47th Scientific Symposium Program of the UJNR Aquaculture Panel

Marine Aquaculture in a Changing Environment

Okinawa Industry Support Center, Room 304

1831-1 Oroku, Naha, Okinawa

November 12th and 13th, 2019

Photo credit: Atsushi Nanami

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Aim of the Symposium

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 47th UJNR Aquaculture Symposium is the final symposium of a 3-year cycle with the theme Marine Aquaculture in a Changing Environment. Environmental changes impact aquaculture in many ways. Nutrient pollution is driving eutrophication and dead zones; ocean acidification is changing water chemistry, and climate change is already influencing our food supply, freshwater availability, weather and way of life. Aquaculture will be impacted by, and can also impact, these environmental changes over various scales. Aquaculture of finfish, shellfish and seaweed have different threats, benefits and opportunities related to environmental change.

Over the last two years, we discussed the potential of aquaculture to mitigate impacts of environmental change (e.g., carbon sequestration, bioextraction of nutrients and CO_2, antacidity, and oxygen production), impacts of environmental change on aquaculture production(e.g., effects of ocean acidification on shellfish aquaculture), and science to mitigate these impacts (countermeasures). This is the final year of the three year-plan, and the symposium theme is the Application of Aquaculture Technology to Provide Sustainable Seafood and Reduce Impacts of Environmental Change. This theme includes development of technology to increase marine aquaculture production to offset seafood deficit due to loss of capture fisheries impacted by environmental change, and to augment food deficits due to impacts of environmental change on inland agriculture.

Program

Tuesday, November 12, 2019

Registration 9:00-9:15

Welcome and Aim of the Symposium

Hideaki Aono, Japan Panel Chair, Japan Fisheries Research and Education Agency 9:15 - 9:25

New techniques to assess and mitigate impacts of environmental changes on aquaculture

(Moderators: Simona Augyte and Natsuki Hasegawa)

Bacterial communities in marine sediments; a biological parameter to evaluate coastal environment

Tomoko Sakami, Research Center for Aquaculture Systems, National Research Institute of Aquaculture, FRA 9:25 - 9:55

Applications of environmental DNA data in support of aquaculture

Tom Noji, Northeast Fisheries Science Center, NOAA Fisheries 9:55 - 10:25

Stable isotopic approach to investigate nitrogen pathway in coastal aquaculture area

Satoshi Watanabe, Research Center for Aquaculture Systems, National Research Institute of Aquaculture, FRA 10:25 - 10:55

Establishment of immunological assays and baseline profile of hemocytes in the hard clam *Mercenaria mercenaria* as evaluation biomarkers for environmental stresses

Huiping Yang, School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida 10:55 - 11:25

Purple urchin barrens: an opportunity for aquaculture and fisheries to work together to solve an environmental issue

Luke Gardner, California Sea Grant 11:25 - 11:55

(PM: UJNR Aquaculture Panel Business Meeting)

Reception 18:00 - 20:00

Wednesday, November 13

Impact assessment of environmental changes and conservation of fisheries environment in coral reef areas

(Moderators: Luke Gardner and Masakazu Hori)

Impacts of ocean acidification on Japan coastal water and marine fisheries

Haruko Kurihara (Invited speaker), University of the Ryukyus 9:15 - 9:45

Sustain seafood resources in the U.S. affiliated Pacific islands- status and strategies

Cheng-Sheng Lee, Center for Tropical and Subtropical Aquaculture, United States Department of Agriculture 9:45 - 10:15

Towards effective coral community restoration for sustainable fishery of a coral reef grouper *Epinepherus ongus*: implication of ecosystem-based management

Atsushi Nanami, Research Center for Sub-tropical Fisheries, Seikai National Fisheries Research Institute, FRA 10:15 - 10:45

Scaling up coral restoration to meet the demands of a collapsing ecosystem

Tali Vardi, ECS for NOAA Fisheries Office of Science & Technology 10:45 - 11:15

Sustainable large-scale coral restoration by establishing "artificial spawning hotspot"

Go Suzuki, Research Center for Sub-tropical Fisheries, Seikai National Fisheries Research Institute, FRA 11:15 - 11:45

Lunch Break (75 min)

Production management under environmental changes in bivalve aquaculture

(Moderators: Cheng-Sheng Lee and Atsushi Nanami)

The Influence of Climate and Environment on the Growth and Survival of Pacific Oyster Seed in US West Coast Estuaries

Brett Dumbauld, Agricultural Research Service, United States Department of Agriculture 13:00 - 13:30

Comparative Study of the Impact of Environmental Changes on Oyster Culture between USA and Japan, as Collaborative Research under UJNR

Natsuki Hasegawa, Stock Enhancement and Fisheries Oceanography Department, Hokkaido National Fisheries Research Institute, FRA 13:30 - 14:00

Oyster aquaculture using seagrass beds as a climate change countermeasure

Masakazu Hori, National Research Institute of Fisheries and Environment of Inland Sea, FRA 14:00 - 14:30

Coffee Break (15 min)

Aquaculture technologies to respond to environmental changes; Seaweed breeding and feed development

(Moderators: Brett Dumbauld and Satoshi Watanabe)

Kelp, *Saccharina* spp, population genetics in the Northwest Atlantic for guiding a breeding program of thermally resilient strains

Simona Augyte, Dept. of Ecology and Evolutionary Biology, University of Connecticut 14:45 - 15:15

Cell selection technique for establishment of low salinity tolerance strain in *Pyropia tenuipedalis*

Mahiko Abe, Applied Aquabiology, National Fisheries University, FRA 15:15 - 15:45

Improvement of dietary effect on juvenile *Ruditapes philippinarum* using the dietary-supplements and new diet microalga

Yasuhiro Yamasaki, Applied Aquabiology, National Fisheries University, FRA 15:45 - 16:15

Exploration of alternative protein sources in the development of a sustainable Japanese white trevally *Pseudocaranx dentex* juvenile diet

Jonas Miller, Uragami Station, Aquaculture Research Institute, Kindai University 16:15 - 16:45

Science Symposium Closing

Michael Rust, US Panel Chair, NOAA Fisheries Office of Aquaculture 16:45 - 17:00

List of Participants

Abe, Mahiko	Japan Fisheries Research and Education Agency (FRA), National Fisheries University (NFU)
Aono, Hideaki	Japan Panel Chair, FRA Headquarters
Augyte, Simona	University of Connecticut
Chiba, Toshinari	Akita Prefectural Department of Agriculture, Forestry and Fisheries, Institute of Fisheries Promotion
Dumbauld, Brett	United States Department of Agriculture, Agricultural Research Service
Gardner, Luke	California Sea Grant
Hasegawa, Natsuki	FRA, Hokkaido National Fisheries Research Institute
Hori, Kazumasa	FRA, National Research Institute of Fisheries and Environment of Inland Sea
Ishikawa, Takanori	Okinawa Prefectural Fisheries Research and Extension Center（OPFREC）
Kishimoto, Kazuo	OPFREC
Kurihara, Haruko	University of the Ryukyus
Lee, Cheng-Sheng	United States Department of Agriculture, Center for Tropical and Subtropical Aquaculture (College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa)
Miller, Jonas	Kindai University
Miwa, Satoshi	FRA, National Research Institute of Aquaculture (NRIA)
Nanami, Atsushi	FRA, Seikai National Fisheries Research Institute (SNFRI)
Nakata, Yuji	OPFREC
Noji, Tom	NOAA Fisheries, Northeast Fisheries Science Center
Okumura, Takuji	FRA, NRIA
Oomine, Risako	OPFREC
Rust, Michael	US Panel Chair, NOAA Headquarters
Saito, Yoho	FRA, Headquarters
Sakami, Tomoko	FRA, NRIA
Suzuki, Go	FRA, SNFRI
Takano, Masatsugu	FRA, Headquarters
Vardi, Tali	NOAA Fisheries Office of Science & Technology
Watanabe, Satoshi	FRA, NRIA
Yamasaki, Yasuhiro	FRA, NFU
Yang, Huiping	University of Florida

Bacterial communities in marine sediments: a biological parameter to evaluate coastal environments

Tomoko Sakami[1*], Toru Udagawa[2] and Hiroshi Yagi[3]

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Environmental monitoring of marine coastal areas is becoming increasingly important because of the accelerated global climate change and rapid industrial development. Understanding certain biological parameters is fundamental to assess the environmental conditions of fisheries ground. However, much effort is required for the examination of community change because species identification must be done manually by well-trained professionals who may not be available whenever necessary. In this context, the recently developed metagenomic analysis of bacterial communities may be useful as a mechanism to obtain much data with less effort. Bacterial communities in coastal sediments are known to be affected by environmental conditions. We examined the bacterial community composition in coastal sediments collected around Cape Inubo, where the cold Oyashio mixing water and the warm Kuroshio current influence the northern and southern areas of the cape, respectively. The purpose of this study was to understand how bacterial communities vary in areas under the influence of different types of ocean currents. Surface sediments were collected from seabed at water depths of 10 m and 30 m in the northern and southern areas, and 16S rRNA gene profiles were examined using the Illumina Miseq platform. The bacterial community composition was different between the two water depths, and the bacterial communities at 10 m depth was different between the northern and southern areas. The chlorophyll *a* content and temperature were indicated as the major environmental parameters influencing the composition of the bacterial communities. Characteristic taxonomic groups were identified from each area. At 10 m depth in the southern area, operational taxonomic units (OTUs) of *Bacteroides*, which is known as a high molecular weight organic matter degrader, were abundant, whereas OTUs of *Nitrosomonas* and *Nitrospira*, which are related to the nitrification process, were less abundant. This implies that the decomposition and mineralization process of the sediment organic matter in this area may differ from those in the other three areas. In fact, at 10 m depth in the southern area, the total organic matter content was high but chlorophyll *a* content was low as compared to the other areas, suggesting a low input

of fresh organic matter. In conclusion, the bacterial community composition in coastal sediments around Cape Inubo varied with water depth. At 10 m depth, the bacterial communities differed between the two areas. These differences were concomitant with the differences in some environmental parameters. Moreover, the characteristic taxonomic groups found in each area suggested that the difference seemed to be related to properties of the sediments' organic matter. Metagenomic information of benthic bacterial communities is useful to monitor coastal environmental changes because not only does it reflect the environmental conditions, but its taxonomic features indicate the ecological functions of the community.

Annotated Bibliography of Key Works

Sakami, T., Kakehi, S. 2019. Distribution and community composition of ammonia-oxidizing archaea and bacteria in coastal sediments in response to sediment material gradients at Sendai Bay, Japan. Gojobori, T. et al. (eds.), Marine Metagenomics, Springer Nature Singapore Pte Ltd, pp 161-181.

The distributions of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were determined along an environmental gradient from the coastal mud to the offshore coarse sand at Sendai Bay, Japan. The abundance of AOA ammonia monooxygenase alpha subunit gene (*amoA*) was high in the coastal muddy areas and low in the offshore sandy areas. There was a strong positive correlation between AOA-*amoA* abundance and ammonia content in the sediment. The distribution of AOB-*amoA* was similar to that of AOA-*amoA* in July, but remarkably low in the muddy sediments in December. Clone library analysis indicated that the community composition for both types of organisms differed in sandy and muddy sediments and that the diversity was considerably lower in the muddy sediments. These results suggest that the abundance of ammonia-oxidizing organisms was controlled by the ammonia levels in the sediment. However, there are some inhibitive conditions for AOB: presumably, the low organic matter supply to the surface oxic layer during autumn in the muddy sediment in Sendai Bay.

Applications of environmental DNA data in support of aquaculture.

Thomas Noji[1*], Daniel Wieczorek[1], Beth Phelan[1], Yuan Liu[2], Lisa Milke[2], Renee Mercaldo-Allen[2], Julie Rose[2], Lauren Sassoubre[3] and Bruce Nash[4]

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With advances in analytical and computational technologies the data for environmental DNA or eDNA are becoming rapidly and increasingly available. eDNA data have been applied successfully to assess presence of fish species, impacts of human activities on benthic biota and more recently to a limited extent to assess biomass. Because of the relative ease with which eDNA data can be collected, the number of proposed applications is increasing rapidly; this includes applications to support aquaculture.

The NEFSC Sandy Hook Lab was instrumental in development of a NOAA Fisheries Strategic Initiative to explore the potential use of eDNA to assess fish stock size and distribution. In 2017, with an expanded team of eDNA experts we began discussions to conduct experiments to test whether environmental DNA can be used as a quantitative tool to support fisheries stock assessments. We are the only group in NOAA performing seawater laboratory experiments to support this research. These experiments are conducted in a closed recirculating, to determine species-specific shedding and decay rates of eDNA (Sassoubre et al. 2016) under different environmental conditions. This is particularly important for the assessment of stocks which cannot easily be surveyed by standard trawl surveys. To date we have run experiments on adult black sea bass, winter flounder, and summer flounder (Sassoubre et al. in prep) and in 2020 several new species will be investigated. One of the important next steps to making this a quantitative survey tool is to develop eDNA particle transport models (Andruszkiewicz et al. 2017). These models are intended to indicate the geographical origin of collected eDNA and to estimate the biomass (Kelly et al. 2019) of the corresponding fish stock. Further, the NEFSC Milford Laboratory is now in its third year of conducting field experiments on the effect of caged oyster on the diversity and behavior of finfish. The investigation measures diversity with eDNA, as well as visual corroboration using underwater video and sonar. Observed in 2018 using eDNA were 17 species of finfish; 14 species were observed visually. Only about half of the species were recorded in both sets of observations. The

investigation sites with caged oyster had a greater diversity of finfish than comparable sites without cages (Liu et al. submitted).

This talk will present data from these two investigations. Further the talk will explore ways that eDNA data can help to address some of the challenges related to aquaculture. Some of the applications to be discussed include measurement of eDNA as a surveillance tool for shellfish and finfish pathogens and nuisance organisms such as sea lice, as an indicator of benthic impacts, as an indicator of recovery rates for augmenting natural populations, and other uses.

Annotated Bibliography of Key Works

Sassoubre, L.M., K.M. Yamahara, L.K. Gardner, B.A. Block and A.B. Boehm. Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. 2016. *Environ. Sci. Technol.* 50: 10456−10464.

A key publication on experiments for shedding and decay rates of eDNA from marine finfish. Also includes a much cited conceptual model for processes in the field affecting concentrations of eDNA.

Andruszkiewicz, E.A., H.A. Starks, F.P. Chavez, L.M. Sassoubre, B.A. Block, A.B. Boehm. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS ONE* 12(4): e0176343. https://doi.org/10.1371/journal.pone.0176343

This paper is the first to my knowledge to apply numerical modeling to predict the origin of eDNA collected in the field. The outputs include levels of uncertainty for the calculations.

Kelly, R.P., A.O. Shelton and R. Gallego. 2019. Understanding PCR Processes to Draw Meaningful Conclusions from Environmental DNA Studies. *Scientific Reports* (2019) 9:12133 | https://doi.org/10.1038/s41598-019-48546-x

This paper presents guidelines for the use of PCR for the successful application of eDNA data to estimate biomass. The investigation is a modeling approach and describes how the proportional indices of amplicon reads capture trends in taxon biomass with high accuracy

Liu, Y., G.H. Wikfors, J.M. Rose, R.S. McBride, L.M. Milke, R. Mercaldo-Allen. Submitted. Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. *Frontiers in Marine Science*.

Describes the field investigations addressing the beneficial effect of caged oyster on finfish biodiversity in Long Island Sound, USA.

Sassoubre, L.M., D. Wieczorek, B. Phelan, B. Nash, A. Kirtane, T. Noji. In Preparation.

Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish in Support of Quantitative Field Investigations.

Describes the results from laboratory experiments on one pelagic fish species and two flatfish. Also relates these data to field surveys for these species with comparisons to trawl data.

Stable isotopic approach to investigate nitrogen pathway in coastal aquaculture area

Satoshi Watanabe

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Coastal eutrophication due to increasing anthropogenic activities and consequent environmental deterioration, such as anoxia, harmful algal bloom and hydrogen sulfide emission have been problematic in many parts of the world, sporadically causing mass mortality of aquatic organisms. While this holds true for Japan, oligotrophication and resulting reduction in coastal fisheries and aquaculture productivity have also been a recognized problem in some parts of Japan. Some studies argue that ever-dwindling fisheries production of coastal resources, as well as unfed aquaculture production of inorganic and organic extractive species is partially attributable to the reduced nutrient levels in the coastal waters in recent years. Eutrophication mitigation efforts have reduced the load of nitrogen and phosphorus of terrestrial and aquaculture origins to the coastal environment over the past forty years in Japan. Allegedly excessive oligotrophication has reduced primary productivity and hence carrying capacity or coastal ecosystems supporting coastal fisheries and aquaculture. However, complex nutrient flows within coastal ecosystems are not necessarily well understood.

Fisheries production, inclusive of bottom culture, of the Manila clam, *Ruditapes philippinarum*, for instance has reduced by 95% over the past three decades. Insufficient food supply is one amongst many speculated factors causing the reduction. Food availability to the clam is often represented by chlorophyll *a* level in a water column collected far from the bottom water the clams inhale and filter feed. Our stable isotopic study has suggested that the food source of the clam may differ depending on the bottom topography of the habitat. Many filter feeding bivalves are generally held to be phytoplanktivorous; however, they also feed on benthic microalgae. Provisional ratio of the planktonic to benthic microalgae seems to be affected by the extent of resuspension of the latter from the sediment to the bottom water to make them available to the clam. As a result, clams inhabiting the same tidal flat only 10 m apart from each other can have a different carbon stable isotopic ($\delta^{13}C$) signature, indicating the presence of small-scale difference in nutrient sources for the clam.

Anthropogenic nitrogen is known to have higher nitrogen stable isotopic ($\delta^{15}N$) signature than naturally available nitrogen. For example, sewage treated water and agricultural fertilizers contain dissolved inorganic nitrogen (DIN) with high $\delta^{15}N$, which acts as an indicator of the level of anthropogenic nitrogen loads to coastal waters. The

$\delta^{15}N$ in the soft tissues of the Manila clam was found to be positively correlated to DIN concentration in the bottom water in tidal flats in several areas in Japan. The higher $\delta^{15}N$ in the clam is considered to be attributable to higher $\delta^{15}N$ in the food particles, especially those in sediment surface, and $\delta^{15}N$ in the food particles was associated with that in DIN. This demonstrates that elevated $\delta^{15}N$ in DIN in coastal waters due to anthropogenic nitrogen loads is reflected in the $\delta^{15}N$ in the clam. Thus, pervasive effects of terrestrial nutrient load to the food availability to the clam may be ascertained by $\delta^{15}N$.

Integrated multi-trophic aquaculture (IMTA) is a technique to use unfed aquaculture of inorganic and organic extractive species to consume effluent from fed aquaculture of finfish. Although IMTA is proposed as a mitigation measure for eutrophication, it can alternatively enhance unfed aquaculture production in an oligotrophic environment. In order to trace nutrient flow within an IMTA system, it is necessary to know how stable isotopic signatures differ between the aquaculture feed and fish feces, both of which are a nutrient source for the organic extractive species, such as for example sea cucumber. We compared the $\delta^{13}C$ and $\delta^{15}N$ of feces collected from five different finfish species (*Lutjanus argentimaculatus, Chanos chanos, Siganus guttatus, Trachinotus blochii* and *Lates calcarifer*) fed the same formulated feed. While the feces $\delta^{15}N$ showed no significant difference among the species, $\delta^{13}C$ were different presumably depending on the digestibility of the plant ingredients. Herbivorous and omnivorous species may be able to digest and assimilate plant ingredients that cannot be used by carnivorous species. Therefore, stable isotopic technique may not be used to trace carbon flow within an IMTA system.

Annotated Bibliography of Key Works

Satoshi Watanabe, Satoshi Katayama, Masashi Kodama, Naritoshi Cho, Kaoru Nakata and Masaaki Fukuda (2009) Small-scale variation in feeding environments for the Manila clam *Ruditapes philippinarum* in a tidal flat in Tokyo Bay. *Fisheries Science* 75: 937–945.

The relative contribution of particulate organic matters (POMs) in water column and sediment as a food source for the Manila clam, *Ruditapes philippinarum*, was studied using carbon and nitrogen stable isotopic method ($\delta^{13}C$ and $\delta^{15}N$) in a tidal flat in Yokohama, Japan. Comparisons of $\delta^{13}C$ and $\delta^{15}N$ among *R. philippinarum* and POMs in surface water, bottom water, and sediment surface indicated that *R. philippinarum* larger than 5 mm shell length (SL) mainly assimilated benthic POM, and individuals smaller than 5 mm SL assimilated benthic and pelagic POM. Continuous measurements of chlorophyll concentrations in the bottom water revealed tide-driven resuspension of the benthic phytopigments. *R. philippinarum* showed differences in $\delta^{13}C$ and $\delta^{15}N$ along an inshore–offshore transect, indicating small-scale spatial differences in POM provision in the tidal flat. These findings suggest that POM in the bottom water, supposedly inhaled by *R. philippinarum*, is a mixture of a larger proportion of resuspended benthic POM

and a smaller proportion of pelagic POM, and that the mixing ratio of the POMs may be affected by the hydrodynamics of flooding water associated with tidal flat topography. Some stable isotopic studies overly emphasize the importance of benthic POM as a food source for benthic communities; however, relative importance of benthic and pelagic food source should be argued quantitatively.

Satoshi Watanabe, Masashi Kodama and Masaaki Fukuda (2009) Nitrogen stable isotope ratio in the manila clam, *Ruditapes philippinarum*, reflects eutrophication levels in tidal flats. *Marine Pollution Bulletin* 58: 1447–1453.

Understanding the effects of anthropogenic eutrophication on coastal fisheries may help in the enhancement of fishery production by effective utilization of sewage effluents, as well as in the consequent reduction of eutrophication. In this study, it was revealed that the nitrogen stable isotope ratio ($\delta^{15}N$) in the soft tissues of the manila clam, *Ruditapes philippinarum*, can be used as an indicator of anthropogenic eutrophication levels in tidal flat environments. Investigation of $\delta^{15}N$ in dissolved inorganic nitrogen (DIN), particulate organic matter (POM), sedimentary organic matter (SOM) and soft tissues of the clam in five tidal flats in Japan with different levels of DIN concentration was completed. In addition, it was found that the acid insoluble fraction of the shell organic matrix, conchiolin, can be used as a proxy for the soft tissues in $\delta^{15}N$ analyses. This will contribute in easier storage handling and the expansion of chances for sample acquisition. Oligotrophication of the coastal waters started to draw attention after publication of this article.

Establishment of immunological assays and baseline profile of hemocytes in the hard clam *Mercenaria mercenaria* as evaluation biomarkers for environmental stresses

Yangqing Zeng and Huiping Yang*

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Environmental stresses, such as temperature, salinity, dissolved oxygen, pollutions, and algal toxins and are important factors to affect shellfish aquaculture by disrupting physical and physiological conditions. In general, vertebrate animals respond to environmental stresses (and diseases pathogens) through physical barriers, such as skin-fur system, and a complex network of circulating cells and molecular regulations by neurological, endocrinological, and immunological systems. As invertebrates, aquaculture shellfish bivalves can usually close their shells tight or move away (for species with this capability) as immediate responses to environmental stresses. On neurological or endocrinological levels, the responses are limited due to their primitive nervous system and endocrine organs. On immunological level, shellfish bivalves possess an open circulatory system with hemolymph circulating through vessels and sinuses throughout soft tissues and the internal organs to deliver nutrients and oxygen. Through hemocytes in circulatory system, shellfish bivalves can use innate immunity, a primitive nonspecific immunological system which all animals have, to provide internal defensive functions against various pathogen and environmental stresses, and may also use adaptive immunity, an acquired sophisticated immunological system which was used to recognize only in vertebrates but now in bacteria and invertebrates, to recognize and destroy specific invaders.

Shellfish aquaculture in the U. S. is a $325 million industry and involves in several hundreds of farms along the coastal states. Environmental stresses often occur in the inshore farming sites, such as high temperatures in hot summers, especially during low tides with shallow water, dramatic salinity changes with river rundown in rainy season, and harmful algal bloom. While facing these stresses, hemocytes immune system of shellfish would make responses. With prolonged exposure, shellfish could be subsequently susceptible to pathogens, increase disease outbreaks and eventually suffer heavy mortality. Similar to the blood tests as diagnostic tool for health evaluation in human and livestock, it is hypothesized that hemocyte assays in shellfish could be used as effective parameters to evaluate the impact of the environmental stresses, serve as criteria for genetic breeding, and provide diagnosis tools to guidance the aquaculture operation management.

The goal of this study was to develop hemocyte immunological assays and establish baseline profile for hemocyte in the hard clam *Mercenaria mercenaria*, one major aquaculture species. The immunological assays established in this study will include hemolymph osmotic pressure, pH, hemocyte count, cell type, viability, apoptosis, phagocytosis, and reactive oxygen species (ROS) production. Cell count showed a concentration of 0.809-5.310 × 106/ml (n = 10) with three different types – agranulocytes, granulocytes and blast-like agranulocytes. Cell viability stained with SYBR green I and propidium iodide indicated a percentage of 71.6 ± 12.4% alive cells (n =10). This study is currently ongoing, and more data will be further reported.

Annotated Bibliography of Key Works

Anisimova, A. A. (2013). *Morphofunctional parameters* of hemocytes in the assessment of the physiological status of Bivalves. *Russian Journal of Marine Biology* 39(6): 381-391.

This is the comprehensive review publication on shellfish hemocyte immunology. The basic morphology, cell type, cell function and dynamic changes of hemocytes in shellfish bivalves were summarized. The effects of temperature, season, salinity, annual cycle, food quality availability, toxin algae, bacteria and virus, pollutions, and heavy metals on hemocyte morphology, number and function were reviewed and documented.

Donaghy, L., et al. (2009). Flow cytometry studies on the populations and immune parameters of the hemocytes of the Suminoe oyster, *Crassostrea ariakensis*. *Fish & Shellfish Immunology* 27(2): 296-301.

This publication studied the immunological activities and morphology of hemocytes in the Suminoe oyster *Crassostrea ariakensis* using flow cytometry and light microscopy. Three types of hemocyte types were identified, including hyalinocyte, granulocyte and blast-like cells. Cell count, survival, mortality, phagocytosis, and reactive oxygen species (ROS) production were evaluated using flow cytometer with different staining methods. It revealed that the granulocytes are most active in the cell phagocytosis and the hyalinocytes showed a certain level of the phagocytosis and oxidative activity, and the blast-like cells did not show any phagocytosis or oxidative activity.

Vieira, G. C., et al. (2017). Morphological and functional characterization of the hemocytes from the pearl oyster *Pteria hirundo* and their immune responses against Vibrio infections. *Fish & Shellfish Immunology* 70: 750-758.

This paper tested most hemocyte parameters including morphological characterization through light & electron microscopy and flow cytometry. Same as that in Sumino oysters, three types of the hemocytes were identified. Assays of phagocytosis and reactive oxygen species (ROS) production was performed by use of flow cytometer. Furthermore, hemocyte responses with exposure to a Vibrio pathogen was evaluated.

Hegaret, H., et al. (2003). Flow cytometric analysis of haemocytes from eastern oysters, Crassostrea virginica, subjected to a sudden temperature elevation II. Haemocyte functions: aggregation, viability, phagocytosis, and respiratory burst. *Journal of Experimental Marine Biology and Ecology* 293(2): 249-265.

This paper studied the effects of temperature increase on hemocyte functions of eastern oysters, including aggregation, viability, phagocytosis, and ROS production. This is one of a series of publications on oyster hemocyte functions and detailed protocols were documented for readers to understand the analysis procedure. The results indicated that temperature increase (from 20 to 28 degree) caused no significant change in hemocyte aggregation, decreased the phagocytosis of all hemocyte types, induced significant hemocyte mortality in all hemocyte types, and increased, although not significantly, the ROS production.

Jauzein, C., et al. (2013). Flow cytometric characterization of hemocytes of the sunray venus clam *Macrocallista nimbosa* and influence of salinity variation. Fish & Shellfish Immunology 35(3): 716-724.

Salinity is one the most important factor potentially affecting shellfish physiology, especially in the inshore area with river flows. This paper estimated the hemocyte types and cellular parameters (oxidative activity, lysosomal content, phagocytosis capacity) in sunray venus clams, a potential aquaculture species in Florida. After exposure to salinities of 18, 21, 25, 30, 35 and 38 ppt for 7 days, hemocyte samples were collected and analyzed the parameters along with estimation of physiological status of clams, including mortality, valve closure, and filtration activity. It was interestingly found that hemocytes of sunray venus clam appeared as a unique population, both in terms of morphology and intracellular parameters. Clams after transferring to 18 and 21 ppt resulted in valve closure, mortality and decreased filtration activity. Low salinities highly impacted hemocyte functions as follows: increased cell and lysosomal compartment volumes, decreased phagocytosis capacity, and increased oxidative stress and mortality.

Purple urchin barrens: an opportunity for aquaculture and fisheries to work together to solve an environmental issue

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Sea urchin barrens and can stretch over 1000s of kilometers and last decades at a time. They are characterized by a predominance of urchins and coralline algae where kelp forests once existed. In contrast to barrens, kelp forests provide habitat supporting thousands of vertebrates, invertebrates and plant species. Because kelp forests are keystone hosts, their presence is vital to sustaining commercial and recreational industries, including fishing and tourism. However, these kelp forests can collapse and shift to alternate stable states whereby urchin barrens persist. Over the last 4 decades, transitions between kelp beds and sea urchin barrens have been widely reported along temperate coastlines globally. During a kelp forest phase, urchin predation is the primary mechanism keeping sea urchin populations in check. However, due to various factors including climate change, predator densities can be reduced leading to shifts toward urchin barrens. Development of urchin fisheries has been implicated several times in recent history as a driver to return urchin barrens to kelp forests. However, this driver most recently has not worked in California where a large barren is persisting. California already had a urchin fishery, but it has been uneconomical for the fishery to operate given urchins in the barrens had little gonad development or undesirable human consumption traits necessary for commercialization. Aquaculture in the US has potential to restore kelp forests by collaborating with fisheries to harvest wild urchins from barrens and fatten them in an aquaculture setting prior to sale. This is of particular interest in California given U.S fisheries and environmental groups have long opposed aquaculture development due to concern of competition and environmental damage respectively. A collaboration between aquaculture and fisheries to positively impact the environment via urchin barren restoration would help to develop aquaculture in the US and California. Although urchin ranching operations exist, there are still technical limitations to the activity, primarily being availability of macroalgae diets given seasonality and the propensity of urchin barrens to deplete kelp forests. Development of sustainable alternative diets for urchins

is necessary for future commercial urchin aquaculture. In this student lead study, a preliminary replicated diet trial was performed for ranching purple sea urchins (*Strongylocentrotus purpuratus*) collected from California barrens using 4 diet treatments including giant kelp (*Macrocystis pyrifera*), ogo (*Gracilaria pacifica*), formulated commercial diet (Urchinomics) and an unfed control. During the 10 week study duration, gonadal somatic index (GSI) was measured in a subset of urchins from each replicate tank every 2 weeks. Baseline GSI at the beginning of the trial was <0.5%. A GSI of 10% was reached most rapidly in the formulated diet treatment at 6 weeks, followed by ogo and kelp at 8 and 17 weeks, respectively. This study was a preliminary examination of the feasibility of urchin ranching in California, showing biological potential for alternative diets to develop urchin gonads with a view to restore kelp forests and develop a nascent aquaculture industry in California.

Annotated Bibliography of Key Works

Filbee-Dexter, K., Scheibling, R.E., 2014. Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine Ecology Progress Series* 495: 1–25. https://doi.org/10.3354/meps10573

This paper provides an excellent description of sea urchin barrens as to what they are comprised of and their extent both temporally and spatially across the world. The authors examine and list the drivers of phase shifts between barrens and kelp forests. They describe different thresholds for forward (to barrens) and reverse (to kelp beds) shifts, in accordance with alternative stable-state dynamics. They surmise that accelerating climate change and increasing anthropogenic impacts play important roles in altering alternative stable-state dynamics and triggering phase shifts.

Heflin, L.E., Makowsky, R., Taylor, J.C., Williams, M.B., Lawrence, A.L., Watts, S.A., 2016. Production and economic optimization of dietary protein and carbohydrate in the culture of juvenile sea urchin *Lytechinus variegatus*. *Aquaculture* 463: 51–60. https://doi.org/10.1016/j.aquaculture.2016.05.023

This manuscript is a through collection of urchin feeding experiments to understand the nutrient requirements of urchins for aquaculture. The paper creates predictive models of growth, production and efficiency outcomes and generates economic analysis models in relation to these dietary outcomes for urchins held in culture. The models compare dietary requirements and growth outcomes in relation to economic costs and provide insight for future commercialization of sea urchin aquaculture

Unuma, T., Sakai, Y., Agatsuma, Y., Kayaba, T., 2015. Sea Urchin Aquaculture in Japan, in: Echinoderm Aquaculture. John Wiley & Sons, Ltd, pp. 75–126. https://doi.org/10.1002/9781119005810.ch5

This is the most recent review of urchin aquaculture in Japan. Japan is the foremost

consumer of urchins and significant producers of urchins both from ranching and closed life cycle aquaculture. The chapter details the history of urchin fisheries in Japan and the rise of urchin barrens and urchin aquaculture. The review discusses the diver mediated destruction of urchins to bring back kelp beds as well as reseeding efforts to restore overfished urchin grounds. The review also discusses the development of full life-cycle aquaculture to meet both reseeding and commercial production requirements. Also the movement of urchins from barrens to other kelp grounds and aquaculture facilities for commercial fattening are detailed.

Impacts of ocean acidification on Japan coastal water and marine fisheries

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Increase of anthropogenic atmospheric carbon dioxide (CO_2) is now causing change of seawater carbon chemistry, which is known as ocean acidification (OA, IPCC 2007). As the excess atmospheric CO_2 dissolves into the seawater, seawater pCO_2 rises while seawater pH and calcium carbonate saturation state (Ω) decrease (Orr et al. 2000). Several studies have demonstrated that change of seawater carbonate chemistry can affect the physiology of marine organisms, particularly reducing calcification of marine calcifiers such as mollusks, echinoderms and corals (Fabry et al. 2008, Kurihara 2008, Doney et al. 2009). In addition to these global climate impacts, principally in coastal waters, land derived stresses such as eutrophication and hypoxia can affect coastal ecosystems and marine organisms (Gattuso et al. 2015). Hence, there is now a strong need for understanding the coastal seawater chemistry and its potential impact on marine resources. Considering the high reliance on marine resource as a food source for tourism, here in this study, I will highlight present scientific knowledge and gaps of ocean acidification impacts in coastal waters of Japan, including Okinawan reefs.

Annotated Bibliography of Key Works

Kurihara H (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series* 373: 275-284
This paper first reviewed the effect of ocean acidification (OA) on the early developmental stages of marine calcifiers including mollusks, sea urchins and corals. Results highlight that future changes in ocean acidity will potentially impact the population size and dynamics, as well as the community structure of calcifiers, and will therefore have negative impacts on marine ecosystems.

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science* 1: 169-192
This paper has highlighted that global warming and the increase of atmospheric CO_2 can cause another problem in the ocean called ocean acidification. This paper reviewed the effect of increase atmospheric CO_2 on ocean carbon system, the biological effects of ocean acidification on marine organisms and the potential impacts on marine ecology and biogeochemistry.

Gattuso J-P, Magnan A, Bille R, Cheung WWL, Howes EL, Joos F, Bopp L, Cooley SR et al. (2015) Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. *Science* 349 (6243)

This paper reviewed the potential impacts of climate change including ocean acidification on the ocean ecosystem and its services under high and stringent emission scenario (RCP 8.5 and 2.6). Results suggest that services, including coastal protection and fish capture, will be in high risk at RCP 8.5 scenario.

Sustain seafood resources in the U.S. affiliated Pacific islands- status and strategies

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The U.S.-affiliated Pacific Islands (U.S.A. PI) include American Samoa, the Republic of the Marshall Islands (RMI), the Federated States of Micronesia (FSM), the Commonwealth of the Northern Mariana Islands (CNMl), Guam, and the Republic of Palau (Palau). This region, composed of thousands of tiny islands widely spread between the latitudes of 15° N to 14° S and the longitudes of 134° E to 170° W, extends across an area as large as the continental United States. With less than 2558 km2 in total land mass and extended Exclusive Economic Zone (EEZ), the primary source of dietary protein for Pacific islanders has come from the Ocean. The per capita seafood consumption exceeded global average. In 2015, per capita seafood consumption in Oceania was 25.0 kg/year vs 15.5 kg/year worldwide (FAO 2018).

Seafood comes from capture fisheries and aquaculture but capture fisheries are the main seafood source for the U.S.A. PI. Climate change and overfishing are two major threats to the sustainability of yield from capture fisheries. Although there were optimistic reports on status of tuna stocks in the Pacific, foreign fishing companies have exported majority of their catch to consumers outside the islands. Islanders have relied on subsistence catch to meet their demand. As the harvest from nearshore fishery declined, they have to consider secure their seafood supplies via aquaculture. However, the islands of the Pacific is still the least developed region in terms of aquaculture worldwide as concluded at 2010 FAO Global Conference on Aquaculture in Thailand. On the other hand, this region enjoys superior natural resources, such as pristine water, year-round warm weather, and isolated condition for disease prevention. However, it has to overcome constraints to aquaculture development such as small land area, natural hazards for some islands, lack of knowledge base, short of available capital, distant markets, and poor transportation systems. Other than natural hazards for some islands, the other constraints are solvable. A good strategic development plan is essential to reveal aquaculture potential in the region.

This report discusses the potential threat of climate change to fisheries, reviews the current status and challenges of aquaculture, and finally presents some suggestions on future development.

Annotated Bibliography of Key Works

Adams, T., Bell, J. and Labrosse, P. 2001. Current status of aquaculture in the Pacific Islands. In: R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 295-305. NACA, Bangkok and FAO, Rome.

In the book "Aquaculture in the Third Millennium", this chapter "Current status of aquaculture in the Pacific Islands" gave an overview the status of aquaculture in the Pacific Islands at the turn of 20th century. It served as the base to assess any new development in 21st century. Through the new wave of international cooperation, it is expected to increase sustainable use of aquatic resources to meet the goal of food security. Food and Agriculture Organization of the UN (FAO) and Network of Aquaculture Centres in Asia-Pacific (NACA) co-organized this conference in 2000.

Lee, C.S. and Awaya, K. 2003. Viable aquaculture development in the U.S. affiliated islands – lessons from giant clam and sponge farming. *Aquaculture Economics & Management* 7 (1&2):125-135.

This paper reviewed the farming technology for giant clam and bath sponges. Then, it used giant clam and bath sponges as an example to discuss the challenges of technology transfer, which include biological, technological, environmental, and socioeconomic, to Pacific Islands. Finally, some recommendation was made for the successful technology transfer.

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. 227pp.

An important of aquaculture and fisheries status report published by FAO every two years. It highlights the critical importance of fisheries and aquaculture for the food, nutrition and employment of millions of people, many of whom struggle to maintain reasonable livelihoods. Data and graphics presented in this publication are widely used by research groups to assess the progress and to propose future works.

Bell, J.D., Johnson, J.E., Ganachaud A.S., Gehrke, P.C., Hobday A.J., Hoegh-Guldberg, O., Le Borgne, R., Lehodey, P., Lough, J.M., Pickering, T., Pratchett, M.S. and Waycott, M. 2011. Vulnerability of Tropical Pacific Fisheries and Aquaculture to Climate Change: Summary for Pacific Island Countries and Territories. Secretariat of the Pacific Community, Noumea, New Caledonia. 386pp.

The book entitled Vulnerability of Tropical Pacific Fisheries and Aquaculture to Climate change provides the region with the understanding needed, and the adaptations, policies and investments recommended to reduce the likely impacts of climate change on fisheries and aquaculture. It also gives the sector a roadmap for capitalising on the opportunities expected to arise from the changing climate. This book is the product of a

partnership that started between the Australian Agency for International Development (AusAID) and the Secretariat of the Pacific Community (SPC), and then grew to embrace contributions from 36 institutions. This summary, which is a companion to the book, provides this vital information in an accessible form for each Pacific Island country and territory. It is a quick access to understand projected changes to surface climate and the ocean, to oceanic fisheries, to coastal fisheries, to freshwater and estuarine fisheries, to aquaculture, economic and social implications, and adaptations and suggested policies. It is a valuable document to have a quick overview of potential climate change impacts and suggested remedy policy.

Charlton, K. E., Russell, J., Gorman, E., Hanich, Q., Delisle, A., Campbell, B., and Bell, J. 2016. Fish, food security and health in Pacific Island countries and territories: a systematic literature review. BioMedCentrak *Public Health* 16: 285

This paper discussed the importance of fish to Pacific Island Countries and territories (PICTs) in both food security and health related concerns based on the review of 29 studies. However, there is a paucity of research aimed at assessing how maintaining and/or improving fish consumption benefits the diets and health of Pacific Islanders. Instead of fresh seafood, there is an increasing demand for packaged imported foods, such as canned meats, instant noodles, cereals, rice, and sugar-sweetened beverages, with subsequent decreased consumption of locally-produced plants and animals.

Gillett, R., and Tauati, M. I. 2018. Fisheries in the Pacific Regional and national information. FAO Fisheries and Aquaculture Technical Paper No. 625. Apia, FAO. 401pp.

This paper discusses the important species, the status of the resources, and the fisheries management under offshore fishing and coastal (or nearshore) fishing. This report also provides information on the fisheries in each of the 14 independent Pacific Island countries (including Federated States of Micronesia, Marshall Islands, and Palau) in the following categories:

- Overview and main indicators
- Production sector
- Post-harvest sector
- Socio-economic contribution of the fishery sector
- Trends, issues and development
- Institutional framework
- Legal framework

Towards effective coral community restoration for sustainable fishery of a coral reef grouper *Epinepherus ongus*: implication of ecosystem-based management

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Spawning aggregation of coral reef fishes are very vulnerable to fishing since only conspecific individuals gather at particular sites in restricted seasons and lunar phases. Since capturing spawning aggregation has a significant negative impact for both local stock and reproductive success, protection of the spawning aggregation has been a recently urgent action in coral reefs all over the world. Furthermore, conservation of habitat in non-spawning season is also essential for the species that form spawning aggregation since habitat loss at their home ground would also lead to reduce their population.

In the present presentation, I will focus on a coral reef grouper, *Epinephelus ongus,* that is a very important fishery species and forms spawning aggregations in an Okinawan coral reef and discuss effective coral community restoration to establish a sustainable fishery of the species. My previous studies have shown: (1) their home ground reached several kilometers from the spawning ground; (2) their home range was very limited around a coral colony in non-spawning period; (3) juveniles preferentially used corals with fine structures (e.g. bottle-brushed acroporid corals), whereas adults were mainly found at corals with coarse structures (e.g. massive corals and staghorn acroporid corals); (4) very precise returning ability was observed after spawning migration, i.e., the species could return to the coral colonies that were used before the spawning migration.

These results suggest that: (1) conservation of coral community around the spawning ground is indispensable; (2) coral species that is preferentially used by *E. ongus* should be selected for coral community restoration; (3) the area that is several kilometers around the spawning ground should be the proposed area for coral community restoration. Since the spawning ground of the species has been already assigned as a marine protected area, coral community restoration around the spawning ground would be useful to enhance the *E. ongus* stock.

Annotated Bibliography of Key Works

Almany GR et al. 2013. Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. *Current Biology* 23: 626-630.

This study examined the dispersal distance of larvae of a coral reef grouper, *Plectropomus areolatus,* from the spawning ground. The parentage analysis revealed that 50% of the larvae settled within 14 km of the spawning ground. The noteworthy point of the study is that the spawning ground protection would be effective at a local scale (within several-tens of kilometers) for larval dispersal. The method described in the present study is greatly helpful to test the actual manner of larval dispersal in my study site.

Nanami A et al. 2013. Microhabitat association in white-streaked grouper *Epinephelus ongus*: importance of *Acropora* spp. *Marine Biology* 160: 1511-1517.
Microhabitat associations are considered to be important for juvenile survivorship and growth of coral reef fishes. The aim of this study was to quantify microhabitat associations for juvenile and adult white-streaked grouper *Epinephelus ongus*. Underwater observations revealed that most juveniles were found in bottlebrush *Acropora* spp., staghorn *Acropora* spp. and coral rubble and there was a significant positive use of bottlebrush *Acropora* spp. and a significant negative use of coral rubble. For adults, most individuals were found in bottlebrush *Acropora* spp. and staghorn *Acropora* spp., and there was a significant positive use of staghorn *Acropora* spp. and significant negative use of coral rubble. A habitat choice experiment by using pre-settlement individuals revealed that both bottlebrush *Acropora* spp. and staghorn *Acropora* spp. were used as settlement sites, whereas coral rubble was rarely used as a settlement site. Field results of the study suggest that juvenile and adult *E. ongus* showed significantly positive microhabitat associations with bottlebrush *Acropora* spp. and staghorn *Acropora* spp., respectively. Bottlebrush *Acropora* spp. has smaller interbranch spaces than staghorn *Acropora* spp., which could drive patterns of microhabitat associations. In addition, post-settlement processes such as predation may influence the spatial distribution of juveniles.

Nanami A et al. 2015. Estimation of spawning migration distance of the white-streaked grouper (*Epinephelus ongus*) in an Okinawan coral reef system using conventional tag-and-release. *Environmental Biology of Fishes* 98: 1387-1397.
The white-streaked grouper (*Epinephelus ongus*) is an important fisheries species in the region around Okinawa. This species forms spawning aggregations at specific spawning grounds in the last quarter of the moon in April and/or May. Using a tag-and release method, we estimated the migration distance and the degree of unified movement of groupers associated with the spawning migration. In total, 1157 *E. ongus* individuals were tagged and released at their home grounds in the non-spawning period and 350 were tagged at a known spawning ground (Yonara Channel) in the spawning period. For the fish that were released at the home grounds, 23 individuals were recaptured at the spawning ground during the spawning periods. For the individuals that were released at

the spawning ground, six individuals were recaptured outside of the spawning ground. The estimated migration distances from the home ground to the spawning ground ranged from 2.2 to 8.8 km.

Nanami A et al. 2017. Spawning aggregation of white-streaked grouper *Epinephelus ongus*: spatial distribution and annual variation in the fish density within a spawning ground. *PeerJ* 5: e3000
The aims of the present study were to investigate the ecological characteristics of annual spawning aggregations such as 1) spatial variations in the density of *E. ongus* at the spawning ground, 2) the relationship between fish density and environmental variables, 3) inter-annual variations in the spawning aggregation, 4) the proportion of males to females at the spawning ground for several days pre and post-spawning and 5) the relationship between male density and female density at the protected spawning ground, based on observations over five years at an Okinawan coral reef. Although the protected spawning ground area was large (ca. 2500 m × 700 m), high density of *E. ongus* (over 25 individuals per 100 m^2) was found in a limited area (within c.a. 750 m × 50 m). Current velocity and coverage of rocks had significant positive effects on the spatial distribution of *E. ongus* at the spawning ground. Inter-annual variation in the degree of aggregation was found and this variation was explained by the annual variation of mean seawater temperature 40 days before the spawning day. The male-female ratio (male : female) at the spawning ground was ca. 3 : 1 for three years (May 2012, May 2014 and May 2015) whereas >13 : 1 for one year (May 2013). Significant positive relationships between male density and female density were found at the aggregation sites. It is suggested that *E. ongus* use aggregation sites with greater current velocity to reduce the risk of egg predation and seawater temperature is one of the main factors that is responsible for determining the degree of aggregation. It is also suggested that females possibly select sites with a greater density of males and this selection behavior might be the reason why females arrived at the spawning ground after the arrival of the males. For effective management of spawning grounds, precise site selection as well as the duration of the protection period are suggested to be key aspects to protect the spawning aggregations of *E. ongus*, which have been currently achieved at the spawning ground.

Nanami A et al. 2018. Diel variation in home range size and precise returning ability after spawning migration of a coral reef grouper *Epinephelus ongus:* implications for effective marine protected area design. *Marine Ecology Progress Series* 606: 119-132.
Marine protected areas (MPAs) are regarded as effective tools for protection of marine organisms. Precise estimation of home range size as well as diel differences in home ranges are essential to consider appropriate MPA size. In addition, for setting MPAs, behavioral characteristics of the spawning migration should also be considered for species that form spawning aggregations. The purpose of the present study is to clarify

the diel variation in home range size and the degree of precision for returning ability of white-streaked grouper *Epinephelus onus* by acoustic telemetry. Seventeen individuals were studied, and night-time home range sizes that were calculated by 50% and 95% kernel utilization distributions were 5.9-times and 5.5-times greater than the respective daytime home ranges. The average inter-center distance of home ranges between the two time periods ranged from 3.0 m to 67.9 m (22.5 m on average), suggesting that day-night home range shift in the home ground varied individually. Returning ability for 10 individuals that showed clear spawning migration behaviour was also analysed and average inter-center distance of home ranges between the period before and after the spawning date was 8.1 m. Eight out of the 10 individuals showed precise returning after the spawning migration to the patchy coral substrates that were used before the spawning migration. The present study suggests that appropriate setting position of the home ground can establish long-term protection of the species due to their precise returning ability after the spawning migration.

Scaling up coral restoration to meet the demands of a collapsing ecosystem

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Coral reefs feed millions of people on earth and are critical to the livelihoods of millions more. They house approximately a quarter of the ocean's biodiversity and are critical to fisheries worldwide. Their importance and fragility to humans cannot be overstated. According to climate models, in about thirty years half of all coral reefs are predicted to disappear due to bleaching caused by unprecedented ocean warming. If political and economic forces begin to seriously address emissions, corals will still need help maintaining enough abundance and diversity to rebuild reefs by the end of the century when ocean temperatures may begin to stabilize. With the tacit assumption that emissions will be curbed, an explosion of coral restoration and research into coral "interventions" has taken place over the past two years. Coral aquaculture is a pivot point of any advancement in scaling-up restoration or implementing a new intervention - as any and all applications involve the delicate care and propagation of corals in the water or on land. NOAA has taken a lead role in coordinating coral restoration efforts throughout the US and the globe, primarily via the Coral Restoration Consortium and by commissioning the National Academies of Sciences to review coral interventions. I will highlight recent restoration and intervention successes in the U.S. and globally and describe NOAA's proposed research and action plan on coral interventions. I will also describe my own research on coral population modelling, highlighting how results can be incorporated into decision-making processes that help managers and practitioners implement and increase the success and scale of coral restoration programs.

Annotated Bibliography of Key Works

National Academies of Sciences, Engineering, and Medicine. 2019. A Research Review of Interventions to Increase the Persistence and Resilience of Coral Reefs. Washington, DC: The National Academies Press AND National Academies of Sciences, Engineering, and Medicine. 2019. A Decision Framework for Interventions to Increase the Persistence and Resilience of Coral Reefs. Washington, DC: The National Academies Press.
NOAA commissioned the U.S. National Academies to review the science behind next generation coral restoration techniques. Most of these techniques rely on coral aquaculture in one form or another. The Research Review groups 23 interventions types (e.g. assisted evolution, marine shading to prevent bleaching, genetic engineering) into

four categories and describes the benefits, potential scale of application, current feasibility, risks, limitations, and infrastructure needs for each. The Decision Framework outlines an adaptive management strategy by which interventions can be evaluated against each other, additional research needs, and recommendations for the Caribbean. Research on coral interventions is progressing very rapidly and these reports thoroughly capture the current state of the science and make some solid recommendations about how the field should move forward in the near future.

Mary Hagedorn, Christopher A. Page, Keri O'Neil, Daisy M. Flores, Lucas Tichy, Valérie F. Chamberland, Claire Lager, Nikolas Zuchowicz, Kathryn Lohr, Harvey Blackburn, Tali Vardi, Jennifer Moore, Tom Moore, Mark J. A. Vermeij, Kristen L. Marhaver. 2018. Successful Demonstration of Assisted Gene Flow in the Threatened Coral Acropora Palmata Across Genetically-Isolated Caribbean Populations using Cryopreserved Sperm. BioRxiv.

Rescuing genetically depauperate coral populations by increasing genetic diversity from nearby populations, is one of the most feasible coral interventions proposed. This study demonstrated for the first time that viable juveniles of an endangered coral can be created by artificially inseminating cryo-preserved sperm from one population, with eggs of another regionally-distinct population. This project highlighted the importance and need for additional expertise in coral larval rearing and early life-stage aquaculture. Currently, there are only a handful of such experts worldwide. As corals continue to be threatened with extinction primarily due to climate change, these techniques will become increasingly necessary for the long-term persistence of coral reefs which feed hundreds of millions of people and provide habitat to 25% of marine fisheries globally.

Darling, E. S., McClanahan, T. R., Maina, J., Gurney, G. G., Graham, N. A., Januchowski-Hartley, F., ... & Puotinen, M. (2019). Social–environmental drivers inform strategic management of coral reefs in the Anthropocene. *Nature Ecology & Evolution*.

Data from more than 2500 reefs across the Indo-Pacific Ocean were analyzed to delineate key drivers and suggest appropriate management at three different levels: protection, recovery, and transformation. Over 50% of surveyed reefs would benefit from recovery which includes some form of restoration in addition to traditional management, and, most importantly, reduction in ocean warming. Using comprehensive studies like this and the Global Coral Reef Monitoring Network's reports, should help nations prioritize coral research, restoration, and management efforts.

Iliana B. Baums, Andrew C. Baker, Sarah W. Davies Andréa G. Grottoli, Carly D. Kenke,l Sheila A. Kitchen, Ilsa B. Kuffner, Todd C. LaJeunesse, Mikhail V. Matz, Margaret W. Miller, John E. Parkinson, Andrew A. Shant. 2019. Considerations for maximizing the adaptive potential of restored coral populations in the western Atlantic. *Ecological*

Applications.

This paper is a succinct guide to maximizing genetic diversity using various coral aquaculture techniques for restoration. Despite differences in species and environmental conditions, restoration is happening throughout the globe. Although this paper uses a Caribbean example, the recommendations serve as a helpful starting point for coral aquaculture and restoration elsewhere. The paper explicitly recommends the number of genets per area that should be planted to maintain or maximize diversity. Because bleaching, coral gardening, and assisted evolution have the potential to reduce genetic diversity, having clear guidelines that managers and practitioners can understand is paramount for proper aquaculture and coral reef restoration techniques.

Sustainable large-scale coral restoration by establishing “artificial spawning hotspots”

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Coral reefs have degraded over time and, recently, severe bleaching events during 2015-2017 caused further damage to coral communities worldwide. Because coral reefs are an important source of coastal fish and invertebrates in tropical coasts, these fisheries resources are decreasing as well. For sustainable use of these resources, therefore, it is important to preserve coral communities that act as fisheries grounds and nurseries. Coral transplantation is known as a positive restoration method. However, large-scale transplantation requires a great deal of labor and, even if it succeeds once, the transplanted corals are vulnerable to extermination by only a single disturbance such as bleaching or an outbreak of crown-of-thorns starfish (COTS). Hence, we need to enhance annual larval recruitment for sustainable large-scale coral restoration.

Two key factors are crucial for sustainable coral restoration by enhancing reproduction. The first factor is establishment and maintenance of “artificial spawning hotspots” that consist of densely populated conspecific adult colonies. The second factor is improvement of initial survivability by collecting eggs and sperms at spawning and rearing larvae until settlement. For “artificial spawning hotspots”, safeguards are required against predation by COTS and the use of shading is desirable against bleaching during the high seawater temperature season. In addition, a special gamete and larval collector, “larval cradle”, was developed to consistently perform bundle collection, fertilization, and larval rearing. Eventually, we aim to develop simple techniques that can be easily handled by any users such as fishermen and leisure divers.

Annotated Bibliography of Key Works

Zayasu Y, Suzuki G (2019) Comparisons of population density and genetic diversity in artificial and wild populations of an arborescent coral, *Acropora yongei*: implications for the efficacy of “artificial spawning hotspots”. *Restoration Ecology*: 27: 440-446
The authors assessed population density and genetic diversity of a wild, arborescent coral, *Acropora yongei*, and compared these parameters with those of an artificially established *A. yongei* population in the field. The population density of wild arborescent corals was only 0.27% of that in the artificial population, even in a high-coverage area. Genetic diversity was also low in the wild population compared with the artificial population, and approximately 10% of all wild colonies were clones. Based on these

results, the larval supply in the artificial population was estimated to be at least 1,400 times higher than that in wild *A. yongei* populations for the same area of adult population.

Suzuki G, Yamashita H, Kai S, et al. (2013) Early uptake of specific symbionts enhances the post-settlement survival of Acropora corals. *Marine Ecology Progress Series* 494: 149-158.
The authors tested the hypothesis that early acquisition of symbionts enhances post-settlement survival. Symbiotic and aposymbiotic *Acropora* larvae were prepared in the laboratory and settled on experimental plates in the field. The survival of settlers was monitored for 15 months and the results showed that more larval-stage settlers harbouring symbionts survived than those without. The higher survival rate of 'early uptake' corals was more pronounced on shaded plates. These results suggest that the early uptake of specific symbionts enhances post-settlement survival in dark places such as reef crevices, which are sites commonly settled by coral larvae.

Suzuki G, Kai S, Yamashita H, et al. (2011) Narrower grid structure of artificial reef enhances initial survival of in situ settled coral. *Marine Pollution Bulletin* 62: 2803-2812.
The authors demonstrated through field experiments that the design of artificial grid plates may influence the initial survival of *Acropora* corals, with narrower grids being the most effective. In fact, grid plates with a 2.5-cm mesh presented the highest recorded survival rate (14%) at 6 months after settlement (representing approximately 50 corals per 0.25 m^2 of plate). This was the first study where such high survival rates, matching those of cultures under aquarium conditions, were obtained in the field without using additional protective measures, such as guard nets against fish grazing after seeding. Therefore, their results provide a foundation for establishing new and effective coral restoration techniques for larval seeding.

The influence of climate and environment on the growth and survival of Pacific oyster seed in US west coast estuaries

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Pacific oysters *Crassostrea gigas* were introduced to the US west coast in the early 1900's and were initially raised directly from seed (juveniles that had set naturally on cultch shell) imported from Japan. Oysters regularly spawned and became "naturalized" in only several selected West coast locations, such as Willapa Bay, Washington, where conditions allowed for both adult oyster spawning and larval survival, retention and settlement. The shellfish industry relied on "natural or wild" caught seed from these locations or continued seed imports until the advent of hatchery technology in the late 1970's. Recent larval mortality events in hatcheries have been linked directly to changes in seawater chemistry with high pCO_2 conditions and acidified water associated with seasonal upwelling along the U.S. West Coast. These conditions may have also resulted in reduced natural oyster sets in Willapa Bay, but estuarine gradients in water chemistry and temperature add complexity making this more difficult to assess. Shellfish hatcheries have adapted to these conditions by measuring seawater carbonate chemistry, buffering incoming water, and adjusting the timing of larval production cycles. While there appear to be larval fitness traits that are genotype-dependent, the potential of breeding programs to improve OA specific traits remains uncertain. Experiments have rarely been conducted that distinguish success at important physiological transitions in the larval life history and potentially also at the juvenile seed stage when these oysters are out-planted to estuaries with variable conditions.

We present results from experiments where survival of oyster larvae raised from crosses of "wild" parental broodstock collected in Willapa Bay was compared to that of larvae raised from controlled crosses with improved lines created by the Molluscan Broodstock Program (MBP) at Oregon State University. The MBP breeding program was designed to enhance valuable field traits including growth and survival of juvenile and adult oysters, but to date has not explicitly addressed larval traits. Nonetheless, MBP larvae produced more than twice the number of settled spat compared with wild larvae under commercial hatchery conditions (with buffered seawater, pH ~8.3 and Ω_{arag} >2). This advantage also occurred, but to a lesser degree, under unfavorable high pCO_2)

conditions. Separate experiments were conducted to evaluate survival of juvenile seed along the estuarine gradient and inside and outside eelgrass (*Zostera marina*) which also has the potential to modify local seawater chemistry. Growth and survival of juvenile oyster seed varied along the estuarine gradient with fastest growth, but lower survival occurring near the estuary mouth and there was no apparent effect of seed source. Results from experiments where MBP seed was planted both inside and outside eelgrass along these same gradients, suggest that this plant can reduce seed growth, especially at locations away from the estuary mouth.

Annotated Bibliography of Key Works

Barton, A., G.G. Waldbusser, R.A. Feely, S.B. Weisberg, J.A. Newton, B. Hales, S. Cudd, B. Eudeline, C.J. Langdon, I. Jefferds, T. King, A. Suhrbier, and K. McLaughlin. 2015. Impacts of Coastal Acidification on the Pacific Northwest Shellfish Industry and Adaptation Strategies Implemented in Response. *Oceanography* 28: 146-159.

This is a review of the history and science underpinning the effects of changing seawater chemistry on bivalve shellfish larvae and the impacts that have already taken place to the commercial shellfish aquaculture industry on the US West Coast. Multiple authors contributed to this review which addresses a broad audience but covers the leading research on direct effects to bivalve larvae as well as monitoring seawater conditions and adapting to these changes.

Durland, E., G. Waldbusser, and C. Langdon. 2019. Comparison of larval development in domesticated and naturalized stocks of the Pacific oyster Crassostrea gigas exposed to high pCO_2 conditions. *Marine Ecology Progress Series* 621: 107-125.

Two replicated experiments were conducted where Pacific oyster larvae from "wild" parent crosses and those from selected stocks (OSU Molluscan Broodstock Program) were raised and set under favorable hatchery conditions (buffered seawater, pH ~7.8 and Ω_{arag} ~2) and unfavorable (pH ~7.4 and Ω_{arag} ~1) conditions. Early larval development was inhibited by acidified seawater and it affected the timing, but not magnitude of larval mortality. The effect on metamorphosis (setting) was variable, but MBP larvae produced more and larger spat in ambient and high pCO_2 seawater, respectively.

Hales, B., A. Suhrbier, G.G. Waldbusser, R.A. Feely, and J.A. Newton. 2017. The carbonate chemistry of the "Fattening Line," Willapa Bay, 2011-2014. *Estuaries and Coasts* 40: 173-186.

The authors present detailed data on seawater chemistry (especially PCO_2 and aragonite saturation state) for Willapa Bay, Washington where Pacific oysters have been the mainstay of the oyster aquaculture industry for almost 100 years and there is a long term record of spawning and setting. They reconstruct this record for a longer historical period and their data suggest that recent conditions provide a smaller window of optimal

conditions (low aragonite saturation state and warm enough temperatures for oyster spawning) than occurred historically. While they did not sample larvae and therefore cannot confirm effects, they substantiate the complexity of measuring these effects and attributing them to a single cause in an estuary where conditions are variable.

Lowe, A.T., J. Kobelt, M. Horwith, and J. Ruesink. 2019. Ability of eelgrass to alter oyster growth and physiology is spatially limited and offset by increasing predation risk. *Estuaries and Coasts* 42: 743-754.

The authors planted juvenile seed oysters and measured water chemistry inside and outside of eelgrass (*Zostera marina*) at several locations in Willapa Bay and in the Salish Sea in Washington state. They demonstrate that while eelgrass modified carbonate chemistry, it did not appear to be the primary variable influencing juvenile oyster growth and survival and, instead, predation was higher and thus survival lower inside eelgrass beds where surviving oysters also grew slower.

Ruesink, J.L., G.C. Roegner, B.R. Dumbauld, J.A. Newton, and D.A. Armstrong. 2003. Contributions of coastal and watershed energy sources to secondary production in a Northeastern Pacific estuary. *Estuaries* 26: 1079-1093.

These authors measured growth of juvenile Pacific oyster seed deployed at numerous locations throughout Willapa Bay and used stable isotopes to distinguish marine versus terrestrial energy sources. Results demonstrated a very distinct along estuary gradient with oysters growing faster near the mouth and slower at greater distance from the mouth and away from the mouth. Oysters also displayed distinct stable isotope ratios that reflected a strong marine signal near the mouth (-18 $\delta^{13}C$) and a stronger terrestrial signature at distance (-22 $\delta^{13}C$). Oysters grown just off the bottom grew slower than those off-bottom at any given tidal elevation.

Comparative study of the impact of environmental changes on oyster culture between USA and Japan, as collaborative research under UJNR

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Several oyster species are cultured globally, and the Pacific oyster, *Crassostrea gigas*, is the most widely cultured species both in Japan and the USA. In Japan, aquaculture production of the Pacific oyster is decreasing slightly for multiple reasons including large die-offs of adults during and after the reproductive season due to a delay in the reproductive season and poor post-spawning recovery, poor wild spat collection, and a labor shortage for both operation of aquaculture and post-harvest processing. These problems may be aggravated by environmental changes such as global warming and oligotrophication around Japan's coastal areas. FRA is investigating the causes of the die-off during the reproductive season and attempting to establish countermeasures. The Pacific oyster is native to Japan but was introduced to the USA for aquaculture in the early 1900's. Aquaculture production in the USA has now exceeded that in Japan. Nonetheless there are large differences in the culture system, habitats and environmental conditions between the two countries. A comparative study was initiated to evaluate oyster reproduction in the two countries in order to understand the effects of habitat and environment on future success of aquaculture given predicted environmental change. Oyster culture experiments were conducted in intertidal and subtidal zones just inside and outside seagrass habitat in Hiroshima Bay in Japan and Willapa Bay in USA during the reproductive season (March to June 2019 and February to July 2019, respectively). We focused on elucidating the effects of habitat and environment on the energy allocation of the oyster between reproduction and somatic growth.

Annotated Bibliography of Key Works

Akashige, A., Hrata, Y., Takatsuji, Y., Aida, S. 2006. Occurrence of mass mortality in oyster culture with relation to seawater temperature and rainfalls in Hiroshima. *Bulletin of the Hiroshima Pref. Fisheries & Marine Technology Center* 1: 9-13.

Relationships between mass mortality in Pacific oyster, *Crassostrea gigas*, occurred in 1979, 1994, 2001 and 2002 and surface water temperature or rainfall in Hiroshima Bay were analyzed using a dataset from 1970 to 2004. Mass mortality occurred in years with high water temperature (i.e. the number of days over 20℃ was greater than the mean + SD) and small rainfall (i.e. cumulative total rainfall from July to October was smaller than the mean - SD) except in 2001. Extraordinary mass mortality of spat occurred in 2001 which were introduced from Miyagi Prefecture which has colder climate than Hiroshima Prefecture. Therefore, there is a high risk of mass mortality in local oyster in years with high water temperature and small rainfall.

Akashige, A., Hrata, Y., Takayama, K., Sorammoto, K. 2005. Seasonal change in oxygen consumption rates and filtration rates of the cultured Pacific oyster *Crassostrea gigas*, *Nippon Suisan Gakkaishi* 71(5): 762-767.

The oxygen consumption rate (OCR) and filtration rate (FR) of cultured Pacific oyster, *Crassostrea gigas*, of different sizes were measured in still water systems at different seawater temperature in various seasons. The OCR had clear relationships with water temperature (t, ℃) and dry body flesh weight (W_d g) expressed by the formula: OCR (mg O^2 / hr / ind) = (0.072t - 0.64) $W_d^{0.75}$ and there was no difference among seasons. The FR was expressed by the formula: FR (L / h / ind) = (0.70t - 6.6) W_d in non-spawning seasons. However, in the spawning season, the FR were expressed by the formula: FR = 4.9 W_d, which was lower than the value expected by the formula applied on non-spawning season. These results clearly showed that feeding activities dropped during the spawning season, but oxygen demand (weight base and temperature dependent) was constant throughout the year. Oysters would be especially vulnerable during the spawning season when metabolic activities would be devoted to gamete production.

Cheney, D.P., 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* 19: 353-359.

Summer mortality has been a recognized problem in Pacific Coast oyster aquaculture on the west coast of the USA since the mid 1950's, and these authors related some of these incidences to stress during the reproductive season combined with environmental factors. Nonetheless triploid oysters experienced higher mortality than diploids.

Oyster aquaculture using seagrass beds as a climate change countermeasure

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In the framework of the Sustainable Development Goals (SDGs) led by the United Nations, it is required to develop coastal management methods to achieve both sustainable food production and environmental conservation as a climate change countermeasure. Oyster farming is an important food production method now being developed in coastal areas around the world though, in certain instances, oyster farming has caused local environmental degradation. As a typical example, local ecosystem functioning of the coastal area has been often overused by oyster aquaculture at an excessive oyster density derived from economic priority without consideration for ecosystem sustainability. Recently, climate change has caused several negative effects on oyster aquaculture such as poor spat collection due to oligotrophication, ocean acidification, and poor spat growth and survival due to frequent anoxic events derived from high seawater temperature. These effects gradually shifted the oyster farmers' concern from high production aquaculture to sustainable aquaculture. In response to the recent consideration for environmentally friendly aquaculture, collaborative research between France and Japan has started to achieve both sustainable oyster aquaculture and environmental conservation which aims for climate change mitigation and adaptation.

The oysters cultivated in many regions of the world are intertidal species inhabiting intertidal zones such as sandy/muddy tidal flats and estuaries, where seagrass beds are often distributed in adjacent subtidal areas. Seagrass vegetation is one of the most important ecosystems functioning as a countermeasure for global climate change. Not only does it mitigate green-house gas emission by sequestration and storage of blue carbon derived from atmospheric CO_2, but it also functions as an adaptation measure utilizing the buffer function against ocean acidification and moreover as water quality improvement.

Based on returning to aquaculture using the natural ecosystem interactions between oysters and seagrass beds, our project is now demonstrating whether aquaculture techniques that take into account both mitigation and adaptation to climate change are effective for both sustainable use of coastal areas and coastal environmental conservation.

We will present the results from field experiments conducted in both the French Mediterranean Sea and the Seto Inland Sea of Japan, to clarify the effect of eelgrass beds on (1) natural oyster spat collection and (2) growth and survival of oyster spat.
The results of our experiments revealed that spat recruitment was significantly higher in the intertidal zone where there was no eelgrass distribution in the subtidal zone, while spat growth and survival rate after settlement was significantly higher in eelgrass beds even when anoxic events occurred. In addition, the organic carbon storage in the sediment was larger in the eelgrass meadows with oyster aquaculture, suggesting that oyster aquaculture using seagrass meadows would be a good practice for the climate change adaptation and mitigation.

Annotated Bibliography of Key Works

Hori, M, Hamaoka, H, Hirota, M, Lagarde, F, Vaz, S, Hamaguchi, M, Hori, J, Makino, M. 2018. Application of the coastal ecosystem complex concept toward integrated management for sustainable coastal fisheries under oligotrophication. *Fisheries Science* 84 (2): 283-292.

Harmonizing coastal fisheries with water-quality improvement has become an essential factor for the sustainable use of coastal ecosystem services. Here, we present the scope of our study based on an interdisciplinary approach including ecological actions, socio-economic actions and socio-psychological actions. We chose to focus on the interaction between oyster aquaculture and seagrass vegetation as a typical ecological action using the coastal ecosystem complex (CEC) concept. Coastal organisms have adapted their traits to the environment over a long period of time, so that restoration of the CEC represents reconstruction of the original process of coastal production. Subtidal seagrass vegetation with intertidal oyster reefs is the original CEC in Japan, which would be expected to enhance coastal production by improving the production efficiency without adding nutrients. A simple field experiment examining carbon and nitrogen contents and stable isotope ratios revealed that oyster spats cultivated on a tidal flat adjacent to seagrass beds had higher nitrogen contents and higher $\delta^{13}C$ ratios than spats cultivated in an offshore area using only pelagic production. This result suggests that utilization of the CEC, which enables oysters to use both pelagic and benthic production, has potential to sustain a food provisioning service for humans, even in oligotrophic conditions.

Lagarde, F, Richard, M, Bec, B, Roques, C, Mortreux, S, Bernard, I, Chiantell, C, Messiaen, G, Nadalini, J, Hori, M, Hamaguchi, M, Pouvreau, S, d'Orbcastel, ER, Trenblay, R. 2018. Strophic environments influence size at metamorphosis and recruitment performance of Pacific oysters. *Marine Ecology Progress Series* 602: 135-153.

Reproduction and recruitment of benthic invertebrates are influenced by the climate and

by the ecological structure of marine ecosystems, along with local anthropogenic pressures such as eutrophication or oligotrophication. Using the Pacific oyster, *Crassostrea gigas*, as a biological model, we tested the hypothesis that the variability in prodissoconch II (PII) size (i.e. size at metamorphosis) depends on ecological functioning. Settlement and recruitment were assessed at 5 sampling sites in the French Mediterranean shellfish farmed Thau lagoon during the main summer recruitment events in 3 consecutive years (2012−2014). Hydrobiological and planktonic analyses were conducted at 3 sampling sites. Our results showed that recruitment was extremely heterogeneous, ranging from 0 to 260 ± 27 SE ind. dm^{-2} throughout the ecosystem and was linked with variability in PII size, which ranged from 180 to 296 µm. The annual temporal pattern of PII sizes appeared to be controlled by temperature during the settlement period, whereas the spatial pattern depended on phytoplankton biomass and on the trophic functioning of the ecosystem. Smaller PII sizes were significantly correlated with the highest phytoplankton biomass, while larger PII sizes were positively correlated with mixotrophic cryptophyte abundance. We found an inverse relationship between PII size and survival after metamorphosis, showing that recruitment success was associated with smaller PII sizes. Regional climate conditions and local trophic functioning appear to be key factors in metamorphosis and consequently contribute to recruitment heterogeneity. Further studies should be performed in other ecosystems following an oligotrophication trajectory to generalize this result.

Kelp, *Saccharina* spp, population genetics in the Northwest Atlantic for guiding a breeding program of thermally resilient strains

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The cold-water sugar kelp, *Saccharina latissima* has a circumboreal distribution and in the Northwest Atlantic is at its southern distributional limits in Long Island Sound. An understanding of genetic diversity of natural kelp populations is critical for making recommendations for breeding and cultivation efforts of the growing seaweed aquaculture sector in the US. An important component of the ARPA-E's MARINER project is selectively breeding *Saccharina* spp. in order to improve overall productivity for biofuels, feeds and food.

Historical records indicate the presence of regional kelp ecotypes based on physiological tolerance, specifically temperature. We made collections of 13 wild *Saccharina* spp. populations via SCUBA along the New England coast. These parental populations were also used to make over 300 hybrid crosses that were planted at several farm locations. We then used genome-wide single nucleotide polymorphism data to explore the biogeographic diversity of the kelp.

An assessment of the sequence diversity revealed distinct genetic variation between the Gulf of Maine and Southern New England, confirming that Cape Cod acts as a barrier to kelp gene flow. Furthermore, based on the analysis of molecular variance (AMOVA), we found the largest variance (58%) within sites. We also observed admixture among five sub-populations and isolation by distance in the Gulf of Maine.

Future steps for this project include skim sequencing the haploid phase of the kelp life cycle to identify trait heritability and phenotypic diversity observed for both morphological traits and tissue composition. Furthermore, we plan to place our sequence

data into a larger context to include samples from sites in Europe and Asia.

Annotated Bibliography of Key Works

Langton, R., S. Augyte, N. Price, J. Forster, T. Noji, G. Grebe, A. St. Gelais and C. J. Byron. 2019. An Ecosystem Approach to the Culture of Seaweed. NOAA Technical Memo. NMFS-F/SPO-195, 24 pp. url: https://spo.nmfs.noaa.gov/content/tech-memo/ecosystem-approach-culture-seaweed

Seaweeds are a significant component of current marine aquaculture production and will play an increasing role in global food security as the human population increases rapidly over the next 30 years. Seaweed farming is analogous to plant-based agriculture except that the crop is cultured in a marine environment. It differs from agriculture in that seaweeds do not require tillable land, fertilization or freshwater, which are resources that may ultimately constrain the expansion of agriculture. Seaweeds are converted into a variety of goods, such as food and nutritional supplements for humans and livestock, fertilizer, unique biochemical and biofuels. Wild and cultured seaweed also offer multiple ecosystem services, such as bioremediation for coastal pollution, localized control of ocean acidification, mitigation of climate change and habitat for other marine organisms. Incorporation of seaweeds into marine aquaculture farms in the United States (U.S.) is, however, not without its challenges. Seaweed is an unconventional food which necessitates establishing product acceptability, creating a sustained market and then balancing demand with a consistent supply for long term economic profitability. Seaweed farms also need to be developed in a manner that is compatible with wild capture fisheries, marine mammal migrations and other users of the marine environment. A comprehensive understanding of the role that cultured seaweeds play in the marine ecosystem is necessary in order to determine not only the economic value of the goods produced but also the ecosystem services offered by marine farming activities. This will result in a better understanding of how an ecosystem approach to aquaculture incorporates the role and need for both the goods and services these macroalgae will provide.

Augyte, S., Umanzor, S., Yarish, C., and S. Lindell. 2018. Enhancing marine ecosystem services via kelp aquaculture. International Society of Applied Phycology News 1. December-2018;10–14.
https://docs.wixstatic.com/ugd/e9f50b_f3edc9d188f248a18061e09cfa25b5d9.pdf

For centuries, humans have harvested different seaweeds for food, medicinal purposes, feed, and more recently as raw materials for industrial processes. Currently, wild harvested seaweed account for less than 5% of the total worldwide supply (FAO 2018). The majority of seaweed production is provided via aquaculture, with 99% of the production taking place in Asia including China, Korea, Japan, Indonesia, and the Philippines, worth US $11.7 billion annually (FAO 2018). Although seaweed

aquaculture is a fast expanding industry, the global demand for seaweed-based products is surpassing the supply. Such demands necessitate either domesticating new species or further expanding the productivity of the existing leading seaweeds. According to the FAO, only a few species dominate seaweed farming, including two brown kelps, *Saccharina japonica* and *Undaria pinnatifida* (Buschmann et al., 2018). To meet the present market requirements and to contribute in reducing the over-exploitation of wild stocks, experimental trials worldwide have assessed the performance of newly farmed seaweeds as potential products, particularly kelp species that have been traditionally harvested from wild populations.

Augyte, S., C. Yarish, and C. D. Neefus. 2019. Thermal and light impacts on the early growth stages of the kelp *Saccharina angustissima* (Laminariales, Phaeophyceae). *Algae* 34: 1-10. https://doi.org/10.4490/algae.2019.34.5.12.

Anthropogenic disturbances, including coastal habitat modification and climate change are threatening the stability of kelp beds, one of the most diverse and productive marine ecosystems. To test the effect of temperature and irradiance on the microscopic gametophyte and juvenile sporophyte stages of the rare kelp, *Saccharina angustissima*, from Casco Bay, Maine, USA, we carried out two sets of experiments using a temperature gradient table. The first set of experiments combined temperatures between 7-18°C with irradiance at 20, 40, and 80 µmol photons m^{-2} s^{-1}. The second set combined temperatures of 3-13°C with irradiance of 10, 100, and 200 µmol photons m^{-2} s^{-1}. Over two separate 4-week trials, in 2014 and again in 2015, we monitored gametogenesis, the early growth stages of the gametophytes, and early sporophyte development of this kelp. Gametophytes grew best at temperatures of 8-13°C at the lowest irradiance of 10-µmol photons m^{-2} s^{-1}. Light had a significant effect on both male and female gametophyte growth only at the higher temperatures. Temperatures of 8-15°C and irradiance levels of 10-100 µmol photons m^{-2} s^{-1} were conditions for the highest sporophyte growth. Sporophyte and male gametophyte growth were reduced at both temperature extremes—the hottest and coldest temperatures tested. *S. angustissima* is a unique kelp species known only from a very narrow geographic region along the coast of Maine, USA. The coupling of global warming with high light intensity effects might pose stress on the early life-history stages of this kelp, although, as an intertidal species, it could also be better adapted to temperature and light extremes than its subtidal counterpart, *Saccharina latissima*.

Cell selection technique for establishment of low salinity tolerance strain in *Pyropia tenuipedalis*

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Pyropia tenuipedalis is characterized by reddish thalli and direct budding from the shell substratum. The distribution of this species is limited to coastal areas in Seto Inland Sea, Ise Bay and Tokyo Bay in Japan. In Yamaguchi Prefecture, this species has been used for direct human consumption for its nutritional value. Alanine and glutamic acid contents of *P. tenuipedalis* are three times and two times higher, respectively, than those of *P. yezoensis* which is commonly referred to as "nori". Yamaguchi Prefectural Fisheries Research Center began development of mariculture techniques for *P. tenuipedalis* in 2002 and, in 2007, they succeeded in commercial cultivation of this species. However, production levels have continued to decrease since 2012. More recently, extensive disappearance of thallus has been observed from January to February since 2016. One of the reasons for the production decrease is thought to be low salinity seawater due to recent heavy rain fall supposedly associated with global climate change. The aim of this study was to develop low salinity tolerance strains of *P. tenuipedalis* with cell selection techniques. Disks of thallus were cultured in a 10% seawater medium diluted with distilled water with 1/2SWM-III for one to four months. Cells that survived in this medium were then cultured in a 100% seawater medium with 1/2SWM-III. Surviving cells divided and regrew to thallus, and stock culture strains were established with self-fertilization. A field culture trial of the established low salinity tolerance strain and the conventional strain was carried out in Koto River estuary from December 2018 to February 2019. The low salinity tolerance strain showed better growth than the conventional strain, suggesting the efficacy of the cell selection technique for breeding. However, some of the thalli growing on the culture plates suddenly shortened or disappeared in early January 2019. Time-lapse observation with an underwater camera revealed that the disappearance of the thalli was due to predation by blackhead seabream, *Acanthopagrus schlegelii*. Immediate establishment of not only strains resistant to the changing environment but also effective measures for predation are necessary to increase production of *P. tenuipedalis*.

Annotated Bibliography of Key Works

Abe, M., Murase, N., Hatama, T., Shikano, Y., Kanai, T. 2015. New record of *Pyropia*

tenuipedalis from Koto river estuary, Yamaguchi prefecture. *J. Nat. Fish. Univ.* 63, 244-248.

We collected reddish foliose thalli from Koto River estuary, Yamaguchi Prefecture. In order to identify the species, we carried out field collections of the foliose thalli, morphological observation in culture and PCR-RFLP analysis using the two regions of the partial mitochondrial DNA. As for the morphological survey, the spherical cells were formed at the tips of conchocelis and developed to foliose thalli. The fragment patterns of this species in the PCR-RFLP analysis matched with *Pyropia tenuipedalis*. In the present study, the foliose thalli collected at Koto River were identified as *P. tenuipedalis*, which is an endangered species distributed along the coast of Japan. This species was newly recorded from Koto River estuary, Yamaguchi Prefecture.

Nakayama, T., Abe, M., Murase, N., Shikano, Y. 2017. Influence of salinity on growth of red alga *Pyropia tenuipedalis* and *Pyropia yezoensis* foliose thallus. *Aquacult. Sci.* 65, 321-330.

Pyropia tenuipedalis is a new culture species in Yamaguchi Prefecture, Japan. The habitat of this species is more brackish as compared with that of *P. yezoensis*, which is the common species in Japanese nori mariculture. In this paper, the authors investigated the relationship between salinity and growth of *P. tenuipedalis* and *P. yezoensis*. It was revealed that *P. tenuipedalis* tolerates lower salinity in comparison with *P. yezoensis*.

Abe, M., Murase, N., Hatama, T., Shikano, Y., Kanai, T. 2017. Environmental characteristics of *Pyropia tenuipedalis* (Miura) Kikuchi et Miyata growing at Yamaguchi Bay, Yamaguchi Prefecture. *J. Nat. Fish. Univ.* 65, 19-29.

This study surveyed environmental characteristics of *Pyropia tenuipedalis* foliose thallus growing in Yamaguchi Bay, Yamaguchi Prefecture from November to March, 2010-2014. Young foliose thallus of this species appeared in December and grew to maximum length from January to March. In March, mature thalli were observed. Water temperature from November to March was usually within the range of 6 - 16°C, but the temperature was lower in early December 2012 than other years. Light reaching the growing depth of this species was 6.2 ± 3.1% of the level at water surface at high tide. The concentration of dissolved inorganic nitrogen at the sampling site was within the range of 8.8 - 68.3 μmol L^{-1}, that was approximately 10 times higher than the level that is assumed to cause the discoloration of *P. yezoensis*. Water temperature falling rate from November to December in 2012-2013 was 0.33°C day^{-1}, which was higher than those in other years. In this study, it was suggested that the growth of thalli from spherical cell was inhibited by a long or short term rapid water temperature decrease, which occurred from November to December.

Abe, M., Murase, N., Nakae, M., Nakayama, T., Nakagawa, M., Shikano, Y. 2018.

Water temperature characteristics in growth of filamentous thallus and formations of spherical cell, uniseriate and foliose thallus of *Pyropia tenuipedalis* (Miura) Kikuchi et Miyata. *J. Nat. Fish. Univ.* 66, 81-88.

We investigated the water temperature effects on growth of filamentous thallus and formations of spherical cell, uniseriate and foliose thallus of *Pyropia tenuipedalis* with culture experiments. Optimal growth of filamentous thalli was observed at 20ºC. Moreover, optimal water temperatures for formations of spherical cells, uniseriate and foliose thalli were 20ºC, 15–20ºC and 15ºC, respectively. Optimal water temperatures of each life stage were different. At 10ºC, the formations of uniseriate and foliose thalli were suppressed. At 25ºC, almost all thalli had morphological abnormalities. Furthermore, decreasing temperature to 15ºC from 20ºC enhanced formation of uniseriate and foliose thalli. It was thought that low production in 2012 resulted from suppressed formation of uniseriate and foliose thalli at less than 10ºC.

Murase, N., Abe, M., Fukudome, K., Nakagawa, M., Shikano, Y. 2018. Influence of temperature on the growth of red alga *Pyropia tenuipedalis* thalli. *J. Nat. Fish. Univ.* 66, 215-220.

This study was designed to clarify the optimal temperatures for growth in uniseriate thalli and foliose thalli of *Pyropia tenuipedalis* in the laboratory culture at 5ºC interval from 10ºC to 25ºC or 30ºC. The optimal temperature for uniseriate thalli developed from a spherical cell at two-cell stage were 15ºC and 20ºC. The optimal temperature for foliose thalli allowed to develop from young blade with the length about 6 cm was 15ºC. It was suggested that the shift in the optimal temperatures from uniseriate to foliose thalli stages was related to the seasonal reduction of water temperature from autumn to winter.

Improvement of dietary effect on juvenile *Ruditapes philippinarum* using the dietary-supplements and a new diet microalga

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The Manila clam, *Ruditapes philippinarum* (Adams and Reeve, 1850), is not only a commercially important bivalve but also one of the most ecologically important bivalves in the world. However, the annual catch of this species in Japan continues to decrease drastically, and several causative factors have been reported, such as the degradation of bottom sediment, mass depletion of egg production and larvae, predation, and overfishing. In addition, these factors may be related in some way to the relatively rapid global environmental change in recent years. To conserve the clam resources, there have been many studies investigating the development of seed production and juvenile clam culture. In these circumstances, a persistent problem is insufficient food supply for juvenile clams exceeding a shell length of 1 mm, which is a size when the clams have high food requirements, especially during winter and spring where low temperature slows the growth of the diet microalgae. Therefore, the development of dietary-supplements and/or new species of diet microalgae that contain the essential nutritional properties and are capable of rapid growth under low water temperatures will lead to a breakthrough for juvenile clam culture and consequently to the conservation of the clam resource in the coastal environment. This talk introduces the results of enhancement of dietary effect on juvenile *R. philippinarum* using alginate, an acidic polysaccharide known as a dietary-supplement, and the euglenophyte *Eutreptiella eupharyngea* as a new diet microalga. Future challenges on development of a process for practical utilization of the dietary-supplements and the new diet microalga for clam culture will also be discussed.

Annotated Bibliography of Key Works

Yamasaki, Y., Taga, S., Kishioka, M. 2015. Preliminary observation of growth-promoting effects of alginate hydrolysates on juvenile Manila clams, *Ruditapes philippinarum*. *Aquaculture Research* 46, 1013-1017. DOI: 10.1111/are.12246.

Several studies have been suggested that certain types of sugars are potentially a good supplement for growth of bivalves such as *Ruditapes philippinarum*. We observed the dietary effects of a harmful raphidophyte *Heterosigma akashiwo* on juvenile clams and suggested that the acidic sugars in phytoplankton might be an important factor determining the shell-length growth of clams because total sugar and acidic sugar content of *H. akashiwo* were higher than other diet microalgae. Therefore, we focused

on alginate known as one of the acidic polysaccharides, and showed that shell-length growth of juvenile clams (average shell length: 432 to 507 μm) was significantly promoted by supplementing the diatom *Chaetoceros neogracile* (40,000 to 80,000 cells/mL) with alginate-hydrolysates (AHs) of at least the concentration of 1 mg/L. In addition, the most effective concentration of AHs was 2 to 4 mg/L.

Yamasaki, Y., Taga, S., Kishioka, M., Kawano, S. 2016. A metabolic profile in *Ruditapes philippinarum* associated with growth-promoting effects of alginate hydrolysates. *Sci. Rep.* 6, 29923. DOI: 10.1038/srep29923.
We demonstrated that shell-length growth of *Ruditapes philippinarum* (average shell length: 15.7 mm) was significantly promoted by supplementing the diatom *Chaetoceros neogracile* (80,000 cells/mL) with alginate-hydrolysates (AHs) at the concentration of 4 mg/L. Furthermore, metabolomics indicates that clams in the groups given *C. neogracile* with AHs at the concentration of 4 mg/L actively utilized excess carbohydrate for the development of reproductive tissue. On the other hand, clams in the groups given *C. neogracile* only were actively growing through the use of their adequate carbohydrate resources. Thus, supplementation of AHs with the algal diet may be an effective way to shorten the rearing period of clams.

Yamasaki, Y., Ishii, K., Taga, S., Kishioka, M. 2018. Enhancement of dietary effect of *Nannochloropsis* sp. on juvenile *Ruditapes philippinarum* clams by alginate hydrolysates. *Aquaculture Rep*. 9, 31-36. DOI: https://doi.org/10.1016/j.aqrep.2017.11.006
The eustigmatophyte *Nannochloropsis* sp. is widely used in the aquaculture industry because this species can be produced on a large scale at low cost. However, *Nannochloropsis* sp. has less dietary effect on juvenile bivalves compared with other diet algae such as the diatom *Chaetoceros neogracile* and the haptophyte *Diacronema* (=*Pavlova*) *lutheri*. In this study, the use of alginate-hydrolysates (AHs) to enhance the dietary effect of *Nannochloropsis* sp. on juvenile *Ruditapes philippinarum* (average shell length: 1,090 μm) was attempted. As a result, enhancement of the dietary effect on shell-length growth of juvenile clams was observed in the groups given *Nannochloropsis* sp. (300,000 cells/mL) with AHs at the concentration of 4 mg/L. Hence, the enhanced dietary effect of a combination of *Nannochloropsis* sp. and AHs will be useful to shorten the rearing period of *R. philippinarum*.

Yamasaki, Y., Ishii, K., Hikihara, R., Ishimaru, M., Sato, F., Taga, S., Kishioka, M., Matsunaga, S., Shikata, T., Abe, M., Kato, S., Tanaka, R., Murase, N. 2019. Usefulness of the euglenophyte *Eutreptiella eupharyngea* as a new diet alga for clam culture. *Algal Res*. 40, 101493. DOI: https://doi.org/10.1016/j.algal.2019.101493.
Microalgae are an essential feed source for seed production of bivalves such as *Ruditapes philippinarum*. However, there is a deficiency of microalgae that can provide

a stable supply of nutrient-rich feed at low water temperatures during the winter and spring. To develop a new diet of microalga that can grow well outdoors at low water temperatures and possesses the essential nutritive constituents, we focused on the euglenophyte *Eutreptiella* eupharyngea, which was isolated from a pond used for extensive phytoplankton cultivation at the Yamaguchi Prefectural Fisheries Research Center (Yamaguchi, Japan) in January 2013. As a result, this species grew well at water temperatures of 10–25 °C, but could not grow at 30 °C. Furthermore, the dietary effect of *E. eupharyngea* per dry weight on juvenile *Ruditapes philippinarum* (average shell length: 1,426 μm) exceeded that of the diatom *Chaetoceros neogracile*. These findings are attributable to the high nutritional value of *E. eupharyngea* as typified by its high protein and sugar content and high content ratio of n-3 fatty acids such as eicosapentaenoic and docosahexaenoic acid and n-6 fatty acids such as arachidonic acid.

Exploration of alternative protein sources in the development of a sustainable Japanese white trevally *Pseudocaranx dentex* juvenile diet

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White trevally (*Pseudocaranx dentex*) is a novel marine finfish species that is being cultured commercially in Japan. Few nutritional studies have been conducted on white trevally using alternative protein sources to replace the marine ingredients fish meal (FM), the primary protein source in marine fish diets, and the flavor attractant krill meal (KM). The plant protein soybean meal (SBM) is widely produced and has a good amino acid (AA) balance for aquaculture diet formulations. Poultry byproduct meal (PBM) is made from combining the byproducts originating from poultry processing plants and also contains a strong AA balance. Three feeding trials were conducted to evaluate the potential of SBM as a replacement of FM and PBM as a replacement for KM in an effort to develop an eco-friendly juvenile diet for white trevally.

In trial 1, six iso-energetic diets were formulated; FM 560 g kg^{-1} diet (Control, diet C), and FM protein in diet C was replaced at 10 (S-10), 20 (S-20), 30 (S-30), 40 (S-40) and 50% (S-50) by SBM, and compared to a commercial reference. Fifteen juveniles (42.7 g) were randomly distributed into each of twenty-one 500 L tanks, set in triplicate and fed for 10 weeks. In trial 2 seven iso-energetic diets were formulated, and FM protein was replaced at 30 (S-30), 35 (S-35), 40 (S-40), 45 (S-45) and 50% (S-50) by SBM. Another diet was designed by omitting binders carboxymethyl cellulose and guar gum from diet C and referred to as C-CG. Thirty juveniles (3.68 g) were randomly distributed into each of twenty-one 300 L tanks, set in triplicate and fed for 6 weeks. In trial 3, diet C-CG from trial 2 was used as the control. Five iso-energetic diets were formulated as follows: FM protein from diet C-CG was replaced at 50 (S-50) and 70% (S-70) and KM from diets S-50 and S-70 was replaced by PBM and referred to as P-50 and P-70, respectively, and a FM-free diet (FMF). Fifteen juveniles (31.8 g) were randomly distributed into eighteen 500 L tanks, set in triplicate and fed for 8 weeks.

In trial 1, all SBM-based diets outperformed the control and commercial diet in terms of final weight gain and feed efficiency. The results of trial 2 revealed no significant differences between diets C and C-CG, indicating that binder was not the cause of poor growth performance of diet C in trial 1. Although there were no significant differences

among SBM-based diets (P>0.05), S-50 exhibited the greatest final weight gain out of all treatments. In trial 3, there were no significant differences in the growth performance among SBM-based diets (P>0.05). While there were no statistical differences between diets C-CG and FMF, fish fed with diet S-70 showed significantly higher growth performance than those fed with both diets (P<0.05). The results of all three feeding trials indicate that SBM can effectively replace FM up to 70% with the addition of KM, methionine, lysine, taurine and phytase supplementation. Additionally, PBM has the ability to effectively replace KM in up to 50% FM replacement diets by SBM.

Annotated Bibliography of Key Works

Biswas, A., H. Araki, T. Sakata, T. Nakamori, K. Kato, and K. Takii. 2017. Fish meal replacement by soy protein from soymilk in the diets of red sea bream (*Pagrus major*). *Aquaculture Nutrition* 23: 1379-1389.

The results of this study introduce the ecological benefits of replacing FM by soy-based products in juvenile red sea bream, such as reduced phosphorus discharge to the environment. This feeding experiment was also carried out at the Aquaculture Research Institute of Kindai University, Japan using the same methods that were utilized in the research presented in our abstract.

Jirsa, D., A. Davis, K. Stuart, and M. Drawbridge. 2011. Development of a practical soy-based diet for California yellowtail, *Seriola lalandi*. *Aquaculture Nutrition* 17: e869-e875.

This study was carried out in an effort to develop an environmentally sustainable and cost-effective diet for California yellowtail juveniles using soy products to replace fish meal. This research used a similar approach in terms of feed formulation, specifically investigating the utility of soybean meal and soy protein concentrate. Interestingly, fish fed diets containing soybean meal outperformed fish fed soy protein concentrate, which further influenced our choice of testing the utility of conventional soybean meal on white trevally juveniles. This research was carried out in the United States.

Kader, A.Md., M. Bulbul, S. Koshio, M. Ishikawa, S. Yokoyama, B.T. Nguyen, and C.F. Komilus. 2012. Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea beam, *Pagrus major*. *Aquaculture* 350-353: 109-116.

The authors found that in red sea bream juveniles, nutrient utilization, body composition and blood parameters were improved or not significantly affected by replacing fish meal with soybean meal in combination with flavor attractant supplementation. Growth performance results from this experiment express a similar trend to the results presented in our abstract, in which soybean meal diets outperformed the fish meal control diets in white trevally. This research was carried out in Japan.

Novriadi, R., G. Salze, A. Abebe, T. Hanson and D.A. Davis. 2019. Partial or total replacement of fish meal in the diets of Florida pompano *Trachinotus carolinus*. *Aquaculture Research* 2019: 1-12.

This research demonstrates the high utility of soybean meal in combination with other alternative protein sources other than fish meal. The results of this study revealed that 466 g/kg conventional soybean meal in combination with enzyme treated soybean meal and 80 g/kg corn protein concentrate can be used to reduce dietary fish meal from 15 to 9 g/kg in practical diets for Florida pompano. This research was carried out in the United States.

Waldemar, R. Jr. and D.A. Davis. 2012. Replacement of fish meal with poultry by-product meal in the diet of Florida pompano *Trachinotus carolinus* L. *Aquaculture* 338-341: 160-166.

The authors of this research found that poultry byproduct meal in combination with soybean meal proved to be favorable, even in diets containing up to 50% conventional soybean meal, 9.8% poultry byproduct meal, and 5% fish meal. This research influenced our decision to explore the use of poultry by-product meal as replacement for krill meal, in combination with soybean meal in the feed formulations used in trial 3. This research was carried out in the United States.