Symposium Schedule

52nd Scientific Symposium of the UJNR Aquaculture Panel

"Next Steps for Sustainable and Resilient Aquaculture"

Three-Year Theme
"A New Era for Sustainable Aquaculture - The Next 50 Years of Research, Education and Collaborations"

Sinfonia Technology Hibiki Hall Ise
1-13-15 Iwabuchi
Ise, Mie 516-0037

November 5th and 6th, 2024

52nd Scientific Symposium of the UJNR Aquaculture Panel
"*Next Steps for Sustainable and Resilient Aquaculture*"
November 5th and 6th, 2024
Sinfonia Technology Hibiki Hall Ise
1-13-15 Iwabuchi, Ise, Mie

Aim of the Symposium

The Japan Fisheries Research and Education Agency will host the 52nd UJNR Aquaculture Panel Scientific Symposium in Ise, Mie, Japan. The UJNR Aquaculture Panel is a cooperative research exchange between the U.S. and Japan, jointly addressing environmental and technical issues that affect the aquaculture industries of both nations.

The UJNR Aquaculture Panel has been interacting with each other for more than 50 years. The global aquaculture industry has continued to develop and expand during this time. Making aquaculture a sustainable industry has become an important issue in recent years. Therefore, in the 12th Three-Year Plan, we have decided to discuss research, education, and collaborations over the next 50 years to make aquaculture sustainable regarding fishery resources and energy and to ensure a permanent supply of animal protein to humanity. This long-term vision inspires hope and a sense of purpose as we embark on this journey. The 52nd UJNR Aquaculture Scientific Symposium is the first year of the current Three-Year Plan, and we will discuss how to make the aquaculture industry sustainable through alternative feeds, breeding, health management, seaweed culture, and ecosystem management.

Program

Tuesday, November 5, 2024

Registration 12:00 - 13:00

Welcome and aim of the symposium
Natsuko Miki, Japan Panel Chair, Japan Fisheries Research and Education Agency
13:00-13:15

Plenary Lecture
(Moderator: Kazumasa Ikuta)

Building capacity for land-based aquaculture production in the US-national academia-industry-federal partnerships

Yonathan Zohar, University of Maryland, Institute of Marine and Environmental Technology & Department of Marine Biotechnology

13:15-13:45

Alternative Feeds

(Moderators: Caird Rexroad and Akiyuki Ozaki)

Next-generation sustainable aquaculture systems employing insect protein-based feeds

Kazumasa Ikuta, Japan Fisheries Research and Education Agency 13:45-14:00

Multifunctional utilization of insects in aquaculture

Takeshi Miura, Ehime University 14:00-14:30

Validation of the suitability of full fat and defatted black soldier fly meals in diets for rainbow trout

Wendy Sealey, Bozeman Fish Technology Center, Agricultural Research Service, United States Department of Agriculture 14:30-15:00

Protein assimilation from larvae of black soldier fly *Hermetia illucens* in diets for red seabream *Pagrus major*

Tadashi Andoh, Fisheries Technology Institute, Japan Fisheries Research and Education Agency 15:00-15:30

Break 15:30-15:45

Omics analysis of red seabream (*Pagrus major*) fed a soybean meal-based diet

Hazuki Yoshinaga, Fisheries Technology Institute, Japan Fisheries Research and Education Agency 15:45-16:15

Frass from black soldier fly larvae as an aquafeed ingredient: Nutritional value and potential health benefits

Mediha Aksoy, Aquatic Animal Health Research Unit, Agricultural Research Service, United States Department of Agriculture 16:15-16:45

Effect of feeding black soldier fly larvae diets on growth and culture condition of Kuruma prawn

Katsutoshi Ito, Fisheries Technology Institute, Japan Fisheries Research and Education Agency 16:45-17:15

Wednesday, November 6, 2024

Registration 08:30 – 09:00

Genetics/Selective Breeding and Monosex Breeding
(Moderator: Luke Gardner and Shohei Takuno)

Current status of artificial seed production and selective breeding in the Japanese yellowtail *Seriola quinqueradiata*: The progress achieved by FRA
Kenta Adachi, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 09:00-09:30

Population structure and selective breeding program for the growth of farmed rainbow trout (*Oncorhynchus mykiss*) in Japan
Tsubasa Uchino, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 09:30-10:00

Advancing monosex breeding technology for sablefish (Gindara) aquaculture
Adam Luckenbach, Fisheries Northwest Fisheries Science Center, NOAA 10:00-10:30

Break 10:30-10:45

Health Management
(Moderators: Caird Rexroad and Tomofumi Kurobe)

Developing vaccination strategies for prevention of atypical furunculosis in sablefish (*Anoplopoma fimbria*)
Kenneth D Cain, Northwest Fisheries Science Center, NOAA Fisheries 10:45-11:15

Hygiene management is important to prevent red sea bream iridovirus transmission between net pens: Insights from a case study that assessed cross-contamination
Yasuhiko Kawato, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 11:15-11:45

Disease control measures in hirame juvenile hatchery: The case of hirame aquareovirus
Tomoki Maeda, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 11:45-12:15

Lunch break 12:15-13:30

Seaweed Culture and Ecosystem Management
(Moderators: Luke Gardner and Satoshi Watanabe)

Opportunities and challenges for Alaska kelp aquaculture
Jordan Hollarsmith, Alaska Fisheries Science Center, NOAA Fisheries 13:30-14:00

The present status and future scope of seaweed aquaculture in Japan
Hiromori Shimabukuro, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 14:00-14:30

Production improvement of Nori aquaculture using biostimulants
Mahiko Abe, National Fisheries University,
Japan Fisheries Research and Education Agency 14:30-15:00

Seaweed seedling culture technique using LEDs and feeding behavior of herbivorous fish to suppress fouling seaweed - In the case of "*hiziki*" *Sargassum fusiforme*-
Tsutomu Noda, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 15:00-15:30

Break 15:30-15:45

Advantages of small-sized macroalgae in seaweed bed restoration in waters with high feeding pressure from herbivorous fishes
Tatsuru Kadota, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 15:45-16:15

Pacific oyster condition and mortality in a U.S. Pacific coast estuary: Can relationships with climate, food and reproductive state be utilized to sustain future production?
Brett Dumbauld, Pacific Shellfish Research Unit, Agricultural Research Service,
United States Department of Agriculture 16:15-16:45

Image analysis for estimating soft body mass from shell morphology in the Pacific oyster, *Crassostrea gigas*
Junpei Shinji, Fisheries Technology Institute,
Japan Fisheries Research and Education Agency 16:45-17:15

Scientific symposium closing
Janet Whaley, US Panel Chair, NOAA Fisheries Office of Aquaculture 17:15-17:30

52nd Scientific Symposium of the UJNR Aquaculture Panel

"Next Steps for Sustainable and Resilient Aquaculture"

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BUILDING CAPACITY FOR LAND-BASED AQUACULTURE PRODUCTION IN THE US - NATIONAL ACADEMIA-INDUSTRY-FEDERAL PARTNERSHIPS

Yonathan Zohar

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The United States is the world's largest importer of seafood, which contributes over $17 billion to its trade deficit annually. For example, over 90% of the ~500,000 metric tons of Atlantic salmon consumed annually in the US are imported from overseas, for a value of around $3.6 billion. As the challenges surrounding fish farming in floating coastal net-pens mount, we have witnessed increased interest and major investments in the US in land-based production of salmon, yellowtail, seabass and other fish species, using recirculating aquaculture systems (RAS). Responding to these trends, NOAA/National Sea Grant and USDA-NIFA have funded national collaborative projects, consisting of academic, industry and federal partners, to build capacity and promote the development of innovative, environmentally sustainable land-based, RAS aquaculture in the US. The benefits of RAS are that it is fully contained, generates near-zero environmental waste, prevents escapees of farmed fish, is devoid of environmental contaminants and pathogens, can be tailored to meet the physiological requirements of any fish of interest to enable optimal performance (thus is generic), and can be located anywhere, thereby reducing the carbon footprint of fish farming.

The Recirculating Atlantic Salmon Network (RAS-N; 2019-2021) and the Sustainable Aquaculture Systems Supporting Atlantic Salmon (SAS^2; 2021-2026), funded by NOAA/National Sea Grant and USDA-AFRI, respectively, were created as national multi-disciplinary, public-private-federal partnerships with the overarching goal to support and enable the rapidly emerging US land-based aquaculture industry. RAS-N and SAS^2 are holistic hubs of knowledge and expertise that integrate research and development with extension, outreach, education, training and workforce development to promote the successful growth, stability and economic feasibility of the salmon RAS sector and, more broadly, US aquaculture of this and other species. Engaging with industry stakeholders, we first identified gaps in knowledge and technology impediments to the development of the land-based aquaculture in the US and generated a list of prioritized R&D needs to overcome them.

The following 8 stakeholder-driven objectives were identified:

1. Understand and mitigate off-flavors in RAS platforms
2. Establish domestic, year-round egg production from North American strains
3. Understand/reduce early maturation, develop methods for reproductive sterility
4. Develop RAS-specific and alternative feeds
5. Study/optimize microbiomes in RAS for efficient bio-filtration, waste treatment and conversion to value-added products, better containment, fish health and environmental compatibility.

6. Engage in economic and market analysis
7. Develop education programs - K-16, RAS certificates, workforce development
8. Create effective extension programs – outreach, community engagement, public awareness, technology transfer

SAS^2, established in 2021, is a synergistic, "hands-on" partnership in which leading aquaculture scientists, in collaboration with all major US producers, carry out research focusing on the above industry-identified barriers to the expansion of the salmon (and other species) RAS industry. This presentation will provide highlights of progress made by SAS^2 partners on the above objectives, towards enhancing environmentally and economically sustainable US aquaculture.

Yonathan Zohar: Annotated Bibliography

Zohar, Y., 2023. Innovative Aquaculture Technologies – Recirculating Aquaculture Systems. The Journal of Ocean Technology, 18 (2): 114-115. https://www.thejot.net/article-preview/?show_article_preview=1446

This short paper describes the emergence of innovative, environmentally sustainable and economically feasible platforms of aquaculture - land-based recirculating aquaculture systems (RAS). In view of some of the challenges facing sea cage (net-pen) aquaculture production, intensive research and development efforts have been invested in developing land-based RAS fish farms, both marine and freshwater, that are fully contained, recycle over 99% of their water, have near-zero discharge, and optimally produce high density yields of commercially important fish. Beneficial microbes are used to eliminate dissolved waste such as ammonia, nitrites and nitrates, as well as to convert solid/organic waste to fuel-grade biomethane (which is used to offset some of the energy cost of the operation). All environmental conditions in the tanks are controlled to meet the physiological requirements of the fish of interest (marine or freshwater) for optimal performance. Such RAS platforms do not generate environmental waste and fish are not exposed to environmental contaminants or pathogens. RAS technology can also be implemented to enable containment of floating aquaculture cages, thus reducing adverse effects on the environment.

Ben-Asher, R., Zohar, Y., Stromberg M. and Lahav, O., 2024. A new approach for eliminating off-flavors from RAS fish, as part of the normal grow-out period. Water Research, 249: 1-9. https://doi.org/10.1016/j.watres.2023.121015

One of the major challenges in RAS fish farming is preventing off-flavor in the harvested fish. As the water exchange is reduced, undesirable organic compounds in the water accumulate in the fish muscle and give them an “earthy” or “muddy” taste, which greatly undermines the marketability of the fish. The two most abundant off-flavor compounds in RAS are Geosmin and 2-methyl-isoborneol (MIB). These compounds, produced by microorganisms naturally present in the environment as well as in RAS, are usually removed from the fish tissues pre-harvesting by purging (depurating) them in dedicated flow-through tanks while keeping the fish unfed. In many fish species, especially cold-water species like salmon and trout, purging must continue for 12-14 days to ensure that all off-flavor is flushed from the fish muscle. This practice uses large volumes of water that must be discharged, involves labor and the unfed fish lose weight, all of which result in financial losses to the farmers. This paper describes a new and efficient off-flavor mitigation technology, using electro-oxidation of the culture water. Geosmin and MIB were efficiently removed, within 10 days, from rainbow trout grown at high density in 20 ppt, 14^{o}C water, while maintaining the fish on feed in their original production tanks and continuing to recycle the water. The authors conclude that RAS farms can increase both their annual production and their income by more than 10%, if they implement the suggested technology as part of the fish production period.

Zohar, Y., 2021. Fish reproductive biology - reflecting on five decades of fundamental and translational research. General and Comparative Endocrinology, 300: 113544 https://doi.org/10.1016/j.ygcen.2020.113544

Intensive, efficient and cost-effective aquaculture, in the sea or on-land, relies on full control of the fish reproductive cycle and overcoming reproduction-related bottlenecks. This paper reviews five decades of fundamental and applied research in fish reproductive biology, using both models such as zebrafish and medaka, as well as a variety of aquaculture important species, demonstrating how basic research leads to overcoming reproductive barriers and to making aquaculture more reliable, environmentally sustainable and economically feasible. Research and development efforts are discussed that led to closing life cycles of farmed fish, enabling predictable, year-round production of good quality eggs and juveniles, controlling sex determination and differentiation, advancing or delaying puberty, and enhancing overall reproductive competence and success. The integration of traditional fish physiology approaches with the most advanced platforms of genomics and biotechnology, as well as the multidisciplinary and collaborative nature of the scientific community, have been essential to the tremendous progress and success of the field and the implementation of the research in industry.

Wong, T.T. and Zohar, Y. 2024 (in press, draft attached). Reproductive technology: sex control and sterilization in fish. In: Encyclopedia of Reproduction, 3rd Ed, Elsevier.

Many farmed fish reach sexual maturation before attaining harvest size, which results in slower growth rate, deterioration of flesh quality and, in some cases, mortalities. Fish grown in RAS experience optimal environmental conditions and often exhibit precocious (early) maturation, which significantly reduces the economic profitability of the farms. In some fish species, one gender is more desirable for farming than the other. Also, farmed fish escaping from cage production often propagate in the wild and/or inter-breed with wild stocks, affecting the genetic signature of wild populations and posing a major threat to the marine biodiversity. This paper reviews sex control and sterility induction technologies that are key reproductive strategies to enhance environmentally-friendly and cost-effective aquaculture practices by 1. Promoting controlled reproduction and constant and voluminous seed production; 2. Offering monosex populations with the most desirable performance traits; 3. Providing reproductively sterile populations to ensure genetic containment and better performance; and 4. Avoiding precocious maturation to enhance fish growth, health, and market value.

Quinn, B.M, Apolinario, E.A, Gross, A. and Sowers, K.R., 2016. Characterization of a microbial consortium that converts mariculture fish waste to biomethane. Aquaculture 453: 154-162. http://dx.doi.org/10.1016/j.aquaculture.2015.12.002

Innovative land-based RAS aquaculture, marine or freshwater, is now reusing over 99% of its production water. At high fish densities of 60-100 kg/m^3, significant dissolved and solid fish waste accumulates in the water and must be treated without discharge to the environment. Microbial communities have been successfully used in nitrifying and denitrifying biofilters to close the nitrogen cycle and convert toxic ammonia, nitrites and nitrates to free nitrogen.

Hydrogen sulfide, produced mainly in marine systems, is removed by sulfide oxidizing bacteria present in the denitrifying biofilters. However, organic/solid waste remained a main challenge. RAS systems generate very large volumes of solid waste (~20 metric tons daily for every 1,000 metric tons of fish produced annually). Traditionally, these volumes of sludge have been used as soil amendments (which is not possible for salty sludge) or removed and buried in landfills. Neither practice is environmentally-responsible. This paper describes the development of a microbial consortium that very efficiently converts marine aquaculture solid/organic waste to fuel-grade biogas. The consortium includes two anaerobic fermentative bacteria that hydrolyze and ferment the raw sludge into smaller molecules, which serve as substrate for the three other members of the consortium- methanogenic microorganisms that convert the waste into fuel-grade biogas (bio-methane). Inoculating this consortium into anaerobic digesters in marine RAS growing high densities of gilthead seabream, European seabass and Atlantic salmon, efficiently converted over 90% of the organic/solid waste into biogas that can be (and has been) used on the farm to offset up to 10% of its energy needs.

Next-Generation Sustainable Aquaculture Systems Employing Insect Protein-Based Feeds

Kazumasa Ikuta

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As seafood consumption continues to increase world-wide, aquaculture production is also expanding rapidly, and has for the first time, recently exceeded capture fisheries production. However, the aquaculture industry is simultaneously facing the serious problems of a lack of fish meal and oil which serve as the main components of aquaculture feed, and the associated rising prices of these materials. At the same time, the so-called “protein crisis”, which means that the predicted consumption of animal protein will exceed its actual production is postulated to occur in 2030, due to the expansion of the world’s population. Therefore, the FAO has strongly recommended to extend the utilization of edible insects as a protein source.

In this context, the Japanese government commenced the Moonshot Research and Development Program for innovative research to build future society in 2050. Under the auspices of this program, we have been promoting R&D to develop innovative aquaculture feeds using alternative protein sources and oils produced from insects and microalgae. In the present session, sustainable aquaculture food supply chains using alternative insect protein cultured under food recycle systems will be discussed

Multifunctional Utilization of Insects in Aquaculture

Takeshi Miura* and Chiemi Miura

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Insects are being investigated as a sustainable alternative to fishmeal, a conventional component in aquaculture feed, due to their environmental advantages. However, the higher production cost of insect-based meal compared to fishmeal remains a significant challenge. To facilitate its widespread adoption, it is essential to highlight the additional functional benefits of insect meal beyond sustainability, while simultaneously reducing production costs.

Research has demonstrated that housefly (*Musca domestica*) pupae can enhance disease resistance in fish, suggesting the presence of immunostimulatory compounds within the pupae. These compounds, which activate phagocytic cells, are not exclusive to houseflies but are found in various insect species. Studies have successfully isolated immunostimulatory substances from several insects, including the melon fly (*Bactrocera cucurbitae*), black soldier fly (BSF; *Hermetia illucens*), Japanese oak silk moth (*Antheraea yamamai*), and silkworm (*Bombyx mori*). One such substance, Silkrose, derived from the silkworm, has been extensively studied and shown to improve not only disease resistance in fish but also muscle structure, stress tolerance, and heat resistance.

Gene expression analysis in fish treated with Silkrose revealed modifications in the expression of genes associated with immune function, redox balance, lipid metabolism, and protein processing. These genetic changes indicate that Silkrose mitigates stress, enhances disease resistance, improves thermal tolerance, and potentially enhances meat quality in fish. Notably, similar gene expression patterns have been observed in other species, including mice, crustaceans, and bivalves, suggesting that the effects of Silkrose are not limited to fish but extend across a broad range of vertebrates and invertebrates.

Other insect species, such as BSF, crickets, and mealworms, also contain similar bioactive substances, implying that they may offer comparable benefits. Insects, already recognized as a promising protein source for food and feed, are gaining increasing global attention. Their biofunctional properties are expected to further elevate their value as feed ingredients, potentially positioning them as superior to traditional fishmeal. Future research efforts will focus on maximizing the utility of these bioactive compounds to fully exploit the potential of insects as an alternative protein source in aquaculture.

As insect-based meals become more cost-competitive and their functional benefits are better characterized, they could play a pivotal role in advancing sustainable aquaculture. By enhancing fish health, improving stress resilience, and increasing product quality, insect-derived feeds may surpass fishmeal in both efficacy and environmental compatibility, supporting the growing demand for eco-

friendly and nutritious aquaculture solutions.

Annotated Bibliography of Key Works

Ido, A., Iwai, T., Ito, K., Ohta, T., Mizushige, T., Kishida, T., Miura, C. and Miura, T. 2015. Dietary effects of housefly (*Musca domestica*) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (*Pagrus major*) (Perciformes: Sparidae). *Appl. Entomol. Zool.* Vol:50: 213-221.

This paper investigates the potential use of housefly larvae and pupae as aquaculture feed. In this study, we were the first to reveal the immunostimulatory properties of housefly meal.

Ohta, T., Kusano, K., Ido, A., Miura, C. and Miura, T. 2016. Silkrose: A novel acidic polysaccharide from the silkmoth that can stimulate the innate immune response. *Carbohydr. Polym.* Vol: 136: 995-1001.

This paper reveals that the pupae of the Japanese oak silk moth, a species of Lepidoptera, contain immunostimulatory substances and demonstrates that these substances are acidic high-molecular-weight polysaccharides.

Ali, M. F. Z., Yasin, I. A., Ohta, T., Hashizume, A., Ido, A., Takahashi, T., Miura, C. and Miura, T. 2018. The silkrose of *Bombyx mori* effectively prevents vibriosis in penaeid prawns via the activation of innate immunity. *Scientific Reports* 8(1): 8836.

This paper reports the isolation and purification of an immunostimulatory polysaccharide from silkworm pupae, clarifies its monosaccharide composition, and demonstrates its ability to enhance immunostimulation and disease resistance in kuruma shrimp (*Marsupenaeus japonicus*) and whiteleg shrimp (*Litopenaeus vannamei*).

Ali, M. F. Z., Ohta, T., Ido, A., Miura, C. and Miura, T. 2019. The dipterose of black soldier fly (Hermetia illucens) induces innate immune response through Toll-like receptor pathway in mouse macrophage RAW264.7 cells. *Biomolecules* Vol:9 (11): 677.

This paper identifies and elucidates the structure of a functional polysaccharide with immunostimulatory properties isolated from the larvae of the black soldier fly (*Hermetia illucens*).

Ali, M. F. Z., Kameda, K., Kondo, F., Iwai, T., Kurniawan, R. A., Ohta, T., Ido, A., Takahashi T., Miura, C. and Miura, T. 2021. Effects of dietary silkrose of *Antheraea yamamai* on gene expression profiling and disease resistance to Edwardsiella tarda in Japanese medaka (*Oryzias latipes*). *Fish & Shellfish Immunology* Vol:114: 207-217.

This paper demonstrates that the immunostimulatory polysaccharide Silkrose, derived from the Japanese oak silk moth, enhances disease resistance in fish, and investigates the mechanism of this resistance at the molecular level.

Validation of the Suitability of Full Fat and Defatted Black Soldier Fly Meals in Diets for Rainbow Trout

Wendy M. Sealey[1]*, Ken Overturf[2], Gary Burr[3], and Kari Madden[4]

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*Presenting author, email: Wendy.Sealey@usda.gov

Improved insect stocks, culture methods, and mitigation of safety concerns have increased availability and use of commercial black soldier fly (BSF) meals as animal feedstuffs. Both full-fat and defatted BSF can have beneficial applications in aquatic animal feeds; defatted BSF protein can serve as a high-quality amino acid source that compliments feeds with reduced fish meal and increased amounts of plant ingredients while the full-fat BSF lipid is rich in medium-chain fatty acids, is a readily utilized energy source, and can upregulate fish immune function. However, variation in BSF meal protein digestibility and quantity and quality of the BSF meal lipid occurs due to differences in culture and processing. Thus, to effectively utilize black soldier fly meals in rainbow trout (*Oncorhynchus mykiss*) feeds, thorough characterization of the nutrient content of BSF meals and assessment of their effects on immune function are necessary to validate their suitability.

To accomplish this goal, an *in vivo* digestibility trial was conducted in juvenile trout to determine the available nutrient content of commercially sourced full fat and defatted BSF meals. Based on digestibility data, a 3 X 2 factorial design was created by supplementing a practical-type rainbow trout diet with three levels (0, 5, or 10%) of either full fat or defatted BSF meal; an additional test diet was created by top-coating the 0% BSF meal with black soldier fly lipid at a level equivalent to the 10% full fat BSF diet. All diets were formulated to contain 44.8% DP and 15% CL, and balanced to available lysine, methionine, threonine and phosphorus to targets of 3.82, 1.30, 2.14 and 0.6, respectively prior to cooking extrusion. For the growth trial, fifteen rainbow trout (10.4 ± 0.2g, initial weight) were randomly stocked into quadruplicate tanks (400 L each) and fed their respective diets for 12 weeks to assess effects on growth efficiency and immune function.

Growth results indicated no significant effects of BSF level, type, or their interaction on final fish weight (P=0.672; P=0.303; P=0.752, respectively), feed conversion ratio (P=0.184; P=0.259; P=0.610, respectively), feed intake (P=0.617; P=0.856; P=1.000, respectively) or body condition indices (hepatosomatic index: P=0.184; P= 0.608; P=0786, respectively; Intraperitoneal fat ratio: P=0.902; P=0259; P=0.610, respectively; Fillet ratio: P=0.617 P=0.865 P=0.740, respectively).

Evaluation of expression of the immune related genes, IL-10, IL-1β, TNFα, HSP70, Defensin β3, IL-4 like, and UDP glucose 6-dehydrogenase (UDPG6D) found no significant changes in the fish fed full fat or defatted BSF meal. However, significant upregulated expression of IL-10, IL-1β, TNFα, IL-4 like gene, HSP70, and UDPG6D were detected in the intestine of fish fed the 0% BSF meal with black soldier fly lipid.

These data suggest that both full-fat and defatted BSF meals are suitable for rainbow trout feeds when diets are formulated on an available nutrient basis that accounts for differences in protein availability and endogenous lipid content. Further, the effects of BSF lipid top coating on immune gene expression requires further study but may indicate sensitivity of the immunomodulating BSF lipid components to degradation during feed manufacturing.

Annotated Bibliography of Key Works

English, G., Wanger, G. and S. Colombo. 2021. A review of advancements in black soldier fly (*Hermetia illucens*) production for dietary inclusion in salmonid feeds. Journal of Agriculture and Food Research, Volume 5: 100164. https://doi.org/10.1016/j.jafr.2021.100164.

The authors summarize the main findings on the advancement on black soldier fly (BSF) production methods and their use in salmonid aquaculture and highlight the importance of BSF rearing procedures and processing on the suitability of BSF as a nutritious protein source for salmonids. Further, the authors identify areas for future research regarding optimizing rearing and processing procedures for BSF destined for aquafeeds.

Gasco, L., Bellezza Oddon, S., Vandenberg, G.W., Veldkamp, T. and I. Biasato. 2023. Factors affecting the decision-making process of using insect-based products in animal feed formulations. Journal of Insects as Food and Feed. Volume 0: Pages: 1–12. https://doi.org/10.3920/JIFF2022.0164

The authors discuss the various factors to consider when including insect-based products in animal feeds. In particular, the authors identified the importance of insect meal form, insect species, rearing substrates and production processes. The authors highlighted how the increased use of additives during insect processing has created additional sources of variation in commercial insect products. The authors also discussed how feed manufacturing method was an important consideration when using insect-based products in animal feeds.

Koutsos, E., Modica, B., and T. Freel. 2022. Immunomodulatory potential of black soldier fly larvae: applications beyond nutrition in animal feeding programs. Translational Animal Science, Volume 6: txac084. https://doi.org/10.1093/tas/txac084

The authors review recent research that demonstrates the potential for the immunomodulatory activity of various components of black soldier fly (BSF)-derived ingredients. The authors focus this review on the actions of three important BSF components that have been shown to alter immune function and disease resistance in companion animals, poultry and livestock including: antimicrobial peptides that are present

in BSF hemolymph; lauric acid, a predominant BSF fatty and, the chitin/chitosan components of the insect exoskeleton.

Weththasinghe, P., Øvrum Hansen, Torunn Mydland, L., and M. Øverland. 2022. A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. Reviews in Aquaculture. Volume 14: Pages 938-956. https://doi.org/10.1111/raq.12635

The authors discussed how black soldier fly (BSF) has gained attention as a sustainable novel protein source in fish feed due to its high nutritional value and low environmental impacts and conducted a meta-analysis to compile and systematically quantify the effect of BSF in diets for salmonids on growth performance and nutrient utilization. The authors presented results that demonstrate that overall dietary inclusion of BSF did not compromise the specific growth rate, feed conversion ratio, feed intake, protein digestibility and protein efficiency ratio in salmonids. Importantly, the authors reported that when the published literature reviewed was sorted according to the replaced protein source(s) that replacing fishmeal by BSF decreased growth rate and feed intake in salmonids but replacing non-fishmeal sources improved growth rate and feed conversion.

Protein Assimilation from Larvae of Black Soldier Fly *Hermetia illucens* in Diets for Red Seabream *Pagrus major*

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Fishmeal (FM) is a major feed ingredient for fish aquaculture. Japan depends on imported FM at approximately 50%. Recent increases in imported FM prices substantially impact aquaculture in Japan, and finding an alternative proteinic ingredient would be an essential solution. Black soldier fly larvae meal (BSF) is a candidate as an FM substitute for aquaculture. Recently, several private companies started producing BSF food residues in Japan. However, higher BSF formulations resulted in relative growth retardation in several fish species. Using conventional growth, stable isotope, and metabolomic analyses, we investigated protein assimilation from BSF by red seabream (*Pagrus major*). A 42-day feeding experiment was conducted to assess the performance of BSF-fed fish. Diets were isocalorically designed with FM or BSF in ratios of 100:0 (Group 1), 50:50 (Group 2), and 22:78 (Group 3). Group 1 exhibited a significantly higher specific growth rate (2.92% ± 0.22%/day) than Group 3 (2.54% ± 0.22%/day), while Group 2 (2.77% ± 0.21%/day) displayed no significant difference. Stable isotope analysis suggested a lower BSF assimilation in Groups 2 and 3, as indicated by their δN15 ratios. In vitro digestibility evaluation revealed a significant deficiency in essential amino acids (EAAs) in Group 3 compared to Group 1. This result suggests that a diet comprising up to 50% BSF does not affect the growth rate of red seabream, but an EAA deficiency may retard growth in Group 3. Therefore, while BSF can support red seabream growth, further improvements are needed for higher BSF formulation rates.

Annotated Bibliography of Key Works

Andoh, T., Yasuike, M., Ishihara, K., Fujiwara, A. 2023. Effects of heating duration on the digestibility of fish protein powders at 99°C *in vitro* using yellowtail *Seriola quinqueradiata* digestive enzymes. *Fish. Sci.* 89, 671–685.

The effects of heating duration on the digestibility of the muscle powder of three fish (pollock [WP], yellowtail [YT], and mackerel [CM]) were assessed in vitro using a digestive enzyme complex (EC) extracted from yellowtail pyloric caeca. This research will contribute to establishing a theoretical

basis for feed processing conditions for yellowtail. The powder samples were heated at 99 °C for 0, 3, 20, 72, and 240 min, followed by hydrolysis with the EC to assess the heating effects. After 3 min of heating WP, YT, and CM, the average production of essential amino acids (EAAs) decreased to 81.3%, 72.0%, and 66.9%, respectively, compared to the non-heated controls. The production further decreased with increased heating duration from 3 to 240 min, although most of the decrease happened within the first 3 min in WP, YT, and CM.

Takakuwa, F., Tanabe, R., Nomura, S., Inui, T., Yamada, S., Biswas, A., Tanaka, H. 2022. Availability of black soldier fly meal as an alternative protein source to fishmeal in red sea bream (*Pagrus major*, Temminck & Schlegel) fingerling diets. *Aquaculture Res.* 53, 36–49.
The authors replaced fishmeal (FM) in red sea bream (*Pagrus major*) diets with black soldier fly meal (BM) to investigate the effects of the diet on growth and feed utilization. Six isonitrogenous and isolipidic experimental diets were prepared by substituting 0%, 20%, 40%, 60%, 80%, and 100% FM proteins with BM (control, BM20, BM40, BM60, BM80, and BM100, respectively). After an eight-week feeding trial, final body weight, weight gain, specific growth rate, and feed efficiency decreased linearly with increasing BM levels ($p < 0.05$). The results suggest that BM can replace a maximum of 41.7% of FM in the red sea bream diet without compromising growth performance or feed efficiency for 56 days.

Ido, A., Ali, MFZ, Takahashi, T., Miura, C., Miura, T. 2021. Growth of yellowtail (*Seriola quinqueradiata*) fed on a diet including partially or completely defatted black soldier fly (*Hermetia illucens)* larvae meal. *Insects* 12, 722. https://doi.org/10.3390/insects12080722
Yellowtail, the most popular farmed fish in Japan, is carnivorous; its diet requires a high proportion of fishmeal (FM). This study represents the first example of yellowtail fed on a diet including BM (BSF) as an FM replacement. Partially defatted BSF meal (PDBM) comprised 49.0% crude protein and 23.2% crude fat, while completely defatted BSF (CDBM) contained less than 10% crude fat, and the same level as FM was achieved by defatting PDBM using hexane. In feeding trials, fish growth was reduced following PDBM content: 10%, 20%, and 30%. Therefore, even 10% of partially or completely defatted BSF meal inhibited juvenile yellowtail growth.

Omics Analysis of Red Seabream (*Pagrus major*) Fed a Soybean Meal-Based Diet

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In response to the increasing demand for seafood, aquaculture production is expanding worldwide in recent years. On the other hand, high demand and unstable supply of fishmeal (FM) make it difficult for feed manufacturers to use it as the main ingredient in aquaculture feeds. Soybean meal (SBM) is one of the most promising plant protein sources as a substitute for fishmeal in aquaculture feeds because of its economic advantages and nutritional properties. Although SBM has a relatively high protein content and balanced amino acid composition, it has been reported that dietary intake of large amounts of SBM has adverse effects on the physiology and growth of fish. To facilitate the development of more effective aquaculture feeds comprising of plant protein sources, it is required to investigate the effects of dietary SBM on fish nutritional metabolism in detail. In this study, comprehensive approaches, transcriptomics and metabolomics analyses were employed to investigate the effects of SBM on nutritional metabolism in red seabream, an important aquaculture species in Japan.

Red seabream were fed an SBM-based diet (SBMD) and FM-based diet (FMD) for eight weeks, and the hepatopancreas collected at the 2nd, 4th, and 8th week were used for transcriptome analysis. Growth retardation, and several abnormal physiological conditions, such as lower serum total cholesterol content and degeneration in the distal intestine, were observed in the SBMD group. Transcriptome sequencing analysis using a next-generation sequencer identified 454, 353, and 566 differentially expressed genes between the SBMD and FMD groups at the 2nd, 4th, and 8th week, respectively. The transcriptome analysis showed increased expression levels of genes involved in the terpenoid and steroid biosynthesis pathways, suggesting that hepatic cholesterol biosynthesis was up-regulated in the SBMD group. The hepatopancreas from fish fed each diet for eight weeks was also

used for metabolome analysis using a capillary electrophoresis time-of-flight mass spectrometry, and the 301 quantifiable metabolites were identified. The results of metabolome analysis coupled with the transcriptome analysis suggest that the SBMD affects glutathione and glycine metabolism. Additionally, transcriptome sequencing of the rectum from red seabream at 8th week revealed changes in the expression levels of genes related to cell cycle control, cholesterol metabolism and immune response.

In recent years, various ingredients have been proposed as substitutes for fishmeal, and it is practically more effective to mix a variety of alternative ingredients into aquaculture feeds relative to using a large amount of a single ingredient alternative to fishmeal. On the other hand, detailed experimental investigations into the effects of a single ingredient on gene expression and metabolites, like this study, could clarify the characteristics specific to each ingredient. In this study, the findings provide valuable information that enhances our understanding of the effects of dietary SBM on red seabream metabolism, which leads to relieving the abnormal physiological conditions. Findings obtained from omics analyses could be used to identify the indices of negative effects of alternative ingredients and thereby lead to improvement in the quality of future aquaculture feeds.

Annotated Bibliography of Key Works

Lu, F. et al. 2015 Effects of replacing fish meal with rendered animal protein and plant protein sources on growth response, biological indices and amino acid availability for rainbow trout *Oncorhynchus mykiss*. *Fisheries Science* 81, 95-105

The authors reported the effects of diets where fish meal was replaced by several protein sources on rainbow trout growth and showed that a combination of some protein sources could be substituted for fish meal. The result of biological indices and amino acid availability also support this conclusion. The report is one of the papers that shows the necessity of combining multiple alternative protein sources instead of using a single protein source as a substitute of fish meal.

Murashita, K. et al. 2018. Effects of dietary soybean meal on the digestive physiology of red seabream *Pagrus major*. *Aquaculture* 493, 219–228.

The authors reported acute and chronic effects of dietary SBM on the digestive physiology of red seabream. Red seabream were fed fishmeal and soybean meal-based diets, and the authors investigated the gastric transit, digestive enzyme activity and gene expression levels of digestive enzymes. This paper is one of the reports that clearly shows the SBM effects on fish nutritional physiology in detail.

Roques. S et al. 2020. Metabolomics and fish nutrition: a review in the context of sustainable feed development. *Reviews in Aquaculture* 12, 261-282.

The authors have described the utilization of metabolomic approaches for the development of fish feed and the analysis of fish nutritional metabolism in this review. It is expected that it will be a require to improve feeds using plant resources and other alternative raw materials as substitutes for

fish meal and fish oil in the feature. In this review, previous studies related to fish nutrition metabolism using metabolomic analysis are summarized, and future applications of metabolomics for study in fish nutrition and development of aquaculture feed were discussed.

Yoshinaga et al. 2023. Multi-omics analysis of hepatopancreas of red seabream (*Pagrus major*) fed a soybean meal-based diet. *Aquaculture* 574. 739631.

The authors analyzed the hepatopancreatic transcriptome and metabolome in red sea bream fed an SBM diet for 8 weeks. Fish fed the SBM diet showed delayed growth and physiological abnormalities. The results of omics analyses suggest that the SBM diet affects the metabolism of cholesterol, glutathione, and glycine.

Frass from Black Soldier Fly Larvae as an Aquafeed Ingredient: Nutritional Value and Potential Health Benefits

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Insects have gained global research interest as potential protein sources and producers scaling up insect production to meet the increasing demand for protein. Insect meal production generates a large quantity of frass -a term used to describe the larval waste, leftover food, and remaining exoskeletons after the larvae are removed from breaking down a compost source. Two separate trials were conducted to explore the nutritional value of frass generated from black soldier fly larvae, *Hermetia illucens* fed dried distillers' grains for channel catfish, *Ictalurus punctatus* and hybrid tilapia, Nile x Mozambique (*Oreocromis niloticus* x *O. mozambique*). The purpose of our studies was to evaluate the influence adding various levels of frass to the diets has on overall fish performance and health. Five isocaloric practical-type diets containing frass from 0% to 30% (as partial replacement of a combination of soybean meal, cottonseed meal, wheat short and corn meal on an equal protein basis) were fed to catfish (5.24 ± 0.04g) and tilapia (2.6 ± 0.035g) for 10 weeks. Diets were fed to four replicate tanks of fish per treatment (50 fish/tank). After that time, fish were evaluated for growth, feed utilization, body proximate, and mineral composition. Representative fish were bled for hematological and serological assays and challenged to determine the effects of dietary frass on disease resistance. Final weight gain significantly increased in catfish fed diets containing frass at levels from 10% to 30%. Catfish fed diets without frass, and 30% frass, showed the lowest and highest feed intake, respectively. Feed and protein efficiencies, however, were significantly lower in catfish fed frass at levels of 20% and higher compared to the control diet. On the other hand, final weight gain significantly increased in tilapia fed the diet containing the highest level of frass (30%). Tilapia fed diets containing frass (5% to 30%) had significantly higher protein efficiency than the control group. Feed intake and feed utilization efficiency of tilapia were not significantly affected by dietary treatments. Survival, whole-body composition and mineral content of both fish species were not affected by frass. In catfish, hematological parameters (red blood cell count, hemoglobin, and hematocrit) were improved with the inclusion of frass. In the same species, dietary frass lowered serum glucose and increased cholesterol level than fish on the control diet. Hematological parameters and serum biochemistry of tilapia were not affected by dietary treatment. In both species, dietary frass enhanced serum alternative complement activity and improve disease resistance from fish pathogenic bacteria. Diets containing frass at levels 20% or more showed significantly higher survival rates against *Flavobacterium covae* than that of control catfish or fish on diets with lower levels of frass. Tilapia fed the diets containing frass showed significant dose-dependent trends in survival against both *F. covae* and *Streptococcus. iniae* challenges. Insect frass has potential

for use as a feed ingredient for enhancing palatability of feed and improving the growth of channel catfish and hybrid tilapia as well as the overall resistance of fish against pathogenic bacteria.

Annotated Bibliographies of Key Works

Romano, N., S.N. Datta, G.S.J. Pande, A.K. Sinha, F.Y. Yamamoto, B.H. Beck, C.D. Webster. 2023. Dietary inclusions of black soldier fly (*Hermetia illucens*) larvae frass enhanced production of channel catfish (*Ictalurus punctatus*) juveniles, stevia (*Stevia rebaudiana*), and lavender (*Lavaridula angustifolia*) in an aquaponic system. Aquaculture, Vol 575,2023,739742. https://doi.org/10.1016/j.aquaculture.2023.739742.

The authors for the first time examined the potential of frass to enhance fish and plant production using a dietary approach in an aquaponic system. Channel catfish (*Ictalurus punctatus*) juveniles were fed with, or without, 10% black solider fly *(Hermetia illucens*) larvae frass for 8 weeks. The authors used 2x2 factorial design, with the main effects of dietary frass inclusion and media type at two different plant bed types. Overall, the authors reported improvement on both fish growth and plant growth in an aquaponic system with dietary frass. They predicted that increased catfish growth was likely due to the upregulation of genes responsible for growth, mitigating intestinal inflammation, and significantly enhancing diet intake. Additionally, they reported that dietary frass provided more water-borne nutrients for the plants thus leading to better growth.

Romano, N., F. Yamamoto, S.D. Rawles, C.D. Webster. 2024. Type of black soldier fly (*Hermetia illucens*) larvae frass influences the nutritional value when included in a prepared diet for Mozambique tilapia (*Oreochromis mossambicus*). Aquaculture, Vol 589, 2024, 740946, https://doi.org/10.1016/j.aquaculture.2024.740946.

The authors for the first time present the nutritive value of frass from black soldier larvae fed on different substrates to Mozambique tilapia (*Oreochromis mozambicus*). They hypothesized that initial substrate would influence composition of frass and thus nutritive values to fish. They examined, frass from larvae fed on either expired fish diet (45% crude protein) or a combination of fruits/vegetable peels (9.3% crude protein) at 5% or 10% dietary inclusion level for 8 weeks. They showed that expired fish diet-based frass at 10% significantly enhanced tilapia growth compared to fish fed the control diet. Further the authors provide liver and intestine histological scoring associated with feeding dietary frass with mild liver inflammation but improve intestinal histomorphology. Implications of the initial substrate provided to the black soldier fly larvae to its nutritive value and liver and intestine histological scoring associated with feeding can be derived from the information presented in this paper.

Yildirim-Aksoy, M., R. Eljack, B.H. Beck, and E. Peatman. 2022. Nutritional evaluation of frass from black soldier fly larvae as potential feed ingredient for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture Reports. Vol 27, 2022,101353, https://doi.org/10.1016/j.aqrep.2022.101353

The authors for the first time present nutritional value and health benefits of frass derived from the larvae of black solder flies fed dried distillers' grains to shrimp. They examined inclusion of frass at

levels up to 30% in the diets of Pacific white shrimp, *Litopenaeus vannamei*. Overall, the authors showed a quadratic trend of growth with increasing dietary levels of frass with being 5% frass highest, and 30% frass lowest weight gain. Feed efficiency and body composition were also provided. Further the authors evaluate the health benefits associated with feeding dietary frass by testing serum samples for immune parameters and antibacterial activities. Implications of feeding shrimp at different levels of frass in can be found from the information presented in this paper.

Banavar, A., S.K. Amirkolaei, L. Duscher, B.H. Khairunisa, B. Mukhopadhyay, M. Schwarz, S. Urick, and R. Ovissipour. 2022. Nutritional Evaluation of Black Soldier Fly Frass as an Ingredient in Florida Pompano (*Trachinotus carolinus* L.) Diets. Animals (Basel). Vol 12:18. Pp 2407. doi: 10.3390/ani12182407. PMID: 36139267; PMCID: PMC9495079.

The authors for the first time assess the potential of frass as a dietary ingredient in a carnivorous fish species. They evaluated effect of three levels of dietary frass (8%, 12%, and 18%) on Florida Pompano (*Trachinotus carolinus* L.) growth performance and feed utilization. They suggest that pompano cannot fully utilize frass leading to the reduction in fish growth performance with increasing levels of frass. Further the authors provide the intestinal microbiome analysis that showing the highest diversity of the gut flora in the control diet, while the frass diets showed signs of community imbalance. Overall, they concluded that frass diets are not ideal for carnivorous fish diets.

Sankappa, N.M., M.D. Lange, M. Yildirim-Aksoy, R. Eljack, H. Kucuktas, B.H. Beck, J.W. Abernathy. 2024. Transcriptome analysis and immune gene expression of channel catfish (*Ictalurus punctatus*) fed diets with inclusion of frass from black soldier fly larvae. Front Physiol. 2024 Jan 9;14:1330368. doi: 10.3389/fphys.2023.1330368. PMID: 38264328;

The authors reported both systemic and mucosal tissue gene expression, especially in regard to growth and immune-related genes of channel catfish (*Ictalurus punctatus*) fed various levels of frass for 10 weeks. They analyzed liver, head kidney, gill, and intestine samples for gene expression analyses. Further, they identified differential expression of genes using targeted quantitative PCR panels for both innate and adaptive immune genes from channel catfish. Overall, the authors showed alteration of global gene expression and activation of innate and adaptive immunity of channel catfish feeding diet with frass.

Effect of Feeding Black Soldier Fly Larvae Diets on Growth and Culture Condition of Kuruma Prawn

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Despite not being commonly used, insect-based feeds are attracting significant attention as an alternative source relative to traditional fish-based diets in aquaculture industries. The insect-based feed could be a potential replacement for fish-based feed in aquaculture due to its implication in enhancing fish growth and disease resistance. The effects of different feeds used in aquaculture, including insect-based diets on the environment should be assessed because the knowledge is limited. The environmental impact assessment should be addressed in advance to implement insect-based feeds more frequently on a larger scale. To fill the knowledge gap, this research will introduce the effect of black soldier fly (BSF) *Hermetia illucens* larvae as insects-based feed on the growth and culture condition of Kuruma prawn *Marsupenaeus japonicus* instead of traditional fish-based feed.

Feeding experiments were conducted using juvenile Kuruma prawns (i.e., body weight: 106 mg±9.5 mg), using diets with 0% BSF (i.e., 100% fishmeal), 50% BSF treatment (i.e., 50% of fishmeal substituted with BSF), and 80% BSF treatment (i.e., 80% of fishmeal substituted with BSF). Each prawn was kept in a glass container/vial, and fed once every two days. The test was conducted at 20°C.

The body weight of the prawns in the 50% BSF was significantly higher than that in the 0% BSF after one week. There was no significant difference between the growth in the 0% BSF and the 80% BSF. The concentration of dissolved inorganic nutrients in seawater was measured in each test plot during a one-week feeding period. The concentration of dissolved ammonium nitrogen was lower with increasing BSF content ($p<0.05$). On the other hand, phosphate concentrations tended to be higher in BSF treatments, but no significant difference was found between 0% BSF (100% fish meal) and 50% BSF treatment, where higher prawn growth was documented.

Oxidative conditions of the bottom sediment are important for monitoring the benthic organisms such as, Kuruma prawn. Therefore, we monitored the redox potential of the bottom sediment in real time using the method we have reported previously [1]. The redox potentials were found to be highest in 80% BSF, lowest in 0% BSF, and 50% BSF found in the intermediate level (i.e., 80% BSF>50% BSF>0%).

These results signify that for prawn culture 50% BSF can be a potential alternative to fish meal,

increasing the growth with low environmental impact.

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Annotated Bibliography of Key Works

Shono, N., Ito, M., Umezawa, A., Sakata, K., Li, A., Kikuchi, J., Ito, K., & Nakamura, R. (2022). Tracing and regulating redox homeostasis of model benthic ecosystems for sustainable aquaculture in coastal environments. *Front. Microbiol.,* 13, 907703. https://doi.org/10.3389/fmicb.2022.907703.

Aquaculture in coastal environments has an increasingly important role in the world's food supply; however, the accumulation of organic compounds on seafloors due to overfeeding harms benthic ecosystems. To assess the ecological resilience of aquafarms to nutrient influx, we investigated the redox homeostasis of benthic ecosystems using a marine oligochaete as a model benthic organism in aquaculture fields. Real-time monitoring of the redox potential of a model benthic ecosystem constructed in an electrochemical reactor allowed evaluation of the homeostatic response of the system to nutrient addition. Although the detrimental effects of overfeeding were confirmed by irreversible potential changes in the sediment, redox homeostasis was reinforced through a cooperative relationship between oligochaetes and sediment microorganisms. Specifically, the oligochaetes exhibited reversible changes in metabolism and body position in response to dynamic changes in the sediment potential between -300 and 500 mV, thereby promoting the decomposition of organic compounds. The potential-dependent changes in metabolism and body position were reproduced by artificially manipulating the sediment potential in electrochemical reactors. Given the importance of benthic animals in sustaining coastal ecosystems, the electrochemical monitoring and physiologic regulation of marine oligochaetes could offer an intriguing approach toward sustainable aquaculture.

Current Status of Artificial Seed Production and Selective Breeding in the Japanese Yellowtail *Seriola quinqueradiata*: The Progress Achieved by FRA

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The Japanese yellowtail *Seriola quinqueradiata* is one of the most important commercial fish in Japan. The cultivation of yellowtail has been practiced since the 1920s, with naturally caught juveniles in the Pacific coast of southern Japan. However, the catch of juveniles is occasionally not stable probably due to environmental factors. Besides, as the best time to harvest and sell the yellowtail cultivated from the juveniles, which can be obtained only in early spring, is from late fall to winter, the distribution quantity of the yellowtail in the market significantly decreases from May to September. Hence, artificial seeds should be employed to overcome these problems, and they are currently being used.

In this situation, a family lineage or strain with fast growth and disease resistance traits would be worthful. Therefore, the production of strain via selective breeding and popularization of artificial seed production has been eagerly desired in Japan.

In FRA, the seeds obtained via artificial spawning were raised to approximately 5 cm and then cultivated to harvest size in aquaculture farms. Thereafter, we randomly selected 2,000 adults (approximately 60 cm) and obtained the fish length, body weight data, and fin chip for pedigree reconstruction from the individuals. Finally, based on estimated breeding values using pedigree-based BLUP and inbreeding coefficients, 200 individuals were selected as broodstock candidates. Thus far, the correlations of breeding value between the first and second generations were shown in both fish length and body weight, showing that our breeding program is promising.

Annotated Bibliography of Key Works

Shimada, Y., Yoshida, K., Ozaki, A., Hotta, T., Noda, T., Akita, K., Chujo, T., Shinoda, R., Fujinami, Y., Okouchi, H., Oda, K. and K. Okuzawa. 2019. Evaluation of family composition and genetic diversity of artificial seedlings in Yellowtail *Seriola quinqueradiata*, and estimation of heritabilities of growth-related traits at early developmental stage. *Fish Genet. Breed. Sci.* Vol:49(1). pp 7-18.

To investigate the family composition of artificial seedlings in the yellowtail *Seriola quinqueradiata* and estimate the genetic factors for growth-related traits, we produced 30 families from more than 2,000 samples collected at different developmental stages. Heritability was estimated for total length, body weight, and condition factor as 0.375, 0.335, and 0.098, respectively, and they were all significantly higher than zero. The authors showed that it is important to understand the family composition of broodstocks to control inbreeding coefficient and adopt a sustainable breeding program for yellowtail.

Population Structure and Selective Breeding Program for the Growth of Farmed Rainbow Trout (*Oncorhynchus mykiss*) in Japan

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Rainbow trout (*Oncorhynchus mykiss*) is one of the most cultured salmonid species worldwide. Selective breeding programs of rainbow trout targeting growth rate and disease resistance have successfully produced rainbow trout with improved heritable traits that benefit aquaculture production. Native to North America, this species has been introduced multiple times to Japan since 1877. In Japan, it has been traditionally cultured in freshwater and, more recently, saltwater. However, the genetic background of Japanese rainbow trout populations remains poorly understood because of multiple introductions, subsequent breeding within populations, and local adaptation to rearing environments. To investigate the genetic diversity and population structure, we collected tissue samples from 24 rainbow trout strains cultured in Japan. Using a 57K SNP array, we verified its effectiveness in the Japanese-farmed rainbow trout population. Assessments of genetic diversity using various indices indicated restricted genetic variation within each strain. Population structure analyses demonstrated that each strain clustered separately, suggesting they were genetically isolated populations. To explore the potential for selective breeding in Japanese farmed rainbow trout, we first established a base breeding population by crossing nine strains of Japanese farmed rainbow trout. We evaluated the heritability and prediction accuracy of estimated breeding values for growth traits in both freshwater and seawater environments. Heritability estimates for body weight were moderate, indicating potential for genetic improvement. When comparing the prediction accuracy of genomic BLUP (GBLUP) to pedigree-based BLUP, GBLUP consistently outperformed pedigree-based BLUP, with the highest accuracy increase (+10%) observed for body weight.

Annotated Bibliography of Key Works

Paul, K., Restoux, G., and F. Phocas. 2024. Genome-wide detection of positive and balancing signatures of selection shared by four domesticated rainbow trout populations (*Oncorhynchus mykiss*). *Genetics Selection Evolution*, Vol:56(1). pp 1-20.

This study provides insights into the genetic basis of domestication in rainbow trout. The aim of this work is to discover genomic regions with a high level of homozygosity (positive selection) or heterozygosity (balancing selection). Researchers studied four genetically distinct populations and found 13 regions under selection. These regions contained numerous genes that were involved in essential biological functions.

Bernard, M., Dehaullon, A., Gao, G., et al. 2022. Development of a high-density 665 K SNP array for rainbow trout genome-wide genotyping. *Frontiers in Genetics*, Vol: 13. pp. 941340.

This study describes the development of a high-density SNP array (HD chip) for rainbow trout. The authors used variant datasets from USDA, a French commercial line and INRAE isogenic lines. The 664,531 SNPs on the HD chip successfully genotyped 463 individuals across two French commercial populations. The high-density nature of the 665K SNP array enables researchers to conduct more detailed studies, including population structure analysis, GWAS or genomic selection.

Uchino, T., Tabata, J., Okamoto, H., et al. 2021. Population structure analysis using SNP-chip in Japanese hatchery rainbow trout (*Oncorhynchus mykiss*). *Fish Genetics and Breeding Science*, Vol:50(1 of 2). pp. 37-51.

This study aimed to investigate the genetic diversity and population structure of Japanese farmed rainbow trout using a 57K SNP array. The results provide valuable information for selective breeding of Japanese rainbow trout.

Advancing Monosex Breeding Technology for Sablefish (Gindara) Aquaculture

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The process of sexual differentiation (i.e., development of ovaries or testes) in fishes is much more plastic than it is in humans or other mammals. Even in fish species for which genetic sex determination systems have been well described, exogenous factors such as high temperature or steroid exposure may override those genetic controls and induce sex reversal–a mismatch between genetic and phenotypic sex. Sablefish *Anoplopoma fimbria*, also known as black cod (gindara in Japan), is an important U.S. fishery and emerging marine aquaculture species, and a focal species for intensive aquaculture research & development at NOAA. Upon initiating our work with sablefish, it was recognized that it exhibits sexually dimorphic growth, with females growing significantly faster and attaining a larger body size than males, a phenomenon seen in many other species as well. This led to a major goal to develop a non-GMO method to produce monosex female (100% female) sablefish stocks for aquaculture to capitalize on the faster growth of females. A series of studies was conducted to gain an understanding of the basic processes of sex determination and differentiation (e.g., When do the gonads differentiate? What molecular and morphological changes in the gonads signal its occurrence?) and to develop an effective method for feminization of sablefish populations. The labile period of sex differentiation was successfully characterized and indirect feminization was achieved: First, sex differentiation of XX-genotype fish was redirected towards testicular development instead of ovarian development using dietary 17 alpha-methyltestosterone (MT) treatment during the labile period. Second, putative neomale (i.e., XX-genotype male) broodstock were obtained and ultimately bred with normal female broodstock. Offspring resulting from neomale x female crosses were 100% female, while those from control male x female crosses were ~50% female. Sperm from neomale sablefish broodstock could then be routinely utilized to generate all-female populations without the use of hormones, allowing for evaluation of their performance in an aquaculture setting. A semi-commercial scale trial was conducted with industry and academic partners which highlighted the importance of this monosex breeding technology to the economics of sablefish aquaculture production, including reducing time to harvest. Current research seeks to address common reproductive problems observed in neomale sablefish broodstocks through development of more natural alternatives to MT treatment. In summary, the advancement of monosex breeding technology for sablefish not only enhances the economic viability of sablefish aquaculture but also sets a precedent for sustainable practices in industry. The successful development of hormone-free methods to produce all-female populations represents a significant step toward optimizing growth performance while minimizing potential environmental impacts. This research contributes valuable insights that can be applied to other aquaculture species, supporting the industry's shift toward more sustainable and efficient production practices.

Annotated Bibliography of Key Works

Martínez, P., Vinas, A.M., Sanchez, L., Diaz, N., Ribas, L., and F. Piferrer (2014). Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. Frontiers in Genetics 5, 340. https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2014.00340/full

This article reviews the state-of-the-art for fish sex determination and approaches to control sex ratios in aquaculture in order to capitalize on enhanced performance characteristics of either females or males. The primary characteristic of interest in aquaculture is growth, which may differ between sexes. This phenomenon, known as sexually dimorphic growth or sexual growth dimorphism, is common in fishes and may greatly influence the time to harvest, uniformity of harvested fish, or other aspects of aquaculture. The authors begin with an introduction on the main features of early gonadal development, including the processes of sex determination and gonadal sex differentiation (i.e., differentiation of the gonads into either ovaries or testes), as well as gonadal gene expression and morphological changes observed in most fish species (summarized in Fig. 1). They also highlight the complex interplay between environmental and genetic factors that influence sex determination, with particular focus on how manipulating environmental conditions (e.g., temperature, hormones) or employing genetic breeding schemes can be used to control sex ratios. These approaches are of particular interest for improving productivity in aquaculture settings, where the selective production of a single sex can lead to faster growth rates or other desirable traits. A number of examples of sex-control strategies for various species, such as turbot, European sea bass, and tilapia are provided. Finally, the authors discuss the potential ethical and ecological implications of these methods, offering a comprehensive view of both the benefits and challenges of sex ratio manipulations in aquaculture.

Luckenbach, J.A, Kikuchi, K., Iwamatsu, T., Nagahama, Y., and R.H. Devlin (2023). The lasting impact of Toki-o Yamamoto's pioneering chapter on fish sex determination and differentiation: A retrospective analysis of its contributions to reproductive biology and influences on aquaculture and fisheries sciences. In: D.J. Randall, A.P. Farrell, C.J. Brauner, and E.J. Eliason, eds. Fish Physiology Vol 40A, Chapter 11, pp. 401-419.
https://www.sciencedirect.com/science/article/abs/pii/S1546509823000031?via%3Dihub

This chapter, developed in collaboration with Japanese and Canadian colleagues, provides a retrospective analysis of Dr. Toki-o Yamamoto's foundational work on fish sex determination and its continuing influence on modern aquaculture practices. While honoring Yamamoto's 1969 chapter in the famous *Fish Physiology* book series edited by Hoar and Randall, the authors place significant emphasis on how Yamamoto's research has informed current strategies for sex control and genetic selection in aquaculture. The text outlines key advancements in understanding the genetic and environmental mechanisms governing fish sex differentiation, offering insights into the development of techniques such as hormonal sex reversal and breeding of monosex populations. These methods, derived from Yamamoto's early findings, are critical for enhancing fish production in aquaculture by enabling the manipulation of sex ratios to produce all-male or all-female stocks, depending on the desired traits (e.g., faster growth rates in one sex). The chapter also highlights the application of these approaches in various species and the potential for integrating genetic selection techniques to improve growth performance and disease

resistance. Overall, the analysis underscores the enduring relevance of Dr. Yamamoto's work for optimizing genetic outcomes in aquaculture, making it a good resource for researchers and practitioners focused on sustainable fish production.

Luckenbach, J.A., Fairgrieve, W.T. and E.S. Hayman (2017). Establishment of monosex female production of sablefish (*Anoplopoma fimbria*) through direct and indirect sex control. Aquaculture 479, 285-296. https://www.sciencedirect.com/science/article/abs/pii/S0044848617306361

This paper focuses on the development of methods for both direct and indirect feminization of sablefish populations for aquaculture and establishes for the first time that sablefish have an XX/XY system of sex determination. For direct feminization, the authors administered estradiol-17 beta (E2) via the diet starting just after weaning for two or four months. They found that E2 treatment successfully feminized genetic males, as fish sampled at both time points all developed ovaries. For indirect feminization, 17 alpha-methyltestosterone (MT) was used, with the intention to masculinize genetic females. After four months of MT treatment, fish had all developed testes, while a two-month treatment resulted in intersex gonads, indicating incomplete sex reversal. Once sexually mature, the "neomales" (genetic females with masculinized traits) were crossed with females and their offspring were all female, confirming that sablefish have an XX/XY sex determination system. The study demonstrates that indirect feminization using neomales can yield all-female populations of sablefish for aquaculture without the need for hormone treatment of food fish. This approach, which avoids direct hormone treatment of market fish, offers significant advantages to the aquaculture industry by improving production and ensuring consumer safety.

Hartley M.L., D.M. Schug, K.F. Wellman, B. Lane, W.T. Fairgrieve, and J.A Luckenbach (2020). Sablefish aquaculture: An assessment of recent developments and their potential for enhancing profitability. NOAA Technical Memorandum NMFS-NWFSC-159.
https://doi.org/10.25923/cb0y-n468

This article provides a comprehensive assessment of the history of sablefish aquaculture and recent advancements in research, and evaluates their potential to improve the profitability of the industry. The authors, some of which are economists, summarize technological innovations and biological research aimed at overcoming key production challenges, such as feed efficiency, broodstock management, and disease resistance. In particular, they emphasize developments in monosex female sablefish production, and the use of novel feeds, which have the potential to reduce production costs and enhance growth rates. The authors report findings from the first economic simulation of monosex (all female) compared to mixed-sex (both females and males) grow-out for sablefish, which demonstrated a significant increase in the internal rate of return to growers. Additionally, the article explores market factors, such as consumer demand and pricing trends, offering insights into how improved production practices could meet market needs and increase profitability. This resource is important to stakeholders seeking to enhance the sustainability and economic viability of sablefish farming. Many of the principles therein may apply broadly to aquaculture of other fish species.

Developing Vaccination Strategies for Prevention of Atypical Furunculosis in Sablefish *Anoplopoma fimbria*

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Sablefish (black cod) represent a promising and high-value species for marine aquaculture. Research efforts to optimize culture strategies and methods have been ongoing for more than two decades. This species is commercially produced using land-based RAS in combination with net-pen grow out in BC, Canada. To set the stage for expanded production of farmed sablefish in the US, NOAA Fisheries has prioritized research projects and partnerships to address remaining challenges. One such challenge that impacts production of this species is the disease furunculosis. This is currently the primary disease threat for sablefish and is caused by atypical strains of *Aeromonas salmonicida*, a gram-negative bacterium. Although antibiotic treatments can be administered to reduce mortality following an outbreak, disease prevention through vaccination is desirable and has been identified as a high-priority need for further development of black cod aquaculture. Recent vaccination projects that have explored practical delivery strategies (oral and immersion) to prevent furunculosis will be highlighted. These include an oral vaccination study that assessed the potential for utilizing a simple killed *A. salmonicida* vaccine administered orally via feeding alginate/gelatin micro-particles to juvenile fish. Another ongoing project involves the development and testing of attenuated atypical *A. salmonicida* strains that could be administered via immersion during early juvenile stages. To produce such attenuated vaccine candidates, known virulent *A. salmonicida* strains were repeatedly passaged on TSA plates containing increasing concentrations of the antibiotics rifampicin and novobiocin. Three isolates that grew in high concentrations of these antibiotics have been confirmed as fully attenuated. These three vaccine candidates are being assessed for their potential to protect sablefish from disease following immersion or injection immunization. Finally, results from another study that assessed the efficacy and adjuvant effect following a primary immersion immunization and injection booster will be discussed. This delivery strategy represents the most practical approach currently available to provide long-term protection of sablefish from furunculosis.

Annotated Bibliography

Goetz FW, Anulacion BF, Arkoosh MR, et al. Status of sablefish, *Anoplopoma fimbria*, aquaculture. *J World Aquac Soc.* 2021;1–40. https://doi.org/10.1111/jwas.12769

This is a key review article on sablefish, *Anoplopoma fimbria* (also called black cod), which is a long-lived marine species that is found in the Pacific from Baja California to Alaska, the Bering Sea, and through to the eastern coast of Japan. The value and feasibility of commercial aquaculture development along with important research needs are discussed. Advances in many research areas have been significant over the last 20 years and there are a few companies producing sablefish. Research advances include early life stage rearing along with production of all-female monosex offspring that grow faster than male or mixed sex populations.

Econometric models suggest that internal rates of return are 11–15% higher for monosex relative to mix-sex stocks over a 10-year period under typical cage culture conditions. Work showing that sablefish are susceptible to diseases (furunculosis and vibriosis) brought on by atypical *Aeromonas salmonicida* and *Vibrio anguillarum* is highlighted, but commercial vaccines (developed for salmonids) are only protective when given by injection. Long-term protection offered by vaccination has not been defined. Key takeaways from this paper are that more research is needed in relation to effective vaccine development and that improvements in methods for vaccine delivery would be beneficial.

Arkoosh MR, Dietrich JP, Rew MB, Olson W, Young G, Goetz FW. Exploring the efficacy of vaccine techniques in juvenile sablefish, *Anoplopoma fimbria*. *Aquac Res*. 2017;00:1–12. https://doi.org/10.1111/are.13449

This paper evaluates delivery strategies (immersion and injection) and tests multivalent vaccines for their ability to protect against atypical furunculosis for sablefish. Sablefish vaccinated by immersion at ~1.5 or ~4.5 g with a whole-cell multivalent vaccine were not protected against either typical or atypical *Aeromonsa salmonicida*. However, the relative percent survival (RPS) or potency of the whole-cell multivalent vaccine injected i.p. in juvenile sablefish at ~50 g against typical and atypical *A. salmonicida* was 94.3% and 81.7%, respectively. The high RPS values indicated that the vaccine successfully initiated an immune response in sablefish.

Ignacio Vasquez, Trung Cao, Ahmed Hossain, Katherinne Valderrama, Hajarooba Gnanagobal, My Dang, Robine H.J. Leeuwis, Michael Ness, Briony Campbell, Robert Gendron, Kenneth Kao, Jillian Westcott, A. Kurt Gamperl, Javier Santander, *Aeromonas salmonicida* infection kinetics and protective immune response to vaccination in sablefish (*Anoplopoma fimbria*), *Fish & Shellfish Immunology,* Volume 104, 2020, Pgs 557-566, ISSN 1050-4648, https://doi.org/10.1016/j.fsi.2020.06.005

This study addresses the need for effective vaccine programs against *Aeromonas salmonicida*, which have been identified as a high priority area for sablefish (*Anoplopoma fimbria*) aquaculture. This study established an *A. salmonicida* infection model to evaluate commercial vaccines and an autogenous vaccine preparation. Using a clinical isolate of *A. salmonicida* (J410) they estimated a median lethal dose (LD_{50}) of ~3×10^5 CFU/dose, and determined that the relative percent survival (RPS) for the autogenous bacterin mix was 65.22%, for commercial Forte Micro 4® vaccine it was 56.52%, and for Alpha Ject

Micro 4® it was 30.43%. The RPS trends agreed with *A. salmonicida* tissue colonization levels at 10 days post-challenge. They measured total IgM titers, which peaked at 6–8 weeks post-immunization, but determined that the *A. salmonicida* A-layer binds to immunoglobulins F(ab)' in a non-specific fashion and affects immune assays and potentially vaccine efficacy. These results show that vaccine design influences sablefish immunity and provides a guide for sablefish vaccine programs.

Jones, E.M., Oliver, L.P., Ma, J., Leeuwis, R.H.J., Myrsell, V., Arkoosh, M.R., Dietrick, J.P., Schuster, C.M., Hawkyard, M., Gamperl, K.A. and Cain, K.D. 2022. Production of a monoclonal antibody specific to sablefish *(Anoplopoma fimbria*) IgM and its application in ELISA, western blotting, and immunofluorescent staining. *Fish and Shellfish Immunology*, 130 (2022) 479–489, https://doi.org/10.1016/j.fsi.2022.09.038

This work addresses the issues with polyclonal antibodies and their limitations for important assays designed to monitor specific antibody kinetics or identify important immune cells in tissues. Sablefish (*Anoplopoma fimbria*) are an emerging aquaculture species and such new tools are needed to determine antibody response following vaccination or disease outbreaks. In this paper, a monoclonal antibody, UI-25A, specific to sablefish IgM was produced in mice. Western blotting confirmed that UI-25A recognizes the heavy chain of IgM and does not cross-react to proteins or carbohydrates in serum of four other teleost species. An ELISA was developed to measure *Aeromonas salmonicida* specific IgM in the plasma of sablefish, and UI-25A was used in Western blot analyses to identify immunogenic regions of *A. salmonicida* recognized by IgM from vaccinated sablefish. Immunofluorescent staining also demonstrated the ability of UI-25A to recognize membrane-bound IgM and identify IgM + cells (presumably B cells) in sablefish head kidney. Results demonstrate the usefulness of UI-25A as a tool to improve the understanding of antibody-mediated immunity in sablefish. This product will be valuable for vaccine development and the expansion of sablefish aquaculture efforts.

Hygiene Management Is Important to Prevent Red Sea Bream Iridovirus Transmission Between Net Pens: Insights from a Case Study that Assessed Cross-Contamination

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Red sea bream iridovirus, which is currently listed as a notifiable disease by WOAH, has caused significant economic damage in Japanese mariculture since the 1990s. Although formalin-inactivated vaccines are commercially available to control RSIV outbreaks, fish farmers do not use the vaccine when the vaccination cost is not acceptable compared to the market value of cultured fish. Therefore, basic biosecurity management such as hygiene procedures could be important to control RSIV outbreak. Nevertheless, in the case of semi-open system aquaculture, the hygiene procedures have been considered less effective than land-based aquaculture because net pens or cages have no physical barrier to prevent moving pathogens via environmental water between net pens. Our latest study suggested that RSIV transmission via seawater is highly associated with the distance between net pens and that the environmental water could function as a potential barrier to prevent viral transmission. Hence, we hypothesized that hygiene management effectively reduces the risk of RSIV transmission even in semi-open system aquaculture where environmental water can move freely.

In the present study, we assessed risk factors for transmission of RSIV between net pens in a case study to identify effective hygiene management in semi-open system aquaculture. Cross-contamination of RSIV in gears and facilities in a fish farm was investigated by surface swabbing tests and an environmental DNA (eDNA) technique. Our investigation during the RSIV outbreak in the fish farm demonstrated that landing nets and gloves associated with collecting dead fish (carcasses) were highly contaminated with RSIV. Based on the results, we proposed mitigation measures against RSIV transmission between net pens to the fish farm and the following actions were initiated. A daily operation for collecting dead fish started from the net pens where the disease had not occurred and moved to the net pen where RSIV outbreak occurred to ensure that RSIV was not transmitted to the other net pens by cross-contamination. In addition, the landing nets used for collecting fish carcasses was disinfected at the end of each day to avoid carryover of the virus to the following day. As a result, RSIV was not transmitted to the other net pens in the fish farm for more than 30 days. However, once the RSIV outbreak occurred in the net pen upstream in the operation for collecting dead fish, RSIV was transmitted to all net pens in 1 week, suggesting that the transmission was caused by cross-contamination. This study

indicated that appropriate hygiene management is important to reduce the risk of RSIV transmission between net pens, even in semi-open system aquaculture.

This study was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (grant number JPJ007159).

Annotated Bibliography of Key Works

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

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

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

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

Kawato, Y., Takada, Y., Mizuno, K., Harakawa, S., Yoshihara, Y., Nakagawa, Y., Kurobe, T., Kawakami, H., Ito, T., 2023. Assessing the transmission risk of red sea bream iridovirus (RSIV) in environmental water: insights from fish farms and experimental settings. *Microbiol Spectr*, 11:e0156723.

This study aimed to understand the actual transmission risk of RSIV through environmental water among fish farms. The results indicated that the viral loads in the seawater were low, except for the net pens where RSIV outbreaks occurred. Furthermore, our experimental infection model indicated that the infection risk of RSIV-contained seawater with less than 10^3 copies/L was extremely low. These results suggest that the transmission of RSIV among fish farms via seawater is highly associated with the distance between the net pens, and the environmental water is not always an infection source for the transmission of RSIV between fish farms.

Kawato, Y., Mizuno, K., Harakawa, S., Takada, Y., Yoshihara, Y., Kawakami, H., Ito, T., 2024. Risk assessment of wild fish as environmental sources of red sea bream iridovirus (RSIV) outbreaks in aquaculture. *Dis Aquat Organ*, 158:65–74.

RSIV in wild fish near aquaculture installations was surveyed to evaluate the risk of wild fish being an infection source for RSIV outbreaks in cultured fish. In total, 1102 wild fish, consisting of 44 species, were captured from 2 aquaculture areas in western Japan between 2019 and 2022. Eleven fish from 7

species were confirmed to harbor the RSIV genome using a real-time PCR assay. Based on the diagnostic records of RSIV in the sampled area of wild fish, the RSIV-infected wild fish appeared during or after the RSIV outbreak in cultured fish, suggesting that RSIV detected in wild fish was derived from the RSIV outbreak in cultured fish. Therefore, wild fish populations near aquaculture installations may not be a significant risk factor for RSIV outbreaks in cultured fish.

Disease Control Measures in Hirame Juvenile Hatchery: The Case of Hirame Aquareovirus

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Hirame or Japanese flounder *Paralichthys olivaceus* is a widely produced fish species, mainly for stock enhancement, in Japan. However, outbreaks of various diseases, such as viral nervous necrosis, birnaviral infection, and bacterial enteritis, have been impacting production of hirame juveniles for stocking. Besides, mass mortality events associated with reovirus-like pathogens have been reported in the hirame hatcheries since the 2000s, which drastically hampered the juvenile production. Our research team identified the causative agent as hirame aquareovirus (HAqRV) and developed several diagnostic tools. Through our work, we demonstrated that HAqRV was highly pathogenic to hirame juveniles, and the intestinal tract was the main target organ of the virus. Furthermore, we obtained data suggesting that the vertical transmission from broodstock to juveniles was a primary transmission route, resulting in mass mortality events in juveniles.

Wild fish are used as broodstock to maintain genetic diversity. Our previous data revealed that HAqRV was detected in approximately 30% of the wild fish caught for the broodstock. In general, hirame broodstocks are maintained in a tank throughout the year so that we speculated that most of the broodstock are horizontally infected with HAqRV and become carriers of the viral agent. The risk of vertical transmission is increased by this process as well. Fertilized eggs, naturally spawned in a broodstock tank, are collected from the water, and hence, the fertilized eggs could be contaminated by HAqRV released from carrier fish. Based on these presumptive risks, we developed a series of control measures against the HAqRV infection.

Firstly, we developed a sublethal sampling method for broodstock to detect and eliminate heavily virus-infected broodstock. A medical swab was used to collect intestinal epithelium and mucus in the digestive tract by inserting it from the anal, allowing the virus testing without sacrifice the broodstock. Secondly, we determined the optimal condition for disinfection of egg surfaces with electrolyzed seawater. HAqRV was demonstrated to be inactivated by the electrolyzed seawater at an oxidant concentration of 0.25 mg/mL for 1 minute, while hirame fertilized eggs were stable and showed little impact on hatching success at an oxidant concentration of 0.75 mg/mL for 5 minutes. The results

suggest that the virus-contaminated fertilized eggs can be decontaminated by electrolyzed seawater without disrupting embryonic development. These newly developed methods have been incorporated into a conventional disease control protocol, such as use of UV-treated seawater and appropriate zoning in hatcheries. We confirmed that HAqRV outbreak was successfully prevented by the application of the control measures, indicating that the measure significantly reduced the risk of HAqRV transmission in a hatchery.

Annotated Bibliography of Key Works

Nishioka, T., Furusawa, T., and Younosuke Mizuta Y. 1997. Diseases Occurring in Marine Fish and Shellfish Hatcheries in Japan (1989-1994). *Aquacult. Sci.* 45(2): 285-290.

This valuable paper summarizes diseases that have occurred in juvenile hatcheries in Japan. The highest number of cases was reported in flatfish, and the diseases that occur are diverse.

Nishioka, T., Fujimoto, H., Oka, M., and Arimoto, M. 2009. Diseases of Marine Fish and Shellfish in Hatcheries in Japan. *J. Fish. Sci. Technol*. 2(1): 57-65.

As above article, this paper also summarized diseases that have occurred in juvenile hatcheries in Japan. The highest number of reports remained in hirame, indicating that the diseases of hirame continue to be a problem.

Kawato, Y., Mekata, T., Nishioka, T., Kiryu, I., Sakai, T., Maeda, T., Miwa, S., Koike, K., Sadakane, M., and Mori, K. 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 Japanese flounder (hirame) and determined complete genome of this virus. A comparison of the entire genome between the new virus and other aquareoviruses suggested that HAqRV is likely a new aquareovirus species. Virulence of HAqRV was demonstrated by experimental infection using hirame juveniles and HAqRV were reisolated from diseased fish. In immunohistochemistry, syncytial cells in intestinal tract and liver of the experimentally infected fish were stained with antiserum against HAqRV.

Kawato, Y., Maeda, T., Nishioka, T., Kiryu, I., Mekata, T., Matsuyama, T., Tensha, K., Yamashita, I., Kawamura, Y., Raku, A., Senbokuya, K., Yanagi, S., Hayashi, K., Kumagai, A., and Mori, K. 2022. Asymptomatically Infected Broodstock are a Potential Infection Source for Aquareovirus Outbreaks in Hatchery-reared Japanese Flounder *Paralichthys olivaceus*. *Fish Pathol*. 57(1): 11-19

This paper estimates the route of infection in hirame Japanese flounder hatcheries where outbreaks occurred due to HAqRV. Quantitative PCR analysis of organs from broodstock revealed that more viral genes were detected in the intestinal tract than in the gonads. In addition, the viral sequences from broodstock and juveniles died by HAqRV were consistent among facilities. These results suggest that vertical transmission was the primary route of infection, and the virus released from the intestinal tract of the broodstock might be the source of infection.

Opportunities and Challenges for Alaska Kelp Aquaculture

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Kelp aquaculture is a nascent and growing industry in Alaska where cold, nutrient-rich, and uncontaminated waters result in high quality biomass. There is considerable support from regional and federal initiatives to expand the blue economy sector, and in Alaska much of that support is focused on kelp and shellfish aquaculture. This presentation will review the opportunities, challenges, and ongoing research in three select topics that are key to sustainable industry expansion: (1) aquaculture site suitability, (2) ecosystem interactions with aquaculture, and (3) kelp stabilizing and processing. Regarding site suitability, there are ample opportunities for aquaculture expansion in the extensive coastal zone of Alaska, however low population densities and vast distances between inhabited communities result in little infrastructure and logistical challenges for both cultivating kelp and getting it to target markets. Investigations are underway that will inform optimized farm placement from both marine spatial planning and organismal physiology perspectives. Ecosystem interactions can constitute a service or a detriment depending on the nature of the interaction, which may also vary spatially and temporally. We are conducting research to understand how species of interest are interacting with kelp aquaculture, and developing *in situ* strategies and permitting tools to maximize benefits while minimizing interactions seen as harmful, such as Pacific herring spawning on cultivated kelp. Finally, stabilizing cultivated kelp in a cost effective manner has proved challenging in Alaska's cold and wet climate. Research is underway to develop processing methods suitable to the climate and economies of Alaska, and local products are being developed to incorporate kelp into local manufacturing that may require less transport and stabilization. The expansion of the kelp aquaculture industry is full of exciting possibilities and formidable challenges. Knowledge exchanges with regions that have mature kelp aquaculture industries, such as Japan, will likely aid in sector growth in Alaska.

Annotated Bibliography of Key Works

1. General overview of US kelp aquaculture industry development:

Kim J, Stekoll M, Yarish C. 2019. Opportunities, challenges and future directions of open-water seaweed aquaculture in the United States. Phycologia 58:446–461. Taylor & Francis.

This paper provides an overview of research and development of seaweed aquaculture in different regions of the US from the 1970s to the 2010s, including challenges and lessons learned by species and region. The initial motivation for seaweed aquaculture development was for energy production in the 1970s by converting cultivated *Macrocystis pyrifera* biomass (California) and *Saccharina latissima* biomass (New York) to methane; the program was discontinued in 1991 due to declining concerns about available oil and gas. Cultivation of *M. pyrifera* was also pursued in Alaska in support of the herring roe-on-kelp fishery, though relocation of the fishery to Southeast Alaska halted those efforts as cultivation

was no longer necessary for access to desired kelp. Knowledge exchange with Japanese experts in the 1980s resulted in successful attempts to cultivate *Pyropia* spp. in Washington, including endemic-US and Japanese species, however social resistance stymied industry establishment. Attempts in Maine to grow *Py. yezoensis* resulted in hatchery development in the 2000s but progress stalled at grow-out due to environmental conditions that did not favor the Japanese species, though efforts may have resulted in accidental naturalization of the species to Long Island Sound. The conchocelis phase was successfully cultivated for *Py. torta* in Alaska in the 2000s along with some successful outplants, however obstacles in scaling up production and remaining questions regarding site suitability meant commercialization was never reached. Recent attempts at kelp cultivation started again in the 2010s with the successful commercial production of *S. latissima, Laminaria digitata,* and *Alaria esculenta* in Maine, now known as Atlantic Sea Farms. *Gracilaria tikvahiae* was also cultivated in the US Northeast, though scaling up of this species is difficult as the summer growing season competes with other industries and uses of the nearshore coastal zone. In addition to food production, kelp aquaculture in the northeast is also in support of nutrient remediation. Kelp in this region is processed and stabilized by freezing. In Alaska, commercial kelp farming began in 2015 with successful trials of *S. latissima* and *A. marginata.* Additional species cultivated initially included *Nereocystis luetkeana* and *Eualaria fistulosa*. In order to minimize potential impacts to wild kelp populations, regulations require that all sorus material be collected within 50 km of the farm site and from 50 different fertile individuals; selective breeding is prohibited. Future steps for macroalgae aquaculture likely include developing off-shore cultivation potential for bioenergy production, gametophyte banking to develop temperature-tolerant strains of kelp (especially in the Northeast), and expansion to other non-kelp species.

2. Site suitability:

Stephens T, Li Y, Yarish C, Rogers MC, Umanzor S. 2024. Does Seawater Nitrogen Better Predict the Baseline Farmed Yield for Sugar Kelp (Saccharina latissima) Rather than the Final Yield? *Phycology* 4:370–383.

Seawater nutrient levels are often used in site selection for kelp aquaculture, therefore this study assessed the influence of seawater nitrogen availability on the yield of *Saccharina latissima* across 5 farms on the US East (4) and West Coasts (1) over two seasons. Nutrient samples (NO_3^-, NH_4^+, and total N) were collected starting in March, and final biomass (kg/m) data were collected at time of commercial harvest, between April and June. Tissue samples were also collected at the tip, mid-section, and base of fronds, and analyzed for carbon-to-nitrogen molar ratios as proxies for the nutritional status and overall health of the kelp. They found that seawater nitrate and total N varied across time and space, and that time of harvest was influenced by total N. While linear regression indicated a weak relationship between total N and harvested biomass, quartile regression showed a stronger relationship at the 10th percentile relative to the median, indicating a potential threshold effect. Total N and line spacing together explained 50% of the variation. Therefore, seawater nitrogen may be a more useful metric in predicting baseline kelp yields (lowest 10th percentile for the expected yield) rather than realized yields, and production above that baseline is likely more dependent on other factors that may or may not interact with seawater nitrogen, such as farm design or local flow environment. Other factors that were not measured but likely influenced

the results were temperature, light, and the genetics of the parental stock. General site suitability recommendations presented by the authors based on their findings include: (1) selecting sites where the total seawater nitrogen does not dip below 0.6–1.0 μM during the farming season and (2) when farming in a site that experiences seasonal nitrogen limitation, increase the spacing between the grow lines to maximize the flow and nutrient transfer (1.5 m in moderate-flow sites; 3 m in low flow sites).

3. Ecosystem interactions:

Theuerkauf SJ, Barrett LT, Alleway HK, Costa-Pierce BA, St. Gelais A, Jones RC. 2022. Habitat value of bivalve shellfish and seaweed aquaculture for fish and invertebrates: Pathways, synthesis and next steps. *Reviews in Aquaculture* 14:54–72.

This paper is a review and meta-analysis to understand the habitat effects of shellfish and seaweed aquaculture. Mechanisms of habitat impact of aquaculture identified by the authors include disease transmission risk; disadvantage of species associated with soft-sediment, such as wading birds; and reduction in submerged aquatic vegetation. Aquaculture infrastructure can also reduce nutrient levels and increase water clarity, and provide habitat to organisms drawn to hard- or algal-created structure, though whether such a habitat increases or reduces fecundity (source or sink) may vary between sites, farming methods, and species. When native species are cultivated, reproduction from the farm environment can also contribute to species recovery in the wild. The meta-analysis found that aquaculture was associated with higher fish and invertebrate abundance and species richness compared to controls. Mussel and oyster farms yielded the largest increases in species abundance and richness, respectively. Seaweed farming was associated with the smallest increase in wild fish and mobile invertebrate abundance and the largest and most variable increase in species richness, though notably the meta analysis included mainly tropical seaweed farms.

The Present Status and Future Scope of Seaweed Aquaculture in Japan

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Japan is a maritime country surrounded by seas and oceans. There are approximately 1,500 species of seaweed growing along the coast, and they have been used since ancient times. Seaweed is a traditional and indispensable ingredient in Japanese cuisine. Natural seaweed beds are essential nurseries for various organisms, including fish and shellfish. Recently, seaweed has attracted much attention as a carbon sequestrant in the ocean, known as blue carbon. However, seaweed beds in Japan have faced various threats in recent years. Since 2000, seaweed has been dying due to rising seawater temperatures, and seaweed beds have been disappearing due to grazing by herbivorous fish and sea urchins. Research is underway to address these issues from a conservation and restoration perspective. Zoospores of autochthonous seaweeds are being released into the sea, and the growth of seaweeds is being studied on substrates where seaweeds are likely to grow. However, not only do seaweed beds not grow back, but seaweed vegetation does not move north with the rising seawater temperature due to climate change, leaving barren grounds. This indicates that the current rate of climate change is faster than the ability of seaweed to spread and reforest. Although efforts are being made to develop technology to restore the original seaweed beds, it is impossible to grow seaweed and create seaweed beds using traditional methods that depend on the natural environment. The use of aquaculture techniques to produce edible seaweed and seaweed beds in natural marine areas has recently attracted attention. Green lettuce (green algae, *aonori*), a seaweed widely used in Japan, rarely grows at the mouth of the Shimanto River in Kochi Prefecture, a major seaweed production area. The advancement of land-based aquaculture technology is noteworthy for the production of *aonori*. Land-based aquaculture does not depend on the marine environment, making it possible to stably produce *aonori* under controlled conditions. In addition, land-based aquaculture leads to the development of local economies. Algae farming is a technology that achieves a sustainable food supply with low environmental impact; therefore, land-based aquaculture techniques are expected to continue to be established in the future for many types of algae. It is difficult to restore the original seaweed vegetation that has disappeared due to rising water temperatures and increasing grazing pressure; therefore, efforts are being made to ensure the supply of seaweed spores released by seaweed in the ocean using the aquaculture techniques that Japan has developed to date, such as rope culture of large, inedible brown algae and cage culture in the sea.

Annotated Bibliography of Key Works

Terada, Ryuta, et al. 2021. Japan's nationwide long-term monitoring survey of seaweed communities known as the "Monitoring Sites 1000": Ten-year overview and future perspectives. *Phycological Research* 69: 12-30.

"*Monitoring Sites 1000*" – Japan's long-term monitoring survey was established in 2003, based on the Japanese Government policy for the conservation of biodiversity. Ecological surveys have been conducted on various types of ecosystems at approximately 1000 sites in Japan for 15 years and are planned to be carried out for 100 years. Since 2008, seaweed communities had been monitored at six sites, featuring the kelp (e.g., *Saccharina* and *Ecklonia*; Laminariales) and *Sargassum* (Fucales) communities in the subarctic and temperate regions of Japan. Annual surveys were carried out during the season when these canopy-forming seaweeds are most abundant. A non-destructive quadrat sampling method, with permanent quadrats placed along transects perpendicular to the shoreline, was used to determine species composition, coverage, and vertical distribution of seaweeds at these sites. In contrast, destructive sampling was done every 5 years to determine biomass. The occurrence of canopy-forming species *Saccharina japonica* (var. *japonica*) and *Ecklonia cava* have appeared to be stable at the Muroran (southwestern part of Hokkaido Island) and Shimoda (Pacific coast of middle Honshu Island) sites, respectively, whereas the coverage of *Ecklonia radicosa* (= *Eckloniopsis radicosa*) at the Satsuma-Nagashima site in southern part of Kyushu Island was highly variable until its sudden disappearance from the habitat in 2016. Thalli of *E. radicosa* lost most of their blades through browsing by herbivorous fish, and thus, this may be one of the causes of the decline. A shift in the community structure related to environmental changes had also been observed at some other sites. Pre- and post-disaster data revealed the impact of the 2011 earthquake and *tsunami* disasters, including a shift in the vertical distribution of *Ecklonia bicyclis* (= *Eisenia bicyclis*) to shallower depths at the Shizugawa site in the Pacific coast of northern Honshu Island due to seafloor subsidence.

Hiraoka, Masanori, and Naohiro Oka. 2008. Tank cultivation of Ulva prolifera in deep seawater using a new "germling cluster" method. *Journal of Applied Phycology* 20: 97-102.

A new "germling cluster" method is proposed for tank cultivation of seaweed in a free-floating form. This method was applied to the tank cultivation of *Ulva prolifera* using deep seawater (DSW) pumped up from over 300 m depth off the cape of Muroto in southwest Japan. Numerous zoids of *U. prolifera* were induced by cutting thalli into 1–2 mm long pieces. Three days after fragment production, the zoids were released. The zoid suspension was concentrated to a density of more than 10^4 zoids per mL medium and placed in a Petri dish for culture. The dense, germinating zoids began to adhere to each other and form aggregations. The germling aggregations were then removed from the bottom of the dish and torn into a large number of small "germling clusters" using an electric mixer. Each cluster contained 10–100 germlings. Once the germling clusters had attained more than 5 mm diameter in culture, they were transplanted as free-floating forms to a 500 L outdoor tank with continuous aeration, to which DSW was supplied at an exchange rate of 3 volumes per day. As a result, the average daily growth rate (DGR) in the tank throughout the year was 37%, though the DGR fluctuated with seasonal temperature changes.

Production Improvement of Nori Aquaculture Using Biostimulants

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Nori aquaculture is one of the most significant fisheries industries in Japan. However, the production of nori sheets has kept decreasing since 2000's due to high water temperature in the early aquaculture season (Oct. to Nov.), oligotrophication through the season and predation by herbivorous fishes and birds. The genetic diversity of nori strains used for aquaculture is low, making it difficult to breed new beneficial strains against climate change. So, we focused on "Biostimulants," which give many plants some tolerance to abiotic stress. In our experimental series, amino acids were used as biostimulants. We conducted three experiments based on the scheme of land plants using biostimulants: 1) screening of direct effective amino acids to axenic nori protoplasts, 2) laboratory culture experiments under the condition of bacteria existence and 3) field aquaculture trials. Moreover, to prepare for field trials, we considered the method of soaking in seawater dissolved each amino acid to avoid using a lot of amino acids, such as spraying them into aquaculture waters. As a result, nori protoplasts added arginine or ornithine had rapid growth. Under the bacteria's existence, these two amino acids enhanced the adherence strength of nori just once soaking for 15 hours. For field trials, soaking in arginine or ornithine for 24 hours enhanced not only the adherence strength, but also showed high growth and increased production by 1.1 to 1.3 times. We clarified that arginine and ornithine were useful biostimulants in nori aquaculture, especially enhancing adherence strength and growth in early aquaculture season and improving production.

Annotated Bibliography of Key Works

Abe, M., C. Tara, S. Fujiki, S. Kawasaki and N. Murase. 2021. Effects of four organic nitrogen in survival and growth on *Neopyropia yezoensisn* protoplasts – preliminary study-. *Journal of National Fisheries University*, vol. 70: 2. pp 55-61. (in Japanese with English abstract)

In order to research useful organic nitrogen, survival and growth of *Neopyropia yezoensis* protoplasts were examined using L-arginine (Arg), L-glutamic acid (Glu), Inosine (Ino) and taurine in 0.1, 1.0 and 10 mM. For survival rates for 1 week in culture, there were no significant differences except Glu in 1.0 and 10 mM. For growth for 3 weeks in culture, Arg in 10 mM was grown over two-times higher compared with control. On the other hand, growth in Glu and taurine were suppressed under every

condition. Moreover, growth in Ino in 10 mM was suppressed. Our data suggested that *Neopyropia yezoensis* use directly organic nitrogen and has a potential to induce growth using organic nitrogen.

Abe, M., C. Tara, S. Fujiki, S. Kawasaki and N. Murase. 2021. Screening of effective amino acids in survival and growth of *Neopyropia yezoensis* protoplasts. *Journal of National Fisheries University*, vol. 70: 2. pp 63-68. (in Japanese with English abstract)

In order to identify effective amino acid species, survival and growth of *Neopyropia yezoensis* protoplasts cultured using 18 amino acids were examined. Survival rates of each protoplast cultured for 1 week with arginine, asparagine, ornithine and tyrosine were similar to that of the control condition. Other amino acids significantly reduced the survival rates than the control. Growth of each protoplast cultured for 2 weeks with β-alanine, arginine, glutamine, histidine, lysine, ornithine and phenylalanine were significantly higher than that of the control. Histidine might be useful to make only thallus grow, although survival rate was very low. Arginine and ornithine had potentials to be able to promote the growth without negative influences on the survivals of nori cells.

Abe, M., C. Tara, S. Fujiki, S. Kawasaki and N. Murase. 2022. Effects of soaking in Arginine or Ornithine immediately after conchospores adhere to substrate in adherence strength and growth of *Neopyropia yezoensis*, *Aquacul. Sci.*, vol. 70: 2. pp 179-191. (in Japanese with English abstract)

In order to clarify the soaking effects of arginine and ornithine solutions to conchospores of *Neopyropia yezoensis*, the adherence strength for preventing losses and the growth for increasing yield of thalli were examined with laboratory culture experiments and field aquaculture trials, respectively. From the results of laboratory cultures, the adherence strength with soaking in ornithine for 1 hour or more were enhanced. From the results of field aquaculture trials, the enhances of the adherence strength and the growth promoting were showed with soaking in arginine and ornithine. Additionally, yields with soaking in arginine and ornithine increased by more than 10% per net a day. It was considered that the just once soaking in arginine or ornithine at the time of seedling collection, not only can contribute the enhance of the adherence strength for preventing thalli losses, but also can the increase of yields of lavers.

Umanzor, S., Han, S., Song, H.-I., Park, J.-S., Critchley, A., Yarish, C. and Kim, J. K. 2022. Ascertaining the interactions of brown seaweed-derived biostimulants and seawater temperature on spore release, germination, conchocelis, and newly formed blades of the commercially important red alga *Neopyropia yezoensis*? *Algal Research*, vol. 64. 102692.

Seaweed-derived extracts are commonly used as biostimulants in agriculture and, more recently, to cultivate other types of seaweeds. Here, we examined if Ascophyllum marine plant extract powder (aka AMPEP) and Kelpak® influenced changes in the life history of the commercially important, Neopyropia yezoensis. N. yezoensis was cultured in von Stosch enriched (VSE) medium with different concentrations (0, 0.001, and 1 mL L^{-1}) of AMPEP and Kelpak® for up to six days. Cultivars were then allowed to grow in VSE at either 15, 20, or 25 ºC, 12:12 L:D photoperiod, and 100 µmol photon m^{-2} s^{-1} of PAR for up to 42 days. Spore release, germination, and appearance of newly formed

blades per treatment were tracked and quantified using photographs. Results showed that temperature was the main factor driving the transition between developmental stages. However, interactive effects were observed. The concentration of 0.001 mL L^{-1} Kelpak® seemed to promote early germination and formation of conchocelis tufts at 15 ºC. The same concentration of AMPEP and Kelpak® at 20 ºC appeared to have a delayed positive effect on blade formation. These preliminary results do not show significant evidence that the application of Kelpak® and AMPEP, as tested, could affect the transition between life stages or provide enhancements on thermal tolerance.

Umanzor, S., Shin, S., Marty-Rivera, M., Augyte, S., Yarish, C. and Kim, J. K. 2019. Preliminary assessment on the effects of the commercial seaweed extract, AMPEP, on growth and thermal tolerance of the kelp *Saccharina* spp. from the Northwest Atlantic. *J. Appli. Phycol.* Vol. 31, 3823-3829.

Acadian Seaplants, Ltd., marine plant extract powder (AMPEP), is a commercially available extract derived from the brown seaweed, Ascophyllum nodosum. This extract is widely applied to increase the performance of land crops and has been reported to enhance growth of some seaweed crops. To assess the effects of AMPEP on the growth and thermal tolerance of *Saccharina latissima* and *S. angustissima* cultivars, we performed two experiments. First, juvenile sporophytes were dipped (i.e., 30 or 60 min) in AMPEP solutions of different concentrations (0.001, 0.005, 0.05, 1, and 5 g L^{-1}). Sporophytes were then cultivated in half-strength Provasoli's enriched seawater (PES) and allowed to grow at different temperatures (12, 16, 19, 23, and 25 ± 1 ºC) for 20 days using a temperature gradient table. Photoperiod was maintained at 12:12 L:D with photosynthetically active radiation (PAR) of 90 ± 10 µmol photons m^{-2} s^{-1}. Results show a differential response between the two species tested. Sporophytes of both species cultured at 23 and 25 ºC died during the first 7 days post dipping in AMPEP. After the 20-day period, the surviving sporophytes were transferred to an incubator set at 18 ± 1 ºC (i.e., LT50 after 20 days). These sporophytes remained for 14 additional days to assess for any delayed effect on thermal tolerance, with treated sporophytes showing a higher percentage of survival and growth than control sporophytes never exposed to AMPEP. Furthermore, after the 14-day incubation at 18 ºC, treated sporophytes of both species showed blades more than three times thicker than control sporophytes. These preliminary results indicate that AMPEP may enhance the growth capacity of *S. latissima* and *S. angustissima* when exposed to suboptimal temperatures, allowing them to overcome heat stress more effectively while maintaining growth.

Seaweed Seedling Culture Technique Using LEDs and Feeding Behavior of Herbivorous Fish to Suppress Fouling Seaweed - In the Case of "*Hiziki*" *Sargassum fusiforme* -

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The brown seaweed *Sargassum fusiforme,* called "*hiziki*" in Japanese, is distributed on rocky shores in the intertidal zones of Japan, Korea, and China. *Hiziki* is an important fishery resource in Japan. However, the production of natural *hiziki* is decreasing due to changes in the coastal environment. *Hiziki* has been produced by aquaculture mainly using natural seedlings, which has a negative impact on natural resources. The artificial seedling production method of *hiziki* has been studied, and fouling of seaweeds during the seedling production is problematic. In this study, we developed an efficient *hiziki* seedling culture technique using LEDs and the feeding behavior of herbivorous fishes, Largescale blackfish *Girella punctata* and Smallscale blackfish *G. leonina*.

In June 2021, we collected germlings from mature *hiziki* in tanks and cultured them on concrete blocks (about 10,000 germlings/block, 39×19×10 cm). These germlings were cultured for 30 days under four different colors of LED light (green: central wavelength of 520 nm, blue: 450 nm, red: 632 nm, and white: complex light as a control; 100 μmol/m^2/s; 12L:12D light regime). At the end of the experiment, the size of these germlings and the cover of fouling seaweeds were measured. The mean sizes of the germlings used in the green, blue, red, and white LED light treatments were 1.95 mm, 1.44 mm, 0.25 mm, and 1.46 mm, respectively. The mean cover of fouling seaweeds was 29.1%, 96.8%, 29.1%, and 87.2%, respectively. These results show that green LED light is adequate for high growth of *hiziki* and suppression of fouling seaweeds.

The fouling seaweeds, however, started to increase during the 30 days of cultivation with green LED light. In July 2021, tank experiments were conducted to remove these fouling seaweeds using herbivorous fish. We placed germlings of *hiziki* and fouling seaweeds propagated on concrete blocks with 10 juvenile Largescale blackfish (60.4±9.0 mm) or 10 juvenile Smallscale blackfish (62.1±5.9 mm) in a tank. We measured the cover of fouling seaweeds, and the size of *hiziki* on the block, *hiziki* detached from the block by browsing of the fish and *hiziki* in the stomach content of the fish after 4.5 hours. The cover of fouling seaweeds decreased from 23.5% to 0.5% in the Largescale blackfish tank, and 15.5% to 0.6% in the Smallscale blackfish tank. The size of *hiziki* on the block, detached from the block and in the stomach content of the fish was 2.04±0.71 mm, 1.47±0.53 mm, and 0.86±0.20 mm in the Largescale blackfish tank, and 2.37±0.85 mm, 1.54±0.61 mm and 1.25±0.11 mm in the

Smallscale blackfish tank, respectively. More than 80% of the *hiziki* detached or eaten by the both species of blackfishes were less than 2 mm. These results showed that the larger the *hiziki*, the less susceptible they were to browsing by blackfishes. Thus, blackfishes were found to be effective in removing fouling seaweeds in the seedling production of *hiziki*.

Annotated Bibliography of Key Works

Tsutomu Noda, Tatsuru Kadota, Keiichiro Shimaoka and Yuichiro Fujinami. 2022. Removal of fouling seaweed on artificial substrate for Sargassum fusiforme seedling culture by juvenile *Girella punctata* grazing. *Aquaculture Science*, 70(1), 113-117.

Tank experiments were conducted to develop a technique to remove fouling seaweeds from seedling production of *Sargassum fusiforme*. We set a substrate with *S. fusiforme* and fouling seaweeds (Ulva australis and U. intestinalis), and 12 juvenile *Girella punctata* in replicate tanks (n = 4). Cover and length of the fouling seaweeds decreased as time proceeded, while those of *S. fusiforme* did not decrease. In addition, *S. fusiforme* were not observed in the stomach contents of *G. punctata*. These results suggest that *G. punctata* can remove fouling seaweeds without apparent damage to *S. fusiforme* in seedling production.

Noboru Murase, Mahiko Abe and MikioNoda. 2018. Growth and Maturation of Gametophyte in *Undaria pinnatifida* under Different Light Quality from Light Emitting Diodes (LEDs). *Journal of National Fisheries University*, 67(2), 91-97.

The effects of light quality on growth and maturation of gametophyte in *Undaria pinnatifida* were examined in an indoor culture at 20°C, 12h light-12h dark cycle and 50μmol/m^2/s using four different light emitting diodes (LEDs) and a fluorescent light. The relative growth rates of the male and female gametophytes under green LEDs showed high values, but those under red LEDs showed low values. The female gametophyte matured quickly under blue LEDs and a fluorescent light. On the other hand, they matured more slowly under white and green LEDs. Under red LEDs condition, the maturation of female gametophytes was not observed at all.

Takeshi Ito, Yudai Iino, Shizuko Nakai, Shiro Itoi, Haruo Sugita, Noriyuki Takai. 2018. Distribution patterns of settlement-stage juveniles of *Girella punctata* and *Girella leonina* on the rocky coast of the Kanto–Izu region, Japan. *Fisheries Science*, 84, 627-640.

The early life history of girellid fishes in Japanese waters is unclear, and little is known about their species-specific reproductive strategies. We examined seasonal changes of distribution patterns for settlement-stage juveniles of *Girella punctata* and *Girella leonina* on the rocky shore in the regions of Kanto and Izu, Japan, to infer the influence of the Kuroshio Current on their reproduction. We collected 813 settlement-stage juveniles mainly in Sagami Bay and genetically identified the species. The juveniles of *G. punctata* were collected on the rocky shore in Sagami Bay from April to August, with abundant catches in May and June. Thus, we infer that juvenile *G. punctata* ubiquitously inhabit the rocky shore in the area in spring and summer. By contrast, juveniles of *G. leonina* were rarely

collected in Sagami Bay, with a total catch of only 66. Notably, no juveniles were collected during the wintertime in Sagami Bay, although an abundant catch of *G. leonina* had been previously reported for Sagami Nada off Sagami Bay during January to March. This clear-cut difference between the areas likely reflects the difference in proximity to the path of the Kuroshio Current. We expect that the Kuroshio Current strongly influences the reproductive success of *G. leonina*.

Advantages of Small-Sized Macroalgae in Seaweed Bed Restoration in Waters with High Feeding Pressure from Herbivorous Fishes

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Seaweed beds composed of large-sized macroalgae (i.e., *Sargassum* spp. and *Ecklonia* spp.) have been declining along the coast of western Japan, especially in Kyushu. Feeding by herbivorous fishes is likely responsible for the loss of macroalgal beds, but we have yet to develop techniques to remove these fishes. Recent research in Kyushu has started to focus on the role and function of small-sized macroalgae in the restoration of seaweed beds, because small-sized macroalgae may be less susceptible to herbivory and may have value as feed or habitat for fishery benthos such as the purple sea urchin, *Heliocidaris crassispina*. This review collates recent research on the benefits of small-sized macroalgae in seaweed bed restoration in Japan and highlights:

1) the susceptibility of small-sized macroalgae feeding by herbivorous fishes.
2) the functionality of small-sized macroalgae as feed for *H. crassispina*.
3) the effectiveness of using small-sized macroalgae in the restoration of seaweed beds in environments with high feeding pressure from herbivorous fishes.

The susceptibility of small-sized macroalgae to feeding by the brown chub *Kyphosus bigibbus*, Japanese parrotfish *Calotomus japonicus*, and mottled spinefoot *Siganus fuscescens* were examined in tank experiments. Small-sized macroalgae were less susceptible than large-sized macroalgae to feeding by all fish species. In addition, susceptibility to feeding varied among species of small-sized macroalgae: *Gelidium elegans* and *Dichotomaria falcata* were the least susceptible among small-sized macroalgae. Tank experiments also revealed that many small-sized macroalgae were useful as feed for *H. crassispina*, although the gonad index (GI) of sea urchins fed any of the small-sized macroalgae was lower than that of sea urchins fed large-sized macroalgae. In addition, the GI of the sea urchins fed *G. elegan*s or *Palisada intermedia* was higher than that of sea urchins fed other species of small-sized macroalgae. Attempts to restore seaweed beds using small-sized macroalgae were conducted at two sites (Nagasaki and Kagoshima) in Kyushu and succeeded in increasing small-sized macroalgae such as *G. elegans* and *Chondrophycus undulatus* by removing sea urchins. However, large-sized macroalgae such as *Sargassum* spp. did not increase at either site. Furthermore, the GI of *H. crassispina* increased in restored seaweed beds at Kagoshima. These studies suggest that the use of small-sized macroalgal species would be an effective means of restoration of seaweed beds to increase fishery resources such as *H. crassispina* in waters with high feeding pressure from herbivorous fishes.

Annotated Bibliography of Key Works

Kadota, T., Kiyomoto, S., Masuda, Y., Miyano, T., Yoshimura, T. 2022. Restoration of a small-sized macroalgal bed through the removal of sea urchins in Kashiyama, Nagasaki Prefecture. *Nippon Suisan Gakkaishi*, Vol 88:2. pp49–57 (in Japanese with English abstract)

In this study, a small-sized macroalgal bed was restored by removing sea urchins at a barren site in Kashiyama, Nagasaki Prefecture. Sea urchin densities decreased from 13.9 to 0.7 individuals/m^2 from June to November 2015. By the following spring, small-sized macroalgae such as Gelidiaceae spp. and *Chondrophycus undulatus* increased, while large-sized macroalgae did not. Tank experiments showed that many of the small-sized macroalgae examined (*Dictyopteris undulata*, *D. prolifera*, *Gelidium elegans*, and *Dichotomaria falcata*) were less vulnerable to feeding by *Siganus fuscescens* and *Kyphosus bigibbus* than large-sized macroalgae (*Sargassum alternato-pinnatum*). Our results indicate that seaweed beds composed of small-sized macroalgae can be more easily restored than those composed of large-sized macroalgae simply by removing sea urchins, even under high browsing pressure from herbivorous fish.

Noda, M., Kadota ,T. 2024. Diurnal feeding patterns, seasonal changes in feeding rate and feeding selectivity of the herbivorous fish *Calotomus japonicus* held in aquaria. *Aquaculture Science*, Vol:72:1. pp59–68 (in Japanese with English abstract)

The browsing behavior of the parrotfish, *Calotomus japonicus*, was examined under tank-rearing conditions to help improve the efficiency of longline fishing. Browsed rates for morning and daytime were higher than for evening. Furthermore, the amount of *Ecklonia cava* subsp. *kurome* browsed by the fish is relatively high from June to August during the season of rising water temperature. In addition, the six seaweed species, *Sargassum fusiforme*, *Padina arborescens*, *Dictyopteris undulata*, *Palisada papillosa*, *Gelidium elegans* and *Codium fragile*, were fed to the fish. The browsed rate of *S. fusiforme* was higher than the other seaweeds. Based on these results, the time period for longline fishing to remove *C. japonicus* is preferable during the morning to daytime in spring to early summer, and the suitable bait is *S. fusiforme*.

Igari, T., Tojo T., Takasugi, T., Ichiki, T., Manabe, M., Hirae, T. 2022. Efforts to restore large-sized macroalgae by removing sea urchins under feeding pressure from herbivorous fishes. *Bulletin of Kagoshima Prefectural Fisheries Technology and Development Center*, Vol:8. pp1–7. (in Japanese)

Removal of sea urchins at a barren site in Kagoshima Prefecture resulted in the restoration of a small-sized macroalgal bed, but no increase in large-sized macroalgae. Additionally, gonad index values for *Heliocidaris crassispina* and recruitment rates for *Panulirus japonicus* increased in the restored seaweed beds.

Pacific Oyster Condition and Mortality in a U.S. Pacific Coast Estuary: Can Relationships with Climate, Food and Reproductive State Be Utilized to Sustain Future Production?

Running Title: Influence of Climate on Pacific Oyster Condition and Mortality

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Pacific oysters (*Crassostrea gigas)* were introduced to the US Pacific coast beginning in the early 1900's and have become the predominant cultivated shellfish species contributing substantially to US domestic production. While they were widely transported amongst locations, they only regularly spawned and became "naturalized" in several discrete estuarine locations like Willapa Bay, WA. Though culture methods differ, the US industry, like that in Japan, relied on "natural" set at these locations until the large scale adoption of shellfish hatcheries in the late 1970's. A monitoring program was therefore established to examine both larval set and the condition of adult oysters in Willapa Bay during the mid 1950's and has been maintained since that time. The gametogenic cycle for oysters is relatively well studied and has been previously correlated with environmental triggers like temperature, salinity, and food which influence oyster growth but also act as stressors and are linked to gametogenesis and summer mortality events. We summarize the results of an investigation into broad decadal temporal scale and Pacific Ocean basin spatial scale environmental forcing factors that influence oyster condition in Willapa Bay and link these to summer mortality events that have occurred within this estuary and seasonal shifts in oyster condition that have occurred on an annual temporal scale. A comparison with similar observations and environmental data from the Seto Inland Sea suggests that multiple stressors are likely involved, but these could differ by location. We provide a brief proposal and framework to further examine metabolic or energetic characteristics (glycogen content or anaerobic capacity) that could be used to either breed oysters for resilience to such stressors and/or provide the shellfish industry the ability to adapt to and mitigate for the effects of an uncertain future climate.

Annotated Bibliography of Key Works

Cheney, D., B.F. MacDonald, and R.A. Elston. 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *Journal of Shellfish Research* 18: 456-473.

This manuscript presents the results of a study conducted to investigate Pacific oyster summer mortality events in Puget Sound, Washington, USA where no specific disease factors appeared responsible but instead mortality events linked to multiple environmental stressors including temperature extremes, low oxygen conditions and phytoplanton as food. An initial comparison of mortality amongst diploid and triploid oysters was also made.

Dumbauld, B.R., X.N. Du, M. Hunsicker, and Z. Forster. 2023. Multi-decade changes in the condition index of adult Pacific oysters (*Crassostrea gigas*) in response to climate in a US west coast estuary. *Journal of Sea Research* 193.

The authors present an analysis of almost seven decades of oyster condition data collected in Willapa Bay, Washington USA. They identify two important and coherent shifts in oyster condition that can be associated with changes in ocean climate at basin wide and more local scales. They also charactrize patterns in oyster condition within Willapa Bay that have long been recognized by industry participants and associated with gametogenesis and spawning.

George, M.N., O. Cattau, M.A. Middleton, D. Lawson, B. Vadopalas, M. Gavery, and S.B. Roberts. 2023. Triploid Pacific oysters exhibit stress response dysregulation and elevated mortality following heatwaves. *Global Change Biology* 29: 6969-6987.

These authors examined the physiological response of Pacific oysters to environmental conditions that are potentially responsible for summer mortality. Physiological assays included metabolic depression due to a reduction in sodium pump activity and dysregulated expression of genes associated with glucose metabolism and mitiochondrial function.

Hasegawa, N., B. Dumbauld, M. Hori, S. Watanabe, M. Rust, and Z. Forster. 2021. Comparative study of the impact of environmental change on oyster clture between USA and Japan, as collaborative research under UJNR. *Bulletin of the Japanese Fisheries Research Education Agency* 50: 115-121.

This is an introduction to the collaborative study initiated between the USA and Japan. Data was collected, but some analyses have been delayed primarily due to the pandemic.

Samain, J.F. 2011. Review and perspectives of physiological mechanisms underlying genetically-based resistance of the Pacific oyster to summer mortality. *Aquatic Living Resources* 24: 227-236.

This review of multiple years of data collected for the "Morest" project in France remains one of the important references that suggests a link between summer mortality events, physiological mechanisms like reproductive effort and stressors like temperature. The authors also suggest avenues for investigating genetically – based resistance to stress.

Image Analysis for Estimating Soft Body Mass from Shell Morphology in the Pacific Oyster, *Crassostrea gigas*

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Oyster farming is one of the oldest forms of aquaculture and is now one of the largest in the world. Oysters are sessile organisms whose shell morphologies are highly variable to strongly adhere onto the substrate. The relationship between the shell morphology and the amount of soft body in the oyster is poorly understood because of the difficulty in the morphological analysis of the complex-shaped shell. The "ideal" shell shape for aquaculture production has not been determined in its more than 2,000 years of history.

In this study, we report a new analytical approach to estimating the soft body mass from the shell morphology in the Pacific oyster, *Crassostrea gigas*. We assumed that the shell morphologies should be directly translated into numerical values that characterize the soft body mass rather than using the traditional major size indices that measure linear distances, such as those represented by shell length and shell width. To do this, we first developed the apparatus to easily capture the shell morphologies by backlighting. Using this apparatus, we collected the photographic data taken from the side and the top of the shells. These data were transformed into values representing the morphological components by the Fourier contour analysis. From these, we screened out the morphological components that change the relative soft body mass using the generalized linear model. Using this analytical result, we developed a numerical model to evaluate the shell morphologies in terms of oyster meat production. Our results will help to establish a new criterion to assess the quality of the oyster as a seafood to promote current oyster farming.

Annotated Bibliography of Key Works

Iwata, H., and Y. Ukai (2002) SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. Journal of Heredity 93: 384-385.

In this article, the authors have developed a software that transforms the morphologies of target objects from photographic data by the Fourier contour analysis. This software has enabled easy analysis using the elliptic Fourier descriptors.

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ARS: Agricultural Research Service