRA

**Emergent Pathogens in Aquaculture: How Should We Identify Them?** 20:45-21:05 (E)
Linda Rhodes, Northwest Fisheries Science Center, NOAA 09:45-10:05 (J)

## Wednesday, November 3, 2021 (Eastern); Thursday, November 4, 2021 (Japan)

### Session 2: Education and Disease Management

(Moderators: Satoshi Miwa, Luke Gardner)

**The Role of National Fisheries University and Studies of Fish Disease** 19:00-19:20 (E)
Shinya Yasumoto, National Fisheries University, FRA 08:00-08:20 (J)

**Aquaculture Medicine in the 21st Century: Training the Veterinary** 19:20-19:40 (E)
**Community to Meet the Needs of a Global Industry** 08:20-08:40 (J)
Iskande Larkin, University of Florida

**Epitope Mapping of Anti-RSIV Monoclonal Antibody Using a Phage** 19:40-20:00 (E)
**Display Library** 08:40-09:00 (J)
Tomokazu Takano, Fisheries Technology Institute, FRA

**Managing the Threat of Emerging Diseases in Bivalve Mollusc** 20:00-20:20 (E)
**Aquaculture** 09:00-09:20 (J)
Ryan Carnegie, Virginia Institute of Marine Science, College of
William & Mary

**Advancing Epidemiological Tools for Marine Disease Management –** 20:20-20:40 (E)
**Modeling Dispersion of OsHV-1μvar in Pacific Oyster Populations** 09:20-09:40 (J)
Meghana Parikh, Northeast Fisheries Science Center, NOAA

**Using a OneHealth Approach to Evaluate How Marine Pathogens and Parasites That Are Infectious to Cultured Finfish, Shellfish, and Macroalgae Within the United States May Be Affected by Climate Change** 20:40-21:00 (E) 09:40-10:00 (J)
Michelle Rub, Northwest Fisheries Science Center, NOAA

**Science Symposium Closing** 21:00-21:05 (E)
Hideaki Aono, Japan Panel Chair, FRA 10:00-10:05 (J)

## List of Participants

**UNITED STATES:**

| Name | Affiliation |
|---|---|
| **Panel** | |
| Dr. Michael Rust | NOAA Fisheries, Office of Aquaculture |
| Dr. Luke Gardner | California Sea Grant |
| Dr. Caird Rexroad | USDA, ARS |
| Mr. Clete Otoshi | NOAA Fisheries, Office of Aquaculture |
| **Speakers** | |
| Dr. Linda Rhodes | NOAA Fisheries, NWFSC |
| Dr. Michelle Rub | NOAA Fisheries, NWFSC |
| Dr. Meghana Parikh | NOAA Fisheries, NEFSC |
| Dr. Miles Lange | USDA, ARS, Aquatic Animal Health Research Unit |
| Dr. Tim Welch | USDA, ARS, National Center for Cool and Cold Water Aquaculture |
| Dr. Ryan Carnegie | College of William & Mary, Virginia Institute of Marine Science |
| Dr. Iske Larkin | University of Florida, College of Veterinary Medicine |
| **Invited to Attend** | |
| Ms. Danielle Blacklock | NOAA Fisheries, Office of Aquaculture |
| Mr. David O’Brien | NOAA Fisheries, Office of Aquaculture |
| Dr. Seth Theuerkauf | NOAA Fisheries, Office of Aquaculture |
| Ms. Marifrancis Moschopoulos | NOAA Fisheries, Office of Aquaculture |
| Dr. Janet Whaley | NOAA Fisheries, Office of International Affairs and Seafood Inspection and Office of Aquaculture |
| Dr. John Shelley | NOAA Fisheries, Office of International Affairs and Seafood Inspection |
| Dr. Penny Swanson | NOAA Fisheries, NWFSC |
| Dr. William Fairgrieve | NOAA Fisheries, NWFSC |
| Mr. Kellen Parrish | NOAA Fisheries, NWFSC |
| Dr. Kathleen Hartman | USDA, APHIS |
| Dr. Alicia Marston | USDA, APHIS |
| Dr. Thomas Waltzek | USDA, APHIS |
| Dr. Marvirstine Briggs-Fisher | USDA, APHIS |

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|---|---|
| Dr. Lori Gustafson | USDA, APHIS |
| Dr. Christine Ellis | USDA, APHIS |
| Dr. Tim Leeds | USDA, ARS |
| Dr. Gary Richards | USDA, ARS |
| Dr. Michael Deshotel | USDA, ARS |
| Dr. Brett Dumbauld | USDA, ARS |
| Dr. Dina Proestou | USDA, ARS |
| Dr. Neil Thompson | USDA, ARS |
| Dr. Benjamin LaFrentz | USDA, ARS |
| Dr. Ben Beck | USDA, ARS |
| Dr. Timothy Sullivan | USDA, National Institute of Food and Agriculture |
| Dr. Jennifer Matysczak | FDA, Center for Veterinary Medicine |
| Dr. Paul Wills | Florida Atlantic University, Harbor Branch |

NOAA Fisheries: National Oceanic and Atmospheric Administration, National Marine Fisheries Service
NWFSC: Northwest Fisheries Science Center
NEFSC: Northeast Fisheries Science Center
USDA: United States Department of Agriculture
ARS: Agricultural Research Service
APHIS: Animal and Plant Health Inspection Service

**JAPAN:**

| **Name** | **Affiliation** |
|---|---|
| **Panel** | |
| Dr. Aono Hideaki | FRA, FTI |
| Dr. Gen Kouichiro | FRA, FTI, Planning and Coordination Department |
| Dr. Ozaki Akiyuki | Ministry of Agriculture, Forestry and Fisheries, Fisheries Agency |
| Dr. Takano Masatsugu | FRA Headquarters, General Planning and Coordination Department |
| Dr. Oikawa Hiroshi | FRA, FTI, Planning and Coordination Department |
| Arai Daisuke | FRA, FTI, Planning and Coordination Department |
| Dr. Yamano Keisuke | FRA, FTI, Aquaculture Research Department |
| Dr. Nakayasu Chihaya | FRA, FTI, Aquaculture Research Department |
| Dr. Okumua Takuji | FRA, FTI, Aquaculture Research Department |
| Saito Yoho | FRA Headquarters, General Planning and Coordination Department |
| Dr. Kamaishi Takashi | FRA, FTI, Aquaculture Research Department |
| Dr. Ito Takafumi | FRA, FTI, Aquaculture Research Department |
| Dr. Miwa Satoshi | FRA, FTI, Aquaculture Research Department |
| Ishihi Yuka | FRA, FTI, Management Department |
| Dr. Shibuno Takuro | FRA, FTI, Management Department |
| **Speakers** | |
| Dr. Kawato Yasuhiko | FRA, FTI, Aquaculture Research Department |
| Dr. Matsuura Yuta | FRA, FTI, Aquaculture Research Department |
| Dr. Shimahara Yoshiko | FRA, FTI, Aquaculture Research Department |

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| Dr. Yasumoto Shinya | FRA, NFU |
| Dr. Takano Tomokazu | FRA, FTI, Aquaculture Research Department |
| **Invited to Attend** | |
| Dr. Urawa Shigehiko | FRA, FRI, Salmon Research Department |
| Dr. Sakai Takamitsu | FRA, FTI, Planning and Coordination Department |
| Dr. Ishida Noriko | FRA, FTI, Environment and Fisheries Applied Techniques Research Department |
| Dr. Matsuyama Tomomasa | FRA, FTI, Aquaculture Research Department |
| Yoshino Tomoaki | FRA, FTI, Aquaculture Research Department |
| Dr. Sato Jun | FRA, FTI, Aquaculture Research Department |
| Dr. Maeda Tomoki | FRA, FTI, Aquaculture Research Department |
| Nakagawa Yukihiro | FRA, FTI, Aquaculture Research Department |
| Dr. Umeda Kousuke | FRA, FTI, Aquaculture Research Department |
| Dr. Kiryu Ikunari | FRA, FTI, Aquaculture Research Department |
| Dr. Yuasa Kei | FRA, FTI, Aquaculture Research Department |
| Dr. Kurobe Tomofumi | FRA, FTI, Aquaculture Research Department |
| Dr. Mekata Toru | FRA, FTI, Aquaculture Research Department |
| Dr. Inada Mari | FRA, FTI, Aquaculture Research Department |
| Dr. Yoshinaga Tomoyoshi | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Dr. Itoh Naoki | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Dr. Karakawa Nanae | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Kawahara Miku | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Kuboyama Azusa | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Vince Neil Fuertes | Graduate School of Agricultural and Life Sciences, The University of Tokyo |
| Dr. Sano Motohiko | Tokyo University of Marine Science and Technology |
| Dr. Hirono Ikuo | Tokyo University of Marine Science and Technology |
| Dr. Kondo Hidehiro | Tokyo University of Marine Science and Technology |
| Dr. Kato Goshi | Tokyo University of Marine Science and Technology |
| Dr. Matsumoto Moe | Tokyo University of Marine Science and Technology |
| Lah Lik Ming | Tokyo University of Marine Science and Technology |
| Dr. Shirakashi Sho | Aquaculture Research Institute, Kindai University |
| Dr. Matsuo Kazutoshi | Animal Products Safety Division, Ministry of Agriculture, Forestry and Fisheries |
| Daikai Takateru | Food Safety Policy Division, Ministry of Agriculture, Forestry and Fisheries |
| Ota Chiharu | Food Safety Policy Division, Ministry of Agriculture, Forestry and Fisheries |
| Nobuhisa Tsuchiya | International Research Collaboration Division, Agriculture, Forestry and Fisheries Research Council Secretariat |
| Asato Masaki | Fishery and Ocean Technology Center, Okinawa Prefecture |
| Omine Risako | Fishery and Ocean Technology Center, Okinawa Prefecture |

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| Mizutani Kouta | Ibaraki Prefectural Fisheries Research Institute |

FRA: Japan Fisheries Research and Education Agency
FTI: Fisheries Technology Institute
NFU: National Fisheries University
FRI: Fisheries Resource Institute

## Abstracts and Annotated Bibliographies

### Abstract Index

# Isolation and Characterization of Hirame Aquareovirus (HAqRV): A New Aquareovirus Isolated From Diseased Hirame *Paralichthys olivaceus*.

Yasuhiko Kawato*[1], Toru Mekata[1], Toyohiro Nishioka[2], Ikunari Kiryu[1], Takamitsu Sakai[3], Tomoki Maeda[4], Satoshi Miwa[4], Kanae Koike[5], Masahiro Sadakane[6], Koh-ichiro Mori[3]

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Hirame or Japanese flounder (*Paralichthys olivaceus*) is an important flatfish for aquaculture in Japan. As a part of stock enhancement effort, large numbers of hirame juveniles are released annually by public corporations, and hence, a total of over 20 million juveniles are produced every year in more than 100 hatcheries in Japan. Mass mortalities of hirame larvae or juveniles due to reovirus-like infection have been reported since the 2000s. Currently no effective protection measures against the disease have been established because the causative agent has not been identified so far. The goal of present study is to identify the virus causing the mass mortality in hirame juveniles by Koch's postulates. We successfully isolated a novel *Aquareovirus* (hirame aquareovirus: HAqRV) from diseased fish using hirame natural embryo (HINAE) cell line. A cytopathic effect characterized by aggregation of the virus-infected cells was observed 20 days after virus inoculation. In electron microscopy, the spherical virion (75 nm in diameter) with multi-layered capsid structure was observed. The viral genome consisted of 11 segments, and regions encoding 7 virion structural proteins (VP1 - VP7) and 5 non-structural proteins (NS1 - NS5) were predicted. The deduced amino acid sequences of those proteins were highly similar to those of the viruses of genus *Aquareovirus*. The terminal sequences (5'-GUUUUA and UCAUC-3') of each segment were conserved among the 11 segments and they were identical to those of *Aquareovirus A* and *G*. However, the similarities of the complete genome sequence between the HAqRV and other aquareoviruses were less than 60% at nucleotide level. Phylogenetic analyses based on the deduced amino acid sequences of VP2 (RNA dependent RNA polymerase), VP7 (outer capsid protein), and NS5 (fusion associated transmembrane protein) suggest that the HAqRV is not classified into the known species of *Aquareovirus*. Pathogenicity of HAqRV was clearly demonstrated in accordance with Koch's postulates by by experimental challenge tests using hirame juveniles at 20 days post hatching. HAqRV had specific tissue tropism to the epithelium of the digestive tract including hepatocytes and caused syncytia in the infected tissues. The results suggest that the HAqRV is likely a new *Aquareovirus* species which is highly virulent for the hirame at early life stages. We believe that the data from this study will significantly contribute to develop protection measures against HAqRV, which is a big threat to hirame hatcheries in Japan.

For example, qPCR assay developed in this study can be used for rapid diagnosis and investigating the route of infection. The cell culture system can be used for validating the disinfectant conditions. This project is ongoing, and we have been working hard to achieve a goal of development of the protection measures against the disease and improve hirame production in Japan.

**Annotated Bibliography of Key Works**

Isshiki, T., Nagano, T., Abe, M., Miyazaki, T. 2003. Histopathological changes probably associated with a virus in larval and juvenile marbled sole *Pleuronectes yokohamae*. Fish. Pathol., 38:143–149.

The seed production of marbled sole *Pleuronectes yokohamae* has been attempted at a hatchery in Kagawa Prefecture, Japan since 1989, while a subacute disease with a considerable amount of mortality occurred in larval and juvenile fish (24-69 days old). The evident histopathological features were the formation of syncytia and necrosis in the liver, renal tubules, pancreas, and intestinal epithelium. Electron microscopy of affected hepatocytes revealed formation of inclusion bodies consisting of crystalline arrays of virus particles (75-80 nm in diameter) which morphologically resemble reovirus. Although aquareovirus infection was suspected in the reported cases, the causative agent could not be identified as virus isolation failed.

Blindheim, S., Nylund, A., Watanabe, K., Plarre, H., Erstad, B., Nylund, S. 2015. A new aquareovirus causing high mortality in farmed Atlantic halibut fry in Norway. *Arch. Virol.*, 160:91–102.

A new aquareovirus (Atlantic halibut reovirus: AHRV) was isolated from cultured Atlantic halibut *Hippoglossus hippoglossus* fry at a facility in Norway where massive mortalities had occurred during the start-feeding phase. The virus replicated in BF-2 and CHSE-214 cell cultures and produced syncytia and plaque-like cytopathic effects. AHRV was associated with necrosis of the liver and pancreas, and syncytium formation in these tissues in halibut fry. Transmission electron microscopy revealed that the viroplasm contained virions, non-enveloped, icosahedral particles approximately 70 nm in diameter with a double capsid layer, amorphous material, and tubular structures. The RNA-dependent RNA polymerase (RdRp) gene from the AHRV isolates showed the highest amino acid sequence identity (80 %) to an isolate belonging to the species *Aquareovirus A*. A similar disease of halibut fry has also been described in halibut production facilities in Canada and Scotland.

Kawato, Y., Mekata, T., Nishioka, T., Kiryu, I., Sakai, T., Maeda, T., Miwa, S., Koike, K., Sadakane, M., Mori, KI. 2021. Isolation and characterization of hirame aquareovirus (HAqRV): A new Aquareovirus isolated from diseased hirame *Paralichthys olivaceus*. Virology, 559:120–130.

We isolated a novel *Aquareovirus* (hirame aquareovirus: HAqRV) from hirame or Japanese flounder *Paralichthys olivaceus* suffering from reovirus-like infection. Complete genome sequence of the isolated virus suggested that HAqRV is likely a new *Aquareovirus* species. Pathogenicity of HAqRV was clearly demonstrated in accordance with Koch's postulates by experimental infection using hirame juveniles. The present study would contribute to develop protection measures against the infection with HAqRV, which is a big threat to the hatchery production of Japanese flounder in Japan.

# Weissellosis: An Emerging Disease of Farmed Rainbow Trout

Timothy J. Welch

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Weisselosis, caused by the gram-positive lactic acid bacterium *Weissela ceti*, is an emerging infectious disease of farmed rainbow trout. This disease was first recognized in China in 2007 but was soon after found in Brazil in 2008, in the United States and Japan both in 2011, and more recently in Mexico in 2015, Colombia in 2016, Canada in 2017, and Peru in 2018. This disease is of major concern as losses can be as high as 40-80% of production and thus results in significant loss of animals with coinciding economic impact. Genome sequence analysis of *W. ceti* strains from the US, Brazilian, and Japanese outbreaks revealed striking genetic similarity between strains from these geographically diverse sites and suggests emergence from a common source. This is surprising given the geographical distance between these sites and a lack of obvious epidemiological links between farms and countries. In contrast, the Canadian outbreak strain is distinct from the US, Japanese and Brazilian isolates and suggests that this strain represents an independent emergence of this pathogen. Injection vaccination with formalin-killed whole-cell preparations of *W. ceti* provides excellent protection against experimental challenge when tested under laboratory conditions. Recent work has also demonstrated that vaccination induces a robust IgM antibody response and that serum factors provide protection against experimental challenge, suggesting a role for specific IgM in vaccine-induced protection. In practice, vaccination has been highly successful in controlling this disease in both North Carolina and Canada. Gaining a better understanding of the origins of this pathogen and the epidemiological pathways that have facilitated its rapid dissemination to highly disparate locations will be critical for controlling its further spread.

## Annotated Bibliography of Key Works

Welch, TJ, Good, CM. Mortality associated with Weissellosis (Weissella sp.) in USA farmed rainbow trout: Potential for control by vaccination. Aquaculture. 388-391:122-127.

This was the first report of this emerging pathogen in North Carolina, USA. This paper also reported the development and validation of an efficacious vaccine against *W. ceti*. This vaccine was additionally shown to be effective when mixed with another vaccine used in North Carolina thus demonstrating that these vaccines can be delivered together minimizing the increased production costs and negative effects caused by multiple injections. Vaccination has been used to control *W. ceti* in North Carolina since 2012. Early detection of this pathogen and rapid development and implementation of this vaccine aided in control efforts and reduced the likelihood of further pathogen dissemination in the US.

Figueiredo HC, Soares SC, Pereira FL, Dorella FA, Carvalho AF, Teixeira JP, Azevedo VA, Leal CA. Comparative genome analysis of *Weissella ceti,* an emerging pathogen of farm-raised rainbow trout. BMC Genomics. 2015.

This paper presents whole genome comparisons between Brazilian and US strains of *W. ceti* and was first to demonstrate the remarkable similarity in the genetic structure of these *W. ceti* strains despite their lack of epidemiological linkage. Several genomic islands and putative virulence genes were also identified including adhesins, hemolysins, and antibiotic resistance genes. This work provided important insight into the pathogenesis, evolution, and epidemiology of this emerging pathogen.

Snyder AK, Hinshaw JM, Welch TJ. Diagnostic tools for rapid detection and quantification of Weissella ceti NC36 infections in rainbow trout. Lett Appl Microbiol. 2015 Feb; 60(2):103-110. This paper describes the development and validation of rapid and quantitative PCR-based assays for the identification of *W. ceti* from diseased fish and other environmental samples. The assays were validated in a controlled experimental challenge and in confirming the diagnosis of Weissellosis in a commercial rainbow trout farm during an outbreak investigation. The assays allow rapid Weissellosis diagnosis, enhanced pathogen surveillance for epidemiological studies.

Mitomi K, Hoai TD, Nishiki I, Yoshida T. First isolation of *Weissella ceti* responsible for outbreaks of weissellosis in farmed rainbow trout in Japan. J Fish Dis. 2018 May; 41(5):847-850. These authors describe the identification of *W. ceti* in cultured trout in Japan. It was revealed that Japanese outbreaks coincided with outbreaks occurring in the US and that the strains were phenotypically very similar to US strains.

## Development of a Method to Quantify Endogenous IFNγ Protein in Amberjack Species

Yuta Matsuura*, Tomokazu Takano, Tomomasa Matsuyama

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Interferon gamma (IFNγ) is a pivotal cytokine that promotes and orchestrates adaptive cell-mediated immunity against intracellular pathogens. The capacity of T lymphocytes in mammals to produce IFNγ has been measured using specific antibodies to analyze cell-mediated immune responses against infection or immuno-stimulants such as vaccines. However, measurement of fish IFNγ protein has not been possible due to a lack of research tools. The development of vaccines for intracellular fish pathogens that are thought to be targeted by cell-mediated immunity has been a difficult task in such a situation. Serious economic losses due to Nocardiosis caused by an intracellular bacterium "*Nocardia seriolae*" have occurred in the aquaculture of amberjack species in Japan. The method to quantify IFNγ of amberjack species is required to develop vaccines for Nocardiosis.

To develop the method for quantification of IFNγ, we firstly generated antibodies that react with endogenous amberjack IFNγ. A monoclonal antibody (mAb) and a polyclonal antibody (pAb) that specifically react with endogenous IFNγ produced by Japanese amberjack *Seriola quinqueradiata* and greater amberjack *Seriola dumerili* were established. We then constructed a sandwich enzyme-linked immunosorbent assay (ELISA) for the IFNγ of the species using the antibodies. The sandwich ELISA could detect the endogenous IFNγ at concentrations less than 100 pg/mL in leukocytes culture supernatant without cross-reactivity with another cytokine (IL-4/13). Using the ELISA, IFNγ produced by Japanese amberjack leukocytes were analyzed. The level of IFNγ secreted by stimulated leukocytes isolated from *N. seriolae*-infected fish was significantly higher than that secreted by cells of naïve fish ($p<0.05$) and was lasted a long time (at least eight months) following the infection. A portion of the fish that survived for eight months after infection were re-challenged with *N. seriolae*, and all re-challenged fish survived, whereas 100% of control fish died of the infection. Thus, the fish that had recovered from *N. seriolae* infection were considered to have acquired cell-mediated immunity to the organism.

In conclusion, we established a new sandwich ELISA method that enables for the first time the quantification of IFNγ at the protein level in teleost. Our results obtained using the method suggest that the developed method would help evaluate the capacity of cell-mediated immunity to respond against intracellular pathogen infection. Assessment of IFNγ expression at the protein rather than genetic level could provide information that would enhance understanding of the immunologic process and contribute to vaccine development.

### Annotated Bibliography of Key Works

Matsuura, Y., Takano, T., Matsuyama, T., Sakai, T., Terashima, S., Nakayasu, C. 2020. Development of a method to quantify endogenous IFNγ protein in amberjack species. Fish Shellfish Immunol., 107 (A):251-259.

The authors established the method to quantify a cytokine IFNγ, an important marker of cell-mediated immune responses in fish for the first time. The established method could contribute to the vaccine development against Nocardiosis that has caused serious damages in amberjack farming in Japan.

Matsuura, Y., Takasaki, M., Miyazawa, R., Nakanishi, T. 2017. Stimulatory effects of heat-killed *Enterococcus faecalis* on cell-mediated immunity in fish. Dev. Comp. Immunol., 74:1-9.
The paper reported that heat-killed lactic acid bacteria *Enterococcus faecalis* enhanced cell-mediated immunity in fish. The heat-killed bacteria triggered the induction of Th1 responses and increases in $CD4^+$ Th lymphocytes and effector cells including $CD8^+$ lymphocytes and macrophages. Thus, the heat-killed one is a promising vaccine adjuvant to induce cell-mediated immunity in fish and could contribute to the studies of vaccines against Nocardiosis.

Matsumoto, M., Araki, K., Hayashi, K., Takeuchi, K., Shiozaki, K., Suetake, H., Yamamoto M. 2017. Adjuvant effect of recombinant interleukin-12 in the Nocardiosis formalin-killed vaccine of the amberjack *Seriola dumerili*. Fish Shellfish Immunol., 67:263-269.
The authors succeeded production of the recombinant interleukin-12 cytokine that is an inducer of cell-mediated immunity. Furthermore, they demonstrated that the cytokine had an adjuvant effect against Nocardiosis. The work could contribute to the development of vaccines against Nocardiosis.

# Use of a *Flavobacterium columnare* DnaK Recombinant Protein Vaccine to Guard Against Columnaris Disease in Channel Catfish

Miles D. Lange

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The incidence of different bacterial diseases and their ongoing contribution to on-farm losses including the cost of therapeutants during the production cycle persists among the U.S. catfish industry accounting for as much $17 million in overall annual lost revenue. *Flavobacterium columnare* causes substantial losses among cultured finfish species. The Gram-negative bacterium is an opportunistic pathogen that manifests as biofilms on the host's mucosal surfaces as the disease progresses. We previously established that the dominant mucosal IgM antibody response to *F. columnare* is to the DnaK protein found in the extracellular fraction (Lange et al. 2016). Recent advances in recombinant protein technology have made the production and subsequent testing of individual immunogens quite effective. To establish the efficacy of using recombinant protein technology to develop a new vaccine against columnaris disease, we are reporting on several vaccine trials using a recombinant *F. columnare* DnaK protein (rDnaK). In the first study, three groups of channel catfish were immunized by bath immersion with a live attenuated *F. columnare* isolate, rDnaK or sham immunized. After six weeks, an *F. columnare* laboratory challenge showed a significant increase in survival in both the live attenuated and rDnaK vaccines when compared to the non-immunized control. A rDnaK-specific ELISA revealed significant levels of mucosal IgM antibodies present in the skin of catfish immunized with rDnaK at four- and six-weeks post immunization. In the second study, three groups of channel catfish were bath immunized with rDnaK alone or with rDnaK after a brief osmotic shock or sham immunized. In study two, after six weeks a laboratory challenge with *F. columnare* was conducted and showed a significant increase in survival in the rDnaK and in rDnaK with osmotic shock when compared to the non-immunized control. The rDnaK ELISA demonstrated significant levels of mucosal IgM antibodies in the skin of catfish groups immunized with rDnaK at six weeks post immunization. To further understand the processes which have conferred immune protection in the rDnaK group, we conducted RNA sequencing of skin samples from the non-immunized and rDnaK treated channel catfish at one-week and six weeks post immunization. Differential gene expression was identified between the non-immunized and immunized skin and gene ontology analyses (Lange et al. 2019). We next confirmed and extended these findings by evaluating the efficacy of the recombinant *F. columnare* DnaK protein vaccine when using different immersion adjuvant strategies. The results demonstrate significant protection of channel catfish at after 6 to 8 weeks post vaccination (Lange et al. 2021). Experimental methods to enhance the catfish immune response to recombinant *F. columnare* DnaK protein under different strategies continues as this vaccine remains a promising candidate for experimental trials in a production setting.

Funding: USDA Research Projects #6010-32000-027-00-D and #6028-32000-007-00-D.

**Annotated Bibliography of Key Works**

Lange, M.D., B.H. Beck, J.D. Brown, B.D. Farmer, L.M. Barnett, and C.D. Webster. 2016.

Missing the target: DNAk is a dominant epitope in the humoral immune response of channel catfish (*Ictalurus punctatus*) to *Flavobacterium columnare*: Fish and Shellfish Immunology. 51:170-179.

This paper was the first to demonstrate that an *F. columnare* 70 kDa extracellular protein, identified as a DnaK chaperonin, is the dominant antigen during a mucosal IgM response to infection. This study also demonstrated that mucosal catfish IgM antibodies generated after *F. columnare* infection are cross reactive to DnaK proteins identified from other Gram-negative bacteria.

Lange, M.D., J. Abernathy and B.D. Farmer. 2019. Evaluation of a recombinant *Flavobacterium columnare* DnaK protein vaccine as a means of protection against columnaris disease in channel catfish (*Ictalurus punctatus*). Frontiers in Immunology. 10:1175 https://doi.org/10.3389/fimmu.2019.01175

This paper was the first to test the efficacy of a new *F. columnare* recombinant protein vaccine and demonstrated that protection could be conferred after immunization with a single antigen. The study also identified adaptive immunity processes using high-throughput RNA sequencing through which protection was likely achieved in the skin post-immunization.

Lange, M.D., J. Abernathy, B.D. Farmer and B.H. Beck. 2021. Use of an immersion adjuvant with a *Flavobacterium columnare* recombinant protein vaccine in channel catfish. Fish and Shellfish Immunology. 117: 136-139.

This paper demonstrated the effectiveness of using a bath immersion formulated adjuvant to reduce the amount of immunization time required to confer protection against *F. columnare*.

## α-glucosidase Activity of *Nocardia seriolae* Isolated From Seawater Fish in Japan

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Nocardiosis caused by a bacterial pathogen *Nocardia seriolae* has been confirmed in a variety of marine fishes, including yellowtail *Seriola quinqueradiata*, amberjack *S. dumerili,* Japanese flounder *Paralichthys olivaceus*, mackerel *Scomber japonicus*, bluefin tuna *Thunnus orientalis* in Japan. *N. seriolae* is divided into two types based on the presence or absence of α-glucosidase activity (α-glu), a carbohydrate-hydrolase, and strains isolated in Japan between 2000 and 2005 were α-glu negative except for some strains from amberjack (Shimahara et al., 2008). In this study, α-glu activity, DNA polymorphism, and pathogenicity of *N. seriolae* strains isolated from 2006 to 2014 in Japan were determined. Of the 103 newly collected s trains isolated from yellowtail, amberjack, and Japanese flounder, 26 strains (25%) were positive for α-glu, which was a significant increase from the surveillance in 2000-2005 (6%). Many of the α-glu positive strains were isolated from amberjack cultured in southern Kyushu, but also confirmed in yellowtail and Japanese flounder raised in the same area. DNA polymorphism analysis by pulsed-field gel electrophoresis (PFGE) showed that the α-glu positive and negative strains were divided into 8 (type 1-8) and 9 pulsotypes (type 9-17), respectively. In experimental infection, mortality of amberjack challenged with 3 α-glu positive strains were 25%, 88%, and 100%, while those challenged with 3 α-glu negative strains were and 0%, 0% and 25%. Although outbreaks of nocardiosis with α-glu positive strains were confirmed only in southern Kyusyu, it is possible that α-glu positive strains, which shows high pathogenicity to amberjack, expand to many areas in Japan in future.

### Annotated Bibliography of Key Work

Shimahara, Y., Nakamura, A., Nomoto, R., Itami, T., Yoshida, T. 2008. Genetic and phenotypic comparison of *Nocardia seriolae* isolated from fish in Japan. J. Fish Diseases, 31:481-488.

The authors investigated phenotypic and genetic characterizations of fish pathogen determined by α-glucosidase activity and biased sinusoidal field gel electrophoresis (BSFGE) analysis, respectively. There was no α-glucosidase activity in strains isolated from 2000–05 (n=50) with a few exceptions (n =3), while all strains isolated from 1970–90 (n=8) were positive. In BSFGE analysis, all restriction patterns obtained from 50 strains isolated during 2000–05 were unrelated to those obtained from eight strains isolated during 1970–90, with the exception of two strains isolated during recent outbreaks. Based on the phenotypic and genetic characterizations, recent outbreaks of nocardiosis in Japan are suggested to be epidemiologically unrelated to earlier outbreaks in Japan.

## Emergent Pathogens in Aquaculture: How Should We Identify Them?

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Identification of the causes of infectious disease has relied on demonstrating a relationship that exceeds simple correlation. The Henle-Koch's postulates have served as guidelines for establishing causation, but over time, they have needed augmentation as awareness of etiologic agents expanded to viruses, polymicrobial infections, and evolving technologies (Rivers, 1937, Huebner, 1957, Evans, 1976, Fredricks and Relman, 1996).

There are several case studies demonstrating the complexities in establishing causation of disease in aquatic organisms. One example is heart and skeletal muscle inflammation (HSMI) of Atlantic salmon, a disease linked to a novel piscine orthoreovirus (PRV) through deep sequencing of affected tissues (Palacios *et al.,*, 2010). However, molecular genetic screening reveals the presence of PRV sequences in animals exhibiting no signs of HSMI (Garseth *et al.,*, 2013, Purcell *et al.,*, 2020). Another example is the difference in virulence in infectious salmon anemia virus (ISAV) strains (McBeath *et al.,*, 2014), including possible strain evolution genetically and in pathogenicity (Christiansen *et al.,*, 2017, Markussen *et al.,*, 2008).

Broad spectrum screening using high throughput sequencing technologies further complicates identification of true pathogens and can generate misconceptions about the prevalence of pathogens (Mordecai *et al.,*, 2019). For aquatic animal health researchers and practitioners as well as reviewers of scientific publications, it is important to establish clear criteria for pathogen identification for both scientific accuracy and correct public understanding.

**Annotated Bibliography of Key Works**

Christiansen DH, McBeath AJA, Aamelfot M, Matejusova I, Fourrier M, White P, Petersen PE, Falk K. 2017. First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus. J Gen Virol 98: 595-606.

This publication reports on the first evidence that the avirulent HPR0 strain of ISAV could evolve into a virulent phenotype under cultured fish conditions. Furthermore, the emergent subtype possessed genetic changes that were consistent with virulence in other strains of ISAV. The work provided the first support for HPR0 as a reservoir for virulent strains of ISAV.

Evans AS. 1976. Causation and disease: The Henle-Koch postulates revisited. Yale J Biol Med 49: 175-195.

This contains a nice historical timeline of the evolution of the Henle-Koch's postulates. It includes an acknowledgment that new technologies will detect microorganisms that are not connected to a disease.

Fredricks DN, Relman DA. 1996. Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. Clin Microbiol Rev 9: 18-33.
An early but still highly relevant review of the role of sequence analysis in identifying microbial pathogens. One of the authors, David Relman, is a leader in the human microbiome initiative in the U.S., and he continues cutting edge research in this area.

Garseth AH, Fritsvold C, Opheim M, Skjerve E, Biering E. 2013. Piscine reovirus (PRV) in wild Atlantic salmon, *Salmo salar* L., and sea-trout, *Salmo trutta* L., in Norway. J Fish Dis 36: 483-493.
This publications reports on surveillance for PRV in wild-caught salmon, trout, and char in Norway. It uncovered relatively high prevalences (13 - 55%) of PRV with nearly no evidence of disease or inflammation.

Huebner RJ. 1957. Criteria for etiologic association of prevalent viruses with prevalent diseases; the virologist's dilemma. Ann N Y Acad Sci 67: 430-438.
In this publication, Huebner suggested that an epidemiological aspect could be added as a tool in identifying and validating an etiologic agent.

Markussen T, Jonassen CM, Numanovic S, Braaen S, Hjortaas M, Nilsen H, Mjaaland S. 2008. Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus. Virology 374: 515-527.
This paper reports a detailed full genome analysis of the avirulent strain of ISAV, HPR0. It provides genetic characterization of a virulence determinant by identifying a marker site that has been pivotal in studying ISAV virulence.

McBeath AJ, Ho YM, Aamelfot M, Hall M, Christiansen DH, Markussen T, Falk K, Matejusova I. 2014. Low virulent infectious salmon anaemia virus (ISAV) replicates and initiates the immune response earlier than a highly virulent virus in Atlantic salmon gills. Vet Res 45: 83.
The publication reports on salmon immune responses to experimental exposure to strains of ISAV with differential virulence. Low virulence strains replicated and disseminated more quickly, stimulating a more rapid host response. In contrast, high virulence strains replicated less efficiently, and the less effective host response ultimately allowed a higher viral load and greater mortality. The study provided a mechanistic explanation for differences in pathogenicity.

Mordecai GJ, Miller KM, Di Cicco E, Schulze AD, Kaukinen KH, Ming TJ, Li S, Tabata A, Teffer A, Patterson DA et al. 2019. Endangered wild salmon infected by newly discovered viruses. Elife 8:347615.
This publications reports on a viral discovery screening of moribund and dead hatchery and wild salmon. Many viral sequences were identified, including arenaviruses, nidoviruses, and 3

reoviruses. The publication implied these represented widespread sources of viral diseases, but in the absence of evidence of disease being present in the sampled fish. The publication generated considerable public concern about fish viruses and the role of aquaculture in amplifying and spreading fish viruses.

Palacios G, Lovoll M, Tengs T, Hornig M, Hutchison S, Hui J, Kongtorp RT, Savji N, Bussetti AV, Solovyov A et al. 2010. Heart and skeletal muscle inflammation of farmed salmon is associated with infection with a novel reovirus. PLoS One 5: e11487.
The first study to credibly inplicate PRV as a causative agent in HSMI, based on genetic sequencing and bioinformatics.

Purcell MK, Powers RL, Taksdal T, McKenney D, Conway CM, Elliott DG, Polinski M, Garver K, Winton J. 2020. Consequences of piscine orthoreovirus genotype 1 (PRV-1) infections in chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*) and rainbow trout (*O. mykiss*). J Fish Dis 43: 719-728.
This publication reports on experimental challenges of Pacific salmon with the PRV genotype that causes HSMI in Atlantic salmon. The outcomes demonstrated that although PRV-1 could establish in Pacific salmon, there was no establishment of disease.

Rivers TM. 1937. Viruses and Koch's postulates. J Bacteriol 33: 1-12.
As president of the Society of American Bacteriologists, Thomas Rivers recognized that viral etiology could not be demonstrated by strictly following Koch's postulates, especially when co-infections occurred or in carrier states. He proposed that a appropriate timing of the appearance of the putative agent and that a host response (i.e., antibodies) could be incorporated when investigating causal relationships.

## The Role of National Fisheries University and Studies of Fish Disease

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Two topics have been included in this presentation. The first topic is introduction of National fisheries university (NFU) and second one is my research activities in NFU.

NFU has 80 years of history. Ministry of Agriculture, Forestry and Fisheries has jurisdiction over NFU. Therefore, NFU has different policies (diploma, curriculum, and admissions policy) from other universities which are under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology. NFU consists of four departments, advanced course, and graduate school, and is a school that educates students to be the industry's future leaders. NFU contributes to the fisheries industry by producing many talents to the fisheries related company, government office and academic.

In our laboratory, diseases of ornamental fish were studied mainly, and we are focusing on koi carp (Nishikigoi; *Cyprinus carpio*) among them. Koi carp is internationally popular as an ornamental fish, and the industry is unique. Product value is influenced by a number of factors, including size, shape, color, and variety, and has recently been increasing. Many types of diseases, including Koi herpesvirus (CyHV-3), have been reported in koi carp, and idiopathic diseases are known among farmers and hobbyists.

Recently, we are studying about "Sinking disease" (swim bladder disorders) which has become a large issue in the field of koi carp breeding. This disease was an idiopathic disease. Diseased fish sink, become immobile, and stay at the bottom of a pond or tank with lethargy, but have no external signs. Therefore, we herein examined the gross and histological features of diseased fish from farms. At necropsy, their swim bladder was filled with liquid (swim bladder fluid, SBF). Histopathologically, fish had hyperplastic connective tissue in the tunica intima of the swim bladder, and some were accompanied by inflammation. Furthermore, some species of bacteria were isolated from SBF samples of diseased fish. To reproduce the disease, experimental infections using the three major isolates (*Streptococcus* sp., *Pseudomonas aeruginosa*, and *Mycobacterium* sp.) were also attempted. Immobility and the accumulation of SBF occurred in the live bacteria-injected groups. Based on these results, we concluded that the major cause of sinking disease is the accumulation of SBF due to bacterial invasion in the swim bladder and, as a result, a loss of buoyancy. Future studies are needed to elucidate the mechanisms underlying the accumulation of SBF and develop measures to prevent and treat sinking disease in koi carp.

### Annotated Bibliography of Key Words

Yasumoto, S. 2018. Remedy. In "Nishikigoi A to Z" (ed. by S. Mano). Shin Nippon Kyoiku Tosho, Shimonoseki, pp 128-158.

This is the textbook of koi carp breeding. We introduced more than 30 kinds of diseases (non-communicable, viral, bacterial, fungal, and parasitic disease) and explained those diagnostic and treatment methods. This textbook is red by farmers and hobbyists and used in our lectures for NFU students.

Yasumoto, S., Okada, M., Moroi, H., Shimizu, H., Kondo, M. 2021. A Preliminary Study on Sinking Disease in Koi Carp. Fish. Pathol., 56(3):107-114.

Sinking disease has become a major issue in the field of koi carp breeding. Although many diseased fish did not exhibit any significant changes in their appearance, some had ulcers and erosions on the abdomen and at the base of the fins due to physical scratching caused by being at the bottom of a pond for a prolonged period of time. At necropsy, the swim bladder of the fish was filled with fluid (swim bladder fluid, SBF) and very little gas. Histopathologically, fish had hyperplastic connective tissue in the tunica intima of the swim bladder, and some were accompanied by inflammation. Furthermore, one to three species of bacteria were isolated from six out of fourteen SBF samples of diseased fish. To reproduce this disease, two experiments were performed using the three major isolates (live or formalin-killed bacteria) from SBF as well as SBF itself. Immobility and the accumulation of SBF occurred in the live bacteria-injected groups. Based on these results, we concluded that the major cause of sinking disease is the accumulation of SBF due to bacterial invasion in the swim bladder and, as a result, a loss of buoyancy.

# Aquaculture Medicine in the 21st Century: Training the Veterinary Community to Meet the Needs of a Global Industry

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Globally, aquaculture is rapidly growing, diverse, and increasingly important as a source of protein and maintaining food security. In the United States, aquaculture was valued at $1.5 B in 2018, with the most valuable products produced being shellfish (oysters and clams) and Atlantic salmon (NOAA Fisheries 2021). The loss of livestock by disease is a major threat to production, consequently there is a significant need for well-trained veterinarians and aquatic animal health specialists. We will describe how we have addressed this need by providing elective training for veterinary students and graduate veterinarians.

Florida currently ranks 9th in aquaculture production by US states, with annual farm gate value of approximately $72M in 2018 (Florida Department of Agriculture and Consumer Services, 2020). Florida aquaculture is very diverse, with the major aquaculture product consisting of hundreds of species and varieties of ornamental fish sold for the pet trade and valued at $28.7M in 2018. Shellfish production is the major aquatic food product, valued at $15.5M in 2018. Other species reared in Florida include food and game fish, alligators and other reptiles, and aquatic plants. The types of production systems used in Florida are also very diverse and include pond, raceways, and recirculating systems as well as aquaponics and off shore "bottom" leases for clams. This diversity creates an exceptional teaching opportunity for veterinarians and veterinary students in aquaculture medicine. Further, Florida has famous theme parks and oceanaria that include large collections of aquatic organisms, and a very engaged state fish and wildlife agency; the Florida Fish and Wildlife Conservation Commission. These entities collectively create an exceptional teaching opportunity for veterinary students, graduate veterinarians, and para-professionals including veterinary technicians and biologists.

In the United States, there are currently 33 colleges of veterinary medicine that are accredited by the American College of Veterinary Medicine. Of these, approximately 10 offer some elective training to students in aquatic animal health. The University of Florida's training programs are globally recognized for excellence in this specialized area. Although some aspect of aquatic medicine has always been offered to students at the University of Florida, specific training in aquaculture was initiated in the 1980s, and enhanced in the 1990s with the development of the UF Tropical Aquaculture Laboratory (UF-TAL) that included a full-service veterinary diagnostic laboratory. In 2000, through a state legislative appropriation for "Training in the care, treatment, and rehabilitation of marine mammals" with an emphasis for species within the state of Florida, especially the Florida manatee (*Trichechus manatus*), the existing program was enhanced to include learning opportunities with species beyond Florida's aquaculture industries. Expanded elective training for students included on-site clinical experiences at UF – TAL and with many of our industry partners, including theme parks and government agencies.

The UF College of Veterinary Medicine (CVM) offers a unique 15-credit hour elective certificate to veterinary students in *Aquatic Animal Medicine*. This is a popular program and has an average of 8 students per year completing the training. This certificate is restricted to UF veterinary students and includes two required courses (Sea Vet and Diseases of Warm Water Fish) and elective on-line course work, clinical externships, and research opportunities. Many students who have earned the certificate have continued on to complete clinical residencies or graduate programs and are emerging as leaders in the aquatic health field. For non-UF students and graduate veterinarians, a new 12-credit hour certificate program entitled *Care and Conservation of Aquatic Animals* has recently been approved.

Clinical training is also available but limited in scope. An annual clinical internship takes one veterinarian each year and provides 12-months of training that includes fish and marine mammal medicine. Advanced training is available through a 3-year residency program accredited by the American College of Zoological Medicine (ACZM) but only one candidate is selected every three years. Since 2004, 6 veterinarians have completed this program and a seventh is currently enrolled. Five of our residents have been board certified by ACZM as specialists in aquatic medicine.

One of the most effective ways to provide post veterinary school training is through our distance education program. The College of Veterinary Medicine and the School of Forest, Fisheries and Geomatic Sciences collaborate and offer comprehensive on-line educational programs for practicing veterinarians and working professionals. The development of strong distance education programs has created significant learning opportunities for veterinarians and para-professionals from around the globe. A fully on-line Master of Fisheries and Aquatic Sciences (MFAS) degree program is very popular with veterinarians seeking additional course work and an academic credential in aquatic science. The MFAS degree requires 32 credits of course work and a technical paper reviewed by a supervisory committee and presented to the public. For veterinarians who are not ready to commit to 32 hours of course work there is a certificate in *Aquaculture and Fish Health.* This program consists of 4 graduate level courses. Required courses are Aquaculture 1 and Diseases of Warm Water Fish, and then the student can select from a menu of courses to complete the program. A veterinarian can complete the Aquaculture and Fish Health certificate and apply the academic credits to the MFAS degree if he/ she wishes to continue.

In 2017 the USDA provided funding for a national fellowship program to train practicing veterinarians so that they could provide veterinary services to aquaculture farms in their community. Eight aquaculture fellows have been trained and all are providing some level of support to aquaculture industries. The fellows benefitted from academic and experiential training in aquaculture production techniques, fish and shellfish disease diagnosis and health management, and training to help them navigate the complex regulatory environment in the United States. All fellows completed the *Aquaculture and Fish Health* certificate mentioned above as well as an on-site practicum offered at the UF-TAL in 2019. These veterinarians are willing and able to work with aquaculture businesses and apply principles that have resulted in improved efficiency and disease prevention in traditional livestock production to aquatic clientele. For maximum benefit, aquaculture producers have to realize the benefits of establishing a veterinary-client-patient relationship with well-trained veterinarians. Simply calling a veterinarian when they need a health certificate signed or a prescription issued will not be sufficient. Regular veterinary involvement in decision-making on aquatic farms will optimize production and profitability while minimizing the loss of livestock.

## Annotated Bibliography of Key Works

Hartman, K., R.P.E. Yanong, C.A. Harms, G.A. Lewbart. 2006. The future of training for aquatic veterinarians. Journal of Veterinary Medical Education 33(3):389-393. https://doi.org//0.3138/jvme.33.3.389
The article provides an overview of employment opportunities and continuing education programs for veterinarians in aquatic animal health.

DeHaven, W.R. and A.D. Scarfe. 2011. Professional education and aquatic animal health: a focus on aquatic veterinarians and veterinary paraprofessionals. Proceedings OIE Global Conference on Aquatic Animal Health, June 28030 2011, Panama, Pp 139-154.
This article provides a global perspective on the need for a “well educated and competent veterinary workforce” to meet the needs of expanding aquaculture production and the threat of livestock loss through disease. It also describes several programs designed to provide supplemental education to practicing veterinarians so they can develop the knowledge base required to do this work.

Sanders, J. 2018. Aquatic ambulatory practice. Vet Clin Exot Anim 21: 609-622. https://doi.org/10.1016/j.cvex.2018.05.003
This article is a description of how a practicing veterinarian can adapt their business to provide service to aquaculture and pet fish owning clients. The emphasis is on practice management and individual animal care.

# Epitope Mapping of Anti-RSIV Monoclonal Antibody Using a Phage Display Library

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Red sea bream iridoviral disease (RSIVD) spreads readily throughout the marine environment because of its wide variety of host species. Use of a rapid diagnostic method, such as the immunofluorescence antibody test (IFAT), helps to control the disease, so the anti-RSIV monoclonal antibody M10 (MAb M10) has been developed. The IFAT test with MAb M10 for tissue smears from the diseased fish is introduced as a simple method in the OIE manual of diagnostic tests for aquatic animals (CHAPTER 2.3.8. RED SEA BREAM IRIDOVIRAL DISEASE). MAb M10 is available from our laboratory, which is part of the activities of the OIE reference laboratory of the RSIVD. However, the antigens recognized by MAb M10 have not been characterized. Therefore, in order to define the specificity of IFAT, it is important to identify the antigen and epitope of this antibody.

In the present study, we carried out epitope mapping using the phage display method to identify the antigen of MAb M10. A phage display RSIV peptide library was constructed to cover the entire genome of RSIV, then a total of 17 phage clones recognized by the antibody were selected by biopanning. The selected clones harbored partial fragments of the laminin-type epidermal growth factor-like domain (LEGFD) gene. N-terminal and C-terminal deletion peptides were then prepared from the deduced amino acid sequence of the smallest fragment obtained from the biopanning to precisely determine the epitope. Finally, seven amino acids, EYDCPEY, located in the extracellular domain of the LEGFD protein were determined to be the epitope. Identical residues of the epitope were also identified from the LEGFD protein in other megalocytiviruses including the infectious spleen and kidney necrosis virus (ISKNV) and turbot reddish body iridovirus (TRBIV). MAb M10 is considered to be widely available for the diagnostics of these megalocytivirus infections.

## Annotated Bibliography of Key Works

Nakajima, K., Sorimachi M. 1995. Fish Pathology, 30(1):47-52.
A hybridoma clone secreting monoclonal antibody (MAb M10) recognizing an RSIV protein was established.

Nakajima, K., Maeno, Y., Fukudome, M., Fukuda, Y., Tanaka, S., Matsuoka, S., Sorimachi, M. 1995. Fish Pathology, 30(2): 115-119.
IFAT with MAb M10 was shown to be a rapid and effective method for diagnosis of the iridovirus infection in cultured marine fishes.

Nakajima, K., Maeno, Y., Yokoyama, K., Kaji, C., Manabe S. 1998. *Fish Pathology*, 33(2):73-78.
No cross-reactivity with other fish iridoviruses (EHNV, SFIV and GIV) was observed in the IFAT and immunoprecipitation using MAb M10.

Takano, T., Matsuyama, T., Kawato, Y., Sakai, T., Kurita, J., Matsuura, Y., Terashima, S., Nakajima, K., Nakayasu C. 2020. Fish Pathology, 54(4):83-92.
Seven amino acids located in the extracellular domain of the LEGFD protein were determined to be the epitope of MAb M10.

# Managing the Threat of Emerging Diseases in Bivalve Mollusc Aquaculture

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The history of diseases impacting, and sometimes devastating, fisheries and aquaculture for marine bivalve molluscs spans more than a century, and the full global geography of these industries. Yet even today, despite advances for example in biotechnology for pathogen detection, we struggle to detect and manage emerging diseases, as exemplified most recently by the emergence and spread over the last decade of the ostreid herpesvirus OsHV-1 microvariants in Europe and other areas. Because of our recognized challenges, and lack of success, with emerging pathogens, the shellfish health community has begun to focus attention on identifying and mitigating our greatest areas of weakness, through work at the regional level in the U.S.A., as well as internationally through the International Council for the Exploration of the Sea (ICES) Working Group on Pathology and Diseases of Marine Organisms (WGPDMO).

Initial work identified three areas of disease management that would appear to be strengths, but which instead may represent structural flaws in our management paradigm. The use of notifiable pathogen lists and advanced molecular diagnostics such as polymerase chain reaction assays (PCR) are central to disease management, but, paradoxically, create blind spots with regard to emerging diseases that are not listed or not targeted by the diagnostics (Carnegie et al. 2016). Uncertainty about pathogen distributions and poorly studied aspects of pathogen biology can create regulatory paralysis that, while perhaps unnecessarily restrictive of reasonable commerce, at least should maintain a high level of biosecurity, with commerce and thus pathogen transfers limited; unless, paradoxically, this has the effect of reducing biosecurity by driving commerce to surreptitious channels.

Subsequent work by the ICES WGPDMO, through a focused Workshop on Emerging Mollusc Pathogens (WKEMOP, ICES 2019), identified thirty-six wide ranging priorities for improvement in regional to national and international aquatic animal health management, in four primary areas: improving communication and frameworks, essential infrastructure and expertise, key research priorities, and the central role of husbandry. Highlights among the thirty-six priorities were a continued relevance of foundational fields such as pathology, bacteriology and virology, as opposed simply to molecular diagnostics; the critical need to maintain laboratory capacity, especially at provincial governmental and academic institutions more deeply connected to local environments and industries than major national laboratories would be; the great importance of communication and information and data sharing at all levels, for example among labs and between regulators, the scientific community, and industry; and the need to better align funding with the value of collecting long-term, continuous data for diseases and environments has value beyond.

This presentation will synthesize work in this area and discuss as well an additional contemporary challenge, our need to better understand and manage the strain-level diversity in aquatic pathogens, differences in virulence or pathogenicity potentially important contributors to outbreak risks.

**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 unique perspective on the troubled response to OsHV-1.

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 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.

Carnegie, R.B., S.E. Ford, R.K. Crockett, P.R. Kingsley-Smith, L.M. Bienlien, L.S.L. Safi, L.A. Whitefleet-Smith, and E.M. Burreson. 2021. A rapid phenotype change in the pathogen *Perkinsus marinus* was associated with a historically significant marine disease emergence in the eastern oyster. Scientific Reports 11: 12872. http://doi.org/10.1038/s41598-021-92379-6
This new paper provides entirely new perspective on the great intensification of oyster parasite *Perkinsus marinus* in the 1980s, revealing it to be caused by emergence of a new, highly virulent parasite phenotype. It highlights the importance of understanding sub-species or strain dynamics of pathogens, which may be more important than we appreciate in driving disease dynamics in fisheries and aquaculture.

Ben-Horin, T., C.A. Burge, D. Bushek, M.L. Groner, D.A. Proestou, L.I. Huey, G. Bidegain, and R.B. Carnegie. 2018. Intensive oyster aquaculture can reduce disease impacts to sympatric wild oysters. Aquaculture Environment Interactions 10: 557-567. http://doi.org/10.3354/aei00290
This recent paper presents counter-intuitive results from disease modeling, that aquaculture may reduce, rather than exacerbate, disease impacts of certain pathogens. The results are relevant to our consideration of how aquaculture "fits" into ecosystems and potentially interacts with fisheries and restoration activities for wild natural populations of resource species.

## Advancing Epidemiological Tools for Marine Disease Management - Modeling Dispersion of OsHV-1μvar in Pacific Oyster Populations

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Ostreid herpesvirus-1 microvariant (OsHV-1 μvar), a significant pathogen of the Pacific oyster in Europe, Australia, and New Zealand, poses a major threat to the US oyster aquaculture industry. Introduction of the virus into naive populations could leave growers vulnerable to a widespread epidemic accompanied by mass mortalities and major financial losses. For OsHV-1μvar and other emerging pathogens, there is a great need for risk assessment to inform prevention and management strategies. Classic statistical  methods and models are regularly employed for this purpose, and offer an opportunity to describe how specific factors influence disease outcomes based on observational or experimental data. For marine diseases, this is sometimes unable to be accomplished due to lack of sufficient data. Perhaps more importantly, these methods may not always capture the complexity of marine disease dynamics, resulting in uncertainty in their application to management practices.  Mathematical simulation models offer a solution to these challenges in understanding and managing aquatic animal diseases. Significant advantages of this approach include the ability to investigate processes where observational data is limited, as well as the ability to incorporate uncertainty into the starting conditions. There have been several studies detailing the construction of mathematical models for marine disease, and these are typically particle tracking models which estimate an area's risk of exposure based on the hydrodynamic connectivity of the region. There is great need to couple these models with epidemiological models to better answer questions about disease risk, potential outcomes, and implications of various management strategies. In this study, I will further discuss the need for advancement of these modeling tools for decision making, and I will present our plans for such a model simulating spread of OsHV-1μvar in Pacific oyster populations in the United States.

### Annotated Bibliography of Key Works

Ben-Horin, T., Bidegain, G., de Leo, G., Groner, M. L., Hofmann, E., McCallum, H. & Powell, E. (2020). Modelling marine diseases. In D. C. Behringer, B. R. Silliman & K. D. Lafferty (Eds). *Marine Disease Ecology* (pg. 223–255). Oxford University Press. DOI: 10.1093/oso/9780198821632.003.0012

In this review the authors discuss various modelling methods that have been used to describe marine disease dynamics. They note that while the foundational principles apply to both terrestrial and marine diseases, the physical ocean environment adds a layer of complexity that requires an altered approach to disease transmission. Of particular interest is section 12.7 which references many of the most well-known examples incorporating ocean hydrodynamics into their modeling methods.

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

This report discusses the threat of emerging pathogens to the growth and sustainability of shellfish aquaculture, and it identifies key priorities related to infrastructure, expertise, and research necessary for the management of these diseases. In particular, the report highlights the devastation caused by OsHV-1µvar globally as well as the rapid assessment and preparation required to manage the pathogen in North America, where most Pacific oyster populations are currently unaffected.

Lupo, C., Dutta, B.L., Petton, S., Ezanno, P., Tourbiez, D., Travers, M.-A., Pernet, F., Bacher, C. Spatial epidemiological modelling of infection by Vibrio aestuarianus shows that connectivity and temperature control oyster mortality. Aquaculture Environment Interactions. Vol.12 (511-527) 19 Nov 2020, https://doi.org/10.3354/aei00379
The aim of this study was to develop a spatial epidemiological model coupling hydrodynamic connectivity with a traditional epidemiological compartment model to estimate disease spread and resulting mortality caused by *Vibrio aestuariansu* in Pacific oysters. Sensitivity analyses were conducted to evaluate the relative importance of each parameter to the model outcomes. The authors propose that their methods can be used to evaluate the efficacy of proposed management strategies. This model is one of the first to pair dispersion and epidemiological models, and it represents a significant advancement in marine modeling methods.

Peeler, EJ, Taylor, NG. The application of epidemiology in aquatic animal health -opportunities and challenges. Veterinary Research vol. 42 (94). 11 Aug. 2011, https://doi.org/10.1186/1297-9716-42-94
This review discusses epidemiological approaches to disease management and applications to aquatic animal diseases. The paper details the short history of aquatic animal epidemiology, method employed, and challenges to implementation. The author emphasizes that modern epidemiological approaches are integral to addressing questions within complex aquatic disease systems, and they note particle tracking models as a valuable means of studying disease spread. A lack of available data and systems for data collection is identified as a key constraint to adaptation of many epidemiological methods.

# Using a OneHealth Approach to Evaluate How Marine Pathogens and Parasites That Are Infectious to Cultured Finfish, Shellfish, and Macroalgae Within the United States May Be Affected by Climate Change.

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Climate change has led to an increase in the frequency of extreme events such as marine heatwaves and harmful algal blooms (HABs). Such events can lead to catastrophic loss of cultured and wild marine species. Due to their high visibility, these phenomena are well documented and studied. However, there are more subtle effects of climate change that may prove to be just as devastating to U.S. aquaculture in the future as these ‘extreme events’ are today. One such example includes the various impacts that marine pathogens and parasites can have on cultured finfish, shellfish, and seaweeds.

Predicted climate related changes in ocean temperature, dissolved oxygen, salinity, pH, ocean currents, estuarine flushing patterns, wind speed and direction, precipitation and freshwater nutrient runoff (to name a few) will most certainly influence the virulence and spatial reach of marine bacteria, viruses, and parasites. Novel pathogens and parasites may be ‘introduced’ into a given area through expansion of their host species’ range or through other mechanisms. Changes in the physical environment of a cultured species can induce stress or alter immune responsiveness, and this may influence their susceptibility to disease. These changes could result in impacts to cultured animal health, reduced quality of cultured seafood products, or impacts to human health.

In order to be better prepared for these climate related impacts, researchers from NOAA Fisheries Office of Aquaculture, the NOAA Climate Program Office, NOAA Fisheries Northwest Fisheries Science Center, and the US Global Change Research Program (USGCRP) are working together using a OneHealth approach to identify current and emerging marine pathogens and parasites that are important to cultured species and that are likely to be affected in either their scope of influence/range or in their severity by climate change.

Information collected through this effort will be used to prioritize support for research, to identify adaptive measures that can be taken to prepare for climate change related aquaculture impacts, to inform aquaculture drug development and approval, and it will be used to inform the permitting process and management of Aquaculture Opportunity Areas (AOAs). Our goal in presenting our approach to is to learn how countries with a more developed marine aquaculture industry maintain biosecurity on their farms. Additionally, we hope to learn about how others are incorporating climate change science into their aquaculture strategies and tactics.

## Annotated Bibliography of Key Works

Callaway R, Shinn AP, Grenfell SE, Bron JE and others. 2012. Review of climate change impacts on marine aquaculture in the UK and Ireland. Aquat Conserv 22: 389−421.

This paper provides a comprehensive review of the various impacts that climate drivers such as sea-level rise, storm damage, increased precipitation, and increasing ocean acidification may have on marine aquaculture in the UK and Ireland. Key to this paper is an extensive section describing the potential effects of climate change on the health of finfish and shellfish. In particular, this paper covers climate induces changes in the virulence and range of parasite and microbial pathogens, changes in the host species, and changes in contact and interaction between host species and pathogens.

Chiaramonte L, Munson D, Trushenski J. 2016. Climate change and considerations for fish health and fish health professionals. Fisheries 41: 396−399.

This paper is short and succinct. The authors describe how a specific combination of pathogen, host, and environmental conditions is required in order for fish to succumb to disease. They further describe how climate change might influence this relationship and warn that although isolated changes in the aquatic environment such as increased temperature can alone influence fish health, fish health experts should prepare themselves for even more complex scenarios in the future.

Marcogliese DJ. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. Rev Sci Tech 27: 467−484.

This paper presents a comprehensive look at how climate change is likely to impact the virulence and spread of aquatic pathogens and parasites. The authors include predictions of environmental changes and the biological effects associated with global warming from the perspective of the host-pathogen-parasite relationship as well as illustrations to describe the mechanisms behind these changes. The role of temperature in virulence is specifically discussed. Both marine and freshwater systems are covered, and diseases of marine invertebrates are highlighted. The authors also discuss how interactions between climate drivers and other stressors such as pollution, hypoxia, and habitat loss/fragmentation influence the disease process. Finally, there is discussion of the effects of climate change on human health in relation to diseases that are transmitted through water.