United States-Japan Natural Resources (UJNR)
Panel on Aquaculture
41st Scientific Symposium

"Advanced Aquaculture Technologies"

October 9-10, 2013
Sapporo, Hokkaido

Program and Abstracts

Photo: Spawning sea cucumbers (*Apostichopus japonicus*) induced with “cubifrin” pictured by Dr. K. Yamano (FRA)

Program

The 41st Scientific Symposium of the UJNR Aquaculture Panel

Advanced Aquaculture Technologies

Wednesday, October 9th, 2013

Registration 13:00-17:00

Welcome and opening remarks (Masaaki Fukuda, Director, Hokkaido National Fisheries Research Institute, FRA) ・・・・・・・・・・・・・・・・・・・・ 13:30-13:45

Aim of the Symposium (Katsuma Hanafusa, Japan Panel Chair) ・・・・・・・13:45-14:00

Session Ⅰ. Overview of Aquaculture in the US and Japan

(Moderators: P. Olin & Y. Machiguchi)

1. The Fisheries Agency's policy directions for aquaculture
   Kazumasa Ikuta (Counselor, Resource Enhancement Promotion Department Japan Fisheries Agency, Ministry of Agriculture Forestry and Fisheries)
   ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・14:00-14:20
2. Aquaculture in the US: The First Annual National Report on Aquaculture
   Marie Fujitani (NOAA Office of Aquaculture)・・・・・・・・・・・14:20-14:40
3. The Challenge of Reconstructing Coho Salmon Aquaculture after the Great East Japan Earthquake and Tsunami in 2011
   Ikutaro Shimizu (National Research Institute of Fisheries Science, FRA)
   ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・14:40-15:00

Discussion・・・・・・・・・・・・・・・・・・・・・・・・・・・・・15:00-15:10

Break・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・15:10-15:20

Session Ⅱ. Fish Diet and Nutrition

(Moderators: K. Webb & K. Murashita)

4. Development of a new type of fish diet, non-fish meal extruded-pellet
   Noriko Ishida (National Research Institute of Fisheries Science, FRA)
   ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・15:20-15:40
5. The importance of taurine and n-3 fatty acids in cobia nutrition
   Aaron Watson (University of Maryland)
   ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・15:40-16:00

6. Availability of fisheries by-product materials with cadmium removal treatment as a feed ingredient for fingerling black rockfish *Sebastes schlegeli*

Nobukazu Satoh (Fisheries Research Department Mariculture Fisheries Research Institute, Hokkaido Research Organization) ・・・・・・・・・・16:00-16:20

7. Development and characterization of several open formula reference diets for marine fish larvae

Ken Webb (NOAA, Northwest Fisheries Science Center)

・・・・・・・・・・・・・・・・・・・・・・・・・・・・・16:20-16:40

8. Effects of diets supplemented with herb essential oils on ectoparasitic protozoan infections on chum salmon (*Oncorhynchus keta*) fry

Shinya Mizuno (Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization) ・・・・・・・・・・・・・・・・・16:40-17:00

Discussion ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・17:00-17:20

Thursday, October 10th, 2013 Registration 9:00-9:20

Session Ⅱ. Fish Diet and Nutrition; continued

(Moderators: A. Watson & N. Satoh)

9. Effect of feed ingredients on digestive enzyme secretion in fish

Koji Murashita (National Research Institute of Aquaculture, FRA)

・・・・・・・・・・・・・・・・・・・・・・・・・・・・9:20-9:40

10. Cholecystokinin and trypsin responses of larval red drum (*Sciaenops ocellatus*) in response to algae, live prey, and inert particles

Michael B. Rust (NOAA) (Presented by Ken Webb) ・・・・・・・・・・9:40-10:00

Discussion ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・10:00-10:10

Session Ⅲ. Management, Social and Economic Issues of the Aquaculture Industry

(Moderators: M. Fujitani & I. Shimizu)

11. An analysis of the causality between the market price of imported fishmeal and market price of marine farmed fish

Yoshifumi Takahashi (Tohoku National Fisheries Research Institute, FRA)

・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・10:10-10:30

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

Stanley Serfling (Food and Drug Administration Office of Food Safety)

(Presented by M. Fujitani) ・・・・・・・・・・・・・・・・・・・・・・10:30-10:50

13. Modeling intraspecific genetic effects for Management of Aquaculture Programs

Jason Volk (ICF International) ・・・・・・・・・・・・・・・・・・10:50-11:10

Discussion · 11:10-11:20
Group Photo Session · 11:20-11:40
Lunch Break · 11:40-13:00

Session Ⅳ. Techniques of Aquaculture Production

(Moderators: J. Volk & K. Okuzawa)

14. Specific monovalent and divalent ion supplementation for culture of marine species
    Chris Green (Louisiana State University Agricultural Center) · · · · · 13:00-13:20
15. Genetically modified salmon in aquaculture: well regulated and safe
    Paul G. Olin (UC SD/ Scripps Institution of Oceanography) · · · · · 13:20-13:40
16. Reproductive dysfunction in cultured sablefish (*Anoplopoma fimbria*)
    Jose Guzman (NOAA) · 13:40-14:00

Discussion · 14:00-14:10

Session Ⅴ. Invertebrate Aquaculture I: Echinoderm

(Moderators: S. Eddy & T. Unuma)

17. Induced spawning in the sea cucumber *Apostichopus japonicus* by neuropeptide, cubifrin
    Keisuke Yamano (National Research Institute of Aquaculture, FRA)
    · 14:10-14:30
18. Mass production of artificial seed of the Japanese common sea cucumber (*Apostichopus japonicus*) in Hokkaido, Japan
    Yuichi Sakai (Fisheries Research Department Mariculture Fisheries Research Institute, Hokkaido Research Organization) · · · · · · · · · · · · 14:30-14:50

Break · 14:50-15:00

(Moderators: D. Morse & K. Yamano)

19. Fluorochrome marking of out-planted green sea urchins, *Strongylocentrotus droebachiensis*, for sea ranching and restocking programs in the Gulf of Maine, USA
    Stephen Eddy (University of Maine, Center for Cooperative Aquaculture Research) · 15:00-15:20
20. Improving the food quality of sea urchin gonads by suppressing gametogenesis
    Tatsuya Unuma (Hokkaido National Fisheries Research Institute, FRA)
    · 15:20-15:40

21. 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

Takaaki Kayaba (Kushiro Fisheries Research Institute, Hokkaido Research Organization) ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・15:40-16:00

Session Ⅵ. Invertebrate Aquaculture II: Mollusc

(Moderators: C. Green & Y. Sakai)

22. Long-term outcomes in the tech transfer of scallop spat collection techniques, from Aomori Prefecture, Japan to Maine, USA

Dana Morse (Maine Sea Grant) ・・・・・・・・・・・・・・・・・・・・16:00-16:20

23. Relationship between spat density and environmental factors in cultured Japanese Scallop, *Mizuhopecten yessoensis* in Funka Bay, Japan.

Katsuhisa Baba (Hakodate Fisheries Research Institute, Hokkaido Research Organization) ・・・・・・・・・・・・・・・・・・・・・・・・・・・16:20-16:40

24. Oyster culture in Hokkaido, Japan

Natsuki Hasegawa (National Research Institute of Aquaculture, FRA) ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・16:40-17:00

Discussion ・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・17:00-17:10

Closing remarks (Paul Olin, U.S.A. Panel Chair) ・・・・・・・・・・17:10-17:20

Abstracts

THE FISHERIES AGENCY'S POLICY DIRECTIONS FOR AQUACULTURE

Kazumasa Ikuta

Counselor, Resource Enhancement Promotion Department
Japan Fisheries Agency, Ministry of Agriculture Forestry and Fisheries

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.

U.S. FIRST ANNUAL REPORT ON AQUACULTURE FOR THE U.S.-JAPAN NATURAL RESOURCES PANEL ON AQUACULTURE

Marie Fujitani

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The first U.S. National Report on Aquaculture for the 41st U.S. Japan Natural Resources Panel on Aquaculture will overview major policy items, current trends in aquaculture production, and other important developments affecting contemporary U.S. aquaculture.

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 is currently reviewing the proposed regulations that would implement the Gulf Council's Fishery Management Plan for Regulating Offshore Marine Aquaculture in the Gulf of Mexico. This would permit aquaculture in federal waters in the Gulf of Mexico, and would be a first for the U.S. as there is currently no framework for permitting aquaculture anywhere in our Exclusive Economic Zone. We expect final regulations to be effective sometime in 2014.

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. consists of trout, tilapia, catfish, crawfish, shrimp, salmon, oysters, mussels, and clams. For the period from 2005-2010, the value of U.S. aquaculture showed 3% average year-over-year growth, though total volume fell by an average of 1.8%. Marine aquaculture (salmon and shellfish), on the other hand, showed robust 14.6% year-over- year average (by volume) growth in the same period. 2010 is the last year published aquaculture statistics are available. From 2009 to 2010, total U.S. aquaculture grew 4% by volume (to 341,568 metric tons) and 10% by value (to 1.28 billion dollars). Marine aquaculture grew 22% by volume (to 40,823 metric tons) and 28% by value (to 312 million dollars). Most of the growth in value for marine aquaculture is from salmon (*Salmo salar*) and oyster (mainly *Crassostrea gigas* and *Crassotrea virginica*) production.

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]

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Aquaculture of coho salmon (*Oncorhynchus Kisutsh*) is one of the most important fisheries in Miyagi prefecture, Tohoku, where they had annually produced more than 10,000 tons until 2010. 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 since then. One year later, farmed coho salmon started being landed again by the support of national funding. Fortunately, the tsunami did not affect the juveniles of inland coho salmon. 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 in 2012 (less than 200 yen/kg). The higher price (beyond 370-380 yen/kg) is necessary to maintain the farming facilities for management. It is necessary for stakeholders to clarify the causes of the slump to make a reconstruction plan for Sanriku salmon aquaculture. This paper aims to clarify 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.

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 since the autumn in 2011 reduced the domestic wholesale price, (2) Low sea water temperature in the winter 2012 suppressed the growth of Sanriku coho salmon, (3) Late landing of Sanriku coho salmon in the spring 2012 affected centralized 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 farmed coho salmon price, and (6) The discontinuation of the supply of Sanriku coho salmon in 2011 changed consumers' purchasing pattern from Sanriku to imported salmon.

The price of Sanriku coho salmon has synchronized with the price of Chilean frozen

farmed coho salmon, and has decreased since the 1980s. One of the most important characteristics of Sanriku coho salmon aquaculture is differentiation by fresh products. In addition, the spring supply of fresh seafood has been less than other seasons in the Sanriku region. It is important to supply fresh salmon for consumer markets by the Golden Week holidays (from late April to early May). It is necessary to improve the traditional aquaculture system to consolidate a new brand value for Sanriku coho salmon. Sales promotion has been expected to drive the fresh coho salmon price 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.

Key words:
Sanriku coho salmon aquaculture, Great East Japan Earthquake, Reconstruction, Wholesale price, Chilean coho salmon

DEVELOPMENT OF A NEW TYPE OF FISH DIET, NON-FISH MEAL EXTRUDED-PELLET

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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 business conditions of Japanese aquaculture farmers because fish meal for Japanese aquaculture feed is dependent on imports. Therefore, in Japan a new type of diet that is independent of fish meal is strongly desired. Low or non-fish meal extruded-pellets have been developed. First of all, 10% fish meal containing diets were prepared, and fed to cultured yellowtail, and the fish growth performances were compared to those of 50% fish meal containing diet. It was observed that fish growth was similar in both diet groups. Then, three experimental non-fish meal extruded-pellets were designed, and were fed to one-year-old yellowtail (immature, rapidly growing fish).

The control diet contained 50% fish meal and the fish growth was compared with yellowtail reared on three kinds of non-fish meal diets that replaced the fish meal with commercially available plant and animal ingredients. Soy protein concentrate, defatted soy bean meal, and corn gluten meal for plant material and pork meal, feather meal, and blood meal for animal material were employed in this study.

It is known that yellowtail become less responsive to a diet as the amount of fish meal included is reduced. Therefore, two commercially available materials, a skipjack peptide and a fish sauce were supplemented to maintain the palatability of the non-fish meal diet. The skipjack peptide is concentrated from the cooking water of skipjack and tuna. The fish sauce is made from mackerel gut using a patent-pending method of rapid production.

Non-fish meal diet No.1 contains plant meals and the skipjack peptide.
Non-fish meal diet No.2 contains animal meals and the skipjack peptide.
Non-fish meal diet No.3 contains plant meals and the fish sauce.

One-year-old yellowtail that originated from seeding production in Goto Island, Nagasaki Prefecture were used. 55 fish with an average body weight of 753g. were

transported to 4 m x 4 m net cages on the sea surface, and fed the trial diets from July till March for 8 months.

Five months later, fork length and body weight were measured and compared. Average fork length and body weight were 476 mm and 2068 g in the control diet, 474 mm and 2131 g in the No. 1 diet, 476 mm and 2233 g in the No.2 diet, and 480 mm and 2152 g in the No.3 diet. Differences between control and non-fish meal diets were not observed and the growth was almost the same. There was no difference between daily weight gain (%/day), daily feeding rate (%/day), and weight gain ratio (%), 0.58 %, 2.24 %/day, 2.83 in the control, 0.58 - 0.59 %, 2.21 - 2.26 %/day, 2.76 - 2.83 in the non-fish meal diet groups.

These results demonstrate that non-fish meal diets have the potential to support the growth of one-year-old yellowtail. Now, the effect of these diets on fish meat quality as a method of enhancing the meat quality control is being determined.

This work was supported by Research and Development Projects for application in promoting new policy of agriculture, forestry and fisheries from the Ministry of Agriculture, Forestry and Fisheries of Japan.

Key words
yellowtail (*Seriola quinqueradiata*), non-fish meal extruded pellet.

THE IMPORTANCE OF TAURINE AND N-3 FATTY ACIDS IN COBIA NUTRITION

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In the wild, fish evolve to their diet by down regulating or eliminating biosynthetic pathways for nutrients found in abundance in their diets. Upon domestication 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 polyunsatured 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. These roles include photoreceptor protection, osmolyte, antioxidant, feed attractant, and as a bile salt conjugate. 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 fishmeal replacement.

We examined the effects of graded levels of taurine addition (0%, 0.5%, 1.5%, 5.0%) to both a fishmeal based diet and a plant protein based diet with complete fishmeal replacement formulated and manufactured by the USDA-ARS. At the conclusion of a growth trial, RNA was extracted from liver, muscle, and brain tissue for quantitative-RT-PCR analysis of the genes involved in taurine synthesis. Cysteine sulfinate decarboxylase (CSD), cysteine dioxygenase (CDO), cysteamine dioxygenase (ADO), and taurine transporter (TauT) 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 growth rates.

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.

AVAILABILITY OF FISHERIES BY-PRODUCT MATERIALS WITH CADMIUM REMOVAL TREATMENT AS A FEED INGREDIENT FOR FINGERLING BLACK ROCKFISH *SEBASTES SCHLEGELI*

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The price of fish meal, a major ingredient for fish feed, has been at high levels since 2006 due to the increasing demand worldwide. Moreover, fish meal production in Chile and Peru has gradually declined because of fishery regulations for the purpose of sustaining the fishery for anchovy (Engraulidae). These difficulties have affected the increase of feed cost, which is predominant cost in aquaculture production. Therefore, the importance of studies on alternative protein sources for fish meal in diets has been increasingly realized. However, it is widely recognized that high inclusion of alternative protein source in diets results in poor palatability, particularly at low temperatures.

Squid liver 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. However, the squid liver and scallop mid-gut glands contain cadmium (Cd) at 34 and 39 mg per kg wet weight on average, respectively. Cd is generally known as a harmful heavy metal for human health. Wakasugi et al. 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 1.5-2.0 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 can be substituted for 60% of sardine meal in diets for juvenile 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. Moreover, based on the merit that supplementation of methionine and lysine is not necessary with the inclusion of dCSVM, we concluded that dCSVM can be used as an alternative protein source in diets for fingerling black rockfish.

Recently, we are investigating availability of scallop mid-gut gland 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. Moreover, we confirmed that the Cd concentrations in fish muscle of all treatment groups were below the detection limit (<0.01 mg/ kg dry matter).

Our results demonstrate that proper inclusion of dCSVM 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 dCSVM and dCSMGE as a feed ingredient for aquaculture fish species other than black rockfish.

The research was financially supported by a Research Project for Utilizing Advanced Technologies in Agriculture, Forestry, and Fisheries (grant no. 18031), Ministry of Agriculture, Forestry, and Fisheries, Government of Japan and partly by Hokkaido Government.

Key words
Black rockfish (*Sebastes schlegeli*), Squid viscera meal, Cadmium, Growth performance, Alternative protein, Scallop mid-gut gland,

DEVELOPMENT AND CHARACTERIZATION OF SEVERAL OPEN FORMULA REFERENCE DIETS FOR MARINE FISH LARVAE

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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.

Feed	Moisture (%as fed)	Proximate Composition (% dry weight)			Fatty Acid Concentrations (mg fatty acid/100mg total fatty acids)			Protein Leaching $t_{1/2}$ /% lost (sec)/(%)	Sinking Rate (cm/sec)
		Protein	Lipid	Ash	ARA (n-6)	EPA (n-3)	DHA (n-3)		
MEM	5.7	53.8	30.5	8.4	0.4	7.5	5.7	98/23.0	0.84±0.010[a]
PARA	5.3	54.2	29.7	8.9	0.4	7.4	5.6	94/30.2	0.34±0.001[c]
F	9.0	75.9	10.9	7.3	1.0	6.0	15.2	20/44.3	0.33±0.001[c]
F-MEM	13.3	75.6	13.4	7.5	1.0	5.3	13.0	50/41.8	0.78±0.060[a]
F-PARA	11.0	75.6	12.6	7.3	1.0	6.0	15.2	33/47.2	0.61±0.001[b]
Artemia	91.3	NA	15.3	13.7	5.6	12.1	7.1	NA/0.1	NA
Rotifers	90.1	49.6	15.5	17.7	1.5	5.7	23.3	NA	NA

EFFECTS OF DIETS SUPPLEMENTED WITH HERB ESSENTIAL OILS ON ECTOPARASITIC PROTOZOAN INFECTIONS ON CHUM SALMON (*ONCORHYNCHUS KETA*) FRY

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Chum salmon *Oncorhynchus keta* are artificially propagated to enhance their stock for the coastal fishery in Hokkaido, northern Japan. Ectoparasitic infection of ciliates, *Trichodina* sp. and flagellates, *Ichthyobodo* sp. causes a high mortality in chum salmon fry cultured at salmon hatcheries for release into the river as artificial seed in the propagation. These ectoparasitic protozoans are exterminated by soaking the infected fry into diluted vinegar or salt solution. However, the treatments debilitate the fry physiologically and increase cost for required effort. As a breakthrough of this problem, we need to develop techniques to prevent the protozoan infections. It is reported that essential oil of herbs including mint and lavender have an effect of insect proofing. The present study was aimed to examine suppressive effects of dietary supplement with herb essential oils on infections with two ectoparasitic protozoans by two experiments.

In the first experiment, hatchery-reared chum salmon fry infected with no ectoparasitic protozoan were separated into 5 groups on May 21, 2010. Each group (n=200 fish) was stocked in a 60 l -acrylic tank and cultured using running spring water for 35 days. Each of the five groups were fed either commercial trout crumble diet (control) or diets supplemented with essential oils of original Japanese (OJ-) mint, breeding Japanese (BJ-) mint, peppermint or lavender, respectively. Herb oil was added to respective diets at 500 ppm. Total amount of the supplied diets were equalized among the 5 groups. Fry were randomly sampled from each group during the experiment and examined for the total parasite number of *Trichodina* and for quantitative analysis of small subunit ribosomal RNA gene of *Ichthyobodo* by real-time PCR. In the second experiment, uninfected hatchery-reared chum salmon fry were divided into 2 groups: control and 500 ppm OJ-mint groups on February 21, 2013. Each group (n=85,000 fish) was taken in a hatchery pond and reared for 64 days. Methods of fry culture, fry sampling and protozoan analysis followed those of the first experiment.

In the first experiment, dietary supplement with all herb oils significantly restrained *Trichodina* infection up to 21 and 35 days and *Ichthyobodo* infection up to 35 days. Especially, *Trichodina* infection was not completely found in the OJ-mint group for 35 days. Effects of herb oils on suppression of protozoan infections were high in the following order: OJ-mint>lavender=BJ-mint>peppermint. In the second experiment, the OJ-mint

dietary supplement significantly suppressed *Trichodina* infection up to 15 and 40 days and *Ichthyobodo* infection up to 40 and 55 days, whereas it was not able to prevent protozoan infections perfectly. In consequence, the present study suggested that dietary supplement of mint and lavender oils suppressed protozoan infections on chum salmon fry and those effects depend on variation of herb species.

Key words
chum salmon (*Oncorhynchus keta*), *Trichodina*, *Ichthyobodo*, herb essential oil, mint, lavender

EFFECT OF FEED INGREDIENTS ON DIGESTIVE ENZYMES SECRETION IN FISH

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In response to limitations 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 cause growth retardation in most aquacultured fish species. Feed must be digested for their utilization, and 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 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 soybean meal based diet (SBM) fed fish were lower than those of fish meal based diet (FM) fed fish. Also, lower gene expression levels of the digestive enzymes in hepatopancreas were observed in SBM fed fish compared with FM fed fish. These data indicate that SBM does not fully stimulate the secretion/synthesis of the pancreatic digestive enzymes. In other words, FM strongly stimulates the digestive enzymes secretion/synthesis. Then, we tried to identify the stimulation factor in fish meal. Administration of a FM water-soluble fraction increased the gene expression of pancreatic digestive enzymes, 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 will improve the utilization of plant based diets in aquacultured fish species.

This work was partly supported by JSPS Grant-in-Aid for Scientific Research (B) (Grant Number: 24380117) to H. Fukada and K. Murashtia.

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

CHOLECYSTOKININ AND TRYPSIN RESPONSES OF LARVAL RED DRUM (SCIAENOPS OCELLATUS) IN RESPONSE TO ALGAE, LIVE PREY, AND INERT PARTICLES

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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, PPY, 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 (DPH) 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.

AN ANALYSIS OF THE CAUSALITY BETWEEN THE MARKET PRICE OF IMPORTED FISHMEAL AND MARKET PRICE OF MARINE FARMED FISH

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In Japan, many piscicultural famers are facing difficulties due to the impact of falling fish prices and the rising cost of feed. These are serious problems because they cause a great reduction in profits for many piscicultural farmers. According to statistics produced by the Japanese Corporation of Feed for Pisciculture (Nihon Yougyo Shiryou Kyoukai), the production of feed reached 360 thousand tons in 2011, the majority of which was for yellowtail and sea bream. Moreover, this feed was made from raw ingredients such as fishmeal and starchy food. Fishmeal was especially important, making up 50% or more of the feed content. Therefore, piscicultural farmers who farm yellowtail and sea bream always worry about a rising market in imported fishmeal.

The market price of imported fishmeal is determined by a number of factors, such as global competition among importing countries, and the gap between internal and external prices. The three main exporting countries are in South America - Peru, Ecuador and Chile – and account for 65-80 percent of the domestic market share. The market price of imported fishmeal has been rising lately because many importing countries are struggling to obtain larger amounts of fishmeal to maintain their piscicultural industries. In this case, South American countries have stronger price leadership than importing countries. Importing countries (including Japan) have to accept the market price determined by the countries with price leadership. A rise in the price of imported fishmeal affects the cost of pisciculture, and ultimately the “cost-push inflation” phenomenon may affect the equilibrium price of farmed fish.

This research investigates the extent of influence on the market price of pisciculture when the imported fishmeal price changes, because there has been no research about that in Japan. The purposes of this paper are as follows: 1) to reveal the causality among prices in the imported fishmeal market, yellowtail market and sea bream market, and 2) to calculate the impact on market prices of those fish products in landing area when the price of imported fishmeal is increasing. I used the Granger causality test and impulse response functions to conduct my analysis.

The results of the Granger causality test and impulse response functions are as follows: 1) there is causality between the price of imported fish meal and the market price of sea bream in landing area, but no causality linked to the market price of yellowtail. 2) When the price of imported fishmeal changes it has a positive impact on the market price of sea bream in landing area, and this positive impact is decreasing. Finally, the positive impact converges to zero at around the sixth month

Key words
Granger causality test, Impulse response functions, Cointegration test, market price of imported fishmeal, market price of yellowtail and sea bream in landing area

GOOD AQUACULTURE PRACTICES TO REDUCE THE USE OF CHEMOTHERAPEUTIC AGENTS, MINIMIZE BACTERIAL RESISTANCE, AND CONTROL PRODUCT QUALITY

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The need to minimize antimicrobial use in aquaculture is widely understood by academia as well as the aquaculture industry. Many fish and shrimp farmers, particularly in Asian countries, face production problems due to disease and may use chemotherapeutic agents in an effort to save stock. These agents also may pose food safety risks and have the potential for immediate and long-range human health consequences that may include carcinogenic and mutagenic effects, and increasing prevalence of antibiotic-resistant microorganisms.

In all aspects of health care for food producing animals the first step towards minimizing risk of disease is prevention. In aquaculture, the preventive measures intended to reduce the risk of disease occurring can be called many things one being good aquaculture practices. They all have the intent of preventing disease from occurring and then having to use a chemotherapeutic agent to treat the animals. Therefore, developing and implementing a preventive measures program is the first step in prudent and responsible use of veterinary medicines (antimicrobials) in aquatic food production.

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. Therefore, if infectious agents are reduced along with stress, disease outbreaks are less likely to occur. From this concept there are a number of physical, chemical, and biological precautionary measures that fish and shrimp farmers may practice to minimize disease outbreaks.

Biosecurity plans for aquaculture should include the following:

- *Hazard and risk analysis* (identify the hazard associated with the product)
- *Identify Critical Control Points* (a step at which controls can be applied to prevent of eliminate a hazard or reduce it to an acceptable level)
- *Establish Critical Limits* (a maximum and/or minimum value to which a biological or chemical hazard must be controlled to an acceptable level)
- *Monitor each critical control point* (accurate monitoring indicates when there is a lack of control at a Critical Control Point)
- *Establish corrective actions* (identify corrective action, implement and document)
- *Establish verification procedures* (the system is operating according to the plan)
- *Record keeping* (establish record keeping and documentation procedures)

Good aquaculture practices are an essential component of sustainable aquaculture. These practices can improve the quality and safety of aquaculture products by reducing bacteria and filth and the use of chemotherapeutic agents. Ensuring the safety of the food supply is a priority for the United States Food and Drug Administration.

MODELING INTRASPECIFIC GENETIC EFFECTS FOR MANAGEMENT OF AQUACULTURE PROGRAMS

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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 quinqueradiata*), salmon (*Oncorhynchus* spp.), or any other aquaculture candidate species, and to identify opportunities to validate model results.

SPECIFIC MONOVALENT AND DIVALENT ION SUPPLEMENTATION FOR CULTURE OF MARINE SPECIES

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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 production relevant, biochemical, and molecular levels.

Separate four week trials were conducted exposing newly hatched Gulf killifish to concentration gradients of potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). The K^+ supplementation consisted of 0.3, 1.3, and 2.9 mM. Treatment groups for Ca^{2+} consisted of 0.2, 1.1, 1.5, and 2.1 mM Ca^{2+}, while trials using Mg^{2+} consisted of 0.1, 2.7, 5.1, and 10.4 mM Mg^{2+}. 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^+, Mg^{2+}, Cl^-), Na^+/K^+-ATPase (NKA) activity, dry weight, and expression/localization of osmoregulatory 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 7 dph, differences in intestinal NKA and CFTR staining were observed and NKA mRNA expression was found to be higher in the 0.3 mM [K^+] group than in other treatment groups. Survival was significantly influenced by both Mg^{2+} and Ca^{2+} concentration ($p \leq 0.05$). Highest survival (71.1%) in the Ca^{2+} trial was noted in the 0.2 mM [Ca^{2+}] treatment. In the Mg^{2+} trial, highest survival was noted in the 2.7 mM [Mg^{2+}] treatment (82.9%). NKA enzyme activity was reduced and delayed peaks were observed in whole body ion composition in the Mg^{2+} treatments. In addition, decreased CFTR intensity was observed at the gill and intestinal epithelium for the 0.05 mM [Mg^{2+}] treatment at 1 dph, indicating that Mg^{2+} deficiency has a possible effect on larval ormoregulatory 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.

GENETICALLY MODIFIED SALMON IN AQUACULTURE: WELL REGULATED AND SAFE

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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.

Figure 1. AquAdvantage® and non-GM Atlantic salmon of similar age

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 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.

REPRODUCTIVE DYSFUNCTION IN CULTURED SABLEFISH (*ANOPLOPOMA FIMBRIA*)

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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 over the past few years. Due to its high market value and growth rate, sablefish has been identified as an excellent candidate for marine aquaculture in the US. However, efforts to establish sustainable and efficient production of sablefish have been constrained by reproductive problems in F1 females (i.e., those produced in captivity), which fail to mature in captivity even when they reach a similar body size and age as wild, reproductively mature females. This situation hampers the creation of captive broodstock for a selective-breeding program.

Current research at the Northwest Fisheries Science Center (NOAA, Seattle, US) is integrating basic and applied biology to gain knowledge on the reproductive endocrine system of sablefish and processes underlying the reproductive dysfunction of F1 female sablefish. As part of our basic line of research, we are characterizing the reproductive cycle in wild females caught off the Washington coast (US). Cortical alveolus stage ovarian follicles (i.e., developmental stage that precedes the onset of vitellogenesis) were first observed in May, whereas the first vitellogenic ovarian follicles were found during late summer, concomitant with increases in plasma sex steroids and gonadosomatic index. These data suggest that late spring-summer is a critical period of time for the initiation of ovarian vitellogenic grown, and therefore, key to the onset of puberty (i.e., first sexual maturation) in cultured broodstock. Also in support of this idea, wild females captured in the fall successfully completed gametogenesis in captivity, whereas females captured three months earlier, during summer, failed to mature. Interestingly, these wild non-maturing females exhibited similar reproductive features as prepubertal F1 females, including low levels of pituitary gonadotropin subunit and ovarian receptor mRNAs and plasma sex steroids, and ovarian follicles arrested at the perinucleolus stage (i.e., primary oocyte growth). These data suggest that rearing conditions impair vitellogenic growth of ovarian follicles in sablefish, compromising the reproductive success of broodstock. Using an in vitro ovarian tissue culture system, we demonstrated that fragments of prepubertal F1 sablefish ovaries incubated with either human-chorionic gonadotropin or testosterone increased the production of estradiol, the main regulator of vitellogenin production and ovarian growth, in a dose-dependent manner. This indicates that ovaries of F1 females are equipped to synthesize and release sex steroids critical for vitellogenesis under the appropriate hormone

stimulation, and that the failure to initiate vitellogenesis, and thus undergo puberty is likely a lack of adequate gonadotropin signaling. Finally, we found that F1 female sablefish express higher levels of dopamine receptor in the brain and pituitary than wild-caught maturing females. These data provided the foundation for the development of an initial phase of hormone treatments aimed at inducing puberty for the first time in F1 female sablefish. This experiment is currently underway.

INDUCED SPAWNING IN THE SEA CUCUMBER *APOSTICHOPUS JAPONICUS* BY NEUROPEPTIDE, CUBIFRIN

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In hatcheries, the induction of spawning in sea cucumbers has been typically carried out by the 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 in *in vitro* assay with ovarian fragments, from the buccal tissues containing the nerve ring. 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 that 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, oocyte maturation

MASS PRODUCTION OF ARTIFICIAL SEED OF JAPANESE COMMON SEA CUCUMBER (*APOSTICHOPUS JAPONICUS*) IN HOKKAIDO, JAPAN

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Seed production and releasing of *Apostichopus japonicus* is one of the major projects 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 released juveniles from natural ones. Due to this, some hatcheries discontinued *A. japonicus* enhancement projects.

On the other hand, due to the 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 strongly interested in *A. japonicus* stock enhancement by releasing artificial seeds. Thereafter sea urchin and abalone hatcheries began to produce *A. japonicus* seed after 2006.

This paper will introduce the recent mass production techniques developing in Hokkaido.

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

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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Fluorescent batch tagging by immersion can be used to identify sea urchins released onto the ocean bottom for sea ranching aquaculture and stock enhancement. Here, we present information on the use of fluorochrome tagging to estimate returns of green sea urchins, *Strongylocentrotus droebachiensis*, out-planted onto two sea ranching lease sites in the Gulf of Maine, USA. We tagged a hatchery cohort of 31,000 green sea urchin juveniles (≈5 mm test diameter) with the fluorochrome marker tetracycline prior to out-planting onto 400m^2 study areas located at each lease. The release areas were surveyed seven times by SCUBA divers at 3-5 month intervals for up to 27 months post-release to measure growth and recovery rates of out-planted juveniles. The growth and recovery data was used to extrapolate returns to the lease holder from out-planting the entire lease area (.80937 ha) with hatchery seed urchin numbers proportional to those used for the study area Juveniles from the same hatchery cohort were simultaneously reared in a land-based recirculating aquaculture system as a benchmark to assess sea ranching returns.

Our results show that the fluorochrome tetracycline can be detected for at least 27 months in sea urchins released into the field. Tagged urchins were consistently found at both sites and at every dive survey, showing that there is potential for out-planting programs in the Gulf of Maine to succeed. A disproportionate number of urchins recovered from the two out-planting areas were smaller than the average release size, suggesting that size dependant mortality or dispersal of out-planted urchins had occurred. There were significant differences in growth and recovery rates between the two sites, but recovery rates were sufficiently high at one of the sites to indicate that sea ranching could be economically viable under certain conditions. Growth and recovery rates in the land-based recirculating aquaculture system were significantly higher than those seen at either ocean lease site, and provided a benchmark against which future out-planting efforts could be compared.

The results presented here offer a case study of how fluorochrome marking can be used in sea ranching and out-planting studies to compare different sites and estimate returns. This study also illustrates some of the limitations of this approach, but recent advances in tagging methods and visualization offer promise that these limitations can be overcome. In all likelihood, fluorochrome marking will be a requirement for sea ranching urchins in the Gulf of Maine, as it is presently the only practical means of differentiating between hatchery and wild stocks.

IMPROVING THE FOOD QUALITY OF SEA URCHIN GONADS BY SUPPRESSING GAMETOGENESIS

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The quality of sea urchin gonads as food products gradually 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 without copious gametes have a higher commercial value. Thus, suppressing gametogenesis by controlling environmental conditions is advantageous for sea urchin aquaculture. In the present study, we investigated the effect of water temperature on the size and quality of the gonads of *Strongylocentrotus nudus*. Rearing *S. nudus* under a low temperature between summer and autumn suppressed the progress of gametogenesis and alleviated the quality deterioration caused by maturation, without sacrificing the gonad size.

Key Words
gametogenesis, ovary, quality, sea urchin, temperature, testis

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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The short-spined sea urchin *Strongylocentrotus intermedius*, which is distributed on the northern coast of Japan, is a valuable commercial species and one of the most popular and expensive seafoods in Japan. The edible part of the sea urchin is the gonads, and its quality as food varies dramatically with gonadal growth and maturation. In general, the superior quality of gonads, defined by attractive taste 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. Therefore, the best season for harvesting *S. intermedius* is very short (less than three months per fishery region) in spite of continuous 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.

Serving local *S. intermedius* to visitors during the summer tourist season in Rausu, located in a world natural heritage site “Shiretoko,” has long been hoped. However, it has not been feasible during the sea urchin spawning season (July to September), resulting in quality degradation in gonads due to maturation. In several echinoderms, water temperature has been shown to have a strong effect on the progress of gametogenesis. Fortunately, a facility for collecting deep-sea water, which has properties for stable low temperature, is available in Rausu. Therefore, taking advantage of the available facility, 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 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. Whereas in groups reared at low temperatures (2.1–5.1°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 *S. intermedius* is effectively suppressed by rearing sea urchins under low temperature conditions. Additionally, we also investigated 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 under low temperature, while suppressing the progress of gametogenesis. Moreover, the results of chemical analysis (free amino acids) and tasting test proved that the quality of gonads were very excellent in sea urchin fed with live *Saccharina diabolica*. Consequently, we demonstrate that low temperature rearing, supplemented with feeding live *Saccharina 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 the Rausu Fishery Cooperative.

This study was conducted as part of a research project, "Research and Development Projects for Application in the New Policy of Agriculture, Forestry and Fisheries," funded by the Ministry of Agriculture, Forestry and Fisheries, Japan.

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

LONG-TERM OUTCOMES IN THE TECH TRANSFER OF SCALLOP SPAT COLLECTION TECHNIQUES, FROM AOMORI PREFECTURE, JAPAN TO MAINE, USA

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In 1999, a delegation from Maine traveled to their sister-state of Aomori Prefecture in Japan, to gain a better firsthand view of the world-famous scallop industry there. The delegation included representatives from the fishing sector, processing, management, aquaculture, science and outreach, and the hopes were to learn from the Japanese experience such that the scallop industry in Maine could be strengthened. While the trip was tremendously informative from many angles, the detail that had most immediate and broadest application was the use of so-called 'spat bags' to capture competent larvae, and retain them for later stock enhancement and intensive aquaculture. Over the next years following the delegation, over 100 fishermen in Maine used spat bags to capture juveniles of our local scallop species, the Giant Scallop (*Placopecten magellanicus*). The practice allowed us to build relationships with counterparts in Atlantic Canada and through southern New England as well, as we sought expertise in the technique, and in determining the effectiveness in augmenting wild populations of scallops.

Although we have not been successful in documenting strong stock enhancement success (it is suspected, but not verified), there are several other areas in which the use of spat collection has proven to be extraordinarily valuable. For example, the current progress made in the aquaculture sector of sea scallops is largely due to the excellent success in collecting spat as a reliable seed supply. There is activity and great interest in linking source populations for settled juveniles, spat collectors provide excellent access to juveniles for shell composition analysis, and are a component in understanding the oceanographic and behavioral components of larval dispersal. Area closures - sometimes at the request of the fishermen - also involve 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, and have been so 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.

RELATIONSHIP BETWEEN SPAT DENSITY, FOOD AVAILABILITY, AND GROWTH OF SPAWNERS IN CULTURED JAPANESE SCALLOP (*MIZUHOPECTEN YESSOENSIS*) IN FUNKA BAY: CONCURRENCE WITH ENSO

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To elucidate the factors that influence the interannual variation in the density of cultured Japanese scallop (*Mizuhopecten yessoensis*) spat, we analyzed the relationship between spat density (*Ds*), monthly Chl-*a* concentration, water temperature, and adductor muscle weight of spawners over 15 years (1992–2006) in Funka Bay on the western North Pacific Ocean. The interannual variation of spat density was best explained by a campaniform model that used chlorophyll a concentration in February (Chl_{Feb}) and a categorical variable, which indicates whether growth of spawners in a year is low or not, as independent variable ($R^2 = 0.91$). The gonad-somatic index increased fastest in February. Low growth years were detected as outliers in the *Ds*– Chl_{Feb} relationship, and were characterized by an average weight of adductor muscles in February of < 12 g. Therefore, food availability during gonadal development and growth conditions of spawners were the main factors determining spat density. The proportion of ovary necrosis was high in the years of low Chl_{Feb} and low growth. Those years corresponded with El Niño and La Niña years, respectively. Thus, global climatic anomalies apparently affect reproduction of the scallop in Funka Bay.

Key words
scallop, spat, food availability, ovary necrosis, ENSO.

OYSTER CULTURE IN HOKKAIDO, JAPAN

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The Pacific oyster, *Crassostrea gigas* is the one of the most commercially important aquaculture species in the Japanese fisheries industry with around 200, 000 metric tons a year, the same as Japanese scallop *Mizuhopecten yessoensis*. The Oyster is cultured using hanging method in various Japanese coastal areas including Hokkaido. The Saroma Lake and the Akkeshi Bay with estuary, which face Ohotsuku Sea and Pacific Ocean, respectively are both major oyster production areas in Hokkaido with total annual production of about 700 tons a year. In the early days Hokkaido, then popularly called Yezo (pre-1869), natural oyster beds were grown in these areas and the oysters were harvested. However, after loss of natural oyster resources in these areas, the aquaculture of oysters has depended on seedling spat supplied from Miyagi, Japan. Miyagi seedlings are widely used in Japanese oyster production areas, not only Hokkaido. Therefore, when the catastrophic tsunami on March 11, 2011 damaged the Miyagi fisheries, many oyster culturing areas were heavily affected.

Most Miyagi seedlings are naturally collected using scallop shells and reared by the specialized fishermen. The seedlings have fast growth potential as reported by several studies, and thus are preferred by many oyster farmers. However, the hardship caused by the tsunami exposed the risks of excessive dependence on the Miyagi seedlings. Moreover, introduction of seedlings from distant areas have risks of invasion of diseases and alien organisms as hitchhiking species. For example, the paramyxean parasite, *Marteilioides chungmuensis*, has had negative impacts on the oyster industry in some Japanese production areas, and invasive ascidian such as *Molgula manhattensis* and *Ascidiella aspersa* were also recorded in some bivalve culturing areas. Moreover, the potential transport of harmful algae via relocation of bivalve molluscs has been reported. Therefore, the techniques to reduce these risks have to be applied with efforts for maintaining local populations of oysters when the seedlings grown in different locations are relocated. In addition, using seedlings that originated from local populations in each area is also one of the approaches for decreasing some risks. In the Akkeshi area, the artificial seedlings spats collected from the locally survived adults are also used for aquaculture, although there is little genetic variation. Akkeshi Bay faces the Pacific Ocean where a cold subarctic ocean current “Oyashio” flows, but Akkeshi-ko estuary connected to the bay is shallow and its water temperature increases during summer enough to enhance oyster growth and

maturation. Culture of single-seed oysters in hanging baskets is more suitable in this kind of shallow area than seedlings attached to scallop shell. This is because more oysters can be cultured by basket hanging than scallop shell hanging in a shallow area, and the amounts of oysters and extraneous organisms falling to the sea bottom is smaller in cage culturing than in culturing with seedlings on scallop shell. Moreover, single-seed oysters have deep shell breadth, which are marketed as value-added oysters with shell and are popular among consumers as the local special products. However, the yield of single-seed oysters was lower than that of Miyagi-seedling oysters at the beginning of this culturing, because the culturing methods for Miyagi seedlings were not suitable for single-seed oysters. After the crisis of Miyagi seedlings, the preliminary use of local populations is beginning in some production areas. In these attempts, it is important that the existing culturing management is suitable for culturing of local population, which might have original characteristics.

This research was partly supported by annual research grants " Study of coastal shellfish fisheries for supports the recovery from tsunami damage in eastern Hokkaido" and grants-in-aid for Scientific Research " Analysis and future prediction of coastal ecosystem" provided by the Hokkaido National Fisheries Research Institute, Fisheries Research Agency of Japan.

Key words
Pacific oyster (*Crassostrea gigas*), Miyagi seedlings, Saroma Lake, Akkeshi Bay, single-seed