

46th Scientific Symposium of the UJNR
Aquaculture Panel

Marine Aquaculture in a Changing Environment

Hyatt Place Mystic
224 Greenmanville Ave
Mystic, CT
November 14th and 15th, 2018



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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 46th UJNR Aquaculture Symposium is the second year of the current three-year theme of Marine Aquaculture in a Changing Environment. Environmental change impacts 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, fresh water 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. This year's presentations focus on impacts to aquaculture production due to environmental change (e.g., ocean acidification impacts on shellfish aquaculture) and science to mitigate these impacts (counter measures).

Program

Wednesday, November 14, 2018

Registration 13:00 - 13:30

Welcome and Aim of the Symposium

Michael Rust, US Panel Chair, NOAA Fisheries Office of Aquaculture 13:30-13:45

Production Technologies

(Moderators: Simona Augyte and Masakazu Hori)

Distribution patterns of the cysts of the paralytic shellfish poisoning plankton *Alexandrium tamarense* and *A. catenella* off the Pacific coast of eastern Japan

Tomoko Sakami, National Research Institute of Aquaculture
Fisheries Research and Education Agency 13:45-14:10

Update on the progress of the scallop farming industry in Maine
Dana Morse, Maine Sea Grant College Program 14:10-14:35

Trends in aquaculture production in Japan
Satoshi Watanabe, National Research Institute of Aquaculture
Fisheries Research and Education Agency 14:35-15:00

Establishment of oyster tetraploid breeding stocks for the fast-growing oyster industry in the Gulf region
Huiping Yang, School of Forest Resources and Conservation Institute of Food and Agricultural Sciences, University of Florida 15:00-15:25

Development of environmentally friendly, cost-effective and nutritionally balanced alternative protein based diets for high value marine fish culture in recirculating aquaculture system
Md Shah Alam, Center for Marine Science, Aquaculture Program, University of North Carolina Wilmington 15:25-15:50

Symposium Reception: One on one discussions continue
Hyatt Place Mystic 16:00-18:00

Thursssday, November 15, 2018

Temperature Resilience

(Moderators: Dana Morse and Tomoko Sakami)

Seaweed aquaculture in a warming environment - building resilience with *Saccharina angustissima* (Laminariales, Phaeophyceae)
Simona Augyte, Department of Ecology and Evolutionary Biology,
University of Connecticut 9:00-9:25

On the application of bio-stimulants to enhance growth and thermal resistance of sugar kelp, *Saccharina angustissima*, for aquaculture production
Schery Umanson, Department of Ecology and Evolutionary Biology,
University of Connecticut 9:25-9:50

***Vibrio coralliilyticus* induction of virulence toward larval oysters and corals at elevated seawater temperatures and potential mitigation treatments**

Gary Richards, Agricultural Research Service,
United States Department of Agriculture 9:50-10:15

Break 10:15-10:30

Ocean Acidification

(Moderators: Schery Umanzor and Satoshi Watanabe)

Chemical changes in the environment: what does this mean to shellfish?
Shannon Meseck, Northeast Fisheries Science Center Milford Lab, NOAA 10:30-10:55

The current trends in pH and ocean acidification in an aquaculture sea area in Gokasho Bay, Japan
Masayuki Minakawa, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 10:55-11:20

Ocean acidification experimentation in seagrass-oyster ecosystems using a novel system to conduct in-situ CO₂ enrichment
Masakazu Hori, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 11:20-11:45

Effects of ocean acidification on the early developmental stages of the commercially important gastropods, Ezo abalone and horned turban, in Japan
Ryo Kimura, Fisheries Research and Education Agency, Headquarters 11:45-12:10

Lunch Break 12:10-13:10

Responses to Environmental Impacts

(Moderators: Shannon Meseck and Masayuki Minakawa)

A slow growing perspective on multi-generational responses to future change
Coleen Suckling, Animal and Veterinary Sciences, University of Rhode Island 13:10-13:35

The influence of climate and environment on the spawning, condition, and larval set of naturalized Pacific oyster *Crassostrea gigas* in a US west coast estuary
Brett Dumbauld, Agricultural Research Service,
United States Department of Agriculture 13:35-14:00

The influences of environmental changes on Japanese Nori mariculture
Mahiko Abe, National Fisheries University,
Fisheries Research and Education Agency 14:00-14:25

The potential of seaweed aquaculture to reduce methane emissions in Californian livestock

Luke Gardner, University of California, San Diego,
California Sea Grant

14:25-14:50

Break

14:50-15:05

Ecological Interactions

(Moderators: Brett Dumbauld and Ryo Kimura)

Condition index and fecundity of Manila (Asari) clam *Ruditapes philippinarum* related to habitat environment

Natsuki Hasegawa, National Research Institute of Aquaculture
Fisheries Research and Education Agency

15:05-15:30

Ecological interactions of Horseshoe crabs and shellfish aquaculture: a case study from New Jersey

Daphne Munroe, Haskin Shellfish Research Laboratory, Rutgers University

15:30-15:55

Use of GoPro cameras to document fish on aquaculture gear

Gillian Phillips, Northeast Fisheries Science Center Milford Lab, NOAA

15:55-16:20

Science Symposium Closing

Fuminari Ito, Japan Panel Chair, Fisheries Research and Education Agency

16:20-16:30

List of Participants

Abe, Mahiko	Japan Fisheries Research and Education Agency (FRA), National Fisheries University
Alam, Md Shah	North Carolina State University
Augyte, Simona	University of Connecticut (UCONN)
Cowperthwaite, Hugh	Coastal Enterprises Incorporated
Dumbauld, Brett	United States Department of Agriculture (USDA), Agricultural Research Service (ARS)
Eddy, Steve	University of Maine
Gardner, Luke	California Sea Grant
Hasegawa, Natsuki	FRA, National Research Institute of Aquaculture (NRIA)
Hori, Masakazu	FRA, National Research Institute of Fisheries and Environment of Inland Sea
Ito, Fuminari	Japan Panel Chair, FRA, NRIA
Kimura, Ryo	FRA Headquarters
Meseck, Shannon	NOAA Northeast Fisheries Science Center Milford Lab
Minakawa, Masayuki	FRA, NRIA
Morse, Dana	Maine Sea Grant
Munroe, Daphne	Rutgers University
Okumura, Takuji	FRA, NRIA
Olin, Paul	California Sea Grant
Phillips, Gillian	NOAA Northeast Fisheries Science Center Milford Lab
Rexroad, Caird	USDA, ARS
Richards, Gary	USDA, ARS
Rust, Mike	US Panel Chair, NOAA Headquarters
Sakami, Tomoko	FRA, NRIA
Takano, Masatsugu	FRA Headquarters
Umanzor, Schery	UCONN
Watanabe, Satoshi	FRA, NRIA
Yang, Huiping	University of Florida
Yarish, Charles	UCONN

46th Scientific Symposium of the UJNR Aquaculture Panel

Aquaculture in a Changing Environment

Symposium Abstracts

1. *Distribution patterns of the cysts of the paralytic shellfish poisoning plankton Alexandrium tamarense and A. catenella off the Pacific coast of eastern Japan*

Tomoko Sakami^{*1}, Tetsuroh Ishikawa², and Toru Udagawa³

Presenting author*

¹National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency, 422-1 Nakatsuhamaura, Minami-ise, Mie, 516-0193, Japan, *Email: sakami at affrc.go.jp

²Miyagi Prefectural Government, 3-8-1, Honcho, Aoba-ku, Sendai, Miyagi, 980-8570, Japan

³National Research Institute of Fisheries Engineering, Japan Fisheries Research and Education Agency, 7620-7, Hasaki, Kamisu, Ibaraki, 314-040, Japan

Abstract

Shellfish poisoning occurs as bivalves consume poisonous phytoplankton and the poison accumulates in their tissues. Monitoring systems for bivalve products have improved in Japan, and food poisoning damage has rarely occurred in recent years. However, contaminated shellfish is detected nearly every year and presents a notable challenge to fishermen who have to voluntarily regulate their shipments. Two dinoflagellate plankton species of the genus *Alexandrium* mainly cause paralytic shellfish poisoning in Japan. Highly toxic *A. tamarense* is found in low-temperature waters, whereas less toxic *A. catenella* is found in warmer waters. Both species produce cysts that settle at the bottom of the sea and become the population source for the following years. A cyst distribution survey was conducted to evaluate the risk of shellfish poisoning. Cyst counting can be done efficiently using a fluorescent microscope; however, visual distinction of the cysts of the two species is difficult because of their similarity in shape. A molecular biological method has been developed to distinguish between the species. In this study, we aimed to determine the difference in the distribution of the cysts between the two species by detecting genes from the sea floor sediments using quantitative PCR. We estimated the gene copy numbers of *A. tamarense* and *A. catenella* in the sediment collected off the eastern Pacific coast of Japan. Both species were detected in some ria-type inner bays in the Sanriku region. However, only *A. tamarense* was detected from Sendai Bay to Kashima Nada, which open widely to the Pacific Ocean. In addition, only *A. catenella* was detected south of Cape Inubo, which is strongly influenced by the Kuroshio current. These results suggest that the distribution patterns of the two toxic phytoplankters differ depending on topography and the ocean current system.

Annotated Bibliography of Key Works

Nagai, S. 2011. Development of a multiplex PCR assay for simultaneous detection of six *Alexandrium* species (Dinophyceae). *J. Phycol.* 47, 703–708.

In Japan, the blooming seasons of two toxic dinoflagellate species, *Alexandrium catenella* (Whedon et Kof.) Balech and *A. tamiyavanichii* Balech, sometimes overlap with those of three nontoxic *Alexandrium* species, *A. affine* (H. Inouye et Fukuyo) Balech, *A. fraterculus* (Balech) Balech, and *A. pseudogoniaulax* (Biecheler) T. Horig. ex Y. Kita et Fukuyo. In this study, a multiplex PCR assay was developed to enable simultaneous detection of six *Alexandrium* species on the basis of differences in the lengths of the PCR products. The accuracy of the multiplex PCR system was assessed using 101 DNA templates from the six target *Alexandrium* species and 27 DNA templates from 11 non-target species (128 DNA templates in total). All amplicons obtained from the 101 DNA templates of the target species were appropriately identified, whereas all 27 DNA templates of the non-target species were not amplified. Species-specific identification by the multiplex PCR assay was possible from single cells of the target species.

Natsuike, M., Yokoyama, K., Nishitani, G., Yamada, Y., Yoshinaga, I., Ishikawa, A. 2017. Germination fluctuation of toxic *Alexandrium fundyense* and *A. pacificum* cysts and the relationship with bloom occurrences in Kesenuma Bay, Japan. *Harmful Algae* 62, 52-59. While cyst germination may be an important factor for the initiation of harmful/toxic algal blooms, in situ assessments of the fluctuation in the occurrence of phytoplankton cyst germination from the bottom sediments to the water column are rare due to lack of technology to detect germinated cells in natural bottom sediments. This study introduces a simple mesocosm method, modeled after previous in situ methods, to measure the germination of plankton resting stage cells. Using this method, seasonal changes in germination fluxes of toxic dinoflagellate resting cysts, specifically *Alexandrium fundyense* (*A. tamarensis* species complex Group I) and *A. pacificum* (*A. tamarensis* species complex Group IV), were investigated at a fixed station in Kesenuma Bay, northeast Japan, from April 2014 to April 2015. This investigation was conducted in addition to the typical samplings of seawater and bottom sediments to detect the dinoflagellate vegetative cells and resting cysts. Bloom occurrences of *A. fundyense* were observed in June 2014 and February 2015 with maximum cell densities reaching 3.6×10^6 cells m^{-2} and 1.4×10^7 cells m^{-2} , respectively. The maximum germination fluxes of *A. fundyense* cysts occurred in April 2014 and December 2014 and were 9.3×10^3 cells $m^{-2} day^{-1}$ and 1.4×10^4 cells $m^{-2} day^{-1}$, respectively. For *A. pacificum*, the highest cell density was 7.3×10^7 cells m^{-2} during the month of August, and the maximum germination fluxes occurred in July and August, reaching 5.8×10^2 cells $m^{-2} day^{-1}$. Thus, this study revealed the seasonal dynamics of *A. fundyense* and *A. pacificum* cyst germination and their bloom occurrences in the water column. Blooms occurred one to two months after peak germination, which strongly suggests that both the formation of the initial population by cyst germination and its continuous growth in the water column most likely contribute to toxic bloom occurrences of *A. fundyense* and *A. pacificum* in the bay.

Ishikawa, T., Kusaka, K., Oshino, A., Nishitani, G., Kamiyama, T. 2015, Distribution patterns of *Alexandrium* vegetative cells and resting cysts, and paralytic shellfish poisoning in Kesenuma Bay, after the Great East Japan Earthquake. *Nippon Suisan Gakkaishi* 81(2), 256-266. In 2013, paralytic shellfish poisoning (PSP) occurred in Kesenuma Bay, northern Japan, for the first time in 24 years. From April to early May, *Alexandrium tamarensis* increased in the inner part of the bay (up to 136,200 cells/L), expanded to the whole bay, and caused PSP. In contrast, *A.*

catenella increased in early September (up to 1,310 cells/L) in the center of the bay but did not cause PSP. Resting cysts of *A. catenella/tamarensis* ranged from 30 to 6,383 cysts/cm³ and dominated the inner part of the bay. A meta-analysis of temporal trends in the density of *A. catenella/tamarensis* cysts in Kesennuma Bay revealed that the cysts in the surface bottom sediment drastically increased after the Great East Japan Earthquake. High PSP toxicity was observed in four bivalves: *Chlamys farreri* (130 MU/g), *Mytilus galloprovincialis* (110 MU/g), *Ruditapes philippinarum* (74 MU/g), and *Mizuhopecten yessoensis* (66 MU/g).

2. Update on the progress of the scallop farming industry in Maine

Hugh Cowperthwaite¹ and Dana L. Morse*²

Presenting author*

¹Coastal Enterprises, Inc. 30 Federal Street, Brunswick, ME, 04011, USA

²*Maine Sea Grant College Program, and University of Maine Cooperative Extension. Darling Marine Center, 193 Clark's Cove Road, Walpole, Maine, 04573, USA, *Email: dana.morse@maine.edu

Abstract

Climate change has profoundly impacted capture fisheries in the Gulf of Maine. Notably, the largest fishery in the United States is in flux: landings of lobster (*Homarus americanus*) have shifted northeast in recent years. These challenges - not unique to Maine and are experienced by the Japanese fisheries - have caused great uncertainty among fishermen in Maine. Consequently, some are turning to aquaculture, to compliment fishing incomes. Maine's fishing and aquaculture industries have benefited from exchanges with Japan's well-established scallop industry, and we continue to learn directly from the Japanese.

Coastal Enterprises Inc. (CEI), Maine Sea Grant (MESG) and others are working to introduce commercially-viable scallop aquaculture into the state of Maine, building on a 20-year-old foundation of expertise. Maine's sister-state relationship with Aomori Prefecture is central to this process, with an origin dating to the wreck of the Bath-built *Chesborough*, which foundered off the coast of Aomori in 1889.

Trips to Aomori in 1999, 2010 and 2016 have focused on many aspects of scallop culture and fisheries; spat collection, nursery and growout techniques, product development, and environmental interactions (Beal et al 1999).

Currently, several projects are being carried out in Maine, developing the culture sector for scallops in coastal waters. An aquaculture cooperative in Maine focused on scallops is now in place. Optimization of spat collection has been ongoing work over several years. Biotxin monitoring in scallop tissues enables some producers to access the live market. A dedicated license for scallop spat collection is underway. Altogether, fishermen, farmers, economic development groups, scientists and regulators are engaged, in pursuit of the developing Maine's

scallop aquaculture industry. These collaborations - combined with expertise from Japanese colleagues - is a powerful aggregation of expertise to solve the many problems involved. Ex-vessel value for Maine scallops has risen for over a decade, though state and federal landings have increased, reflecting the value that consumers place on Maine scallops specifically; and the trend is expected to continue (Hale Group 2016). Trials by CEI and collaborators indicates that the Japanese production machinery appears to work well, and growth rates from ongoing studies and older works appear to fall within acceptable limits, often in excess of 25mm/year shell height (Cowperthwaite, unpublished; Morse, unpublished, Davidson et al 2014, Pottle and Hastings 2001). Lastly, all indications point to a growout period from spat (seed) to commercially viable harvest size between two and three years.

Aquaculture practitioners in Maine desire to incorporate Japanese expertise, so the industry can ramp up effectively and provide new sources of protein. We seek ongoing advice with lease site set-up and husbandry, growout techniques, biofouling and predators, equipment and machinery, handling, processing, food safety and packaging.

This innovative and unique project offers a one-of-a-kind exchange experience that is helping to further strengthen ties between the United States and Japan. The introduction of Japanese technical knowledge and equipment to our Maine scallop industry is the first of its kind in the United States and is groundbreaking for the American aquaculture community.

Annotated Bibliography of Key Works

Beal, B.F., M. Hastings, W. Hopkins, S. Inches, D. Morse, K. Porter, T. Pottle, S. Rappaport, L. Taylor, J. Trenholm. 1999. The culture of Japanese scallops (*Patinopecten yessoensis*). Report of the Maine delegation to Aomori Prefecture, Japan, May 14-21, 1999. The Maine Aquaculture Innovation Center, 49p.

Beal et al summarizes the delegation from Maine to Aomori in 1999, which focused principally on spat collection. This trip was important in advancing the tech-transfer process and getting fishermen in Maine involved in culture-related issues on scallops in state waters.

Davidson, L-A., R. Nowlan and M. Niles. 2014. Open-ocean sea scallop (*Placopecten magellanicus*) culture trials in Chaleur Bay, Canada: Comparing culture gears and husbandry practices. *Can. Tech. Rep. Fish. Aq. Sci.* #3121. 26p.

Davidson et al reviews culture techniques in northern regions of North America; these are all applicable to scallop production in Maine state waters. Canadian researchers, producers and those in the regulatory/equipment communities have all contributed substantially to the understanding and progress by Maine participants.

Hale Group, 2016. Farmed Shellfish Market Analysis. A report to the Gulf of Maine Research Institute, Portland, Maine. 65p.

The Hale Group report is important and current information in understanding the market possibilities for cultured scallops in Maine.

Pottle, T., and M. Hastings. 2001. Sea Scallop Demonstration Project. Final Programmatic Report Submitted to National Fish and Wildlife Foundation. Maine Aquaculture Innovation Center, Orono, ME. 10p.

This project run by fisherman Tom Pottle was important to establish some growth rates, equipment use, siting and leasing issues and access to the market for whole scallops; a model which is still in use today.

<http://www.mainebiz.biz/article/20161003/NEWS01/161009997/maine-fisheries-experts-head-to-japan-to-learn-scallop-practices-buy-machinery>

This news piece summarizes the nationally-groundbreaking work by Coastal Enterprises Inc. in scallop production, and some of the relevant partnerships involved.

3. Trends in Aquaculture Production in Japan

Satoshi Watanabe* and Tomoko Sakami

Presenting author*

National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency, 422-1 Nakatsuhamaura, Minamiise, Mie, 516-0193, Japan, *E-mail: swat@affrc.go.jp

Abstract

A wide variety of aquatic organisms are commercially cultured in Japan. For marine species, there are national statistical data published by the Ministry of Agriculture, Forestry and Fisheries on the aquaculture production of seven species of finfishes (*Oncorhynchus kisutch*, *Seriola* spp., *Trachurus japonicus*, *Pseudocaranx dentex*, *Pagrus major*, *Paralichthys olivaceus*, *Tetraodontidae* spp., *Thunnus orientalis*), two species of bivalves (*Mizuhopecten yessoensis* and *Crassostrea* spp.), one species of prawn (*Marsupenaeus japonicus*), one species of tunicate (*Halocynthia roretzi*), and four species of aquatic plants (*Saccharina* spp., *Undaria* spp., *Pyropia* spp. and *Nemacystus* spp.). Four freshwater species of finfishes (*Oncorhynchus mykiss*, *Plecoglossus altivelis*, *Cyprinus carpio* and *Anguilla japonica*) are also included in the statistics. There are also miscellaneous groups and some species have been deleted from the census due to budgetary constraints of the related agencies. Despite the historically strong affinity of the Japanese to seafood, production of aquaculture and capture fisheries are on a long-term moderate decreasing trend largely due to socio-economic factors in Japan. The total marine aquaculture production decreased from 1.28×10^6 t in 1996 to 1.03×10^6 t in 2016. Bivalve aquaculture production was relatively constant around 450×10^3 t. The production of *Crassostrea* spp. (oysters) and *M. yessoensis* (common scallop) decreased substantially due to the Great East Japan Earthquake in 2011, and harvest has not recovered to the level prior to the earthquake. Finfish and aquatic plant productions are on a continuous decreasing trend. Finfish production was not severely affected by the earthquake, except for *O. kisutch* (Coho salmon), production of which has quickly recovered. Although the production of some aquatic plants such as *Saccharina* spp. (kelps), *Undaria* spp. (*wakame*), *Pyropia* spp. (laver or *nor*i) was devastated by the earthquake not only in the Tohoku area but many other parts of Japan, the long-term decreasing trend is not attributed to the earthquake. One of the causes for the production declines is considered to be the reduced number of management bodies due to aging of the operators. The number of management bodies for *nor*i culture, for instance, decreased from 51,354 in 1963 to 3,819 in 2013. Merger of the management bodies is one of the reasons for the decrease, but the number of farmers is also

decreasing. On the other hand, aquaculture production of finfish and bivalves is increasing in value in recent years. The increase in finfish production is attributable to new inclusion of *T. orientalis* (Pacific bluefin tuna), which entered the major product species list in 2012. For bivalves, production of the common scallop leaped from JPY 25.7×10^9 in 2012 to JPY 62.4×10^9 in 2016. In freshwater aquaculture, *A. japonica* (eel) has by far the largest production in volume and value. The seeds for eel aquaculture are all wild caught, however, and the low availability of glass eel has been problematic in recent years. Prompt establishment of mass production technologies for eel seeds is desired.

Annotated Bibliography of Key Works

e-Stat, statistics of Japan (<https://www.e-stat.go.jp/en>)

The statistical data presented in this paper are available on line; however, please note that not all data are available in English.

Demura, M. 2010. Trend in fishmeal price and its effects on aquaculture. *Norinkinyu* 2010.10, 45-49. (in Japanese, <https://www.nochuri.co.jp/report/pdf/n1010jo1.pdf>)

Despite the increasing global demand on aquaculture products, aquaculture business management is not necessarily sustainable in Japan. Some aquaculture companies producing pufferfish and red seabream went bankrupt in 2010 due to low fish price and increasing feed price. The author analyzed the supply and demand and price change trends of fishmeal and discussed their effects on aquaculture business in Japan. The author urged the importance of counter measures for insufficient fishmeal supply, such as use of unutilized fish and development of low fishmeal feed.

4. Establishment of oyster tetraploid breeding stocks for the fast-growing oyster industry in the Gulf region

Huiping Yang

School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32653, USA, *Email: huipingyang at ufl.edu*

Abstract

In the Gulf region, oyster farming is rapidly increasing in recent years to meet the seafood market needs due to the decline of fishery landing since 2012. Based on the official registration information from the Florida Department of Agriculture and Consumer Services, a total of 180 leases (about 413 acres) are now farming oysters in 2017. For oyster farming industry, triploids are recognized as the preferred products because of their fast-growth, better meat quality, and year-round harvest. With the fast-growing of oyster farming industry, the demands of oyster triploid seed are growing rapidly. Seed shortage has occurred in the past several years and is becoming major constraint to the industry. To address the demand of triploid oyster seed, establishment of tetraploid breeding stocks is needed because tetraploids are the key for commercial all-triploid seed production. With support from the Gulf States Marine Fisheries Commission, one project focusing on establishment of tetraploid breeding stocks was initiated in 2017. In Year 1, chemically-induced triploids were produced by using Florida wild populations. In Year 2, triploid individuals were screened and identified, and the triploid females were used

for induction of tetraploid founders by using the ever-patented technique (which is to fertilize the eggs from triploids with sperm from diploids following by inhibition the first polar body release). Currently, this project is ongoing, and data collected in 2017 and 2018 are in process of analysis. It is expected that establishment of tetraploid breeding stocks will be useful for triploid seed production with diversified genetic resources to support the oyster industry.

Annotated Bibliography of Key Works

Tetraploid production for all-triploid seed production

- 1) Guo, X. & Allen, S. 1994. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body I in eggs from triploids. *Molecular Marine Biology and Biotechnology*, 3, 42-50.
- 2) Guo, X., DeBrosse, G. A. & Allen, S. K. 1996. All-triploid Pacific oysters *Crassostrea gigas* (Thunberg) produced by mating tetraploids and diploids. *Aquaculture*, 142, 149-161.

Tetraploid production has been a challenge in shellfish due to their poor survival to spat and adult stage although there have been huge efforts in this research topic since 1980s. The publication in 1994 by Guo and Allen reported a unique methodology for tetraploid induction in the Pacific oyster, and the tetraploids survived to adult stage and were used for all-triploid seed production by crossing with normal diploids (Guo et al., 1996). The innovative method reported for viable tetraploids spat and adult production is to use oocytes from triploid females to fertilize with haploid sperm and followed by inhibition of the first polar body (PB 1) (Guo and Allen 1994). Later, this method has been applied to other oyster species and led the production of viable tetraploids in the Pearl oyster *Pinctada margaritifera*, eastern oyster *Crassostrea virginica*, and Somonie oysters *Crassostrea ariakensis* (He et al. 2000, Guo et al. 2002, Allen et al. 2005). This unique methodology has been patented by Rutgers University (US5824841A) and adopted by a private company named 4Cs Breeding Technologies, Inc. (4Cs, www.4cshellfish.com). So far, tetraploid breeding stocks have been successfully established mostly in oyster species (including Pacific oysters, eastern oysters, Sydney rock oysters, and Suminoe oysters) by using this method and applied for commercial triploid seed production.

Eudeline, B., Allen, S. K., & Guo, X. M. (2000). Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. *Aquaculture*, 187(1-2), 73-84. doi:10.1016/S0044-8486(00)00302-1

The publication modified the method for tetraploid induction (Guo and Allen, 1994) by using oocytes from individual females, thus the fertilized eggs would develop with a more synchronized way to allow polar body inhibition effectively to increase tetraploid occurrence. This is an important improvement considering the difficulty to find triploid females and the few oocytes from triploid females.

McCombie, H., Ledu, C., Phelipot, P., Lapegue, S., Boudry, P. & Gerard, A. 2005. A complementary method for production of tetraploid *Crassostrea gigas* using crosses between diploids and tetraploids with cytochalasin B treatments. *Marine Biotechnology*, 7, 318-33. Complementarily, another novel approach has been reported in the Pacific oyster by using tetraploid founders (McCombie et al. 2005) to produce viable tetraploids by fertilizing oocytes from diploids with sperm from tetraploids followed by inhibition of the polar body 2 (PB2). This method offers an alternative way for producing more tetraploids after production of initial founders and avoids the use of oocytes from triploid females which is always a big challenge.

This is an approach to diversify, change, and enrich the genetic background of tetraploid populations (50% from mother in the 1st generation, and probably increase to 100% from mother in the second generation depending on the chromosome segregation in sperm formation).

Guo, X., Wang, Y., Xu, Z. & Yang, H. 2009. Chromosome Set Manipulation in Shellfish. Pages 165-194 in G. Burnell & G. Allen editors. *New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management*. Woodhead Publishing Limited, Abington, Cambridge.

This book chapter reviewed systematically the shellfish polyploid breeding, including triploid and tetraploid production, concepts, mechanism, history, updates, and future application on aquaculture industry. In this publication, two comprehensive tables are included to summarize the triploid production, growth, and performances, and the tetraploid induction and breeding. In addition, there are several figures included in this review to describe the chromosome segregation mechanism for triploid, tetraploid and aneuploid production.

5. Development of environmentally friendly, cost-effective and nutritionally balanced alternative protein based diets for high value marine fish culture in recirculating aquaculture system

M.S. Alam*, W. O. Watanabe and P.C. Carroll

Presenting author*

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Abstract

With very little domestically grown seafood, along with increasing exploitation of wild stocks, the US seafood trade deficit now exceeds \$14 billion. Economics, environmental issues and fish feed are important factors vital to the sustainability of the aquaculture industry in both the US and Japan. These factors increase the pressure on aquaculturists to develop eco-friendly and sustainable aquaculture practices. One of the challenging tasks remaining in the aquaculture industry is how to prevent the pollution of water by artificial feed. Fish meal is the main protein source used in aquafeed that made from small pelagic species of forage fish. Two nutrients in fish meal that have a great impact on environment are nitrogen and phosphorus and greater amounts of phosphorus and nitrogen result in eutrophication of farm areas. Fish meal could contain polychlorinated biphenyls (PCBs), dioxins, and other harmful chemicals and becomes a vector of contamination in farm-raised finfish. Alternate protein sources such as terrestrial animal and plants protein source can reduce the amount of wild fish used as protein, and potentially reduce the nutrient levels in effluent waste. One of the goals of our research is to develop sustainable marine finfish feed with less inclusion of fish meal in recirculating aquaculture system (RAS) that are widely considered to be “environmentally friendly”. At the University of North Carolina Wilmington-Center for Marine Science, a series of experiments were conducted to test the effects of different dietary levels of soybean meal, ultra-low gossypol based glandless cottonseed meal, poultry meal in the diet of southern flounder, red

porgy and black sea bass. Fish (juveniles, pre adults and grow-out stages) were fed the respective test diets in aquaria, semi-pilot and commercial scale RAS systems for 8-10 weeks, 4 and 10 months, respectively. The growth performance, feed utilization, digestibility and biochemical composition of fish tissues were evaluated.

Results suggests that about 70, 40 and 35% fish meal protein could be replaced by soybean meal and 80, 100 and 70% of fish meal could be replaced by poultry meal in the diets of black sea bass, southern flounder and red porgy, respectively. Results also suggest that fish meal could be replaced up to 100 and 75% by glandless cottonseed meal in black sea bass and southern flounder diets, respectively. The digestibility, proximate composition, amino acid and fatty acid composition of fish tissues will be presented. These findings could be used to develop environmentally-sound and cost-effective pre-dominantly alternative protein based diets for high value marine fish culture in USA and Japan.

My 8 years higher education and research on aquaculture in Japan (Masters, doctoral and JSPS post-doctoral research in Kagoshima University 1997-2005) and my research at University of North Carolina Wilmington, USA (from 2005 to date) inspired me to propose collaborative research between Japan and USA to reduce fish meal by alternative protein sources. Professor Dr. Yutaka Haga, Lab of Fish Nutrition, Tokyo University of Marine Science and Technology, Japan expressed his interest for collaborative research between our institutions in this topic area.

Annotated Bibliography of Key Works

Alam, M.S., Watanabe, W.O., Carroll, P.M., Gabel, J.E., Corum, M.A., Seaton, P., Wedegaertner, T.C., Rathore, K.S., Dowd, M.K. 2018. Evaluation of genetically-improved (glandless) and genetically-modified low-gossypol cottonseed meal as alternative protein sources in the diet of juvenile southern flounder *Paralichthys lethostigma* reared in a recirculating aquaculture system. *Aquaculture*, 489: 36–45.

One of the biggest operational expenses in marine recirculating aquaculture systems (RAS) is high quality feed. Traditionally, fishmeal (FM) is used as the main source of protein in marine fish diets due to its high protein content, amino acid profile, digestibility, and palatability. Unfortunately, as the aquaculture industry continues to grow it is creating a higher demand for FM worldwide. Limited supplies along with increasing demand are causing the price of FM to continually increase. In an aim to search the replacement of FM by alternative protein sources in southern flounder diets glandless cottonseed meal was tested at our University of North Carolina (UNCW)-Aquaculture Facility. Cottonseed meal (CSM) proteins from genetically-improved (glandless) low-gossypol seed (GI-CSM, 52.1% crude protein, CP), genetically-modified low-gossypol seed (GMO-CSM, 56.0% CP) and from an untreated regular (glanded) seed (R-CSM 49.9% CP) were evaluated to replace fish meal (FM) protein (59.5% CP) in juvenile southern flounder *Paralichthys lethostigma* diets. The results suggest that up to 75% of fish meal protein may be replaced by GI- or GMO-CSM protein in the diet of juvenile flounder without adverse effects on growth performance and body composition. Cottonseed meal is a potentially cost-effective alternative plant protein source for use in aquafeeds. These ultra-low gossypol cottonseed flour proteins could be inexpensive protein sources for the commercial culture of southern flounder and other finfish species.

Alam, M.S., Watanabe, W.O., Sullivan, K.B., Rezek, T.C., Seaton, P.J. 2012. Replacement of menhaden fish meal protein by solvent extracted soybean meal protein in the diet of juvenile black sea bass *Centropristis striata* supplemented with or without squid meal, krill meal, methionine and lysine. *North American Journal of Aquaculture*, 74:2, 251-265

Three experiments were conducted at our UNCW-Aquaculture Facility to determine the extent to which menhaden fish meal protein (FMP) can be replaced by solvent - extracted soybean meal protein (SBP) in the diet of juvenile black sea bass *Centropristis striata*. Diets were formulated replacing FMP by SBP at 0, 10, 20, 30, 40, 50, and 60% (experiment 1) and 0, 60, 70, 80, 90, and 100% (experiment 2), with supplementation with squid meal, krill meal, and attractants in both experiments. Experiment 3 was designed to replace FMP by SBP at 40, 50, 60, 70, and 80% without supplemental squid and krill meal and at 60% and 70% with supplemental methionine and lysine. Diets were fed twice daily to triplicate groups of fish ($N = 15$ per group) in 75 - L tanks containing recirculating seawater. Fish were fed for 6, 10, and 8 weeks in experiments 1, 2, and 3, respectively. No significant differences in body weight gain, feed efficiency, and survival were observed among treatments in experiment 1. In experiment 2, no significant differences in percent weight gain were observed among fish fed diets replacing FMP at 0, 60, and 70%. In experiment 3, body weight gain was not significantly different for fish fed supplemental methionine and lysine in 70% SBP diets compared with fish fed 0% SBP diets. No significant differences were observed in whole - body n - 3 polyunsaturated fatty acids among treatments in experiment 2. Broken - line regression of the specific growth rate data suggested that the maximum level of FMP replacement with SBP in black sea bass diets was 67.6–68.4% with 75 g/kg squid meal and 50 g/kg krill meal in the diet and 57.2–58.0% without squid and krill meal supplementation.

Alam, M.S., Watanabe, W.O., Dawson, M., Carroll, P. 2015. Pilot, commercial-scale testing of promising diets for intensive cultivation of black sea bass and southern flounder. *World Aquaculture*, Dec 2015, 34-40.

In order to replace fish meal in the diet of a marine finfish, the protein source must be high enough in protein, have a similar amino acid profile, cannot harm the fish in any way, must be palatable for the fish, needs to be cost effective, and must be accepted by the consumer. To reduce cost in commercial fish feed industries, soybean meal is considered to be one of the most suitable and stable supplies of an alternative ingredient for replacing fish meal. However, there was no published information on utilization of soybean meal as an alternative protein source in the diets of southern flounder and black sea bass in pilot commercial scale, two high-value marine finfish in the Atlantic coastal region of the U.S. Therefore, commercial scale feeding trials were conducted in three commercial aquaculture farms in North Carolina and Virginia for about 10 months in order to investigate the replacement of fish meal (0, 50 and 30%) by soybean meal protein in pre adult and grow-out southern flounder and black sea bass. Result suggests that a substantial amount of fish meal protein (50% for black sea bass and 30% for southern flounder diets) could be replaced in the diets of grow-out black sea bass and southern flounder without any adverse effect on growth and survival.

Alam, M.S., Watanabe, W.O., Myers, A.R., Rezek, T.C. Carroll, P.M., Skrabal, S.A. 2015. Effects of dietary salt supplementation on growth, body composition, tissue electrolytes, and gill and intestinal Na⁺K⁺ATPase activities of black sea bass reared in low salinity water. *Aquaculture*, 446, 250-258.

Two feeding trials were conducted to investigate the effects of dietary sea salt supplementation on growth and survival of black sea bass *Centropristis striata* reared under sub-optimal salinity conditions at 15 and 10 g L⁻¹. Irrespective of dietary salt treatment, black sea bass juveniles maintained good growth and survival and normal whole body electrolyte concentrations at rearing salinities of 10 and 15 g L⁻¹, indicating that these fish are good osmoregulators and could be potentially cultured under these low salinities. These findings may enable black sea bass to be grown in recirculating aquaculture systems in low-salinity brackish water. The results indicate that dietary salt supplementation may be used to avert mortality when a producer is faced with adversely low salinity conditions due to weather and tides as may occur in coastal black sea bass fish farms sourcing brackish water from tidal creeks.

Alam, M.S., Teshima, S., Yaniharto, D., Koshio, S., Ishikawa, M. 2002. Influence of dietary amino acid patterns on growth and body composition of juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 210:359-369.

Since essential amino acids are of vital importance, these must be found in the sources of protein used to create finfish diets and in amounts similar to those required by the fish. If not, they must be supplemented to the diet in such a way that they meet the required balance for the particular species of fish. As per “ideal protein concept” “if the amino acid profile of the feed mimics the whole-body amino acid profile of the animal being fed, protein utilization and growth should be maximized.”

A feeding trial using five semi-purified diets (50% crude protein) was conducted to investigate the effects of different dietary amino acid patterns on growth and body composition of juvenile Japanese flounder. The control diet contained casein and gelatin as intact protein sources and four other diets contained 30% casein–gelatin (2:1, w/w) and 20% crystalline amino acids (CAA). CAA were added to the diets to simulate the amino acid pattern found in red sea bream egg protein (REP), Japanese flounder larvae whole body protein (FLP), Japanese flounder juvenile whole body protein (FJP), and brown fish meal protein (BFP), respectively. The test diets were fed to triplicate groups of juveniles (2.75±0.05 g) twice a day for 40 days to evaluate weight gain, survival, feed conversion efficiency (FCE), protein efficiency ratio (PER), and apparent protein utilization (APU). The apparent retention of total dietary amino acids in the whole body and A/E ratios of the whole body were also evaluated. The highest weight gain was observed in fish fed the diet containing the dietary amino acid pattern of BFP followed by fish fed the control, FJP, FLP and the REP diets. Percent survival, FCE, PER and APU were also significantly ($P<0.05$) affected by the amino acid pattern in the diets, indicating the highest value in fish fed the BFP diet. Except for a few amino acids, the amino acid composition of the whole body did not show marked differences with different dietary amino acid pattern. Results suggest that BFP could be more suitable as a reference amino acid pattern in the diet of juvenile Japanese flounder compared to the amino acid pattern of FLP, FJP or REP.

6. Seaweed aquaculture in a warming environment – building resilience with *Saccharina angustissima* (Laminariales, Phaeophyceae)

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Abstract

Locally sourced, high quality sea vegetables, in particular kelp, are sparking consumer interest and demand in New England. The narrow-bladed sugar kelp, *Saccharina angustissima*, with one narrow (1.6 ± 0.7 cm wide) and one long (average 1.8 ± 0.96 m, up to 4.4 m) blade is morphologically and genetically distinct from *Saccharina latissima*, which grows much wider (20-30 cm) and is usually shorter (up to 3.5 m) in length. In the intertidal, *S. angustissima* is adapted to withstand extreme hydrodynamic forces an order of magnitude higher than the subtidal populations of sugar kelp that are usually found in more sheltered locations. It is restricted in distribution in mid-coastal Maine and occurs in the low intertidal, exposed to high ocean swells. In collaboration with kelp farmers from Maine Fresh Sea Farms and Maine Coast Sea Vegetables, we successfully domesticated this kelp growing it to full maturity on longlines to be used commercially for its desirable culinary traits. Based on harvest results from two growing seasons, biomass yields of this kelp were up to $24.1 (\pm 6.3)$ kg m⁻¹ of line with a plant density of 400 plants m⁻¹ of line. The phenotypic characteristics that make this kelp exceptionally adapted to extreme sea conditions were preserved at the open-water farm sites. Experiments with the microscopic gametophyte stages of *S. angustissima* show that it has the capacity to withstand some temperature fluctuations. Overall, this domesticated kelp species has great potential as an economically valuable sea vegetable in the Gulf of Maine. Nevertheless, because of its restricted geographic distribution, care must be taken to protect the donor population. Although additional work needs to be done to bring this kelp to market, growing it also offers a suite of ecosystem services including nutrient bioextraction and temporary habitat formation for sea animals.

Annotated Bibliography of Key Works

Augyte, S., Yarish C., S.Redmond, & J.K.Kim. 2017. Cultivation of a morphologically distinct strain of the sugar kelp, *Saccharina latissima* forma *angustissima*, from coastal Maine, USA, with implications for ecosystem services. *Journal of Applied Phycology* 29: 4. 10.1007/s10811-017-1102-x.

Consumer interest and demand for North Atlantic sourced sea vegetables drives opportunities for aquaculture development in the northeast USA. The unique morphology and desirable culinary traits of the wild narrow-bladed kelp, *Saccharina latissima* forma *angustissima*, were successfully translated into a cultivated crop on two geographically distinct open-water farms in Maine, USA. Environmental conditions, growth, and tissue analysis were quantified. Peak meristematic growth rates for blade length occurred from March through April at $2.85 (\pm 0.34)$ cm day⁻¹. The kelp was harvested from May through June with yields of up to $17 (\pm 4.4)$ kg m⁻¹ of line and plant density of 330 plants m⁻¹ of line at the Bristol farm and yields of $13.3 (\pm 6.2)$ kg wet weight m⁻¹ line and a plant density of 400 plants m⁻¹ of line at the Sorrento farm. Second season yields at Sorrento were on average $24.1 (\pm 6.3)$ kg m⁻¹ of line. Both farms grew

significantly narrower blades of *f. angustissima* than of the sugar kelp, *S. latissima*. Common garden experiments with the two morphotypes identified trait stability for length and width, while blade ruffles and thickness varied with the environment. Calculations estimating the nutrient bioextraction capability of the cultivated *f. angustissima* kelp harvested in June reveal N removal of 88.7 kg ha⁻¹ and C removal of 1666.7 kg ha⁻¹ (combined farm site averages). Overall, this unique kelp form has the potential as a new aquaculture crop for the Gulf of Maine while providing several coastal ecosystem services.

Augyte, S., L. Lewis, S. Lin, C. D. Neefus, and C. Yarish. 2018. Speciation in the extremely exposed intertidal: the case of *Saccharina angustissima* (Collins) Augyte, Yarish & Neefus comb. nov. et stat. nov. (Laminariales, Phaeophyceae). *Phycologia* 57: 100-112.

Saccharina latissima is a perennial kelp with a circumboreal distribution from the North Pacific to the North Atlantic coasts. Our study clarified the taxonomy of the morphologically distinct *Saccharina latissima* forma *angustissima* (Collins) A. Mathieson from the low intertidal zone on exposed islands and ledges of Casco Bay, Maine, USA. To identify genetic divergence between the two morphotypes, *S. latissima* and *S. latissima f. angustissima*, we used a multilocus phylogenetic approach. Genetic analysis suggested low divergence between the two forms. However, there was as much or more genetic divergence between *S. latissima* and *S. latissima f. angustissima* as there were between other taxonomically accepted species of *Saccharina*. To investigate sexual compatibility between the two forms, we made reciprocal crosses of the gametophytes and observed sporophyte formation. All crosses were successfully grown to the juvenile sporophyte stage, suggesting that the two are reproductively compatible in vitro. It is unknown if the two populations freely hybridize in the field. Last, we compared wave action, the ecological factor most likely driving the unique morphology, at exposed sites with *S. latissima f. angustissima* and protected sites with *S. latissima*. The mean wave force at the exposed site was over 30 times higher in magnitude than at the protected site respectively, during the summer. The significant differences in morphology, the lack of specimens with intermediate morphologies, and the results of a common garden experiment suggest that the morphological differences in *S. latissima f. angustissima* are heritable with a genetic basis. Therefore, on the basis of our molecular evidence coupled with ecological studies, we are elevating *S. latissima f. angustissima* (Collins) A. Mathieson to specific rank as *S. angustissima* (Collins) Augyte, Yarish & Neefus comb. nov. & stat. nov

J. K. Kim, G.P. Kraemer, & C. Yarish 2015. Use of sugar kelp aquaculture in Long Island Sound and the Bronx River Estuary for nutrient extraction. *Marine Ecology Progress Series* 531:155-166.

The present study revealed an even higher nutrient bioextraction capacity in the cold-water species *Saccharina latissima* at 3 sites – the mouth of the Bronx River Estuary (Bronx, NY; BRE), western Long Island Sound (Fairfield, CT; WLIS) and central Long Island Sound (Branford, CT; CLIS), during winter and spring of the 2012–2013

growing season. These sites differ in temperature (BRE > CLIS > WIS), salinity (BRE < WLIS = CLIS) and nutrients (BRE >> WLIS = CLIS). It was estimated that *S. latissima* could remove up to 180, 67 and 38 kg N ha⁻¹ at BRE, WLIS and CLIS respectively, in a hypothetical kelp farm system with 1.5 m spacing between longlines. In the same hypothetical kelp farm system, the estimated carbon sequestration values are 1350 (BRE), 1800 (WLIS) and 1100 (CLIS) kg C ha⁻¹. The potential monetary values of N sequestration by the sugar kelp are up to \$1600 (BRE), \$760 (WLIS) and \$430 (CLIS) ha⁻¹, if incorporated in the State of Connecticut Nitrogen Credit Trading Program and a carbon-pricing scheme. The potential economic values of C sequestration are \$30–300 (BRE), \$40–400 (WLIS), and \$24–240 (CLIS) ha⁻¹. These results suggest that seaweed aquaculture is a useful technique for nutrient bioextraction in urbanized coastal waters, such as LIS and BRE. Alternation of the warm- and cold-water species would maximize nutrient bioextraction and augment other ecosystem services, producing economic benefits for the region while helping to manage non-source eutrophication.

7. On the application of bio-stimulants to enhance growth and thermal resistance of sugar kelp, *Saccharina angustissima*, for aquaculture production

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Abstract

Macro- and microalgae are good sources of bioactive compounds, nutrients, and bio stimulants that are being used in agriculture and horticulture to mitigate abiotic and biotic stresses to improve plant productivity and food production. We tested commercially available algal extracts to see if they can enhance growth and thermal resistance of the sugar kelp, *Saccharina angustissima* under laboratory conditions. We subjected kelp juvenile sporophytes to different temperatures, periods, and varying concentrations of extract solutions. Blade growth was measured on a weekly basis for a four-week period. Preliminary results show that the commercial extracts tested do not have a direct effect on growth but a delayed effect on thermal resistance and blade growth. These results suggest that algal bio-stimulants could enhance the

performance of seaweed cultures on open-water farms subjected to environmental stress, particularly in light of ocean warming and global climatic change.

Annotated Bibliography of Key Works

Craigie, J.S. 2011. Seaweed extract stimuli in plant science and agriculture. *J Appl Phycol.* 23:371–393

This contribution derives from the author's presentation at the "XX International Seaweed Symposium". Craigie provides an overview of the utilization of seaweeds by humans, and more relevant, a summary of seaweeds uses in agriculture. Furthermore, this paper details with examples how the application of seaweed as bio-stimulants enhances land crops in different ways. The author includes brand names, countries of origin, and nutritional content of various commercially available products.

Tibubos, K.R., Hurtado, A.Q., Critchley, A.T. 2017. Direct formation of axes in new plantlets of *Kappaphycus alvarezii* (Doty) Doty, as influenced by the use of AMPEP K⁺, spindle inhibitors, and plant growth hormones. *J Appl Phycol.* 29:2 3 –245 349

This is a recent contribution describing the effect of AMPEP K⁺ on the formation of vegetative shoots of *Kappaphycus alvarezii*. AMPEP K⁺ is a Canadian commercial extract of the brown seaweed *Ascophyllum nodosum* with enhanced levels of potassium. The authors tested this extract in combination with colchicine or oryzalin, in addition to synthetic plant growth regulators. They found that 5 mg L⁻¹ of AMPEP K⁺ with plant growth regulators produced the longest direct axis shoots (9.6 ± 0.33 mm), followed by 0.1 mg L⁻¹ of AMPEP K⁺ combined with 1.0 mg L⁻¹ oryzalin and plant growth regulators (8.7 ± 0.00 mm). Their results suggest that using AMPEP K⁺ could improve shoot formation of *K. alvarezii* plantlets in land-sea-based nursery cultivation.

Hurtado, A.Q. and Critchley, A.T. 2018. A review of multiple biostimulant and bioeffector benefits of AMPEP, an extract of the brown alga *Ascophyllum nodosum*, as applied to the enhanced cultivation and micropropagation of the commercially important red algal carrageenophyte *Kappaphycus alvarezii* and its selected cultivars. *Journal of Applied Phycology* <https://doi.org/10.1007/s10811-018-1407-4>

The majority of publications related to using seaweed extracts on the aquaculture of other seaweeds have assessed the effect of *Ascophyllum nodosum* extracts on *Kappaphycus alvarezii*. This review mainly summarizes studies made on *K. alvarezii* exposed to different treatments of *A. nodosum* extracts. *A. nodosum* extracts are utilized to mitigate biotic and abiotic stressors of *K. alvarezii* subject to farming conditions. The authors provide examples related to micropropagation, field cultivation, endophyte mitigation, and impacts on the resulting carrageenan qualities of different *K. alvarezii* cultivars. In addition, the authors also mention about alternative seaweed extract that has been applied to boost the growth of another cultivated seaweed. Altogether, this review paper provides evidence of the favorable results of using *Ascophyllum* extracts as biostimulants for *Kappaphycus* aquaculture.

8. *Vibrio coralliilyticus* induction of virulence toward larval oysters and corals at elevated seawater temperatures and potential mitigation treatments

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Abstract

Recent research has advanced our understanding of the role pathogenic vibrios play in disease of various aquaculture species. *Vibrio coralliilyticus*, formerly thought to infect just corals, is now known to infect larval oysters, causing major losses in hatchery settings. The negative effect of *V. coralliilyticus* on wild-type oyster production remains uncertain, but circumstantial evidence suggests it may be substantial. We identified eight strains of *V. coralliilyticus* that infect both Eastern oyster (*Crassostrea virginica*) larvae and Pacific oyster (*Crassostrea gigas*) larvae in U.S. East and Pacific coast hatcheries, respectively. West Coast hatcheries have experienced major production losses over the years, due to the presence of *V. coralliilyticus*. Some of the largest hatcheries have reported losses of up to 80%, resulting in shortages in seed oysters needed for commercial oyster production. Losses can exceed 10 billion larvae per year in the larger hatcheries. The U.S. East Coast hatcheries are considerably smaller, but also experience losses in Eastern oysters, possibly from *V. coralliilyticus*, but also from the known shellfish pathogen *V. tubiashii*. The infection and death of corals by *V. coralliilyticus* **is known to be enhanced at seawater temperatures $\geq 27^{\circ}\text{C}$. In our studies, multiple strains of *V. coralliilyticus* produced high larval oyster mortalities with LD_{50} 's ranging from 3.8×10^3 to 4×10^4 CFU/ml of seawater, depending on the *V. coralliilyticus* strain and the oyster species (Eastern vs. Pacific oysters). Studies with knockout mutations demonstrated that the transcriptional regulator ToxR and the outer membrane protein OmpU were important in larval oyster (and coral) infections caused by *V. coralliilyticus*. Gross pathological changes occur in *V. coralliilyticus*-infected larvae, initially in the velum and cilia and then in internal organs which become liquefied. Infection of larval oysters is likely induced by stress and a lowering of resistance to these pathogens. Stressors, particularly in hatcheries, likely include incorrectly adjusted seawater temperatures, salinities, pHs, and dissolved oxygen levels or inadequate nutrition, over stocking, high overall bacterial loads, etc. To begin to mitigate vibrios in hatcheries and other venues, we identified a variety of predatory bacteria which, in nature, help to modulate *Vibrio* levels in seawater and/or shellfish. They include several predatory bacteria such as *Pseudoalteromonas piscicida*, which secrete digestive enzymes that inhibit and kill many kinds of bacteria including vibrios; and *Halobacteriovorax*, a Bdellovibrio and Like Organism (BALO) which infect Gram-negative bacteria like vibrios and replicate within them, killing the vibrios in the process. Together these bacteria may serve as probiotics in reducing hatchery mortalities and in killing vibrios and other bacterial pathogens in a variety of other aquaculture settings. Bacteriophages (phages) are another means to inactivate many undesirable pathogens in the environment and are increasingly being used in aquaculture. To date, we isolated phages against *V. coralliilyticus* and *V. tubiashii* and showed the practical application of phage therapy to reduce or eliminate mortalities from *V. coralliilyticus* in larval oysters.**

Annotated Bibliography of Key Works

Richards, G.P., M.A. Watson, D.S. Needleman, K.M. Chruch and C.C. Häse. 2015. Mortalities of Eastern and Pacific oyster larvae by the pathogens *Vibrio coralliilyticus* and *Vibrio tubiashii*. *Appl. Environ. Microbiol.* 81:292–297.

Vibrio tubiashii has been a well known pathogen in Eastern oyster (*Crassostrea virginica*) larvae on the United States (U.S.) East Coast for many years and has also been associated with Pacific oyster (*Crassostrea gigas*) larvae on the U.S. West Coast. *Vibrio coralliilyticus*, a well-known coral pathogen and a cause of coral bleaching, has also been associated with disease of Pacific oyster larvae, particularly in oyster hatcheries. It became evident that some of the outbreaks on the West Coast that were thought to be from *V. tubiashii* were actually caused by *V. coralliilyticus*. In this study, we evaluated two strains of *V. tubiashii* and four strains of *V. coralliilyticus* in both Eastern and Pacific oyster larvae to determine their ability to cause larval mortalities. We showed that *V. tubiashii* strains infected primarily the East Coast oyster larvae while all four of the *V. coralliilyticus* strains infected the Eastern and Pacific oyster larvae. The LD₅₀ values for the *V. coralliilyticus* strains ranged from 1.1×10^4 to 3×10^4 CFU/ml of seawater in Eastern oysters larvae. In Pacific oyster larvae, the LD₅₀ range was 1.2×10^4 to 4×10^4 CFU/ml of seawater. Together, these studies defined the host specificity of these *Vibrio* strains with regard to Eastern and Pacific oyster larvae and demonstrated the ability of coral-associated pathogens to also infect and kill larval oysters. Subsequent research (Ushijima et al., 2018, see abstract below) showed similar results with other strains of *V. coralliilyticus* and an up-regulation of mortalities at elevated seawater temperatures.

Ushijima, B., G.P. Richards, M.A. Watson, C.B. Schubiger and C.C. Häse. 2018. Factors affecting infection of corals and larval oysters by *Vibrio coralliilyticus*. PLOS ONE.

<https://doi.org/10.1371/journal.pone.0199475>

Vibrio coralliilyticus is a naturally occurring marine bacterium which infects and kills corals and larval shellfish. It is best known as the cause of coral bleaching, which has contributed to the loss or damage to coral reefs worldwide. We determined that *V. coralliilyticus* strains also infect and kill larval oysters and other shellfish, particularly in shellfish hatcheries, causing shortages of seed oysters needed for commercial shellfish operations. We determined that larval oyster mortalities were significantly higher at an elevated seawater temperature (27°C) compared to a lower temperature (23°C). Thus, multiple coral pathogens were found to infect larval oysters in a temperature- and dose-dependent manner. Also identified were virulence factors that promote the infection of both coral and oyster larvae. This work demonstrates for the first time that elevated seawater temperatures enable *V. coralliilyticus* to more readily infect oyster larvae. It also serves to warn hatchery operators to maintain seawater temperatures below 27°C.

Richards, G.P., M.A. Watson, D.S. Needleman, J. Uknalis, E.F. Boyd and J.P. Fay. 2017.

Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. Appl. Environ. Microbiol. 83:e00175-17, <https://doi.org/10.1128/AEM.00175-17>

Pseudoalteromonas are marine bacteria that are known to secrete antimicrobial compounds which inhibit competing bacteria in the marine environment. We identified a second method by which some *Pseudoalteromonas* (*P. piscicida*) kill competing bacteria. It involves the direct transfer of digestive vesicles from the surface of the *Pseudoalteromonas* to the surface of competitors, digestion of holes in the competitor's cell wall by proteolytic enzymes associated with the vesicles, and apparent feeding of the *Pseudoalteromonas* off the nutrients released by the digested bacterium in a predatory fashion. Among the *P. piscicida* enzymes identified were aminopeptidase B, a trypsin-like serine protease, a chymotrypsin-like serine protease, and a cysteine protease. *Pseudoalteromonas piscicida* inhibited and killed the pathogens *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae*, *Photobacterium damsela*, *Shewanella algae*, and

Staphylococcus aureus. Together, this data indicates that *Pseudoalteromonas piscicida* produce important antibacterial compounds that have a potential role in the probiotic treatment of aquaculture products and in reducing biofilm formation.

Richards, G.P., J.P. Fay, J. Uknalis, M. Olanya and M.A. Watson. 2016. Purification and host specificity of predatory *Halobacteriovorax* isolates from seawater. **Appl. Environ. Microbiol.** 82: 922–927.

Halobacteriovorax are small predatory bacteria found in the marine environment and may serve as biocontrol agents against human, fish and shellfish pathogens. They are within a group of predatory bacteria known as the *Bdellovibrio* and Like Organisms (BALO). *Halobacteriovorax* were isolated from seawater and had broad specificity toward five strains of *Vibrio parahaemolyticus* while two additional *Halobacteriovorax* strains isolated from low salinity seawater readily infected and killed the human pathogens *E. coli* O157:H7 and *Salmonella* Typhimurium DT104. The replication rate of *Halobacteriovorax* against *E. coli* and *Salmonella* increased as salinities decreased. It is likely that *Halobacteriovorax* could readily be isolated against a broad range of Gram-negative seafood pathogens. Improved methods were also developed to purify infectious *Halobacteriovorax* from their host cells. Overall, the use of *Halobacteriovorax* in various aquaculture applications may serve as an environmentally friendly, non-antibiotic treatment to reduce pathogens in fish and shellfish.

Richards, G.P., J.P. Fay, K.A. Dickens, M.A. Parent, D.S. Soroka and E.F. Boyd. 2012.

Predatory bacteria as natural modulators of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seawater and shellfish. **Appl. Environ. Microbiol.** 78:7455–7466.

Levels of the human pathogenic *Vibrio parahaemolyticus* and *Vibrio vulnificus* are increasing in shellfish harvesting areas in many parts of the world due, in part, to elevated seawater temperatures. A means to reduce these vibrios in shellfish is needed. When seawater and oysters were spiked with *V. parahaemolyticus* or *V. vulnificus*, the *Vibrio* levels rapidly diminished over a 3-day period when natural seawater was used, but counts quickly climbed when sterile seawater was used. In an effort to determine what was inhibiting the vibrios in natural seawater, tests showed the presence of *Vibrio* predatory bacteria, later classified as *Halobacteriovorax* species. The *Halobacteriovorax* in natural seawater readily eliminated vibrios that were added to the seawater. In contrast, oysters spiked with vibrios showed some initial increase in *Vibrio* counts within the first 24 h, followed by a rapid decline to baseline levels. Thus, it appears that *Halobacteriovorax* are one of nature's tools to modulate bacterial levels in shellfish. Harnessing this tool may provide a means to reduce or eliminate pathogens in aquaculture operations and in the seafood industry.

9. Chemical changes in the environment: what does this mean to shellfish?

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Abstract

Coastal areas, estuaries, and river deltas are experiencing an increase in carbon dioxide from human activities, resulting in what is termed “coastal acidification.” Approximately 30% of atmospheric CO₂ dissolves in the oceans. As this CO₂ dissolves, it reacts with water and produces carbonic acid (H₂CO₃), which dissociates into bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), and hydrogen ions (H⁺). With increased CO₂ in the water, there are multiple ways that shellfish may be affected: (1) food availability; (2) physiological responses, and (3) sediment acidification.

Food availability may be influenced by changes in phytoplankton community structure and nutritional value. For example, increased carbon dioxide may cause a shift in phytoplankton biomass and community composition depending upon species’ efficiency of inorganic carbon acquisition. Shifts in phytoplankton communities, may favor less – or more -- nutritional phytoplankton that support production of human food.

In addition to potential changes in food supply, coastal acidification may be causing physiological changes in marine bivalves that affect how they feed. Bivalve gills are constantly exposed to ambient water, with the gill cilia moving water through the shell for respiration and feeding. Depending upon environmental conditions, physiological changes to the heart, gills, gonads, and general metabolism regulation may occur. Physiological changes could result in slower growth, higher mortality, or inability to maintain shell production, or the opposite responses in some cases. The influence that coastal acidification has on bivalve physiological functions is important to understand so that aquaculture may be modified to respond to a changing environment.

Finally, the same reaction that occurs in water during coastal acidification occurs at the sediment-water interface, where bivalves settle, following bacterial decomposition of settling organic matter. As particles settle, aerobic and anaerobic reactions, combined with redox processes, result in the production of CO₂ in sediment porewater. Corrosive and hypoxic sediment can impede successful settlement, recruitment, and abundance in bivalve populations. Nutrient-enriched, coastal areas in the United States are already experiencing sediment acidification, and this might be affecting bivalve recruitment at the sediment-water interface. The research presented here will focus on the multiple ways that coastal acidification may affect marine bivalves.

Annotated Bibliography of Key Works

Clements, J.C. and H.L. Hunt. 2018. Testing for sediment acidification effects on within-season variability in juvenile soft-shell clam (*Mya arenaria*) abundance on the Northern Shore of the Bay of Fundy. *Estuaries and Coasts*. 41. Pp. 471-483.

The authors conducted a field study assessing the relationship between juvenile soft-shell clam abundance and spatial, temporal, and environmental variables in the Bay of Fundy. Sediment pH, water temperature, location, and date was monitored in 2012 during the settlement season for *Mya arenaria* at four different study sites along the shore. Using Akaike’s information criterion models that incorporated environmental variables alone sediment pH and minimum air

temperature best predicted bivalve recruitment. By removing temporal effects, there was a significant positive relationship with sediment pH and grain size. These results suggest that carbonate geochemistry might influence bivalve settlement.

Meseck, S.L., R. Mercaldo-Allen, C. Kuropat, P. Clark, R. Goldberg. 2018. Variability in sediment-water carbonate chemistry and bivalve abundance after bivalve settlement in Long Island Sound, Milford, Connecticut. *Marine Pollution*. 135. Pp. 165-175.

The authors conducted field experiments in 2013 to assess the relationship between pore water sediment carbonate chemistry (pH, Alkalinity, dissolved inorganic carbon), grain size, and bivalve abundance at two sites in Long Island Sound (LIS), Connecticut, USA. Akaike's linear model was used to predict total bivalve community abundance and specific species abundance when appropriate. Over the entire season, 29% of bivalve abundance at the sites could be explained by grain size, salinity, and pH. When temporal effects were removed, up to 71% of *Nucula* spp. abundance could be accounted for by pH, phosphate, and silica concentrations in the porewater. These results suggest that bivalve abundance in LIS may be influenced by carbonate geochemistry and grain size.

Bermúdez, R., M. Winder, A. Stühr, A. Almén, J. Engström-Öst, and U. Riebesell. 2016. Effect of ocean acidification on the structure and fatty acid composition of a natural plankton community in the Baltic Sea. 2016. *Biogeosciences*. 13. Pp. 6625-6635.

The authors conducted mesocosm perturbation experiments in coastal waters to investigate the response of phytoplankton to increased carbon dioxide with respect to community composition and fatty acid composition (nutritional status). These results found little change in plankton community composition from the natural condition (~347 μatm) to predicted levels for the year 2100 (~1333 μatm). The fatty acid composition was influenced by community composition, which was driven by phosphate and silica availability. The results presented here suggest that phytoplankton community composition is influenced more by nutrient availability than by CO_2 , with little change in nutritional composition.

King, A. L., B.D. Jenkins, J.R. Wallace, Y. Liu, Y., G.H. Wikfors, L.M. Milke, and S.L. Meseck, S. L. 2015. Effects of CO_2 on growth rate, C: N: P, and fatty acid composition of seven marine phytoplankton species. *Marine Ecology Progress Series*. 537. Pp. 59-69.

The authors conducted laboratory-based experiments on 7 species of phytoplankton common in coastal estuaries of the North Atlantic where carbon dioxide was manipulated to 4 levels ranging from the glacial minimum (<290 ppm) to geological maximum levels (>2900 ppm). Results indicated that carbonate system-driven changes in growth rate did not result in changes to elemental composition of the species studied. Fatty acid composition was not affected by elevated CO_2 . The results showed little sensitivity of common coastal marine phytoplankton species to elevated CO_2 that will result in changes in nutritional status.

10. The current trends in pH and ocean acidification in an aquaculture sea area in Gokasho Bay, Japan

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Abstract

Coastal environments are influenced by various human-induced stressors. Recently, the global reduction of ocean pH, referred to as ocean acidification (OA), has arisen as a consequence of oceanic uptake of anthropogenic CO₂ from the atmosphere. OA has become a significant environmental stressor to diverse marine ecosystems. OA impacts not only marine phytoplankton production but also fisheries and seafood industries. Shellfish are farmed in many coastal and bay areas in Japan. It is likely that shellfish aquaculture will be impacted by OA in the near future since the shells are composed of calcium carbonate whose deposition is controlled by carbonate chemistry and the pH of seawater. While there are many studies on pH in open ocean and continental shelf waters, effects of OA are not well understood in many shellfish growing areas due to a large variation of pH associated with many biogeochemical processes. To obtain basic information on the water quality in an aquaculture sea area in Gokasho Bay, Mie, Japan, monthly hydrographical observations have been recorded since November 2016. Seawater samples were collected at three depths by a 1.3 L Kitahara's water sampler at each of 20 stations in the bay. The samples were collected in 25 ml co-stoppered glass test tubes for measurement of pH to avoid the contamination of CO₂ from atmosphere and in 250 ml polyethylene bottles for measurements of total alkalinity and nutrients. A CTD system (RINKO profiler, JFE Advantech) was also used for getting hydrographical data. The pH of seawater was measured more precisely using a glass electrode (MM-60R, DKK-TOA Co.) (± 0.003 pH). The glass electrode was standardized against the JIS buffer scale (NBS scale) and any drift in pH was corrected against the seawater buffer scale. The total alkalinity was measured by using a one-point titration method (± 0.006 meq/L). The calcium carbonate saturation state of seawater (Ω) was calculated from the values of pH, total alkalinity, salinity and seawater temperature. In the winter season, the value of pH was almost constant (8.02 - 8.13) across the sampled range in the bay and was approximately the same level as in surface water off the southern coast of Japan. In contrast, pH values varied greatly during the summer seasons (7.75 - 8.60). The lower value was thought to be caused by the remineralization of organic matter in the seabed, and the higher value probably resulted from high phytoplankton productivity. The pH measurements currently recorded were generally lower than those recorded during observations in the 1980s. The total alkalinity (TA) levels suggested that the riverine water in the bay was mixed rapidly with the outer bay waters. The values of Ω (> 3) calculated were relatively high across the bay. Although the mineral calcium carbonate is supersaturated in the current environment of the bay, if OA advances in offshore surface water around Japan in the near future, the degree of Ω in the bay water would drop with reduction of pH and could result in negative impacts on the calcareous ecosystem of the bay.

Annotated Bibliography of Key Words

Ishii, M., Koasugi, N., Sasano, D., Saito, S., Midorikawa, T., Inoue, H.Y. 2011. Ocean acidification off the south coast of Japan: A result from time series observation of CO₂ parameters

from 1994 to 2008. *J. Geophys. Res.* 116, C06022.

Japan Metrological Agency. Long-term trend of pH in surface water of the Pacific Ocean.

http://www.data.jma.go.jp/kaiyou/shindan/a_3/pHPac/pH-pac.html

The authors determined that a significant trend in ocean acidification is superimposed on the large seasonal and interannual variabilities of acidity in surface waters off the south coast of Honshu Island, Japan, based on their observation of the carbonate system (partial pressure of CO₂, total inorganic carbon and pH). Multiple regression analysis of these parameters showed that pH and the aragonite saturation state (Ω_{arago}) are decreased at a rate of -0.020 ± 0.007 pH/decade, and -0.12 ± 0.05 Ω_{arago} /decade, respectively. If future atmospheric CO₂ levels keep increasing as predicted by IPCC scenario A1FI, which postulates tentative fossil fuel use associated with very rapid economic growth, a further reduction of -0.8 pH and -0.1 Ω_{arago} is likely in the next 50 years. The authors suggested that such a rapid reduction of Ω_{arago} could have negative impacts on a variety of calcareous organisms.

Kubota, K., Yokoyama, Y., Ishikawa, T., Suzuki, A., Ishii, M. 2017. Rapid decline in pH of coral calcification fluid due to incorporation of anthropogenic CO₂. *Nature*, 7, 7694, DOI:10.1038/s41598-017-07680-0.

Based on Boron isotopic ratio ($\sigma^{11}\text{B}$) measurements of corals, the authors show clear evidence that ocean acidification is affecting the pH of the calcification fluid (pH_{CF}) in Porites corals within the western North Pacific Subtropical Gyre at two separate locations, Chichijima Island (Ogasawara Archipelago) and Kikaijima Island. A comparison with the pH of the ambient seawater (pH_{sw}) near these islands, estimated from a large number of shipboard measurements of seawater CO₂ and atmospheric CO₂, indicated that pH_{CF} is sensitive to change in pH_{sw}. The authors suggested that the calcification fluid of corals will become less supersaturated with respect to aragonite by the middle of this century (pH_{CF}~8.3 when pH_{sw}~8.0 in 2050), earlier than previously expected, despite the pH_{CF}-unregulating mechanism of corals.

Suga, H. P, Sakai, S., Toyofuku, T., and Ohkouchi, N., 2013. A simplified method for determination of total alkalinity in seawater based on the small sample one-point titration method. *JAMSTEC Rep. Res. Dev.*, 17, 23-33.

Measurement of pH and total alkalinity is the most convenient way to describe the carbon system in seawater samples. In this paper, the authors introduced a modified method for determination of total alkalinity focused on reduced volume seawater samples (1 mL) and based on the one-point titration method. The precision of this method is 0.1 - 0.2% (relative standard deviation). On the other hand, with no correction, the precision was 0.1 - 1.0%. Even though values showed a systematic error, precision was maintained through the correction based on concurrent measurement of commercially available standards. This method allows us to describe the carbonate system in a small amount of water with sufficient precision and accuracy to investigate the mechanism of calcification using only a pH meter.

Fujii, T., Komai, Y., Fujiwara, T. 2001. Carbon dioxide dynamics in coastal regions of Osaka Bay. *Japan Sci. Civil Eng. Papers B2*, 67, 911-915.

In coastal regions where the biological production is far larger than that in the open ocean and the short-term change of hydrographical conditions is significant, the authors also conducted continuous measurements of salinity, pH and dissolved oxygen (DO) at three stations in Osaka Bay. Estimates of carbonate species in bay water were calculated by using the values of pH and

total alkalinity. The results showed that dissolved inorganic carbon and DO fluctuated synchronously with high correlation ($R^2=0.97$), and the records of DO and partial pressure of CO₂ (pCO₂) indicated prominent diurnal variations which correspond to diurnal irradiation variations that are due to a high productivity in the bay.

11. Ocean acidification experimentation in seagrass-oyster ecosystems using a novel system to conduct in-situ CO₂ enrichment

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Abstract

Coastal ecosystems are under the influence of global environmental changes that threaten their ecological status and the sustainability of seafood production. Seagrass beds, which are widely distributed along coastlines around the world, are highly important ecosystems offering various types of ecosystem services to human beings. Especially, seagrass beds are quite important for environmental change mitigation and adaptation via carbon storage, acidification mitigation, and water quality improvement, as well as providing a nursery for marine coastal organisms. Therefore, seagrass beds are essential for harmonizing coastal fishery/aquaculture with environmental improvement, resulting in the sustainable use of ecosystem services. Here, we developed a novel system of in-situ manipulation of CO₂ and nutrient enrichment to demonstrate seagrass mitigation functions that combat against environmental changes. The system is called FORTES, which stands for the Free-Ocean Real-Time Experimental System. In addition, we developed non-environmental risk deterrents from natural algae, specializing in arthropods and molluscs, which can also be manipulated by FORTES. Now, we focus on the interaction between oyster aquaculture and seagrass vegetation as a common practice to achieve better coastal management in a changing environment. Using this system, we can demonstrate not only the mitigative effects of seagrass beds on oyster aquaculture, but also the direct effects of environmental changes such as rising pCO₂/pH, eutrophication, and agrochemical inputs on oysters.

Annotated Bibliography of Key Works

Campbell, J.E., Fourqurean, J.W. 2013. Effects of in situ CO₂ enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. Mar. Biol. 160, 1465-1475.

Seagrasses commonly display carbon-limited photosynthetic rates. Thus, increases in atmospheric pCO₂, and consequentially oceanic CO₂(aq) concentrations, may prove beneficial. While addressed in mesocosms, these hypotheses have not been tested in the field with manipulative experimentation. This study examines the effects of in situ CO₂(aq) enrichment on the structural and chemical characteristics of the tropical seagrass, *Thalassia testudinum*. CO₂(aq) availability

was manipulated for six months in clear, open-top chambers within a shallow seagrass meadow in the Florida Keys (USA), reproducing forecasts for the year 2100. Structural characteristics (leaf area, leaf growth, shoot mass, and shoot density) were unresponsive to CO₂(aq) enrichment. However, leaf nitrogen and phosphorus content declined on average by 11% and 21%, respectively. Belowground, non-structural carbohydrates increased by 29%. These results indicate that increased CO₂(aq) availability may primarily alter the chemical composition of seagrasses, influencing both the nutrient status and resilience of these systems.

Gattuso, J.P., Kirkwood, W., Barry, J.P., Cox, E., Gazeau, F., Hansson, L., Hendriks, I., Kline, D.I., Mahacek, P., Martin, S., McElhany, P., Peltzer, E.T., Reeve, J., Roberts, D., Saderne, V., Tait, K., Widdicombe, S., Brewer, P.G. 2014. Free-ocean CO₂ enrichment (FOCE) systems: present status and future developments. *Biogeosciences* 11, 4057-4075.

Free-ocean CO₂ enrichment (FOCE) systems are designed to assess the impact of ocean acidification on biological communities in situ for extended periods of time (weeks to months). They overcome some of the drawbacks of laboratory experiments and field observations by enabling (1) precise control of CO₂ enrichment by monitoring pH as an offset of ambient pH, (2) consideration of indirect effects such as those mediated through interspecific relationships and food webs, and (3) relatively long experiments with intact communities. Bringing perturbation experiments from the laboratory to the field is, however, extremely challenging. The main goal of this paper is to provide guidelines on the general design, engineering, and sensor options required to conduct FOCE experiments. Another goal is to introduce xFOCE, a community-led initiative to promote awareness, provide resources for in situ perturbation experiments, and build a user community. Present and existing FOCE systems are briefly described and examples of data collected are presented. Future developments are also addressed as it is anticipated that the next generation of FOCE systems will include, in addition to pH, options for oxygen and/or temperature control. FOCE systems should become an important experimental approach for projecting the future response of marine ecosystems to environmental change.

Kline, D.I., Teneva, L., Schneider, K., Miard, T., Chai, A., Marker, M., Headley, K., Opdyke, B., Nash, M., Valetich, M., Caves, J.K., Russell, B.D., Connell, S.D., Kirkwood, B.J., Brewer, P., Peltzer, E., Silverman, J., Caldeira, K., Dunbar, R.B., Koseff, J.R., Monismith, S.G., Mitchell, B.G., Dove, S., Hoegh-Guldberg, O. 2012. A short-term in situ CO₂ enrichment experiment on Heron Island (GBR). *Scientific Reports* 2, Article number: 413.

Ocean acidification poses multiple challenges for coral reefs on molecular to ecological scales, yet previous experimental studies of the impact of projected CO₂ concentrations have mostly been done in aquarium systems with corals removed from their natural ecosystem and placed under artificial light and seawater conditions. The Coral-Proto Free Ocean Carbon Enrichment System (CP-FOCE) uses a network of sensors to monitor conditions within each flume and maintain experimental pH as an offset from environmental pH using feedback control on the injection of low pH seawater. Carbonate chemistry conditions maintained in the -0.06 and -0.22 pH offset treatments were significantly different than environmental conditions. The results from this short-term experiment suggest that the CP-FOCE is an important new

experimental system to study in situ impacts of ocean acidification on coral reef ecosystems.

12. Effects of ocean acidification on the early developmental stages of the commercially important gastropods, Ezo abalone and horned turban, in Japan

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Abstract

Commercially valuable shellfish species will be affected more seriously by ocean acidification (OA) in conjunction with overfishing. We investigated the effects of OA on fertilization, larval development, metamorphosis, and post-larval growth of valuable gastropods, Ezo abalone *Haliotis discus hannai* and turban shell *Turbo cornutus*, using a highly accurate CO₂ manipulation system, AICAL pCO₂ control system (Kimoto Electric Co., Ltd, Japan). No effects of exposure to <1000 µatm pCO₂ seawater were observed in fertilization, malformation, or mortality rates in both gastropods. However, fertilization and hatching rates decreased by exposure to >1500 µatm pCO₂ seawater. The malformation rates increased significantly at >1000 µatm, and the diameter of larval shells exposed to >800 µatm pCO₂ was significantly smaller than those in the control seawater (400-450 µatm pCO₂) in both species. Shell growth rates of post-larval *H. discus hannai* were significantly smaller at >1500 µatm than at <1000 µatm pCO₂. Scanning electron microscopic images of post-larval shells exposed to >1000 µatm pCO₂ showed abnormal development, suggesting problems with shell deposition and/or increased shell dissolution. These results indicate that pCO₂ >1000 µatm reduce developmental performance of these gastropods in early life stages. The habitats of these gastropods are characterized by kelp beds. Photosynthesis and respiration of the macroalgae create marked pCO₂ diel fluctuations, which are larger than pCO₂ change owing to OA projected for open ocean waters by 2100. We also investigated the effects of OA in relation with diel cycles of seawater pCO₂ levels projected for the near and distant future on the early development of *Haliotis discus hannai* in the laboratory. Shell length, abnormality and mortality of larval abalone were examined under different pCO₂ levels with mimicked diel cycle using the AICAL pCO₂ control system. Adult abalone with fully matured gonads were induced to spawn and gametes were artificially fertilized at concentrations of ca. 400 (atmosphere-balanced, control treatment), 800 or 1200 µatm pCO₂. Hatched larvae 16 hours post-fertilization were exposed to running seawater adjusted to a constant pCO₂ level [ca. 400, 800 or 1200 µatm pCO₂] or fluctuating pCO₂ on a diel cycle [800±400 (400-1200) or 1200±400 (800-1600) µatm pCO₂] in 4-day experimental periods. There were no significant differences in the mortality rate, abnormality rate and shell length of the larvae among the 400, 800, and 400-1200 µatm pCO₂ treatments. In

contrast, the abnormality rate was significantly higher in the 1200 and 800-1600 $\mu\text{atm } p\text{CO}_2$ treatments than in the control treatment. Larval shell length in the 800-1600 $\mu\text{atm } p\text{CO}_2$ treatment was significantly smaller than that in the other $p\text{CO}_2$ treatments. The exposure duration exceeding the aragonite saturation state would be a critical factor determining the negative impacts on the larval development of abalone.

Annotated Bibliography of Key Works

Kimura, R., Takami, H., Ono, T., Onitsuka, T., Nojiri, Y. 2011. Effects of elevated $p\text{CO}_2$ on the early development of the commercially important gastropod, Ezo abalone, *Haliotis discus hannai*. Fish. Oceanogr. 20, 357-366.

This is one of the first papers investigating the effect of ocean acidification on commercially important gastropod species in the world. This is also a very early paper using an AICAL system that precisely controls the $p\text{CO}_2$ concentration during the experiment.

Kurihara, H. 2008. Effects of CO_2 -driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275-284.

This review paper describes the effects of ocean acidification on the early development of many marine invertebrates, including bivalves (oyster and mussel). It provides an overview of the effects of carbon dioxide concentration on the development of marine invertebrates.

Onitsuka, T., Takami, H., Muraoka, D., Matsumoto, Y., Nakatsubo, A., Kimura, R., Ono, T., Nojiri, Y. 2018. Effects of ocean acidification with $p\text{CO}_2$ diurnal fluctuations on survival and larval shell formation of Ezo abalone, *Haliotis discus hannai*. Mar. Env. Res. 134, 28-36.

This paper assessed the effects of constant and diurnally fluctuating $p\text{CO}_2$, mimicking the rhythm of actual ocean conditions on development and shell formation of larval abalone, using an AICAL system which can precisely control $p\text{CO}_2$. It is suggested that the effects of ocean acidification on development and shell formation of larval abalone can be determined by intensity and exposure duration to $p\text{CO}_2$ over the threshold associated with aragonite saturation state (Ω -aragonite).

13. A slow growing perspective on multi-generational responses to future change

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Abstract

Our oceans are changing, becoming increasingly undersaturated with respect to carbonates as atmospheric CO_2 concentrations increase. Predicting how organisms will respond to these changes has become a major area of research, particularly for those species needing to maintain homeostatic and biomineralizing processes. In recent years there has been a shift in focus from shorter (hours-weeks) towards assessing responses over longer term exposure periods (months-years) advancing our understanding in this field. Given that predicted changes span across years and decades, and that organisms will be producing offspring, a greater consideration of multi-generational response is needed to better understand how organisms will respond under future climates. For many species,

this is still in its infancy with the majority of focus on organisms with rapid life cycles. In an experimental context this is convenient as numerous generations can be achieved within weeks/months. However, they cannot be a substitute for higher trophic level organisms that have much more complex life cycles, developmental and physiological processes. High trophic levels typically comprise of slower growing organisms with longer life cycles and deferred maturity (i.e. years). With these, rearing multiple generations becomes time-consuming, difficult and almost unfeasible within normal grant time-scales (i.e. 3 years). This presentation will address this knowledge gap by discussing the physiological, energetic, somatic and reproductive responses of a slow growing benthic invertebrate, the European Green Sea Urchin (*Psammechinus miliaris*), bred across several generations under IPCC predicted CO₂ conditions. This sea urchin species currently has a low level of commercial interest within Europe, but our results indicate that these animals will become much more marketable under future CO₂ conditions.

Annotated Bibliography of Key Work

Suckling, C.C., Clark, M.S., Peck, L.S., Cook, E. 2014. Experimental influence of pH on the early life-stages of sea urchins I: different rates of introduction give rise to different responses. *Invertebrate Reproduction and Development*, 58 (2): 148-159.

The authors identified that focusing on only the early life stages within an organism's life-cycle to determine marine invertebrate responses to future climate change is not a suitable approach. When this focus is taken, often the larvae are derived from present-day ambient conditions and then directly transferred to low pH or high CO₂ (~1000 ppm). We showed that this elicits a gross negative response which will consequently skew our predictions. It was identified that this focus instead provides important information on present day phenotypic flexibility which can provide insights into natural selective pathways. The authors also identified that the focus on early life stages is too narrow because contrasting responses were found between planktotrophic larval stages and juveniles.

Suckling, C.C., Clark, M.S., Beveridge, C., Brunner, L., Hughes, A., Cook, E., Davies, A.J., Peck, L.S. 2014. Experimental influence of pH on the early life-stages of sea urchins II: increasing parental exposure times gives rise to different responses. *Invertebrate Reproduction and Development*. 58 (3): 161-175.

Suckling, C.C., Clark, M.S., Richard, J., Morley, S.A., Thorne, M.A.S., Harper, E.M., Peck, L.S. 2015. Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. *Journal of Animal Ecology*, 84 (3): 773-784.

These studies (2 and 3) further challenge the suitability of directly transferring present day ambient sourced early life stages to future conditions (see study 1 above). We widened the focus across the entire life cycle by pre-exposing parents prior to assessing offspring responses to future climate change conditions. The authors for the first time present temporal models to identify when marine invertebrates are able to acclimate to future ocean acidification conditions. It was shown that it takes at least 42 days exposure to for temperate European Green Sea Urchins (*Psammechinus miliaris*) adults and 8 months for the Antarctic Sea urchin (*Sterechinus neumayeri*) to acclimate to IPCC predicted climate change conditions. These studies are important because they highlight the need to utilise longer exposure times in predictive experimental designs – by needing to incorporate at least one full gametogenic cycle within experimental designs for predicting

organismal responses to future changes to determine whether acclimation is possible. This time frame will differ depending on the latitudinal source of your study animals.

Kelly, M.S., Carboni, S., Cook, Hughes, A. 2015. Sea urchin aquaculture in Scotland. In: Echinoderm Aquaculture (Eds. Brow, N. Eddy, S.). Wiley Blackwell, New Jersey. pp. 211-224. This chapter outlines the echinoderm aquaculture effort in Scotland (my previous work place). It highlights that the model species (*Psammechinus miliaris* – the European Green Sea Urchin) I have utilised in my climate change studies (e.g. see studies 1-3 above) has been identified as a good candidate for aquaculture. It provides an overview of examples to support its use in aquaculture – i.e. gonad deposition and colour can be positively influenced via pigmented finishing diets.

14. The Influence of Climate and Environment on the Spawning, Condition, and Larval Set of Naturalized Pacific Oysters *Crassostrea gigas* in a US West Coast Estuary

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Abstract

The Pacific oyster *Crassostrea gigas* was introduced to the US west coast in the early 1900's where it replaced the native oyster *Ostrea lurida* and has become the mainstay for the shellfish aquaculture industry. Pacific oysters only regularly spawned and became “naturalized” in several discrete estuarine locations where conditions allowed for both adult oyster conditioning and spawning and larval survival, retention and settlement. The shellfish industry relied on “natural” set at these locations and/or continued to import seed from Japan until the advent of hatchery technology in the late 1970's. Since that time the industry has gradually shifted to hatchery production of oyster larvae with large operators establishing their own in-house hatcheries and smaller companies incorporating remote setting facilities and purchasing product from hatcheries that specialize in producing late stage eyed larvae. Most operators continued to take advantage of “wild” set until a prolonged set of failures in larval production termed the seed crisis occurred in both hatcheries and in these estuaries like Willapa Bay, Washington where wild set was and continues to be a feature of management on state established oyster reserves. The failures at hatcheries have been clearly linked to water chemistry and ocean acidification where aragonite saturation state is reduced and larval oysters are unable to deposit shell, but evidence for direct effects of carbonate chemistry in estuaries like Willapa Bay is more equivocal due to complex interactions with other factors. Variables like temperature, salinity, and phytoplankton as food as well as other sources of larval mortality are still related to the proximity of the ocean and cold upwelled water, but have been less studied in this estuary and some of these factors also influence gametogenesis and spawning in adult oysters. We

summarize historical records for spawning and setting in this naturalized Pacific oyster population including a long term record of oyster condition. We propose a new effort to evaluate some of these additional factors and compare these records with data from locations in Japan where these oysters were originally sourced and other locations with similar climate records where Pacific oysters have been introduced in order to understand and potentially mitigate for the effects of anthropogenic changes to these systems and an uncertain future climate.

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 the most recent 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.

Dumbauld, B.R., J.L. Ruesink, A.C. Trimble, and B.E. Kauffman. 2011. The Willapa Bay oyster reserves in Washington State: Fishery collapse, creating a sustainable replacement, and the potential for habitat conservation and restoration. *Journal of Shellfish Research* 30: 71-83. The authors review the history of the state oyster reserves in Willapa Bay, Washington where Pacific oysters were introduced in 1928 and one of a few locations on the US West Coast where a naturalized population became established. They review the record of larval set in this estuary and attempt to link this to broad scale oceanographic conditions (but see Hales et al 2017 and Ruesink et al. 2018 below for a more detailed discussion of water chemistry within this estuary).

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 PCO₂ 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 (see Ruesink et al 2018 below) and therefore can't confirm effects, they substantiate the complexity of measuring these effects and attributing them to a single cause in an estuary where conditions are variable.

Ruesink, J.L., A. Sarich, and A.C. Trimble. 2018. Similar oyster reproduction across estuarine regions differing in carbonate chemistry. *Ices Journal of Marine Science* 75: 340-350. These authors measured seawater chemistry in Willapa Bay, Washington, but unlike Hales et al (2017), they also present simultaneously collected data on four cohorts of Pacific oyster larvae that were collected over three summers. The southern end of Willapa Bay has two arms which create distinctly different characteristics because one is much more affected by riverine conditions that cause reduced aragonite saturation relative to the other. Oyster settlement differed greatly

between cohorts, but they did not find differences they could attribute to this different water chemistry and instead found thermal conditions were perhaps more important.

Weisberg, S.B., N. Bednarsek, R.A. Feely, F. Chan, A.B. Boehm, M. Sutula, J.L. Ruesink, B. Hales, J.L. Largier, and J.A. Newton. 2016. Water quality criteria for an acidifying ocean: Challenges and opportunities for improvement. *Ocean & Coastal Management* 126: 31-41. This review represents a call for developing ecologically relevant water quality criteria for acidification and augmenting coastal water quality monitoring efforts to characterize this at appropriate temporal and spatial scales. Historical data for oyster larval recruitment in Willapa Bay is one of two case examples presented that demonstrate the challenges involved in relating biological endpoints to appropriate water chemistry criteria in estuaries where conditions are variable.

15. The influences of environmental changes on Japanese Nori mariculture

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Abstract

Nori mariculture has a long history of over one hundred years and is one of the most important fisheries industries in Japan. Recently, nori production has decreased due to environmental changes, such as seawater temperature rise and low nutrient levels. Seawater temperature rise delays the start of nori mariculture because water temperature stays above 23°C in autumn and spores begin to be released, thereby shortening the season. Low nutrient levels in the ambient waters cause bleaching of the thalli, reducing the quality and price of nori. Now, we are trying to find new culture species and new strains of nori around the coasts of Japan. The thallus has a simple structure, and cells within the thalli sometimes have mutations. Mutant cells may have potential for tolerance of high water temperatures and low nutrient levels, as well as other favorable characteristics. More recently, shortening of the thalli is observed frequently. One of the causes is predation by herbivorous fishes (i.e. *Acanthopagrus schlegelii*, *Mugil cephalus cephalus*) and ducks (i.e. *Anas penelope*, *Anas acuta*). In Japan, it has been reported that herbivorous fishes cause disappearance of marine forests, which is referred to as “isoyake”. The duration of isoyake caused by herbivorous fish has become longer than before because of water temperature rise. The isoyake caused by activities of the fish is reduced below 17°C. On the other hand, the herbivorous fishes eating nori are still active at low water temperature. Even if some measures are taken to control herbivorous fish, birds would alternatively eat nori. It is necessary to develop effective measures to control these herbivores immediately.

Annotated Bibliography of Key Works

Abe, M., Kobayashi, M., Fujiyoshi, E., Tamaki, M., Kikuchi, N., Murase, N. 2013. Use of PCR-RFLP for discrimination of Japanese *Porphyra* and *Pyropia* species (Bangiales, Rhodophyta). *J. Appl. Phycol.* 25, 225-232. DOI. 10.1007/s10811-012-9856-7

In this paper, the authors developed a PCR-RFLP method useful for discrimination of 16 Japanese and two non-Japanese *Porphyra* and *Pyropia* species. These species have simple morphological features, making morphological species identification difficult. The two primer sets used in this study were able to amplify with PCR single fragments on the mitochondrial DNA (partial mitochondrial DNA related to *ATP6* gene and *trnC – rns*). All 18 species were successfully distinguished using a combination of five restriction enzymes (*TaqI*, *SspI*, *AciI*, *Cfr13I* and *AluI*). It was concluded that PCR-RFLP analysis is a useful tool for discrimination of wild strains of *Porphyra* and *Pyropia* species for potential use in mariculture.

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 *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., Fujita, Y., Kobayashi, M., Fujiyoshi, E., Tamaki, M., Fukui, Y., Satomi, M., Murase, N. 2015. Effects of antibiotics on survivals and growth for *Pyropia yezoensis* protoplasts. *Aquacult. Sci.* 63, 1-8.

The nori thallus sometimes has cell mutations. For the breeding of *Pyropia* species, it was necessary to develop an axenic cell culture method. In this paper, we investigated a way of isolating axenic protoplast of *P. yezoensis* and the effects of twelve antibiotics on the survival and growth of the protoplasts. The axenic protoplasts could be isolated by a series of treatments with sodium citrate seawater. The growth rates of the protoplasts treated with FRM and ABPC were 1.1–2.7 times higher than that of the control, while PCG and SM suppressed growth. The authors succeeded in developing an axenic culture method of *Pyropia* species, but the survival, growth and morphogenesis of the cells in the axenic culture varied. Improvement of the methodological stability is necessary.

16. Potential of seaweed aquaculture to reduce methane emissions in Californian livestock

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Abstract

Release of greenhouse gases into the atmosphere is a major environmental, social, and economic threat worldwide. Methane is a particularly potent greenhouse gas that is 25 times more efficient at trapping heat in the atmosphere than carbon dioxide. It is well documented that ruminant livestock contribute significantly to the global release of methane via digestion. The United States is the fourth largest cattle producer in the world and cattle contribute 26% of the total U.S. methane emission. Dairy cattle are the largest methane producers among livestock. California is the dairy industry's most productive state and has 1.4 million dairy cows that account for the majority of the enteric methane emission. Consequently, California recently enacted legislation in 2016 mandating a 40% reduction from present day methane emissions by 2030 (2016). Several methane reductions are expected to come through existing technologies associated with landfills and energy producers. However, the state's largest contributor to methane production is cattle and methods to reduce gases produced from cattle have largely been unexplored in California. Elsewhere, recent research has shown that some species of sub-tropical seaweed can reduce methane production in cattle by up to 99% when used as a feed additive at 2% inclusion rates of organic matter. The mode by which seaweed reduces methane production is still unclear, but most recent research attributes the reductions to the bioaccumulation of halogenated methane analogues produced as algal secondary metabolites. The advantages of seaweed over other dietary manipulations is that seaweed has shown the most significant methane reductions with minimal declines in digestibility, and seaweed production does not compete with terrestrial food production systems. Temperate species have not yet been assessed for methane reducing properties, but many of the species present in the California Current Ecosystem are closely related to these sub-tropical species. The nutrient-rich waters off the Californian coast are ideal for the development of seaweed aquaculture, offering an excellent opportunity to address the underdevelopment of aquaculture in California and the reduction in methane emissions.

Annotated Bibliography of Key Works

Machado, L., Magnusson, M., Paul, N. A., de Nys, R., Tomkins, N., 2014. Effects of Marine and Freshwater Macroalgae on In Vitro Total Gas and Methane Production. PLOS ONE. 9, e85289. The authors for the first time assess an array of different sub-tropical seaweeds for methane reducing properties in cattle using *in vitro* techniques. They find that a red seaweed species called *Asparagopsis taxiformis* is the most effective seaweed tested reducing methane production by up to 99%.

Maia, M. R., Fonseca, A. J., Oliveira, H. M., Mendonca, C., Cabrita, A. R., 2016. The Potential Role of Seaweeds in the Natural Manipulation of Rumen Fermentation and Methane Production. Sci Rep. 6, 32321.

This research is important in that it assesses the effect of the basal feed source (substrate) on methane reduction for a number of different seaweed species. The authors find that there is a significant effect on methane reduction based on whether the basal feed is either grass or corn highlighting the need to further examine the seaweed interaction with different cattle feeds.

Li, X., Norman, H. C., Kinley, R. D., Laurence, M., Wilmot, M., Bender, H., de Nys, R., Tomkins, N., 2018. *Asparagopsis taxiformis* decreases enteric methane production from sheep. *Animal Production Science*. 58, 681-688.

The authors for the first time assess the effect of seaweed on methane reduction using a whole animal experimental design. The article reports that sheep feed a 3% diet of seaweed *Asparagopsis taxiformis* reduce their methane emissions by 81% without any decrease in weight gain compared to the control treatment.

17. Condition index and fecundity of Manila (asari) clam *Ruditapes philippinarum* related to habitat environment

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Abstract

Reproductive traits are important information for maintenance or recovery of Manila (asari) clam *Ruditapes philippinarum* fisheries production, which has dropped considerably in Japan over the past half century. Relationships between condition index and the number of eggs (fecundity) of the asari clam were assessed at several clam habitats in Ise Bay, Japan, during spring and autumn spawning seasons in 2015. Condition index was calculated from the shell size and soft body wet weight. Fecundity was quantified using an indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody specific to an asari clam yolk protein. Land elevation (i.e. subtidal or intertidal), association with river input (river mouth or not), closedness of the shore and chlorophyll *a* concentration in the water column were the habitat parameters analyzed in this study. A correspondence analysis revealed that the condition index in both spawning seasons was positively correlated ($r = +0.48$ to $+0.80$) with the 1st and 2nd correspondence analysis scores on various habitat parameters. On the other hand, the fecundity, which is summed for each spawning season (three sampling days) and normalized to 30 mm shell length, was negatively correlated with the 2nd habitat score (spring: $r = -0.64$, autumn: $r = -0.80$). These results show that the nutritional condition as determined by the condition index is not the sole factor affecting fecundity of the clam. Actually, the fecundity was high in the intertidal zones and enclosed river mouth areas, whereas the condition index was higher in the subtidal zones and open non-river mouth areas. The information obtained about asari clam reproduction would be useful for stock enhancement by improving reproduction in the populations. This study was conducted as part of a research project

for recovery of asari populations by Fisheries Agency of Japan.

Annotated Bibliography of Key Works

Toba, M., and Y. Miyama 1994. Relationship of size to gonadal maturation and spawning in artificially conditioned Manila clams. *Nippon Suisan Gakkaishi*, 60 (2), 173-178.

In this study, the authors observed sexual maturation and measured the number of eggs released per spawned female subjected to thermal spawning stimuli in breeding experiments for evaluating the size dependent batch fecundity of asari clam. Smaller clams at 0 yr old (groups A and B) produced as many eggs per soft tissue weight as larger clams at ≥ 1 yr (groups D and E). No significant differences in egg diameter, percent fertilization, and larval survival from straight-hinge to pediveliger stage were observed among the clam size groups.

Kakino, J. 1996. Growth and survival of Japanese littleneck clam *Ruditapes philippinarum* in cage test related to the sharpness index. *Nippon Suisan Gakkaishi*, 62 (3), 376-383.

The author evaluated the variation in asari clam growth in a tidal flat in Tokyo Bay, Japan, by using the “sharpness index” defined as width / length of shell. The author concluded that juveniles of the clam offshore grew faster than those that were shoreside, but the juvenile survival rate in the intermediate zone of the flat was higher than those in the offshore and shoresides. Juveniles having a high sharpness index tended to show a high survival rate. At the same age, the juveniles with a high sharpness index grew faster than or equal to those with a low sharpness index.

18. Ecological interactions of horseshoe crabs and shellfish aquaculture: a case study from New Jersey

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Abstract

Globally, shellfish aquaculture is growing rapidly. For the first time in history, global seafood supply from aquaculture exceeded that of wild fisheries. Although shellfish culture is viewed as an ecologically sound industry, as farm production expands it faces key challenges assuring ecological and social sustainability. Understanding the ways shellfish farms interact with mobile coastal wildlife, particularly species of special concern, is among those challenges (Barrett et al., 2018; Collier et al., 2018).

Farms for Eastern oyster (*Crassostrea virginica*) are commonly located in shallow coastal areas along the US east coast, use a range of equipment, and involve regular access to care for and harvest livestock. In some cases, these farms are located in areas used by American Horseshoe

Crabs (*Limulus polyphemus*) as they come ashore during spring to spawn along sandy Atlantic Coast beaches. Horseshoe crabs are economically and ecologically important; their blood is highly valuable in the medical industry and migratory shorebirds including the red knot (*Calidris canutus rufa*), a threatened migratory shorebird, feed on crab eggs when stopping in Delaware Bay.

Limited studies have been done to examine the interactions among horseshoe crabs and intertidal oyster farms (Kwan et al., 2018 and Munroe et al., 2017), and recently concern has been raised about horseshoe crab ability to traverse oyster farms to reach spawning habitat. A case study examining potential farm interactions with Horseshoe Crabs was done in Delaware Bay, New Jersey, during the 2018 crab spawning season. Experiments included a range of controlled experiments and surveys during high and low tide using sonar and walking respectively, to observe crab behavior at farm and non-farm sites. In all cases, results indicate that crabs can successfully traverse farms and reach spawning beaches, and crabs do not differentially use farm versus non-farm areas of the Delaware Bay flats. These results provide important context for developing frameworks for managing ecological interactions among farms and species of concern.

Annotated Bibliography of Key Works

Barrett, Luke T., Stephen E. Swearer, and Tim Dempster. 2018. Impacts of marine and freshwater aquaculture on wildlife: a global meta - analysis. *Reviews in Aquaculture*. 1-23. doi: 10.1111/raq.12277

In this review, authors from the University of Melbourne in Australia demonstrate that evidence in support of higher wildlife biomass and diversity is found around aquaculture farms. They argue that to properly understand the possible impacts of farms on wildlife, studies have thus far failed to address issues of impacts on behavior, reproduction or fitness. If animals that tend to aggregate around farms suffer poor fitness relative to those occupying non-farm habitats, the farm becomes an 'ecological trap'.

Callier, M.D., Byron, C.J., Bengtson, D.A., Cranford, P.J., Cross, S.F., Focken, U., Jansen, H.M., Kamermans, P., Kiessling, A., Landry, T. and O'beirn, F., 2017. Attraction and repulsion of mobile wild organisms to finfish and shellfish aquaculture: a review. *Reviews in Aquaculture*. The ways in which finfish and shellfish farms act as attractant or repulsive areas for mobile marine species is reviewed. At finfish farms, food inputs and physical structure of the farm tend to drive aggregating effects. Likewise, at shellfish farms, the farm structure (both farm gear and shellfish themselves) and increased feeding opportunities via biodeposition, the shellfish crop or fouling organisms tend to attract wildlife to farms. In general, the authors note that a great deal of variability in attractiveness or repulsiveness exists when considering the suites of habitats, farms, and mobile fauna that have been studied thus far.

Kwan, Billy KY, Hoi Kin Chan, and Siu Gin Cheung. 2018. Habitat use of globally threatened juvenile Chinese horseshoe crab, *Tachypleus tridentatus* under the influence of simulated intertidal oyster culture structures in Hong Kong. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28: 124-132.

The authors conducted this study to test if juvenile Chinese horseshoe crabs differ in abundance and if their feeding trails differ at oyster farm spat collection locations. They performed their test at a shallow mudflat in Hong Kong which is home to a large population of juvenile horseshoe

crabs. Addition of oyster spat collectors (bricks on the mudflat) lead to a significant reduction in the number of juvenile horseshoe crabs, and a reduction in the length of feeding trails.

Munroe, D., Bushek, D., Woodruff, P., Calvo, L. 2017. Intertidal rack-and-bag oyster farms have limited interaction with Horseshoe Crab activity in New Jersey, USA. *Aquaculture Environment Interactions*, 9: 205-211. <https://doi.org/10.3354/aei00227>

In this study, the authors sought to assess whether intertidal oyster farms altered the distribution of mature horseshoe crabs as they came ashore to spawn in the lower Delaware Bay. In total, they used a combination of field surveys of crabs on mudflats and stranded along the upper shore, and controlled tests to determine if crabs can move past farm gear. Results show no evidence that horseshoe crab distribution within this area is altered by the presence of oyster farms.

19. Use of GoPro Cameras to Document Fish on Aquaculture Gear

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Abstract

Oyster cages are an increasingly common style of aquaculture gear in Long Island Sound. Cages are used to contain and protect shellfish during grow-out but also provide three-dimensional structure for the local wild fish community. Understanding the ecosystem services provided by aquaculture gear may inform regulatory decisions and increase public understanding of shellfish farming. Point-of-view (GoPro) cameras attached to oyster cages were used to visually assess fish activity near a high-density cage farm as well as adjacent single cages. To compare fish interactions with cages to natural structured rock-reef habitat, t-platform stands were fabricated to mount cameras adjacent to boulders while minimizing additional structure. Seawater samples were collected for environmental DNA (eDNA) analysis to detect fish species that may not be observed in video. Video collected by cameras attached to oyster cages and near boulders facilitated visualization and quantification of the wild fish communities along with behavioral interactions between animals and these physical elements of habitat. Preliminary results suggest that aquaculture cages support a higher abundance and greater diversity of fish species and life stages than did the natural rock-reef habitat. Analysis of eDNA detected differences in fish community composition between sites.

*The Federal Government does not endorse the use of GoPro™ cameras

Annotated Bibliography of Key Works

Watson D.L., Harvey E.S., Anderson M.J., and Kendrick G.A. 2005. A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. *Marine Biology*. Vol 144. Pp 415-425.

The authors deployed underwater stereo-video cameras to sample the relative density and species richness of temperate reef fishes in Southwest Australia. This study compared diver deploy, unbaited remote and baited remote cameras. Their definition of MaxN “Species presence and the maximum number of individuals belonging to each species in the field of view at one time (MaxN).” This metric avoids repeated counting of fish within a given time frame and gives a conservative estimate of relative density relating to area of survey.

Dealteris J.T., Kilpatrick B.D., and Rheault R.B. 2004. A comparative evaluation of the habitat value of shellfish aquaculture gear, submerged aquatic vegetation and a non-vegetated seabed. *Journal of Shellfish Research*. Vol 23:3. Pp 867-874.

The authors set out to evaluate habitat value of shellfish aquaculture gear (SAG) submerged aquatic vegetation (SAV) and shallow non-vegetated seabed (NVS) over one year in Port Judith, RI, USA. To identify the community around oyster cages lift were diver deployed under oyster cages and left to soak for two weeks. Submerged aquatic vegetation and non-vegetated seabed sites were sampled with quadrats, drop nets, and venture-driven suction dredge. Sessile invertebrate growth was documented on eelgrasses and on oysters and oyster cages. All fish >5mm were collected. The authors documented that the physical habitat of shellfish aquaculture gear has greater surface area compared with the other two habitat types as well as had a significantly higher abundance and species richness of organisms per meter squared throughout the year. The authors concluded that shellfish aquaculture gear has greater habitat value compared to their control sites.

Tallman J.C. & Forrester G.E. 2007. Oyster grow-out cages function as artificial reefs for temperate fishes. *Transactions of the American Fisheries Society*. Vol 136:3. Pp 790-799.

The authors compared fish habitats within Narragansett Bay, RI, USA using trap surveys. They compared three oyster grow-out sites, six natural rock reefs and one artificial reef built for fish habitat looking to identify patterns in fish density, growth and disappearance rates. Their traps were encompassing juvenile and adult fish in the summer and fall. The study showed that oyster cages provide habitat for fish associated with hard bottom habitats including scup, tautog and black sea bass.

Dumbauld, B.R., J.L. Ruesink, and S.S. Rumill. 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. *Aquaculture* Vol 290. Pp 196-223.

This review article focuses on bivalve shellfish aquaculture on the US West Coast. Much like natural rock reefs, cages become colonized by epibenthic, emergent, and encrusting organisms and may provide sheltering fish with camouflage, refuge from predation, respite from high current flow, and a source of food. Multi-tiered off-bottom cages, an increasingly popular method for growing oysters on a smaller footprint, offer vertical three-dimensional structure and surface area similar to that afforded by natural reefs. In traditional on-bottom shellfish culture, where live oysters and shell cultch are distributed on sediments for grow-out and spat collection, oysters act as ecosystem engineers creating hard bottom irregular substrate on otherwise

featureless seafloor. Evaluation of habitat services provided by oyster aquaculture can be challenging. Shellfish farms vary in spatial size and bottom area covered, variety and quantity of aquaculture gear, and have a seasonally variable footprint as gear is relocated or harvested. Aquaculture of oysters, whether in cages or grown on the seafloor, may represent valuable estuarine habitat.

Closing remark of 46th scientific symposium of UJNR

Dr. Fuminari Ito, Japan chair

Good afternoon, everyone.

Now, we come to close the 46th Scientific Symposium of the UJNR Aquaculture Panel, “Marine Aquaculture in a Changing Environment, impacts to aquaculture production due to environmental change and science to mitigate these impacts”.

First of all, I would like to congratulate all of you for the successful conduct of this Scientific Symposium. I am quite sure that we would be able to have another good result of cooperative activities among the Aquaculture Panel, one of the most active UJNR panels.

The 10th Three-Year Plan is “Marine Aquaculture in a Changing Environment”. The Special Report on Global Warming of 1.5°C (SR15) was accepted during the 48th session of the Intergovernmental Panel on Climate Change (IPCC 48) held in Korea this October. There has been an increasing interest in risks, adoptability and sustainability of varied systems which could possibly be influenced by global climate change. In the field of aquaculture, it is also important to further conduct research and to develop technologies to tackle issues associated with environmental change.

Last year, the first scientific symposium was held under the theme “potential of aquaculture to mitigate impacts of environmental change” in Hiroshima, Japan. We exchanged information and discussion on potentials for sustainable seafood production through aquaculture of a variety of species in different environments. Today we discussed in detail the effects of ocean acidification on aquaculture organisms, and so on. (当日の感想を挿入)

Next year will be the final year of the three-year plan, and the theme for the symposium, which is planned to be held in Japan, will be “Application of Aquaculture Technology to Provide Sustainable Seafood and Reduce Impacts of Environmental Change.”

In the next year's symposium, we will be able to share scientific knowledge about the latest research results for the future of aquaculture.

Through these three-year symposium presentations and discussion, I believe that we will successfully deepen the issue of counter measures for global environmental change, which are expected to contribute in the development of the sustainable aquaculture industry in not only the US and Japan, but also the rest of the world.

Finally, I would like to extend my deep appreciation to Dr. Rust, Dr. Olin, Mr. Otoshi, who could not unfortunately join the symposium, and all the staff concerned for their great contributions, which led to the success of this symposium.

Again, I would like to express my heartfelt congratulations and declare this symposium closed. Thank you very much for your cooperation, and good afternoon.