Preface

The 41st Joint Meeting of the UJNR Aquaculture Panel was conducted from October 2013 (October 9th – 13th at Sapporo and Hakodate, Hokkaido) through December 2013 (December 10th – 13th at Yokohama, Kanagawa and Miyako, Iwate). Through the long history of UJNR, Aquaculture Panel has contributed to development of aquaculture researches of both countries by means of various cooperative activities, i.e. the exchange of scientists, the exchange of literatures and promoting joint research projects. It is understood that the Aquaculture Panel is one of the most active UJNR Panels by both countries. Further positive action is expected to the UJNR Aquaculture Panel for a solution of the varied problems presenting in aquaculture of both countries.

The present special issue of Bulletin of Fisheries Research Agency is the proceedings of the 41st UJNR Aquaculture Panel Symposium “Advanced Aquaculture Technologies”. This Symposium was a venue to exchange original, high-quality research on new and developing technologies that give promise to improve or revolutionize aquaculture, based on the key bottleneck issues identified at the 39th Scientific Symposium “The Present and Future of the Aquaculture Industry”.

Further development of aquaculture is highly desired as a production source of important foods for human being all over the world. Stable supplies of safe and economical feed ingredients are requisite for the sustainable development of aquaculture. Nevertheless, in recent years we recognized some serious problems, which threatened the use of important feed ingredients like fish meal and oil. It is my great pleasure that the present UJNR proceedings containing high quality papers of the selected American and Japanese aquaculture scientists will help integrating aquaculture and fisheries technologies to optimize value from coastal resources, zoning for aquaculture, use of biotechnology in aquaculture and effects on natural population, and improvement of public perception.

Finally I would like to express my sincere gratitude to the colleagues involved in the UJNR Aquaculture Panel for their efforts to prepare and organize the symposium. I also would like to deeply thank the editorial board members for publishing the proceedings.

Fuminari Ito  
Chair of UJNR Aquaculture Panel  
Executive Director  
Incorporated Administrative Agency,  
Fisheries Research Agency
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The 41st Scientific Symposium of the UJNR Aquaculture Panel

October 9th - 10th, 2013

Advanced Aquaculture Technologies

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The Fisheries Agency’s Policy Directions for Aquaculture

Kazumasa IKUTA*1

Abstract: Aquaculture is an important industry in Japan, and production levels occupy approximately 20% in terms of yield, and 30% in terms of market value, of the country’s total fisheries production. However, the economic aspects of aquaculture have been precarious due to the low price of certain products and increasing costs of production. In addition, the guarantee of food safety, minimization of environmental impact, and management of natural stock populations are highly necessitated in order to achieve the sustainability of the industry.

In order to address these problems, the Fisheries Agency held a series of committee meetings during 2013 in order to develop ideal projections for the aquaculture industry. Thereafter, policy directions for aquaculture were put forth as follows:

1) Measures to improve unstable business practices of aquaculture should be implemented; this includes the expansion of mutual-aid systems, conversion of feed resources to alternative usages of protein, maintenance of carrying capacity, improvement of systems to compensate for increases in feed costs, and the promotion of planned production including the enhanced exportation of products.

2) Measures to improve production techniques should be implemented; this includes achieving improved food safety based on production systems that have high traceability, risk management and the usage of vaccines, conservation of the environment in and around aquaculture grounds, a shift from the use of wild fish seedlings and natural feed resources to the use of artificially-produced seedlings and feeds, the development of enclosed recirculating aquaculture systems, and R&D for advanced aquaculture technologies that will reduce costs and enhance productivity such as offshore aquaculture systems, enhanced breeding methodologies, and raft aquaculture systems for new bivalve species.

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Policy- April 2013 saw the release of the National Ocean Policy Implementation Plan (NOP-IP), a document to translate President Obama’s 2010 National Ocean Policy (Executive Order 13547 –Stewardship of the Ocean, Our Coasts, and the Great Lakes) to specific federal actions. Aquaculture featured prominently in the Implementation Plan. The Joint Sub-committee on Aquaculture (JSA) was re-named the Interagency Working Group on Aquaculture (IWG-A), and was tasked with identifying and supporting milestones in the NOP-IP. The NOP-IP also supported the National Shellfish Initiative to increase shellfish production and restoration in U.S. waters. NOAA and the Gulf of Mexico Fishery Management Council are working on a Fishery Management Plan to permit aquaculture in federal waters in the Gulf of Mexico. The U.S. currently does not have a framework to permit aquaculture in the EEZ, and this would be a first for the U.S. if it goes into effect.

Developments- For the first time in some years, in 2013 many new sites for shellfish aquaculture have been permitted. This is attributed to increased interest in shellfish aquaculture, particularly in the northwest and northeast, federal interagency and state efforts on behalf of the Washington Shellfish Initiative, and state-level successes at streamlining permitting. In September 2013, the California Shellfish Initiative was launched. A program is underway to permit and restore Hawaiian fishponds, a form of traditional Hawaiian aquaculture, for cultural heritage, subsistence, and possibly commercial production.

Production Trends- Aquaculture production in the U.S. is largely composed of catfish, crawfish, trout, salmon, oysters, mussels, clams, tilapia, and shrimp. For the 15-year period from 1996–2011, the value of U.S. aquaculture showed 3% average year-over-year growth, though total volume fell by an average of 0.5%. Mariculture (salmon and shellfish), on the other hand, showed robust 4% by volume and 5% by value year-over-year average growth in the same period. 2011 is the most current year published aquaculture statistics are available. From 2010 to 2011, total U.S. aquaculture went down ~18.8% by volume (to 277,335 metric tons) but grew 4.2% by value (to 1.34 billion dollars). From 2010 to 2011 mariculture decreased by ~12.4% by volume (to 35,739 metric tons) while growing 0.3% by value (to 314 million dollars). However, in the previous year from 2009–2010, mariculture grew 21.6% by volume (to 40,823 metric tons) and 28.5% by value (to 312 million dollars). Most of the growth in value for marine aquaculture was from salmon (*Salmo salar*) and oyster (mainly *Crassostrea gigas* and *Crassotrea virginica*) production.

Keywords: aquaculture policy, aquaculture production, annual report
Background

The U.S. imports over 90 percent of its seafood, half of which is a product of foreign aquaculture. While aquaculture produces 50 percent of all seafood eaten worldwide, in 2011 U.S. domestic aquaculture production was only 9% of total domestic landings. Total domestic aquaculture in the U.S. provided around 5% of the seafood supply; mariculture was the source of less than 1% of U.S. seafood.

Policy

The Department of Commerce, NOAA Fisheries, and Aquaculture: Marine aquaculture is an important part of the missions of the Department of Commerce (DOC) and the National Oceanic and Atmospheric Administration (NOAA). The DOC sees aquaculture as a way to create jobs and economic activity. A federal agency under the DOC, NOAA sees aquaculture as a critical component to meeting increasing global demand for seafood and maintaining healthy ecosystems.

The NOAA Office of Aquaculture is within the National Marine Fisheries Service (NMFS), a branch of NOAA. NMFS is involved in aquaculture from both science and policy perspectives: science to foster efficiency and sustainability and policy to enable marine aquaculture while ensuring environmental responsibility. Marine aquaculture is an increasingly important part of the NOAA NMFS mission. Marine aquaculture is providing a growing amount of seafood to U.S. consumers, and supports commercial and recreational fisheries and helps restore species and habitat.

Aquaculture is garnering increasing attention from Commerce, members of Congress, and the private business sector for its ability to provide economic opportunities (especially in fishing communities) and potential to grow more seafood domestically in a safe, sustainable way. In 2011, NOAA and the Department of Commerce released complimentary aquaculture policies (http://www.nmfs.noaa.gov/aquaculture/policy/2011_policies_homepage.html) supporting aquaculture to enable domestic production of seafood, maintain and restore healthy marine ecosystems, and create employment and business opportunities.


The aquaculture-specific actions in the NOP-IP relate to increasing efficiencies in the aquaculture permitting processes, coordinating agency participation, and providing jobs and economic value by protecting and restoring coastal wetlands, coral reefs, and other natural systems. Along with goals for commercial aquaculture, restoration is also specifically mentioned in the NOP-IP, to improve coastal and estuarine restoration efforts through better monitoring, coordination, and planning.

Agency Abbreviations:
- NOAA: National Oceanic and Atmospheric Administration
- USDA: U.S. Department of Agriculture
- ARS: Agricultural Research Service
- NIFA: National Institute of Food and Agriculture
- APHIS: Animal and Plant Health Inspection Service
- EPA: Environmental Protection Agency
- USCG: U.S. Coast Guard
- DOC: Department of Commerce
- DOE: Department of the Interior
- DOL: Department of Labor
- USFWS: U.S. Fish and Wildlife Service
- FERC: Federal Energy Regulatory Commission
- DOE: Department of Energy
- HHS: Department of Health and Human Services
- FDA: Food and Drug Administration
- ACOE: The U.S. Army Corps of Engineers

NOP-IP Actions Relating to Aquaculture, and Responsible Agencies:
By the end of 2013
- Develop and implement permitting regulatory efficiencies for aquaculture. [NOAA, USDA, EPA, USACE, USCG, DOI (USFWS)]
• Establish an interagency aquaculture initiative that supports jobs and innovation through the National Science and Technology Council’s Interagency Working Group on Aquaculture and other partnerships. [DOC, NOAA, USDA (ARS, NIFA)]

• Through leveraging existing research priorities, provide scientific information on the environmental health effects of finfish aquaculture to streamline permitting and improve water quality monitoring. [Aquaculture Regulatory Task Force]

• Through the National Shellfish Initiative develop pilot projects to identify ways to both maximize the environmental sustainability and ecosystem benefits (e.g., nutrient filtration, carbon sequestration, fish habitat) and the commercial value of shellfish aquaculture. This would help develop a comprehensive plan to sustainably increase shellfish production and restore populations in U.S. waters. [NOAA, USDA (ARS, NIFA)]

By the end of 2015
• Identify and make available best management practices to inform and improve Federal permitting processes for aquaculture. [NOAA, USDA, EPA, USACE, USCG, DOI (USFWS)]

• Develop an analysis of the contribution and impacts (including job creation) of emerging uses—including renewable energy, aquaculture, and biotechnology—on the economies of the communities and regions dependent on marine and coastal resources. [NOAA, DOE, DOI, FERC, DOL, DOC]

By the end of 2016
• Develop and incorporate adaptation strategies for coastal and ocean species and habitats into future planning and management processes, such as fisheries, protected species, coral reefs, or shellfish aquaculture. [NOAA, DOI, EPA]

The Inter-Agency Working Group on Aquaculture (IWG-A): The Inter-agency Working Group on Aquaculture (IWG-A; formerly called the Joint Sub-committee on Aquaculture) is an inter-agency group under the auspices of the Life Sciences Subcommittee of the National Science and Technology council. The purpose of the working group is to increase the overall effectiveness and productivity of Federal aquaculture research, technology transfer, and technology assistance programs. Efficient, coordinated permitting processes will allow ocean industries, including commercial shellfish and finfish aquaculture, to save time and money and encourage economic growth without compromising federal agency responsibilities to protect health, safety, and the environment. Improved interagency coordination and reduced redundancy will decrease administrative waste, the burden on federal agencies, and the regulatory burden for industry.

Under the IWG-A, an Aquaculture Regulatory Task Force (hereafter ‘task force’) was created in 2013 under the auspices of the National Aquaculture act of 1980, and will address specific milestones and activities in support of the Implementation Plan of the National Ocean Policy in consultation and partnership with the National Ocean Council and the IWG-A. The task force consists of the following federal agencies: The USDA: ARS, NIFA, and APHIS; DOC: NOAA; HHS: FDA; DOI: USFWS; EPA; and the ACOE. The task force also includes the following organizations in the Executive Office of the President: National Ocean Council;Office of Management and Budget; and Office of Science and Technology Policy. The task force will operate from 2013–2015 unless renewed.

In 2013 the task force assembled a draft charter to support aquaculture milestones in the NOP-IP, as well as a draft work plan identifying specific actions towards task force goals. Progress has been made on facilitating inter-agency communication, coordination to streamline multi-agency permitting processes, and increasing the ease of access to information about the permitting process.

The Gulf of Mexico Fishery Management Plan: The U.S. currently does not have a framework to permit long-term aquaculture projects in the Exclusive Economic Zone (federal waters), 3 to 200 miles (5 to 322 kilometers) offshore. This is despite the huge potential for offshore aquaculture in the U.S. A recent publication from the Food and Agricultural Organization (FAO) of the United Nations (Kapetsky et al., 2013) listed the US as number one in the world for offshore marine production potential. Japan also
ranked highly (fifth) in this category. The rankings were obtained by accounting for the area in each country’s Exclusive Economic Zone with appropriate depth, currents, temperature, and distance to ports for finfish (e.g. salmon and cobia) and shellfish (e.g. mussels) production.

An ongoing development for U.S. offshore aquaculture is the Fishery Management Plan for Regulating Offshore Marine Aquaculture in the Gulf of Mexico (Aquaculture FMP). The Gulf of Mexico Fishery Management Council proposed regulations for an Aquaculture FMP, the first time that a regional fishery management council has approved a comprehensive regulatory program for offshore aquaculture in U.S. federal waters. NOAA is currently reviewing the proposed regulations that would implement the Aquaculture FMP. The Fishery Management Plan authorizes NOAA Fisheries to issue permits to culture species managed by the Gulf of Mexico Fishery Management Council, with the exception of shrimp and corals. We expect final regulations to be effective sometime in 2014.

Recent Key Developments in U.S. Aquaculture

**National and Regional Shellfish Initiatives:** The National Shellfish Initiative is a policy created in conjunction with the 2011 DOC and NOAA Aquaculture Policies. The goal of the initiative is to support increases in shellfish production and restoration in U.S. waters. In 2013 the Office of Aquaculture and aquaculture regional coordinators supported a number of actions for the National Shellfish Initiative in partnership with other NOAA offices, industry, the restoration community, and state and tribal partners in California, Washington State, the Northeast, and Hawaii.

The Washington State Shellfish Initiative, through the actions of the Washington State Shellfish Initiative Inter-agency Working Group, completed several products to streamline the site permitting process. Due to many factors (for example regulatory complexities and social license), for a number of years there have been difficulties with permitting new sites for shellfish aquaculture in some regions of the United States, particularly in the Pacific Northwest. Actions by the Washington State Shellfish Initiative Inter-agency Working Group to coordinate and streamline the permitting process in that state led to 7 new shellfish site permits, the first issued by the ACOE in six years.

The Washington State Shellfish Initiative also led to the Ocean Acidification Blue Ribbon panel report and new funding from the State of Washington for shellfish ocean acidification research. The Blue Ribbon Panel gave a summary of ocean acidification research, and a summary of recommendations for shellfish hatcheries and growers. Ocean acidification is of major concern on the west coast, particularly in the Pacific northwest. In previous years before ocean acidification was identified as the culprit and mitigation measures were employed, some hatcheries experienced up to 80% larval mortality. Research led to the finding that acidic water from upwelling put stress on larval shellfish, rendering them unable to build shells in a critical point in their development. Hatcheries now have sensors and only draw seawater when the pH level is ‘safe’ for larvae. However, this does not help with shellfish in the wild, and some bays have not seen a natural set in almost ten years. The Washington Shellfish initiative also led to funding construction of a hatchery in Manchester, WA, for native Olympia oyster seed.

In September 2013, the California Shellfish Initiative was launched with a kickoff meeting involving agencies and stakeholders to identify key actions for expanded shellfish farming and restoration to support and conserve California’s unique ecosystem. Over 70 people attended. One outcome of this meeting was coordination between NMFS staff and the Humboldt Harbor District on shellfish aquaculture in Humboldt Bay, California. The Harbor District is pursuing an innovative approach for permitting, where the Harbor District owns a regional permit for the entire bay, bears the cost of the permitting process, and then issues leases to individual growers. This will allow small businesses to participate in shellfish aquaculture, because the permitting process can be too expensive and uncertain for a small business to navigate alone.

The Northeast U.S. has been very successful in streamlining the permitting process, and is seeing impressive growth in the shellfish sector. For example, over the last two years in Maryland, 1,100
acres have been leased for shellfish aquaculture, and 1,500 more acres are in the process. Over the same period in Virginia, over 5,000 oyster acres were permitted. The New England region (Maine, Vermont, New Hampshire, Massachusetts, Connecticut, and Rhode Island) is working on a general regional permit to streamline the permitting process for new shellfish aquaculture operations. In the state of Maine, state agencies and the ACOE are also pursuing a streamlined permitting system. Further, due to economic and fishery conditions, there are a growing number of fishermen and fishing families in the Northeast who are starting aquaculture in addition to or instead of fishing.

In Hawaii there is growing interest in raising oysters. The Hawaii State Department of Health just developed a process to certify waters to raise shellfish. The growth rate for oysters in Hawaii is high, taking only 6–8 months to reach market size. In fact, there is at least one major land-based oyster hatchery operating in Hawaii, which benefits from these high growth rates.

**Native Hawaiian Aquaculture Site Permitting:** Fishponds built by the original inhabitants of Hawaii were among the most sophisticated examples of aquaculture used by pre-historic native Pacific peoples. Rock walls were built along the shoreline to form ponds. The walls were made of porous lava rock, and allowed for saltwater from the ocean to enter and exit the ponds, cleaning them through tidal flushing while preventing fish from escaping. The most widely cultivated species were flathead mullet (*Mugil cephalus*), milkfish (*Chanos chanos*) and moi (*Polydactylus sexfils*). There were once hundreds of fishponds in use across the Hawaiian Islands, but by the turn of the 20th century only about a hundred remained. Today, most fishponds are in disrepair.

The State of Hawaii, the NOAA Pacific Regional aquaculture coordinator, and various federal agencies and the environmental non-governmental organization Conservation International are collaborating on a general permit to make it possible to restore Hawaiian fishponds for cultural heritage, subsistence, and commercial production. The next step is to develop a general permit from the ACOE to allow pond restoration.

Part of the goal of this project is to introduce a broad segment of the population to aquaculture, through cultural and outreach activities, and foster grass-roots familiarity and support for aquaculture in Hawaii, a state with great marine aquaculture potential.

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**Fig. 1.**
Production Trends

The main species for aquaculture production in the U.S. are trout, tilapia, catfish, crawfish, shrimp, salmon, oysters, mussels, and clams. The value of U.S. aquaculture showed 3% average year-over-year growth over the last 15 years, though total volume fell by an average of 0.5%. The last year data are available is for 2011. From 2010 to 2011, total U.S. aquaculture went down ~18.8% by volume (to 277,335 metric tons) but grew 4.2% by value (to 1.34 billion dollars).

By volume, most of U.S. aquaculture is from land-based ponds growing catfish, crawfish, and trout. Marine species make up a very small proportion of aquaculture production by volume. However, marine species make up a disproportionately large part of the value of aquaculture in the U.S. Shellfish in particular are produced in relatively smaller quantities but are high-value products.

Mariculture*: Major U.S. marine species are Atlantic salmon (Salmo salar), mussels (mainly blue mussels, Mytilus edulis), clams (largely hard clams Mercinaria mercinaria and Manila clam Venerupis philippinarum, and geoduck Panopea generosa), and oysters (mainly Crassostrea gigas and Crassotrea virginica). Saltwater shrimp are excluded from mariculture because they are raised in inshore ponds.

From 2010 to 2011 mariculture went down ~12.4% by volume (to 35,739 metric tons) while growing 0.3% by value (to 314 million dollars). However, from 2009–2010, mariculture grew 21.6% by volume (to 40,823 metric tons) and 28.5% by value (to 312 million dollars). The 15-year average growth for mariculture is 4% by volume and 5% by value.

1. Finfish

Atlantic salmon mariculture in the U.S. takes place in Maine and Washington State. Salmon from hatcheries are raised in tanks until they reach 40-120 grams, and then are transferred to marine net-pens for grow-out. From 2010–2011 salmon production decreased ~4.8% to 18,595 metric tons, and grew 5.1% by value to 104 million dollars. The average year-over-year growth over the last 15 years is 6% by volume and 11% by value.

* We define Mariculture in this section as species cultured in the marine environment (e.g. oceans, bays, salt-water estuaries).
Other marine finfish species are cultured, but due to issues such as the small number of farms, consistent data are not available. To protect the privacy of businesses, the USDA ARS cannot report detailed statistics on farms where there are so few of them that it would be possible to identify individual farms from the data. White sturgeon are cultured (Acipenser transmontanus). Species in the early stages of commercialization in research and development include sixfinger threadfin (Polydactylus sexfiliis), commonly known in Hawaii as mo, cobia (Rachycentron canadum), yellowtail amberjack (Seriola rivoliana), Atlantic cod (Gadus morhua), yellowtail amberjack (Seriola lalandi), red drum (Sciaenops ocellatus), sablefish (Anoplopoma fimbria), California flounder (Paralichthys californicus), summer flounder (Paralichthys dentatus), yellowfin tuna (Thunnus albacares) and Florida pompano (Trachinotus carolinus).

2. Shellfish
Clam culture in the U.S. is largely hard clam (Mercinaria mercinaria) and Manila clam (Venerupis philippinarum), with some native goodek clams (Panopea generosa) grown in the Pacific Northwest. Hard clam culture in the U.S. involves seed production, nursery stage, and final grow-out. Producers rely on seed from hatcheries. At the nursery stage, juvenile clams have a protected environment where they can grow to a size that provides optimum success for survival once placed into a natural setting for grow-out. Once juvenile clams are 7–15 mm, they are placed into the final grow-out system until they reach a harvest size of 50 mm. For grow-out, clams are placed into different structures (e.g. trays, pens, bags) and secured to the substrate of intertidal or subtidal areas. Harvesting occurs by either collecting trays/bags or by raking the substrate.

Geoduck seed are produced in hatcheries, and juveniles are raised in the hatchery until they reach about 5–12 mm in size. Geoduck are next raised in a nursery where they grow large enough for survival outdoors. Geoduck seed are planted on intertidal beach in PVC pipes. The pipe is placed in the sediment, about 30 cm deep, with 10 cm left at the surface. Juvenile geoducks are very susceptible to predators and the pipe protects them until they are large enough to evade predators on their own. On many farms, a net is placed over all of the tubes to provide further protection from predators. Two to three juveniles are placed into each pipe. While protected, the geoduck will grow and burrow itself into the sediment. After 1–2 years, the netting is removed since the geoducks are large and deep enough to be safe from predators. The grow-out stage can take 4 to 7 years for a geoduck to reach harvest size of 2 pounds.

From 2010–2011 clam production increased 12% to 4,683 metric tons, and grew 9.3% by value to 104 million dollars. The average year-over-year growth over the last 15 years is 18% by volume and 13% by value.

Oyster culture in the U.S. mainly consists of two species. The Pacific oyster (Crassostrea gigas) is the predominant oyster grown on the west coast, and the Virginia oyster (Crassostrea virginica) is the most commonly raised oyster on the east coast. However, on both coasts other species are raised in smaller numbers, such as the native Olympia oyster (Ostrea conchaphila) on the west coast. The majority of oyster farms rely on hatchery production of seed, though wild seed collection also occurs. Oysters are grown by on-bottom, off-bottom, or suspended-culture techniques. On-bottom culture is used when a site has a suitably firm substrate in an intertidal or sub-tidal area. Off-bottom culture requires seed to be put into mesh bags or trays and attached to rope and wood frames in the intertidal zone to be
grown to market size. Suspended culture is where oyster seed is hung from longlines and attached to horizontal lines or rafts in deeper waters. Harvest typically occurs at a size of around 75mm.

From 2010–2011 oyster harvest dropped ~27.8% to 12,062 metric tons, and ~11.9% by value to 98.4 million dollars. However, in the previous year, compared to 2009, 2010 saw 15.0% growth by volume to 16,721 metric tons, and 26.4% growth by value to 111.7 million dollars. The average year-over-year growth over the last 15 years is 6% by volume and 6% by value.

Mussels are farmed either on- or off-bottom. On-bottom mussel farming involves seeding sea bottom areas, and dredging mussels for harvest. There are several methods of off-bottom farming, including longline culture and raft culture. From 2010–2011 mussel production decreased ~0.7% to 402 metric tons, and grew 9.3% by value to 7.2 million dollars. The average year-over-year growth over the last 15 years is 8% by volume and 20% by value.

Inland Aquaculture*: Major U.S. inland finfish aquaculture species are channel catfish (*Ictalurus punctatus*), Rainbow trout (*Oncorhynchus mykiss*), hybrid striped bass (*Morone saxatilis × M. chrysops*), and tilapia (*Tilapia spp.*). Farmed shellfish include the red swamp crawfish (*Procambarus clarkii*) and whiteleg shrimp (*Litopenaeus vannamei*).

From 2010 to 2011 inland aquaculture went down 19.7% by volume (to 241,596 metric tons) while growing 7.2% by value (to 737 million dollars). The average year-over-year growth over the last 15 years is ~0.3% by volume and 3% by value.

1. Finfish

Channel catfish (*Ictalurus punctatus*) are the predominant aquacultured species in the U.S., and only Channel catfish can be sold as ‘catfish’ in U.S. markets. Catfish are grown primarily in embankment or levee ponds, where the earth that is removed to dig the ponds is used to build levees or embankments to encircle the ponds. Ponds range in size from 0.04 km² and 2m deep to 0.1 km² and 1m deep.

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* In this section, inland aquaculture refers to the culture of freshwater species as well as marine species grown in land-based systems.
From 2010 to 2011 catfish production went down 27.3% by volume (to 157,942 metric tons) while growing 42% by value (to 391 million dollars). The average year-over-year growth over the last 15 years is -1% by volume and 1% by value.

Rainbow trout (*Oncorhynchus mykiss*) are predominantly raised in raceways, though some farms use ponds or recirculating systems. Raceways, also known as flow-through systems, are essentially artificial streams. Gravity flows water from natural streams through the concrete raceways, and the water is often filtered to remove waste products before being released back into the natural stream. Steelhead trout (*Oncorhynchus mykiss*) are sea-run rainbow trout, and can be raised in net-pens in rivers and the marine environment. From 2010 to 2011 trout production went down -1.8% by volume (to 15,112 metric tons) while increasing 7.9% by value (to 51 million dollars). The average year-over-year growth over the last 15 years is -3% by volume and 0% by value.

Hybrid striped bass are produced in the hatchery by fertilizing female white bass (*Morone chrysops*) eggs with sperm from male striped bass (*Morone saxatilis*). Hybrid striped bass have greater tolerance to extremes in temperature and dissolved oxygen than either of its parents and is thus better suited for pond culture. The majority of hybrid striped bass producers in the southern region raise their fish in freshwater ponds, but cages and tanks are also used for hybrid striped bass production.

From 2010 to 2011 hybrid striped bass production decreased 9.1% by volume (to 3,516 metric tons) while growing 1.4% by value (to 28 million dollars). The average year-over-year growth over the last 15 years is 1% by volume and 3% by value.
Tilapia are grown in ponds or tanks in the U.S. and production did not change between 2010 and 2011, though there was an increase in value of 1.7%. In 2011, 9,979 metric tons of tilapia worth 54 million dollars were produced. The average year-over-year growth over the last 15 years is 2% by volume and 7% by value.

2. Shellfish

Crawfish are the predominant cultured crustacean in the U.S. and are cultivated and consumed for food in several southern states. Louisiana dominates the aquaculture and wild harvest crawfish industry. Crawfish are grown in shallow ponds 20 to 60 cm deep, and ponds are flooded and drained each year. Crawfish in the U.S. are not fed formulated feeds, and instead primarily consume plant material as forage. In order to provide crawfish with plant material to feed upon, they are often double-cropped or rotated with field crops, most commonly rice but also soybeans and sorghum. From 2010 to 2011 crawfish production grew 0.9% by volume (to 53,435 metric tons) while growing 15.9% by value (to 206 million dollars). The average year-over-year growth over the last 15 years is 13% by volume and 17% by value.

Shrimp in the U.S. are mostly wild-caught. However, both marine (penaeid) and freshwater shrimp species are grown in ponds, tanks, and recirculating systems. Whiteleg shrimp (Litopenaeus vannamei) are the most commonly cultured shrimp species, though culture of the Malaysian prawn (Macrobrachium rosenbergii) in land-based systems is increasingly popular. From 2010 to 2011 shrimp aquaculture grew 19% by volume (to 1,612 metric tons) while growing 3.2% by value (to 6.1 million dollars). The average year-over-year growth over the last 15 years is 5% by volume and ~0.6% by value.

3. Other land-based culture of marine species

There are several ongoing experimental and commercial land-based marine finfish culture operations in the U.S.: notably barramundi (Lates calcarifer) has been commercialized in a large land-based facility in Massachusetts. Several non-native species of sturgeon (Acipenser baeri, A. gueldenstaedtii) have been cultured in commercial and demonstration projects in Florida (e.g., Mote Marine Laboratory). Additionally, work to commercialize cobia (Rachycentron canadum) and black sea bass (Centropristis striata) continues in land based recirculating systems in Virginia.

Annotated Bibliography


The annual Fisheries of the United States is the official published source of fisheries statistics for the US. The 2012 report gives preliminary 2012 data on US commercial fisheries, and the final report for
2012 on US recreational fisheries. US aquaculture statistics are reported for 2011. Summary statistics are also provided on US production of processed fishery products, domestic supply and per capita consumption, foreign trade, and world fisheries production.


The FAO produced this document to highlight that mariculture, specifically offshore aquaculture, offers significant opportunities for sustainable food production but is underutilized worldwide. The report states that offshore aquaculture could foster development of many coastal communities, especially in regions with limited freshwater and arable land. This report measures and compares for all maritime nations the current status and potential for offshore mariculture development from a spatial perspective, and identifies nations with high but unrealized offshore potential.
The Challenge of Reconstructing Coho Salmon Aquaculture after the Great East Japan Earthquake and Tsunami in 2011

Ikutaro SHIMIZU*1, Tsuyoshi TANAKA*2, Hideki MIURA*3, and Kohichi SAOTOME*4

Abstract: The wholesale price of farmed salmon in local fish markets showed higher prices before the tsunami in 2011. Though farmed coho salmon started being landed again by the support of national funding in 2012, the price of the farmed salmon slumped. A higher price is necessary to maintain the farming facilities for management. This paper aims to clarify the causes of the price drop of Sanriku coho salmon and the issues in the reconstruction process of coho salmon aquaculture. The most important cause of the price drop of Sanriku coho salmon was that consumers’ purchasing patterns changed from Sanriku coho salmon to imported salmon due to the lack of Sanriku coho salmon in 2011. We hope to supply fresh Sanriku coho salmon at prices between imported fresh Atlantic salmon and frozen rainbow trout in consumer markets. It is necessary to improve the traditional aquaculture system and to develop brand value for Sanriku coho salmon. Sales promotion is expected to drive the price of the fresh coho salmon up to a fair level.

Key words: coho salmon, aquaculture, Miyagi prefecture, tsunami, reconstructing, and price analysis

Aquaculture of coho salmon (Oncorhynchus Kisutsh) is one of the most important fisheries in Miyagi prefecture. Tohoku, where the annual production was more than 10,000 tons until 2010 (Fig. 1). However, the Great East Japan Earthquake hit the Pacific coast area of eastern Japan on March 11 in 2011. The tsunami destroyed nearly all the farming facilities, local fish markets, and processing firms in the Sanriku region, and suspended coho salmon farming. One year later, farmed coho salmon started being landed again with help from national funding. Unfortunately, the tsunami did not affect the coho salmon juveniles which were located inland. While the wholesale prices of the farmed salmon in local fish markets showed higher prices (more than 400 yen/kg) before the tsunami, the price of the farmed salmon slumped (less than 200 yen/kg) in 2012 (Fig. 2). Prices higher than 370 ~ 380 yen/kg is necessary to maintain the farming facilities and bread even. Understanding the causes of the slump is necessary to reconstruct Sanriku salmon aquaculture.

This paper clarifies the causes of the price drop of Sanriku coho salmon and the issues in the reconstruction process of coho salmon aquaculture. We conducted interviews at fisheries cooperatives, farming facilities, local fish markets, seafood processing firms, and wholesale markets in Miyagi prefecture in September and October 2012, and general merchandising stores in the Tokyo area in November 2012. In addition, the relational expressions of salmon price and fishmeal price were

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measured with the software R (http://www.r-project.org).

**Process of coho salmon aquaculture in Japan**

Domestic eyed eggs of coho salmon are supplied from inland hatcheries (Koshimizu and Sarabetsu) in Hokkaido and a part of eyed eggs are imported from a company in the US (AquaSeed Corporation in Washington) to inland fish farms in the Tohoku district. Coho salmon fry are produced by inland fish farms in Iwate and Miyagi prefectures from December to the next October (Fig. 3). Coho salmon fry (total length: 140 –180 cm) are
transported to marine cages in the Sanriku region. The fish farmers begin to land coho salmon after they grow bigger than 1.5 kg body weight from April until early August. Landed farmed coho salmon are transported to processing facilities and they are supplied to consumer markets around Japan.

**Causes of the price drop of Sanriku coho salmon**

Based on the interviews, the causes of the price drop in domestic farmed coho salmon (Sanriku coho salmon) can be as follows:

1. The mass imports of Chilean farmed coho salmon starting autumn 2011 reduced the domestic wholesale price.
2. Low seawater temperature in the winter 2012 suppressed the growth of Sanriku coho salmon.
3. Late landing of Sanriku coho salmon in the spring 2012 affected supply in June.
4. The spread of rumors about radiation caused by the accident at Fukushima nuclear power plant decreased the demand for Sanriku coho salmon.
5. Avoiding prolonged cold storage and increase in fresh supply of Sanriku coho salmon reduced the price of the farmed coho salmon, and
6. The discontinuation of the supply of Sanriku coho salmon in 2011 changed consumers’ purchasing pattern from Sanriku coho salmon to imported salmon.

**Issues about coho salmon aquaculture**

The price of Sanriku coho salmon is synchronized with the import price of frozen farmed coho salmon and frozen farmed rainbow trout from Chile, and has decreased since the 1980s (Fig. 4). The import price of frozen Chilean coho salmon (IPCCS) is synchronized with the import price of frozen Chilean rainbow trout (IPCRT). IPCCS (yen/kg/year) was explained by the imports (tons/year) of frozen Chilean coho salmon (IQCCS) and IPCRT (yen/kg/year) from 1989 to 2010. The data was used by the
Fig. 4. Prices of fresh Sanriku coho salmon, frozen imports of coho salmon and rainbow trout from Chile between 1976 and 2012. The prices of fresh Sanriku coho salmon were supplied from JF-Miyagi and import prices were provided by the trade statistics, Ministry of Finance of Japan.

This study employed a conventional double-log demand equation model with the estimation results as follows:

$$\log(PCCS) = 1.395 - 0.077\log(IQCCS) + 0.910\log(IPCRT)$$

$$(1.308) (-2.104)^* (7.752)^***$$

Adjusted $R^2 = 0.928$, DW = 2.54, n = 22

The statistical significant code: ***, **, and * suggests p-value under 0.005, under 0.01, and under 0.1, respectively. DW is Durbin-Watson ratio.

The price (yen/kg/year) of fresh Sanriku coho salmon (PSCS) was explained by the import price (yen/kg/year) of frozen Chilean rainbow trout (IPCRT) from 1989 to 2010. The data was used by the local market price of JF (Japan Fisheries Cooperatives) – Miyagi and the trade statistics (http://www.customs.go.jp/toukei/). Ministry of Finance of Japan.

This regression model was estimated by step AIC as follows:

$$\log(PSCS) = 2.494 + 0.578\log(IPCRT)$$

$$(4.114)^** (5.911)^***$$

Adjusted $R^2 = 0.618$, n = 22

The result shows the import price of frozen Chilean coho salmon increases by 0.91% as the import price of frozen Chilean rainbow trout increases 1%. In addition, the price of fresh Sanriku coho salmon increases by 0.58% as the import price of frozen Chilean rainbow trout increases 1%.

In the Japanese salmon market, the highest priced salmon is fresh Atlantic salmon imported from Norway (Fig. 5). The second highest priced salmon are frozen sockeye salmon imported from Alaska, frozen rainbow trout or frozen coho salmon imported from Chile. The lowest priced salmon are domestic chum salmon and export chum salmon to China. One of the most important characteristics of Sanriku coho salmon aquaculture is market differentiation by being a fresh product. In addition, the supply of fresh seafood in spring has been less than other seasons in the Sanriku region. We hope to move the
price of fresh Sanriku coho salmon to be between fresh Atlantic salmon and frozen rainbow trout in consumer markets.

**Issue about fishmeal in Japan**

One of the common issues about Sanriku coho salmon aquaculture system (from eyed eggs production, and fry production, to seawater farming) is the impact on feed costs due to import price of foreign produced fishmeal. The price increase of imported fishmeal has impacted the economics of inland and seawater fed aquaculture. The import price (yen/kg/year) of fishmeal in Japan (JFIP) was explained by the export quantity (tons/year) of Peruvian fishmeal (PFEQ), the fishery production (tons/year) of Peruvian anchovy (PAFQ) and the import quantity of fishmeal in China (CFIQ). The data was used from 1984 to 2009 of the FAO statistics, FishStatJ*. This model with estimation results by step AIC is as follows:

\[
\text{Log (JFIP)} = 8.860 - 0.071 \text{Log (PAFQ)} \\
(7.336)** (-1.465) \\
- 0.410 \text{Log (PFEQ)} + 0.182 \text{Log (CFIQ)} \\
(-3.226)** (3.427)**
\]

Adjusted \( R^2 = 0.635, n = 26 \)

In addition, the import quantity (tons/year) of fishmeal in Japan (JFIQ) was explained by the fish aquaculture production (tons/year) in Japan (JAPQ) and the export quantity (tons/year) of Peruvian fishmeal (PFEQ). The data was used from 1979 to 2009 of the FAO statistics, FishStatJ. This demand model is defined below:

\[
\text{Log (JFIQ)} = 27.001 + 2.389 \text{Log (JAPQ)} \\
(-2.057)^* (2.089)^*
\]

The import price of fishmeal in Japan decreases by 0.41% as the export quantity of Peruvian fishmeal increases 1%. The import quantity of fishmeal in Japan increases by 2.4% as the fish aquaculture production in Japan increases 1%. We hope fishmeal production would be stable in the near future. At the same time, the development of feed with low fishmeal rate will be expected.

Conclusions

It is important to supply fresh salmon for consumer markets by the Golden Week holidays (from late April to early May) in Japan. It is necessary to improve the traditional aquaculture system and to create a new brand value for Sanriku coho salmon. Sales promotion is expected to drive the price of fresh coho salmon up to a fair level. We believe that the development of Sanriku coho salmon aquaculture contributes to the reconstruction of regional industries in Miyagi prefecture. All of the Chilean farmed coho salmon and rainbow trout are imported frozen. We believe that one of the most important characteristics of Sanriku coho salmon for market differentiation is freshness (Fig. 6).
Development of a New Type of Fish Diet, Non-Fish Meal Extruded-Pellet

Noriko ISHIDA *,1, Tomohiko KOSHIISHI *,2, Tatsuo TSUZAKI *,3, Koji MAENO *,4, Satoshi KATAYAMA *,5, Minoru SATOH *,5, and Shuichi SATOH *,6

Abstract: We developed a non-fish meal diet using plant and/or animal protein materials. Three kinds of non-fish meal diets and a control diet containing 50% fish meal were processed. In the non-fish meal diets, the fish meal was replaced with commercially available plant or animal materials and supplemented with taurine and materials for maintaining palatability. These diets were fed to one year old yellowtail (BW 753 ± 96g) in net cages. There was no difference in growth, daily weight gain, daily feeding rate, feed conversion ratio and protein efficiency ratio among the diets. Non-fish meal diets were processed in a factory and dietary properties were studied such as uptake, stomach evacuation rate, and comparative disease resistance in fish fed the experimental diets. In addition, palatability of each substitute protein material for fish was examined and materials to enhance palatability of the non-fish meal diets were clarified. Non-fish meal diets have the potential to support the growth of one year old yellowtail.

Key word: Fish diet, Non-fish meal, Extruded-pellet, Yellowtail, Palatability, Substitute protein material

In Japan, fish meal is the main material in fish diets used in aquaculture and is contained at a level of over 50%. Over 90% of the fish meal used in fish diets for aquaculture is imported. Globally, the price of fish meal has increased dramatically in recent years due to reduced production caused by fishery regulation in South America and the increased worldwide demand for aquacultured fish. The increasing fish meal price threatens the viability of production for Japanese aquaculture farmers. Therefore, a new type of diet that is independent of fish meal is strongly required. Low or non-fish meal extruded-pellets have been developed. In our preliminary study, we prepared diets containing 10% fish meal, and fed them to cultured yellowtail to compare the fish growth performances to a diet containing 50% fish meal. It was observed that fish growth was similar in both diet groups. Three experimental non-fish meal extruded-pellets were designed and fed to one year old yellowtail which were at an immature and rapidly growing size. We also studied dietary, uptake, stomach evacuate rate, palatability, and comparative disease resistance of fish. Then the meat quality fed the experimental diets was evaluated using a panel sensory test.

Materials and Methods

Experimental diets and processing: The control diet and three kinds of non-fish meal diets were prepared using a variety of ingredients (Table 1) (Tsuzaki et al., 2013 and 2014). The control diet (FM) contained 50% fish meal to provide a suitable balance of amino acids. Fish meal was replaced with commercially available plant (PP) and animal (AP) materials in the non-fish meal diets. Soy protein concentrate, defatted soy bean meal, and corn gluten meal were used for plant materials and pork meal, feather

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### Table 1. Formulation of the experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td>Fish meal 1</td>
<td>50.0</td>
</tr>
<tr>
<td>Soy bean concentrate 2</td>
<td>0</td>
</tr>
<tr>
<td>Defatted soy bean meal 3</td>
<td>3.5</td>
</tr>
<tr>
<td>Corn gluten meal 4</td>
<td>0</td>
</tr>
<tr>
<td>Krill meal</td>
<td>0</td>
</tr>
<tr>
<td>Pork meal 5</td>
<td>0</td>
</tr>
<tr>
<td>Feather meal 5</td>
<td>0</td>
</tr>
<tr>
<td>Blood meal 6</td>
<td>0</td>
</tr>
<tr>
<td>Fish oil 7</td>
<td>15.4</td>
</tr>
<tr>
<td>Wheat</td>
<td>13.0</td>
</tr>
<tr>
<td>Defatted rice brain 8</td>
<td>8.5</td>
</tr>
<tr>
<td>Tapioca starch 9</td>
<td>6.6</td>
</tr>
<tr>
<td>Vitamin mixture 10</td>
<td>2.0</td>
</tr>
<tr>
<td>Mineral mixture 10</td>
<td>1.0</td>
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<tr>
<td>Taurine</td>
<td>0</td>
</tr>
<tr>
<td>Amino acid mixture 11</td>
<td>0</td>
</tr>
<tr>
<td>Calcium phosphate 12</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme mixture 13 (external addition)</td>
<td>0</td>
</tr>
<tr>
<td>Skipjack peptide 14 (external addition)</td>
<td>0</td>
</tr>
<tr>
<td>Fish sauce 15 (external addition)</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Fish meal was made from anchovy meal as the main raw material, imported from Peru.
2. Soycomil: Archer Daniels Midland Japan Ltd.
3. Nisshin Oillio Co., Ltd.
4. Japan Corn Starch Co., Ltd.
5. Kagoshima Pure Foods Co., Ltd.
6. APC Japan Co., Ltd.
7. Tsuji Oil Mills Co., Ltd.
8. Suzuki Oil Mills Co., Ltd.
9. Sanguan Wongse Industries Co., Ltd.
10. See Takagi et al. (2005).
11. See Aoki et al. (2000b).
12. Onoda Chemical Industry Co., Ltd.
14. Marubeni Nisshin Feed Co., Ltd.
15. Sakaioex Co., Ltd.
meal, and blood meal were used for animal materials in this study. Taurine was added to the non-fish meal diets at a 1% composition. Experimental diets were carefully shaped using an extruder to have suitable physical properties in the sea and sink at an appropriate rate rather than float.

It is known that yellowtail become less responsive to a diet as the amount of fish meal included is reduced (Aoki et al., 2000a and 2000b). Therefore, two commercially available materials, a skipjack peptide (SP) and a fish sauce (FS) were supplemented to maintain the palatability of the non-fish meal diet. The skipjack peptide is concentrated from the cooking water of skipjack. The fish sauce is made from mackerel gut using a patent-pending method for rapid production. An enzyme solution and amino acid chelated trace metals were added to the non-fish meal diets to improve digestibility and absorption. These palatability materials and enzyme solution were added by a spray method after the extruder processing to minimize the volume (Fig. 1).

Therefore, the three kinds of non-fish meal diets were as follows:

- PP-SP was a plant protein based diet with the skipjack peptide as a material to enhance palatability.
- PP-FS was a plant protein based diet with the fish sauce as a material to enhance palatability.
- AP-SP was an animal protein based diet with the skipjack peptide as a material to enhance palatability.

Two sizes of experimental diets, 13mm and 15mm grain diameter, were prepared at the Kagoshima plant of Marubeni Nissin Feed Co., Ltd. There were minimal differences in the general composition among the four experimental diets. Small differences were seen in the fatty acid composition. AP-SP had 16% of n-3 PUFA while the other diets ranged from 22–27%. AP-SP also had 41% of total monoenes, higher than the 30–35% found in the other diets (Table 2).

**Feeding experiment:** One year old yellowtails which originated from seedling production in Goto Island, Nagasaki Prefecture were used in the experiments (Tsuzaki et al., 2013 and 2014). The average body weight of 220 fish was 753 ± 96g. Four groups of 55 fish fed different experimental diets to satiation three times a week for six months from July to January were reared in four 4 m x 4 m replicate net cages on the sea surface. After January fish continued to be fed with the leftover experiment diet until March.

The feeding experiment was divided into two periods, summer season (July to September) and autumn season (October to December). Sampling and analysis were performed for each period.

Body weight and fork length were monthly measured for 20–25 live fish at random from every group. At the end of the experiment, all the fish were sampled and weighed and the following data were measured or calculated.

\[
\text{Condition factor} = \left( \frac{W}{FL^2} \right) \times 10^2
\]

\[
\text{Weight gain ratio (\%)} = \frac{(TW_t + SDW - TW_0)/TW_0 \times 100}{(TW_t + SDW - TW_0)/2 \times t \times 100}
\]

\[
\text{Daily weight gain ratio (\%/day)} = \frac{(TW_t + SDW - TW_0)/2 \times t \times 100}{(TW_t + SDW - TW_0)/2 \times t \times 100}
\]

\[
\text{Daily feeding rate (\%/day)} = F/(TW_t + SDW - TW_0)
\]

\[
\text{Feed conversion ratio (FCR)} = F/(TW_t + SDW - TW_0)
\]

\[
\text{Protein efficiency ratio (PER)} = (TW_t + SDW - TW_0)/P
\]

\[
W: \text{Body weight (g)}
\]

\[
FL: \text{Fork length (cm)}
\]

\[
TW_0: \text{Initial total body weight (g)}
\]

\[
TW_t: \text{Final total body weight (g)}
\]

\[
SDW: \text{Total body weight of sampled fish and dead fish (g)}
\]

\[
N_t: \text{Number of final fish}
\]

\[
F: \text{Total feed intake (g)}
\]

\[
t: \text{Rearing days}
\]
<table>
<thead>
<tr>
<th>Analysis</th>
<th>Diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
</tr>
<tr>
<td><strong>Proximate composition (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>7.6</td>
</tr>
<tr>
<td>Crude ash</td>
<td>9.1</td>
</tr>
<tr>
<td>Crude protein</td>
<td>39.8</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>18.9</td>
</tr>
<tr>
<td><strong>Phosphorus content</strong></td>
<td></td>
</tr>
<tr>
<td>P (mg/g)</td>
<td>15.4</td>
</tr>
<tr>
<td><strong>Fatty acid composition (%)</strong></td>
<td></td>
</tr>
<tr>
<td>14: 0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>16: 0</td>
<td>18.3</td>
</tr>
<tr>
<td>18: 0</td>
<td>3.8</td>
</tr>
<tr>
<td>16: 1n-7</td>
<td>5.8</td>
</tr>
<tr>
<td>18: 1n-9</td>
<td>16.0</td>
</tr>
<tr>
<td>20: 1n-9</td>
<td>2.6</td>
</tr>
<tr>
<td>20: 1n-11</td>
<td>2.2</td>
</tr>
<tr>
<td>22: 1n-11</td>
<td>4.0</td>
</tr>
<tr>
<td>18: 2n-6</td>
<td>4.5</td>
</tr>
<tr>
<td>20: 4n-6</td>
<td>1.0</td>
</tr>
<tr>
<td>18: 3n-3&lt;sup&gt;<strong>2</strong>&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>18: 4n-3</td>
<td>1.8</td>
</tr>
<tr>
<td>20: 4n-3</td>
<td>0.6</td>
</tr>
<tr>
<td>20: 5n-3 (EPA)</td>
<td>8.0</td>
</tr>
<tr>
<td>22: 5n-3 (DPA)</td>
<td>2.0</td>
</tr>
<tr>
<td>22: 6n-3 (DHA)</td>
<td>13.5</td>
</tr>
<tr>
<td>Others</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>n-3 HUFA</strong></td>
<td>25.9</td>
</tr>
</tbody>
</table>

<sup>*</sup>1 Left figure represents the number of carbon atoms and right figure after colon represents the number of carbon unsaturated bonds.

<sup>**2**</sup>“n-3” represents the omega-3 fatty acid.

n-3 HUFA = 18: 4n-3 + 20: 4n-3 + EPA + DPA + DHA.

Experimental diets are shown in Table 1.
P. Total protein intake (g)
Blood, muscle, and liver samples were collected from 5 fish in every group at the start (initial), late summer (intermediate), and late autumn (end). The ratio of liver weight per body weight was calculated. Hematocrit value and plasma component levels were analyzed. General content, phosphorus content, fatty acid composition, and amino acid composition of muscle were analyzed.

The statistical differences between trial diets were determined by Tukey’s multiple range comparison test (p<0.05).

**Experimental diet uptake, stomach evacuate rate, and comparative disease resistance of fish fed experimental diets:** Nutritional absorption was analyzed from fish feces collected from fish reared in a conical tank (Fig. 2) using special diets containing chrome oxide as an internal standard material (Satoh et al., 2013).

Fish were anesthetized and administered the diet at 2% of body weight directly into the stomach. Stomach residue was analyzed over time by the Kjeldahl nitrogen method and stomach evacuation rate was estimated (Tohata et al., 2012).

Disease resistance was carried out using *Streptococcus dysgalactiae*, an infectious streptococcal bacterium. Fish were reared on each diet in indoor tanks for 3 months and fed the bacterium-containing feed for 5 days. Fish mortality was observed over a 40 day period (Ishida et al., 2013).

**Diet palatability:** Fish behavior was divided into five responses after placing the diets into a fish tank described as recognition, ingestion, orientation within the mouth, swallowing, or spitting out (Fig. 3). The actions were analyzed by using a camera to film red seabream, yellowtail, and coho salmon using single dry pellets made from only one material (Fig. 4) (Ninuma et al., 2012, Yamauchi et al., 2012).

**Meat quality of raw sashimi:** A panel test using the order method, color-difference meter analysis, and electronic nose system analysis were performed for each experimental fish fed PP-SP, PP-FS, AP-SP, and control diet (Tohata et al., 2013).

**RESULTS**

**Processing:** All the experimental diets were processed using a double-screw extruder. At the beginning in the autumn season, plant protein based diets did not sink at a suitable rate and flowed out from the net cage compared to the other diets. Therefore these diets were re-made. Palatability ingredients and an enzyme solution were sprayed onto diets to minimize materials used to 0.05% from the original 2% required in the blending method (Fig. 1).

**Growth performances and biological indices in yellowtail:** The growth performances in both the summer and autumn period are shown in Table 3.

In the summer period when the water temperature was 22.8 - 29.2 °C all diet groups

![Fig. 2](image-url) The conical tank used to collect feces of fish fed the experimental diets to analyze nutritional absorption.

![Fig. 3](image-url) Fish behaviors for the diet.
showed a similar total feed intake ranging from 113 to 118 kg and a similar body weight gain (Fig. 5). In the autumn period when the water temperature was 11.5 °C lower than during the summer period total food intake of all groups was less than in the summer ranging from 90 to 103 kg. No seasonal difference in weight gain was seen among diet groups.

Final fork length and body weight were 48.4 cm and 2,242 g in the control (FM), 48.6 cm and 2,178 g in the PP-SP, 49.0 cm and 2,234 g in the PP-SF, and 48.5 cm and 2,205 g in the AP-SP. Differences between control and non-fish meal diets were not observed and the growth was almost the same. There was no seasonal difference between daily weight gain (%/day), daily feeding rate (%/day), and
weight gain ratio (%). For example, feed conversion ratio in the autumn season was 3.33 in the control (FM) and 3.91 in PP-SP, 3.76 in PP-FS, and 3.39 in AP-SP.

Table 4 shows the results of the biological indices of cultured fish (n=5). The liver to body weight ratio in each group was 1.2 to 1.3 and no significant difference was seen. The average hematocrit value of 5 fish in each group was 46.2 to 50.6 with no significant difference observed. There were three, two, and one mortalities of fish in AP-SP, PP-FS, and FM respectively. Green liver, which is thought to be a sign of taurine deficiency (Takagi et al., 2005), was not seen in any of the dead fish.

Plasma components measured included glucose, total protein, total cholesterol, glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP). No significant difference was observed in plasma components between the treatments in the summer period. Total cholesterol exhibited a significant seasonal variability between the treatments with lower values of 246 and 266 mg/dl in PP-SP and PP-FS respectively (p<0.05) (Table 4).

Characteristics such as uptake, stomach evacuate rate, and disease resistance of fish fed the experimental diets: The uptake of protein from non-fish meal diets was reduced by 4-9 % compared to the FM control (Satoh et al., 2013). Evacuation of feed from fish stomachs was observed for all three diets. The order of the rate of evacuation was PP-SP > AP-SP > FM (Fig. 6).

Survival rate at 42 days after the challenge test was 80% in FM group, and 95% in PP-SP and AP-SP group (Ishida et al., 2013).

Diet palatability: Fish spitting out feed was almost zero for single dry fish meal pellet. The spitting rate was higher in single dry pellet made from plant protein than those made from animal protein. To improve palatability of PP and AP pellets we washed the original materials with 80% ethanol to remove the extractive component and made each single dry pellet from those pre-treated materials. The spitting rate in single dry pellet made from pre-

---

![Fig. 5. Growth curves of yellowtail fed the experimental fish meal diet (FM : ●●●) and non-fish meal diet (PP-SP : - - - , PP-FS : □□□, and AP-SP : - - - ) in net cages for 6 months. Experimental diets are detailed in Table 1.](image-url)

---

**Table 4.** Changes in hepatosomatic index, hematocrit value, and hematochemical characteristics in yellowtail (n=5) fed the experimental diets

<table>
<thead>
<tr>
<th>Rearing season (days)</th>
<th>Initial</th>
<th>Summer (90 days)</th>
<th>Autumn (91 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>PP-SP</td>
<td>PP-FS</td>
</tr>
<tr>
<td>Hepatosomatic index (%)</td>
<td>1.1 ± 0.0</td>
<td>1.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematocrit value (%)</td>
<td>53.6 ± 3.4</td>
<td>61.5 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.8 ± 4.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematochemical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>144 ± 25.2</td>
<td>237.8 ± 51.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>262.6 ± 30.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.0 ± 0.5</td>
<td>4.3 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>349.4 ± 63.0</td>
<td>282.0 ± 30.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203.4 ± 21.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPT (IU/l)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>25.4 ± 2.6</td>
<td>32.4 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.2 ± 44.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP (IU/l)&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>148.6 ± 33.8</td>
<td>156.4 ± 34.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.8 ± 14.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n=5). Values having different superscript letters are significantly different (p<0.05).

<sup>a</sup> Ratio of liver weight to body weight.  
<sup>b</sup> Glutamate-pyruvate transaminase.  
<sup>c</sup> Alkaline phosphates.  
Experimental diets are shown in Table 1.
treated plant protein decreased. On the other hand, the opposite result was shown in pre-treated animal proteins that the spitting rate increased (Ninuma et al., 2012; Yamauchi et al., 2012).

**Fish meat analysis:** Table 5 shows the results of the fish meat analysis of cultured fish. In the proximate composition of all the diet groups, moisture content declined and fat content increased until the end of experiment. No difference was seen in the phosphorus content among the diet groups. The EPA, DHA and n3HUFA content of fish fed AP-SP pellets was always lower than in fish fed the other diets, at times significantly less (p<0.05) in both summer and autumn. These results were similar to the analysis of experimental diets.

**Discussion**

It has long been thought that fish meal was a necessary ingredient in fish feed. Generally, the formulated feeds for yellowtail contain about 50% fish meal. Recently, formulated feeds containing lower amounts of fish meal such as 30% have been produced as a commercial product. However a fish meal content of less than 30% would have considerable financial benefits for aquaculture enabling continued economically viable production. Therefore, we developed non-fish meal feeds and evaluated those in a field experiment with a similar aquaculture environment. We used one year old yellowtail which is an intermediate size during culturing and shows rapid growth requiring high feed costs. Experimental periods included the higher feeding activity season of summer and the lower feeding activity season of winter.

In the present study (Tsuaki et al., 2014), the plant protein based diets of PP-SP and PP-SP showed a similar daily feeding rate and daily weight gain, better feed conversion ratio and protein efficiency ratio than the control FM in summer season. On the other hand, the animal protein based diet of AP-SP showed a higher daily feeding rate and feed conversion ratio, lower protein efficiency ratio demonstrating lower protein uptake than others. We also studied uptake of nutrient in these non-fish meal diets and FM by analysis of yellowtail feces. It is clear that the uptake of protein in these non-fish meal diets were reduced by 4 - 9 % compared to the control FM.

Then, in the autumn season, the plant protein based diets of PP-SP and PP-FS showed a low performance because of the higher daily feeding rate and feed conversion ratio, lower daily weight gain. These results are thought to be due to the physical properties of the diets. Because these plant protein based diets did not sink appropriately and flowed out from the net cage compared to the other diets. From these results, more studies are needed for the improvement of uptake for animal protein based diets and adjustment of the physical characteristics for plant protein based diets. However, these results demonstrate that non-fish meal diets have the potential to support the growth of one year old
Yellowtail compared to the results of Takagi et al. (2013).

For substitute materials, plant protein material such as soy bean concentrate has been studied (Aoki et al., 2000a, 2000b). We also used soy bean concentrate and confirmed its performance as a substitute protein for fish meal in the present study. In the field, it is sometimes believed that plant protein materials stay longer in the stomach than fish meal. However, in our experiment, the plant protein based diets did not stay longer among the diet groups. Fish meal based diet was the first to be evacuated from the stomach. In addition, it is sometime believed that plant protein fed yellowtail is more susceptible to epidemic disease than fish meal based diet. However, in our experiment, those phenomena were not seen (Ishida et al., 2013). We also compared the stomach evacuation rate and the in vitro digestion using only one protein material diet, and found minimal difference (Tohata et al., 2012). More studies are needed in future using low cost materials such as defatted soy bean meal, corn gluten meal and canola oil.

As for substitute animal protein materials, there are meat meal, chicken meal, feather meal, and pork meal. Feather meal and meat meal were reported to be useful for yellowtail (Shimeno et al., 1993a, 1993b, 2000). However, the animal protein studies are fewer than plant protein studies. There is potential to use chicken meal and pork meal. As there are few studies about pork meal, we tried to use pork meal in the present study. Pork meal has less DHA, EPA and n3HUFA of fatty acid than FM. A similar tendency was seen in the whole fish body and the meat of yellowtail fed by pork meal based diet. However, there is no significant effect on growth and

### Table 5. Proximate composition, phosphorus content, and fatty acid composition of muscle in yellowtail (n=5) fed the experimental diets

<table>
<thead>
<tr>
<th>Rearing season (days)</th>
<th>Initial</th>
<th>Summer (90 days)</th>
<th>Autumn (91 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FM</td>
<td>PP-SP</td>
<td>PP-FS</td>
</tr>
<tr>
<td></td>
<td>FM</td>
<td>PP-SP</td>
<td>PP-FS</td>
</tr>
<tr>
<td>Moisture</td>
<td>71.9 ± 0.8</td>
<td>64.7 ± 1.7</td>
<td>64.5 ± 1.4</td>
</tr>
<tr>
<td>Crude ash</td>
<td>2.3 ± 0.5</td>
<td>1.3 ± 0.0</td>
<td>1.3 ± 0.0</td>
</tr>
<tr>
<td>Crude protein</td>
<td>22.1 ± 0.2</td>
<td>21.4 ± 0.6</td>
<td>20.3 ± 1.1</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>3.7 ± 0.9</td>
<td>11.5 ± 2.1</td>
<td>12.8 ± 2.6</td>
</tr>
<tr>
<td>Phosphorus content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (mg/g)</td>
<td>15.5 ± 4.4</td>
<td>4.2 ± 1.7</td>
<td>5.5 ± 2.8</td>
</tr>
<tr>
<td>Fatty acid composition (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>4.2 ± 0.4</td>
<td>4.0 ± 0.3</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>16:0</td>
<td>19.6 ± 0.9</td>
<td>18.0 ± 0.8</td>
<td>16.2 ± 1.3</td>
</tr>
<tr>
<td>18:0</td>
<td>5.6 ± 0.5</td>
<td>4.2 ± 0.3</td>
<td>4.0 ± 0.1</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n=5). Values having different superscript letters are significantly different (P<0.05).

Experimental diets are shown in Table 1. Fatty acid compositions are shown in Table 2.
survival. Only slightly less protein digestion in pork meal based diet was seen than the plant protein based diet. And feeding activity on pork meal based diets was active. From these results, pork meal was thought to be available as a substitute material.

The biggest problem has been shown to be the reduction of feeding activity when fish are fed low fish meal content diets (Aoki et al., 2000a, 2000b, 2000c). However, it is reported that an added palatability ingredients improved the feeding activity in eel (Takii et al., 1984), yellowtail (Takii et al., 1994), and tiger puffer (Takii et al., 1998). In the present study, we found that fish did not especially prefer plant protein to animal protein and fish meal. However, after washing the feed ingredients to remove the extractive component, the spitting ratio decreased in plant ingredient feeds and increased in animal protein feeds, which suggested that the extractive components might affect feeding activity (Niinuma et al., 2012, Yamauchi et al., 2012). Then, we added some palatability ingredients to the plant based diet, fish swallowing ratio was increased and spitting ratio was decreased. From these results, we used the skipjack peptide (SP) and fish sauce (FS) as palatability ingredients in order to improve the feeding activity. In the results, higher palatability was seen when using both ingredients. The biggest problem of reduction of feeding activity in non-fish meal diets was thought to be almost resolved by the addition of palatability ingredients.

The meat quality of yellowtail fed with non-fish meal diets were compared to control yellowtail by a panel test, which showed a difference in color and smell. Then we used colorimeter and Electronic Nose System of Alpha MOS (http://www.alpha-mos.co.jp/products/sensory.html). The fish fed by non-fish meal diets had whiter muscle and a less fishy smell (Tohata et al., 2013). Now, the effect of non-fish meal diets on fish meat quality and as a method of enhancing the meat quality control is being determined.

As described above, we alternated fish meal to plant or animal protein materials to make non-fish meal diets, and fed them to one year old yellowtail, and got similar growth, body and blood compositions. The main reason for the similar growth rates was thought to be the addition of palatability ingredients and enzyme mixtures (Table 1). As we used both plant and animal materials, we suggest the possibility of many materials for non-fish meal diets. More study is needed to improve low cost diets and produce higher quality meat.

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References


Shimeno S., Matsumoto T., Hujita T., Mima T., and


The Importance of Taurine and n-3 Fatty Acids in Cobia, *Rachycentron canadum*, Nutrition

Aaron M. WATSON*, Frederic T. BARROWS*, and Allen R. PLACE*

Abstract: In the wild, fish can adapt to their diet by down regulating or eliminating biosynthetic pathways for nutrients found in abundance in their prey. Upon domestication for aquaculture these fish may now have unique dietary requirements which must be met through supplementation. To examine this hypothesis and aid in determining potential minimum requirement levels for aquaculture production, we have examined the synthetic capacity for taurine and polyunsaturated fatty acids in cobia, *Rachycentron canadum*, through molecular methods and growth trials, respectively.

Taurine, a non-protein amino acid, is found in high concentrations in the natural diet of cobia and plays a variety of physiological roles. The taurine biosynthesis pathway in vertebrates is well known; however it appears to be nonfunctional in some terrestrial (Felidae) and marine (Rachycentridae) carnivores, thereby necessitating dietary supplementation. Taurine is not found in plant protein sources used for replacing fishmeal in aquaculture feeds, an increasingly important priority.

We examined the effects of graded levels of taurine addition (0%, 0.5%, 1.5%, 5.0%) to plant protein and fishmeal based diets formulated and manufactured by the USDA-ARS. At the conclusion of a feeding trial, RNA was extracted from liver, muscle, and brain tissue for quantitative-RT-PCR analysis of the genes involved in taurine synthesis. Cysteine dioxygenase (CDO), cysteamine dioxygenase (ADO), and taurine transporter (TaTu) activity and expression levels were examined and no differences in transcript abundance was detected within the tissues between the dietary taurine levels. Increasing dietary taurine resulted in increased tissue taurine concentrations.

To examine the effects of alternative sources completely replacing fish oil, two replacements were examined as the lipid sources in a fishmeal free, plant based feed (USDA-ARS) and compared to a fish oil version of the diet. A thraustochytrid meal plus soybean oil (TM+SOY) and a canola oil with exogenous docosahexaenoic (DHA) and arachidonic (ARA) acids (CO+EFA) were utilized. At the conclusion of an eight week growth trial, whole body and fillet fatty acid profiles were examined.

The TM+SOY diet worked equivalently to the USDA-ARS control diet, however the CO+EFA diet resulted in significantly lower growth and survival as well as an increased feed conversion ratio. Whole body fatty acid profiles revealed a significant reduction in total essential fatty acid (EFA) concentration in the CO+EFA fed fish, indicating the supplemented levels of DHA and ARA were insufficient to meet requirements. Although all three diets were sufficient in the precursors for EFA synthesis, this reduction in growth, survival, and whole body EFA concentration suggests cobia have limited synthetic capacity for DHA and ARA. Both alternative lipid diets were devoid of eicosapentanoic acid (EPA) supplementation, which does not appear to be essential for cobia as the TM+SOY diet performed equivalently to the fish oil control.

Key words: Cobia, Taurine, Docosahexaenoic Acid, Fishmeal and Fish Oil Replacement

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World fish supply has grown on average 3.2 percent per year over the last five decades, with much of the growth occurring in various aquaculture sectors (FAO, 2012). With worldwide capture fisheries holding relatively static over the last few decades, and expected to remain at current levels, aquaculture must continue to expand rapidly to meet the protein demands of the ever increasing world population. Traditionally, fishmeal and fish oil acquired through reduction of wild capture fisheries have served as major protein and lipid sources, respectively, for compounded fish feeds utilized for many species in culture. In order for aquaculture to continue to grow at current and desired rates, the inclusion levels of these ingredients must be continually reduced or eliminated, as their abundance is finite. The reduction of fishmeal and fish oil use as well as alternate protein and lipid sources have been studied extensively in many species and has been established as a priority for research efforts and the aquaculture industry as a whole (Rust et al., 2011).

Reductions in productivity including decreased growth rates, increased feed conversion ratios, low feed intake, and negative health impacts have been observed in many species when reducing fishmeal below species specific threshold levels (Glencross et al., 2011; Kader et al., 2012; Luo et al., 2006; Thiessen et al., 2004; Wacyk et al., 2012; Xie et al., 1998). This is often related to deficiencies in amino acids, fatty acids, vitamins, or minerals that are not present in sufficient quantities in sources utilized as fishmeal replacements. This is especially true when utilizing plant proteins (wheat flour, corn gluten, soybean meal, barley, etc) as plants have substantially different amino acid profiles when compared to fishmeals or other meat based protein meals. Many of these deficiencies have been identified (lysine, methionine, threonine) and can be corrected for by simple crystalline amino acid addition to feed formulations. One challenge brought about by reducing fishmeal in favor of plant proteins is in identifying deficiencies on a species specific basis and then determining minimal and optimal supplementation levels. Similarly, when reducing or replacing fish oil in favor of alternative lipid sources, some specific fatty acids critical to growth and health are lacking in terrestrial oil sources when compared to fish oil.

Taurine, a non-protein amino acid, is found in high concentrations in fishmeal, wild prey items, and meat based meals but is devoid from plant protein sources. Taurine plays multiple important physiological roles as an osmolyte, antioxidant, photoreceptor protectant, and as a bile salt conjugate (Schuller-Levis and Park, 2003). Multiple studies have shown advantages to supplementing taurine when reducing fishmeal in a variety of species (Chatzifotis et al., 2008; Kim et al., 2005, 2003; Lunger et al., 2007; Pinto et al., 2010).

Long chain polyunsaturated fatty acids, such as docosahexaenoic (DHA) and arachidonic (ARA) acids are also not typically observed in terrestrial oils, and the ability of teleost species to convert precursors into these important fatty acids is typically low and highly variable, rendering them essential for many marine species. As with taurine and several other compounds, these fatty acids in particular must be supplemented to feeds utilizing alternative protein or lipid sources.

Cobia, Rachycentron canadum, is a fast growing marine carnivore whose natural diet is high in fish and crabs (Arendt et al., 2001; Franks et al., 1996) and is therefore likely sufficient in both taurine and the essential fatty acids DHA and ARA. Cobia is a promising species for intensive aquaculture as they can be spawned and maintained in captivity, are highly fecund serial spawners, and have high quality flesh suitable for multiple consumer types (Holt et al., 2007). A great deal of research has focused on cobia nutrition and reducing and replacing both fishmeal and fish oil with alternative sources to develop sustainable feeds (Lunger et al., 2006; Salze et al., 2010; Trushenski et al., 2012; Watson et al., 2012).

Here, several studies were carried out with juvenile cobia to determine the potential synthetic capacity for taurine present in various tissues and to evaluate two fishmeal and fish oil free diets.

Materials and Methods

To examine the effects of dietary taurine on growth and performance, as well as the taurine synthetic capacity of juvenile cobia, four diets
Table 1. Dietary formulations for the diets with graded levels of taurine. From Watson et al. 2014.

<table>
<thead>
<tr>
<th>Ingredient (g kg⁻¹)</th>
<th>FM1</th>
<th>FM2</th>
<th>FM3</th>
<th>FM4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menhaden Fish Meal</td>
<td>345</td>
<td>345</td>
<td>345</td>
<td>345</td>
</tr>
<tr>
<td>Corn Protein concentrate</td>
<td>44.3</td>
<td>44.3</td>
<td>44.3</td>
<td>44.3</td>
</tr>
<tr>
<td>Poultry by-product meal</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td>118</td>
</tr>
<tr>
<td>Wheat Flour</td>
<td>242.7</td>
<td>237.7</td>
<td>227.7</td>
<td>192.7</td>
</tr>
<tr>
<td>Soybean meal, solvent extracted</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Blood meal, spray dehydrated</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Menhaden Fish Oil</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Vitamin Pre-mix¹</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Choline CL</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Stay-C</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Trace mineral pre-mix²</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mycozorb</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Taurine</td>
<td>0</td>
<td>5</td>
<td>15</td>
<td>50</td>
</tr>
</tbody>
</table>

¹Contributed per kg diet; vitamin A, 9650 IU; vitamin D₃, 6.6 IU; vitamin E, 132 IU; menadione sodium bisulfite, 4.7 mg; thiamine mononitrate, 9.1 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 13.7 mg; pantothenate, DL-calcium, 101.1 mg; cyanocobalamine, 0.03 mg; niacin, 21.8 mg; biotin, 0.33 mg; folic acid, 2.5 mg.

²Contributed in mg kg⁻¹ of diet; zinc 37; manganese, 10; iodine, 5; copper, 1.

were formulated to contain 0.0%, 0.5%, 1.5%, and 5.0% supplemental taurine to a fishmeal based diet (Table 1). Juvenile cobia (~125g) were housed in a recirculating aquaculture system at the Institute of Marine and Environmental Technology in Baltimore, Maryland, USA and fed for 8 weeks. At the conclusion of the eight week feeding trial, growth and feed conversion ratios were calculated, and tissues (muscle, liver, and brain) were preserved in RNA later for quantitative-PCR analysis of several genes involved in taurine synthesis. Plasma, liver, and muscle tissues were also taken for taurine content analysis. Full materials and methods are available in Watson et al. (2014). A parallel study was conducted with a fishmeal free, plant protein based formulation also containing 0.0%, 0.5%, 1.5%, and 5.0% supplemental taurine and similar analyses were conducted at the conclusion of the trial.

To examine the effects of fish oil replacement in a fishmeal free, plant protein based diet similar methods were employed. Two experimental diets were formulated and manufactured by the USDA-ARS (Table 2). One experimental diet utilized canola oil plus exogenous DHA (~2.85 g kg⁻¹) and ARA (~0.32 g kg⁻¹) (CO+EFA diet) while the other experimental diet utilized a thraustochytrid meal plus soybean oil (TM+SOY) as the lipid source. The control diet consisted of the same plant protein base as the experimental diets, but utilized fish oil as the lipid source. An eight week growth trial was conducted beginning with ~130g juvenile cobia and at the conclusion growth and feed conversion ratio were calculated, and fatty acid profiles of fillets were determined through gas chromatography (GC). Full materials and methods are available in Watson et al., 2013a.

**Results**

Full results of both studies are available in the cited references, only a brief synopsis is presented here. Taurine supplementation to a fishmeal (~35%) diet did not have significant effects on growth rate, protein efficiency ratio, total bile salt concentration, or feed conversion ratio (Table 3). Transcript abundances of CDO, ADO, and TauT were also not affected by dietary taurine input level in any of the tissues examined (Fig. 1), however the rate limiting step of the reaction, cysteinesulfinate decarboxylase (CSD) was not detected through the q-PCR methods utilized. Tissue taurine levels for plasma, liver, and muscle did increase with increasing dietary taurine.

In contrast, taurine supplementation to the plant protein based diet did have significant effects on growth rate and feed conversion ratio although overall growth was significantly lower in all treatments of the plant protein formulation when compared to the fishmeal based series (Table 4). Similarly to the fishmeal series, transcript abundances for CDO, ADO, and TauT were unaffected by dietary taurine input (Fig. 1).

In the fish oil replacement study, the TM+SOY diet performed similarly to the ARS control fish oil diet while the CO+EFA diet significantly underperformed in regards to growth rate, feed conversion ratio, and survival (Table 5; Watson et al., 2013a). The different lipid sources utilized in the diets did affect whole body and fillet lipid profiles, with direct similarity to the profiles of the diets (Fig. 2; Watson et al., 2013a). The alternate lipid
Table 2. Diet formulations and fatty acid compositions of the diets used for fish oil replacement. From Watson et al., 2013a.

<table>
<thead>
<tr>
<th>Ingredient (g kg⁻¹)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TM+SOY</td>
</tr>
<tr>
<td>Soy Protein Concentrate</td>
<td>269.3</td>
</tr>
<tr>
<td>Corn Gluten</td>
<td>211</td>
</tr>
<tr>
<td>Wheat Flour</td>
<td>226.5</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td></td>
</tr>
<tr>
<td>Solvent Extracted</td>
<td>121</td>
</tr>
<tr>
<td>Menhaden Oil</td>
<td>0</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>5</td>
</tr>
<tr>
<td>Algalac 3050</td>
<td>79</td>
</tr>
<tr>
<td>Canola Oil + DHA + ARA</td>
<td>0</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>23.7</td>
</tr>
<tr>
<td>Vitamin Pre-mix¹</td>
<td>10</td>
</tr>
<tr>
<td>Lysine-HCL</td>
<td>13.5</td>
</tr>
<tr>
<td>Choline CL</td>
<td>6</td>
</tr>
<tr>
<td>Trace Mineral Pre-mix²</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>0.5</td>
</tr>
<tr>
<td>Stay-C</td>
<td>3</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>5.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.1</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>5.6</td>
</tr>
<tr>
<td>Taurine</td>
<td>15</td>
</tr>
<tr>
<td>Fatty Acid (g 100g⁻¹)³</td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>0.15</td>
</tr>
<tr>
<td>14:0</td>
<td>4.33</td>
</tr>
<tr>
<td>16:0</td>
<td>17.17</td>
</tr>
<tr>
<td>17:0</td>
<td>0.16</td>
</tr>
<tr>
<td>18:0</td>
<td>2.77</td>
</tr>
<tr>
<td>20:0</td>
<td>0.28</td>
</tr>
<tr>
<td>22:0</td>
<td>0.24</td>
</tr>
<tr>
<td>SFA³</td>
<td>25.10</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>0.23</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>0.87</td>
</tr>
<tr>
<td>20:1n-9+10</td>
<td>13.86</td>
</tr>
<tr>
<td>20:1n-15+cis-8</td>
<td>0.06</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>0.14</td>
</tr>
<tr>
<td>24:1n-9</td>
<td>0.0</td>
</tr>
<tr>
<td>MUFA⁴</td>
<td>15.15</td>
</tr>
<tr>
<td>16:3n-4</td>
<td>0.05</td>
</tr>
<tr>
<td>16:4n-1</td>
<td>0.04</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>33.27</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>3.94</td>
</tr>
<tr>
<td>20:2n-6</td>
<td>0.04</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.70</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.42</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>6.09</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.16</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>14.93</td>
</tr>
<tr>
<td>PUFA⁵</td>
<td>59.75</td>
</tr>
<tr>
<td>n-3⁶</td>
<td>19.45</td>
</tr>
<tr>
<td>n-6⁷</td>
<td>40.10</td>
</tr>
<tr>
<td>n-3:n-6</td>
<td>0.49</td>
</tr>
</tbody>
</table>

¹Contributed per kg diet; vitamin A, 13510 IU; vitamin D, 9.2 IU; vitamin E, 184.4 IU; menadione sodium bisulfite, 6.6 mg; thiamine mononitrate, 12.7 mg; riboflavin, 13.4 mg; pyridoxine hydrochloride, 19.2 mg; pantothenate, DL-calcium, 141.5 mg; cyanocobalamine, 0.04 mg; nicotinic acid, 30.5 mg; biotin, 0.46 mg; folic acid, 3.5 mg.
²Contributed in mg kg⁻¹ of diet; zinc 37; manganese, 10; iodine, 5; copper, 1.
³Saturated fatty acids = sum of all fatty acids without double bonds.
⁴Monounsaturated fatty acids = sum of all fatty acids with a single double bond.
⁵Polyunsaturated fatty acids = sum of all fatty acids with two or more double bonds.
⁶Sum of all n-3 fatty acids.
⁷Sum of all n-6 fatty acids.
Table 3. Performance characteristics from the graded taurine in a fishmeal diet growth trial. Within a row, different letters indicate significant differences (P<0.05). From Watson et al. 2014.

<table>
<thead>
<tr>
<th>Diet</th>
<th>FM1</th>
<th>FM2</th>
<th>FM3</th>
<th>FM4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight (mean ± SD)</td>
<td>127.44 ± 13.31</td>
<td>122.70 ± 14.97</td>
<td>130.64 ± 12.37</td>
<td>127.36 ± 14.52</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Weight Gain (g fish-1)</td>
<td>227.83 ± 18.15</td>
<td>297.62 ± 24.20</td>
<td>313.47 ± 31.36</td>
<td>290.08 ± 33.11</td>
</tr>
<tr>
<td>FCR</td>
<td>1.52 ± 0.10</td>
<td>1.42 ± 0.02</td>
<td>1.43 ± 0.11</td>
<td>1.51 ± 0.05</td>
</tr>
<tr>
<td>SGR</td>
<td>2.1 ± 0.12</td>
<td>2.2 ± 0.04</td>
<td>2.2 ± 0.14</td>
<td>2.1 ± 0.09</td>
</tr>
<tr>
<td>PER</td>
<td>1.35 ± 0.15</td>
<td>1.44 ± 0.04</td>
<td>1.26 ± 0.43</td>
<td>1.29 ± 0.07</td>
</tr>
<tr>
<td>HSI</td>
<td>3.14 ± 0.14</td>
<td>2.99 ± 0.18</td>
<td>2.78 ± 0.45</td>
<td>2.72 ± 0.19</td>
</tr>
<tr>
<td>Total Bile Salts (mM)</td>
<td>28.48 ± 4.79</td>
<td>32.69 ± 6.04</td>
<td>30.02 ± 4.34</td>
<td>29.59 ± 2.32</td>
</tr>
</tbody>
</table>

1 Weight Gain (g fish) = (final weight (avg per fish) - initial weight (avg per fish))

2 Feed Conversion Ratio (FCR) = \( \frac{\text{food fed (g)}}{\text{weight gained (g)}} \)

3 Specific Growth Rate (SGR) = 100 * (ln final weight (g) - ln initial weight (g)) / days of growth trial

4 Protein Efficiency Ratio (PER) = \( \frac{\text{weight gained (g)}}{\text{protein fed (g)}} \)

5 Hepatosomatic Index (HSI) = 100 * (liver weight (g) / body weight (g))

Table 4. Performance characteristics from the graded taurine in a plant based diet growth trial (128.37 g initial weight). Within a row, values that share common superscripts are not significantly different from one another (P > 0.05).

<table>
<thead>
<tr>
<th>Diet (Taurine %)</th>
<th>PP1 (0.02)</th>
<th>PP2 (0.39)</th>
<th>PP3 (1.35)</th>
<th>PP4 (4.08)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Gain (%)</td>
<td>23.35 ± 22.82a</td>
<td>80.57 ± 60.24a</td>
<td>130.87 ± 25.20b</td>
<td>133.82 ± 10.83b</td>
</tr>
<tr>
<td>FCR</td>
<td>6.38 ± 1.49a</td>
<td>2.97 ± 1.17b</td>
<td>1.98 ± 0.19b</td>
<td>2.12 ± 0.23b</td>
</tr>
<tr>
<td>SGR</td>
<td>0.57 ± 0.12a</td>
<td>1.31 ± 0.26b</td>
<td>1.47 ± 0.19b</td>
<td>1.51 ± 0.09b</td>
</tr>
<tr>
<td>Total Bile Salts (mM)</td>
<td>38.76 ± 7.40</td>
<td>28.37 ± 1.94</td>
<td>36.30 ± 5.69</td>
<td>28.75 ± 3.22</td>
</tr>
</tbody>
</table>

1 Feed Conversion Ratio (FCR) = \( \frac{\text{food fed (g)}}{\text{weight gained (g)}} \)

2 Specific Growth Rate (SGR) = 100 * (ln final weight (g) - ln initial weight (g)) / days of growth trial

Table 5. Production characteristics from the eight week grow out trial with fish oil replacement sources. Data from a previous grow out with juvenile cobia on a fish meal and fish oil based, commercially produced diet are included to show overall effects of fish meal and fish oil replacement. From Watson et al. 2013a.

<table>
<thead>
<tr>
<th>TM+SOY</th>
<th>CAN+EFA</th>
<th>ARS Control</th>
<th>Commercial Diet (2009 grow out data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Gain (%)</td>
<td>288a</td>
<td>117b</td>
<td>275a</td>
</tr>
<tr>
<td>Fillet Yield (%)</td>
<td>25.93 ± 3.34</td>
<td>20.88 ± 4.34</td>
<td>25.71 ± 1.90</td>
</tr>
<tr>
<td>FCR</td>
<td>1.42a</td>
<td>2.98b</td>
<td>1.46b</td>
</tr>
<tr>
<td>Plasma Osmolality</td>
<td>358.25 ± 30.59a</td>
<td>311.75 ± 36.82a</td>
<td>327.58 ± 13.83ab</td>
</tr>
<tr>
<td>PCB Content (ng g-1)</td>
<td>8.40 ± 1.54</td>
<td>9.30 ± 3.41</td>
<td>13.2 ± 4.2</td>
</tr>
<tr>
<td>Mercury Content (ng g-1)</td>
<td>30.20 ± 3.53</td>
<td>48.17 ± 16.24</td>
<td>20.14 ± 2.96</td>
</tr>
<tr>
<td>Hepatosomatic index</td>
<td>1.83 ± 0.19a</td>
<td>2.93 ± 0.79b</td>
<td>2.66 ± 0.55ab</td>
</tr>
<tr>
<td>Specific Growth Rate</td>
<td>2.48b</td>
<td>1.42b</td>
<td>2.36a</td>
</tr>
<tr>
<td>Survival</td>
<td>100%</td>
<td>58%</td>
<td>98%</td>
</tr>
</tbody>
</table>

1 Weight gain=(final tank weight - initial tank weight) / initial tank weight*100.

2 Fillet Yield=(fillet weight/body weight)*100.

3 FCR=Feed conversion ratio= grams fed/grams gained.

4 Hepatosomatic index=liver weight/ body weight.

5 SGR=specific growth rate = ((lnBW2-lnBW1)*(days of growth trial)) / 100.

6 Initital Weight 120g, eight week growth trial (Watson et al. 2012).

Values in the same row with different superscripts are significantly different (p<0.05), no superscript indicates no significant difference within a category.
Fig. 1. Relative transcript expression (mean ± SE) of genes involved in taurine synthesis and transport in juvenile cobia, *Rachycentron canadum*, as a percent expression compared to reference gene beta-actin for each diet (% taurine) in liver (1), brain (2), and muscle (3). Equivalent cDNA input (10 ng) for triplicate samples of each tissue and six fish sampled per treatment group and tissue (*n* = 6 per data point). Numbers in parenthesis indicate measured taurine in each diet. Panels A-1, A-2, and A-3 depict results from Watson *et al.* (2014), with fishmeal based diet series and panels B-1, B-2, and B-3 depict results from fishmeal free, plant protein based diet series.
Fig. 2. Fatty acid compositions of fillet and whole body tissues from juvenile cobia fed experimental fish oil replacement diets expressed as a fraction of dietary total lipid profile. Values were calculated from relative fatty acid methyl ester (FAME) composition (Fillet or whole body fatty acid concentration/Diet fatty acid concentration). Based on this calculation, a value of 1 represents equality between fillet and dietary fatty acid composition. From Watson et al., 2013a.
source diets also resulted in lower total lipid content of the fillets with the TM+SOY diet resulting in 5.81 ± 0.77% lipid, the CO+EFA diet resulting in 5.53 ± 3.15% lipid, and the control ARS fish oil diet resulting in 7.90 ± 1.75% lipid.

Discussion

These studies illustrate the importance of determining species specific requirement levels for compounds which need to be supplemented separately to feeds. Results from the taurine supplementation to a fishmeal based diet indicate that diets containing at least 35% fishmeal contain sufficient taurine and do not require additional supplementation. However, the trial with a fishmeal free, plant protein based formulation reveal negative effects of low taurine such as low growth and high feed conversion, which are alleviated by increasing dietary taurine, up to ~5.0% supplementation. These effects, combined with the lack of modulation at the transcript level of genes involved in potential taurine synthesis and transport appear to indicate that juvenile cobia are unable to synthesize taurine in sufficient quantities, if at all. Taurine should therefore be considered essential, especially when reducing fishmeal in favor of plant based proteins.

DHA and ARA levels were most likely insufficient in the CO+EFA diet to meet the nutritional requirements of juvenile cobia. This also potentially indicates limited or no synthetic capacity for these specific fatty acids in this species. Gilthead sea bream, Sparus aurata, in contrast performed equivalently on all three of the exact same diets from this study (Watson et al., 2013b). The difference in performance of these two species on the same diets could highlight the difference in requirement levels for these fatty acids between species, or different synthetic or conversion capabilities between these two carnivores.

Taken together, these studies demonstrate the ability to produce fishmeal and fish oil free diets for a fast growing, high value marine carnivore without significant loss of production characteristics. Targeted supplementation of specific compounds found to be lacking in fishmeal and fish oil replacement sources can alleviate deficiencies. This type of work will allow for the aquaculture industry to continue to expand to meet growing global needs. Species specific work also has the potential to produce optimal diets which not only maximize production but reduce losses due to waste. These two characteristics, high production and low waste, will be critical in expanding aquaculture operations of various scales and intensity while maintaining profitability around the world as global priorities shift to food and water supply safety and the environmental impacts of aquaculture.

References


Availability of Fisheries By-Product Materials with Cadmium Removal Treatment as a Feed Ingredient for Fingerling Black Rockfish

*Sebastes schlegeli*

Nobukazu SATOH*1, Motoomi WAKASUGI*2, and Shigeharu NOBUTA*3

**Abstract:** Squid liver and scallop-mid gut glands, which are generated and discarded as waste from the Japanese common squid and Japanese scallop, are rich in amino acids and lipids. However, the squid liver and scallop mid-gut glands contain cadmium (Cd) which is generally known as a harmful heavy metal for human health. Hirama et al.*4 recently studied a technique for removal of Cd from squid viscera and scallop mid-gut glands and confirmed the efficacy of the acid leaching and electrolysis method to produce the fisheries by-product materials (meal and extract) with Cd removal treatment as a feed ingredient for cultured fish.

So far we have evaluated the nutritional value of squid viscera meal with Cd removal treatment (dCSVM), which contained 1.5–20 mg/kg Cd, as an alternative protein source to sardine meal in diets for fingerling black rockfish *Sebastes schlegeli*. In our study, it was clearly demonstrated that dCSVM with good protein digestibility could be substituted for 60% of sardine meal in diets for fingerling black rockfish without growth retardation, poor palatability and the problem of Cd accumulation. We suggested that dCSVM was superior to commercial squid viscera meal without Cd removal treatment (CSVM) as a high-quality feed ingredient for black rockfish based on the heavy metal accumulation in fish tissues.

Recently, we are investigating availability of mid-gut glands extract with Cd removal treatment (dCSMGE) as a feed ingredient for fingerling black rockfish. In the recent study, we found that weight gain, specific growth rate, and feed efficiency of fish fed the diet containing dCSMGE at 2% were significantly higher than those of the control. Furthermore, we confirmed that test diets containing dCSMGE was safe in terms of accumulation of Cd.

These results demonstrate that proper inclusion of dCSVM and dCSMGE is effective for the improvement of feed quality in practical diets for fingerling black rockfish.

**Key words:** squid viscera, scallop mid-gut glands extract, cadmium, alternative ingredients for fish meal with Cd removal treatment, black rockfish

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The increasing feed cost in aquaculture production due to the increasing price of fish meal has been a serious problem recently. Therefore, the importance of studies on alternative protein sources for fish meal, a major ingredient for aquaculture feed, in diets has been increasingly realized (Gatlin et al., 2007). However, it is widely recognized that high inclusion of alternative protein source in diets may result in poor palatability, particularly at low temperatures.
Squid liver, squid viscera and scallop mid-gut glands, which are generated and discarded as waste from the Japanese common squid Todarodes pacificus and Japanese scallop Mizuhopecten yessoensis, are rich in amino acids and lipids (Sakuta and Shimakage, 2004). Particularly, by-products of squid processing are important feed ingredients in aquaculture shrimps diets, since the diets contain squid by-products are effective for the improvement of growth performances and feed utilization in prawn species (Hertramp and Piedad-Pascual, 2000). In Hokkaido, Japan, these fisheries wastes are generated and wasted every year, and the volumes of squid viscera and scallop mid-gut glands were 9,324 and 30,533 metric ton in the 2010 fiscal year.

However, the squid liver and scallop mid-gut glands contain cadmium (Cd) at 19 and 39 mg per kg wet weight on average, respectively (Wakasugi et al., 2005; Kruzenski, 2004). Cd is generally known as a harmful heavy metal for human health (Mai et al., 2006). Wakasugi et al. (2009) recently studied a technique (Fig. 1) for removal of Cd from squid viscera and scallop mid-gut glands and confirmed the efficacy of acid leaching and electrolysis to produce fisheries by product materials (meal and extract) with Cd removal. This material was evaluated as a feed ingredient for cultured fish (Wakasugi, 2009). Consequently, they have developed squid viscera meal scallop mid-gut glands extract with Cd removal treatment, dCSV and dCSMGE, respectively.

Black rockfish, Sebastes schlegelii is an important species in Japan (Nakagawa et al., 2007) and Korea (Bai, 2009) that is cultured for stock enhancement and aquaculture. However, information on the alternative protein sources of fish meal in diets for aquaculture is still limited. Aso et al. (1999) reported that scallop viscera meal with Cd removal treatment could substitute 10 % of brown fish meal in the diets for fingerling black rockfish. Lim et al. (2004) showed that dehulled soybean meal could replace fish meal up to 20% without supplementation of methionine and lysine and 30% with these amino acids being supplemented in fingerling and growing black rockfish. Therefore, we studied availability of dCSVM and dCSMGE as a feed ingredient for fingerling black rockfish. The present article reviews information on the feed values of dCSVM and dCSMGE for black rockfish.

### Availability of squid viscera as a feed ingredient for cultured-fish

Availability of various by-products in fisheries as a feed ingredient for aquaculture fish species was studied recently by many researchers, because by-products from fisheries hold promise as potential protein substitutes for fish meal (Li et al., 2004).

It has been reported that extruded diets containing 20-30 % raw squid liver could be used for rainbow trout Oncorhynchus mykiss, yellowtail Seriola quinqueradiata, and red seabream Pagrus major (Mastuda et al., 2001a, b). Feeding studies have shown that the commercial squid viscera meal

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**Fig. 1.** Original production method of scallop mid-gut glands extract with Cd removal treatment (dCSMGE).
without Cd removal treatment (CSVM) can be used as an alternative protein source in diets for various fish species; In fingerling black rockfish, Satoh et al. (2006) showed that CSVM could be substituted for 30% of sardine meal in a diet when growth performance was used as the dependent variable, while substitution of only 10% of sardine meal could be recommended in terms of the heavy metal accumulation in the liver. Mai et al. (2006) evaluated the effects of dietary CSVM on the growth and Cd accumulation in tissues of Japanese seabass, Lateolabrax japonicus. They demonstrated that growth rates in fish fed diets contained 50 and 100g CSVM per kg diet were significantly higher than that in control fish, and that Japanese seabass did not accumulate Cd in the muscle (edible portion), although Cd concentrations in fish kidneys, livers and gills were at high levels (0.4–5.9 mg per kg dry weight). In the paper, they also pronounced that a long-term feeding trial was necessary to investigate Cd accumulation in cultured Japanese seabass. More recently, Wang et al. (2012) reviewed that several fish diets supplemented with CSVM contained high Cd levels, 51–116 mg/kg diet.

**Availability of dCSVM as a feed ingredient for fingerling black rockfish**

We have evaluated the nutritional value of dCSVM, which contained 1.5-2.0 mg/kg Cd, as an alternative protein source to sardine meal in diets for fingerling black rockfish (Satoh et al., 2013a). The Cd level of dCSVM was less than 3.0 mg/kg, which is the allowable level of Cd in livestock feed ingredients according to the Law Concerning Safety Assurance and Quality Improvement of Feeds in Japan. In this study, it was demonstrated that dCSVM with good protein digestibility could be substituted for 60% of sardine meal in diets for juvenile black rockfish without growth retardation, poor palatability and the Cd accumulation, although feed efficiency gradually decreased with the increase of dCSVM inclusion. In that paper, we suggested that dCSVM was superior to CSVM as a high-quality feed ingredient for black rockfish based on the heavy metal accumulation in fish tissues. Furthermore, we found that appropriate replacement (30–60%) of sardine meal by dCSVM was effective for stimulation of feeding in fingerling black rockfish, and that supplementation of methionine and lysine was not necessary with the inclusion of dCSVM. From these results, we concluded that dCSVM was safe and useful as an alternative protein source in fingerling black rockfish diets.

**Availability of dCSMGE as a feed ingredient for fingerling black rockfish**

We also investigated availability of dCSMGE, which contains about 0.5 mg/kg Cd, as a feed ingredient for fingerling black rockfish. The total free amino acids content (mg/kg dry matter) of dCSMGE is about 30,000, while that of fish meal is about 800. dCSMGE contain amino acids effective for feeding stimulants and growth enhancement in various fish species (Mackie and Mitchell, 1985, Shimizu et al., 1999, Ikeda et al., 2012). In the recent feeding trial for 65 days, we found that weight gain, specific growth rate, and feed efficiency of fish fed the diet containing dCSMGE at 2% of the diet were significantly higher than those of the control. Moreover, we confirmed that test diets containing dCSMGE was safe in terms of accumulation of Cd.

Kumai et al. (1989) found that commercial scallop extract as a flavor in the diet for ocelate puffer Takifugu rubripes promoted feed intake and digestive and absorptive functions in the ocelate puffer. Kikuchi and Furuta (2009) reported that blue mussel extract in diets based on fish and soybean meals for tiger puffer would be an effective feeding stimulant. We observed that dCSMGE improved feeding activity of barfin flounder Vraspseudomeris in the short-term feeding experiment at low temperatures. dCSMGE may also be an effective...
feed ingredient for the improvement of growth performance, feed utilization, and feeding activity in fish.

Krill extract and meal are often added to aquaculture feed to improve of platability (Shimizu, 1999) and digestibility (Sato, 2003), but the supplementation in the diets is mostly limited for larval and juvenile fish because of the high price. To date, we estimate the cost of producing dCSMGE (semi-liquid form) will be below half of cost of krill ingredients. On the other hand, the producing cost of dCSMGE in powder form is higher than that in semi-liquid form, since producing of dCSMGE powder requires energy to dry. Interestingly, Kader et al. (2012) found that fermented soybean meal and squid by-product blend (1:1) could be substituted for about 40% of fish meal protein in the diet of Japanese flounder. Likewise, fermented soybean meal and scallop by-product blend (1:1) was able to replace at least 30% fish meal protein in red seabream diet (Kader et al., 2011). These blends are effective for producing low-cost and healthy aquaculture feed, and the fermentation technique may be an effective measure of producing dCSMGE powder.

Conclusion

Our results demonstrate that proper inclusion of dCSV and dCSMGE is effective for the improvement of feed quality in practical diets for fingerling black rockfish. Future research is needed to clarify the nutritional value of dCSV and dCSMGE as a feed ingredient for aquaculture fish species other than black rockfish.

Acknowledgements

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References


Fisheries By-Product Materials as a Feed Ingredient for Fingerling


Cholecystokinin and Trypsin Responses of Larval Red Drum (Sciaenops ocellatus) in Response to Algae, Live Prey, and Inert Particles

Ken WEBB*1

Abstract: In an attempt to better understand the problems in weaning larval fish to artificial diets, our lab has begun to investigate the role of the digestive hormone cholecystokinin (CCK). While there are a number of other labs also investigating CCK and other digestive hormones such as bombesin, PYY, and gastrin, research into the roles of these hormones in fish is still in its infancy. Previous research with red drum larvae suggests that some component of rotifers and algae enable red drum larvae to more efficiently utilize microparticulate diets than when these are not included in the culture system. The current work investigated the impact of soluble components of rotifers and algae on the CCK and trypsin responses of larval red drum at 6 and 10 days post hatch (DHH) as well as the response of red drum larvae to ingestion of inert polystyrene particles at 10 DPH. Introduction of homogenized rotifers was shown to significantly increase whole body CCK levels, CCK mRNA, and trypsin activity in 6 DPH red drum larvae, but not in 10 DPH larvae. Homogenates of Isochrysis galbana did not significantly affect CCK or trypsin at either age. Ingestion of the polystyrene particles was increased in response to the presence of rotifer homogenate and both CCK mRNA and trypsin activity was increased as well. This research suggests that there is a soluble component of rotifers that can upregulate digestive function in larval red drum, at least in 6 DPH larvae, as well as influence consumption.

Annotated Bibliography


The author provides an excellent review of the current understanding of the role of Cholecystokinin (CCK) in satiety and to a lesser extent, digestion. The author focuses on the role of CCK in the cephalic phase and reviews current knowledge of both CCK activators and targets. The author also discussed the role of Leptin in potentiating the effect on CCK on vagal afferent neurons. Of particular note in this manuscript, the author mentions the role of GPR40 and long-chain fatty acids in the secretion of fatty acids while much of the other literature focuses on protein hydrolysate / amino acid roles in promoting CCK secretion.


The authors investigate the role of the PepT1 transporter as the direct mediator of Cholecystokinin (CCK) secretion in response to protein hydrolysate. Before this work, PepT1 was considered a likely mediator of direct mediation of CCK secretion due to previous work that showed a synthetic dipeptide, Gly-Sar, used in PepT1 kinetic studies caused a dose-dependent inhibition of gastric motility consonant with CCK secretion. The work demonstrated that while Gly-Sar did inhibit gastric motility it had no effect on eliciting CCK secretion from CCK-eGFP

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cells. Based on this work, the authors concluded that protein detection by intestinal I cells likely includes both direct and indirect detection but that PepT₁ does not have a direct role in CCK secretion. The authors do however suggest that PepT₁ may function indirectly by stimulating a diazepam-binding inhibitor.


The authors in this work examine the role of protein hydrolysate from blue whiting on CCK secretion in STC-1 cells as well as on long and short-term food intake of rats fed blue whiting muscle hydrolysate (BWMH). The authors found that in the *in vitro* studies on STC-1 cells, peptides from the BWMH stimulated CCK secretion in a dose-dependent response similar to that seen from other types of protein hydrolysate. In the *in vivo* study, the authors found that BWMH produced short-term reductions in food intake but that this reduction was not reflected in the long term. The *in vivo* study did show that blood CCK and GLP-1 levels were more than doubled in rats fed 100 and 250mg of BWMH following a 24h fast lending credence that these may be involved in the short-term decrease in consumption.
Effect of Feed Ingredients on Digestive Enzyme Secretion in Fish

Koji MURASHITA*1, Haruhisa FUKADA*2, Noriyuki TAKAHASHI*2, Noriko HOSOMI*2, Hiroyuki MATSUNARI*1, Hiromi FURUITA*1, Hiromi OKU*1, and Takeshi YAMAMOTO*1

Abstract: In response to the limitation in the global supply of fish meal, the traditional protein source used in aquaculture feed, efforts have increasingly been focused on the use of alternative protein sources of plant origin. However, plant ingredients may cause growth retardation in aquaculture fish species. Feed nutrients must be digested for their utilization, and so pancreatic digestive enzymes have essential roles for the digestion. Also, cholecystokinin (Cck) is known to be a hormone that stimulates the secretion of digestive enzymes in vertebrates. To improve the utilization of plant-based diets in fish, we investigated the effects of various feed ingredients on secretion of digestive enzymes in red seabream Pagrus major and yellowtail Seriola quinqueradiata, which are commercially important aquaculture species in Japan.

We first investigated the effect of soybean meal on digestive enzyme secretion in red seabream. Activities of pancreatic digestive enzymes (trypsin, chymotrypsin, lipase, amylase) in the intestinal content of a soybean meal-based diet (SBM) fed fish were lower than those of a fish meal-based diet (FM) fed fish. Also, lower gene expression levels of the digestive enzymes in the hepatopancreas were observed in the SBM-fed fish compared with the FM-fed fish. These data indicate the FM diet stimulated the secretion/synthesis of pancreatic digestive enzymes to a greater degree than the SBM diet. Then, we tried to identify the stimulation factor in fish meal. Administration of a FM water-soluble fraction increased the gene expression of trypsin, lipase, cck and cck receptor (cck-1r) in yellowtail, suggesting the enzyme stimulation factor may exist in the water-soluble fraction of FM. Supplementation of the enzyme stimulation factor, although not identified yet, may improve the utilization of plant-based diets in aquaculture fish species.

Key words: red seabream (Pagrus major), yellowtail (Seriola quinqueradiata), pancreatic digestive enzymes, fish meal, soybean meal

Fish meal (FM), which is primarily produced from small pelagic fish such as jack mackerel and anchovy, has long been used as the major protein ingredient for fish feeds in aquaculture. However, as the wild stocks of these pelagic fish species become increasingly limited, development of alternative feeds is considered essential to the expansion of sustainable aquaculture worldwide. In response to such a limitation in the global supply of FM, efforts have increasingly been focused on the use of alternative protein sources of plant origin. Especially, soybean meal (SBM) is known to be the most common fish meal-replacement due to its price, high availability in the market and relatively well-balanced amino acid profile. However, fish fed with diets containing high concentrations of plant protein often show low growth performance and digestibility of feed (Gaylord et al., 2008; Glencross et al., 2007).

Feed nutrients must be digested for their utilization, and pancreatic digestive enzymes have essential roles for the digestion; trypsin and chymotrypsin are the main pancreatic proteases, lipase is the major pancreatic lipolytic enzyme, and amylase is known as the major pancreatic digestive

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enzyme for carbohydrates. The exocrine pancreatic enzymes secretion is controlled by both neuronal and hormonal factors (Konturek et al., 2003). In most vertebrates, a peptide hormone cholecystokinin (Cck) is known to be one of the key physiological regulators of pancreatic secretion (Einarsson et al., 1997; Kofuji et al., 2007). Recently, the cck gene has been identified in several fish species such as Atlantic herring Clupea harengus (Kamisaka et al., 2005), Atlantic salmon Salmo salar (Murashita et al., 2009), yellowtail Seriola quinquerguadiata (Murashita et al., 2006) and red drum Sciaenops ocellatus (Webb et al., 2010), and is mainly expressed in the anterior part of the intestine (including the pyloric caeca) (Murashita et al., 2006). Further, the cck receptor (cck-1r) gene has also been identified in yellowtail and is principally expressed in the pyloric caeca (Furutani et al., 2013).

To improve the utilization of plant based diets in fish, we investigated the effects of various feed ingredients on secretion of digestive enzymes in red seabream Pagrus major and yellowtail, which are commercially important aquaculture species in Japan.

Materials and Methods

Effect of soybean meal on digestive enzymes secretion in red seabream: Juvenile red seabream were used for this experiment. Isonitrogenous and isolipidic FM based and SBM based diets were prepared (diet FM and diet SBM). The fish were fed the experimental diets for 6 weeks, and the hepatopancreas and intestinal content of the fish were sampled (n = 6). Activities of pancreatic digestive enzymes (trypsin, chymotrypsin, lipase and amylase) in the intestinal content (n = 7) were analyzed according to Murashita et al. (2007). Gene expression levels of the enzymes in the hepatopancreas were evaluated by real-time quantitative PCR (qPCR) using the specific primers for red seabream digestive enzymes.

Effect of fish meal on gene expression levels of trypsin, lipase, cck and cck-1r in yellowtail: Watersoluble and water-insoluble fractions of FM were prepared. The same amount of the two FM fractions on crude protein basis were orally administrated to juvenile yellowtail according to Murashita et al. (2008). The pyloric caeca was sampled from the fish.

![Graphs showing enzyme activities](image)

**Fig. 1.** Effects of fish meal (FM) and soybean meal (SBM) based diets on activities of the pancreatic digestive enzymes in intestinal content of red seabream. Values are mean ± SE (n = 7 fish).
and the gene expression levels of trypsin, lipase, cck and cck-1r were analyzed by qPCR according to Furutani et al. (2013).

Results and Discussion

Effect of soybean meal on digestive enzymes secretion in red seabream: Activities of all four pancreatic digestive enzymes examined in the intestinal content of SBM fed fish were lower than those of FM fed fish (Fig. 1), indicating the amounts of enzymes secreted into the intestine in the SBM fed fish were lower than those of the FM fed fish. Also, lower gene expression levels of the digestive enzymes in the hepatopancreas were observed in the SBM fed fish compared with the FM fed fish (Fig. 2), which is in line with the report in yellowtail; orally administrated FM increased the trypsin and lipase gene expressions in the pyloric caeca, but not in fish administrated SBM (Furutani et al., 2012). These data indicate that SBM does not fully stimulate the secretion/synthesis of the pancreatic digestive enzymes, or, FM strongly stimulates the digestive enzymes secretion/synthesis.

Effect of fish meal on gene expression levels of trypsin, lipase, cck and cck-1r in yellowtail: Administration of a FM water-soluble fraction increased the gene expressions of trypsin, lipase, cck and cck-1r in yellowtail, whereas those of fish administrated a FM water-insoluble fraction did not (Fig. 3, brief results), suggesting the enzyme stimulation factor may exist in the water-soluble fraction of FM. We are trying to identify the enzyme stimulation factor from the FM water-soluble fraction.

The main component of the FM water-soluble fraction may be very small peptides and/or free amino acids (data not shown). It is known that some amino acids strongly stimulate CCK release in rodents (Daly et al., 2013); such a mechanism might also exist in fish. Supplementation of the enzyme stimulation factor may improve the utilization of plant based diets in aquaculture fish species.

![Graphs of trypsin, chymotrypsin, amylase, and lipase gene expression](image_url)

Fig. 2. Effects of fish meal (FM) and soybean meal (SBM) based diets on gene expression of pancreatic digestive enzymes in the hepatopancreas of red seabream. Data for each gene is represented as mean calculated copy number normalized against red seabream eif1a copy numbers. Values are mean ± SE (n = 6 fish).


Murashita K., Fukuda H., Rønnestad I., Kurokawa T., and Masumoto T., 2008: Nutrient control

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Fig. 3. Effect of fish meal on gene expression levels of trypsin, lipase, cck and cck-1r in yellowtail. The schematic diagram of the experiment is presented with brief results.


Annnotated Bibliography


Cholecystokinin (Cck) and neuropeptide Y (Npy)-related peptides are the key regulators of pancreatic enzyme secretion in vertebrates. Cck stimulates enzyme secretion whereas peptide Y (Py or Ppyb), a Npy-related peptide, plays an antagonistic role to that of Cck. In fish, very little is known about the effects of different nutrients on the synthesis of Cck and Py in the digestive tract, and the mechanism by which Cck and Py actually regulate digestive enzyme secretion is not well understood. In order to determine stimulating effects of different nutrients on the synthesis of Cck and Py in yellowtail (Seriola quinquerguadiata), cck and py mRNA levels in the digestive tract were measured after oral administration of a single bolus of either phosphate-buffered saline (PBS: control), starch (carbohydrate), casein (protein), oleic acid (fatty acid) or tri-olein (triglyceride). In addition, in order to confirm the synthesis and secretion of digestive enzymes, the mRNA levels and enzymatic activities of three digestive enzymes (lipase, trypsin and amylase) were also analyzed. Casein, oleic acid and tri-olein increased the synthesis of lipase, trypsin and amylase, while starch and PBS did not affect the activity of any of these enzymes. cck mRNA levels rose, while py mRNA levels were reduced in fish administered casein, oleic acid and tri-olein. These results suggest that in yellowtail, Cck and Py maintain antagonistic control of pancreatic enzyme secretion after intake of protein and/or fat.


Cholecystokinin (Cck) is the key regulator hormone that stimulates the secretion of digestive pancreatic enzymes in vertebrates. In fish, little is known about the mechanism of induction of Cck in the digestive tract by different feed ingredients. To investigate the response of cck and digestive enzymes to fish feed ingredients in yellowtail Seriola quinquerguadiata, we performed a series of experiments in which we measured the mRNA levels of cck, trypsin, and lipase after oral administration of a single bolus of various ingredients. We administered fish meal and fish oil in experiment 1; and high and low concentrations of fish meal in experiment 2; and five different dietary protein sources (fish meal, soybean meal, soy protein concentrate, corn gluten meal, and glutamic acid fermentation by-products) in experiment 3. In experiments 1 and 3, only fish meal significantly increased the mRNA level of cck and digestive enzyme. In experiment 2, a high concentration of fish meal [20 % (w/v)] significantly increased the cck and trypsin mRNA levels, but a low concentration of fish meal [1 % (w/v)] did not. These results suggest that high concentrations of fish meal (the protein source in fish feed) has the most potent effect on stimulation of cck synthesis and secretion of digestive enzymes in yellowtail.


In vertebrates, the peptide cholecystokinin (Cck) is
one of the most important neuroregulatory digestive hormones. Cck acts via Cck receptors that are classified into two subtypes, Cck-1 receptor (Cck-1r; formally Cck-a) and Cck-2 receptor (formally Cck-b). In particular, the Cck-1r is involved in digestion and is regulated by Cck. However, very little information is known about Cck-1r in fish. Therefore, we performed molecular cloning of cck-1r cDNA from the digestive tract of yellowtail Seriola quinquergiata. Phylogenetic tree analysis showed a high sequence identity between the cloned yellowtail cck receptor cDNA and cck-1r, which belongs to the cck-1r cluster. Furthermore, the expression of yellowtail cck receptor mRNA was observed in gallbladder, pyloric caeca, and intestines, similarly to cck-1r mRNA expression in mammals, suggesting that the cloned cDNA is the yellowtail cck-1r. In in vivo experiments, the cck-1r mRNA levels increased in the gallbladder and pyloric caeca after feeding, whereas in vitro, mRNA levels of cck-1r and digestive enzymes in cultured pyloric caeca increased by the addition of Cck. These results suggest that Cck-1r plays an important role in digestion stimulated by Cck in yellowtail.
Development and Characterization of Several Open Formula Reference Diets for Marine Fish Larvae

Michael B. RUST*1, Frederic T. BARROWS*2, Mark DRAWBRIDGE*3, Emily R. HART*1, Kevin STUART*1, Ken WEBB*1, Harold J. BARNETT*1, Peter M. NICKLASON*1, and Ronald B. JOHNSON*1

Abstract: A limiting constraint in the development and growth of marine aquaculture is successful hatchery production. Presently, larval finfish are raised using live feeds such as copepods, rotifers and Artemia. Live feeds are expensive, time-consuming, labor intensive, unreliable and nutritionally imperfect. An alternative to live feeds is microparticulate feeds, however, high performance microparticulate diets for larval finfish are typically closed formula commercial diets that make scientific inference difficult or impossible. We describe a series of open formula microparticulate reference diets that have been formulated and produced to facilitate comparisons across species and systems for use by the scientific community. Open formula diets can produce consistent results among trials and species, they can be simply formulated with well-defined ingredients and provide a basic platform for improvement. The reference diets were processed by three methods: flaking (F), microextrusion followed by marumerization (MEM) and particle-assisted rotational agglomeration (PARA). An additional two diets were made by using the flake diet and further processing it using the MEM (F-MEM) or PARA (F-PARA) methods. The F, F-PARA and F-MEM diets had the same formulation and only differed by processing method. PARA and MEM had unique formulations. Each diet was screened to the 400-700 µm range. All microparticulate diets were compared to enriched Artemia and rotifers (Brachionus plicatilis) for chemical analysis. Scanning electron microscopy (SEM) was used to visualize the microparticles between 22x and 4000x magnification. It was clear that micropellet structure, sinking rate and leaching was influenced by both formulation and manufacture method. Basic structure remained largely intact for all but the F-PARA particles even after being immersed in fresh water for 15 minutes. Compositional data, leaching half-life and sinking rates are given in the table below (values in a column with differences at P<0.05 are denoted by superscripts). Data on feeding trials with larval fish indicate that two of the open formula diets performed as well as, or almost as well as, a popular commercial diet (Otohime, Marubeni Nisshin Feed, Tokyo, Japan) with larval yellowtail (Seriola lalandi), white sea bass (Atractoscion nobilis) Pompano (Trachinotus carolinus) and red drum (Sciaenops ocellatus). This performance illustrates that these diets provides a good base on which to make improvements.

Annotated Bibliography


In addition to a review of methods to make older micro bound feeds for aquaculture, methods to
produce microparticulate feeds using micro extrusion and particle assisted rotational agglomeration are explained.

Step by step instructions to produce zein and carrageenan bound diets developed in Japan are explained in English.

Nutrition is particularly important in the healthy development of fish during their early-life stages. Understanding the unique nutritional needs of larval fish can improve the efficiency and quality of fish reared in a culture setting. Larval Fish Nutrition comprehensively explores the nutritional requirements, developmental physiology, and feeding and weaning strategies that will allow aquaculture researchers and professionals to develop and implement improved culture practices. Larval Fish Nutrition is divided into three sections. The first section looks at the role of specific nutrient requirements in the healthy digestive development of fish. The second section looks at the impacts if nutritional physiology on fish through several early-life stages. The final section looks at feeding behaviors and the benefits and drawbacks to both live feed and microparticulate diets in developing fish.

Good review and discussion of several types of microparticulate diets including complex microparticles.

Food uptake follows rules defined by feeding behaviour that determines the kind and quantity of food ingested by fish larvae as well as how live prey and food particles are detected, captured and ingested. Feeding success depends on the progressive development of anatomical characteristics and physiological functions and on the availability of suitable food items throughout larval development. The fish larval stages present eco-morpho-physiological features very different from adults and differ from one species to another. The organoleptic properties, dimensions, detectability, movements characteristics and buoyancy of food items are all crucial features that should be considered, but is often ignored, in feeding regimes. Ontogenetic changes in digestive function lead to limitations in the ability to process certain feedstuffs. There is still a lack of knowledge about the digestion and absorption of various nutrients and about the ontogeny of basic physiological mechanisms in fish larvae, including how they are affected by genetic, dietary and environmental factors. The neural and hormonal regulation of the digestive process and of appetite is critical for optimizing digestion. These processes are still poorly described in fish larvae and attempts to develop optimal feeding regimes are often still on a 'trial and error' basis. A holistic understanding of feeding ecology and digestive functions is important for designing diets for fish larvae and the adaptation of rearing conditions to meet requirements for the best presentation of prey and microdiets, and their optimal ingestion, digestion and absorption. More research that targets gaps in our knowledge should advance larval rearing.
## Open Formula Reference Diets for Marine Fish Larvae

<table>
<thead>
<tr>
<th>Feed</th>
<th>Moisture (% as fed)</th>
<th>Proximate Composition (% dry weight)</th>
<th>Fatty Acid Concentrations (mg fatty acid/100mg total fatty acids)</th>
<th>Protein Leaching $t_{1/2}$ (% lost)</th>
<th>Sinking Rate (cm/sec)</th>
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</thead>
<tbody>
<tr>
<td>MEM</td>
<td>5.7</td>
<td>53.8 30.5 8.4</td>
<td>0.4 7.5 5.7</td>
<td>98/23.0</td>
<td>0.84±0.010a</td>
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<td>PARA</td>
<td>5.3</td>
<td>54.2 29.7 8.9</td>
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<td>94/30.2</td>
<td>0.34±0.001c</td>
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<tr>
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<td>9.0</td>
<td>75.9 10.9 7.3</td>
<td>1.0 6.0 15.2</td>
<td>20/44.3</td>
<td>0.33±0.001c</td>
</tr>
<tr>
<td>F-MEM</td>
<td>13.3</td>
<td>75.6 13.4 7.5</td>
<td>1.0 5.3 13.0</td>
<td>50/41.8</td>
<td>0.78±0.060a</td>
</tr>
<tr>
<td>F-PARA</td>
<td>11.0</td>
<td>75.6 12.6 7.3</td>
<td>1.0 6.0 15.2</td>
<td>33/47.2</td>
<td>0.61±0.001b</td>
</tr>
<tr>
<td>Artemia</td>
<td>91.3</td>
<td>NA 15.3 13.7</td>
<td>5.6 12.1 7.1</td>
<td>NA/0.1</td>
<td>NA</td>
</tr>
<tr>
<td>Rotifers</td>
<td>90.1</td>
<td>49.6 15.5 17.7</td>
<td>1.5 5.7 23.3</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
An Analysis of the Causality between the Market Price of Imported Fishmeal and the Market Price of Marine Farmed Fish

Yoshifumi TAKAHASHI*1

Abstract: The purposes of this paper are to introduce the historical background and price trends of the imported fishmeal market, and to reveal the pattern of causality between prices in the imported fishmeal market and the market prices of yellowtail and sea bream in landing areas. Granger causality test was used in this analysis.

The results were as follows: 1) there is causality between the price of imported fish meal and the market price of sea bream in landing areas, 2) but there is no causality among the market prices of all combinations without imported fishmeal and sea bream.

Key words: Granger causality test, market price of imported fishmeal, market prices of pisciculture

In Japan, many piscicultural farmers are facing difficulties due to the impact of falling fish prices and the rising cost of feed. These are serious problems because they can result in a great reduction in profits for many farmers. According to statistics produced by the Japanese Corporation of Feed for Pisciculture (Nihon Yougyo Shiryou Kyoukai), the production of feed reached 360,000 tons in 2011, the majority of which was for yellowtail and sea bream. These feedstock were made from raw ingredients such as fishmeal, starch, and fish oil. Fishmeal was especially important, making up 50% or more of the feed content. Therefore, piscicultural farming yellowtail and sea bream always worry about soaring imported fishmeal prices. This research investigates the relationship of price changes in imported fishmeal on the market price of piscicultural products.

The purposes of this paper are to introduce the historical background and price trends of the imported fishmeal market, and to reveal the pattern of causality between prices in the imported fishmeal market and the market prices of yellowtail and sea bream in landing areas. Granger causality test was used in this analysis.

The domestic production of fishmeal and the market price of major farmed fishes

The domestic production of fishmeal came mainly from inshore fisheries in northern Japan between 1959 and 1991 (Fig. 1), and the main ingredient, Pollack, was used on the grounds that it was fresh and suitable for processing. However, the catch of Pollack has decreased year by year from then as a result of increasing demand for fish pastes and the impact of fishery treaties such as the 200 mile exclusive fishing zone. As a result, the amount of imported fishmeal has increased gradually to make up for insufficient supplies of local ingredients in Japan. More than half current fishmeal stocks are imported from three countries in South America.

Fig. 1. Domestic production of fishmeal and feed for pisciculture in Japan

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Fig. 2 shows the trend of market prices in imported fishmeal, farmed yellowtail and farmed sea bream during the six years from 2007. The market price changes of yellowtail and sea bream have been subject to violent fluctuations within certain limits. As for only yellowtail, there is an exception that the price of yellowtail soared upwards in June 2008 because of little production. Imported fishmeal has also ranged in price from 90 to 175 yen/kg.

Generally, the market price of a commodity tends to rise when the price of ingredients rises; this phenomenon is called “cost push inflation”. As seen in Fig. 2, there have been price changes over time, but we cannot conclude that there has been any relationship between changes in the price of one commodity and that of another. Therefore we decided to analyze the relationships among prices using statistical approaches.

Data and Method

Data: Time series data about piscicultural products such as yellowtail and sea bream are available from the website of JAFIC (Japan Fisheries Information Service Center). These original data are nominal, so for this paper these were deflated with “yellowtail” and “sea bream” in the CPI (Consumer Price Index). Also time series data of imported fishmeal are available from the website of the MOF (Ministry of Finance), and have also been deflated with “fishmeal” in the IPI (Import Price Index).

The data first had to be tested for “stationarity” or “not stationarity” before processing in the Granger causality test, because time series data in economics has an almost unit root. Regression among data sets having unit roots has no meaning because of the potential for high correlations among those data. The DF-GLS (Dicky-Fuller is Based on GLS Detrending) test is reliable statistical method to check for unit root, so this was applied. The first test with raw data was not suitable for null hypothesis testing. As a countermeasure for the unit root problem, first difference data was used and then retested in the same way. The result of this retest made it possible for null hypothesis testing to function correctly (Table 1). From this, first difference data whose lag is 0 was adopted.

Analysis method: Analysis method used in this paper

Table 1. Result of the DF-GLS test

<table>
<thead>
<tr>
<th>Lag</th>
<th>Yellowtail price lag statistic (5%)</th>
<th>Sea bream price lag statistic (5%)</th>
<th>Imported fishmeal price lag statistic (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.804 *</td>
<td>1.474</td>
<td>1.773</td>
</tr>
<tr>
<td>2</td>
<td>2.863 *</td>
<td>0.812</td>
<td>0.789</td>
</tr>
<tr>
<td>3</td>
<td>3.258 *</td>
<td>0.611</td>
<td>0.499</td>
</tr>
<tr>
<td>4</td>
<td>2.817 *</td>
<td>0.494</td>
<td>0.570</td>
</tr>
<tr>
<td>5</td>
<td>2.401 *</td>
<td>0.496</td>
<td>0.648</td>
</tr>
<tr>
<td>6</td>
<td>2.004 *</td>
<td>0.496</td>
<td>0.638</td>
</tr>
<tr>
<td>7</td>
<td>1.820 *</td>
<td>0.325</td>
<td>0.069</td>
</tr>
<tr>
<td>8</td>
<td>1.249 *</td>
<td>0.448</td>
<td>0.229</td>
</tr>
<tr>
<td>9</td>
<td>0.937 *</td>
<td>0.336</td>
<td>0.305</td>
</tr>
<tr>
<td>10</td>
<td>0.820 *</td>
<td>0.316</td>
<td>0.015</td>
</tr>
<tr>
<td>11</td>
<td>0.303 *</td>
<td>0.136</td>
<td>0.114</td>
</tr>
<tr>
<td>12</td>
<td>0.119 *</td>
<td>0.243</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Note: * indicates statistical significance at the 5% level, ** at 5% level
was the Granger causality test in a VAR (Vector Autoregression) model. An AR (Autoregression) single variable model was used, and current value was determined by past own value and white noise (for more information on the VAR model and Granger Causality Test, see chapter 12 and chapter 13 in Maddla (1992)). The VAR model is an expanded AR model, from single to multivariable cases, although the current values of the multivariables are also determined by past own values and white noise.

In this section, the two variables VAR(1) model that was actually used is described. The values given in parentheses are lag numbers. Here, \( x_t \) and \( y_t \) are the price data of commodity at time \( t \), \( \varepsilon \) denotes error terms, and \( a_i \) is an individual parameter.

\[
\begin{align*}
\begin{cases}
    x_t = a_{11} x_{t-1} + a_{12} y_{t-1} + \varepsilon x_t \\
    y_t = a_{21} x_{t-1} + a_{22} y_{t-1} + \varepsilon y_t
\end{cases}
\end{align*}
\] ..............................(1)

The Granger causality test is examined by equation (1). The basic idea of the Granger causality test is that the past value of variable \( y \) can affect variable \( x \) (or not). It can determine that variable \( y \) can affect variable \( x \) if the null hypothesis of “\( a_{12} = 0 \)” is rejected by \( F \) test. The following equation (3) is the form of equation (2) where “\( a_{12} = 0 \)”.

\[
\begin{align*}
    x_t = a_{11} x_{t-1} + a_{12} y_{t-1} + \varepsilon x_t \\
    x_t = a_{11} x_{t-1} + \varepsilon y_t
\end{cases}
\] ..............................(2)

The residual sum of squares of equation (2) replaces \( RSS \), the residual sum of squares of equation (3) replaces \( RSS_0 \), and we can define the F value using the following equation:

\[
F = \frac{(RSS_0 - RSS)/q}{RSS/(n - r - 1)}.
\]

Where \( n \) is the number of samples, \( q \) is the number of constraint conditions such as “\( a_{12} = 0 \)” and \( r \) denotes the number of variables without a constant term in equation (2).

**Results and Discussion**

The VAR model has a problem with respect to lag length selection, so it is necessary to select optimal lag length to obtain an accurate result before applying the Granger causality test. In this paper, the estimation of lag length selection was carried out with four model types which were “none”, “const”, “trend” and “both”. “None” has no drift and trend, “const” has only drift, “trend” has only trend, and “both” has both of these. The estimation of these four types were judged by using S.C. (Schwarz Information Criteria). Consequently, “none” and “lag length = 1” are selected in all combinations of two variable VAR models.

Fig. 3 shows the results of the Granger causality test. There is no causality of price between yellowtail and sea bream, and this is natural because yellowtail cannot be substituted for sea bream. Also, changes in the price of farmed fish have no influence on the price of imported fishmeal. Why the price of imported fishmeal can affect the market price of sea bream in Japan. We do not have a scientific explanation for the fact that this relationship only affects the price of sea bream. Nevertheless, according to Demura (2010), feed stocks for farmed sea bream are dependent on fishmeal even if the price of imports rises. Conversely, feed stocks for yellowtail can be changed to other ingredients such as raw fish if the price of imported fishmeal rises too high. This situation is probably the explanation.

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Good Aquaculture Practices to Reduce the Use of Chemotherapeutic Agents, Minimize Bacterial Resistance, and Control Product Quality

Stanley SERFLING*

Abstract: A significant challenge to the expansion of aquaculture production is controlling the outbreak of disease. Many farmers who experience the potential loss of stock from disease may choose to use chemotherapeutic agents to minimize their loss. It is generally understood that a disease in aquaculture is a combination of the health of the animal, the condition of the environment and the presence of a pathogen. From this concept there are a number of precautionary measures that farmers may practice to minimize disease outbreaks. The principles of Hazard Analysis Critical Control Point (HACCP) may be useful risk management tools to reduce pathogens, animal stress and the need for chemotherapeutic agents to control disease outbreaks on the farm.

Key Words: good aquaculture practices, chemotherapeutic agents, bacterial resistance, food safety

Introduction

As the global demand for seafood increases, the trend towards high density aquaculture systems grows. These systems referred to as “intensive aquaculture” increasingly rely on inputs of oxygen, formulated feed, and the application of agrochemicals, and antibiotics. This method of culture may also create stressful conditions for the animals and increase the risk of bacterial infections and therefore the use of chemotherapeutic agents (Sapkota et al., 2008).

Shrimp is the single most popular type of seafood in the United States and over 90 percent of shrimp products are farm raised and imported primarily from Southeast Asian countries. The primary hazards associated with aquaculture shrimp are contamination from pathogens and residues from unapproved drugs. The origins for these hazards are from production farms and can remain in or on the product throughout the normal cleaning, rinsing and packaging process.

Between 2001 and 2003 the U.S. Food and Drug Administration analyzed 1,234 samples from 103 shrimp aquaculture farms representing six countries for fecal coliforms, Escherichia coli, and Salmonella. A significant relationship was found between the log number of fecal bacteria and the probability that any given sample would contain Salmonella. The study concluded the occurrence of Salmonella bacteria in shrimp from aquaculture farms is directly related to the concentration of fecal bacteria in the source and production pond water (Koonse et al., 2005).

The importance of chemical and antimicrobial compounds in the protection of animals has been acknowledged, but the negative impact and use of these agents in animals raised for food is a concern.
The detection of certain banned chemotherapeutic agents in fish and crustaceans in international trade during 2001–2002 led to greater attention about the potential health risks from farm raised seafood products (Iddya, 2012).

**Good Aquaculture Practices**

Good aquaculture practices (GAqP) can be defined in many ways. In general it is based on preventive practices that are developed and implemented to meet the needs of the species, culture methods and local environmental conditions. When aquaculture producers take time to identify potential risks to their operation and implement controls to manage the risk, then the potential for disease and the use of chemotherapeutic agents is minimized.

The Hazard Analysis Critical Control Point (HACCP) principles can be used as a preventive risk management system to control the introduction of pathogens at aquaculture facilities. The HACCP approach is based on prevention and requires a hazard analysis that identifies a potential hazard in the system and then a Critical Limit, with a maximum and or minimum point, is set for each component of the system. When monitoring the aquaculture system and a critical limit has been in violation, then a corrective action is taken to bring the system back into compliance (Jahncke et al., 2002).

These HACCP principles provide a step by step approach to identify and control hazards found in the environment and production process for both freshwater and marine aquaculture. The following are the seven basic principles with an example:

1. **Hazard and Risk Analysis**: Collect and evaluate information on hazards associated with the product under consideration to decide the likelihood of the occurrence and the severity of the occurrence. To help identify a potential hazard a farmer should construct a flow diagram with all of the inputs to the farm and then evaluate a control for each potential hazard.
   - See Fig. 3 for a shrimp farm flow diagram.

2. **Identify Critical Control Point (CCP)**: This step is essential to prevent or eliminate a hazard or reduce it to an acceptable level.
   - For example a shrimp farmer may choose critical water quality parameters such as minimum and maximum oxygen and temperature levels.

3. **Establish Critical Limits**: A maximum and/or minimum value to which a physical, chemical or biological hazard must be controlled to prevent, eliminate or reduce to an acceptable level the occurrence of a hazard.
   - For example a critical limit could be stocking density at a hatchery tank and the ratio of live feed to animal.

4. **Establish Monitoring Procedures**: Monitoring is a process that the aquaculture operation relies on to maintain control of a Critical Control Point. Accurate monitoring is important to help determine when a CCP has deviated from the desired range. Failure to properly use and monitor chemotherapeutic agents may result in detectable residues in the tissue of aquacultured products.
   - For example, monitoring a specific water quality parameter every day or more frequently depending on the production system.

5. **Establish Corrective Actions**: The farmer should identify procedures to follow when a deviation from the acceptable range occurs.

![Fig. 2.](image)
Fig. 3. Process flow diagram of an integrated shrimp farm with hatchery.

The farmer will implement a corrective action plan after the deviation has been identified and prepare a long term solution as soon as possible.

- For example if a farmer has identified the feed supply for the hatchery does not conform to required standard, the product is rejected and the farmer contacts an alternate feed supplier for new feed.

6. Establish Verification Procedures: This procedure helps to determine the validity of the operation and verify that the farm is operating according to plan.

- For example the farmer may review daily water quality parameter sand feeding records to determine if the production system is meeting expected output.

7. Establish Record Keeping Procedures: The final step is to document all previous steps, from the Evaluation of Hazards and flow diagram, the identification of Critical Control Points and Critical Limits, and any Corrective Actions taken, and all Verification Procedures.

- Records should be made for all identified Critical Control Points and be accessible to staff in paper or electronic format.

Fig. 3 is an example of an operational flow diagram for an integrated shrimp farm with brood stock, hatchery, nursery, and growout production.
Preventive Measures

A standard operating procedure (SOP) manual is an important document the farmer should assemble. This should include facility design and flow diagram, general husbandry procedures for each step in the production process, restricted access areas, waste management, pest control and staff rules. Good Aquaculture Practices may also include welfare of the animals, environmental integrity and social responsibility.

There are a number of biological, chemical and physical precautionary measures that can be undertaken. The following are examples of how to apply these practices on the farm.

**Biological:** Includes measures to prevent or treat infections such as the proper use of chemotherapeutic agents or the use of vaccines as well as management practices to prevent the spread of pathogens by isolating and testing incoming seed stock. In addition, providing clean water and sanitary facilities for staff and proper farm security help to control the spread of pathogens from humans and animals.

**Chemical:** Includes measures that are used to prevent the introduction of pathogens or vectors by treating materials before they enter the facility. For example, chlorination or ozone can be used to treat incoming water, and iodine and chlorine can be used to treat other potential vectors such as tools, footwear and clothing.

**Physical:** Includes barriers that may be useful to prevent vectors from contaminating a farm site. Physical barriers can be stress reducing practices like proper stocking densities, optimal levels for dissolved oxygen and temperatures, and proper feeding practices.

### Table 1. The following hazard analysis for an intensive marine shrimp farm.

<table>
<thead>
<tr>
<th>Identify Potential Hazard</th>
<th>Is the Hazard Significant</th>
<th>Justification</th>
<th>Preventive Measures</th>
<th>CCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed stock</td>
<td>Yes</td>
<td>Shrimp larvae may contain detectable pathogen</td>
<td>SPF certification for each receipt. Quarantine procedures and test periodically (CCP)</td>
<td>Yes</td>
</tr>
<tr>
<td>Water Source</td>
<td>Yes</td>
<td>Potential pathogens</td>
<td>Provide pre-treatment to ensure water supply is safe (CCP)</td>
<td>Yes</td>
</tr>
<tr>
<td>Feed Source</td>
<td>Yes</td>
<td>May result in and poor production</td>
<td>Certificate from feed supplier to ensure standard (CCP)</td>
<td>Yes</td>
</tr>
<tr>
<td>Stocking Density</td>
<td>Yes</td>
<td>Important to optimize production</td>
<td>Controlled by Standard Operating Procedure (SOP)</td>
<td>Yes and No</td>
</tr>
<tr>
<td>Effluent or Recirculated Water</td>
<td>Yes</td>
<td>Effluent may contain pathogens</td>
<td>Controlled by Standard Operating Procedure (SOP)</td>
<td>Yes and No</td>
</tr>
<tr>
<td>Animal and Human Vectors</td>
<td>Yes</td>
<td>May transfer contaminants and pathogens</td>
<td>Controlled by Standard Operating Procedure (SOP)</td>
<td>No</td>
</tr>
<tr>
<td>Farm Equipment and Sanitation</td>
<td>Yes</td>
<td>Equipment may become contaminated with pathogens</td>
<td>Controlled by Standard Operating Procedure (SOP)</td>
<td>No</td>
</tr>
</tbody>
</table>

SPF – Specific Pathogen Free  
CCP – Critical Control Point  
SOP – Standard Operating Procedure
Conclusion

Good Aquaculture Practices may be applied to many different kinds of aquaculture systems in a variety of management strategies. The key elements can be summarized as reliable sources of stock, good diagnostic and detection methods for disease, disinfection and eradication of pathogens and best management practices. HACCP principles provide a logical step by step approach to help prevent pathogen contamination and the outbreak of disease on the farm site. This management approach allows the farmer to choose the most important process control steps and then choose appropriate critical control points and control measures to minimize risk to the animals and the farm operation and insure that chemotherapeutic agents if needed will be used wisely.

References


Annotated Bibliography


The authors identify how the principles of HACCP (Hazard Analysis Critical Control Point) can be used as a preventive risk management system to control the introduction of pathogens and the outbreak of disease in shrimp aquaculture facilities. The HACCP approach requires a hazard analysis that identifies a potential hazard in the system and then a Critical Limit with a maximum and or minimum point is set for each component of the system. When monitoring the aquaculture system and a critical limit has been in violation, then a corrective action is taken to bring the system back into compliance.

To develop an effective biosecurity program fish and shrimp farmers should follow these principles based on seafood HACCP: 1) Perform Systematic Hazard Analysis, 2) Determine Critical Control Points, 3) Establish Critical Limits, 4) Determine Appropriate Corrective Actions, 5) Establish Monitoring Procedures, 6) Establish Verification Procedures, 7) Establish Record Keeping Systems.


The Food and Agriculture Organization of the United Nations (FAO), the World Health
Organization (WHO) and the World Organization for Animal Health (OIE) organized consultations and technical meetings to review the global seafood trade and the use of antimicrobial agents in aquaculture and develop recommendations. The author outlines how detection of certain banned antibiotics in fish and crustaceans in international trade during 2001–2002 lead to greater attention on the public health risks owing to the use of antimicrobial agents in aquaculture. Most fish importing countries adopt a zero tolerance approach regarding residues of antimicrobials that are banned for use in food animals. In such cases, residue levels that attract regulatory action are based on analytical capability rather than toxicology of the residues.


The authors examined the prevalence of Salmonella and coliform bacteria on shrimp aquaculture farms in several Asian countries to develop guidelines or preventative measures for reducing Salmonella and fecal contamination on products harvested from these farms. A significant relationship was found between the log number of fecal bacteria and the probability that any given sample would contain Salmonella. The likelihood of finding Salmonella in grow-out pond water increased 2.7 times with each log unit increase in fecal coliform concentration and 3.0 times with each log unit increase in E. coli concentration. Salmonella is not part of the natural flora of the shrimp culture environment nor is it inherently present in shrimp grow-out ponds. The occurrence of Salmonella bacteria in shrimp from aquaculture operations is related to the concentration of fecal bacteria in the source and grow-out pond water.


The authors conducted an extensive literature research with 145 peer reviewed papers to identify current global aquaculture practices and the potential health risks from aquacultured seafood. The authors focused on aquaculture production in Asia as having 11 of the top 15 producing countries in the world. The paper identifies chemical and biological contaminants used by the aquaculture industry and the potential impacts on food safety and human health. Tables identify the top 15 countries that use antibiotics and the prevalence of antibiotic resistance in the environment as a potential health concern. As global aquaculture production continues to increase worldwide with an emphasis on intensive systems, exposure to various hazardous chemical and biological compounds is a concern. The authors conclude the increased use of chemotherapeutic agents is contributing to elevated levels of antibiotic residues and antibiotic resistant bacteria.
Modeling Intraspecific Genetic Effects for Management of Aquaculture Programs

Jason D. VOLK*1, Michael B. RUST*2, Gregory R. BLAIR*1, Lars E. MOBRAND*1, Conrad V. W. MAHNKEN*2, and Walton W. DICKHOFF*2

Abstract: Rapid worldwide development of marine finfish cage farming has raised awareness over the possible genetic and ecological effects of escaped fish on wild populations. With increased interest in implementation of marine aquaculture in the United States, NOAA Fisheries and other regulators charged with stewardship of marine ecosystems need tools to understand and mitigate risks presented by aquaculture escapees. To develop an understanding of genetic and ecological effects of escapes and design management strategies to address potential risks to marine resources, NOAA Fisheries has developed a numerical decision-support tool: the Off-shore Mariculture Escapes Genetics/Ecological Assessment (OMEGA) model. The OMEGA model is an extension of concepts from another model, the All-H Analyzer (AHA) that is used successfully in the U.S. Pacific Northwest to evaluate genetic and ecological interactions between hatchery and wild salmon and trout.

OMEGA model input parameters include size and growth characteristics of cultured fish, frequency and magnitude of escape events, survival of escapees in the wild, probability of escapees encountering a conspecific natural population and interbreeding, and population dynamics of the natural population. Model results describe the influence of aquaculture escapees on spawning biomass, juvenile production, and genetic fitness of the composite population. Effects of interactions on fitness and abundance are based on the frequency and relative abundance of cultured fish that escape and survive to encounter a natural population, the difference in survival characteristics between the artificial and the natural environments, and the genetic legacy of the cultured and natural populations.

NOAA Fisheries is using the OMEGA model to identify and evaluate risks of marine aquaculture operations, design sustainable aquaculture programs, explore the effects of regulation, and identify research priorities for areas of uncertainty.

This talk will describe the model and present results for a hypothetical sablefish (*Anoplopoma fimbria*) culture program along the U.S. West Coast. We are interested in speaking with any and all individuals interested in collaborating on the further development of the model, applying the model to other species of interest such as rockfish (*Sebastes* spp.), yellowtail (*Seriola quinquergiata*), salmon (*Oncorhynchus* *spp*), or any other aquaculture candidate species, and to identify opportunities to validate model results.

Key words: escapes, survival, introgression, simulation, fitness, selection, OMEGA.

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Modeling intraspecific genetic effects for Management of Aquaculture Programs

The development of world cage farming of marine fishes has raised concerns over the possible genetic and ecological impact of escaped fish on wild populations. Marine fish can escape from farms for a variety of reasons including: when improper mesh size is used or when holes in webbing develop from normal wear and tear, during transfer from cage to cage or while grading or harvesting fish, from high wind and high sea conditions during severe storms, or when net cages are breached by large predators. Potential effects include the introduction of maladaptive genes and reduced fitness, competition for food and space, and predation on native stocks.

Several factors merit consideration when evaluating the likely ecological and genetic impacts cultured fish may have should they escape. Among them are: the wild population genetic structure and phenotypic variability of a species or among stocks within a species, the size of the local (or affected) population relative to the estimated number and frequency of escapement, the type of breeding program to be used, including selection of the founding stock, and the likelihood that unintentional genetic drift (domestication) related to hatchery practices will occur.

In reality, there is little evidence-based information available that can reliably assign risk to escapes of finfish from aquaculture facilities, therefore, escape standards are, out of necessity, more preventative than prescriptive. Because of uncertainty, locally adapted indigenous species will probably be encouraged over the use of nonnative and genetically modified species unless a compelling argument can be made that the genetic and ecological risks are demonstrably low.

The Offshore Mariculture Escapes Genetic Assessment (OMECA) Model was developed by ICF International (ICF) and NOAA as a tool for use by scientists and resource managers to help with understanding the potential negative impact of farmed escapees on their wild counterparts.

The purpose of OMECA is to identify and weigh environmental risks of escapes of marine aquaculture fish to their wild conspecifics. OMECA is intended to: 1) provide insights about factors affecting risks associated with escapes from aquaculture operations, 2) help identify research priorities, 3) explore options for the design of sustainable aquaculture programs, and 4) inform policy and management decisions related to the genetic and ecological risks of aquaculture.

OMEGA characterizes the aquaculture program by brood source, size and growth of cultured fish, and frequency and magnitude of escape events. Genetic and ecological interactions are calculated from assumptions regarding survival of escapees in nature, their likelihood of encountering conspecifics, breeding success, and the consequence of interbreeding on the fitness and abundance of wild conspecifics. The 100-year model simulation describes the influence of aquaculture escapes on survival and genetic fitness of the natural population. By evaluating different aquaculture operation scenarios and wild stock population dynamics, OMEGA allows the user to compare differences in total abundance trends of escapees and wild fish, as well as the effects of aquaculture program on survival and long-term sustainability of wild fish (ICF 2014).

The modeling concepts of OMEGA have been successfully applied over the past decade using a similar model, the All-H Analyzer (AHA). AHA has been successfully used by the Hatchery Scientific Review Group (HSRG) to evaluate 178 salmon and trout hatchery programs in the Columbia River Basin (Paquet et al., 2011). Using model simulation results, the HSRG formulated a working hypothesis for baseline conditions from which they evaluated strategies to better achieve stated goals for hatcheries and wild populations. The HSRG concluded that through following recommended management and harvest practices, such as broodstock selection and selective harvest, hatcheries can serve dual goals of contributing to harvest while remaining compatible with or contributing to conservation goals. The guiding principles of the HSRG are core to the purpose of the OMEGA model.
**OMEGA Model Structure**

As shown in Fig. 1, the OMEGA input data structure is organized around three components:

- the biology and operations of the cultured population, including number of fish by size classification, broodstock source (domesticated or wild), maturation and growth factors;
- the wild conspecific population, including assumptions of abundance, distribution, survival, age and size at maturity, age composition, and age specific harvest rates; and
- factors affecting the potential for interaction between farmed fish escapees and their wild conspecifics, assumptions of frequency and magnitude of fish escaping from the pens, survival of escapees in nature, location of the mariculture pens relative to the wild population, potential movement patterns of escapees and breeding success of escapees in nature.

OMEGA accounts for the different factors by which fish can escape from cages, such as:

- *Chronic leaks* from cages, which can be considered a baseline level of escape from year to year, assuming there are a certain amount of fish lost due to routine factors such as small tears in nets, transfer between cages, or veterinary maintenance;
- *cage failures* due to a mechanical failure or defect in a cage containing one size class of fish (this can also occur because cage farms attract predators);
- *catastrophic events* due to failure of multiple cages in the farm structure, resulting in a massive loss of fish, such as from a major storm event; and
- spawning in cages, leading to release of *gametes*.

Once cultured fish escape into nature, their survival is described by a number of factors, such as size at time of escape, level of domestication and their ability to survive in nature, and environmental factors. An attrition of the escapes occurs in the model simulation based on these factors, resulting in a fraction of escapees surviving to encounter wild-origin fish. If escapees successfully reproduce in the wild, there are spawners in nature persisting with potentially maladapted traits. There leads to an effect on genetic fitness of wild fish, which

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**Fig. 1. OMEGA Model Components**
consequently affects wild recruitment and harvest.

Included in this module are parameter inputs for the phenotypic fitness model in OMEGA. The equations are those described by Ford (2002) and described in additional detail by Campton (2009) for evaluating relative fitness effects of hatchery salmonids interbreeding with wild populations. The phenotypic trait model is described by two environmental optimum values, an aquaculture optimum and a natural optimum, with selection forcing the respective populations towards their environmental optimum (Fig. 2). The model calculates the shift in phenotype trait value of the wild population based on assumed gene flow between escapees and wild fish. The consequence is a loss in fitness and survival of the wild population as a result of this mixed wild population (the progeny of escapees and wild parents) phenotype.

**OMEGA Model Simulation Example**

This example uses a hypothetical commercial aquaculture operation for sablefish (*Anoplophora fimbria*) along the coast of Washington, Oregon, and California. The purpose of this example is to show how escapes can affect population abundance and long-term viability, and how limiting escapes can greatly reduce impacts to fitness and abundance of the wild population. These scenarios were developed to demonstrate model capabilities and to test model sensitivity to changes in parameter values, and are not intended to describe the operations of an actual sablefish aquaculture industry, or to suggest standards that should be used to regulate operations; they are provided only to describe the functions of the model.

In nature, sablefish reach maturity at 6 years of age and can live up to 80 years. Fish are fully selected to the fishery at 4 to 5 years of age. In culture, sablefish can grow to a harvestable size of 1 kilogram in approximately 52 weeks. In this example, the culture program is assumed to consist of 50 separate offshore pen operations, each containing multiple pens with different size classifications as fish grow to harvestable size. Total culture production is assumed to be 10,000 metric tons per year. For the two scenarios described below, natural production, harvest, culture production, encounter rate, and fitness and interactions including genetic effects are the same. Only escape rates were changed between scenarios (chronic losses, cage failures, and catastrophic events).

In the “low escape scenario”, catastrophic events could occur with a probability of 5% in a given year for the first 25 years. After year 25, it is assumed technology improvements would reduce the probability of catastrophic events (1%) resulting in a loss of up to 20% of cultured stock. The scenario assumes that chronic losses are the same across all fish sizes (0.1% leakage). Cage failure rate is assumed to be the same across all size categories (0.1%). Chronic losses and moderate events together average about 200,000 fish escaping per year.

In the “high escape” scenario, catastrophic events are assumed to occur with a probability of 5-10% on a given year and each event results in up to a 60% loss of cultured stock. Chronic losses depend on fish size classification (3% for the smallest size class, 0.5%
for harvest size fish). Cage failure rate is assumed to be 0.5% for smallest size class and 1.0% for harvest size fish. Chronic losses and moderate events together average about 1.0 million fish escaping per year. This is an extremely high percent of total production (10.0 million fish at harvest size) and would represent an extreme example of escapes due to chronic and moderate events. In the 100-year simulation, five random catastrophic events occurred resulting in even larger losses.

The model response in terms of natural population fitness under both scenarios is shown in Figure 3. Escape rates in “low escapes” resulted in a slight shift in the phenotypic trait value of the wild population toward the aquaculture optimum value over the 100-year simulation.

Consistent with the slight shift in trait value, relative fitness also declined slightly, indicating effects of introgression. However, natural fitness reaches an equilibrium value fairly quickly (~year 25). In contrast, under the “high escapes” scenario, natural fitness responded quickly to the effect of escapes on the wild population and it appears fitness does not approach a lower equilibrium value until year 75.

The response of the wild population in terms of mixed population natural recruits under both scenarios is shown in Figure 4. The dark line represents no-escape scenario (i.e. 0% change) as reference. Under the “low escapes” scenario, there is an initial increase in recruitment (progeny of escapee and wild parents) due to increases spawning biomass relative to no escapes, with the value approaching an equilibrium value of about +0.5% at year 100 of

![Fig. 3. Natural Population Fitness, 100-year simulation](image1)

![Fig. 4. Mixed Population Natural Recruits, 100-year simulation](image2)
the simulation.

The “high escapes” scenario describes the model response if there was greater selective pressure on the population due to a high level of escapes in nature. As in the previous scenario, there is an initial increase in recruitment; more than in the previous scenario but the decline is steep and nearly constant after year 30, approaching negative values in year 100. This trend would likely continue for some years due to a long-term loss in natural fitness.

The results suggest a lag between natural recruitment (Fig. 4) and fitness (Fig. 3). Initially the trend is increasing recruitment while fitness was declining. This lag is likely due to the retention of older, larger and fit adults in the spawning biomass. As more fit fish die out over time the population is shifting to less fit adults and the consequence is a decline in wild production. The results of the “high escape” scenario indicate year-over-year declining fitness and reduced survival of wild fish. The long-lived nature of sablefish is a factor in the delayed effect on wild fish biomass.

OMEGA includes many other parameter inputs that allow for exploration of different scenarios and evaluate model sensitivity due to combinations of parameter value changes (culture and wild population).

Balancing aquaculture goals with conservation goals will require careful evaluation of the risks and benefits. The challenge of decision makers is to find solutions that serve both goals. The purpose of OMEGA is to help identify possible solutions, or if a range of solutions exists, how programs can be managed for the best outcomes.

**Next Steps**

OMEGA is ready for general use. The model is available from NOAA along with a user guide that includes model background and instructions for using the model. The model also includes the scenarios for sablefish discussed in this presentation. We are interested in your feedback and comments. At this time we seek collaborators to develop case studies for currently farmed or considered aquaculture finfish species. Items for future development include completing a sensitivity analysis of scenarios for sablefish or other species, and developing an economic cost/benefit analysis module.

OMEGA is available at: http://www.nmfs.noaa.gov/aquaculture/science/26_omega_model_homepage.html

**References**


**Annotated Bibliography**


The authors broadly discuss the magnitude of the problem of escapes from salmon and cod cage-farming aquaculture operations in Norway and provide specific recommendations to prevent escapes. Current knowledge about the extent of threats presented by escapes in terms of economic...
and ecological impacts are discussed in the context of experiences in the Norwegian aquaculture industry. While escapes occur due to several internal and external factors, reports from fish farming companies indicated that cage failure was by far the most common cause of large-scale escape in Atlantic salmon farming operations. They report studies that show the mechanisms of escape are not the same across species. Atlantic cod may cause more wear to nets and be more likely to escape through tears in the net. Consequences of escape, such as disease transfer, interbreeding, competition, and predation are generally discussed as areas for further research. The main message of this paper is that prevention is the best tool to reduce the risk of escapes. The authors report evidence that the level of escapes from cages was greatly reduced in Norway following legislation that has specific requirements for design of farms and the handling and use of equipment. They recommend countries develop similar measures to reduce escapes such as mandatory reporting of escapes, a process to use these reports to develop better standards, mandatory technical assessments following reported “large” escape events, technical standards for equipment, and finally, identification of key operational components that have a higher potential to cause an escape event, including training of staff to reduce human errors. This paper was used to identify mechanisms of escape through cage failure and operational scenarios, which are central to development of the escapes component of the OMEGA simulation model.


The authors present a study of spawning interactions between cultured and wild Atlantic cod and tested the potential for hybridization between farm escapes and wild conspecifics. Using a spatial and temporal analysis of wild and farmed cod tracked through biotelemetry, positioning of fish based on sex and origin indicated that farmed fish behave differently from wild fish relative to spawning ground location. However, despite these differences, hybridization is likely, especially between farmed females and wild males. The results illustrate that behavioral differences between cultured and wild fish may not preclude spawning interactions. The authors conclude there is a high potential for farmed cod to hybridize with wild fish. They recommend further research should be a priority to further understand the consequences of interbreeding and to identify methods for escape prevention.


Much of the concern surrounding effects of escaped cultured fish involves interbreeding with wild conspecifics and potential loss of genetic fitness of the wild population. Ford presents a single trait phenotypic model that assumes different optimum trait values for the culture and natural environments. The Ford model describes how mean phenotype values of captive and wild fish shift relative to optimum values for the environments based on gene flow between escapes (or captive breeding) and wild fish. The results suggest that cultured fish can have a strong influence on the fitness and sustainability of wild populations depending on the amount of interbreeding. The level of effect depends on the details of the model such as differences in optimum trait value, selection pressure, and trait heritability. Controlling gene flow between wild and cultured fish can potentially reduce the domestication effect in wild populations. Overall outcomes of fitness in the wild also depend heavily on habitat capacity and population dynamics. This model has been used in several other studies to explore the potential consequences to wild population fitness from captive breeding to increase the size of wild populations, and from the unintended straying of cultured fish to wild populations.


The Hatchery Scientific Review Group (HSRG) was established by the U.S. Congress to review salmon and trout hatchery programs in the Pacific Northwest with the goal of recommending hatchery reform guidelines while still retaining the goal of providing fish for harvest and conservation goals for natural populations. This paper presents the approach used and recommendations that included an assessment of 178 hatchery programs and 351 salmonid populations within the Columbia River Basin. This approach included a scientific framework and three principles to guide their assessment: 1) “clear and specific quantifiable goals for harvest and conservation,” 2) “be scientifically defensible,” and 3) “include monitoring and evaluation of benefits and risks.” HSRG used the All-H Analyzer (AHA) model to evaluate dynamics of populations in the Columbia River system through an integrative analysis of several factors related to hatchery operations, and productivity and capacity of wild populations. Using model simulation results, HSRG formulated a working hypothesis for baseline conditions and to evaluate strategies to better achieve stated goals for hatcheries and wild populations. The HSRG concluded that through following recommended management and harvest practices, such as broodstock selection and selective harvest, hatcheries can serve dual goals of contributing to harvest while remaining compatible with or contributing to conservation goals. The guiding principles stated by the HSRG are the core purpose of the OMEGA model.
Application of Physiological Tests to Determine Specific Monovalent and Divalent Ion Supplementation for Culture of Marine Species

Calvin FISHER*1, Charlotte BODINIER*2, Adam KUHL*3, and Christopher C. GREEN*1

Abstract: The culture of marine and euryhaline fishes in low salinity or ion deficient waters has been an area of interest for many aquaculturists due to the expense of marine salt mixes. The Gulf killifish (Fundulus grandis) is a euryhaline teleost found abundantly in coastal marshes along the Gulf of Mexico. The ability to investigate the molecular underpinnings of specific ions in this model species could have a number of implications for other commercially important marine finfish species. Although studies have examined the influence of salinity on adults and juveniles, few have investigated the role of salinity or specific ion concentrations in larvae. These investigations utilize a model teleost to determine the role of specific monovalent and divalent ions at biochemical and molecular levels.

Separate four week trials were conducted exposing newly hatched Gulf killifish to concentration gradients of potassium (K+), calcium (Ca2+), and magnesium (Mg2+). The K+ supplementation consisted of 0.3, 1.3, and 2.9 mM. Treatment groups for Ca2+ consisted of 0.2, 1.1, 1.5, and 2.1 mM Ca2+, while trials using Mg2+ consisted of 0.1, 2.7, 5.1, and 10.4 mM Mg2+. All treatments were maintained at a salinity of 9.5±10% using crystal salt (99.6% NaCl). Each investigation consisted of four 50-L aquariums stocked at 7 larvae per liter for each concentration. Fish were sampled at 0, 1, 3, 7, 10, 14, and 28 d post hatch (DPH). Upon each sampling the standpipe was adjusted to maintain a constant density for each treatment. Collected samples were analyzed for whole body ion concentrations (K+, Na+, Mg2+, Cl−). Na+/K+-ATPase (NKA) activity, dry weight, and expression/localization of ion transport proteins (NKA, Na+/K+/2Cl− cotransporter (NKCC) and cystic fibrosis transmembrane conductance regulator (CFTR)).

Mortality and growth was significantly influenced by K+ concentration (P < 0.05). No differences were observed among treatment groups for NKA enzyme activity, however at 28-d post hatch (dph) there were significant differences in dry weight among K+ treatment. At seven dph differences in intestinal NKA and CFTR staining were observed, and NKA mRNA expression was also found to be higher in the 0.3 mM [K+] group than in other treatment groups. Survival was significantly influenced by both Mg2+ and Ca2+ concentration (P ≤ 0.05). Highest survival (71.1%) in the Ca2+ trial was noted in the 0.2 mM [Ca2+] treatment. In the Mg2+ trial, highest survival was noted in the 2.7 mM [Mg2+] treatment (82.9%). NKA enzyme activity was reduced and delayed peaks were observed in whole body ion composition in the Mg2+ treatments. In addition, decreased CFTR intensity was observed at the gill and intestine epithelium for the 0.05 mM [Mg2+] treatment at 1 dph, indicating that Mg2+ deficiency has a possible effect on larval osmoregulatory capability. The role of intestinal epithelium in ion uptake not only allows the potential for uptake and maintenance of ion balance from dietary sources, but also demonstrates the potential to modulate concentrations of specific ions in prepared waters for euryhaline and marine teleosts.

Key words: Gulf killifish, osmoregulation, potassium, magnesium, euryhaline
The major ions in seawater are: chloride (Cl\(^-\)), sodium (Na\(^+\)), magnesium (Mg\(^{2+}\)), Calcium (Ca\(^{2+}\)), sulfate (SO\(_4\)\(^{2-}\)), and potassium (K\(^+\)), with a variety of other ions present in low concentrations. The three ions Ca\(^{2+}\), Mg\(^{2+}\), and K\(^+\) are necessary for maintaining electrolyte and acid-base homeostasis, and the regulation of osmolality and intracellular fluids (Fielder et al., 2001). The high cost of synthetic marine salt mixes has facilitated interest in alternative low-cost salt sources and supplementing physiologically important ions. Potassium is involved in ion regulation of intracellular fluids and can be added directly to the water, usually in the form of KCl, or supplemented in the diet (Wilson and El Naggar, 1992). Calcium and magnesium both influence the permeability of osmoregulatory membranes making them critical ions in teleost ionic regulation (Silva et al., 2005). Magnesium is responsible for the activation of numerous enzymes making it a necessary cofactor to the transfer of phosphate groups and the activation of ATP-dependent ion pumps such as NKA (Bijvelds et al., 1998). In freshwater fish, Mg\(^{2+}\) deficiency is associated with increased Na\(^+\) and Ca\(^{2+}\) levels as well as low K\(^+\) levels (Bijvelds et al., 1997).

Ion regulation is controlled by ionocytes in which there are several proteins involved in ion and water exchange (Evans et al., 2003; Lorin-Nebel et al., 2006). Previous investigations have focused on Na\(^+\)/K\(^-\)-ATPase (NKA), Na\(^+\)/K\(^+\)/2Cl\(^-\) cotransporter (NKCC), and the cystic fibrosis transmembrane conductance regulator (CFTR) as the three primary proteins involved in ionoregulatory processes (Hirose et al., 2003). The ionoregulatory process is initiated by a basolaterally located NKA which couples two extracellular K\(^+\) with three intracellular Na\(^+\) generating an electrochemical gradient that drives the ions according to expression, location, and abundance of NKCC and CFTR (Bodinier et al., 2009; Kang et al., 2008). NKA is responsible for the lowering of intracellular Na\(^+\) concentrations allowing the basolateral NKCC to import Na\(^+\), K\(^+\), and Cl\(^-\). the excess Cl\(^-\) is then secreted through the chloride channel CFTR. Additional water exchange and Cl\(^-\) ion regulation occurs intestinally (Christensen et al., 2012) and it has also been suggested that substantial Mg\(^{2+}\) uptake occurs intestinally (Marshall and Grosell, 2005). The apically located NKCC2 most likely plays an active role in the reabsorption of ions (Lorin-Nebel et al., 2006). The exact mechanism by which Mg\(^{2+}\) uptake occurs has yet to be described in detail. It is suggested that significant differences in Mg\(^{2+}\) regulation exist among species, knowledge of Mg\(^{2+}\) transport across epithelia and cell membranes is still limited (Bijvelds et al., 1998). By exposing euryhaline teleosts to varying salinities or individual ion concentrations, it may be possible to immunolocalize specific osmoregulatory proteins in the gill filaments and intestinal epithelium to better understand the physiological adaptations these species undergo to cope with environmental challenges.

The Gulf killifish, *F. grandis*, inhabits the estuarine waters of the Gulf of Mexico and Atlantic coast of Florida where it is commonly used as a baitfish by anglers for speckled trout, *Cynoscion nebulosus*, flounder, *Paralichthys lethostigma*, and red drum, *Sciaenops ocellatus*. From Texas to Florida it is referred to by many regional names including but not limited to: mudminnow, mudfish, cocahe minnow, and bull minnow. *F. grandis* are closely related to the mummichog, *F. heteroclitus*, which inhabits the Atlantic coast and is similarly utilized by anglers as a live bait. Both species are characterized as a “hardy” bait tolerant of wide swings in salinity, temperature, and other conditions encountered by live marine bait. *F. grandis* occupy coastal marshes where salinity can change dramatically over a short time period. These fish have the ability to live in salinities that range from freshwater (0 \%) to near double the concentration of seawater (up to 70 ppt) for several days. Exploitation of this salinity tolerance in *F. grandis* allows investigators the ability to illicit physiological responses across a wide range of ion concentrations.

Several studies have investigated the use of low salinity inland seawater or water from brackish aquifers as a source for marine aquaculture. Much of these inland saline waters were found to have ion deficiencies, where specific manipulation of the major ions in saltwater have significantly increased growth and survival (Doroudi et al., 2006; Fielder et al., 2001; Potedar et al., 2008). Many studies have investigated the effects of low salinity waters on juvenile and
adult euryhaline species (Coulon et al., 2012; Doroudi et al., 2006; Fielder et al., 2001; Fotedar et al., 2008; Patterson et al., 2012; Roy et al., 2007). Few studies have attempted to explore the importance of specific ion supplementation in euryhaline teleost larviculture. The objectives of this work were to demonstrate the use of physiological examinations at biochemical and molecular levels to determine specific supplementation of monovalent (K+) and divalent ions (Ca2+ and Mg2+) as replacements for saltwater in the culture of F. grandis larvae.

Materials and Methods

Three separate four-week experiments were conducted to investigate the impact of external K+, Ca2+ and Mg2+ concentrations on larval F. grandis. Newly hatched (< 4 h old) F. grandis larvae were stocked, in triplicate, at a density of 7 larvae per L into 50-L aquaria maintained on a common recirculating system utilizing biological and ultra violet filters. Larvae were fed a diet of Artemia sp. nauplii (30 Artemia fish 1 1-1) for the first 7 d at a rate of three times per day followed by Otohome™ B1 (Reed Mariculture Inc., Campbell, CA, USA) diet fed to apparent satiation 4 times per day for the remainder of the study. After 4 weeks, survival was calculated by draining all aquaria and counting each individual.

Treatments were established by adding crystal salt (Diamond Crystal Solar Salt, 99.6% NaCl) to 10% with ion supplementation. Potassium supplementation consisted of 0.3, 1.3, 2.0 and 2.9 mM (Table 1). This investigation represents [K+] from values similar to

### Table 1. Mean salinity and ion concentrations (± SEM) given for each K+, Ca2+, and Mg2+ Concentration treatment group. Superscript letters denote statistically significant differences in specific ion concentrations among treatment groups (P < 0.05)

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<tr>
<td>K+ (mM)</td>
<td>0.33 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.31 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.06 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.96 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Na+ (mM)</td>
<td>170.19 ± 3.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.52 ± 6.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>151.36 ± 7.03&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>138.01 ± 0.83&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Mg2+ (mM)</td>
<td>0.04 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.55 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Ca2+ (mM)</td>
<td>0.31 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Salinity(‰)</td>
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<td>9.4 ± 0.4</td>
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<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.04 ± 0.03</td>
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<tr>
<td>NO3 (mg/L)</td>
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<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
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<td>186.00 ± 6.55</td>
<td>182.50 ± 6.98</td>
<td>209.83 ± 3.06</td>
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*Reported as CaCO3
<sup>a</sup>Represents reference salt group

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<tr>
<td>K+ (mM)</td>
<td>2.37 ± 0.03</td>
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<td>Na+ (mM)</td>
<td>165.31 ± 3.07</td>
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<td>165.84 ± 2.97</td>
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<td>Mg2+ (mM)</td>
<td>0.11 ± 0.01</td>
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<td>0.20 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>NO3 (mg/L)</td>
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<td>140 ± 4.08</td>
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*Reported as CaCO3
<sup>a</sup>Represents reference salt group

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<tbody>
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<td>2.37 ± 0.03</td>
<td>2.40 ± 0.04</td>
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<td>Na+ (mM)</td>
<td>168.48 ± 3.64</td>
<td>169.96 ± 4.11</td>
<td>162.64 ± 4.83</td>
<td>170.55 ± 4.97</td>
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<td>Mg2+ (mM)</td>
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<td>2.88 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.76 ± 1.16&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Ca2+ (mM)</td>
<td>0.19 ± 0.02</td>
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<td>0.16 ± 0.01</td>
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<td>NO3 (mg/L)</td>
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<td>0.00 ± 0.00</td>
<td>0.02 ± 0.01</td>
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<td>Hardness (mg/L)</td>
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<td>320 ± 44.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>530 ± 29.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1040 ± 36.21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>165 ± 19.53</td>
<td>160 ± 4.71</td>
<td>150 ± 16.62</td>
<td>185 ± 21.83</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reported as CaCO3
freshwater sources (0.33 mM) to concentrations of 2.96 mM, found saline waters at 10 %. Potassium concentration in [Ca\(^{2+}\)] and [Mg\(^{2+}\)] experiments were maintained at 2.35 mM, as determined by a previous study (Fisher et al., 2013). Calcium was supplemented to give final concentrations of 0.20, 1.08, 1.50, and 2.07 mM (Table 1), while Mg\(^{2+}\) was supplemented to give concentrations of 0.05, 2.88, 5.76, and 11.52 mM. The [Mg\(^{2+}\)] experiment also received Ca\(^{2+}\) supplementation to the water in the form of CaCO\(_3\) as determined in the previous experiment (Table 1). Weekly water samples were collected to quantify ion concentrations via ICP analysis by the LSU Agriculture Center, Agriculture Chemistry Department (Baton Rouge, LA, USA).

Fish were sampled at 0, 1, 3, 7, 10, 14, and 28 days post hatch (DPH). Upon each sampling the standpipe was adjusted to maintain a constant density for each treatment. Three replicates of 5 larvae were used to establish an average desiccated dry mass due to the inability to accurately measure individual dry mass of larvae less than 7 dph. Whole body ion concentration (Na\(^{+}\), K\(^{+}\), and Mg\(^{2+}\)) was analyzed using the methods described by (Van Genderen, 2003; Fisher et al., 2013). Three replicates of 5 larvae were collected at each time point to determine Na\(^{+}\)/K\(^{-}\)-ATPase activity using the protocol described in McCormick (1993) and expressed as μmol ADP mg protein\(^{-1}\) h\(^{-1}\).

Six larvae were collected per treatment group at 1, 3, and 7 dph and preserved in Z-fix (Anatech, LTD, Battle Creek, MI, USA). Immunocytochemistry reactions were performed according to methods described in Bodinier et al. (2010) and Fisher et al. (2013) for NKA, CFTR, and NKCC1. Ionocyte length measurements were taken using Image Tool\(^{3}\) version 3.0 (University of Texas Health Science Center, San Antonio, TX, USA). A minimum of 50 cells were measured per sampling period for each treatment.

Results

Growth and survival: The 0.3 mM [K\(^{+}\)] treatment resulted in 100% mortality within 24 h of hatch. No significant differences were seen in survival between the 1.3 and 2.0 mM [K\(^{+}\)] groups (survival ≤ 5%); however the 2.9 mM [K\(^{+}\)] treatment resulted in significantly greater survival after 4 weeks (survival ~60%). Final dry mass of the 2.9 mM [K\(^{+}\)] reference group was significantly higher than both the 1.3 and 2.0 mM [K\(^{+}\)] treatments (P ≤ 0.05). The 2.9 mM [K\(^{+}\)] reference group had a final dry mass of 4.93 ± 0.54 mg while the 1.3 and 2.0 mM [K\(^{+}\)] treatments had dry mass of 2.39 ± 0.19 mg and 2.21 ± 1.55 mg, respectively.

Survival and dry mass were significantly influenced by both [Ca\(^{2+}\)] and [Mg\(^{2+}\)] experiments. Supplementation of Ca\(^{2+}\) showed a decrease in mean final mass and mean survival with increasing concentrations of Ca\(^{2+}\) in the absence of Mg\(^{2+}\) (Fig. 1A). Supplementation of Mg\(^{2+}\) resulted in approximately a 5-fold increase in survival with Mg\(^{2+}\) supplementation as compared to no supplementation, no differences were observed among supplemented treatment groups. Final mass was not significantly
influenced until a concentration of 11.52 mM Mg$^{2+}$ was reached (Fig. 1B).

**Whole-body ion concentrations & Na$^+$/K$^+$-ATPase activity:** No differences were found in Na$^+$/K$^+$-ATPase activity among [K$^+$] treatment groups or among time ($P \geq 0.05$). Whole body Mg$^{2+}$ concentration showed no difference for treatment or treatment × dph interaction; however significant difference was determined for dph ($P \leq 0.05$). There was a significant increase in [Mg$^{2+}$] within the first week followed by a rapid decrease for all three[K$^+$] treatment groups. Whole body K$^+$ concentration had no significant difference in treatment × dph interaction, although significant differences were observed among treatments and for dph ($P \leq 0.05$).

No differences were observed among treatments for whole body ion composition in the [Ca$^{2+}$] study. The [Mg$^{2+}$] experiment showed delayed peaks in whole body [Na$^+$], [Ca$^{2+}$], and [K$^+$] for the 0.05 mM [Mg$^{2+}$] treatment group, with ion concentrations reaching their highest point 7 days later than other treatments. As expected, the Mg$^{2+}$ deficient treatment groups (0.05 and 2.70 mM [Mg$^{2+}$]) also showed a significant decrease in whole body [Mg$^{2+}$].

Na$^+$/K$^+$-ATPase activity in the [Ca$^{2+}$] study was significantly lower at 0 and 1 dph followed by a significant increase in activity throughout the experiment, however no treatment specific differences in activity were determined at any specific sample dates. The [Mg$^{2+}$] study demonstrated significant differences in Na$^+$/K$^+$-ATPase activity between Mg$^{2+}$ deficient treatments and those with sufficient supply at 0 dph followed by minor, but not significant, increases throughout the experiment (Fig. 2).

**Immunocytochemistry & cell volume:** All negative slides, with no primary antibody, showed no immunostaining as expected (not illustrated). In [K$^+$] treatments at 7 dph, no significant differences were determined among treatments for gill ionocyte length. Gill ionocytes from the 1.3 mM [K$^+$] treatment were found to have a significantly larger area than those in the 2.0 and 2.9 mM [K$^+$] treatments. Intestinal CFTR and NKA were present at 7 dph in all surviving [K$^+$] treatment groups. No changes were observed in NKA or CFTR in the gill ionocytes. Intestinal NKA and CFTR staining appeared to show a decrease in intensity as K$^+$ concentration increased (Fig. 3). Very little staining was observed for NKCC both intestinally and at the gill epithelium.

No differences among treatments were observed

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**Fig. 2.** Whole body NKA activity for larvae stocked in [Mg$^{2+}$] treatments after 0, 1, 3, and 7 dph. Letters represent significant differences among treatment groups at that day.
<table>
<thead>
<tr>
<th>[K⁺] Treatment Group</th>
<th>Merged</th>
<th>CFTR</th>
<th>NKA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>![Merged Image]</td>
<td>![CFTR Image]</td>
<td>![NKA Image]</td>
</tr>
<tr>
<td>2.0</td>
<td>![Merged Image]</td>
<td>![CFTR Image]</td>
<td>![NKA Image]</td>
</tr>
<tr>
<td>2.9</td>
<td>![Merged Image]</td>
<td>![CFTR Image]</td>
<td>![NKA Image]</td>
</tr>
</tbody>
</table>

Fig. 3. Representative histological sections from larval intestines after one week in [K⁺] treatment groups cut transversely at 5 µm intervals. Immunofluorescence of NKA (red) and CFTR (green). The 2.9 mmol K⁺ group represents a reference salt used and contains other ions which may be essential for the function of osmoregulatory proteins. L: lumen; v: villi

for NKA or NKCC staining intensity in the gills or intestine, nor were there any significant differences among treatments for gill ionocyte length or quantity at 1, 3, and 7 dph for both the [Ca²⁺] and [Mg²⁺] experiments. In the [Mg²⁺] experiment, a decrease in CFTR staining intensity was observed at 1 dph in both the intestine (Fig. 4) and gill in the 0.05 mM [Mg²⁺] treatment, no other changes in CFTR staining intensity were observed.

Discussion

The chemical gradient generated by the activation of NKA is one of the primary driving forces behind ion regulation, thus the ability to measure whole body NKA activity may allow for the evaluation of larval fish osmoregulation capacity in hyper and hypo-osmotic environments. It is possible that K⁺ is a limiting factor in killifish larval osmoregulation and a deficiency can restrict the ability of NKA to function. The current model for chloride secretion as described by McCormick et al. (2003) suggests a basolateral NKA and NKCC coupled with an apically located CFTR-like protein. Because K⁺ is also used by the chloride cotransporter, NKCC, to assist in the transport of chloride ions, an extracellular K⁺ shortage could reduce a larval fish’s ability to regulate sodium and chloride ion concentrations at critical biological membranes. Despite the fact that there were no statistical differences in NKA activity between any treatment groups, it is still conceivable that sufficient K⁺ was not available for the function of NKCC. If so then the inability of the larvae to regulate either Na⁺ or Cl⁻ ions may possibly have been a factor in the low survival of the 0.3, 1.3 and 2.0 mM [K⁺] groups. The reference group (marine mix salt) was the only group that did not display poor survival and it is possible that the presence of other essential ions such as Mg²⁺ and Ca²⁺ played a significant role in the survival of this treatment group.

Potassium supplementation across the gradient examined in the current study at a constant salinity not only altered survival, but affected differences among molecular components of intestinal epithelium. Although potassium treatments in the current study did not appear to alter whole body Na/K ATPase activity, immunofluorescence
showed increased density of NKA and CFTR proteins as K⁺ concentration decreased. Whole body K⁺ concentration was greater in the reference as compared to treatments with 1.3 and 2.0 mM of K⁺ both of which remained lower throughout the study as expected due to the inability to selectively retain K⁺ ions. It is important to note that the reference group containing the highest concentration of K⁺ was also comprised of greater concentrations of Mg²⁺ and Ca²⁺, which could have modulated Na⁺ and Cl⁻ absorption in the intestine relative to lower K⁺ treatments in the current study that were relatively low in these divalent ions (Marshall and Singer, 2002; O’Grady, 1989). Growth and survival metrics for the reference treatment may reflect combined effects of a full to partial complement of other ions in addition to the K⁺ gradient examined. The role of intestinal epithelium in ion uptake not only allows the potential for uptake and maintenance of ion balance from dietary sources and thus important in aquaculture, but also demonstrates the potential to modulate concentrations of specific ions in prepared waters for euryhaline and marine teleosts.

Calcium and Magnesium are critical in teleost ion regulation, varying concentrations of Ca²⁺ and Mg²⁺ can impact the permeability of osmoregulatory epithelia to both ions and water (Silva et al., 2005). In the sea water adapted eel, removal of Ca²⁺ from the external medium diminished the active excretion of sodium by half, excretion returned to normal level after the reintroduction of Ca²⁺ to the medium (Isaia and Masoni, 1976). Proper development of many euryhaline teleosts is related to environmental Ca²⁺ concentration, for example red drum (Sciaenops ocellatus) fry displayed a drop in blood osmolality when transferred from salt to freshwater, this

<table>
<thead>
<tr>
<th>[Mg²⁺]</th>
<th>DIC with CFTR</th>
<th>CFTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>2.88</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>5.76</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>11.52</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Fig. 4.** Representative histological sections of the intestine from 1 day post hatch (dph) Gulf killifish larvae in [Mg²⁺] treatment groups, cut transversely at 5 μm intervals. Differential interference contrast (DIC) images with immunofluorescence of CFTR: lumen: v: villi
decline in osmolarity was reduced by the addition of Ca\(^2+\) (Wurts and Stickney. 1989). Previous studies have determined that Mg\(^2+\) is critical in the activation of ATP-dependent ion pumps (Bijevelds et al., 1998) making it critical for the activation of NKA. In this study, both the 0.05 and 2.88 mM [Mg\(^{2+}\)] treatment groups displayed significantly reduced NKA activity at hatch.

In the current study both Ca\(^2+\) and Mg\(^2+\) had effects on survival, growth, and molecular components at both the gill filaments and intestinal epithelium. In the absence of Mg\(^2+\) supplementation larval F. grandis had reduced survival, decreased Na\(^+\)/K\(^-\) -ATPase activity, and a delayed peak in whole body compositions for several ions essential for larval development. A decrease in CFTR intensity was also observed in the gills and intestine of Mg\(^2+\) deficient treatment as seen by immunocytochemistry staining at 1 dph. This is an indication that Mg\(^2+\) is a necessity for larval F. grandis growth, survival, and ion regulation. However, the Mg\(^2+\) of 10% seawater is approximately 11.52 mM and the greatest growth and survival were achieved in this study at a concentration of 2.88 mM Mg\(^{2+}\). These results demonstrate that the potential for euryhaline teleost culture in prepared water may only require a fraction of the ion concentrations found in 10% seawater. Also the role of intestinal epithelium in ion uptake allows for the potential for ion maintenance through dietary sources.

Acknowledgements

This work was supported, in part, through grants from the Southern Regional Aquaculture Center and Louisiana SeaGrant. The authors would like to acknowledge the faculty, students and support staff that assisted with these investigations: Paige O’Malley, Emily McArdle, Chris Mariani, Mike Coulon, Josh Patterson, Charlie Brown.

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Annotated Bibliography


This study encompasses results pertaining to the K+ ion manipulation portions of the abstract presented above for the UJNR Scientific Symposium. This investigation increased [K+] from values similar to freshwater sources (0.33 mM) to concentrations of 2.96 mM, found in saline waters at 10%. A number of biochemical and molecular techniques
were performed to examine the effect of this K$^+$ ion gradient, which included: whole body ion composition, Na$^+$/K$^+$ -ATPase (NKA) activity, gill ionocyte morphometrics, relative gene expression (NKA, NKCC, and CFTR), and corresponding immunocytochemistry at the gill and intestinal epithelium. Results more tangible to aquaculturist, such as growth and survival, indicated the presence of a threshold within the gradient examined whereby survival was not different between 1.3 to 2.9 mM [K$^+$]. Utilizing immunocytochemistry, the differences between these seemingly similar treatment groups indicated that the treatments groups between 1.3 to 2.9 mM [K$^+$] was different in terms of gill ionocyte area, NKA and CTFR localization.


These authors investigated a number of physiological parameters pertaining to salinity and temperature in embryos and larvae of red porgy (Pagrus pagrus), which is viewed as a high-market value marine species with good potential as an aquaculture species. Embryos and resulting larvae were reared at four temperatures (17, 19, 21, 23°C) and two salinities (24 and 34%). Larvae (16 dph) were transferred from their respective salinities to 44% to represent a sublethal hyperosmotic challenge. These authors demonstrated significant increases in NKA mRNA expression in individuals acclimated to 24% at 24 h after transferred to increased salinity, while individuals from 34% exhibited no significant changes in NKA expression. Temperature was not observed to influence expression of NKA, while metabolic parameters related to growth were influenced by the temperature gradient in their study. The authors utilized traditional growth metrics including molecular tools to anticipate optimum salinity (24%) and temperature (23°C) conditions that are optimum for larval rearing of this species.


The gilthead sea bream (Sparus aurata) is a commercially important aquaculture species, which spawns in the ocean and whose resulting larvae and juveniles migrate to lower salinity estuaries and lagoons. These authors investigated the development of salinity tolerance in gilthead sea bream from 3, 30, 75, 96, and 300 d post hatch (dph) by challenging them with 9 salinities ranging between freshwater and 45.1%. Utilizing immunohistochemistry, these authors localized the NKA throughout these challenges to document location of ion regulation within respect to this ionoregulatory protein. Initially, immunopositive NKA ionocytes were laoced in the integument along the yolk sac and integumentary folds representing the branchial slits. A functional shift from integument to gills was demonstrated 30 and 70 dph, when both the integument and gills were observed to locally express NKA, whereby from 70 to 300 dph the gills remain the main site of osmoregulation. Increases in osmoregulatory capacity for this species at the intervals examined within this study related to the shifts and patterns observed through immunohistochemistry.


In gill ionocytes, ion transport is activated by the basolaterally located NKA which generates an electrochemical gradient by coupling two extracellular K$^+$ with three intracellular Na$^+$, driving the ions according to expression, location, and abundance of other proteins such as NKCC and CFTR. Christensen et al. investigated changes in alewife physiology and branchial epithelium as individuals were acclimated to freshwater or saltwater and represents the first study of its kind to characterize multiple ion-transport proteins in a non-salmonid anadromous fish. Corresponding
increases in NKA, NKCC1, and CFTR abundance at the gill epithelium with increasing salinity was used to establish a gill model for hypo-osmoregulation. In gill ionocytes, NKA is responsible for lowering intracellular Na⁺ allowing the basolateral NKCC1 to import Na⁺, K⁺, and two Cl⁻ ions. Excess intracellular Cl⁻ is then secreted through the CFTR chloride channel. NKCC1, the secretory isoform is expressed basolaterally in the gill ionocytes, while NKCC2 is identified as an absorptive isoform and is expressed apically along the intestinal and urinary bladder epithelium of saltwater and euryhaline teleosts. The authors determined the key differences between freshwater and seawater acclimated alewifes in the context of ion transporters at the gill epithelium. The implications of these investigation has assisted in increasing the information multicellular complexes of mature ionocytes and the role of salinity and specific ion in the maintenance of homeostasis.
Genetically Modified Salmon in Aquaculture: Well Regulated and Safe

Paul G. Olin *1

Abstract: A Massachusetts company, Aquabounty Technologies, submitted an application to the U.S. Food and Drug Administration (FDA) in 1995 to grow a genetically modified AquAdvantage® Atlantic salmon to be marketed as a food product. Aquabounty proposed to raise the broodstock fish on Prince Edward Island, ship their eggs to a contained inland recirculating production system in Panama to grow, harvest and process the fish, and then ship food grade product back to the United States for sale. The fish for this physically secure production system would be at least 99 percent triploid and all-female, as an additional reproductive-containment measure.

The AquAdvantage® Atlantic salmon carries a Chinook salmon growth hormone gene that results in production of growth hormones that enable the fish to grow to market weight in half the time it normally takes (Fig. 1) This gene is regulated by a segment of DNA from the ocean pout, a blenny-like fish found in frigid waters of the Northwest Atlantic.

The review process used by FDA on the AquAdvantage® salmon involved a team of scientists and subject-matter experts on the Veterinary Medicine Advisory Committee who advise the FDA and the general public on scientific issues as they relate to ensuring public and animal health. The FDA released a 172 page briefing packet and an 84 page environmental assessment containing information relevant to the application in advance of a public advisory committee meeting held in September 2010. This briefing packet summarized their scientific review, and the basic conclusions were that the AquAdvantage® salmon are safe, nutritionally comparable to other Atlantic salmon, and when produced as described in the application do not pose a threat to the environment. The review process used by FDA on the AquAdvantage® salmon was rigorous, detailed and extensive, spanning more than 15 years. Specific conclusions from the report stated that “Food from AquAdvantage® Salmon is the same and as safe to eat as food from other Atlantic salmon.”

In reference to concerns about adverse environmental impacts the report states “There is substantial, reliable information available in the environmental assessment document to conclude that AquAdvantage® Atlantic salmon are not expected to have a significant impact on the environment when raised and reared under the current conditions of physical, biological and geographical/geophysical confinement present at hatchery and grow-out facilities in Canada and Panama. We have a high degree of certainty in our conclusions regarding AquAdvantage® Salmon.”

Despite this rigorous scientific review, there remains considerable public controversy regarding the potential for FDA approval of this Aquabounty application to produce and market a genetically

Fig. 1. AquAdvantage® and non-GM Atlantic salmon of similar age.
modified salmon. As a result, 11 senators signed a request that FDA stop the process for approving genetically modified AquAdvantage® Atlantic salmon. The manner in which this review for approval of a transgenic animal for agricultural production and marketing in the United States has progressed has stymied American research in this promising sector of animal biotechnology.

New technologies to genetically improve food and animal crops are one tool to supply the additional food people will need in the future, improving human health, reducing the use of pesticides and fertilizers, and lessening the carbon footprint of animal and plant agriculture. A 2008 scientific review published in the Journal of the Royal Society of Medicine noted that genetically modified foods had been eaten by millions of people worldwide for 15 years, with no reports of ill effects.

Annotated Bibliography


This paper presents a detailed analysis of the regulatory and review process that FDA used for the AquAdvantage® salmon.


This online debate features, Elliot Entis, whose company has created a genetically modified salmon that may soon be for sale in the U.S., who discusses the environmental and health impacts of this controversial technology with author Paul Greenberg, a critic of GM fish.


In this paper the authors provide an overview of salmon farming and world markets and then analyze three scenarios based on the level of acceptance of GM salmon in the marketplace. The three scenarios were named as 1) ‘no market for GM fish,’ 2) ‘GM salmon for dinner,’ and 3) ‘GM salmon doesn’t take off.’ The authors provide a summary of the main outcomes for each scenario.

FDA 2012. AquAdvantage® Salmon, Draft Environmental Assessment. Center for Veterinary Medicine, United States Food and Drug Administration, Department of Health and Human Services. Washington D.C.

This document provides a detailed environmental assessment of the transgenic AquAdvantage® Salmon.
Reproductive Dysfunction in Cultured Sablefish (*Anoplopoma fimbria*)

José M. GUZMÁN*, J. Adam LUCKENBACH*, Frederick W. GOETZ*, William T. FAIRGRIEVE*, Mollie A. MIDDLETON*, and Penny SWANSON*

**Abstract:** Sablefish *Anoplopoma fimbria* is a ground fish native to the North Pacific Ocean that is considered a promising new species for marine aquaculture in the US. However, efforts to establish sustainable production of sablefish have been constrained by the reproductive performance of females from the first-filial (F1) generation. Although some F1 females may mature after 5+ years of age, some others fail to initiate puberty in captivity. Development of methods to unblock/induce early puberty are necessary to reduce costs associated with rearing F1 female broodstock and reduce generation times for selective breeding.

Current research at the Northwest Fisheries Science Center (NOAA, Seattle, USA) integrates basic and applied biology to gain knowledge on the reproductive endocrine system of sablefish and develop approaches to unblock or reduce the age of puberty in F1 female sablefish. As part of our basic line of research, we compared the pituitary gonadotropin-ovary axis in wild-caught, maturing females and 8-year-old F1 females that had never shown signs of sexual maturation. Wild-maturing females had higher levels of pituitary gonadotropin subunit and ovarian gonadotropin receptor mRNAs and plasma sex steroids compared to F1 females, which were holding at the immature, perinucleolus ovarian stage. Anecdotal evidence from sablefish farms indicates that F1 female broodstock maintained in ~4 °C seawater mature in captivity. We hypothesize that culture conditions that use warmer water (10-15 °C) suppress the pituitary gonadotropin-ovary axis in sablefish, and ultimately block the onset of puberty.

As part of our applied line of research, we conducted a series of studies to determine the ability of exogenous hormones to stimulate the reproductive axis in prepubertal F1 females. Treatments with testosterone or estradiol 17-beta (E2) increased the expression of pituitary luteinizing hormone beta subunit 40- and 185-fold, respectively, relative to control. This finding suggests that pituitaries from immature females are responsive to exogenous hormones, and that sex steroids may be an important part of a hormone therapy to stimulate the reproductive axis of F1 females. In addition, using an *in vitro* ovarian tissue culture system, we demonstrated that fragments of prepubertal sablefish ovaries incubated with human-chorionic gonadotropin increased the secretion of E2. This indicates that ovaries of prepubertal females are equipped to synthesize and release sex steroids critical for vitellogenesis under the appropriate hormone stimulation, and that the failure to initiate puberty is likely due to a lack of adequate gonadotropin signaling. These data provide the foundation for the development of hormone treatments aimed at inducing puberty in prepubertal F1 female sablefish.

**Key words:** sablefish, marine aquaculture, reproduction, gonadotropins
In fishes, as in other vertebrates, reproduction is primarily controlled by the hypothalamic-pituitary-gonadal axis. The hypothalamic neuroendocrine system regulates synthesis and release of the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Both gonadotropins are essential in the endocrine control of reproduction by regulating steroidogenesis and germ cell development through interactions with their respective receptors in gonadal tissues (Levavi-Sivan et al., 2010; Zohar et al., 2010).

Optimal function of the reproductive endocrine system is critical for fish to complete gonadal development, maturation and spawn successfully. Under culture conditions, however, many fish species exhibit some degree of reproductive dysfunction. These can vary from inconsistent spawning to a complete failure to undergo puberty (i.e., the time during which an individual becomes capable of reproducing for the first time) (Mylonas et al., 2010; Taranger et al., 2010). Such a delay in age of puberty poses a major problem for selective breeding in finfish aquaculture and necessitates further research.

Hormone therapies are widely used to control reproduction in cultured fish species. During the past two decades, hormone therapies have been developed to induce puberty in striped bass (Morone saxatilis) (Holland et al., 2002), European sea bass (Dicentrarchus labrax) (Zanuy et al., 1999), European eel (Anguilla anguilla) (Vidal et al., 2004), and grey mullet (Mugil cephalus) (Aizen et al., 2005). Therapies for inducing puberty or to accelerate this process were based on an understanding of how the endogenous reproductive system is hormonally regulated and where the insufficiency/failure occurs in the reproductive system (e.g., brain, pituitary gland and/or gonad) that delays or blocks puberty onset. Therefore, characterization of the reproductive dysfunction in a given species helps to tailor specific strategies to optimize its reproduction in captivity.

Sablefish (Anoplopoma fimbria), known as gindara in Japan, is a groundfish native to the North Pacific Ocean ranging from Baja California to Alaska’s Bering Sea and Japan. The primary market for sablefish is in Japan, where demand and prices are high, but an increasing amount of the wild catch is staying in the US market. Due to its rapid growth rate (1.5 kg after 12-months of growout) and high market value (22 USD/lb. approx. market price), sablefish has been identified as an excellent marine aquaculture species in the US. However, efforts to establish sustainable and efficient production have been constrained by the reproductive performance of females from the first filial (F1) generation (i.e., produced and maintained in captivity). Although some F1 females may mature at 5+ years old in the industry setting, others never initiate puberty in captivity (B. Campbell, Sablefish Canada Inc., personal communication). This situation compromises the development of selective breeding programs and increases costs associated with rearing female sablefish broodstock.

Our approach to this issue integrated a series of basic and applied studies aimed at gaining a basic understanding of the reproductive physiology of sablefish and developing a method that would eliminate the block or reduce the age of puberty in F1 female sablefish.

Materials and methods

Study I: Assessment of the pituitary gonadotropin-ovary axis in wild maturing and F1 non-maturing female sablefish: To understand the endocrine differences that underlie the reproductive impairment of female sablefish propagated in captivity, we first compared the pituitary gonadotropin-ovary axis of two stocks of sablefish with different reproductive status: wild-caught maturing females and 8 year-old F1 females that had never shown signs of sexual maturation.

Wild female sablefish were caught using sportfishing gear near the mouth of the Quinault River (Washington, USA) in October 2010. The fish were transported to the Manchester Research Station (Port Orchard, Washington, USA) and maintained in tanks supplied with flow-through, sand-filtered and UV-treated seawater. During February 2011, the peak spawning period along the Washington coast (Mason et al., 1983), the wild females showed signs of sexual maturation by ultrasound. F1 sablefish (broodyear 2003) were reared at the Manchester Research Station in net-pens until fall 2010 when
they were transported to the Northwest Fisheries Science Center (NWFSC, Seattle, Washington, USA) where they were reared on recirculated seawater. These females were maintained under similar rearing conditions as the wild-caught fish at the Manchester Research Station. In contrast to the wild females, the F1 females did not exhibit signs of ovarian maturation when assessed by ultrasound.

Wild and F1 females were sampled on 28 February 2011 and 8 March 2011, respectively. Three females from each broodstock group were deeply anesthetized with MS-222 and body weight (BW) and fork length (FL) recorded (wild, 6413.0 ± 712.9 g BW and 81.3 ± 31 cm FL; F1, 4160.0 ± 303.3 g BW and 69.8 ± 27 cm FL). Plasma was obtained by centrifugation of whole blood and stored at −20 °C for sex steroid analyses. The pituitary gland and a small piece of ovary (~80 mg) were also collected from each fish, frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Genes of interest included the pituitary gonadotropin subunits (fshb, lhb, cgα) and ovarian gonadotropin receptors (fshr and lhcr). For histology, a middle portion of the ovary was preserved in Bouin’s fixative for 48h prior to storage in 70% ethanol and processing as described elsewhere (Campbell et al., 2006).

**Study II: Effect of sex steroids on pituitary gonadotropin gene expression in vivo:** To determine potential effects of sex steroids on pituitary gonadotropins and whether sex steroids will be an important part of hormone therapies to reduce the age of puberty in sablefish, we evaluated the effect of testosterone (T) and estradiol-17β (E2) on pituitary gonadotropin beta subunit (fshb and lhb) mRNA levels in prepubertal F1 females in vivo.

Twelve 2-year old prepubertal F1 female sablefish (1187.4 ± 49.4 g BW and 469.9 ± 5.3 cm FL) maintained at the NWFSC on 12 °C recirculated seawater were distributed into three groups (n=4 sablefish/group) and implanted with cholesteryl-based pellets (0.2 x 0.5 mm) containing no hormone (control) or containing T at doses of 0.75 or 3.75 mg. Twenty-eight days after implantation, fish were euthanized and their pituitaries removed and snap frozen for later RNA isolation. In a parallel study, 6 females from the same cohort of fish received 6 intramuscular injections of 2 mg E2/kg dissolved in ethanol: 0.9% NaCl (1:7) or vehicle alone (control), every other day. Two days after the last injection, fish were euthanized and pituitaries removed to determine gonadotropin beta subunit gene expression. Doses of T and E2 were selected from previous studies in other fish species (Guzmán et al., 2008; Holland et al., 2002; Vidal et al., 2004).

**Study III: Effect of gonadotropins on ovarian secretion of estradiol in vitro:** To determine whether immature ovaries of F1 female sablefish are responsive to gonadotropin stimulation, we evaluated the effect of two gonadotropin preparations, recombinant coho salmon Fsh analog (sFsha) and human chorionic gonadotropin (hCG), on E2 production in vitro.

For this, a 3-year old prepubertal F1 female sablefish (2910 g BW, 67.6 cm FL) maintained at the NWFSC was euthanized and the ovaries removed. Ovarian fragments (~60 mg each) were cultured in Cortland’s solution alone (control) or containing sFsha (provided by Dr. W.R. Moyle, Robert Wood Johnson Medical School, New Jersey, USA) at doses of 50 and 500 ng/ml or hCG (Sigma) at doses of 1, 10 and 100 IU/ml. After 24 h incubation at 11 °C, the culture medium was collected and stored at −20 °C for E2 analysis. Doses of sFsha and hCG were selected based on previous studies in other species (Luckenbach et al., 2011; Sorbera et al., 2001).

**RNA isolation, cDNA synthesis and quantitative PCR:** Total RNA from sablefish pituitaries and ovarian tissue was isolated with Tri-Reagent (Molecular Research Center) using a TissueLyser II (Qiagen). An aliquot of total RNA was diluted to ~250 ng RNA/μl in nuclease-free water and DNase treated using the DNA Free kit’s “rigorous” protocol (Ambion). RNA yields and quality were assessed by NanoDrop (ND-1000 Spectrophotometer) and gel electrophoresis. For reverse transcription, 1 μg of total RNA of each sample was reverse transcribed in a 20-μl reaction with the Superscript II kit (Invitrogen). Quantitative PCRAs were conducted as previously described (Luckenbach et al., 2011). Reactions consisted of 1x Power SYBR Green PCR master mix (Applied Biosystems), 150 nM of gene-specific primers (see Guzmán et al., 2013 for primer sequences) and 0.5 ng of pituitary or ovarian cDNA template. Assays were run on an ABI 7700 Sequence
Detector using standard cycling conditions.

**Sex steroids**: Plasma levels of E2 were measured by radioimmunoassay and levels of T were quantified by enzyme-linked immunosorbent assay using protocols previously validated for sablefish (Guzmán et al., 2013).

**Statistical analyses**: Statistical analyses were performed using Prism 5 software for Mac OSX (GraphPad Software) with the minimum level of significance set to $P<0.05$. Differences were examined using t-test or one-way ANOVA followed by a Tukey multiple comparisons test. When necessary, data were log or Ln transformed in order to comply with normality and homogeneity of variance, which were tested by Kolmogorov–Smirnov and Bartlett methods, respectively. Data are expressed as mean ± standard error of the mean (S.E.M.).

**Results**

**Study I: Assessment of the pituitary gonadotropin-ovary axis in wild maturing and F1 non-maturing female sablefish**

Endocrine parameters indicated a more active reproductive axis in wild versus F1 female sablefish (Fig. I). At the pituitary level, *lhb* and *ega* mRNA

![Graphs showing relative expression of genes and hormone levels](image)

*Fig. 1.* Relative gene expression of pituitary gonadotropin subunits (A) and ovarian gonadotropin receptors (B), plasma sex steroid levels (C), and representative ovarian histological sections (D) of wild maturing and F1 female sablefish. Gene expression levels were determined by qPCR and normalized to *ef1a*. Levels of E2 were determined by RIA, whereas levels of T were determined by ELISA. Data are expressed as the mean ± SEM ($n=3$). Asterisks indicate significant differences (t-test, $p<0.05$). Maturing follicles with the nucleus migrating to the periphery predominated in the ovaries of wild maturing females, whereas only follicles at the perinucleolus stage were found in ovaries of F1 females (D). Scale bars: 100 μ m. ND, non detectable.
levels were significantly higher in wild fish than in F1 fish. Levels of fshb were also elevated in wild fish relative to F1 fish (7.1-fold), although differences were not statistically significant due to the high variance across individuals. In the ovary, transcripts for both gonadotropin receptors, fshr and lhcgr, were significantly higher in wild than in F1 females. Plasma levels of E2 were also significantly higher in wild than in F1 females, whereas only wild females had detectable levels of T in plasma.

The ovary of wild females consisted of post-vitellogenic preovulatory follicles characterized by the nucleus migrating to the periphery and yolk coalescence, as well as another clutch of follicles at the perinucleolus stage. In contrast, ovaries from F1 females were characterized by the sole presence of follicles at the perinucleolus stage.

**Study II: Effect of sex steroids on pituitary gonadotropin gene expression in vivo**

The effect of treatment with T or E2 on in vivo pituitary gonadotropin beta subunit (fshb and lhb) mRNA levels is shown in Fig. 2. Transcripts for fshb were not significantly affected by treatment with T or E2. In contrast, transcripts for lhb increased significantly in fish treated with the highest dose of T or multiple injections of E2 (40- and 185-fold, respectively, relative to control).
**Fig. 3.** Effect of sFsha or hCG on E2 production by ovarian fragments obtained from prepubertal F1 female sablefish. Ovarian fragments were cultured in Cortland’s solution alone (control, CNT) or containing sFsha at doses of 50 and 500 ng/ml or hCG at doses of 1, 10 and 100 IU/ml at 11 °C for 24h. Levels of E2 were determined by RIA. Bars with different superscript letters are significantly different (ANOVA, p<0.05), whereas asterisks indicate significant differences relative to control (t-test, p<0.05).

**Study III: Effect of gonadotropins on ovarian secretion of estradiol in vitro**

The effect of sFsha or hCG on ovarian secretion of E2 in vitro is shown in Fig. 3. Incubation with sFsha had a subtle effect on the secretion of E2 with only the highest dose of sFsha having a significant effect compared to control. In contrast, treatment with hCG regardless of dose significantly stimulated E2 secretion (>4-fold).

**Discussion**

Currently, the emerging aquaculture industry for sablefish is mostly dependent on spawning wild-caught individuals. The expansion and optimization of this industry requires a closer understanding of the reproductive physiology of this species and the development of protocols to control the age of puberty in cultured broodstock.

In Study I, we demonstrated that transcript levels of pituitary lhb and ovarian lhcgr were higher in wild-caught maturing females than in non-maturing F1 females. This is in accordance with the predominant role of LH in the regulation of gonadal maturation, ovulation and spawning in fishes (Gothilf et al., 1997; Swanson et al., 2003; Yaron et al., 2003). Interestingly, ovarian fshr mRNA levels were also higher in maturing females and a similar tendency was observed for the pituitary fshb mRNA levels. Little is known about specific roles of Fsh during gametogenesis in fishes, although it is accepted that Fsh is important for early gonadal development and vitellogenesis (Lubzens et al., 2010; Luckenbach et al., 2011). The elevated levels of pituitary fshb and ovarian fshr that we observed in wild females may indicate that a population of perinucleolar oocytes within the ovary were preparing for the transition to vitellogenic growth, or that Fsh also plays a role in final oocyte maturation. Since the ovary of the wild mature females had a mixed population of oocytes it is not possible to distinguish these two possibilities. Plasma levels of E2 and T were also higher in wild females than F1 females as expected from the observed differences in pituitary gonadotropin subunit and ovarian receptor mRNA levels. This demonstrates classical gonadotropin-mediated sex steroid secretion (Lubzens et al., 2010) by the ovaries of wild females and indicates a lack of gonadotropin signaling in F1 females.

Although F1 females from Study 1 were 8 years old, they remained arrested in a prepubertal stage. Interestingly, it has been recently demonstrated that some F1 females can initiate vitellogenesis (i.e., onset of puberty) if they are maintained in cold water (~4 °C) after 5 years of age (B. Campbell, personal communication). It is generally accepted that every fish species has an optimal range of water temperatures for vitellogenin synthesis, and both higher and lower temperatures affect the normal progression of vitellogenesis (Guzmán et al., 2008; Kim and Takemura, 2003). Considering that sablefish is a deep-water species and the onset of vitellogenesis may naturally occur at very low temperatures, it is possible that the temperature at which fish were maintained (fluctuated between 8 and 15 °C throughout the year) had a deleterious effect on reproductive axis function, and ultimately on the onset of vitellogenesis in F1 females.

Although some F1 females maintained in cold water can achieve puberty after 5+ years, the development of methods to reduce and synchronize the age of puberty is essential for reliable and cost effective sablefish breeding programs. Hormone
treatments are widely used in aquaculture to improve the reproductive performance of captive fishes, including activation of the reproductive endocrine system and promotion of gonadal development in immature stages (Holland et al., 2002; Zanuy et al., 1999). We conducted Studies II and III to determine the ability of exogenous hormones to stimulate the reproductive axis in prepubertal F1 sablefish.

In Study II we demonstrated that treatment with T or E2 significantly elevated transcripts for pituitary lhb, but did not affect levels of fshb mRNA. Effects of sex steroids on levels of the pituitary gonadotropin subunit mRNAs have been demonstrated in a number of fishes, although results vary among species. For example, treatment with T elevated pituitary transcripts of lhb in European sea bass during the period of “sexual resting,” while T and E2 reduced pituitary fshb mRNA levels (Mateos et al., 2002). In immature European eel (Anguilla anguilla), treatments with E2 but not T elevated transcripts for lhb, whereas no effect was observed on fshb mRNA levels (Aroua et al., 2007). Lastly, treatments with T and E2 increased pituitary transcript levels for fshb and lhb in zebrafish (Danio rerio) (Lin and Ge, 2009). Our results suggest that pituitaries from prepubertal female sablefish are responsive to exogenous hormones, and that sex steroids may be an important part of a hormone therapy to stimulate the reproductive axis of F1 females. In line with this, treatments with sex steroids were necessary to induce puberty in European sea bass (Zanuy et al., 1999), striped bass (Holland et al., 2002) and European eel (Vidal et al., 2004).

In Study III, we demonstrated that treatment with hCG, an Lh-like gonadotropin, stimulates ovarian secretion of E2 in vitro. Treatments with gonadotropin preparations, which act directly on the gonad, have proven effective in the stimulation of gonadal maturation, ovulation and spawning in a number of fish species (Zohar and Mylonas, 2001). Most importantly, in species from the genus Anguilla, captive fish often remain arrested in a prepubertal stage in captivity. However, multiple injections with pituitary extracts can trigger ovarian vitellogenesis, maturation and ovulation in females (Ohta and Tanaka, 1997), while multiple injections of hCG can induce testicular development and spermatiation in males (Dou et al., 2007). Our data suggest that, as in anguillid eels, immature ovaries of F1 female sablefish are equipped with the ability to synthesize and release sex steroids (critical for vitellogenesis) under appropriate hormone stimulation, and that treatment with gonadotropin preparations may effectively stimulate ovarian function in this species.

In conclusion, wild-maturing females had elevated gonadotropin signaling (pituitary and ovarian gene expression and plasma steroid levels) compared to 8 year-old F1 females, which were holding at the immature, perinucleolus ovarian stage. Anecdotal evidence indicates that F1 females maintained in ~4 °C seawater may mature in captivity after 5 years of age. It might be possible that culture conditions that use warmer water (10-15 °C) suppress the pituitary gonadotropin-ovary axis in sablefish, and ultimately block the onset of puberty. Our results indicate that hormone treatments, such as sex steroids and gonadotropin preparations, can stimulate the reproductive endocrine axis in prepubertal females and therefore should be considered as candidates to reduce the age of puberty in this species.

References


Induced Spawning in the Sea Cucumber *Apostichopus japonicus* by Neuropeptide, Cubifrin

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**Abstract:** In hatcheries the induction of spawning in sea cucumbers has been typically carried out by regulation of rearing conditions such as temperature and light intensity. However, this method is relatively ineffective and the rate of spawning is unpredictable. In this study we established an efficient method for inducing spawning in the Japanese sea cucumber *Apostichopus japonicus* by injecting a neuropeptide, cubifrin.

We purified peptides that can induce oocyte maturation from the buccal tissues containing the nerve ring, using liquid chromatography and *in vitro* assay with ovarian fragments in combination. The effective dose of each peptide was evaluated with chemically synthesized peptides. Consequently, the most potent peptide was identified as NGIWy-amide. We also found that synthetic derivatives replaced the third amino acid, isoleucine, with a different basic amino acid could be 10–100 times more potent than the natural hormone.

When injected into the body cavity of sexually matured individuals, NGIWy-amide, or its derivative, induced spawning in both males and females. NGIWy-amide was then named cubifrin after the Japanese word "kubifuri" meaning waving head, which is a reproductive behavior of sea cucumbers. Gamete shedding started about 60 min and 80 min after the injection in males and females, respectively, and was completed almost simultaneously in the two sexes about 2 hours after the administration. The *in vitro* responsiveness of biopsied ovarian fragments was well correlated with the spawning success induced by an injection. Therefore, cubifrin can be used as an excellent detector of maturity as well as an inducer of spawning in *A. japonicus* in a hatchery setting.

**Key words:** reproduction, hormone, aquaculture, echinoderm

The Japanese common sea cucumber *Apostichopus japonicus* is the most commercially-important holothurian species in Japan. Until recent years the annual catch had been about 5,000–10,000 tons (wet weight) but fishing pressure has been increasing mainly because of a growing demand for export to China. Therefore, the sustainable production of the sea cucumber is crucial. For over 30 years, cultured juveniles of this species have been released into fishing grounds to supplement natural stocks. The current situation concerning sea cucumber fisheries makes this activity more important than ever.

In hatcheries, the induction of spawning in sea cucumbers is typically carried out by regulation of rearing conditions such as temperature, water exchange, and light intensity (Battaglene *et al.*, 2002; Sui, 2004; Wang and Cheng, 2004; Liu *et al.*, 2004; Shao *et al.*, 2006). In Japan, wild-caught *A. japonicus* broodstock are induced to spawn in the dark in tanks of seawater at temperatures that are ~ 5°C higher than natural seawater (Ito, 1995). However, these methods are relatively ineffective and the rate of spawning is unpredictable. Therefore, more effective method of spawning induction has been

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desired.

Starfishes have long been used as model animals for the study of reproductive biology after the finding of spawning-inducing activity in a nerve extract in the middle of the 19th century (Chaet and McConaughy, 1959). At long last, the nervous substance was purified and identified as a relaxin-like protein (Mita et al., 2009). Since both starfishes and sea cucumbers belong to the Echinodermata, their reproductive mechanism might be driven by similar endogenous factors. Thus, we started the study to discover a spawning-inducing substance in A. japonicus, following the strategy employed in the study in starfish.

Here, we report a native neuropeptide potent in inducing oocyte maturation and spawning in A. japonicus, and propose a procedure of using the peptide in a hatchery setting.

Materials and Methods

Preparation of neural extract for purification of a spawning-inducing substance: Buccal tissues containing the ring nerve were homogenized in the same volume of ice-chilled 4 N acetic acid containing 0.4 mM β-aminoethyl benzensulfonfyl fluoride, 10 µg/ml leupeptin and 4 µM peptatin A. The homogenates were centrifuged at 15,200 x g for 15 min. at 4°C. The resulting supernatants were then ultracentrifuged at 45,000 x g for 1 hr at 4°C. These supernatants of ultracentrifugation were kept as a crude neural extract in aliquots at −80°C. The crude extract was separated by serial ultrafiltration with AmiconUltra YM30 and YM10 (Millipore). The gonadotropic activity determined by the in vitro maturation assay was mainly recovered in the fraction of less than 10K daltons. After YM10 filtration, the filtrate was lyophilized and preserved for a subsequent purification process.

Liquid chromatography. The lyophilized preparation was dissolved in MilliQ water. After centrifugation, the supernatant was applied to a Develosil C8-UG-5 column (20 x 50 mm) and eluted with 80 % acetonitrile containing 0.1 % trifluoro acetic acid (TFA) for desalting. The desalted effluent was lyophilized. The lyophilized extract was fractionated by reversed-phase high performance liquid chromatography (RP-HPLC) using a Develosil RPAQUEOUS-AR-5 column (10 x 250 mm) with a linear gradient of acetonitrile from 10 to 40 % with 0.1 % TFA. Each fraction was lyophilized, then dissolved in 0.5 ml MilliQ water to detect the gonadotropic activity by the in vitro maturation assay. The fractions containing the gonadotropic activity were separated by a Develosil RPAQUEOUS-AR-5 column (10 x 250 mm) with a linear gradient of acetonitrile from 10 to 30 % containing 20 mM ammonium acetate (pH 6.0). The fractions were lyophilized and assayed as mentioned above. The active fractions were separated by a Develosil RPAQUEOUS-AR3 column (2 x 250 mm) with a linear gradient of acetonitrile from 15 to 22 % with 0.1 % TFA. The gonadotropic activity was detected in fractions 30 and 33 of the last chromatography step.

Determination of chemical structure of gonadotropic substances: The fractions 30 and 33 were analyzed for accurate mass values and amino acid sequences by liquid chromatograph-tandem mass spectrometers (Quattro Premier, Waters; nanoFrontier LD, Hitachi High-Technologies) and a protein sequencer (491 cLC, Applied Biosystems).

Preparation of peptides: NGIWY-amide (cubifrin) and its derivatives were chemically synthesized. A stock solution of 1 mM dissolved in MilliQ water was stored at −80°C. Prior to use, it was diluted with filtered seawater to the desired concentrations.

In vitro maturation assay. A portion of ovary was excised through a short incision in the body wall. The excised ovarian tissue was cut into small fragments about 3 mm long. For the purification of a maturation-inducing substance, ovarian fragments were incubated with each fraction for 1.5 hr at 20°C. The gonadotropic activity was determined by germinal vesicle breakdown (GVBD) and also by the incidence of ovulation of oocytes from ovarian fragments.

For the detection of the responsiveness to peptides, ovarian fragments were incubated either with the peptide solution at 100, 10, 1 nM, or 100 pM in filtered seawater or with filtered seawater alone. The test was duplicated for each individual. The incidence of GVBD were scored on a scale of 0-3 in which 3 indicates GVBD in over two-thirds
of oocytes in the fragment; 2, in over one-third of oocytes; 1, in up to one-third of oocytes; 0, in a few percentage of oocytes; 0, no response of oocytes. The sum of scores of all treated ovarian fragments from one individual (8 fragments for each individual) was regarded as the oocyte maturation score of that individual. After the assay, oocytes in the control ovarian fragments were mechanically separated by gentle pipetting and the diameters of 20 well-developed oocytes were measured.

**Injection of cubifrin and observation of reproductive behavior:** Cubifrin solution (10 μM) or filtered seawater was injected into the body cavity of thesea cucumbers; the injection volume was 0.1% of body weight (v/w). Each sea cucumber was then separately placed on the bottom of a 21-l tank, and its behavior was continuously monitored until the completion of spawning, or for 2 hr if no spawning occurred. We recorded the times at which the underside of the water surface was reached, when head waving commenced, and when spawning was started and completed. After the completion of spawning, the number of spawned eggs in 10 ml of tank water was counted to estimate the total number of spawned eggs for each female.

**Results and Discussion**

**Purification of an oocyte maturation-inducing substance:** The neural extract was separated by three serial HPLCs (Fig. 1). On the third chromatography, the gonadotropic activity was separated into two independent fractions. Fractions 30 and 33 (arrowheads in Fig. 1c). Both fractions were analyzed by mass spectrometry and protein sequencing. The amino acid sequence of Fraction 30 was NGIWy, and Fraction 33’s sequence was QGLFSGV. While the calculated monoisotopic mass values of these sequences were 651.320 and 706.365, analyses by mass spectrometry indicated mass signals of 650.35 and 705.42, respectively. Further de novo sequencing analyses of these mass signals suggested that these had the same sequences as those given through protein sequencing except for anamidation at each C-terminal end of the peptides. With these analyses we determined that the sequences of the components in Fractions 30 and 33 were NGIWy-amide and QGLFSGV-amide, respectively. Unexpectedly, both of these peptides completely differ from a relaxin, which is an oocyte maturation-inducing substance in starfish. NGIWy-amide has been reported as a peptide associated with contractions of muscle, intestine and tentacles in *A. japonicus* (Inoue et al., 1999).
whereas QGLFSGV-amide has not been reported ever.

**In vitro maturation-inducing activity of peptides:** Two peptides, NGIWy-amide and QGLFSGV-amide, were chemically synthesized and GVBD-inducing activity was estimated with **in vitro** maturation assay. NGIWy-amide was extremely potent even at 1 nM or less (Fig. 2). QGLFSGV-amide was less potent in inducing GVBD, with effective concentrations being 1 μM or more. Thus, NGIWy-amide is strongly suspected to be involved in the regulation of oocyte maturation. In contrast, QGLFSGV-amide does not appear to be the primary endocrine regulator of oocyte maturation.

The activities of the two derivatives, NGLWY-amide and NGIWy-COOH, were compared with that of the natural NGIWy-amide (Fig. 3). NGLWY-amide was effective at 10 pM or less, a hundred times more potent than the natural peptide, NGIWy-amide. However, NGIWy-COOH barely induced GVBD at 100 nM. Accordingly, C-terminal amidation of the peptide is indispensable for the bioactivity.

**Effect of NGIWy-amide and NGLWY-amide in inducing spawning:** NGIWy-amide and NGLWY-amide were injected into sexually matured males and females to examine the effect on inducing spawning behavior. Injections of NGIWy-amide induced spawning in males and females at 10 nM. NGLWY-amide more effectively induced spawning in males at 1 nM and in females at 100 pM. NGLWY-amide was therefore at least ten times more potent than NGIWy-amide, as in the case of **in vitro** experiments. Sea cucumbers injected with NGIWy-amide or NGLWY-amide exhibited reproductive behaviors that were independent of sex and typically included: (1) climbing up the side wall of the tank to the underside of the water surface; (2) throwing back and swaying of the head; (3) shedding gametes from the gonopore in the head region. Gamete release occurred 45–60 min after

**Fig. 2.** Concentration dependence of NGIWy-amide and QGLFSGV-amide to induce GVBD in ovarian fragments. Ovarian fragments were incubated with NGIWy-amide (white bars) and QGLFSGV-amide (gray bars) at various concentrations. Bars represent percentages of GVBD (means ± SD of duplicate determinations of four separate experiments). Bars with different labels differ significantly (*P* < 0.05*, *P* < 0.01**). The grouping symbol at the top side of the graph indicates a significant difference between NGIWy-amide and QGLFSGV-amide at each concentration. Significant differences were determined using Wilcoxon’s rank sum test. Reproduced from Kato *et al.* (2009) with permission.

**Fig. 3.** Concentration dependence of NGIWy-amide and its derivatives to induce GVBD in ovarian fragments. Ovarian fragments were incubated with synthetic analogues at various concentrations. NGIWy-amide (white bars), NGLWY-amide (gray bars) and NGIWy-COOH (black bars) were examined. Bars represent percentages of GVBD (means ± SD of triplicate determinations of six different experiments), and bars with different labels differ significantly in each peptide (*P* < 0.05*, *P* < 0.01**). The grouping symbol at top side of the graph indicates a significant difference among two peptides at each concentration. Significant differences between means were determined using Steel-Dwass’s pair-wise comparisons. Reproduced from Kato *et al.* (2009) with permission.
the peptide injections in males and 70-90 min in females, and was completed almost simultaneously in the two sexes about 2 hr after the administration (Fig. 4). This series of actions from climbing to spawning observed in the experiment seemed to be a typical behavior of spawning by sea cucumbers (McEuen, 1988; Battaglene, 2002). NGIWW-amide and NGLWY-amide were named cubifrin and cubifrin-L, respectively, after the Japanese word “kubifuri” meaning waving head, which is a reproductive behavior of sea cucumbers.

Association between oocyte size and responsiveness to cubifrin: The competence of ovarian fragments to undergo in vitro oocyte maturation and the induction of spawning by cubifrin-L stimulation were examined in relation to oocyte size. All ovarian fragments with oocytes >155 μm in diameter (animal nos. 17-25 in Fig. 5) responded to cubifrin-L in vitro, as indicated by their oocyte maturation score, which was a measure of their ability for ovulation and GVBD. In contrast, all fragments but one (animal no. 11 in Fig. 5) with oocytes <155 μm failed to respond to the treatment. The injection of cubifrin-L into the body cavity induced spawning in all females with ovaries that responded to cubifrin-L in vitro. Females with unresponsive ovaries in vitro did not spawn following the injection, with the exception of two females (nos. 12 and 13). The number of spawned eggs per 100 g body weight varied from 765 to 1,826,000, and there was a significant correlation between this value and the oocyte maturation score (r = 0.84). The number of eggs spawned by the two non-responsive females in in vitro experiment was especially low (765 eggs per 100 g body weight in female no. 12 and 23,700 in female no. 13).

These results indicate that potential spawners in broodstock can be selected by in vitro examination of the cubifrin or cubifrin-L responsiveness of ovarian fragments or by measuring oocyte size. Furthermore, the number of spawned eggs can be roughly estimated by in vitro examination.

Procedure of spawning induction in a hatchery: In conclusion, spawning induction by cubifrin is an

![Fig. 4](image_url)

**Fig. 4.** Progress of spawning behavior in females (A) and males (B). Open and closed circles indicate the data point of each NGLWY-amide-injected male and female (n=14 each) and control sea cucumber (n=4 each). Vertical bars show the average of the NGLWY-amide-injected group.
effective and simple method for practical application during the artificial culture of *A. japonicus*. We propose the procedure of spawning induction as follows: (1) preparation of sea cucumbers during the spawning season; (2) sex determination and gross evaluation of maturity from the appearance of biopsied gonad; (3) evaluation of definite maturity with biopsied ovary by *in vitro* test; (4) rearing mature individuals below 15°C to suppress unanticipated natural spawning until spawning induction; (5) spawning induction by the injection of 10 μM cubifrin solution (0.1% of body weight (v/w)); (6) standard fertilization and cultivation of fertilized eggs.

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**Annotated Bibliography**


Extracts prepared from tissues containing buccal ring nerve or longitudinal radial nerve of sea cucumber induce oocyte maturation and ovulation from ovarian tissues. We purified two small peptides, a pentapeptide and a heptapeptide, from the buccal tissues of Japanese common sea cucumber, *Apostichopus japonicus*. Both peptides induced oocyte maturation and gamete spawning. The pentapeptide was identified as NGIWy-amide. This peptide induced *in vitro* germinal vesicle breakdown and ovulation of fully grown oocytes at less than 1 pM and *in vivo* spawning at 10 nM. A synthetic derivative of the pentapeptide, NGLWY-amide, was 10–100 times more potent compared to the natural NGIWy-amide. The heptapeptide was less potent, inducing ovulation at 1 μM. NGIWy-amide and NGLWY-amide induced a characteristic spawning behavior when injected into sexually matured individuals. Mature eggs artificially spawned were fertilized, and developed normally and metamorphosed into young sea cucumbers. The details of the production and the mechanism of action of NGIWy-amide are still unclear, but the high biopotency of the peptide will aid understanding of the neuronal and hormonal control of reproduction of sea cucumber.


The neuropeptide cubifrin-I and its derivative cubifrin-L have recently been demonstrated as potent substances that induce oocyte maturation *in vitro* and spawn in the Japanese common sea cucumber *Apostichopus japonicus*. Here, the reproductive behavior provoked by injection of cubifrin-L into the body cavity of *A. japonicus* was examined with a view to the practical application.
of the peptide for induction of spawning in the hatchery. Ovarian fragments with oocytes larger than 155 μm in diameter responded to cubifrin-L \textit{in vitro}. The \textit{in vitro} responsiveness of ovarian fragments was well correlated with the spawning success induced \textit{in vivo} by a cubifrin-L injection. Mature sea cucumbers injected with cubifrin-L displayed sequential reproductive behaviors, which comprised climbing the side wall of the tank toward the water surface, waving of the head, and shedding of gametes. Gamete shedding started about 60 min and 80 min after the injection in males and females, respectively, and was completed almost simultaneously in the two sexes about 2 hours after the administration. Repeated injections of cubifrin-L at intervals of about 10 days successfully induced multiple spawns in males and females. This study demonstrated that cubifrin-L is an effective inducer of spawning in Japanese sea cucumber cultivation.
Mass Production of Artificial Seed of the Japanese Common Sea Cucumber (*Apostichopus japonicus*) in Hokkaido, Japan

Yuichi SAKAI*1

Abstract: Seed production and release of *Apostichopus japonicus* is a key approach to increase and maintain their natural stocks in Japan. Nevertheless, in many trials conducted mainly in Honshu and Kyushu by the end of the 1990’s, the amount of seed production fluctuated annually, and the survival rate of released artificial juveniles was uncertain because of the difficulty of distinguishing them from wild ones. Due to these issues, some hatcheries discontinued *A. japonicus* enhancement projects. However, due to increase in Chinese demand, the price of *A. japonicus* dramatically increased after 2003 in Hokkaido. Facing the decline in the price of sea urchin and abalone, which are major high-value catches in the coastal areas, fishermen were very interested in *A. japonicus* stock enhancement by releasing artificial seeds. Accordingly, sea urchin and abalone hatcheries in Hokkaido began to produce *A. japonicus* seed after 2006. This paper will introduce the recent mass production techniques developed in Hokkaido.

Key word: *Apostichopus japonicus*, artificial seed production, larval rearing, post-larval rearing, control of predation

Japanese common sea cucumber *Apostichopus japonicus* is found in shallow coastal bottoms of Japan, Kuril Islands, Sakhalin, northeastern China and Korean peninsula. Among these countries, Japanese and Korean people prefer to taste this mainly in raw or picked as a seafood, but Chinese people prefer dried sea cucumber as a tonic rather than a seafood. Due to the economic development of China, demand for this species has increased. Unit price in Hokkaido peaked at 43.2 USD/kg in 2010. Facing the decline in the price of sea urchin and abalone, fishermen were very interested in stock enhancement by releasing artificial seeds.

After Imai et al. (1950) made the first trial of artificial seed production in Japan, many trials were conducted in Honshu and Kyushu. Small juveniles (<2mm) were mainly produced by 1990, and the productions peaked at 26.6 million year<sup>-1</sup>. Thereafter, due to increase of production of larger juveniles, the total number of juveniles decreased to around 2-3 million year<sup>-1</sup> (Fig. 1).

Mass mortality during post-larval rearing resulted in fluctuations in the number and size of seed produced. Furthermore, the survival rate of released artificial seeds had not been properly

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*Fig. 1. Number of seed produced in Japan. Drawn from the statistical data published by Fisheries Agency, Fisheries Research Agency and National Association for Promotion of Productive Seas.*

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evaluated, because of difficulty in distinguishing cultured from wild individuals. Therefore, some hatcheries in Honshu and Kyushu discontinued this stock enhancement project. However the demand for artificial seed production by fishermen, began to increase after 2006 in Hokkaido and the production of artificial seed increased again and reached to 12.3 million in 2011. From this experience and background, the improvement of artificial seed production is necessary for stock enhancement to be successful.

Seed production of *A. japonicus* is divided into 5 processes: broodstock collection, fertilization, larval rearing, settlement and post-larval rearing. I review the recent mass production techniques mainly developed in Hokkaido for each process.

**Brood stock collection**

Genetic impacts due to release of artificially produced organisms are considered in broodstock collection. Polymorphic microsatellite allele frequency of artificial seed tends to decrease to compare with wild ones due to small number of their parents (Sakai and Kanno, 2013). So to keep enough broodstock from wild stocks is necessary especially in the case of survival rate of released seed is high enough to contribute its reproduction on the released bottoms. Furthermore, to maintain genetic diversity, broodstock should not be used repeatedly.

Maturation of this species begins after they grow to ca. 50g in body weight, so larger broodstock are necessary. They are normally collected in the spawning season, late June to August in Hokkaido, to avoid excess labor and cost to rear them.

**Fertilization**

Gametes are obtained from fully matured broodstock by two methods: thermal stimulation or injection of gonadotrophic hormone (CUBIFRIN) through the body wall (Kato *et al.*, 2009; Fujiwara *et al.*, 2010). To induce to release gametes by thermal stimulation, broodstock are placed in 15 L volume containers individually and the seawater temperature is raised by 5–7°C above ambient temperature. Mature broodstock begin to release their gametes into the container ca. 0.5–1.5 hours after the stimulation. Alternatively, broodstock injected with gonadotrophic hormones (CUBIFRIN) will also begin to release their gametes.

For insemination, 10–20 ml of sperm is added to a liter of seawater containing 0.1 – 0.2 million eggs. To prevent polyspermy, sperm concentration should not exceed 1,000,000 sperm ml⁻¹ (Sakai and Konda, 2006). After insemination, eggs are transferred onto a 45μm mesh sieve immersed in a shallow tray and gently rinsed with filtered seawater to wash the excess sperm through the sieve.

**Larval rearing**

Hatched blastulae swim to the surface and are transferred to larval rearing tanks at a density of 1–2 individuals ml⁻¹. About 2 days after hatch, the larvae grow to the auricularia larvae and begin to eat *Chaetoceros gracilis*, unicellular phytoplankton. Daily feeding rate is near to 10 000 cells individual⁻¹. The fully developed auricularia larvae grow to ca. 1 mm body length in 5 to 10 days. After this, they begin to shrink to 0.4–0.5mm doliolaria larvae in 2 days. Then about 1 day after, doliolaria larvae grow to pentactula larvae which are ready to metamorphose to young juveniles and begin to settle onto the tank bottom or collectors.

The growth rate of larvae is regionally different in Hokkaido, fast in southwestern Sea of Japan areas and slow in Pacific areas. It takes only 9 days in southwestern Hokkaido and 14 days after hatch in the Pacific area.

Larval rearing tanks are static through the rearing period to minimize the loss of food and satisfy their food requirement. However, to avoid precipitation of larvae, food and feces on the bottom, aeration is very important; the optimum rate is 1.5–2.0 L min⁻¹. The water temperature should be kept at 18–20°C.

**Settlement**

Doriolaria and pentactula larvae are transferred to post-larval rearing tanks. They settle on corrugated polyvinyl chloride (PVC) plates or balled 1mm mesh polyethylene screens of which surface are covered with natural attached diatoms or algal
powder which are used as the juvenile diet. To avoid the loss of these larvae, water exchange is initiated 10 days after the larvae are stocked, by which time most larvae complete settlement.

Post-larval rearing

Settled juveniles are reared up to 5 to 10 mm body length on corrugated PVC plates and/or polyethylene screens without temperature control. Daily water exchange rate varies from 1–7 times day$^{-1}$ with aeration at the center of the tank to stir the rearing water well.

Various diets during this post-larval rearing have been examined (Yanagibashi et al., 1984; Yanagibashi and Kawasaki, 1985; Ueki and Ikeda, 1989; Konda and Sakai, 2005). The naturally occurring attached diatoms and LIVIC-BW, a commercially sold diet made from a mixture of dried algal powder of Undaria pinnatifida and Ascophyllum nodosum are used as inducers for larval settlement and as food for post-larval stages. Phyt plankton C. gracilis is also a good initial food for juveniles for the first month (Sakai and Konda, 2008). Proper feeding of LIVIC-BW has been examined according to juvenile size (Ikeda et al., 1992; Sakai et al., 2009). Juveniles fed LIVIC-BW grow to 6.0 mm in about 3 months (Sakai et al., 2009). Growth rate affects rearing density (Hatanaka, 1996; Sakai et al., 2009) and food densities (Ito and Kawahara, 1993; Sakai et al., 2009).

During the Great East Japan Earthquake, the factory of LIVIC-BW was destroyed. Argin-Gold (Andes-Trading Co., Ltd.) made from dried powder of Ascophyllum nodosum began to be used. This diet compensates the same growth of juveniles at least 3 month (Sakai, unpublished data). Further experiments are necessary to find the proper diet to increase the growth rate of artificial seeds.

In this stage, the predation of settled juveniles by Tigriopus japonicus, which is commonly found in the coastal area of western north-Pacific Ocean, was a major problem to produce the artificial seeds in Japan (Kobayashi and Ishida, 1984). This species grow to only ca. 1 mm in its body length as adults and produce more than 100 eggs at each spawn (Fig. 2). Even just hatched out nauplius larvae, 50 μm in its body length, begin to eat A. japonicus juveniles.

The predatory impacts by T. japonicus are serious during post-larval rearing. The predation begins after the density of this animals increase to ca. 10–20 individuals 10ml$^{-1}$ (Sakai and Konda, 2008;
Kobayashi and Yamaguchi, 2011; Noguchi and Noda, 2011). To prevent predation of artificial juveniles, it is necessary to decrease the density of *T. japonicus*. Trichlorphon, organo-phosphorous compound at a concentration of 1–2 ppm has been used to control this animal (Kobayashi and Ishida, 1984). However, after 2006, this medicine was prohibited by the Pharmaceutical Affairs Law in Japan.

After this, I developed 3 methods to prevent the predation: to immerse the plates into salt enriched seawater (ca.50%), pumping the rearing water containing this predatory animal and filtered out with a 45μm mesh net to decrease copepod density in the water, and using a balled 1 mm mesh as the settlement material which allows the juvenile sea cucumbers to escape from the copepods (Fig. 3).

**Immersing salt enriched water:** Hypersaline seawater induces paralysis of the copepods and removes them from the plates, thereby decreasing predation (Sakai and Konda, 2008). Daily observation to confirm the increase of *T. japonicus* and to determine the timing of this treatment is necessary. Furthermore, a new tank free of copepods to set the collectors in is also necessary.

**Pumping up to filter copepods:** To reduce labor. I set an underwater pump on the bottom of the rearing tank to pump the copepod rich rearing water. The pumped water discharged into a 45 μm mesh net set at the surface of the rearing tank. *T. japonicus* were filtered out by this method and the density of copepods in the rearing tank decreased automatically.

**Balled screen collector:** The balled polyethylene screen is also available as the collector and rearing device to prevent predation. Sea cucumber juveniles can escape into the center of the balled screen if the

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**Fig. 3.** Three main methods to control copepod density in the post-larval rearing tanks. a: Immersing the plates into salt enriched sea water, b: Pumping up and filter out the copepods, c: Balled screen collector. N: 45μm mesh net, P: under water pump.
density of copepods increases and allows them to escape from predation.

Release of artificial seeds

Juveniles are released onto the reefs by divers. Mitsunaga and Matsumura (2004) reported that large juveniles (26 mm) survive better than smaller ones (14 mm). However, the survival rate one year after releasing was only 3.8% and 0.1%, respectively.

Sakai and Kanno (2013) estimated the residual rate in an experimental area using msDNA marker. The residual rate of 8 mm juveniles was only 3.9% four years after releasing, but the released juveniles had also observed in the catches from the fishing area neighbor to this experimental area.

Future perspective

To succeed in increasing the stock of sea cucumbers, further improvement in seed production, especially improving feeding to promote growth during post-larval rearing, is necessary. In addition, evaluating the effectiveness of artificial seed release strategy is indispensable.

The cheapest juvenile for release onto the fishery ground is the newly settled size, 0.4 mm in body length. Fishermen can produce these small juveniles themselves even in the corner of the port without many devices, technicians or long term labor. We can now distinguish even such a small seed from wild ones by msDNA markers. We will now determine if this small seed contributes to increase the natural stocks. The size dependence of the recapture rate should be evaluated as soon as possible for this project to succeed.

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Fluorochrome Marking of Out-planted Green Sea Urchins, *Strongylocentrotus droebachiensis*, for Sea Ranching and Restocking Programs in the Gulf of Maine, USA

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Abstract: Marking calcified structures with fluorochromes is done in a variety of vertebrate and invertebrate species to tag individuals for growth, population, and ecological studies. Here, we describe the use of the fluorochrome tetracycline to identify hatchery reared green sea urchins released on-bottom onto two aquaculture leases known as Job and Sloop, located in the Gulf of Maine, USA. This was done to examine the viability of sea ranching and stock enhancement by looking at recovery rates and growth of reseeded juveniles over the course of two years. 21,000 hatchery reared green sea urchin juveniles (*Strongylocentrotus droebachiensis*) were marked with the fluorochrome tetracycline when they were at 10 - 20 mm test diameter, and released onto 400m² study areas located at each lease. Juveniles from the same hatchery cohort were simultaneously reared in a land-based recirculating aquaculture system so that sea ranching could be compared with tank farming. The release areas were surveyed by SCUBA divers at 3-5 month intervals for over two years. Urchins were collected from the field, measured, and dissected to remove the jaw structures, which were then examined with fluorescence microscopy. Tetracycline fluorescence was detected for up to 27 months post-release in recaptured urchins. Numbers of recaptured marked urchins fluctuated over time, causing large variability in population survival estimates for each site at each sample interval. Size measurements of recaptured urchins showed a decline in average test diameter at the Job site, but at the Sloop site average test diameter increased during the two year study. Green sea urchins from the same hatchery cohort reared in a land-based tank system had significantly better growth than those recaptured from either lease site. Environmental factors, rather than genetic factors (hatchery source), were likely the cause of the size differences observed between hatchery seed recaptured from the lease sites and those reared in tank culture. Site factors may have resulted in size dependant mortality and/or out-migration of larger urchins. One of the limitations of the mark/recapture approach with sea urchins is that dive surveys need to expand over time to account for urchin movement away from the release area. Given the high cost of such efforts, this may not be practical or cost effective. Because the marked jaw structures were internally located, it was not possible to identify marked sea urchins in the field, and the animals had to besacrsificed for laboratory analysis. Recent advances in fluorochrome marking and visualization could allow field identification of marked urchins. This would enhance the ability of resource managers to evaluate restocking programs in the Gulf of Maine, as well as to assign provenance or ownership of sea ranched urchins.

Key Words: green sea urchins, fluorochromes, sea-ranching
The green sea urchin *Strongylocentrotus droebachiensis* has been an economically important fisheries species in the Gulf of Maine, USA (GOM) since the 1980’s. Catch levels peaked in 1993 at 19,050 metric tons, and the fishery value peaked in 1995 at $33,604,275. However, these large annual harvests couldn’t be sustained, and ecological changes have contributed to a steep decline in wild stocks (Steneck et al., 2004). Since 2008 the annual catch in Maine has averaged about 1,300 mt, with an average value of about $5.5 million. Although the fishery may not return to 1990 levels, resource managers and fishermen believe that wild stocks can and should be rebuilt to allow for increased harvest levels. Several management approaches have been taken to help rebuild natural stocks, mostly based on fishing restrictions. Stock enhancement through release of hatchery seed has also been discussed, but uncertainty regarding its ecological and economic viability has discouraged public funding of any sustained stock enhancement programs in the GOM.

Evaluating the effectiveness and benefits of sea urchin reseeding programs is an essential but complex task. The economic return will be a function of seed production costs, growth and survival of out-planted seed, and market prices at the time of recapture. In Japan, sea urchin stock enhancement, known as reseeding, has been done at large scale (>50 million seed annually) for over 20 years (Agatsuma et al., 2004; Sakai et al., 2004). In Hokkaido it cost 4–10 JPY (4–8 US cents) to produce one seed of 5 mm test diameter, and nearly 3x that for 20 mm seed (Sakai et al., 2004). The economic benefits of reseeding to the Japanese fishery remain uncertain; in some cases catches have declined or remained static despite widespread reseeding (e.g. *Strongylocentrotus intermedius* in Hokkaido) , whereas in other cases reseeding is correlated with improved catch levels (e.g. *S. nudus* at Esan near Hakodate City) (Agatsuma 2014, in publication). Ultimately, the costs of seed production versus the economic return to the fishery must be considered in the context of cultural values and ecological consequences.

Although increased catch levels might imply that restocking has been successful, it is not evidence of a cause and effect relationship. Ecological changes, increased recruitment, or intensified fishing effort can also lead to improved stocks or increased catch levels. Measures of survival, growth, and return to the fishery are needed to assess the cost/benefit of stock enhancement. This can only be done if released stock can be differentiated from wild stock, but there are no discernible external differences between hatchery and wild urchins (Agatsuma et al., 2004). However, sea urchins can be internally marked with fluorochromes, either through injection or bath immersion. Kobayashi and Taki (1969) were the first to use tetracycline to mark the sea urchin *S. intermedius* for growth studies. Since that time, a variety of studies have used fluorescent markers to identify sea urchins in the lab or in the field (Ellers and Johnson, 2009). This paper describes the use of fluorochrome marking to identify hatchery seed of *S. droebachiensis* released onto ocean bottom leases in the GOM in order to evaluate sea ranching. Sea ranching is similar to reseeding, but in this case the juveniles were released onto privately held aquaculture leases. The project was carried out by the Center for Cooperative Aquaculture Research (CCAR), working with industry partner Friendship International (FI), a sea urchin trading company based in Maine. We were interested in ascertaining whether this privatized mode of reseeding could be a viable model for the fishery. To do this we needed to determine if released seed would remain within lease site boundaries, and whether growth and recovery rates would be sufficient to realize a return to the lease site operator or to the fishery.

### Materials and Methods

**Hatchery.** The CCAR is a multi-species aquaculture research and development facility operated by the University of Maine (http://www.ccar.umaine.edu/index.html). Hatchery production for the project was carried out at the CCAR in the spring of 2009 (Feb–June). Green sea urchinswere induced to spawn (N=39 females and 30 males) to provide gametes that were fertilized for larval rearing. Laboratory spawning, fertilization and larval rearing methods for *S. droebachiensis* are similar to those described for many other sea urchin species (McBride, 2005). Larvae were reared in conical bottom 230 L clear
fiberglass vats continuously supplied with fresh seawater at about 12 °C. *Dunalieilla tertiolecta* and *Rhodomonas salina* were the primary algal feeds. The larvae were competent for settlement at 24–30 days post-fertilization. Following settlement, juveniles were reared for an additional period of 8–10 months in a land based nursery system. During the nursery period they were held in plastic hydroponic plant baskets in shallow fiberglass raceways, and fed *ad libitum* with freshly harvested *Saccharina latissima*.

**Fluorochrome marking and visualization:** The juveniles were marked (tagged) with tetracycline about four months before release onto the lease sites, using methods adapted from Ellers and Johnson (2009). Juvenile urchins were graded into perforated baskets, and immersed for 24 hours in tanks filled with 0.2 µm filtered seawater and 37.5 mg per L<sup>−1</sup> tetracycline (Sigma-Aldrich Tetracycline T3258). Urchins were fed to satiation before and during tagging to ensure active growth and uptake of the fluorochrome into the calcareous exoskeleton. Two weeks following marking twenty-five urchins were examined using a fluorescence microscope. The jaws of each individual were removed and placed in a sodium hypochlorite solution to dissolve all organic material, leaving only the calcareous jaws behind. These structures were then examined through a GIB filter using a Zeiss Axio Imager Z1 fluorescence microscope. Oxytetracycline goes through excitation at 390nm and emission at 560nm. Tags appear as a bright line of fluorescence spanning the jaw horizontally, and for the most part were easily identifiable (Fig. 1). 100% of those examined directly after tagging had clearly visible tags.

**Sea ranching:** The tagged juveniles were released at two aquaculture leases located in Penobscot Bay, Maine. Site 1 (Sloop) was located off of Northaven, Maine near Sloop Island (44° 12.2'N 68° 50.1'W) and Site 2 (Job) was off of Camden, Maine near Job Island (44° 13.5'N 68° 50'W). Each site comprised two acres (0.81 ha) of sea bottom, with a mean water depth of about 2–5 m. The leases were marked with buoys to indicate that harvesting urchins by dragging nets across the bottom was prohibited. In February of 2010, 10,500 juveniles were released at each site onto a small study area located approximately within the middle of each lease. The juveniles were transferred in plastic bags by divers onto the bottom and distributed along transect lines laid out to 15 m in all four compass directions, encompassing a total area of 400 m<sup>2</sup>. Between 1,000 and 1,500 juveniles were released at 5 m and 10 m markers along the transects to ensure an even distribution. The juveniles were not enclosed and therefore were free to move. No feeding or any other husbandry activity was conducted during the two years following the release.

**Site surveys:** The sites were characterized in a previous study (Kirchoff et al., 2008), but prior to out-planting an initial transect dive was done to estimate the extent of existing sea urchins, predators, and bottom cover. At each release area a baseline was laid out in a North-South orientation and five transect lines were laid out on a perpendicular (East-West) bearing extending to 10 m. Sample quadrats consisting of a 1 m<sup>2</sup> PVC frame were placed at the 10 m marker in each direction, at the center of the transect, and just over the baseline (0 m on transect), for a total of 15 quadrats per site. During the pre-release survey the bottom substrate was characterized and the numbers of predators (crabs, sea starts, etc) and naturally occurring (pre-existing) urchins were counted. The extent and composition of algal feed was also observed for each site. The out-planted areas were then dive surveyed on six more occasions at 3–5 month intervals over the course of 27 months. All urchins within each

**Fig. 1.** A tetracycline marked green sea urchin *Strongylocentrotus droebachiensis* demipyramid viewed using a WIB filter on a fluorescence compound microscope.
sample quadrat were enumerated and those between 4–30 mm TD were collected in numbered mesh tubes to be taken to the laboratory for measurement and identification (absence/presence of fluochrome marker). Urchins smaller or much larger than the original release size were not collected in early surveys, but during later surveys larger urchins were collected to account for any growth. **Tank culture:** During the two year sea ranching study 9,500 green sea urchins from the same hatchery cohort as the lease site urchins were reared in a tank system at the CCAR, to compare growth and survival of lease site urchins with juveniles reared on land. The juveniles were stocked into raceways assembled to form a slanted V interior profile (V-trough), with a perforated bottom plate to remove wastes. The V-troughs were plumbed into a recirculating seawater aquaculture system equipped with a parabolic filter for solids removal, moving bed biofilter, foam fractionator, oxygen injection, 3 hp chiller, and UV sterilizer. Rearing temperatures were held between 6–16 °C year round and the juveniles were fed high quality formulated diets (Nofima diet from Norway). It was anticipated that sea urchins reared under these conditions would have good growth and survival, to provide a benchmark by which the lease site urchins could be compared. **Specimen analysis:** Specimen bags containing urchins from the sample quadrats were brought back to the lab, drained and frozen until analysis. These were later (within 2–6 weeks) thawed in seawater, and all individuals were blotted dry and weighed to the nearest 0.1g. Test diameter (TD) was measured to the nearest 0.1mm with digital calipers (model CD-6PMX Mitutoyo Corporation, Kawasaki, Japan). Each sea urchin collected from the lease sites was analyzed for the presence of oxytetracycline marking, as described above. In some cases multiple or single bands of auto-fluorescence were seen that appeared atypical or ambiguous (e.g. diffuse). Sources of ambiguity and therefore error in identification included size of the jaws, intensity of the light used to make the tags fluoresce, and ambient light from the surrounding room. If the results were uncertain, then the jaws were either reevaluated or marked as “untagged”. Urchins that were clearly tagged were considered as recaptured (hatchery origin).

Urchins reared in the land-based tank system were sampled at intervals coinciding with the lease site surveys. Thirty urchins from each tank were randomly removed and measured for weight to the nearest 0.1 g, and TD to the nearest 0.1 mm using digital calipers. **Data analysis:** The average number of total urchins (tagged and untagged) per square meter was calculated for each study area and survey date as the total number of urchins collected per site divided by the number of sample quadrats (usually 15). The number of released seed remaining at each site and survey date was estimated as the average number of recaptured (tagged) urchins per sample quadrat (m²) x 400 m² (the size of the release area as a whole). The mean, minimum and maximum test diameter of recaptured urchins was calculated for each site and survey date. Chi squared tests were used to determine whether or not the numbers of tagged and untagged urchins were significantly different from each other. The standard deviation of the mean test diameter was determined to see if the average size of recaptured urchins significantly differed (+/– 1 SD) between the two sites and from the tank reared urchins. Data were plotted to display trends in numbers, average TD, and maximum TD of recaptured urchins at each site over time, and the TD of lease site urchins was compared with that of tank reared urchins.

**Results**

**Site characteristics:** The two sites were less than six nautical miles apart and of comparable depth (2–6 m mean water), but they differed in terms of exposure, current, bottom substrate, and population density of naturally occurring (pre-existing) urchins. The Job Island site had relatively uniform depth, but was subject to periods of extreme slack tide and periods of strong current. The bottom substrate at Job was 80% rock cobble with several small boulders throughout, which were populated with macroalgae, but relatively little drift algae was found. Predators were not found in abundance, with only one large Jonah crab (*Cancer borealis*) observed, and the initial population density of pre-existing urchins at the Job site was 225 animals/m². At the Sloop site,
the bottom substrate was 80% shell hash, which provided abundant refuges for small and medium-sized urchins. The study area was on a sloped ledge, so the depth varied across the area compared with Job, which was more flat. A few small boulders were found on the Sloop site with macroalgae growing on them, and drift algae, mostly kelp, were abundant. The Sloop Island site had an abundance of large urchins and sea stars present on it at out-planting. The initial population density of pre-existing urchins at the Sloop site was 4.5 animals/m².

Recapture rates: Sea urchins were found on both study areas at every survey for over two years. The total number (tagged and untagged) found at each survey ranged from 4 - 674 at the Job site and from 194 - 397 at the Sloop site. Tagged urchins (hatchery origin) were recaptured at both sites and at every dive survey up to the last, 27 months post-release. Recapture rates declined in the first year but then significantly spiked in the summer of the following year at both sites, before again declining in subsequent surveys (Fig. 2). At the Job site 10% to 100% of the urchins collected during each dive survey were determined to be of hatchery origin, and at Sloop 35% to 71% of collected urchins were of hatchery origin. It’s important to note that on the one occasion when 100% of the animals collected at Job were tagged, the entire sample population consisted of just four animals, all very small (<7 mm TD). At the final survey a total of 107 urchins were collected from Job and about 30% of these were tagged. At the final Sloop survey the urchin population showed a significant decline from previous levels, and there was evidence (disturbed grounds, gear tracks, and broken tests) that the site had been recently fished by a dragger boat.

Population estimates: Population estimates of hatchery origin urchins remaining within the 400m² release areas at each survey varied in direct proportion with the recapture rates (Fig. 3). Originally, 10,500 urchins were released at each study area. Extrapolation from dive surveys indicated that the number of hatchery origin urchins remaining within the Job release area at each survey ranged from 45 to 36,894; with 3,306 projected as still remaining at 27 months post-release. At the Sloop site, population estimates of hatchery urchins remaining at each survey ranged from 3,680 to 18,165; with 7,360 projected as still remaining at the final survey, 27 months post-release (Fig. 3).

Average and maximum size: The average test diameter (TD) of hatchery origin urchins recaptured at the Job site declined to 5.1 mm over the course of the study, which was the minimal release size, but TD increased at the Sloop site (Fig. 4). At Job, the average TD declined from 10.6 mm at release to 5.1 mm 27 months post-release, whereas at Sloop the average TD increased from 11.3 mm at release to 18.3 mm at 27 months. The largest marked urchin recaptured from any of the surveys at Job during the course of the study was 19.7 mm (Aug 2010), and at Sloop it was 49.3 mm TD (Sept 2011, 19 months post-release) (Fig. 5). The Job site had a disproportionate number of small urchins remaining on it at every survey throughout the course of the

![Fig. 2. Total numbers of hatchery origin *S. droebachiensis* recaptured from two release sites in Penobscot Bay, Maine at each survey.](image1)

![Fig. 3. Estimated population of hatchery origin *S. droebachiensis* remaining within the study area at each lease site and sample date. Calculated as average number of recaptured urchins per sample quadrat (m²) x 400 m² (total study area).](image2)
study. Of the total number of urchins (sum of six surveys) recaptured from Job, 84% were ≤6mm. At the Sloop Island site, only 1% of the total recaptured urchins were ≤6mm.

Growth rates diverged between land and sea based hatchery urchins within the first year of the study. Sea urchins reared in the land-based culture system were much larger on average at the end of the two year study than those recaptured from either ocean lease site (Fig. 4). After 27 months the largest urchin sampled in the tank culture system was 53.4 mm, and = 1/3 of the tank reared urchins were ≥40 mm.

Fig. 4. Average test diameter of hatchery origin *S. droebachiensis* recaptured at two release sites in Penobscot Bay, Maine at each dive survey, and in tank culture at the CCAR. Error bars = ±1 standard deviation from the mean.

Fig. 5. Maximum test diameter of hatchery origin *S. droebachiensis* recaptured at two release sites in Penobscot Bay, Maine at each dive survey.

Discussion

Few previous studies in North America have monitored survival and growth of tagged sea urchins released into the field. Dumont et al. (2004) released three size groups of green sea urchins tagged with tetracycline onto a small study area. Similar to the present study, they found that recapture rates were size and time specific: 69% for <10 mm and 2% for >15 mm urchins after nine days, and 25% and 0% respectively after forty days. In a study by Rogers-Bennett et al. (1994), red urchin juveniles (*Strongylocentrotus franciscanus*) were tagged and
released onto study areas that varied in depth. Recovery rates after 12 months were 21% from shallow habitats and 11% from deep habitats.

The present study provides evidence that hatchery reared green sea urchins can be successfully out-planted for reseeding or sea ranching in the Gulf of Maine. Success is defined as the ability of seed to survive and grow to legal harvest size (52 mm) within 5 years of release. We saw that released juveniles survived and remained for an extended period (27 months) within each release area. However, recaptured juveniles were disproportionately smaller at one site (Job) than at the other (Sloop). This suggests that site factors modified the size distribution of surviving or remaining out-plants in different ways at the two sites. The Sloop Island site may have had a habitat more favorable for sea urchins, with more and larger refuges, and greater feed abundance.

Following settlement, juvenile green sea urchins take refuge under rocks, in crevices, or under debris as an adaptation to escape predation (Cameron and Schroeter, 1980; Dumont et al., 2004). Here they graze on diatoms, coralline algae and detritus (Raymond and Scheibling, 1987). While both sites in the present study supported urchins, the shell hash at the Sloop Island site was full of cracks, holes and larger spaces, providing refuges for a broader size range of juveniles. The rock cobbles at the Job Island site was, for the most part, flat against the sediment, with fewer and smaller hiding places for juveniles. The rock cobbles had small interstices that were well suited for juveniles at or below 5-6 mm, but too small for larger urchins. Most of the urchins recovered from the Job site surveys, whether wild or tagged, were < 15 mm TD.

The Job site generally had lower recapture rates of tagged seed than the Sloop site, indicating that it was less hospitable for out-planted sea urchins. The notable exception occurred in the summer of the second year, when a large and significant number of small urchins were captured at the June 2011 Job site survey, and subsequently identified as hatchery origin due to presence of the fluorochrome mark. This spike in recapture numbers could have been due to misidentification (e.g. detecting auto or pseudo-fluorescence and attributing it to the tag), or it could have been a sampling artifact. Presumably, misidentification would have occurred equally at Sloop at this survey date, and it did not. Although Sloop had higher recapture numbers (per quadrat and total) at this survey date than at other surveys, they were not significantly different from the other Sloop surveys. Also, we were concerned about this issue and any specimens with atypical fluorescence patterns were considered as unmarked. For these reasons, we believe that the spike in recapture numbers observed at Job during the June 2011 survey was a sample artifact. Every sample quadrat had to be thoroughly and equally searched, often by overturning rocks and shells to find hidden urchins. This effort had to be consistent between sites and survey dates, which in practice was difficult to accomplish. Under varying field conditions of bottom substrate, current, turbidity, ambient light, and temperature, it’s likely that the success rate for finding urchins would vary between sites and dates. In addition, random movement patterns of urchins onto and off of the study areas probably occurred, because urchins move in response to food availability and the presence/absence of predators (Dumont et al., 2007). At about 15mm TD sea urchin juveniles are less vulnerable to predation, and a shift from cryptic to active foraging occurs (Dumont et al., 2004). Migration of urchins larger than 10 mm TD away from the release area in search of feed or refuge might explain the disproportionate numbers of small tagged urchins seen at the Job site.

Active foraging enhances the availability and quality of macroalgae, increasing the growth rate. When there is abundant food sea urchins will aggregate in high densities, and they can remain stationary for several months or longer (Dumont et al., 2007). In the present study, both sites provided feed in the form of encrusting algae and particulate macro-algae. However, the Sloop site was more exposed and had greater currents (Kirchhoff et al., 2008), and urchins at this site thus had access to large pieces of drift algae, mostly kelp, that were carried onto the site by the current. This greater feed availability might explain why recaptured urchins had a larger average and maximum TD at Sloop than at Job. The lack of a substantial food source at the Job site might have encouraged
more of the larger urchins to leave the site, while
also causing slow growth of the small urchins
that remained, due to low food intake. Green sea
urchin growth rates can be highly variable in the
natural environment, primarily in response to feed
availability and type (Nestler and Harris, 1994;
Brady and Scheibling, 2006). Growth can be very
slow and rates of $\leq 0.25$ mm per year have been
documented for urchins found in tide pools (Russell,
1998). We observed that green sea urchins from
the same hatchery cohort reared in the land-based
culture system had significantly better growth than
those recaptured at either lease site. This is further
evidence that growth potential at the lease sites
was limited more by environmental factors than
by genetics or by the fact that the urchins were of
hatchery origin.

In the present study we were able to differentiate
hatchery origin from wild urchins for up to 27
months in the field. Johnson et al. (2013) reported
that tetracycline fluorescence could be detected
for at least two years in green sea urchins held in
the lab, when tetracycline was administered via
injection. The fact that fluorochromes can persist for
such extended periods makes this marking/tagging
method invaluable for long term lab and field studies
of sea urchins, and was essential to carrying out
the research described above. Recent advances in
the application and visualization of fluorochromes
offer further advantages, which could bring down
costs and improve the effectiveness of sea urchin
mark/recapture studies. Ellers and Johnson (2009)
describe methods to create multiple marks on the
demipyramids (e.g. at intervals or with multiple
fluorochromes), which would allow for differentiation
of multiple year classes released into the field.

The same authors also describe visualization of
fluorochromes on external structures such as
the skeletal plates (test) and spines, which allows
tagged individuals to be identified without sacrifice
(Johnson et al., 2013). Ultimately, development of a
field portable device for visualizing fluorochromes
seems feasible, to allow reliable identification of
stocks in situ while minimizing adverse impact on
the population (Johnson et al., 2013). These methods
provide powerful tools for evaluating the results of
future restocking and sea ranching programs for
green sea urchins in the Gulf of Maine.

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Improving the Food Quality of Sea Urchins Collected from Barren Grounds by Short-Term Aquaculture under Controlled Temperature

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Abstract: In west Hokkaido, there are many barren grounds where Mesocentrotus nudus is abundant. These sea urchins have poor commercial value due to their thin gonads, but can become marketable by intensive feeding for gonad enhancement. In general, the quality of sea urchin gonads as food products decreases as gametogenesis progresses. Mature ovaries and testes are not suitable as food products because of the unpleasant taste caused by gamete content and the melting appearance caused by gamete flow via breakage of the gonoduct. Immature to pre-mature gonads that contain predominantly nutritive phagocytes (somatic nutrient storage cells) and not copious gametes have a higher commercial value. Thus, enhancing the nutrient accumulation into nutritive phagocytes plus suppressing gametogenesis is advantageous for sea urchin aquaculture. We are developing short-term aquaculture techniques to improve the food quality of M. nudus collected from barren grounds under controlled temperature to suppress gametogenesis. Rearing M. nudus under a low temperature between summer and autumn has proved to be effective to increase the gonad size without the quality deterioration caused by maturation.

Keywords: aquaculture, food quality, gametogenesis, gonad, sea urchin, temperature

For decades, the loss of seaweed beds and the expansion of barren grounds has been a serious problem for coastal fisheries in Japan (Fujita, 2010). The bare grounds populated by sea urchins are known as urchin barrens (Pearse, 2006; Fujita et al., 2008). In west Hokkaido, there are many urchin barrens, where Mesocentrotus nudus is abundant (Fig. 1). The sea urchins in these barrens have poor commercial value because they have thin gonads (edible portion) due to deficiency of their main food, macroalgae. However, they can become marketable by intensive feeding for gonad enhancement in short-term aquaculture (Agatsuma and Nishikiori, 1991; Unuma and Kayaba, 2015). If the cultured M. nudus of improved quality are sold in autumn, out of the fishing season of this species, higher market price can be achieved.

Unlike the gonads of other animals, sea urchin

Fig. 1. An urchin barren in Sutt-su, west Hokkaido. The barren ground is populated by Mesocentrotus nudus that has poor commercial value because of thin gonads. The photograph was taken by Kazuhiro Takahashi.
ovary and testis (both of which are equally preferred as food) play a role as a nutrient storage organ (Walker, 1982; Walker et al., 2013; Unuma, 2002, 2015). A thorough understanding of the unique characteristics of sea urchin reproduction should permit novel methods to improve the quality of gonads as food products in its aquaculture. We are currently conducting research and development for short-term aquaculture techniques to maximize the value of M. nudus collected from urchin barrens under controlled temperature and harvest them when wild M. nudus is scarce in the market. In this paper, we describe the theoretical background to improve the food quality of M. nudus by manipulating environmental conditions and give a brief overview of our ongoing research.

Features of Gametogenesis

The sea urchin has five gonads attached internally to the test (shell). A lobe of gonad consists of hundreds of gonadal acini and resembles a bunch of grapes (Fig. 2A). There are two major populations of cells inside the acinus (Fig. 2B): germ cells (GCs, from oogonia to ova in the ovary and from spermatogonia to fully differentiated spermatozoa in the testis) and somatic cells called nutritive phagocytes (NPs) that are present in both sexes (Walker, 1982; Walker et al., 2013). NPs store nutrients necessary for gametogenesis and supply it to GCs (Walker, 1982; Unuma, 2002).

During the annual reproductive cycle, gonads of both sea urchin sexes pass through a predictable series of structural changes (Unuma and Walker, 2009; Walker et al., 2013). Fig. 3 shows histological changes in the ovary and testis of M. nudus during gametogenesis as classified into five stages by Fuji (1960a) with modifications (Unuma, 2002).

Stage 1. Immature gonad before gametogenesis: In both sexes each acinus is filled with NPs (eosinophilic cell populations). In ovaries, a few young oocytes are present at the periphery of the acini. Hematoxylin-stained round spots, residue from phagocytized ova (Masuda and Dan, 1977; Tominaga and Takashima, 1987), are occasionally present centrally in the ovarian lumen. In testes, detection of spermatogenic cells is sometimes difficult at this stage in paraffin sections. Instead, many hematoxylin-stained speckles, residue from phagocytized spermatozoa (Kato and Ishikawa, 1982; Reunov et al., 2004), are often present in NPs. These speckles, which are amorphous unlike the round spots observed in the immature ovary, are a useful feature to distinguish testes from ovaries.

Stage 2. Beginning of gametogenesis: Many developing oocytes or clusters of spermatogonia are present at the periphery of the acini, and the gonadal lumina are still filled with NPs.

Stage 3. Middle of gametogenesis: NPs are replaced with ripe ova or spermatozoa in the center of the gonadal lumina. Numerous developing oocytes or clusters of spermatogonia and spermatocytes (Ward and Nishioka, 1993; Walker et al., 2005) are present at the periphery of the acini. NPs are gradually decreasing in size and are present between the GCs.

Stage 4. Fully mature gonads at the end of gametogenesis: The gonadal lumina are filled with ripe ova or spermatozoa. Shrunken NPs, which have already lost nutrients, are present only at the periphery of the acini.

Stage 5. After spawning: The gonadal lumina have numerous empty spaces and a few residual ova or spermatozoa. NPs gradually phagocytize residual gametes and begin to grow as they store nutrients. After this stage, gonads return to Stage 1 and a new cycle starts.
Fig. 3. Histological changes in the ovary (upper panels) and testis (lower panels) of Mesocentrotus nudus during gametogenesis. Paraffin-embedded sections are stained with hematoxylin and eosin. Schematic drawing below the photomicrographs shows structural features that are common between the ovarian and testicular acini. At stage 1, the gonadal lumina are filled with nutritive phagocytes. At stage 2, many developing oocytes or clusters of spermatogonia are present at the periphery. At stage 3, nutritive phagocytes are replaced with ripe ova or spermatozoa in the center of the lumina. At stage 4, the lumina are filled with ripe ova or spermatozoa. At stage 5, the lumina contain a few residual ova or spermatozoa. np = nutritive phagocyte, oc = oocyte, ov = ripe ovum, ro = residual ovum, sg = spermatogonium, sz = spermatozoon, and rs = residual spermatozoon. Inset a, round spot derived from a phagocytized residual ovum. Inset b, amorphous speckles derived from phagocytized residual spermatozoa. Scale bar represents 100 μm.

Seasonal Changes in Gonad Size and GCs/ NPs Proportion

Sea urchin gonads grow in size not only because gametogenesis increases the size or numbers of GCs but also because NPs store extensive nutrient reserves before gametogenesis (Unuma and Walker, 2009; Walker et al., 2013). Fig. 4 shows the seasonal changes in the gonad index (the ratio of gonad weight to total body weight) and in the proportion of GCs and NPs in M. nudus. This species spawns around October and gonad indices rapidly decrease (Fuji, 1960b). After spawning, the gonad index gradually increases until the next spawning. The increase before gametogenesis is attributable to the growth of NPs. NPs accumulate various nutrients, such as proteins (principally the major yolk protein; Unuma et al., 2003, 2011), lipids, and carbohydrates (principally glycogen; Marsh et al., 2013) and increase in size. Proliferation and development of GCs begin

![Graph showing seasonal changes in gonad index and GCs/NPs proportion.]

Fig. 4. Diagrammatic representation of the seasonal changes in the gonad index and in the proportion of nutritive phagocytes (NPs) and germ cells (GCs) in Mesocentrotus nudus. Typical gametogenic stages are indicated below the graph. Spawning occurs around October. The long-term increase in the gonad index before gametogenesis is attributable to the growth of NPs. The best season for eating gonad is around July.
three months before spawning in *M. nudus* (Unuma, 2009). After gametogenesis begins, the size of the gonad continues to increase but the proportion of NPs in the gonad rapidly decreases.

**The Best Season for Eating Gonads**

The best season for eating *M. nudus* gonads is around July, when NPs have grown sufficiently but the proportion of GCs is still smaller (Unuma, 2009). Their gametogenic stage is usually from stage 2 to early stage 3 in July (our unpublished observation). Before this period, the size of the gonad is too small and the color of the gonad is not attractive (brownish). After this period, the proportion of GCs becomes higher and the quality as food gradually deteriorates. The most serious problem for food quality is the melting appearance caused by the flow of gametes (Unuma, 2002; Unuma and Walker, 2009). After ripe gonads are taken out of the test, eggs or sperm flow out from the breakage of gonoduct, and the gonads cannot maintain their consistency (Fig. 5). This phenomenon considerably reduces the commercial value of the gonads. Additionally, the gametmes cause unpleasant tastes such as bitterness in the mature gonads (Unuma, 2002; Unuma and Walker, 2009).

Relationship between the gametogenic stage of gonads and the food quality varies among species. Ovaries of *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus* produce an extremely bitter taste after oogenesis begin (Murata et al., 2002; Murata 2009, Unuma and Walker, 2009). In these species, the season for eating is limited only when their gonads are at stage 1 (Murata 2009; Unuma 2002, 2009). On the other hand, other Japanese sea urchins including *M. nudus* can be eaten until when their gonads are at stage 3, because the maturing gonads do not produce a strong bitterness (Murata 2009; Unuma 2009). In every species, the best season for eating gonads is rather short because of the problems caused by gametogenesis. Low-quality or small gonads can be obtained for a longer period of time, but superior-quality and sizable ones can be harvested for only about one or two months (Unuma, 2002).

**Improving Food Quality by Short-term Aquaculture**

As we have seen, NPs are more important as food than GCs. Thus, when sea urchins are cultured for gonad enhancement, not GCs but NPs should be increased in the gonads. The sea urchins dwelling at urchin barrens cannot store much nutrient in the gonads due to deficiency of food (Agatsuma and Nishikiori, 1991). However, the sea urchins produce appropriate amount of gametes and spawn even if the food availability is low. Therefore, the schema of gonad index and GCs/NPs proportion (Fig. 4) is turned to be depressed in the sea urchins at urchin barrens (Fig. 6A). We are trying to change this schema by short-term aquaculture as shown in Fig. 6B. The aims of the culture are (1) to promote nutrient accumulation into NPs by intensive feeding and (2) to suppress proliferation and development of GCs by manipulating environmental conditions. Combination of these two aims should prolong the season for quality gonad until the spawning season when wild *M. nudus* is not fished due to quality deterioration as well as for resource management.

**Environmental Factors Regulating Gametogenesis**

Gametogenic cycles of sea urchins are regulated by environmental factors, such as photoperiod and water temperature. There have been many reports describing the effects of these factors on gametogenesis of sea urchins as listed in Table 1. Most of these researches were conducted as basic biology to identify factors affecting maturation, or as applied biology to develop techniques for promoting

![Fig. 5. Gamete flow from the mature ovary (left) and testis (right) of Mesocestrotus nudus. After mature gonads are taken out of the test, they appear to be melting because of flowing eggs or sperm from the breakage of gonoduct.](image-url)
Fig. 6. Purpose of the short-term aquaculture of *Mesocentrotus nudus* collected from urchin barrens. Schema of gonad index and GCs/NPs proportion can be changed from (A) to (B) by rearing under optimized conditions. The aims of the culture are (1) to increase the nutritive phagocytes (NPs) by intensive feeding and (2) to decrease the germ cells (GCs) by suppressing gametogenesis. Combination of these two aims should prolong the season for quality gonads.

Table 1. Researches on environmental factors affecting sea urchin gametogenesis

<table>
<thead>
<tr>
<th>Factors investigated</th>
<th>Species</th>
<th>literature</th>
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<tr>
<td>Photoperiod</td>
<td><em>Eucidaris tribuloides</em></td>
<td>McClintock and Watts (1990)</td>
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<td><em>Paracentrotus lividus</em></td>
<td>McCarron <em>et al.</em> (2010)</td>
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<td><em>Strongylocentrotus droebachiensis</em></td>
<td>Walker and Lesser (1998)</td>
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<td>Böttger <em>et al.</em> (2006)*</td>
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<td>Dumont <em>et al.</em> (2006)</td>
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<td>Kirchhoff <em>et al.</em> (2010)</td>
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<td><em>Strongylocentrotus purpuratus</em></td>
<td>Cochran and Engelmann (1975)</td>
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<td>Pearse <em>et al.</em> (1986)</td>
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<td>Bay-Schmith and Pearse (1987)</td>
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<tr>
<td>Temperature</td>
<td><em>Heliocidaris crassispina</em></td>
<td>Sakairi <em>et al.</em> (1989)</td>
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<td><em>Hemicentrotus pulcherrimus</em></td>
<td>Yamamoto <em>et al.</em> (1988)</td>
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<td>Ito <em>et al.</em> (1989)</td>
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<td><em>Pseudocentrotus depressus</em></td>
<td>Sakairi <em>et al.</em> (1989)</td>
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<td>Yamamoto <em>et al.</em> (1988)</td>
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<td>Noguchi <em>et al.</em> (1995)</td>
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<td><em>Strongylocentrotus droebachiensis</em></td>
<td>Garrido and Barber (2001)</td>
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<td><em>Strongylocentrotus intermedius</em></td>
<td>Kayaba <em>et al.</em> (2012)*</td>
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<tr>
<td>Photoperiod and Temperature</td>
<td><em>Paracentrotus lividus</em></td>
<td>Spirlet <em>et al.</em> (2000)*</td>
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<td>Shpigel <em>et al.</em> (2004)*</td>
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<td>Kelly (2001)</td>
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*Conducted from a view point of suppressing gametogenesis*

maturation to obtain gametes out of the spawning season for laboratory use or for seed production. For example, in some sea urchin hatcheries in Japan, out-of-season maturation of brood stock of *Pseudocentrotus depressus*, *Hemicentrotus pulcherrimus* and *Strongylocentrotus intermedius* is promoted by manipulating water temperature (Ito *et al.*, 1989; Masaki and Kawahara, 1995; Noguchi *et al.*, 1995; Sakai, 2015). However, for culturing adult sea urchins to enhance the gonads, not promotion but suppression of gametogenesis is required. To our knowledge, only four studies have been conducted from a view point of suppressing gametogenesis to obtain the gonads of better food quality. In *Strongylocentrotus droebachiensis* (Böttger *et al.*, 2006), *Strongylocentrotus intermedius* (Kayaba *et
al., 2012) and *Paracentrotus lividus* (Spirlet et al., 2000; Shpigel et al., 2004), maturation was delayed by manipulation of photoperiod, water temperature and both of them, respectively. However, the control regime of environmental conditions for culturing *M. nudus* is still to be clarified, because optimum conditions to suppress gametogenesis effectively varies among different species.

**Overview of Our Research**

Our final goal is to develop a land-based aquaculture system, using *M. nudus* collected from urchin barrens, controlling their gametogenic cycle, and harvesting high-quality urchins when their wild counterparts are scarce in the fish market. As an initial step, we investigated the effects of water temperature on the size and quality of the gonads in *M. nudus*.

Adult *M. nudus* collected from an urchin barren in Suttusu, west Hokkaido (Fig. 1), were reared from late June to mid October in three tanks maintained at either a constant temperature (10°C or 15°C) or a variable temperature, similar to their natural environment (control group: 18.0°C to 22.5°C). They were given a surplus of kelp (*Saccharina longissima*). Over the experimental period, gonad indices increased in all treatments from 7% initially to over 20% after three months. Histological observations revealed that after three months, there were no urchins with fully mature gonads in the 10°C group, but 6% in the 15°C group, and 39% in the control group. Sensory tests of the final gonads found that the quality of the control group was inferior to that of the 10°C and 15°C groups, in terms of both appearance and taste. We conclude that rearing *M. nudus* under a low temperature between summer and autumn is effective to increase the gonad size without the quality deterioration caused by maturation. These results are being prepared for publication in the journal. We are further investigating the relationship between the rearing conditions and the size and quality of the gonads to determine the optimum control regime for culturing *M. nudus*.

**Methods to Decrease Water Temperature**

To put our findings into practice, economic methods for decreasing the water temperature in aquaculture farms are required. Using electric cooling devices is a classic method but has high energy costs. It would not be practical to use it in a conventional flow-through system. However, closed or semi-closed recirculating system may minimize the problem of energy costs in electric cooling devices.

Deep seawater drawn from a depth of over 300 m has a very low temperature (Nakasone and Akeda, 2000). Large initial investment costs are required to build a facility to draw the water. However, more than ten facilities capable of drawing deep seawater have been constructed in Japan, where it is used for aquaculture, handling of captured fishes, the food industry, medical treatment, and agriculture (Nakasone and Akeda, 2000; Kayaba et al., 2012); including two in west Hokkaido. Therefore, the use of existing facilities is a practical way to adopt the deep seawater method in sea urchin aquaculture.

The use of salty groundwater for aquaculture, with a salinity comparable to seawater, is a recent development in Japan (Ebata et al., 2006; Imada et al., 2006). It can be obtained from wells bored near to the shore and has a stable temperature year round. In west Hokkaido, the temperature of salty groundwater is about 10°C even in summer, although that of surface seawater rises over 20°C. The advantage of this method is that it requires small initial investment costs. However, this water sometimes contains high levels of ammonium and manganese ions (Ebata et al., 2006). Such groundwater requires treatment using aeration and specified microbes to remove the ions before use (Ebata et al., 2006).

Thus, there are multiple methods for decreasing the temperature of rearing water in summer. The chosen method depends on the location and scale of the aquaculture farm.

**Acknowledgments**

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Maturation Control of the Short-Spined Sea Urchin, *Strongylocentrotus intermedius*, by Low Temperature Rearing Using Deep-Sea Water, with the Aim of Extending the Market Season

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Abstract: The short-spined sea urchin *Strongylocentrotus intermedius*, is a valuable commercial species and one of the most popular and expensive seafoods in Japan. Serving local short-spined sea urchin to visitors during the summer tourist season in Rausu, located in a world natural heritage site “Shiretoko,” has long been desired. However, it has not been feasible during the sea urchin spawning season (July to September), resulting in quality degradation in gonads, the edible part of sea urchins, due to maturation. Therefore, the Hokkaido Research Organization and the Rausu Fishery Cooperative Associations cooperatively investigated the possibility of suppressing gonadal maturation and maintaining high quality sea urchin gonads by low temperature rearing using deep-sea water. Unripe sea urchins captured before the spawning season were reared under two temperature conditions from June to September. In groups reared at ambient temperatures (2-18°C), gametogenesis in both sexes progressed rapidly with increased temperature, and almost all sea urchins reached full maturity by late July. Whereas in groups reared at low temperatures (2-5°C), gametogenesis progressed slowly and over 60% of the sea urchins had not reached maturity even by early September. This result suggests that the progress of gametogenesis in the short-spined sea urchin is effectively suppressed by rearing under low temperature conditions. Additionally, we also examined the effects of feeding on gonadal development in sea urchins reared under low temperature conditions, and revealed that feeding with live *Saccharina diabolica*, which were cultured as food in Rausu, could increase the gonadal volume efficiently to commercially preferable size, while suppressing the progress of gametogenesis. Moreover, the results of chemical analysis and tasting test proved that the quality of gonads were very excellent in sea urchin fed with live *S. diabolica*. Consequently, we demonstrate that low temperature rearing, supplemented with feeding live *S. diabolica*, is effective in suppressing gametogenesis to allow for the harvesting of high quality sea urchins during the summer tourist season. At present, this aquaculture method is being put to practical use by Rausu Fishery Cooperative.

Key words: short-spined sea urchin *Strongylocentrotus intermedius*, gametogenesis, deep-sea water, low temperature rearing, sea urchin aquaculture

The short-spined sea urchin *Strongylocentrotus intermedius*, which is distributed on the northern coast of Japan, is a valuable commercial species and is one of the most popular and expensive seafoods in the country; more than 2000 tons of the sea urchin are caught annually in Hokkaido, the main harvest region for this species. *S. intermedius* has bright orange gonads with a rich taste, and the gonads

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are preferred especially as an attractive raw food material, such as “Sushi” or “Sashimi (Uni-don).” *S. intermedius* has also become one of the important tourist resources of Hokkaido, because many tourists visit Hokkaido in anticipation of the tastes of fresh sea urchins every year.

Since the edible part of the sea urchin is usually restricted to the gonads, its quality as food varies dramatically with gonadal growth and maturation. In general, the superior quality of gonads, defined by attractive taste, color, and shape, is only present during the term of the unripe phase when gametogenesis is limited. The mature gonad has an unpleasant taste due to the gamete contents and a melting appearance caused by the flow of gametes (Unuma, 2002). Therefore, the best season for harvesting *S. intermedius* is very short—less than three months per a fishery region—in spite of an annual market demand. There would be considerable commercial value in developing an aquaculture technique to control the timing of gonadal maturity so as to ensure a supply of high quality sea urchins over a longer period.

Rausu town, located on the Shiretoko Peninsula in east Hokkaido, is a prominent production region of *S. intermedius* in Japan (Fig. 1). After Shiretoko Peninsula was registered as a world natural heritage site in 2005, increasing numbers of tourists have visited Rausu, especially during the summer, from July to September. In Rausu, there is a huge demand for serving *S. intermedius* to visitors during the summer tourist season. However, it has been exceedingly difficult to supply high quality sea urchins during the summer, because the spawning season of sea urchins in Rausu is from July to September and the quality of gonads degrades on maturation. Moreover, in Rausu, as a resource conservation measure, harvesting sea urchins from July to September is legally prohibited by local government fishing regulations. Therefore, it is necessary to develop a method for culturing *S. intermedius* caught before the closed season, so as to maintain their food quality by suppressing maturation.

Annual reproductive cycles of sea urchins are regulated by environmental factors, such as water temperature (Yamamoto et al., 1988; Sakairi et al., 1989), photoperiod (Pearse et al., 1986; McClintock and Watts, 1990; Walker and Lesser, 1998; Böttger et al., 2006), and lunar period (Horii, 1997; Coppard and Campbell, 2005). Water temperature in particular has been shown to have a strong effect on the progress of gametogenesis in sea urchins. In some Japanese urchin hatcheries, temperature control is used to induce broodstocks of *Pseudocentrotus depressus*, *Hemicentrotus pulcherrimus*, and *S. intermedius* to mature out of the normal spawning season (Ito et al., 1989; Masaki and Kawahara, 1995; Noguchi et al., 1995). The gonadal development of wild *S. intermedius* tends to be correlated with a rise in water temperature from spring to summer (Tomita et al., 1984). Accordingly, it may be possible to suppress gametogenesis by rearing sea urchins under low temperature conditions during that period.

Generally, using electric cooling devices in aquaculture is limited because of its high costs. Fortunately, a facility for collecting deep-sea water is available in Rausu. The deep-sea water is defined as seawater that is pumped from a depth of greater than 200 m, and has certain advantageous properties: stable low temperature, low in suspended particles and bacteria, and rich in nutrient salts (Nakasone and Akeda, 2000). More than ten facilities capable of drawing deep seawater have been constructed in Japan, where it is used for aquaculture, handling...
of captured fishes, the food industry, medical treatment, and agriculture (Takahashi, 2006). In Rausu, deep seawater is drawn from a depth of 350 m and has been used mainly as a raw material for refining mineral water and as rearing water to temporarily hold captured salmon. Additionally, there is a sufficient supply of deep-sea water, such that it has been collected constantly and used for aquaculture in Rausu. Fig. 2 indicates variations in water temperature between the deep-sea water and ambient sea water drawn from the surface. The water temperature of deep seawater is stable at lower degrees from March to September compared with the ambient seawater, ranging from 2 to 5°C. Therefore, taking advantage of the available facility, the Hokkaido Research Organization and the Rausu Fishery Cooperative Associations cooperatively investigated the effects of rearing under low temperature conditions on the gametogenesis of *S. intermedius*, in an effort to develop reliable techniques for maintaining gonads in good quality during the summer tourist season in Rausu, as a model (Kayaba et al., 2012). In this proceeding, we describe new aquaculture technique of sea urchin and its possibility in the future, adding further data to the results which have been reported in Kayaba et al. (2012).

The effects of rearing under low temperature conditions on gametogenesis

To determine the effect of water temperature on suppressing gametogenesis, rearing experiments were conducted under two types of water temperature conditions from 2008 to 2010 (Kayaba et al., 2012). Unripe sea urchins (weighing 54.0–81.3 g), were collected from the coastal fishery ground at Rausu in March – May. They were divided into four experimental cages (100 × 100 × 80 mm) at densities of 150 individuals per cage, and transferred to the experimental environment on June 1 of each year. Two cages of sea urchins were maintained with running ambient sea water (2.8 – 19.6°C) until September 1 (ambient temperature group), while the other two cages were maintained with running deep-sea water (2.5 – 4.9°C) over the same period (deep seawater temperature group). In addition, for determining the effects of feeding on gametogenesis and gonad size, individuals in one cage of both temperature groups were reared without feeding (non-feeding cage), while individuals in the other cages were given a surplus of commercial dry wakame (*Undaria sp.* obtained from the Riken Vitamin Company, Tokyo) every day (feeding cage). Both groups of sea urchins were reared in the dark throughout the experimental period, except

Fig. 2. Variations in temperature of the ambient sea water drawn from the surface of the Rausu coast and that of the deep-sea water drawn from a depth of 350 m off the coast of Rausu, in 2009.
for during feeding and cleaning of excreta. Twenty individuals were sampled from each group monthly for histological observation of gonads. Maturational status of ovaries and testes was classified into five stages according to Fuji (1960): stage 1 (before gametogenesis), stage 2 (early gametogenesis), stage 3 (mid-gametogenesis), stage 4 (fully mature) and stage 5 (spent). Gonads at stages 1 to 3 are commercially favorable. On the other hand, the fully mature gonads (stage 4) were not suitable for consumption because of the unpleasant taste caused by the gamete contents and a melting appearance caused by the flow of gametes via breakage of the gonoduct (Unuma, 2002). The spent gonads (stage 5) were also unsuitable for food, because they were shrunken.

Table 1 shows the frequency of maturation stage of female and male sea urchins during the experiment in 2009. In the ambient temperature group, gametogenesis progressed rapidly with increased temperature and almost all sea urchins reached full maturity (stage 4) by 27 July, about 2 months after the initiation of the experiment. In contrast, gametogenesis in both ovaries and testes progressed slowly in the deep seawater temperature group, and about 60% of the sea urchins were maintained at an unripe stage (stages 1–3) until 31 August. In spite of distinct differences in maturity among two rearing temperature conditions, significant differences in the distribution of maturation stage could not be found between sea urchins in feeding cages and non-feeding cages in both sexes. These results strongly suggest that the progress of gametogenesis in S. intermedius is closely related to environmental temperature, and is effectively suppressed by rearing sea urchins under low temperature conditions. Following repeated tests over three years, it was proven that rearing at deep seawater temperatures could extend the harvest season two months or longer than that of the wild population (Kayaba et al., 2012). Therefore, the techniques used in our study, for maintaining unripe sea urchins under low temperature conditions, are likely candidates for an efficient method for inhibiting gonadal maturation and serving sea urchins with good quality during summer tourist season.

Table 1. The frequency of maturation stage of female and male sea urchins reared at ambient temperature or deep seawater temperature during the experiment performed in 2009 (based on data from Kayaba et al., 2012).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Feeding condition</th>
<th>Date</th>
<th>Ambient temperature group</th>
<th>Deep seawater temperature group</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
</tr>
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<td>Female</td>
<td>Feeding</td>
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<td>0.00</td>
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<td>1-Jul</td>
<td>0.00</td>
<td>0.50</td>
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<tr>
<td></td>
<td></td>
<td>27-Jul</td>
<td>0.00</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>31-Aug</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Female</td>
<td>Non-feeding</td>
<td>27-May</td>
<td>0.00</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-Jul</td>
<td>0.00</td>
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<td></td>
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<td>27-Jul</td>
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<td>31-Aug</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Male</td>
<td>Feeding</td>
<td>27-May</td>
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<td>Non-feeding</td>
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<td>31-Aug</td>
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Improvement in quality of short-spined sea urchins reared under low temperature conditions

In general, the sea urchins with larger-sized unripe gonads are commercially preferred for consumption. Therefore, for aquaculture of sea urchins, it is also important to determine the rearing condition by which gonads grow faster to a commercial size. For echinoderms, it has been established that nutritive condition is closely related to gonadal development. Nutrients derived from ingested food are stored in nutritive phagocytes inside the ovary and testis, and are utilized for production or growth of germ cells (Unuma, 2002; Walker et al., 2006). In the rearing experiment mentioned above, obvious increases in gonad volume were observed only in the sea urchins reared with feeding: the gonad indexes (GIs) of sea urchins reared with feeding were significantly higher than those without feeding (Fig. 3). This result indicates obviously that rearing with feeding under low temperature conditions is the best way to accelerate the accumulation of nutrients without proceeding gametogenesis in gonads of S. intermedius.

Accordingly, as a next step, we examined the dietary effects of two available diets in Rausu, commercial dry wakame and live brown macroalgae, *Saccharina diabolica;* the former had the advantage of low price and ready availability, while the later was the primary food plant for wild *S. intermedius* and was flourishingly cultured as food in Rausu (Kayaba et al., 2012). *S. intermedius* were reared with four feeding regimes at deep-sea water temperatures from June 1 to September 1 in 2009. In the first group, the sea urchins were reared without feeding during the experimental period as a control. In the second and third groups, the sea urchins were fed a surplus of dry wakame from June 1 to September 1 (feeding for 3 months) and from August 1 to September 1 (feeding for 1 month), respectively. In the fourth group, the sea urchins were fed an unlimited amount of live brown macroalgae, *S. diabolica* from August 1 to September 1 (feeding for 1 month). In comparing GIs from each treatment, when sea urchins were fed live *S. diabolica,* the growth rate of gonads was three times as fast as that when they were fed dry wakame (Table 2). The daily food consumption for each diet was almost the same, suggesting that for *S. intermedius,* *S. diabolica* may be easier to assimilate. Several feeding experiments have shown that the Laminariales are the most nutritionally valuable algae among several algae distributed along the coast of Hokkaido for promoting gonadal growth and

![Graph showing changes in gonad index (GI) and histological structures of *S. intermedius* reared at deep seawater temperature under different feeding regimes during the experiment performed in 2009. Values represent the mean ± standard deviation (based on data from Kayaba et al., 2012).](image-url)
improving the taste of gonads in the northern sea urchin Strongylocentrotus nudus, a sub-arctic species like the S. intermedius (Agatsuma et al., 1993; Nabata et al., 1999). Accordingly, as demonstrated in our experiments with S. intermedius, it may be possible, by feeding with live S. diabolica, to promote gonadal growth efficiently even under low temperature conditions, while suppressing the progress of gametogenesis.

Moreover, we also investigated the characteristic of taste in sea urchins reared with feeding live S. diabolica by chemical analysis (free amino acids) or tasting test directed to tourists in Rausu. As a result, the gonads of sea urchins fed live S. diabolica contained in rich amount of glutamic acid flavoring “Umami taste” and glycine flavoring “Sweetness”. comparing with those fed dry wakame (Fig. 4). Furthermore, tasting test proved that the taste of gonads in sea urchin fed with live S. diabolica were very preferable to customers (Fig. 5). These results clearly indicate that live S. diabolica is an essential diet for rearing S. intermedius to not only enlarge their gonad volume, but also to improve the quality of gonads. Generally, it has been suggested that the largest limiting factor for the success of sea urchin aquaculture on a commercial scale is the difficulty of obtaining sufficient macroalgae for feeding. Fortunately, in the cultivation of S. diabolica in Rausu town, a large amount of surplus algae is produced every early summer by thinning to accelerate its growth. It will be capable of producing commercially viable sea urchins sustainably and at a
lower cost by using this source of unutilized algae as a diet.

**In conclusion and future prospects**

Our present study was initiated to suppress the gametogenesis of unripe *S. intermedius* captured before the spawning season by low temperature rearing using deep-sea water. We succeeded in extending the harvest season of sea urchins reared with deep-sea water two months longer than that of the wild population, and proved that serving them during the summer tourist season was certainly possible. The best practical use of *S. intermedius* in Rausu would include the following steps: 1) a large number of *S. intermedius* are caught from the wild toward the end of the fishing season (late June), 2) sea urchins are reared in tanks supplied
with deep-sea water to suppress their maturation until harvest, and 3) sea urchins are fed mainly on live *S. diabolica* for one month before harvest to improve gonadal growth (Fig. 6). During August, the peak of the summer tourist market in Rausu, the probability of harvesting sea urchins suitable for consumption is approximately 70% per total reared sea urchins. Further detailed studies concerning the rearing conditions of sea urchins would increase that probability in the future.

At present, this aquaculture method is being put to practical use by Rausu Fisheries Cooperative. There is a plan of serving sea urchins at seafood restaurants, hotels, and summer festivals that are visited by many tourists, and they are expected to be a new, attractive food item which will help to promote the popularity of "Shiretoko" as a tourist destination. Should this trial be successful, it will provide a good case for presenting the potential of sea urchin aquaculture to expand not only local fishery production, but also tourism in the local area.

Recently, the extent of barren grounds has increased in coastal areas around Hokkaido. An excessive grazing of seaweeds by wild sea urchins is suspected as one of cause of this phenomenon (urchin-dominated barren ground, called “uniyake” in Japanese) (Fujita, 2008). To recover the luxuriant growth of seaweed, large numbers of sea urchins have been removed from these barren grounds and destroyed, because they have poor gonads due to a shortage of food and have no commercial value (Agatsuma *et al.*, 1997; Kuwahara *et al.*, 2010). The present culture technique would make it possible to improve the quality of surplus wild sea urchins removed from the barren ground so they are preferred by customers. The technique may also become a useful method to create a balance between the biomass of seaweed and the population of sea urchins in coastal fishing grounds. In the future, it is expected that this culture method will be a valuable tool for conserving the coastal fishing grounds in Hokkaido.

### Acknowledgment

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### Reference


Maturation Control of the Short-Spined Sea Urchin


**Annotated Bibliography**


The sea urchin gonad contains two main types of cells: nutritive phagocytes (NPs) and germ cells (GCs). NPs store nutrients necessary for gametogenesis, such as proteins, carbohydrates and lipids. The most abundant protein in NPs is a glycoprotein called the major yolk protein (MYP), originally identified as the most predominant component of yolk granules in the eggs. The proportion of NPs and GCs varies with the maturation of the gonads. Before gametogenesis, NPs fill the gonadal lumina and increase in size by accumulating nutrients to the developing GCs and
decrease in size. In fully mature gonads, a number of type ova or spermatozoa fill the gonadal lumina, whereas, NPs lose their nutrients and shrink. The quality of the gonad as a food product usually deteriorates while GCs proliferate and develop. Promotion of NP growth and suppression of gametogenesis are prerequisites to achieving high profitability in sea urchin aquaculture.


It has long been hoped that sea urchins could be served to visitors in seafood restaurants, hotels, and summer festivals in Rausu, located in a world natural heritage site “Shiretoko,” during the summer tourist season. However, to date this has not been feasible because of the sea urchin spawning season (July to September), during which the quality of gonads, the edible part of sea urchins, decreases due to maturation. In this study, we examined the possibility of suppressing gonadal maturation and maintaining high-quality sea urchin gonads by low-temperature rearing using deep-sea water. Unripe sea urchins captured before the spawning season were reared under two temperature conditions from June to September. In those groups reared at ambient temperatures (2.8–19.6°C), gametogenesis in both sexes progressed rapidly with increased temperature, and almost all sea urchins reached full maturity by late July. In contrast, in groups reared at low temperatures (2.1–5.1°C), gametogenesis progressed slowly and over 60% of the sea urchins did not reach maturity even by early September. The feeding experiment also revealed that feeding with live *Saccharina diabolia* could increase the gonadal volume efficiently under low-temperature conditions. These results demonstrate that low-temperature rearing, supplemented with feeding, is effective in suppressing gametogenesis to allow for the harvesting of high-quality sea urchins during the summer tourist season.


Sea urchin fisheries are valuable commercial resources in the United States with processed gonads sold in Japanese and American markets and maximum U.S. sales of $150M US dollars in 1996. Wild populations of sea urchins on all coasts of the U.S. have been heavily fished. Aquaculture of sea urchins in land-based facilities can help restore commercial populations and preserve this ecologically important herbivore. In this study, we used invariant summer photoperiod to prevent gametogenesis in the North American green sea urchins (*Strongylocentrotus droebachiensis*) maintained in a land-based aquaculture system and provided a commercially available formulated feed that promotes maximum growth of intra-gonadal somatic nutrient storage cells called nutrient phagocytes. Results were compared with individuals fed the same formulated feed under ambient photoperiod in cages in the ocean. Monthly samples of the gonads from both treatments were evaluated for gonad index, volume fractions of cellular constituents of the germinal epithelium, oocyte diameter and taste. Over the 5 months of this study, gonad indices increased significantly ($p < 0.001$) in both treatments from 4.8% ± 0.9 (all values ± SE) initially to 20.5% ± 2.1 under invariant and 23.2% ± 1.4 under ambient photoperiod with no significant difference between treatments ($p = 0.55$). Volume fractions of nutritive phagocytes increased to 80.3% ± 5.9 (initial 37.9% ± 7.1) in males and 71.0% ± 6.7 (initial 10.3% ± 4.0) in females ($p < 0.001$) only under invariant photoperiod. Nutritive phagocyte lengths increased under both photoperiod treatments, but the volume fraction containing nutrients was higher under invariant photoperiod. Volume fractions of gonial/gametogenic cells increased significantly ($p < 0.001$) only under ambient photoperiod from 20.4% ± 5.5 to 37.8% ± 1.8 in males and 0% to 22.6% ± 3.6 in females. The volume of fraction of residual oocytes from last year’s oogenesis increased under invariant photoperiod while that of both residual and new oocytes increased under ambient photoperiod.
Residual oocyte diameters increase from 56.2 μm ± 2.2 initially to 93.5 μm ± 3.7 under invariant and those of residual and new oocytes to 126.0 μm ± 7.3 under ambient photoperiod. Invariant photoperiod yields gonads in both sexes of *S. droebachiensis* that do not initiate fall gametogenesis but attain large size as their nutritive phagocytes grow substantially in size. A Canadian study of wild-collected *S. droebachiensis* indicated that gonads taste best when they contain pre-dominantly nutritive phagocytes and not copious gametes, however gonad taste in our study was unsatisfactory suggesting that the only commercially available sea urchin diet requires modification to support commercial development of land-based aquaculture.


Rearing experiments of the sea urchin, *Strongylocentrotus nudus*, were carried out during June to July, 1995 and June to August, 1996, using as food marine algae which settled and grew on the coralline flats after the removal of sea urchins. Feeding rate and growth rate at 17°C of the sea urchin fed on *Laminaria*, *Undaria*, and *Costaria* were high and those for *Sargassum*, *Polysiphonia*, *Dictyopteris*, and *Desmarestia* were low in the single food item experiment. Among the algae supplied as food, the daily amount of food eaten was high in large-sized groups of the sea urchins, while the small-sized groups show the highest feeding rate. To examine any effect on gonad growth, we fed 3 algae, *Laminaria*, *Sargassum* and *Polysiphonia* to sea urchins. Two month later, the gonad index was found to be the highest in the *Laminaria* fed group. Among the algae fed, based on the gonad growth, the highest feeding rate and the highest growth rate, we estimated the *Laminariales* are the most nutritionally valuable algae for growth of *Strongylocentrotus nudus*. 
Long-term Outcomes in the Tech Transfer of Scallop Spat Collection Techniques, from Aomori Prefecture, Japan to Maine, USA

Dana L. MORSE*

Abstract: Improved management, aquaculture and stock enhancement of the sea scallop (*Placopecten magellanicus*) in Maine are examples of long-term benefits from a technology transfer project. In 1999, a delegation from Maine traveled to their sister-state of Aomori Prefecture in Japan to gain a firsthand view of the world-famous scallop industry of Mutsu Bay. While the trip was informative from many perspectives, the detail with most immediate application was the use of so-called ‘spat bags’ to capture competent larvae for stock enhancement and intensive aquaculture, and the way in which rotational management could be used to maximize production.

In subsequent years, over 100 fishermen in Maine used spat bags to capture juveniles, and the current progress made in aquaculture of sea scallops is largely due to the excellent success in collecting spat as a reliable seed supply. Maine’s fishery is currently managed in rotation and area closures - sometimes at the request of the fishermen. Fishermen are also involved in discussions of spat collection for the purposes of stock enhancement. In addition, spat collectors have provided an invaluable window for fishermen to observe details of this early life stage, observations that they would not otherwise make. Overall, spat bags have been useful both directly and indirectly for well over a decade.

Many of these outcomes were not foreseen during the visit of 1999. Technology transfer often aims for immediate impact, but it seems likely that the normal case is that impacts and benefits may take many years to be observed. It is important to keep this in mind both in reporting to funders of various tech transfer efforts, but to appropriately gauge the long-term value of contemporary efforts.

Keywords: aquaculture, enhancement, *Placopecten magellanicus*, technology transfer

Maine’s fishery for the sea scallop (*Placopecten magellanicus*) has traditionally been an important option for the state’s fishermen; it was an open-access fishery until recently, and provided much-needed wintertime income. Direct on-the-water employment in the 2012/2013 fishing year amounted to 547 mobile-gear licensees (draggers), and 84 divers (Trish DeGraaf, ME Dept. of Marine Resources, pers. comm.). Landings have varied widely, from a historic high of 3.8 million pounds in 1981, to a low of 33,000 pounds in 2005 (Fig. 1). Only the adductor muscle, or meat, is landed.

Prior to 2007, regulation of the fishery was fairly limited, with no limit on the number of licenses, and a 3.5” (88.9mm) minimum shell height. Prompted by low landings and stimulated by examples of better management - such as discussed below - the Maine Dept. of Marine Resources engaged fishermen and scientists in a process to improve the landings and value of the fishery. Since then, several measures have been adopted, such as a larger minimum shell height of 4” (101.6mm), limited fishing days, a daily landing limit, rotational management, and closed areas, and to date, landings have improved (Fig. 1).

The process of industry engagement and inclusion into the management process was not always

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smooth: the issues were highly contentious, and the bringing-together of industry with science and management meant that there was a good deal of learning to be done, toward and from one another. Conversely, it appears that these interactions have begun to pay dividends for all sides: management is more aware and responsive to industry perspectives, industry is more fluent in the science of scallop management and ecology, and science is being conducted on issues important both to fishermen and regulators.

Before the period reported in this document - 1999 and onward - engagement by fishermen and scientists in enhancement and aquaculture production was extremely limited and variable, with little in the way of planning or broader-scale thinking. Fishermen traditionally have experimented with enhancement through such actions as moving seed - meaning sublegal scallops, usually larger than 2” (5cm) - to a location they felt was protected or productive, or removing predators like crabs and starfish when they came up in the drag. Enhancement with seed smaller than 2” was very limited, and none performed in a structured fashion.

Scallop aquaculture has historically been opposed by many fishermen for two principle reasons: occupation of space that would normally be fished at one point of the year or other; and competition in the marketplace. A few small trials have yielded generally positive results though with several important caveats: work by Pottle and Hastings (2001) and Kuenstner (1996) - Maine polyculture study demonstrated that scallops would grow relatively quickly. The Pottle study also showed that whole scallops were a marketable item, but Kuenstner and Pottle both demonstrated the hurdles posed by algal toxins (saxitoxin, okadaic acid and domoic acid, principally) in selling products other than just adductor muscles.

Attitudes toward aquaculture have changed somewhat for many fishermen, however, with a major reason being the limited opportunities offered on the water in recent decades: limited access to licenses or resources have generally put fishermen ‘in a box’ where moving through several fisheries in the course of the year has been supplanted by access to only one or two fisheries, and therefore highly dependent upon them. The second reason concerns the subject of this paper: technology and information transfer becomes integrated in the knowledge, perspectives and ideas of the people who participate, and can support an adaptive stance toward current and future challenges.
Materials and Methods

Maine enjoys a sister-state relationship with the Prefecture of Aomori, Japan; the northernmost region of the island of Honshu. Delegations have passed with some regularity between Maine and Aomori, addressing such interests as energy, affordable housing, and fisheries. One such delegation traveled to Aomori in May of 1999, with the focus of learning about the highly-productive scallop industry there, one which has its basis in aquaculture techniques, and one that has become a worldwide model of financial and social success. It was hoped that the group could identify aspects of the Japanese industry that would transfer well to the scallop fishery in Maine.

Between the 14th and 21st of May, ten representatives from Maine toured scientific facilities, processing plants, waterfront infrastructure, seafood markets, a fishermen’s cooperative, and sea farms. Not unexpectedly, the delegation was impressed at the diversity, volume and vigor of the farming industry there. A brief list of conditions, opportunities and approaches that could potentially be beneficial to Maine ran as follows:

- A diverse suite of products from scallops would be beneficial for Maine, rather than just deriving income from the adductor muscle.
- Spat collection equipment and practices could be useful to stock enhancement and aquaculture in Maine. Techniques and equipment are directly transferrable.
- Collaboration with and support by scientists is beneficial to all involved; good monitoring for biotoxins is critical
- There is no difference between fisherman and seafarmer in Japan: sea farmers are fishermen.

A full report was delivered to the Maine Aquaculture Innovation Center, detailing all aspects of the trip (Beal et al., 1999).

In the years following the visit, several activities and projects were conducted in Maine, in an effort to build upon the knowledge gained.

**Spat Collection:** From 1999 to 2002 several meetings were held around the state, to familiarize fishermen with spat collection equipment and methodology. The emphasis was on searching for good spat collection areas, transferring captured seed to the wild, and educational connections with citizens and environmental groups (Fig. 2). As expected, fishermen quickly proved their expertise, and discovered that best spat collections were not done in the immediate coastal waters, but further offshore, near the limits of state jurisdiction (Fig 3). In these areas, counts were easily above 1,000 seed per collector, and frequently as high as 3,000. Many areas were found to result in collectors catching nearly exclusively scallops and with low fouling rates, whereas others yielded low scallop catch, high bycatch of other bivalves, and heavy fouling.

Fig. 2. Citizen environmental group aboard the F/V Lindsay Marie (Stonington, ME) to assist and learn about scallop spat collection and stock enhancement.

Fig. 3. Spat collections in 1999-2000 (left) and 2000-2001 in coastal Maine. Note that in the second year, most attempts were made further offshore because of prior success there: fishermen adapted rapidly to new knowledge.
Perhaps more importantly, fishermen found that spat collectors are most successful when they are observed regularly: the coast of Maine is heavily fished for lobster, and entanglements with lobster trap lines were common, unless the collector lines were tended. This was an important observation for the co-existence of spat collection with the lobster and other coastal fisheries. It was also during these years that the inclusion of spat collection, stock enhancement and aquaculture became better known to the state regulatory agency - the Maine Dept. of Marine Resources (DMR); this familiarity has proven to be very beneficial in the long run.

**Stock Enhancement:** Stock enhancement efforts in Maine can be generally characterized as haphazard, with a few exceptions. Efforts by the ME DMR and the Northwest Atlantic Marine Alliance (NAMA) were well coordinated, but of limited scope. Primary lessons from those efforts - which were both done with excellent collaboration between science and industry - were to reinforce the highly mobile nature of juvenile scallops, and the supposed high predation rates on seed less than 20mm or so (Schick and Feindel, 2005; Deese-Riordan, 2005). Unfortunately, these were general observations, and not thoroughly described in the detail desirable, due to limited ability to dive on the reseeded sites with the frequency necessary for such detail.

Other smaller efforts in stock enhancement were undertaken between 1999 and 2004; often amounting to a fishermen taking the spat from his collectors and deploying it in a favorite spot. The benefits of these activities have remained anecdotal and uncertain. The uncertainty over the success of stock enhancement led to the gradual decline of fishermen participating in spat collection. From roughly 2004 and onward, only a handful of fishermen per year participated; this area of study remains to this day one where industry interest would align well with scientific interest.

However, one area that has been of obvious benefit is the way in which spat collectors have allowed fishermen to observe and understand the earlier life phases of scallops; a subject that has been lightly discussed or considered previously. Spat collection led to conversations amongst fishermen and with scientists or managers about oceanography, predation, conservation, water quality and other topics, all of which entered the ensuing discussions about management of the fishery and the scallop resource. Similarly, spat collectors also spurred fishermen’s interest in aquaculture: seeing 2000 small scallops in a spat collector immediately led them to consider what it would be like to raise them in cages. Both of these phenomena have played a large role over time in the evolution of scallop management in Maine, and the beginnings of an aquaculture sector.

**Aquaculture:** The investigation of sea scallops as a candidate for aquaculture in Maine has been a story of stops and starts. From 1999 to 2000, a trial of cage culture for scallops was undertaken by Tom Pottle, who was one of the delegates from the 1999 trip, and a fisherman from the Cobscook Bay region. His project successfully demonstrated reasonable growth rates, useful equipment and husbandry practices, and positive feedback from test sales into the marketplace (Pottle, 2001). It also underscored the importance of thorough testing for toxic algae, and presence of algal toxins in scallop tissues. Mr. Pottle did not continue his project past the funded time frame, though has remained optimistic about the chances for scallop aquaculture in Maine.

From 2000 through approximately 2010, scallop aquaculture was at a virtual standstill in Maine; the risks associated with sales of live or roe-on product were prohibitive for state regulators. In recent years however, conversations between industry and the regulatory community have increased, with both sides joining in an effort to explore the possibility for sales of roe-on or live/whole product. A current project involves five pilot scallop farms, with excellent growth observed thus far (up to 0.19mm/day), and low mortality (Fig. 4). Conversations continue regarding the protocols necessary to guarantee product and public safety - but they are occurring and all parties have felt optimistic for future development.

To date, first sales of live scallops are anticipated in 2014, accompanied by sales of seed from spat collectors to new growers. Project details located at: http://www.seagrant.umaine.edu/research/projects/dv/scallop-trials
items come to mind. The Aomori visit supported an improved understanding of biology and ecology of scallops, compared to the level of understanding prior, particularly by industry. By focusing on the deployment of spat collectors and observing the catches, fishermen started to better understand important factors such as proximity of spawners to one another, the influences of oceanographic conditions (currents, tides, temperatures, etc) on larval dispersal and settlement, and in particular, the challenges faced by very young post-set scallops. In fact, the simple identification of young scallops improved a good deal: fishermen have often mistaken so-called 'jingle shells' (*Anomia simplex*) as young scallops; spat collectors have allowed fishermen access to the very smallest sizes, such that identification is now much more accurate - and questions have naturally followed those observations.

The concepts of rotational management have been reinforced; many had focused on the management of offshore scallops on George's Bank as the primary example, but the Japanese example has figured into management discussion. Today, Maine's inshore scallop fishery is managed in rotation, in some measure due to a changed way of thinking by fishermen, scientists and managers resulting from the Aomori visit (Fig. 5). Other issues such as mandatory stock enhancement and creation of spawner sanctuaries have been included in the management discussion and although not included in regulations, have been a noteworthy step about how fishermen and managers have discussed the responsibilities and possibilities in supporting a healthy resource and fishery.

Production remains an area where the Aomori visit has had a profound influence, all starting with the technology of spat collection. Stock enhancement continues to gather support from fishermen when the subject arises, and it's likely that more work will be done in this area, and with greater scientific scrutiny than before, which would permit a better evaluation of effectiveness. An aquaculture industry has begun to develop in the state as well with small sales of the first crop (and scallop seed from collectors) expected later in 2014. Moreover, the tenuousness of the lobster, shrimp and groundfish industries have made commercial fishermen more

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**Fig. 4.** Scallops growing in a cage during the pilot project; Blue Hill, ME

**Results and Discussion**

Technology transfer clearly yields both short-term and long-term results. While it may be possible to anticipate or predict the near-term benefits and activities that stem from a technology transfer activity, real predictions over the longer term are much less achievable, other than to say that they will exist. Documenting such developments is a worthwhile endeavor however, because of the local relevance but also because of the larger implications for future efforts: funding for travel is usually difficult, and when good documentation is available on the value of such travel, it improves the rationale for proposals involving travel to support technology transfer. Therefore, while it’s very difficult to anticipate long-range outcomes, it is of value to note them as they do become apparent.

In the case of the 1999 trip to Aomori, three principal areas of impact can be easily identified. These topics - which are closely linked - are: biology and ecology, management, and production.

In the category of Biology and Ecology, several
open to the possibility of aquaculture as a way to generate income, and thus the integration of the fishing and aquaculture industries has a pathway for progress, through the culture of sea scallops.

The quantification of impacts is more difficult, though not impossible. Landings for scallops have increased in recent years, and surveys of the areas closed as part of the rotational management approach have provided strong indications that management plays a large role in the improvements. Therefore, landings and licenses are two metrics that can be easily identified. As the aquaculture industry continues to advance, it is straightforward to record economic activity and the number of farmers as measures of success. These data are important in the sense of tracking the influence of the original information.

The natural complement to the numbers themselves (sales, licenses, leases, etc) is the use of anecdotes to tell the story of what happened over time. When taken together, the quantifiable data and the qualitative assessments, the stories can create an understandable and informative landscape of the value of technology transfer in both the short and long terms. In fact, both are necessary to fully understand the impacts. In the case of the Aomori trip of 1999, the payoffs became apparent rapidly, and it appears that benefits will continue well into the future.

References


Oyster Culture in Hokkaido, Japan

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Abstract: The Pacific oyster, Crassostrea gigas is the one of the most commercially important aquaculture species in the Japanese fisheries industry and is cultured in various Japanese coastal areas including Hokkaido. The Saroma Lake and the Akkeshi Bay with estuary, which face the Okhotsuk Sea and Pacific Ocean, respectively, are major oyster production areas in Hokkaido with total annual production of about 700 tons a year. The seedling spat supplied from Miyagi known as Miyagi seedlings are widely used in Japanese oyster culturing. Therefore, when the catastrophic tsunami on March 11, 2011 damaged the Miyagi fisheries, many oyster culturing areas were heavily affected. Moreover, introduction of seedlings from geographically separated population has risks of invasion of diseases and alien organisms as hitchhiking species. Using seedlings that originated from local populations in each area is one of the approaches for decreasing some risks. For example, in the Akkeshi area, the artificial seedling spats collected from the locally protected adults are also used for aquaculture, which are marketed as the value-added oysters with shell “Kaki-EMON” and popular among consumers as local special products.

Key words: Pacific oyster (Crassostrea gigas), Miyagi seedlings, Saroma Lake, Akkeshi Bay

The Pacific oyster, Crassostrea gigas is the one of the most commercially important aquaculture species in the Japanese fisheries industry with harvest of around 200, 000 metric tons a year, the same as the Japanese scallop Mizuhapetens yessoensis (Ministry of Agriculture, Forestry and Fisheries, Minister’s Secretariat, 2012). Although a lot of scallops were exported to other countries including the United States, most oysters are consumed domestically. Oyster culturing in Japan is reviewed by Inui (2013). The Oyster is cultured using raft or long line hanging method. Hiroshima and Miyagi prefectures are the main production areas in Japan, and there are also many other production areas along Japanese coastal area. Most of these areas use oyster seedlings which are naturally collected in Miyagi using scallop shell collectors and are known as Miyagi seedlings. Therefore, when the catastrophic tsunami on 2011 damaged the Miyagi fisheries, many oyster farmers were heavily affected because of uncertainty of seedling supply (Tanabe, 2012)

Hokkaido, a northern island of Japan is one of the premier oyster culturing areas with production of 700 metric tons a year (Marinenet Hokkaido: http://www.fishexp.hro.or.jp/marineinfo/internetdb/index.html), using primarily Miyagi seedlings. Hokkaido has two main production areas, Saroma Lake and Akkeshi Bay and estuary (Fig. 1). Saroma Lake and Akkeshi Bay face towards the Okhotsuk Sea and the Pacific Ocean, respectively. These areas have similar production scales and Oyster industry history. In the early days in Hokkaido natural oyster beds were very productive in these areas and the oysters were harvested from there (Inukai and Nishio, 1937). However, natural oyster resources were lost around

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the 1930s and since then the oyster industry has relied on Miyagi seedlings.

**Features of oyster and scallop culturing in Saroma Lake**

Ground and suspended culture of scallops are the main fisheries in the Abashiri Sub prefecture with Saroma Lake in Hokkaido where production reaches 150,000 metric tons a year. Most areas of Saroma Lake are utilized for scallop culture and unsuitable areas for scallop culture due to shallow depth and high water temperatures during summer are used for oyster culture (Fig. 1). In the lake, scallop culture is a major fishery (> 77% of total catch), however, most culture activities must be stopped
during winter due to lake freezing. Oyster fisheries contribute a little (< 1%) to increase the diversity of fisheries and reduce dependency on scallop culture, but oyster culturing brings jobs to farmers in winter such as shucking because oyster's in this area are mainly composed of age 1+ and so small oysters are shipped as a shucked product. Moreover, older fishermen have special feeling for oyster culturing because oyster fishing had been prospered in the lake until 1928 when the continuous channel was opened between Lake and cold Ohotsuku Sea and the oyster could not reproduce in stable (Nishihama, 1994).

**Features of oyster culturing in Akkeshi Bay and Estuary**

The most important feature of Akkeshi is the topography (Fig. 1). Akkeshi has two different ecosystem types: Akkeshi Bay and the Akkeshi-ko estuary. Akkeshi Bay faces the Pacific Ocean where a cold subarctic ocean “Oyashio” current flows, while the Estuary is shallow (average depth: 1.5 m). The continuous measurement of surface water temperature shows that the temperature in this Estuary increases earlier than in the Bay from spring to summer, reaching temperatures higher than 25 °C. However, water temperature in the Bay is around 20 °C in late summer (Fig. 2).

Culture experiments using single-seedling oysters showed variations of growth rate and nutritional condition index between oysters from the Bay and the Estuary. The ratio of flesh wet weight to whole body weight of oyster, which is one of the indices of oyster nutritional condition was lower in the estuary than the bay throughout the experiments (Fig. 3a and b). The specific growth rate of shell height was higher in the Estuary than the Bay in early summer (Fig. 3c and d). The early increase in temperature from spring to summer enhances oyster growth and maturation and early spawning occurs in the Estuary. These features of both culture areas are recognized by farmers and efficiently utilized. The fishermen use the Estuary as the warmer area for enhancing oyster growth and maturation with early spawning. Otherwise, the Bay has a greater surface area, water volume, and food availability than the Estuary, which contributes to improved body conditions of oysters before shipping seasons.

Therefore, fishermen use these two areas properly, and Akkeshi oysters have some advantages to other culturing areas. But carrying capacity is limited by the topography of the shallow and small estuary. To avoid this problem, single-seed oysters are cultured in Akkeshi, which are suitable for farming in shallow areas. In the hatchery, larvae are collected from local adult spawns and settled on small oyster shell pieces individually as single-seedling oysters. The juvenile

![Fig. 2. Fluctuation of water temperature at Akkeshi Bay and Estuary, Japan during 2012.](image-url)
oysters are cultured in the bay and the estuary using baskets. By using basket rearing, more oysters can be cultured in shallow areas such as Akkeshi-ko estuary. And basket hanging keeps oysters in the water column away from pollution on the estuary bottom. Moreover, through basket hanging, single-seed oysters have deeper shell breadth than oysters on scallop shells. These oysters are marketed as value-added oysters with shell “Kaki-Emon” and are popular among consumers as local special products (Fig. 4). Akkeshi town is aiming to be known as “Oyster town” using single-seed oysters to promote tourism and sightseeing, and to develop brand strength of other fisheries products. However, the yield of single-seed oysters was lower than that of Miyagi-seeding oysters at the beginning of this culturing, because the standard culturing methods for Miyagi seedlings were not suitable for single-seed oysters. Therefore, the culture of single-seeding oysters does not increase as much as the research and promotional organizations expected, even today.

**Fig. 3.** Temporal and spatial variation in ratio of fresh wet weight to total weight to whole weight (a and b) and specific growth rate of shell height (c and d) of *Crassostrea gigas* in Akkeshi Bay and Estuary, Japan.

**Fig. 4.** PR poster of local premium oyster in Akkeshi “Kaki-Emon”.
Problems facing oyster culturing in Hokkaido

Miyagi-seedling has been widely used in Japanese local production areas including Hokkaido because most of the seeding are naturally collected using scallop shells with low costs and a stable source provided by the specialized farmers in Miyagi prefecture. These Miyagi oysters have faster growth potential, and thus are preferred by many oyster farmers. Even though there is wide-scale use of Miyagi-seedlings throughout the area, genetic differences can be detected among some local/wild populations in Japanese local production areas (Usuki et al., 2002). The tsunami in 2011 exposed the risks of excessive dependence on Miyagi seedling supplies. Moreover, introduction of seedlings from geographically separated populations have risks of introducing diseases and alien organisms as hitchhiking species. For example, invasive ascidians were also recorded in some bivalve culturing areas in Japan. In particular, *Asciidiella aspersa* strongly affect the scallop culturing in Funka Bay, Hokkaido (Nishikawa et al., 2014). The paramyxean parasite, *Martellioidea chungmuensis*, has negative impacts on the oyster industry in some Japanese production areas with heavily infected oyster’s showing abnormal tissue (Ito et al., 2002). Moreover, the potential transport of harmful algae together with bivalves has also been reported (Matsuyama et al., 2010). Using local seedlings is one of the approaches to decreasing these risks. After the Miyagi seedling crisis, the use of local populations is beginning in some production areas. However, similar to the experience in Akkeshi, it is necessary to improve culturing techniques and management for culture of local seedlings because they have different characteristics. Moreover, sales strategies of “local oyster” with premium price are also important to promoting use of local seedlings to compensate for additional cost and effort.

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