



**UJNR Aquaculture Panel  
39th Scientific Symposium  
October 25th-26th, 2010**



# 養殖産業の現在と将来

**The Present and Future of the Aquaculture Industry**



**Place: Kagoshima University, Inamori Hall**

**(鹿児島大学稲盛会館)**

**Tel: 099-285-7701**

**Organized by: Fisheries Research Agency (FRA), Japan  
National Oceanic and Atmospheric  
Administration (NOAA), USA  
Kagoshima Univ. (under application)**

# The 39<sup>th</sup> Scientific Symposium of UJNR Aquaculture Panel

October 25<sup>th</sup> – 26<sup>th</sup>, 2010

Kagoshima University Inamori Hall, Kagoshima

## **The Present and Future of the Aquaculture Industry**

### **Aim of the symposium**

Based on the goals of the third five-year-plan of FRA that will begin in 2011 and the NOAA and USDA strategic plans for aquaculture, the focus for this meeting will be to present and discuss issues concerning the history and role of aquaculture as well as future production systems and strategies used by each nation to promote aquaculture production, both from the standpoint of the farmer and the consuming public. The primary focus will be on problems faced by the aquaculture industries of the two nations and strategies and solutions to create competitive new and existing aquaculture industries. We will explore current aquaculture systems that are expanding and aquaculture systems that have the potential to expand in the future. We will consider the research needs to maximize the future potential of these industries by examining aquaculture production systems. Aquaculture systems include the whole process from spawning of a certain species to consuming the product including broodstock/hatchery, cultivation, harvest, processing and distribution as a commodity. From this exchange we expect to highlight key bottlenecks that need to be addressed in future meetings. In particular, we will propose a direction for our aquaculture industries and aquaculture research areas that are useful and necessary to promote effective consumer friendly industries that support our seafood culture and consumption. We will present and discuss issues about the history, role, and processes from producing to consuming, process of making a profit and how aquaculture can meet consumer needs.

### **Program**

#### **Monday, October 25<sup>th</sup>, 2010**

	Registration	11:30-14:00
Opening remarks (Takaji Iida, Japan Panel Chair)	• • • • •	13:00-13:10
Welcome greeting (Tadahide Noro, Dean of the Faculty of Fisheries)	• • • • •	13:10-13:20
Aim of the symposium (Makoto Yamasaki, Deputy Secretary General)	• • •	13:20-13:25

#### **Session I . Overview of Aquaculture Research Policy**

(Moderators: Walton Dickhoff, Hisashi Yokoyama )

##### **1. Course of the Research for Sustainable Aquaculture in Japan**

Fuminari Itoh (National Research Institute of Aquaculture, FRA)	• • • • 13:30-13:48
2. Aquaculture Research Priorities and Opportunities 2010-2015 and beyond: a USDA and Interagency Perspective	
Jeffrey Silverstein (USDA, Agricultural Research Service)	• • • • • 13:48-14:06
3. Current Policy of Aquaculture Industry and Research	
Mitsuyuki Hirai (Fisheries Agency, MAFF)	• • • • • 14:06-14:24
4. Aquaculture Policy and Research Priorities from the NOAA Perspective	
Robert N. Iwamoto (NOAA, Northwest Fisheries Science Center)	• • • • 14:24-14:42
Break	• • • • • 14:42-15:02
<b>Session II. Aquaculture Industry Overview and Research Planning</b>	
(Moderators: Marty Riche, Takashi Iwasaki)	
5. Aquaculture in North America: Present Status and Future Opportunities	
Paul G. Olin (UCSD, Scripps Institution of Oceanography)	• • • • • 15:02-15:20
6. Overcoming Technical Barriers to the Sustainable Development of Competitive Marine Aquaculture in the United States	
John A. Hargreaves (Aquaculture Assessments LLC)	• • • • • 15:20-15:38
<b>Session III. Techniques of Aquaculture Production</b>	
(Moderators: James Sullivan, Susumu Uji)	
7. A Challenge to Technology Development of the Year Round Egg Production in Kan-pachi Amberjack <i>Seriola dumerili</i> : Advanced Spawning Technique	
Kazuhisa Hamada (National Research Institute of Aquaculture, FRA)	• • • • • 15:38-15:56
8. Spawning and Larval Rearing of Yellowtail Amberjack ( <i>Seriola lalandi</i> ) in Southern California	
Kevin Stuart (Hubbs-SeaWorld Research Institute)	• • • • • 15:56-16:14
Break	• • • • • 16:14-16:34
(Moderators: Bob Iwamoto, Akiyuki Ozaki)	
9. The Development of Aquaculture Techniques for Greater Amberjack ( <i>Seriola dumerili</i> ) Using Artificially Produced Seed : Technological Advancement of the Seed	

Production

Hiroshi Hashimoto (Shibushi Station, National Center for Stock Enhancement,  
FRA) . . . . . 16:34-16:52

10. Success of Artificial Completion of the Japanese Eel (*Anguilla japonica*) Life Cycle  
Hitoshi Imaizumi (Shibushi Station, National Center for Stock Enhancement,  
FRA) . . . . . 16:52-17:10

11. Offshore Mussel Farming in Southern New England; Recent Results  
Scott Lindell (Marine Biological Laboratory, Marine Resources Center)  
. . . . . 17:10-17:28

Announcement . . . . . 17:28

Tuesday, October 26<sup>th</sup>, 2010 Registration 9:00-9:30

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

(Moderators: Paul Olin, Fuminari Itoh)

12. Structural Problems of Fish Farming Industry in Japan  
Masaaki Sano (Faculty of Fisheries, Kagoshima University) . . . . . 9:20-9:38

13. The Political Economics of United States Aquaculture  
Gunnar Knapp (Institute of Social Economic Research, University of Alaska)  
. . . . . 9:38-9:56

**Session III. Techniques of Aquaculture Production; continued**

(Moderators: Jeff Silverstein, Mitsuyuki Hirai)

14. Fish Nutrition and Fish Health—Recent Progress in Japan—  
Shunsuke Koshio (Faculty of Fisheries, Kagoshima University) . . . . . 9:56-10:14

15. The Future of Aquaculture Feeds in the United States  
Michael B. Rust (NOAA, Northwest Fisheries Science Center) . . . . . 10:14-10:32

Break . . . . . 10:32-10:52

(Moderators: Cheng-Sheng Lee, Tetsuro Shibuno)

16. Progress of DNA Marker-Assisted Breeding in Mariculture Finfish  
Akiyuki Ozaki (National Research Institute of Aquaculture, FRA) . . 10:52-11:10

17. Biotechnology for Competitive Aquaculture

Walton W. Dickhoff (NOAA, Northwest Fisheries Science Center) • • • 11:10-11:28

## 18. Development and Evaluation of Real-time Loop-Mediated Isothermal Amplification Methods for the Rapid Detection of Penaeid Viruses

Tohru Mekata (National Research Institute of Aquaculture, FRA) . . . . 11:28-11:46

Lunch Break . . . . . 11:50-14:00

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Poster Session

## Session V. Supporting Techniques of Aquaculture

(Moderators: Scott Lindel, Shusaku Kadowaki)

## 19. Bioindicator and biofilter function of *ULVA* spp. in fish farm environments

Hisashi Yokoyama (National Research Institute of Aquaculture, FRA) · · · 14:15-14:33

## 20. Inland Marine Fish Culture in Low-Salinity Recirculating Aquaculture System

Marty A. Riche (USDA, Agricultural Research Service) • • • • • 14:33-14:51

## 21. Landbased Poly-Eco-Aquaculture of Abalone and Seaweed in the Small Scale Recirculating System Using the Recycled Frozen Container

Mohammad M. Rahman (Faculty of Fisheries, Kagoshima University) · · · 14:51-15:09

Break . . . . . 15:09-15:24

(Moderators: Kevin Stuart, Makoto Yamasaki)

22. Techniques for current control in tank; lessons learned from rearing larvae of red spotted grouper *Epinephelus akaara*

Takashi Iwasaki (National Research Institute of Aquaculture, FRA) · · · 15:24-15:42

## 23. Pond-to-Plate Analysis of the US Farm-Raised Catfish Industry

Terrill R. Hanson (Department of Fisheries and Allied Aquacultures, Auburn University) . . . . . 15:42-16:00

## 24. Beneficial Meat Quality Induced by Diets in Yellowtail

Itaru Shioya (Nippon Suisan Kaisha Ltd.)	. . . . . 16:00-16:18
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Break . . . . . 16:18-16:33

(Moderators: Gunnar Knapp, Tohru Mekata)

**Session VI. Stock Enhancement**

25. A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage  
Conditioning for Stocking Japanese Flounder, *Paralichthys olivaceus*, in Wakasa Bay,  
Japan  
Michelle L. Walsh (University of New Hampshire) . . . . . 16:33-16:51
26. Acoustic Conditioning and Ranching of Black Sea Bass *Centropristis striata*  
Scott Lindell (Marine Biological Laboratory, Marine Resources Center)  
. . . . . 16:51-17:09
- Closing remarks (Michael B. Rust, U.S.A. Panel Chair) . . . . . 17:09-  
Announcement . . . . . -17:20

Satellite Symposium in Shibushi

29<sup>th</sup> October 10:30-

At a meeting room in Hotel Volver A Daguri

### **Hatchery, the Present State and Issues**

1. Artificial Completion of the Japanese Eel, *Anguilla japonica*, Life Cycle: Challenge to Mass Production  
Yoshitsugu Masuda (Shibushi Station, National Center for Stock Enhancement, FRA)
2. Spawning and Larval Rearing of Yellowtail Amberjack (*Seriola lalandi*) in Southern California 2  
Kevin Stuart (Hubbs-SeaWorld Research Institute)
3. A Challenge to Technology Development of the Year Round Egg Production in Kan-pachi Amberjack *Seriola dumerili* : Proposal of New Systematized Aquaculture  
Kazuhisa Hamada (National Research Institute of Aquaculture, FRA)
4. Sablefish Hatchery Methods. An Overview of Hatchery Design, Brood Stock Spawning, and Egg and Yolk Sac Larvae Incubation Procedures at the Manchester Research Station  
Michael B. Rust (NOAA, Northwest Fisheries Science Center)
5. Development of A Visualizing Method for Physical Injury to Fish Larvae  
Susumu Uji (National Research Institute of Aquaculture, FRA)

## COURSE OF THE RESEARCH FOR SUSTAINABLE AQUACULTURE IN JAPAN

Fuminari Ito

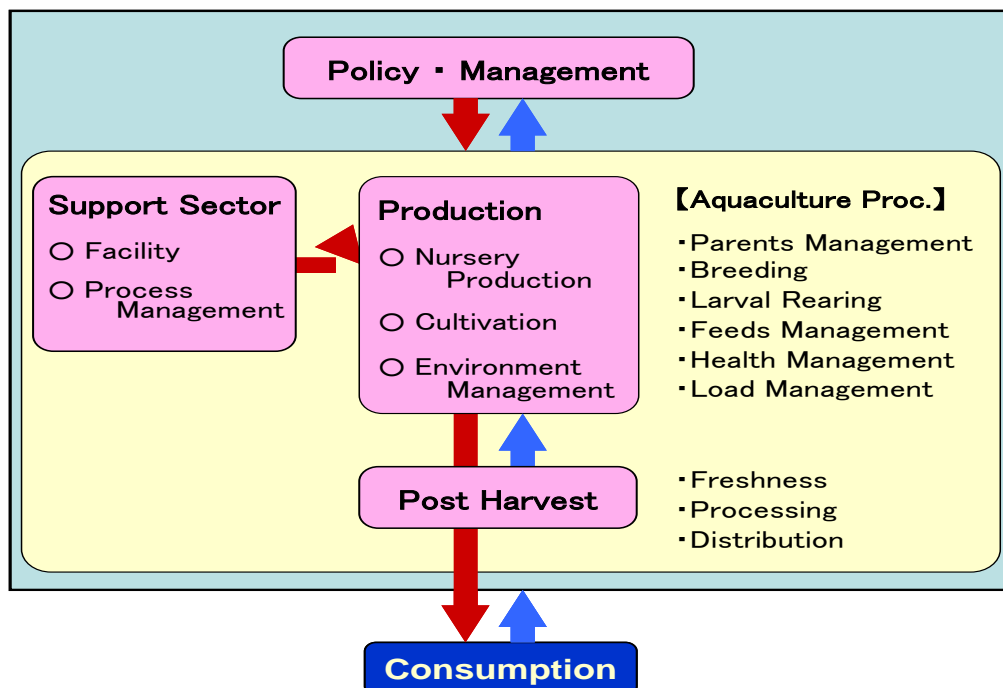
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World aquaculture has grown dramatically in the last 50 years mainly in Asia, and is expected to increase further. On the other hand, aquaculture production of Japan has changed almost stably, and occupies an important position in the entire fishery, but the affairs of that are in very severe. It is due to such as increased production costs of fuel oil and fish meal for raw material of feed, restraint purchasing of high grade fish and sluggish of fish price by economic recession, and damage of red tide and outbreak of disease. From such a situation, aquaculture industry of Japan has fallen into the production structure of high cost and low price.

To improve the management of aquaculture, it is necessary to shift to a sustainable and profitable structure by a review of the entire production process. We organized an overview of aquaculture (aquaculture production system) as shown below, and have discussed research problems and directions of solutions in each category. For sustainable aquaculture, industry must reduce the environmental impact, as well as needs to be profitable. For the low impact on environment, research and technological innovation are needed on artificial seed production that is independent on natural resources, improvement of culture methods and feed quality unaffected to water quality and so on. To increase revenue, it is necessary to practice continual cost savings by analysis each process of aquaculture production and to develop a way to add high value to products. Thus, it is important that aquaculture is regarded as an integrated production system for considering the future research.

### Aquaculture Production System (Illustration of Fish Aquaculture)





## **AQUACULTURE RESEARCH PRIORITIES AND OPPORTUNITIES 2010-2015 AND BEYOND: A USDA AND INTERAGENCY PERSPECTIVE**

Jeffrey Silverstein

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The three federal departments primarily concerned with aquaculture in the United States are Department of Agriculture, Department of the Interior and Department of Commerce. The US Department of Agriculture (USDA) applies expertise in agriculture to support efforts to increase production efficiency and consumer value. Within the Department of Interior, the Fish and Wildlife Service (FWS) creates recreational opportunities and protects freshwater biodiversity. Within the Department of Commerce, the National Oceanographic and Atmospheric Administration (NOAA) enhances commercial, artisanal and recreational fishing opportunities, develops and supports aquaculture for commercial and enhancement goals and protects marine species. The growth of aquaculture in the US was gradual but reliable over the 20 years from 1980 to 2000. Industry growth and stakeholder involvement justified growing budgets for aquaculture research in USDA (ARS and NIFA) and the other agencies to a lesser extent. Nevertheless, over the last decade aquaculture production in the United States has not expanded. The catfish industry, the largest single component of commercial US aquaculture has gone through a sharp decline of approximately 30% over the past 3 years, due to competition from imports, and high production costs. Trout production, the second largest commercial finfish sector has been stable but without growth for the past 3 decades. Other products are seeing some fluctuations; some bright spots include growing salmon production, and increased markets for molluscan shellfish. Within the USDA (Agriculture Research Service, ARS, in particular) research is focused on four major themes: genetic improvement, development of alternative ingredients and improved nutrition, improved health through vaccine and therapeutics development and improving production systems (ponds, raceways and recirculating systems).

Several of these priorities are indeed multiagency, for example there is a joint NOAA-USDA alternative feeds initiative, and work on therapeutic and vaccine development is done in coordination with FWS, Animal and Plant Health Inspection Service (APHIS) and Food and Drug Administration (FDA).

Two clear themes emerging for stimulating growth of aquaculture in the US are strengthening connections along the value chain from hatchery to consumer; and incorporating best new technologies to achieve sustainable production, of high quality, affordable products.

The Joint Subcommittee on Aquaculture is a federal government coordinating body where issues that cross multiple government agencies can be discussed and addressed. Past achievements include development of effluent guidelines for aquaculture in conjunction with Environmental Protection Agency (EPA), and development of a National Aquatic Animal Health Plan. Currently, an aquaculture Research and Technology strategic plan for the federal government is under development.

## **CURRENT POLICY OF AQUACULTURE INDUSTRY AND RESEARCH**

Mitsuyuki Hirai

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The Fisheries Agency (FA) , based on the basic fisheries plan developed in March 2007 and the basic plan for agriculture, forestry and fisheries research developed in March 2010, works to establish sustainable production of fisheries resources and robust fisheries industrial structure by promoting the restoration and management of fishery resources, the preservation of coastal environment including seaweed beds and mud flats, and the prevention of damage to the fisheries industry from harmful organisms such as large jellyfish. Also FA promotes to stabilize the fisheries management through easing of fluctuation in material costs such as fuel oil and decrease of income, and through fostering and securing fisheries employments.

Aquaculture production of Japan is 1.19 million tons (479 billion yen), and accounts for 21% (29%) of total production in 2008. In order to strengthen steadiness and competitiveness of the aquaculture management, FA develops and diffuses new aquaculture technologies suitable for field needs, preserves environment in aquaculture grounds, and secures the safety and reliability of the products by controlling fish disease and introducing the good aquaculture practice (GAP). In particular, as it is recently concerned that the captures of natural juvenile fishes as seeds for aquaculture influence upon the reproduction of fishery resources and also large-scale red tides extensively damage the aquaculture production, FA is especially advancing to develop new technologies for artificial seeds production, e.g., eel or bluefin tuna, and for prevention of damages by red tides.

## **AQUALCULTURE POLICY AND RESEARCH PRIORITIES FROM THE NOAA PERSPECTIVE**

Robert N. Iwamoto

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After 2 years of formulation, a Department of Commerce (DOC)/National Oceanic and Atmospheric Administration (NOAA) aquaculture policy was signed by the Secretary of the U.S. Department of Commerce in 1999. This policy was instrumental in establishing a framework that outlined the DOC vision for U.S. Aquaculture and DOC's Aquaculture Mission to establish a business climate and technological base for industry to develop environmentally sound aquaculture. A significant component of the DOC policy was the declaration of six specific targets or objectives to be accomplished by the year 2025:

- A. Increase the value of domestic aquaculture production from the present \$900 million annually to \$5 billion, which will help offset the \$6-billion annual U.S. trade deficit in seafood.
- B. Increase the number of jobs in aquaculture from the present estimate of 180,000 to 600,000.
- C. Develop aquaculture technologies and methods both to improve production and safeguard the environment, emphasizing where possible those technologies that employ pollution prevention rather than pollution control techniques.
- D. Develop a code of conduct for responsible aquaculture by the year 2002 and have 100% compliance with the code in Federal waters.
- E. Double the value of non-food products and services produced by aquaculture in order to increase industry diversification.
- F. Enhance depleted wild fish stocks through aquaculture, thereby increasing the value of both commercial and recreational landings and improving the health of our aquatic resources.

NOAA, as the nation's ocean agency with its attendant stewardship responsibilities, has a major role in the national strategy to attain those objectives. To provide guidance and a framework for the agency's approach, the NOAA Aquaculture Program developed a 10-year plan in 2007. The plan, while outlining the approaches the agency would take in promoting and achieving the national aquaculture objectives, also identified the many challenges facing commercial aquaculture expansion in the United States within the context of the agency's legislative and regulatory mandates and constraints with budgetary and staffing uncertainties and stakeholder support. Furthermore, in 2009 NOAA announced its intent to develop a new and comprehensive national policy for sustainable marine aquaculture in federal waters. Relying heavily on stakeholder input, the national policy posed seven questions to the public:

1. What opportunities exist for developing sustainable marine aquaculture nationwide? What are the major impediments?
2. What are the most important environmental considerations, and how can these be addressed?
3. Which social and economic consequences or outcomes will be the most important in the next 5 years or in the next 20 years?
4. How can NOAA best support essential research and innovation? What should be the goals of NOAA-funded research related to aquaculture?
5. How can NOAA best communicate with the industry and public on aquaculture issues? What are the opportunities for partnerships?
6. What role should NOAA play with respect to aquaculture issues and initiatives at the international level?
7. What other considerations need to be addressed in NOAA's aquaculture policy?

NOAA has analyzed the public input and is in the process of developing a draft national policy for review and public comment. Once that process is complete, the agency will issue a new NOAA Aquaculture Policy.

This presentation will review the DOC aquaculture policy, NOAA's 10-year research plan, and present salient points regarding the draft NOAA aquaculture policy to provide a perspective of NOAA's aquaculture research priorities as well as the drivers that influence them.

## **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

U.S. Department of Commerce Aquaculture Policy. 1999.  
[http://aquaculture.noaa.gov/pdf/18\\_docaqpolicy.pdf](http://aquaculture.noaa.gov/pdf/18_docaqpolicy.pdf)

NOAA 10-Year Plan for Marine Aquaculture. 2007.  
<http://aquaculture.noaa.gov/about/tenyear.html>

NOAA Draft Aquaculture Policy. 2010.  
<http://www.nmfs.noaa.gov/aquaculture/policy1/#discussion>

## **AQUACULTURE IN NORTH AMERICA: PRESENT STATUS AND FUTURE OPPORTUNITIES**

Paul G. Olin

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This represents a review of the status and trends in aquaculture development in North America. The paper covers current levels of Canadian and American production and species diversity, production trends over the last decade, available resources and governmental support, and projections for future industry growth and development.

The aquaculture industry in North America produced 644,213 metric tonnes of product in 2008 with an estimated value of US\$1,622,460 (FAO 2009). This represents an annual percentage increase over the previous decade of 1.8 percent by volume and 4.5 percent in value. The finfish industry is at the forefront of the aquaculture sector led by production of Atlantic salmon in Canada and channel catfish in the United States. Over the decade from 1997 to 2007, Canadian production increased 54 percent resulting primarily from expansion of Atlantic salmon net pen culture on the Atlantic and Pacific coasts. Canada produced 104,070 metric tonnes of Atlantic salmon in 2008 worth US\$ 663 million (Statistics Canada 2010). This represents 88 percent of total aquaculture revenue. The U.S. experienced a 17 percent increase in the aquaculture sector primarily through increased production of Pacific and Eastern oysters, hard clams, manila clams and red swamp crayfish. The U.S. channel catfish industry has experienced strong competition as a result of significant increases in imports of basa and tra from Southeast Asia and has declined in recent years. In 2008 American catfish growers produced 201,000 metric tonnes of fish valued at US\$389 million. (USDA – NASS 2010)

FAO has projected the need for an additional 40 million metric tonnes of seafood by 2030 to meet anticipated increases in global demand (FAO 2009). There is potential for significant increases in production in North America and both the Canadian and American governments have projections for expansion of their aquaculture industries. Canada estimates that by 2020 production could exceed 308,000 tonnes with farm-gate value of US\$1.6 billion. The United States Department of Commerce estimates that domestic aquaculture production has the potential to increase in value from US\$1 billion to more than US\$3 billion by 2025. In the United States this additional production would be comprised of 760,000 tons of finfish, of which 590,000 tons would be marine finfish, 47,000 tons of crustaceans, and a 245,000 ton increase in mollusc production.

Future significant growth in the North American aquaculture industry will likely follow the successful model demonstrated by the Atlantic salmon industry in Canada and new technologies enabling net-pen culture to move further offshore. Both Canada and the United States have ample areas for this expansion and the Canadian salmon example and pilot projects in the U.S. demonstrate the viability of this approach. However, in some regions there is considerable opposition and whether a significant industry sector develops will depend on the establishment of

a regulatory regime that insures environmental protection while enabling the economic viability of aquaculture ventures.

## References

FAO. 2009. The State of World Fisheries and Aquaculture 2008. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations. Rome, Italy

FAO FishStat Plus. 2010. <http://www.fao.org/fishery/statistics/software/en>

Statistics Canada. 2010. <http://www.statcan.gc.ca/pub/23-222-x/23-222-x2008000-eng.pdf>

USDA – NASS. 2010.

<http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1016>

## ANNOTATED BIBLIOGRAPHY OF KEY WORKS

FAO. 2008. The State of World Fisheries and Aquaculture 2008. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations. Rome, 2009

This is a comprehensive look at world aquaculture and factors influencing aquaculture production. The publication features some of the aspects of fisheries and aquaculture that may receive increasing attention in the future. Examples include climate change, the use of marine genetic resources in areas beyond national jurisdiction, and the proliferation of private standards and certification schemes in the international fish trade. The publication also highlights a few of FAO's special studies. This edition includes the use of wild-fishery resources as seed and feed in aquaculture, reviews the world's shrimp fisheries and the management of marine capture fisheries in the Pacific Ocean.

FAO FishStat Plus. 2010. <http://www.fao.org/fishery/statistics/software/en>

This is a comprehensive database that contains global fishery and aquaculture production statistics by species and country. It is a program that is open domain software, created and maintained by FAO which can be downloaded from the FAO website.

Statistics Canada. 2010. <http://www.statcan.gc.ca/pub/23-222-x/23-222-x2008000-eng.pdf>

This is the aquaculture production database for Canada, created and maintained by the Statistics Canada.

USDA – NASS. 2010.

<http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1016>

This is the aquaculture production database for the United States, created and maintained by the U.S. Department of Agriculture (USDA) National Agricultural Statistics Service (NASS)

## **OVERCOMING TECHNICAL BARRIERS TO THE SUSTAINABLE DEVELOPMENT OF COMPETITIVE MARINE AQUACULTURE IN THE UNITED STATES**

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Technological innovation drives economic growth and development and is a key component of competitive advantage in a global economy, but innovation can be impeded by unresolved measurement barriers. Overcoming measurement barriers requires the development of methods, standards, instruments, and capabilities to evaluate the performance and advancement of technologies. A workshop was held to identify the priority research needs, and where appropriate, the measurement barriers that impede innovation in U.S. marine aquaculture. The workshop focused on the critical technology gaps that affect the sustainable development of a commercial marine aquaculture sector emphasizing four types of production systems: shellfish aquaculture, cage culture of marine finfish, land-based culture of marine finfish, and biofloc-based culture of marine shrimp.

The main subject areas with identifiable technology gaps include: genetic improvement, nutrition, health management, control of reproduction, production of larvae and juveniles, food safety and product quality, environmental performance and impact, system engineering and life-support systems, and economics and marketing.

Technical barriers to innovation can be addressed by strategic investment in high priority areas, particularly those with potential benefits that extend across technology platforms, and those that integrate research among subject areas. Also, cost-effective investments in research to address high-priority information and technology needs, combined with a stable regulatory environment, a streamlined permitting process, and public-private partnerships in demonstration systems can accelerate private-sector investment in the development of a sustainable and competitive marine aquaculture sector in the U.S. economy.

Research needs are summarized and prioritized. Specific measurement gaps are also described. Important non-technical barriers to sector development are also identified. Roadmap implementation focuses on the next steps to promote the advancement of US marine aquaculture.

## ANNOTATED BIBLIOGRAPHY OF KEY WORKS

Browdy, C.L. and J.A. Hargreaves (editors). 2009. Overcoming Technical Barriers to the Sustainable Development of Competitive Marine Aquaculture in the United States. U.S. Department of Commerce, Silver Spring, MD USA. NOAA Technical Memo NMFS F/SPO-100. 114pp.

This publication summarizes the outcome of a workshop convened to identify the barriers to technological development in marine aquaculture in the United States. Specifically measurement barriers related to the lack of technology standards, instrumentation, and methodologies to evaluate system performance are identified. Four marine aquaculture technology platforms are considered: shellfish aquaculture, cage culture of marine finfish, land-based culture of marine finfish, and biofloc-based culture of marine shrimp. Specific research needs are identified and prioritized.

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NOAA. 1998. NOAA's Aquaculture Policy. U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

This represents the guiding document for marine aquaculture policy in the United States as articulated by the lead agency (NOAA). The policy focuses on the roles of: 1) research, development, and technology transfer; 2) financial assistance to businesses; 3) environmental safeguards including regulatory and permit procedures; and, 4) coordination. The research, development, and technology transfer component considers: 1) environmental impacts and standards; 2) systems development; 3) growth and production of marine species; 4) biotechnology; 5) technology transfer; and, 6) coastal management.

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NOAA. 2007. NOAA 10-Year Plan for Marine Aquaculture. U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

The plan was adopted by NOAA as an agency-wide policy document intended to guide a broad, national initiative for marine aquaculture based on four distinct goals: 1) a comprehensive regulatory program for environmentally sustainable marine aquaculture; 2) development of commercial marine aquaculture and replenishment of wild stocks; 3) public understanding of marine aquaculture; and, 4) increased collaboration and cooperation with international partners. The plan also provides an assessment of the challenges NOAA will face in its effort to reach these goals, such as the need for congressional and Executive Branch actions to provide additional legislative and budget support.



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NOAA. 2008. Offshore Aquaculture in the United States: Economic Considerations, Implications & Opportunities. U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

This report explores the economic benefits and challenges associated with offshore aquaculture. This report considers the broad, long-term implications of an established domestic offshore aquaculture industry in the United States. Among the key findings, the report suggests that, over time, the economic potential for offshore aquaculture is likely to grow because seafood demand and prices will increase, there will be more competition for sites closer to shore, and costs will be lower due to improved technology, experience, and economies of scale.

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Pacific Shellfish Institute. 2009. West Coast Shellfish Research and Education 2015 Goals and Priorities. Pacific Shellfish Institute, Pacific Coast Shellfish Growers Association, National Shellfisheries Association / Pacific Coast Section. Olympia, WA.

This report summarizes the goals and research priorities of shellfish producers on the Pacific coast of the United States. The main areas include: 1) domestication of established species; 2) shellfish health, disease prevention and management; 3) shellfish ecology; 4) aquaculture, enhancement, and restoration of native shellfish; 5) management and research of pests and invasive flora and fauna; 6) human health and shellfish; 7) protection, enhancement, and restoration of water quality in shellfish growing areas; 8) aquaculture training, education, and outreach; 9) farming, harvesting, and processing; 10) marketing and promotion; and, 11) policy and regulation.

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U.S. Commission on Ocean Policy. 2004. Setting a Course for Sustainable Marine Aquaculture. Pages 330-336 in An Ocean Blueprint for the 21<sup>st</sup> Century. U.S. Commission on Ocean Policy, Washington, DC.

The U.S. Commission on Ocean Policy's Final Report to Congress called on the Secretary of Commerce to: 1) design and implement national policies for environmentally and economically sustainable marine aquaculture; 2) develop a comprehensive, environmentally, sound permitting, leasing, and regulatory program for marine aquaculture, 3) expand marine aquaculture research, development, training, extension, and technology transfer, including a socioeconomic component; 4) set priorities for research and technology, in close collaboration with the National Sea Grant College Program, states, tribes, academia, industry, and other stakeholders; 5) work with the FAO to encourage and facilitate worldwide adherence to the aquaculture provisions of the Code of Conduct for Responsible Fisheries.

## **A CHALLENGE TO TECHNOLOGY DEVELOPMENT OF THE YEAR ROUND EGG PRODUCTION IN KAN-PACHI AMBERJACK *Seriola dumerili* : ADVANCED SPAWNING TECHNIQUES**

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Amberjack (*Seriola dumerili*, Kan-Pachi in Japanese) is one of the most important species in marine fishery resources in Japan, having recently become a major target of aquaculture production. As aquaculture process, the stable production and distribution of quality eggs are needed to obtain healthy larvae/fry. However, spawning and quality egg production techniques were not yet established.

In Kan-pachi amberjack, spawning season of the wild brood fish is from November to March around the waters of China Kainan islands and Vietnam (the South China Sea). Juveniles grow to about 10- 15 cm in total length with body weights of about 25- 50 g in April in the waters of China, and then they are exported to Japan as aquaculture seeds. On the other hand, in Japan the spawning season of greater amberjack reared in net cages under the natural conditions ranges from May to June. Thus, there is a lag of about six months in the spawning season compared to Chinese wild fish. The delay in the spawning season appears a drawback that it affects directly the initial size of fry at the beginning of the grow-out culture. Consequently, the difference in the initial size would reflect on feed and labor costs and then commodity price. To solve the present issues, therefore, we have developed maturity control techniques by the environmental control based on the technologies with spawning induction in December (winter) of yellowtail (Fisheries Research Agency (FRA), Japan).

We first succeeded in egg production of Kan-pachi amberjack in December (2006) in the world. We have subsequently achieved the early egg production for the last four years. The size of our artificial seeds from the eggs in December reached finally to about 25 cm in the total length and about 250 g in body weight at the end of May in the next year, which is almost equal or superior to imported seeds from China. Furthermore, considering production cost, our artificial seeds from the eggs in December cost 289 to 324 yen for a juvenile, while 350 to 600 yen for a Chinese seed with an annual fluctuation. From the viewpoint of cost in the seed production, we can claim that our seeds produced by the advanced spawning technologies are more effective.

We conclude that the present environmental control technologies in maturity and early spawning established in Kan-pachi amberjack would not only contribute to the establishment of well-planned production and stable supply of quality seeds of Kan-pachi amberjack, but also be applied to the wide-range temperate/tropical marine species.

## **SPAWNING AND LARVAL REARING OF YELLOWTAIL AMBERJACK (*Seriola lalandi*) IN SOUTHERN CALIFORNIA**

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Hubbs-SeaWorld Research Institute (HSWRI) has been rearing yellowtail amberjack (*Seriola lalandi*), known locally as California yellowtail, at its laboratory in San Diego since 2003. The broodstock have been spawning naturally in a 140 m<sup>3</sup> maturation pool since 2002. The sex ratio has been close to 1:1 and spawning typically occurs between 16.5 and 22.0°C (April thru August). The number of adults has ranged from 21 to 35 and associated individual weights from 8.2 to 20.8 kg. Our primary egg quality measures have shown inconsistent spawn quality within a spawning season, with hatch rates ranging from 10 – 90% and survival to first feeding ranging from 5 – 80%. Typically the highest quality spawns are seen early in the season. The numbers of spawning events and eggs per spawn have increased in recent years due to increasing female biomass and improvements to the broodstock feeding regime.

Recent research results on yellowtail have shown no significant differences in growth or survival at 10 dph among egg stocking densities of 50, 100, and 200 eggs per L. Greenwater was demonstrated to be beneficial to larvae up to 16 dph as was 24hr light and high light intensity of 5,000 to 13,000 lux. There were no significant differences in growth or survival when yellowtail larvae were fed at rotifer densities of 15, 30, and 45 rotifers per ml. Larvae were able to consume 1<sup>st</sup> instar Artemia (*Artemia franciscana*) as early as 6 dph. Finally, we determined that greenwater rearing conditions created using either SanoLife ALG (INVE), algae paste, or live algae all produced similar results.

Our current culture protocols for production include 1) stocking eggs at a density of 100 per L; 2) maintaining water temperature at 21.0 – 22.0 °C; 3) providing rotifers (*Brachionus rotundiformis*) from 2 to 9 dph at 20 per ml; 4) moving larvae from tall, narrow 1600L egg incubators to shallow, wide 8,000L tanks at 10 dph; 5) providing 1<sup>st</sup> instar Artemia (*Artemia franciscana*) from 6 to 10 dph and 2nd instar Artemia from 10 to 35 dph; 6) providing 24hr light at 5,000 to 13,000 lux above each tank; and 7) greening the water using SanoLife ALG from 2 to 17 dph.

These procedures yielded survival rates from egg to juvenile of up to 5% with a relatively high degree of variability. High larval mortality, from unidentified causes at around 17 - 20 dph and deformity rates as high as 40% are focal areas for improvement through future research.

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Benetti, D. 1997. Spawning and larval husbandry of flounder (*Paralichthys woolmani*) and Pacific yellowtail (*Seriola mazatlanica*), new candidate species for aquaculture. *Aquaculture* 155, 307-318.

This paper describes the spawning and larval rearing of two new species for aquaculture in Ecuador. Experimental production of these species has been conducted for the first time from eggs in captivity. Survival of *Seriola* throughout larval rearing ranged from 0 – 70%, however high mortalities caused by diseases, cannibalism, and weaning onto artificial diets during and after metamorphosis reduced average survival rates through the juvenile stage to less than 1%. The basic broodstock and larval rearing information provided by the author is a good starting point for the culture of either *Paralichthys* or *Seriola* culture.

Moran, D., C.K. Smith, B. Gara, C.W. Poortenaar. 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). *Aquaculture* 262, 95-104.

The authors describe the spawning behaviour of wild caught brood stock as well as early egg and larval development of yellowtail kingfish (*Seriola lalandi*). Spawning occurred naturally when water temperature was above 17 C. Courtship behaviour involved one male and female, and consisted of a high-speed pursuit punctuated by stalling, nipping and touching. This lasted for approximately 0.5 – 1.5 h until, immediately prior to spawning, the male would nip at the female gonoduct, presumably to induce spawning. Spawning occurred in the early daylight hours at the start of the spawning season, but shifted to around dusk in the latter part. Spawned eggs were positively buoyant, had a high fertilization rate (>99%), ranged 1.33 – 1.50 mm in diameter with a single oil droplet 0.30 – 0.33 mm diameter. Information given in this paper is indicative of a healthy spawning population of *S. lalandi* and can be used as a reference or baseline for culture of this species.

Papandroulakis, N., C.C. Mylona, E. Maingot, P. Divanach. 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. *Aquaculture* 250, 155-161.

The authors apply the mesocosm method for larval rearing to the greater amberjack (*Seriola dumerili*). This paper gives a good description of larval rearing for *Seriola* in terms of weaning schedule, turbidity levels, and live feed levels. Along with the description of the rearing protocols, growth information is provided which is a good template for most *Seriola* species.

Sakakura, Y. and K. Tsukamoto. 1999. Ontogeny of aggressive behaviour in schools of yellowtail, *Seriola quinqueradiata*. *Environmental Biology of Fishes* 56, 231-242

The authors studies ontogenetic changes in social interactions, especially in aggressive behaviour of the yellowtail, *Seriola quinqueradiata*, and compared these to morphological and physiological changes. No agonistic interactions were observed during the larva period until 10 mm in total length (TL) at approximately 20 days after hatching. Typical shivering behaviour with 'J-posture' was observed during metamorphosis, when fin rays and calcification of vertebra were completed and there was an increase of tissue thyroid hormone. The onset of aggressive behaviour was just after metamorphosis to the juvenile period, and coincided with a significant

increase in tissue cortisol levels. The onset of schooling behaviour was at 12 mm TL, slightly after the onset of aggressive behaviour. The authors provided a good basis for determining aggressive behaviour in *Seriola quinqueradiata* which can be used as a model for other *Seriola* species. This paper also presents possible solutions to aggressive interactions found in the culture environment.

Yoseda K., K. Yamamoto, K. Asami, M. Chimuea, K. Hashimoto, S. Kosaka. 2008. Influence of light intensity on feeding, growth, and early survival of leopard coral grouper (*Plectropomus leopardus*) larvae under mass-scale rearing conditions. *Aquaculture* 279, 55-62.

This study investigated the effect of different light intensities on feeding, growth, and survival of early stage leopard coral grouper *Plectropomus leopardus* larvae. Four different light intensities (0, 500, 1000, and 3000 lx) were used and larvae were kept under constant light conditions from 0 day after hatching (DAH) to 5 DAH. The results indicate that coral grouper larvae are visual feeders and their food intake increases with increasing light intensity and that light intensity is the factor affecting larval feeding, growth, and survival. The authors provide a sound experimental design and interesting results which can translate to any species whose larvae are visual predators. The comprehension of optimization of environmental rearing conditions such as light intensity as it relates to first feeding larvae is necessary in establishing stable mass-scale rearing technology for any species.

## **THE DEVELOPMENT OF AQUACULTURE TECHNIQUES FOR GREATER AMBERJACK (*Seriola dumerili*) USING ARTIFICIALLY PRODUCED SEED : TECHNOLOGICAL ADVANCEMENT OF THE SEED PRODUCTION**

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Survival rate in greater amberjack seed production in Japan has been low at 0 to 5%. One reason suggested for this low survival rate is that appropriate feeding schedule and environmental conditions for rearing larvae and juvenile of the greater amberjack have not been clearly defined. In particular, sinking of larvae occurring in an earlier stage up to 20 days post hatching and thrusting behavior resulting from growth heterogeneity between 20 and 25 days post hatching, are the major causes of mass mortality. Overcoming these problems is important for establishing techniques for artificial greater amberjack seed production.

To identify the factors causing mass mortality in the larval stage and to develop techniques for improving the survival rate, we first optimized prey size<sup>1</sup>, fish density<sup>2</sup>, photoperiod and water temperature<sup>3</sup>, using small tanks (500 L). Next, we found that actively removing the oil films from the water surface, increased the swim bladder inflation rate of larvae, resulting in prevention of the sinking death. Furthermore, we showed that accommodating the start of *Artemia* feeding to the smallest individuals could effectively harmonized larval growth, which prevented the mortality due to the thrusting behavior. We then applied these optimum rearing conditions determined in the small tanks to a mass production (80 kL tanks). As the results, we succeeded in producing 110,000 artificial seeds with an average total length of 28 mm in mid-July using fertilized eggs obtained during the regular spawning season (June), and 15,000 artificial seeds with an average total length of 23 mm at the end of January using fertilized eggs obtained in the non-spawning season of December through the environmental control for broodstock rearing.

The seed obtained from the broodstock spawned in December (winter offspring) grew to an average total length of 20 cm and average weight of 100 g by the end of March (3 months post hatching), 2.4 kg by the end of December (1 year post hatching), and reached to a market size of 3.1 kg by the end of next March (1 year and 3 months post hatching). Using the winter offspring, 3-9 months can be reduced for greater amberjack culture relative to the present commercial operation using the summer offspring (the imported seeds obtained from natural waters), which leads to save the aquafarming costs.

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Authors reared greater amberjack larvae to investigate their feeding habits during the early phase of seed production. Larvae were fed on three types of rotifers with different body sizes, and the survival, growth rate and gut contents of the larvae were examined. As the results of this research, authors suggest an appropriate feeding schedule for greater amberjack larvae during the early phase of seed production.

2) Teruya, K., Hamasaki, K., Hashimoto, H., Katayama, T., Tsuruoka, K., Hayashi, T., and K. Mushiake. 2009. Ontogenetic changes of body density and vertical distribution in rearing tanks in greater amberjack *Seriola dumerili* larvae. NipponSuisanGakkaishi, Vol:75. Pp54-63.

To obtain basic knowledge on sinking death syndrome which has been considered to be one of the causes of early mortality during the seedling production of greater amberjack, ontogenetic changes of larval body density were examined. A rearing experiment was conducted to investigate the effects of aeration rate (0.1-2.0L/min) on the vertical distribution, feeding, survival, growth and initial swim bladder inflation of larvae reared in 500-L tanks for 10 days post hatching. Greater amberjack larvae showed negative buoyancy. In tanks with aeration of 0.1-1.0L/min, larvae were distributed in the upper layer in the daytime and on the tank bottom in the nighttime after 3 days post hatching. Excessive aeration (2.0L/min) caused larvae to disperse in tanks. However, feeding amounts, and rates of survival, growth and initial swim bladder inflation of larvae were low in those tanks with rearing seawater of high turbulence. To prevent the larvae from sinking to the tank bottom, rearing techniques with less physical stress on larvae should be developed.

3) Hirata, Y., Hamasaki, K., Imai, A., Teruya, K., Iwasaki, T., Hamada, K., and K. Mushiake. 2009. Effects of different photoperiods and water temperatures on survival, growth, feeding and initial swim bladder inflation of greater amberjack *Seriola dumerili* larvae. NipponSuisanGakkaishi, Vol:75. Pp995-1003.

To determine the appropriate rearing conditions for early stage larvae of greater amberjack *Seriola dumerili*, we investigated the survival, growth, feeding and initial swim bladder inflation of larvae reared in two or three replicates in 500 L tanks under different photoperiods (0L: 24D, 12L: 12D, 18L: 6D, 24L: 0D) and water temperatures (22, 24, 26, 28°C). In the 0L: 24D group, few larvae showed feeding incidence and growth, and total mortality occurred 8 days after hatching. Photoperiod (12-24 hours light condition) and temperature did not affect the larval feeding and swim bladder inflation, whereas larval survival and growth were improved in the 18L: 6D group and larval survival was the best at 22°C.

## **SUCCESS OF ARTIFICIAL COMPLETION OF THE JAPANESE EEL (*Anguilla japonica*) LIFE CYCLE**

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Eel farming is dependent on the natural resources of glass eels. The catches have recently declined, destabilizing the business of eel farming. For that reason, there is an urgent need to achieve the production of juveniles in a hatchery, eliminating dependence on natural resources. However, most of the life cycle of eels has remained unknown. Consequently, there is little knowledge related to the course of maturing and spawning or to the habitat and food in the larvae period, making it necessary to use the process of trial and error in various studies. First, because eels do not mature or spawn under culture conditions, artificial maturation using hormone administration has been developed, which has made fertilized eggs available; the development of techniques for the culture of larvae was started. However, that development was extremely difficult because the culture was not possible merely by the application of existing culture methods other fish. About 30 years after the first hatchlings were obtained; culture up to the stage of glass eels was finally achieved via the discovery of a feed composition available for hatchlings and the development of a feeding method that uses the behavior characteristics of hatchlings.

National Center for Stock Enhancement, Fisheries Research Agency, has been engaged in the mass production of glass eels through improvement of artificial maturing and culture methods, aiming at complete life cycle culture by checking the fertility of hatchery-reared eels with an eye to technical development of breeding that engenders the rearing of parent eels and mass production without depending on wild eels. Shibushi Station prepared 26 hatchery-reared eels in all, including 10 females (313 g mean body weight) and 16 males and sex-unknown individuals (384 g mean body weight), which were artificially hatched hatchlings obtained in 2004–2006. They developed to glass eels and were reared for an additional 4–6 years after being subjected to artificial maturing. Results show that 2,150,000 eggs in all were collected from 9 of 10 females. The fertilized eggs were obtained through artificial insemination. The mean fertility was 65.9%, the mean hatchability was 37.0%, and the mean survival rate at 6 days after hatching was 34.8%. These results of maturing and egg yield were better than those for wild eels in captivity. This study proved that hatchery-born eels can produce artificially hatched larvae of a second generation, achieving complete life cycle culture for the first time in the world. We hope that further research will engender the development of breeding techniques that provide mass production of glass eels as well as development of maturation and egg collection methods without dependence on natural resources.



## **OFFSHORE MUSSEL FARMING IN SOUTHERN NEW ENGLAND; RECENT RESULTS**

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Based on results from a 2008/2009 investigation of mussel growth and pea crab surveys at twelve offshore sites in RI and MA, we installed four subsurface longlines for suspended mussel culture in the fall of 2009. Two long lines were deployed in RI in water depths of 70 feet (off Newport) and 110 feet (off Block Island). Two long lines were deployed in MA in water depths of 100 feet (N. Chilmark) and 120 feet (S. Chilmark). These 500-foot longlines each support 200 ten-foot long mesh socks filled with mussel seed at densities ranging from 100 to 160 seed per foot. The average length of the seed was 24mm when deployed for grow-out in October and November. Growth rates averaged 3 to 3.5 mm per month when calculated at harvest in August 2010. No pea crabs were found in 2 of the 3 sites harvested. One of the sites had a failure of the anchoring system. The net yield of processed and marketable mussels from the gross weight of the harvested socks was 75%. The steamed meat yield of the mussels was 20% in late August versus 30% in early July. The longlines were deployed and are managed by active local fishermen from their lobster and scallop boats. To facilitate the deployment from small boats we used plow anchors (250 kg) instead of more traditional block anchors that weigh 2,000 kg or more. Lessons from our experience in permitting, engineering, socking seed, and managing the lines will be shared.

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*These are some influential Japanese works I know on this topic, too.*

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## **STRUCTURAL PROBLEMS OF FISH FARMING INDUSTRY IN JAPAN**

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The economical situation of Japanese fish farming industry is now very serious. Behind this serious situation, there are some fated structural problems. The first one is the inevitable conflict between the logic of individual fish farming enterprise and the logic of environment. The second one is also the inevitable conflict between the logic of individual fish farming enterprise and the logic of market (price making mechanism). Both are brought from rigid utilization system of fishing ground. This is not valid on these modern days in Japan. But it is very difficult to change this old system. It has been inevitably developed through the long history of fishing villages. The trial to change it without good understandings might break down the whole industry. Furthermore, third structural problem exists behind these two. The products from fish farming must lose their scarcity value through its progress, and must become cheaper. Then the profitability also must decrease inevitably. The serious situation of fish farming industry in Japan has been brought from these structural problems.

## **THE POLITICAL ECONOMICS OF UNITED STATES AQUACULTURE**

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Government leasing, regulatory and other policies are critically important for developing and maintaining competitive aquaculture industries. In much of the United States, local, state or national policies constrain the development of marine aquaculture to a scale far below its economic potential. Two extreme examples are the State of Alaska's ban on all finfish farming, and the absence of an enabling regulatory framework for aquaculture in offshore federal waters. This paper examines how government policies affect the development and scale of United States aquaculture, and the political factors that affect the extent to which policies are favorable or unfavorable towards aquaculture. Aquaculture can best develop with leasing and regulatory policies which are clear, predictable, stable and timely and which provide opportunities and incentives to efficiently take advantage of potential development opportunities. Key political factors affecting aquaculture policies include how many different agencies are involved in making policies which affect aquaculture; whether these agencies are part of local, state or national governments; the agency missions and internal cultures; the extent to which agencies are influenced by different stakeholder groups, and how different stakeholder groups perceive that they are or would be affected by aquaculture.

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## **FISH NUTRITION AND FISH HEALTH -RECENT PROGRESS IN JAPAN-**

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Nutrition is very important to maintain health and recover from illness and to improve the resistance against disease and stress in aquatic animals like other animals. Optimal feeds can provide animals with healthy conditions and greater stress resistance, and optimal nutrients can change immune responses in a positive way.

Since several micronutrients such as vitamins are not synthesized or synthesized less than required in the aquatic animal body to grow normally and maintain or promote health, those should be provided from the feeds. Although recommended dietary levels of many nutrients for avoiding deficiency signs and the maximum growth have been shown in several aquatic species, there are very limited data available on the requirements and/or recommended values in terms of improving health and quality of cultured species. Recently, several studies have demonstrated that over-dosed vitamin intake or administration of some extracts from natural organisms improved the stress tolerance, immunological response, and disease resistance of cultured species.

This paper introduces recent work conducted in Japan on nutritional factors affecting aquatic animal health together with growth parameters, immunological responses, disease resistance and so on. The focuses are as follows:

- Utilization of By-product
- Effects of Micronutrients and Functional Compounds
- Reduction of Oxidative Stress

It can be possible to enhance stress and disease resistance, and therefore aquatic animal health, by taking vitamin concentrations which are higher than those of the requirement, useful bacteria, and functional compounds such as immunostimulants from natural organisms and plants. On the other hand, the universal validity on determining the optimal level of those will depend on the condition such as nutrient types, dosage, period, timing, health status, and genetic history of animals, etc.

## **THE FUTURE OF AQUACULTURE FEEDS IN THE UNITED STATES**

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The development and expansion of farming of carnivorous fish species will be constrained by a limited supply of fish meal and oil for feeds. Fortunately, there is no dietary requirement for specific amounts of fish meal or fish oil for fish, so feeds that lessen the reliance on these limited feedstuffs—such as alternative protein and oil resources—can, and must, be developed. For this reason, the U.S. Department of Commerce (NOAA) and the U.S. Department of Agriculture (USDA) are jointly sponsoring an aquaculture feeds initiative to address those issues. The initiative used expert and public consultations to identify and discuss the future of fish feeds and the benefits to the U.S. by the development of such alternative feeds. The resulting report from these meetings will be released in draft form for comment by the end of 2010 and will be available at <http://aquaculture.noaa.gov>. The report calls for feeds to be evaluated not only for nutritional and economic performance as is done today, but also for environmental and human health performance by taking into account the environmental footprint of feed production and use, and the resulting quality of the product for human consumption. This “triple bottom line approach” – economics, environment and human health – is supported by 20 specific recommendations. The opportunity exists for the international community to comment on the draft before the final report is published by following the process outlined at the web site above.

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The authors for the first time present a comprehensive human health model based on concentrations of mercury, dioxins, polychlorinated biphenyls and long chain n-3 fatty acids for fish and project the impact of increased seafood consumption in the US on the population's health. This model accounts for the increased risks associated with consumption of contaminated seafood along with the benefits from increased consumption of long chain n-3 fatty acids. Overall the authors predict that increasing the per capita consumption of seafood in the United States from 16 to 26 lbs/person (1-2 servings per week of species high in n-3 fatty acids) would result in a decrease in coronary death by 36% and an overall decrease in total mortality of 17%. Further the authors provide the amounts to consume of various species and the cost to provide the benefits associated with seafood consumption. Implications for target nutrient and

contaminate levels in aquacultured fish can be derived from the information presented in this paper.

Rust, M. B., F. T. Barrows, R. W. Hardy, A. Lazur, K. Naughton and J. Silverstein. (In press.) Draft NOAA/USDA Alternative Feeds Initiative: The future of Aquafeeds. Agency Technical Report available at <http://aquaculture.noaa.gov>

This report is the subject of this presentation. More details and recommendations are found on the US approach to future development of diets for aquaculture.

Tacon, A.G.J., and M. Metian. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285:146-148.

This paper provides a peer-reviewed source of information on aquaculture use of fish meal and fish oil up to the mid 2000's with projections in to the future. Environmental implications to the over use of stocks of industrial fish are discussed.

## PROGRESS OF DNA MARKER-ASSISTED BREEDING IN MARICULTURE FINFISH

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The marine products industry has developed based on direct catch from natural resources. However, because of the depletion and gradual restriction of aquatic resources, only recently has breeding been considered as an important research area. With the depletion of aquatic resources, the expectation regarding aquaculture research is increasing. Also genetic improvement of traits for improved culture leading to superior varieties, because we can improve the phenotype to suit aquaculture conditions by domestication of artificially produced fish with each generation.

The essential conditions for DNA marker-assisted selection “MAS” is development of useful resource families to evaluate phenotypes and information about genetic linkage and physical maps, and a large number of polymorphic genetic markers. At the beginning of the study, we mainly aimed to use improved technologies on salmonid fish. But now this research and development are being applied to other kinds of mariculture fish. Some of the cases have already reached a practical stage, and have been used as genetic improvement productions. Two cases of MAS programs have succeeded in developing Japanese flounder resistant to lymphocystis disease (Fuji et al., 2007) and Atlantic salmon resistant to infectious pancreatic necrosis (IPN) (Moen et al., 2009). These cases indicate the validity of the methodology.

MAS breeding is applicable to other target species. However there still remain problems and potential to further improve the methodology. One is markers-assisted introgression (MAI) or QTL pyramiding. Markers-assisted introgression is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits, and introgression hybrid to obtain several economic traits on one strain. We are carrying out research and development about MAI using Japanese flounder (*Paralichthys olivaceus*) as a target species. The other is effective utilization of natural genetic resources. At this point, aquatic resources have more advantages compared with plant and animals, because wild species are not selected and still maintain high genetic diversity. Individuals have high potential for genetic breeding regarding phenotypic characters. Aquaculture species have available those genetic resources in breeding material. We are researching practical applications about selection of economic important traits from natural genetic resources using Japanese amberjack (*Seriola quinqueradiata*) as a target species.

Here I would like to introduce our research on genetic improvement breeding program using DNA marker-assisted selection in mariculture finfish.



## ANNOTATED BIBLIOGRAPHY OF KEY WORKS

Ozaki, A., Okamoto, H., Yamada, T., Matuyama, T., Sakai, T., Fuji, K., Sakamoto, T., Okamoto, N., Yoshida, K., Hatori, K., Araki, K., Okauchi, M., 2010. Linkage analysis of resistance to *Streptococcus iniae* infection in Japanese flounder (*Paralichthys olivaceus*). Aquaculture, Article in press.

Streptococcal disease (streptococcosis) caused by *Streptococcus iniae* is a serious bacterial disease in Japanese flounder (*Paralichthys olivaceus*). Developing streptococcosis-resistant Japanese flounder will reduce the number of outbreaks of this disease as well as reduce the need for medicines and vaccines. Genetic linkage analysis is an effective method for identifying quantitative trait loci (QTL) associated with resistance to a disease. In this study, 159 microsatellite markers selected from genetic linkage maps of Japanese flounder and F1 progeny from crosses between disease-resistant and disease-susceptible parents were used for detection of QTL associated with resistance to this disease. Some loci associated with disease resistance were found in the JF-7, JF-10, JF-11 and JF-17 linkage groups. These QTL regions are candidates for disease resistance against streptococcal infection.

Fuji, K., Hasegawa, O., Honda, K., Kumasaka, K., Sakamoto, T., Okamoto, N., 2007. Marker-assisted breeding of a lymphocystis disease-resistant Japanese flounder (*Paralichthys olivaceus*). Aquaculture 272, 291-295.

An allele of a microsatellite, Poli9-8TUF, has a dominant effect at a single major locus and is responsible for resistance to lymphocystis disease (LD-R) in Japanese flounder. We developed a new population of Japanese flounder produced by marker assisted breeding using this allele. A female that originated from the KP-B inbred line with LD-R that was homozygous for the favorable allele (B-favorable) and a male from a commercial stock bred for higher growth rate and good body shape were selected as parents. A female was selected as the LD-R-bearing parent because the recombination rate of females is lower in the region where the LD-R locus is located. As expected, the B-favorable allele was transmitted as a heterozygote to the progeny (LD-R+ population). The LD-R+ population, when tested at two commercial fish farms that had LD outbreaks, showed no incidence of LD at either farm, while a control population without B-favorable alleles (LD-R-) had incidences of 4.5% and 6.3% at the two farms. These results show that marker-assisted breeding using molecular markers linked to an economically important trait is an efficient strategy for breeding.

Moen, T., Baranski, M., Sonesson, A.K., Kjøglum, S., 2009. Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. BMC Genomics 10:368.

Infectious pancreatic necrosis (IPN) is one of the most prevalent and economically devastating diseases in Atlantic salmon (*Salmo salar*) farming worldwide. The disease causes large mortalities at both the fry- and post-smolt stages. Family selection for increased IPN resistance is performed through the use of controlled challenge tests, where survival rates of sib-groups are recorded. However, since challenge-tested animals cannot be used as breeding candidates, within family selection is not performed and only half of the genetic variation for IPN resistance is being exploited. DNA markers linked to quantitative trait loci (QTL) affecting IPN resistance would therefore be a powerful selection tool. The aim of this study was to identify and fine-map QTL

for IPN-resistance in Atlantic salmon, for use in marker-assisted selection to increase the rate of genetic improvement for this trait.

The QTL confirmed in this study represents a case of a major gene explaining the bulk of genetic variation for a presumed complex trait. QTL genotypes were deduced within most parents of the 2005 generation of a major breeding company, providing a solid framework for linkage-based MAS within the whole population in subsequent generations. Since haplotype-trait associations valid at the population level were found, there is also a potential for MAS based on linkage disequilibrium (LD). However, in order to use MAS across many generations without reassessment of linkage phases between markers and the underlying polymorphism, the QTL needs to be positioned with even greater accuracy. This will require higher marker densities than are currently available.

Rothschild, M. F., Ruvinsky, A., 2007. Marker-Assisted Selection for Aquaculture Species. In: Liu, Z.J. (Ed.), Aquaculture Genome Technologies. Blackwell Publishing, Ames, Iowa, Chapter 12. pp.199-214.

The authors reviewed some background and theory related to MAS and information as it pertains to some other species essential for food production and to present information on recent progress in the field of aquaculture, and introduced recent progression in construction of genetic maps in aquaculture species, also introduced studies of quantitative trait loci (QTL) analysis of complex traits, such as disease-resistance, growth and fecundity, which are important in fish breeding.

## **BIOTECHNOLOGY FOR COMPETITIVE AQUACULTURE**

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Application of biotechnology is critical in developing new species for aquaculture, improving fish culture techniques, and making domestic industries competitive in the global marketplace. Biotechnology tools and approaches in the genome sciences and their applications are advancing rapidly. Next generation sequencing methods are fast, relatively inexpensive and have made it possible to sequence genes, develop DNA markers and obtain sequence reads of thousands to millions from a single sample. Gene expression data can then be used in studies to assess the status of animals in culture, and refine husbandry techniques to improve survival, development, growth and reproduction. Gene information can also be used to assist selective breeding and as diagnostic tools, among other applications. Examples will be given of biotechnological approaches in research on growth, sex control and reproduction of finfish.

## **DEVELOPMENT AND EVALUATION OF REAL-TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHODS FOR THE RAPID DETECTION OF PENAEID VIRUSES**

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Shrimp viral diseases are a major impediment to commercial shrimp farming. The diseases affecting shrimp culture are white spot syndrome virus (WSSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV). Viral diseases are particularly difficult to control after the onset of infection; therefore, prophylaxes to prevent or reduce the losses through vertical and horizontal transmission are most important.

Various diagnostic methods have been developed to detect shrimp viral diseases, including bioassays, histopathology, polymerase chain reaction (PCR), and quantitative real-time PCR. Although the PCR based methods are sensitive and highly specific, they require expensive equipment, costly reagents, and are time consuming. Therefore, a simple, quick and sensitive detection method is urgently needed to prevent the invasion of shrimp viral diseases into Japan from other countries.

The loop-mediated isothermal amplification (LAMP) assay is a novel approach to amplify the nucleic acid with high specificity, sensitivity and rapidity under isothermal conditions, thereby obviating the need for a thermal cyler. Further, during the LAMP reaction, an insoluble by-product, magnesium pyrophosphate, is produced in proportion to the large amounts of the target DNA amplified. Hence, real-time quantification can be achieved by measuring the turbidity of the magnesium pyrophosphate using an inexpensive photometer. This real-time LAMP method allows quantitative analysis of nucleic acids templates (real-time LAMP). In the present study, a comparatively less expensive quantitative real-time LAMP assay was successfully applied for detection of shrimp viral diseases and proven to have high sensitivity and specificity.

## BIOINDICATOR AND BIOFILTER FUNCTION OF *Ulva* spp. IN FISH FARM ENVIRONMENTS

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In order to examine the efficiency of *Ulva* spp. (Chlorophyta) as a bioindicator and biofilter for dissolved inorganic nitrogen discharged from coastal fish farms, field surveys and field cultivation experiments were conducted in Gokasho Bay, central Japan. Naturally occurring green algae *Ulva* spp. were collected in and around a fish farm in February, May and July, and their  $\delta^{15}\text{N}$  and tissue N contents were analyzed. Values of these parameters obtained in May and July decreased with the distance from the fish cages, suggesting that *Ulva* spp. occurring in close vicinity to the fish farm assimilated the fish-excreted N. To evaluate this uptake, we housed round cut pieces of thalli of *Ulva ohnoi* in transparent chambers and cultured them beside a fish cage and at a control station for 2 wks in May, July and September. The thalli cultivated beside the fish cage showed significantly higher specific growth rates (15.8–20.9%) and higher N assimilation rates (4.2–13.9 mg N g dry wt<sup>-1</sup> d<sup>-1</sup>), which are among the highest levels known for seaweed biofilters. The high growth rates during the warm season and easy cultivation indicate the suitability of *Ulva ohnoi* as a biofilter.

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Hirata, H., S. Yamasaki, H. Maenosono, T. Nakazono, T. Yamauchi, and M. Matsuda. 1994. Relative budgets of  $p\text{O}_2$  and  $p\text{CO}_2$  in cage polycultured red sea bream, *Pagrus major* and sterile *Ulva* sp. Suisanzoshoku, Vol:42. Pp 377–381.

Polyculture of fish and seaweeds is a beneficial system in aquaculture. In spite of its importance from the ecological point of view. Little attention has been given in polyculture system. This may be due to the fact that fish and seaweeds have different growth seasons: fish grow in summer and autumn while seaweeds propagate in winter and spring. Previously, culture methods for sterile *Ulva* sp., which grow in summer as do fish was reported. Following those methods, in the present study interrelation between  $p\text{O}_2$  and  $p\text{CO}_2$  in polyculture cages of red sea bream, *Pagrus major* and *Ulva* sp. at the fish culture farm of Azuma-cho Fish Seedling Center was investigated. Two cages, 7 m X 7 m X 7 m, each containing 1750 three years old fish weighting about 654 g were used in this experiment.  $p\text{O}_2$  and  $p\text{CO}_2$  were measured by Radiometer ABL-330. Considering the value of  $p\text{O}_2$  in the control cage as 100%,  $p\text{O}_2$  in the experimental cage stand 109.1%. On the other hand, average  $p\text{CO}_2$  in the control cage as 100%,  $p\text{CO}_2$  in the experiment cage stands 96.1%. From the results mentioned above, it may concluded that the  $p\text{CO}_2$  in the experimental cage decreased to 4% while  $p\text{O}_2$  augmented up to 9% compared to those in the control cage.

Kitadai, Y., and S. Kadowaki. 2004. The growth and N, P uptake rates of *Ulva pertusa* cultured in coastal fish farms. *Suisanzoshoku*, Vol:52. Pp 65–72. (in Japanese with English abstract)

Biological water purification by culturing *Ulva pertusa* in coastal fish farms was estimated as a function of light intensity, dissolved inorganic nutrient and water temperature. *Ulva pertusa* was cultured from 0.5 m to 8 m depths in coastal fish farms from August to November, 2002. The maximum growth of thallus area, wet weight and thickness were recorded at 0.5 m depth. The maximum growth of thallus area reached to 640 cm<sup>2</sup> on August. The maximum growth of thallus thickness reached to 0.11 mm on November. Average contents of total nitrogen (N) and total phosphorus (P) in *Ulva pertusa* reached to 35 mg N/g dry and 2.1 mg P/g dry in October, respectively. The maximum N, P uptake rates of *Ulva pertusa* were 3.6 mg N/m<sup>2</sup>/day and 0.19 mg P/m<sup>2</sup>/day at 0.5 m depth, respectively. Michaelis-Menten's constants of N, P uptake rates were 26 µg N/l and 8.6 µg P/l, respectively. Downward irradiance of 730 µmol/m<sup>2</sup>/s resulted in the maximum N, P uptake rates. N, P uptake rates of *Ulva pertusa* at 25°C were 2.5 mg N<sup>2</sup>/day and 0.13 mg P/m<sup>2</sup>/day, respectively. Water temperature coefficients of N, P uptake rates were 1.076 and 1.084, respectively. The N and P uptake rates of cultured *Ulva pertusa* were formulated from dissolved inorganic nutrients, light intensity and water temperature characteristics found in coastal fish farms, and the calculated values corresponded well with the observed values.

Hiraoka, M., S. Shimada, M. Uenosono, and M. Masuda. 2004. A new green-tide-forming alga, *Ulva ohnoi* Hiraoka et Shimada sp. nov. (Ulvales, Ulvophyceae) from Japan. *Phycological Research*, Vol:52. Pp 17–29.

*Ulva ohnoi* Hiraoka et Shimada sp. nov. (Ulvales, Ulvophyceae) is described from southern and western Japan and is characterized by the following combination of features: (i) the large, fragile, easily torn thalli, which are 30–55 µm thick in the upper and middle regions and often have microscopic marginal teeth; (ii) the production of zoids in the upper marginal region; (iii) a regular alternation of dioecious gametophytes and a sporophyte; (iv) the production of free-floating thalli from torn-off attached thalli, which reproduce vegetatively by fragmentation and form green tides in summer to autumn; (V) disorderly arranged cells that are polygonal or quadrangular in the upper and middle regions; and (vi) the chloroplast covering the outer face of cell, with 1–3 pyrenoids. *Ulva ohnoi* differs from *U. armoricana* Dion et al., *U. fasciata* Delile, *U. reticulata* Forsskål, *U. scandinavica* Bliding and *U. spinulosa* Okamura et Segawa, which all possess microscopic marginal serrations, in thallus shape, cell shape or life history pattern. It is also distinguished from morphologically similar species by sequences of the nuclear encoded internal transcribed spacers and the 5.8S ribosomal RNA gene and the plastid encoded large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase gene. Furthermore, crossing tests demonstrate that there is a reproductive boundary between *U. ohnoi* and the most closely related species, *U. fasciata* and *U. reticulata*.

Yokoyama, H. 2010. Monitoring, assessment and management of fish farm environments in Japan. *Reviews in Aquaculture*, Vol:2. Pp 1–12.

In Japan, coastal fish farming has become a well-established industry. Intensive cultivation of finfish, however, generates large amounts of organic waste and nutrients, resulting in

environmental deterioration in and around aquaculture facilities. Such negative effects have become increasingly conspicuous since the commencement of fish farming in the mid 1960s and its subsequent rapid development during the 1970s and 1980s. Various studies have attempted to measure the material fluxes in fish farms, to assess the potential environmental impacts, to determine criteria the optimization of the location of farms and production levels, and to establish methods for improvement of aquaculture environments. In this review, research conducted during the last five decades in Japan concerning the flux of feed, environmental impacts of fish farming, factors controlling the degree of impacts, the upper limit of production and methods for improvement of environments is reviewed and current concerns for aquaculture environments discussed.

## **INLAND MARINE FISH CULTURE IN LOW-SALINITY RECIRCULATING AQUACULTURE SYSTEM**

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A growing and increasingly health-conscious population, coupled with declining capture fisheries is driving an increased global demand for farm-raised seafood that can only be met through expansion of aquaculture. In 2007, aquaculture represented 33% of total global seafood production and is projected to increase to as much as 71% by 2030. The U.S. aquaculture industry represents a \$1.0 billion/year industry, but is principally based on production of freshwater finfish. In contrast, on a global scale marine aquaculture comprises one-third of farmed production, and cultivation of marine aquatic animals represents the fastest growing segment within aquaculture. This suggests tremendous potential for growth of a developing U.S. marine aquaculture industry. However, development and expansion of marine aquaculture is challenged by the high cost and limited availability of coastal land and water resources, effluent concerns, high production costs, restricted growing seasons, lack of quality seedstock, and inadequate regulatory and permitting processes. Many of these constraints can be addressed using inland marine fish culture in low-salinity recirculating aquaculture systems as a production model. We describe recent and ongoing development of technologies in four principal areas: 1) engineering and system design; 2) year-round production of seedstock; 3) diet development; and 4) physiological adaptation of marine fish to low-salinity environments using genomic approaches. It is anticipated these technologies could find application for rearing euryhaline marine fish throughout approximately 2/3 of the U.S. where lightly saline groundwater is available. Development of technologies for rearing marine species that can be adapted to low-salinity or freshwater environments in recirculating systems will reduce the need to be located near coastal land, reduce saltwater effluent, and reduce the carbon footprint of marine finfish production.

## **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**



Weirich, C.R., P.S. Wills, R.M. Baptiste, P.N. Woodward and M.A. Riche. 2009. Production characteristics and body composition of Florida pompano reared to market size at two different densities in low-salinity recirculating aquaculture systems. *North American Journal of Aquaculture* 71:165-173. doi:10.1577/A08-016.1

The effect of culture density on production characteristics and body composition of Florida pompano *Trachinotus carolinus* reared to market size using recirculating aquaculture systems (RASs) maintained at a salinity of 5‰ was evaluated in a 110-d growth trial (water temperature = 27.0–28.5 °C). Juvenile Florida pompano (mean weight = 259.0  $\pm$  3.0 g) were stocked into two tanks from each of four separate RASs at an initial nominal density of 6.5 (low density) or 13.0 kg/m<sup>3</sup> (high density), equivalent to 200 and 400 fish/tank, respectively. After stocking, fish were fed a 4.7-mm, floating pelleted diet (50% protein, 14% lipid) twice daily at a targeted daily feed rate of 3% body weight per day. At stocking and at 3-week intervals thereafter, 10% of the fish population of each tank was sampled to determine production characteristics, including mean weight, weight gain, specific growth rate, absolute growth rate (AGR), feed conversion efficiency (FCE), biomass, and survival. At termination, fish in all tanks were harvested to determine production characteristics and samples were obtained to determine whole-body composition, energy retention, protein efficiency ratio (PER), protein productive value (PPV), hepatosomatic index, gonadosomatic index, and fillet yield. Results indicated that final weight (570.0 g), weight gain (312.7 g), AGR (2.8 g/d), FCE (25.5%), PER (0.54), and PPV (11.5%) of fish reared at high density were significantly lower than those of fish reared at low density. No differences were observed between density treatments with respect to other measured variables. Additional studies must focus on the development of finishing diets for final stages of Florida pompano production to increase FCE and to determine optimal rearing densities. In addition, research to evaluate the potential of rearing pompano in RASs at salinities lower than 5‰, perhaps through environmental or dietary enhancement of critical ions, is warranted.

Riche, M. and T.N. Williams. 2010. Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in sea water and low-salinity water. *Aquaculture Nutrition* 16:223-230. doi:10.1111/j.1365-2095.2009.00654.x

Two experiments were conducted with Florida pompano, *Trachinotus carolinus* L. at 3 and 28 g L<sup>-1</sup> salinity to determine apparent crude protein digestibility (ACPD), energy digestibility (AED) and amino acid availability (AAAA) from soybean meal (SBM), soy protein isolate (SPI), and corn gluten meal (CGM). Mean AAAA was similar to ACPD. In fish adapted to 3 g L<sup>-1</sup> salinity, they were 81.2 and 81.9% (CGM), 93.6 and 92.2% (SBM), 93.8 and 93.1% (SPI) for AAAA and ACPD, respectively. In fish adapted to 28 g L<sup>-1</sup>, they were 84.5 and 83.4% (CGM), 86.5 and 87.1% (SBM), and 83.4 and 85.0% (SPI) for AAAA and ACPD, respectively. The AED was highest for SPI and lowest for SBM and inversely related to carbohydrate. The ACPD, AED, and AAAA of soy products appeared to be lower in high salinity, whereas CGM was unaffected. The data suggests SBM, SPI, and CGM should be further evaluated as partial fishmeal replacements in Florida pompano diets. Application of the generated coefficients can be used to develop well-balanced, low-cost diets for Florida pompano reared in low salinity or seawater.

Pfeiffer, T.J. and M. Riche. (In press). Evaluation of a low-head recirculating system used for rearing Florida pompano to market size. *Journal of the World Aquaculture Society*.

A low-head recirculating aquaculture system (RAS) for the production of Florida pompano, *Trachinotus carolinus*, from juvenile to market size was evaluated. The 32.4 m<sup>3</sup> RAS consisted of three dual-drain, 3-m diameter culture tanks of 7.8 m<sup>3</sup> volume each, two 0.71-m<sup>3</sup> moving bed bioreactors filled with media (67% fill with K1 Kaldness media) for biofiltration, two degassing towers for CO<sub>2</sub> removal and aeration, a drum filter with a 40 micron screen for solids removal, and a 1 hp low-head propeller pump for water circulation. Supplemental oxygenation was provided in each tank by ultrafine ceramic diffusers and system salinity was maintained at 7.0 g/L. Juvenile pompano (43 g mean weight) were stocked into each of the three tanks at an initial density of 1.7 kg/m<sup>3</sup> (300 fish/tank). After 306 days of culture the mean weight of the fish harvested from each tank ranged from 589 to 655 g with survival ranging from 57.7 to 81.7%. During the culture period the average water use per kg fish was 3.26 m<sup>3</sup> or 1.82 m<sup>3</sup> per harvested fish. Energy consumption per kg fish was 47.2 kwh or 22.4 kwh per harvested fish. The mean volumetric TAN removal rate of the bioreactors was 127.6 ± 58.3 g TAN removed per m<sup>3</sup> media/day with an average of 33.0% removal per pass. Results of this evaluation suggest that the system modifications are warranted to enhance production to commercial levels (>60 kg/m<sup>3</sup>).

Riley, K.L., C.R. Weirich and D. Cerino. 2009. Development and growth of hatchery-reared larval Florida pompano (*Trachinotus carolinus*). *Fishery Bulletin* 107:318-328.

Although the Florida pompano (*Trachinotus carolinus*) is a prime candidate for aquaculture, the problematic production of juveniles remains a major impediment to commercial culture of this species. In order to improve the understanding of larval development and to refine hatchery production techniques, this study was conducted to characterize development and growth of Florida pompano from hatching through metamorphosis by using digital photography and image analysis. Newly hatched larvae were transparent and had a large, elongate yolk sac and single oil globule. The lower and upper jaws as well as the digestive tract were not fully developed at hatching. Rotifers were observed in the stomach of larvae at three days after hatching (DAH), and *Artemia* spp. were observed in the stomach of larvae at 14 DAH. Growth rates calculated from total length measurements were 0.22 ± 0.04, 0.23 ± 0.12, and 0.35 ± 0.09 mm/d for each of the larval rearing trials. The mouth gape of larvae was 0.266 ± 0.075 mm at first feeding and increased with a growth rate of 0.13 ± 0.04 mm/d. Predicted values for optimal prey sizes ranged from 80 to 130 µm at 3 DAH, 160 to 267 µm at 5 DAH, and 454 to 757 µm at 10 DAH. Based on the findings of this study, a refined feeding regime was developed to provide stage- and size specific guidelines for feeding Florida pompano larvae reared under hatchery conditions. Cossins, A.R. and D.L. Crawford. 2005. Fish as models for environmental genomics. *Nature Reviews: Genetics* 6(4):324-333. doi:10.1038/nrg1590.

Genomic approaches using fish promise increased investigative power, and have already provided insights into the mechanisms that underlie short-term and long-term environmental adaptations. Environmental genomics explores how the genome interacts with and integrates cues from the environment to produce both the effects of environmental stress and adaptive responses to this stress. This approach can provide a description of genes involved in metabolic and osmoregulatory functions important for adaptation of Florida pompano and other euryhaline marine species to low-salinity culture conditions. These responses can then be correlated with phenotypic responses at the whole animal level; thus, providing greater insight into the ability of marine fishes in general, and Florida pompano in particular, to adapt to low salinity culture.

## **LANDBASED POLY-ECO-AQUACULTURE OF ABALONE AND SEAWEED IN THE SMALL SCALE RECIRCULATING SYSTEM USING THE RECYCLED FROZEN CONTAINER**

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To minimize environmental impacts, we wanted to develop a poly-eco-aquaculture of abalone and seaweed in the small scale recirculating aquaculture system housed in the recycled frozen container. The main objective was to develop a low cost, environment friendly and land-based integrated aquaculture with hybrid abalone (*Haliotis discus hannai* × *H. sieboldii*) and seaweed (*Ulva* sp.).

We conducted two experiments in two small-scale recirculating systems housed in a recycled frozen container (4.3×1.9×1.9 m). Each system consisted of two biofilters (100 and 200 L) and two abalone culture tanks (each 200 L). Each abalone culture tank contained three plastic baskets (each 50 × 34 × 6 cm, with 12 mm mesh). One of these systems also incorporated a protein skimmer (PS: 100 L).

An air-conditioner (2.8 kw/h) was used to maintain the desired water temperature ( $19.2 \pm 0.8^{\circ}\text{C}$ ). Water circulation was maintained at 43 L per minute. Artificial salt water was used instead of natural seawater. Salinity of water was 32 ppt. The stocking density of abalone was 20 individuals per basket. Pelleted artificial feed was supplied six days per week at 2.3% of abalone body weight per day. Temperature, salinity, dissolved oxygen, total ammonia nitrogen,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$  and bacterial abundance were regularly monitored.

In the first experiment,  $3.5 \pm 0.8$  cm (shell length) abalone were cultured to investigate the effects of protein skimming on water quality, abundance of bacteria and FCR and growth of abalone. Over an experimental period of 87 days, protein skimming resulted in significantly better water quality (lower total ammonia nitrogen,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and bacterial abundance) and abalone growth.

In the second experiment, a seaweed culture tank (100 L) of round and transparent with conical bottom was connected with the system with PS to observe the combined effects of protein skimming and seaweed (PSS) on water quality and abalone growth. In this experiment,  $4.0 \pm 0.2$  cm (shell length) abalone were cultured for 72 days. Seaweed (*Ulva* sp.) was stocked (20 g) in the seaweed culture tank at the beginning of the experiment and harvested 63~95 g per week. We observed significantly higher pH and lower TIN and TIP concentrations in system with PSS than the system without PSS. Bacterial abundance was significantly lower in system with PSS ( $4.1 \times 10^5$  CFU/mL) than the system without PSS ( $6.4 \times 10^5$  CFU/mL). Bacterial abundance in protein skimmer waste was  $2.2 \times 10^7$  CFU/mL. Growth rate of abalone in both length ( $60.0 \pm 8.0$   $\mu\text{m/day}$ ) and weight ( $63.6 \pm 15.5$  mg/day) was better in system with PSS than the system without PSS (length  $40.0 \pm 11.0$   $\mu\text{m/day}$  and weight  $33.8 \pm 7.3$  mg/day). FCR was observed better in system with PSS ( $1.45 \pm 0.45$ ) than the system without PSS ( $2.40 \pm 0.26$ ). The survival of abalone was similar between the system with PSS ( $96.3 \pm 2.5$  %) and without PSS ( $97.5 \pm 2.5$  %).

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Sugawara K., and S. Kadowaki. 2006. Landbased aquaculture system of abalone using the recycled frozen ship container. *Aquaculture Magazine* 555, 70-73.

The authors proposed landbased polyculture of abalone and seaweed using the recycled frozen ship container. They compared the complex poly-eco-aquaculture of abalone, seaweed and bacteria with conventional landbased aquaculture. In the new system, they maintained best abalone growth temperature ( $18 \pm 1.5$  °C) using low cost home air-conditioner. They mentioned that using seaweed to purify water for abalone is a new farming system for future. They achieved faster growth of hybrid (ezo abalone and megai abalone) abalone by supplying artificial feed. This type of aquaculture can supply safe seafood for consumers. They suggested this poly-eco-aquaculture for sustainable production of abalone.

Harris J.O., G.B. Maguire, S. Edwards, and S.M. Hindrum. 1998. Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, *Haliotis laevis* Donovan. *Aquaculture* 160, 259–272.

The authors assessed the chronic toxicity of unionised ammonia in terms of growth rate, food consumption rate and oxygen consumption rate for juvenile greenlip abalone, *Haliotis laevis*. They found that Juvenile greenlip abalone were sensitive to ammonia as indicated by depressed growth rate and food consumption. They observed that free ammonia nitrogen (FAN) concentrations exceeding  $0.031 \text{ mg L}^{-1}$  suppressed abalone (*Haliotis laevis*) growth rates, and FAN concentrations of  $0.041$  and  $0.158 \text{ mg L}^{-1}$  reduced abalone growth (whole weight basis) 5 (EC<sub>5</sub>) and 50% (EC<sub>50</sub>) respectively. Oxygen consumption rate increased to a maximum of 188% of control values at  $0.235 \text{ mg FAN L}^{-1}$  and decreased slightly at the highest concentration of  $0.418 \text{ mg FAN L}^{-1}$ .

Neori A., M.D. Krom, S.P. Ellner, C.E. Boyd, D. Popper, R. Rabinovitch, P.J. Davison, O. Dvir, D. Zuber, M. Ucko, D. Angel, and H. Gordin. 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture* 141: 183- 199.

In this article, Neori et al. examined the integrated culture of fish and seaweed on water quality in landbased mariculture system. They recirculated seawater between intensive fishponds and seaweed ponds. They observed that seaweed removed most of the ammonia excreted by the fish. Water-quality parameters (DO, NH<sub>4</sub>-N, pH and phosphate) remained stable and within safe limits for the fish during experimental period. Therefore, they concluded that their results provided a practical solution to major management and environmental problems of land-based mariculture.

## **TECHNIQUES FOR CURRENT CONTROL IN TANK; LESSONS LEARNED FROM REARING LARVAE OF RED SPOTTED GROUPE *Epinephelus akaara***

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Mass mortality of the early larval-stage (from hatch to about 10 days after hatching) has been one of the big constraints during the process of mass fry production in groupers (*Epinephelus bruneus*, *E. septemfasciatus*), bluefin tuna (*Thunnus orientalis*) or amberjacks (*Seriola dumerili*, *S. quinqueradiata*). A phenomenon associated with the mass mortality is observed that early stage larvae sink to and pile onto tank bottom, and the phenomenon, so called “sinking death syndrome”, has been supposed to be one of the causes of the mass mortality. Many marine fish appear to show ontogenetic change of larval body density, and it is speculated that body density and swimming activity of larvae and current condition of rearing tank mutually influence on the incidence of sinking death. It is indicated that proper current condition is important to prevent the sinking death syndrome, and aeration, submersible pump and/or wave-making device have been practically examined to make the current proper.

In the present study, rearing experiments were conducted in 60 m<sup>3</sup> tanks to investigate the effects of current control by aeration volumes for preventing the sinking death syndrome of red spotted grouper larvae. The aeration was done through four porous air hoses (1.5 m in length, 25 mm in diameter) that were set on the tank bottom along the lower edge of each wall. Larvae were reared different aeration rates (0.25-1.50 L/min/tube), and the distribution, feeding amount, survival and growth of larvae were determined. Larvae in the tank with aeration rate of 0.25 L/min/tube showed clearly the sinking performance to tank bottom. Larvae with aeration rate of 1.50 L/min/tube were not remarkably distributed in the lower layer, showing the best survival without any differences in growth and feeding amounts from larvae in the other treatments. Thus, the present results clearly show that physical environments such as water current inside tanks are crucial factors to the improvement of survival and consequently the establishment of stable/mass fry production technologies in groupers.

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Miyashita, S. 2006. Surfacing and bottoming death in seedling production. Nippon Suisan Gakkaisi, Vol: 72:5. Pp 947-948.

Surfacing death and bottoming death has been considered to the two big causes of early larval mortality in fry production of bluefin tuna. Surfacing death has been thought that due to the stress of aeration or surface tension, and the hypersecretion of mucus causes the loss of respiration and/or motor function of larvae. In order to prevent the surfacing death, surface of the rearing water is covered with feed oil. However, oil slick blocks the initial swim bladder inflation of larvae. On the other hand, bottoming death has been assumed that caused the death by injury at the bottom friction or invasion of bacillus. As the countermeasure to the bottoming death, improving the aeration method, water injection to the tank bottom or development of wave-making device has been trying to make proper water current.

Sakakura, Y., Shiotani, S., Chuda, H., and A. Hagiwara. 2006. Improvement of the survival in the seven-band grouper *Epinephelus septemfasciatus* larvae by optimizing aeration and water inlet in the mass-scale rearing tank. Fisheries Science, Vol: 72:5. Pp 939-947.

The water flow in rearing tanks has been indicated to cause mass mortality of the seven-band grouper larvae. Therefore, authors tried a new aerating method in the 100 m<sup>3</sup> volume rearing tank, in which an aerator was placed at the center of the tank surrounding cylindrical drain to generate the flow field. The survival rate at 10 days after hatching with the new aeration method (61.5 %) was approximately three times higher than the previous methods, in which several aerators were located in the tank (21.2%). The flow environment of tanks was also investigated by quantifying the flow field, and the relationship between the flow field in the rearing tank, behavior of larvae and survival discussed.

Hirata, Y., Hamasaki, K., Teruya, K., and K. Mushiake. 2009. Ontogenetic changes of body density of larvae and juveniles in seven-band grouper *Epinephelus septemfasciatus* and kelp grouper *Epinephelus bruneus*. Nippon Suisan Gakkaisi, Vol: 75:4. Pp 652-660.

Authors investigated the body density of larvae and juveniles of seven-band grouper and kelp grouper to figure out the period when the larvae and juveniles sinking the rearing tank bottom during the fry production. As the results, larvae of both species keep the nearly neutral buoyancy from hatching to growing 3.5-3.9 mm in TL when the buds of the second dorsal and pelvic fin spines appeared. However, body density of both species greatly increased with increasing the length of fin spines and reached plateau at 1.060-1.070 g/cm<sup>3</sup> after the size of around 11 mm TL. Therefore, authors concluded that larvae of both groupers tend to sink to easily bottom of the rearing tank at the initiation of great increase in body density when fin spines begin to elongate.

## **POND-TO-PLATE ANALYSIS OF THE US FARM-RAISED CATFISH INDUSTRY**

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US produced catfish and sales in the US have been declining from 662 million pound of processed catfish in 2003 to 466 million pounds in 2009, while during the same period imports of tilapia, basa, tra and channel catfish were increasing. Several reasons have an impact on this situation, including lower import fish prices, increasing domestic feed/fuel prices, inefficiencies in US catfish production, and quality inconsistencies in domestically produced products. It has been recognized by many US catfish industry representatives that reactive policies and actions of the past have failed to revive the industry and a pro-active focus on industry improvement at all levels is needed. Thus, the Pond-to-Plate project was initiated, with the goals of improving the competitiveness of the US farm-raised catfish industry and evolving it into a modern livestock industry. Auburn University's Fisheries Department has brought in LEAN trainers from the AU College of Business to assist in conducting Pond-to-Plate meetings in West Alabama.

This project uses the LEAN manufacturing and continuous improvement concept, and has been introduced at Pond-to-Plate meetings held in West Alabama. Each meeting includes participant representatives of the value chain, i.e., catfish producers, harvesters, transporters, processors, distributor and consumers. The LEAN enterprise produces more with existing resources by eliminating non-value-added activities. Manufacturers are facing increased worldwide competition and the stakes are high. The winners in this competition work to eliminate overproduction caused by traditional scheduling systems and to only make what customers want when they want it. Lean establishes a systematic approach to eliminate these wastes and create a flow throughout the whole company. It also helps companies develop and implement a long-term plan to streamline their operations for success. Training uses a hands-on approach involving a mixture of company's management and staff members. This approach was modified to address the fact that the US catfish industry is not one company but comprised of independent US catfish producers with few formal ties to processing plants.

Catfish Pond-to-Plate meetings have used Lean principals to address key issues of industry efficiency at each level of the value chain, increasing demand for catfish products, lack of product/value informational flow, final customer/consumer needs/desires, and non- or mis-aligned objectives, product quality needs, and incentives/rewards among value chain members to produce consistently high quality products. Meetings have resulted in articulated vision statements addressing the key issues identified and have focused on activities to reach their stated goals of increasing per capita consumption of US farm-raised catfish.

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Brown, T.W., J. A. Chappell and T.R. Hanson. 2010. Commercial Production of Channel Catfish *Ictalurus punctatus* and Hybrid Catfish *Ictalurus punctatus* x *Ictalurus furcatus* Utilizing an In-pond Raceway System in West Alabama. Abstract printed in the Book of Abstracts of the World Aquaculture Society 2010 meeting, March 1-5, San Diego, CA, USA: p. 142.

**ABSTRACT:** The aim of this project was to improve profitability by demonstrating methods to achieve high levels of feed performance, survival, and efficiency in a commercial farm setting. A commercial-scale modified In-Pond Raceway System (IPRS) was deployed in 2007 on a commercial fish farm in west Alabama. The IPRS was developed and installed in a 6-acre earthen pond with an average depth of about 5.5 feet. Six production cells (raceways) were constructed of concrete blocks on a reinforced concrete pad. Raceway dimensions are 16 feet wide by 37 feet long by 4 feet deep. They are arranged side by side and share common walls. Each cell is equipped with a ½ HP water mover (paddlewheel) at its upstream end which rotates at 1.2 RPM and allows for the exchange of water in each raceway as frequently as once every 4.9 minutes. Each raceway was originally stocked with 12,000 to 30,000 advanced stockers that weighted between 0.13 and 0.92 pounds to simulate a staggered stocking and harvest production method.

Results from the 2008 production season had an 84.3% mean survival across all raceways. The average feed conversion ratio (FCR) for channel catfish and hybrid catfish was 1.78 and 1.36 respectively (range from 1.16 - 2.11 to 1.0) and total catfish harvested was 109,808 lbs (18,301 lbs per acre). An additional 14,003 lbs of tilapia and paddlefish were harvested as co-cultured species (Table 1). IPRS improvements in survival, feed efficiency, disease management and overall production have increased yields, reduced unit production costs, and improved enterprise profitability over traditional multiple-batch pond production systems for catfish.

This paper is important as in-pond raceways increase the amount of control the aquaculturist has over catfish production compared to present open pond production. This can lead to efficiency improvement including lowering of the cost of production, and providing processors with the size fish consumer prefer, that is a market pull rather than a market push approach.



TABLE 1. In-Pond Raceway System - production and economic data for catfish, tilapia, and paddlefish.

<u>Species</u>	<u>Total weight stocked (lbs)</u>	<u>Total weight harvested (lbs)</u>	<u>Production (lbs/acre)<sup>2</sup></u>	<u>Survival (%)</u>	<u>FCR<sup>1</sup></u>	<u>Receipts (\$/lb)</u>	<u>COP (\$/lb)</u>	<u>Estimated Net Returns (\$/lb)</u>
Channel & Hybrid catfish	40,713	109,808	11,516	84.3	1.50	\$ 0.74	\$ 0.71	\$ 0.03
Tilapia	30	8,710	1,447	100.0	-	\$ 3.00	\$ 0.05	\$ 2.95
Paddlefish	<u>505</u>	<u>5,293</u>	<u>798</u>	85.9	-	\$ 2.50	\$ 0.20	\$ 2.30
<b>Subtotal (co-cultured fish)</b>	535	14,003	2,245					
<b>Grand Total (all fish)</b>	41248	123,811	13,761			\$ 0.98	\$0.63	\$ 0.35
<b><u>Projected models</u></b>								
All Channel catfish w/ co-cultured fish	14,562	94,255	13,282	78.7	1.78	\$ 1.07	\$0.66	\$ 0.41
All Hybrid catfish w/ co-cultured fish	22,187	153,367	21,863	89.7	1.36	\$ 0.96	\$0.53	\$ 0.43

<sup>1</sup>FCR cannot be calculated for tilapia and paddlefish as no direct feed was fed.

<sup>2</sup>Production, lb/acre, is based on the 8.0 acre pond area, not the raceway catfish production area.

Li, M.H., E.H. Robinson, D.F. Oberle, P.V. Zimba. 2007. Effects of Various Dietary Carotenoid Pigments on Fillet Appearance and Pigment Absorption in Channel Catfish, *Ictalurus punctatus*. Journal of the World Aquaculture Society, Dec., v. 38, no. 4. Pp. 557-563.

**ABSTRACT:** A study was conducted to evaluate effects of various carotenoids on skin and fillet coloration and fillet carotenoid concentration in channel catfish, *Ictalurus punctatus*. For 12 wk, juvenile catfish were fed one of six experimental diets containing no supplemental carotenoid or 100 mg/kg of one of following carotenoid additions:  $\beta$ -carotene (BCA), lutein (LUT), zeaxanthin (ZEA), canthaxanthin (CAN), and astaxanthin (AST). Visual yellow color intensity score was highest for fish fed LUT, followed by ZEA, AST, and CAN, and lowest for fish fed basal and BCA diets. Skin and tissue Commission Internationale de l'Eclairage yellowness value was the highest in fish fed LUT, followed by fish fed ZEA, AST, and CAN, and lowest for fish fed basal and BCA diets. Fish accumulated the supplemental carotenoids in muscle tissues, but concentrations of different carotenoids in the tissue varied greatly. Approximately 30% of the LUT added was converted to echineone; no conversion was observed among other supplemental carotenoids. Results from the present study indicate that channel catfish can accumulate yellow pigments LUT and ZEA and red or pink pigments CAN and AST in the flesh, resulting in yellow coloration. The yellow pigment BCA does not appear to deposit in skin or flesh at levels sufficient to alter the coloration.

This paper is important to our work as it addresses one of the key fillet quality issues, that is yellow pigmentation of fillets, which is considered a negative in some retail (grocery store) outlets. This paper identifies important carotenoids found in fish feed and pond algae that impart yellow coloration to fillets. An understanding of this may allow us to develop pond production practices or feed formulations that reduce the yellow coloration in catfish fillets.

Misimi, E., J.R. Mathiassen and U. Erikson. 2007. Computer Vision-Based Sorting of Atlantic Salmon (*Salmo salar*) Fillets According to Their Color Level. *Journal of Food Science*, Vol: 72:1. Pp S030–S035, Jan/Feb.

Keywords: Atlantic salmon; color; computer vision; processing line; quality control

**ABSTRACT:** Computer vision method was used to evaluate the color of Atlantic salmon (*Salmo salar*) fillets. Computer vision-based sorting of fillets according to their color was studied on 2 separate groups of salmon fillets. The images of fillets were captured using a digital camera of high resolution. Images of salmon fillets were then segmented in the regions of interest and analyzed in red, green, and blue (RGB) and CIE Lightness, redness, and yellowness (Lab) color spaces, and classified according to the Roche color card industrial standard. Comparisons of fillet color between visual evaluations were made by a panel of human inspectors, according to the Roche SalmoFan™ lineal standard, and the color scores generated from computer vision algorithm showed that there were no significant differences between the methods. Overall, computer vision can be used as a powerful tool to sort fillets by color in a fast and nondestructive manner. The low cost of implementing computer vision solutions creates the potential to replace manual labor in fish processing plants with automation.

This paper is important because of its highlighting how automatic sorting of fish fillets by color can be achieved and may be instrumental to catfish processing plants sorting their fillets by color to meet the different color needs/desires of their clients.

## **BENEFICIAL MEAT QUALITY INDUCED BY DIETS IN YELLOWTAIL**

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Yellowtail *Seriola quinqueradiata* is about 216,000 t for the amount of domestic products, and an important species with a large market scale in Japan. The cultivation production is 160,000 t and wild fish is 56,000 t. Thus, it is features that the proportion of the cultivation production is high in the yellowtail.

The hardness is important to many kinds of fish including yellowtail when eating with raw fish meat such as “sashimi”. The hardness of sashimi decreases contrary to progress of rigor mortis after slaughter. The color change of the red muscle of the yellowtail is caused comparatively fast. The discoloring red muscle remarkably ruins economic merit of the yellowtail. Therefore, maintenance of muscular hardness and delay of color change of the red muscle are important to raise commercial value of the yellowtail.

The meat quality of farmed yellowtail that was fed EP containing red pepper (experimental group) was compared with the same age yellowtail that was fed EP (control group). The hardness of ordinary muscle of the experimental group was significantly large compared with the control group. The color difference ( $\Delta E^*ab$ ) of red muscle was lower in the experimental than the control group. In the yellowtail that fed on red pepper EP, many items were high score in the sensory evaluation compared with the wild yellowtail. These results describe that dietary red pepper is able to raise muscular hardness and to slow down the color change in red muscle of the yellowtail.

**A MULTI-YEAR COLLABORATIVE EFFORT TO EVALUATE AND IMPACT OF ACCLIMATION CAGE CONDITIONING FOR STOCKING JAPANESE FLOUNDER, *Paralichthys olivaceus*, IN WAKASA BAY, JAPAN**

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Japan is the most active country worldwide with respect to flatfish stock enhancement both in the range of species and number of fish released<sup>1</sup>. In Japan, Japanese flounder, or hirame *Paralichthys olivaceus*, is the primary species represented in annual flatfish catch<sup>1</sup>; thus, hirame has been a paramount choice for both aquaculture and stock enhancement for decades, and is, in fact, the most important stocked marine finfish in Japan<sup>2</sup>. A total of approximately 25 million Japanese flounder are released yearly<sup>2</sup> from federal, prefectural (state), local and private hatcheries throughout the country.

Conditioning flatfish to the natural environment before release may increase successful recruitment to the fishery, as fish trained for "wild" conditions may transition more easily and successfully upon release<sup>3</sup>. Since 2008, Obama Station, National Center for Stock Enhancement, has conducted pre-release, experimental acclimation cage conditioning for Japanese flounder (N = 10,000-40,000) in both the Takahama and Obama portions of Wakasa Bay, Japan. Fish were reared via the "Hottoke shi-iku" method, a simplified rearing process that boosts cultivation efficiency of seedlings by reducing rearing time and manpower<sup>4</sup>. Recaptured fish were acquired through a cooperative effort between researchers and local fishermen (both commercial and recreational). To date, more conditioned fish have been recaptured via fishermen's catch than unconditioned fish. Initial observations suggest that non-feeding individuals recaptured near the release sites may be weaker and more likely to be caught by small boat beam trawl (towing speed 1-1.5 knots) than actively feeding, translocating fish. Thus, higher speed shrimp trawlers deeper in the bay (towing speed 3-3.5 knots) and set/fyke nets set kilometers away from the release sites may be better, non-biased indicators of fitness and intermediate stocking success.

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A vital resource - as it summarizes a considerable amount of Japanese literature that is only available in Japanese, especially federal and prefectural documents.

Yamashita Y, Aritaki M (2010) Stock enhancement of Japanese flounder in Japan. In: Daniels HV, Watanabe WO (eds.) Practical Flatfish Culture and Stock Enhancement. Blackwell Publishing, p 237-255.

This review takes a step back and regards the overall impact of stocking Japanese flounder in Japan. The authors compile data from many individual Japan-wide releases and compose "big picture" analyses of statistics such as market return rate and annual catch change rate.

Sparrevohn, C.R. Støttrup, J.G. 2007. Post release survival and feeding in reared turbot. Journal of Sea Research 57, 151-161.

The authors found that conditioning flatfish in predator free enclosures (i.e. acclimation cages) at the release site six days prior to release increased survival, burying behavior, and the ability to feed.

Takahashi Y (1998) Seed Production in Japanese Flounder by the Hottoke Method. Momoshima Station of the Japan Sea-Farming Association. Momoshima. Onomichi, Hiroshima 722-0061, Japan. 55 pp.

The author details "The Hottoke Method" – a low labor/high efficiency rearing strategy for culturing flounder juveniles. Steps to conduct the method are detailed and troubleshooting tips are provided.

## **ACOUSTIC CONDITIONING AND RANCHING OF BLACK SEA BASS** *Centropomus striata*

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Acoustic ranching consists of training fish to school to an area via a sound stimulus that is coupled with a food reward (classical Pavlovian conditioning). It may present an opportunity to raise fish with less environmental impact and at less expense than typical open-ocean fish farms. Some advantages include (i) low capital and operating costs to construct, install, and maintain a feeding and recapture station, (ii) low feed costs because fish have opportunities to forage on wild food as well as formulated diets, (iii) low impact on the environment due to natural dispersion of fish and their wastes, and (iv) the technology could aid stock replenishment efforts by weaning hatchery-raised fish from pelleted diets to fending for themselves in the wild.

This project represents the first attempt to farm marine fish with acoustic ranching in North America. In June 2008 we erected and installed an AquaDome<sup>TM</sup>, a 10 m wide by 5 m high geodesic dome in Buzzards Bay, Massachusetts. The AquaDome was fitted with a feeding tube, an underwater speaker and underwater cameras to monitor and record fish behavior. Approximately 5,000 tagged black sea bass (50 to 80g) were stocked into the AquaDome. The fish were trained in the cage by feeding them twice a day in tandem with a sound cue. Once the training was completed, some mesh on the AquaDome was replaced with 4-inch mesh so that the fish could swim out and set up residency on the nearby rocks.

We present results of laboratory and field trials including efforts to recapture these fish, evaluate their growth and measure the economics of this method of fish production.

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## **ARTIFITIAL COMPLETION OF THE JAPANESE EEL, *ANGUILLA JAPONICA*, LIFE CYCLE: CHALLENGE TO MASS PRODUCTION**

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Development and modification of slurry type diets made from shark-egg powder have enabled glass eel production in captivity by the group of Dr. Tanaka in National Research Institute of Aquaculture<sup>1-3)</sup>. Based on this success, recent investigations have specifically examined establishment of techniques for consistent mass production of glass eels. Now, there are many problems to settle in rearing eel larva, leptocephalus. The current procedures of feeding and maintenance are too troublesome for application to mass rearing. In addition, rearing period is too long and can not be correctly estimated. Moreover, limited supply of diet material, shark-egg, cannot meet mass production of glass eels. To overcome these problems, we suggested colloid-type diet and try to develop automatic system of rearing procedures. We also try to develop the diets made from new materials besides shark-egg. Then we succeeded shortening the rearing period from egg to glass eels. In our presentation, we would introduce these challenges to achieve mass production.

### **Annotated Bibliography of Key Works**

1) Tanaka, H., H. Kagawa, and H. Ohta. 2001. Production of leptocephali of Japanese eel, *Anguilla japonica*, in captivity. *Aquaculture* 201. 51-60.

Despite intensive research on wild and captive eels, no resource has so far provided access to all life cycle stages of the Japanese eel *Anguilla japonica*. The transition from the preleptocephalus, newly hatched larva, to the leptocephalus stage, typical leaf-like eel larva, has, therefore, remained the missing link in the eel life cycle. We recently found that a slurry-type diet made from shark egg powder is suitable feed for captive-bred eel larvae. The larvae were successfully reared with this diet in aquaria for 100 days and raised to 22.8 mm in total length, TL. Age, RL, and body proportions of the reared specimens overlapped with those of wild leptocephali. We revealed for the first time the transition from the preleptocephalus to the leptocephalus stage of the eel.

2) Tanaka, H., H. Kagawa, H. Ohta, T Unuma, and K. Nomura. 2003. The first production of

glass eel in captivity: fish reproductive physiology facilitates great progress in aquaculture. *Fish Physiology and Biochemistry* 28, 493-497.

Weekly injections of salmon pituitary extracts (SPE) were administered to female Japanese eel, *Anguilla japonica* at a dose of 20mg/fish. This induced vitellogenesis and caused oocytes to reach the migratory nucleus stage. Later, a majority of the females that received an injection of SPE at a priming dose, followed 24h later by 17,20 $\beta$ -dihydroxy-4-pregnen-3-one (DHP), ovulated 15 to 18h after the final injection. In cultivated males, repeated injections of human chorionic gonadotropin (hCG) at a dose of 1 IU/g BW/week induced spermatogenesis and spermiation. Since potassium ions were revealed to be an essential constituent for the maintenance of motility in the eel spermatozoa, artificial seminal plasma containing KCl was designed as a diluent of milt, and enabled the preservation of milt for several weeks in refrigeration. As a result, artificial fertilization performed immediately after ovulation with pre-diluted and stocked milt consistently resulted in the production of high-quality gametes. Recently, a slurry-type diet made from shark egg yolk has been found to be a suitable feed for captive-bred eel larvae. Although preleptocephalus larvae can be reared with this diet beyond the depletion of their yolk and oil droplet stores, it remains inadequate because larvae reared under this way cannot be raised to the following stage. Therefore, the diet was improved by supplements of krill hydrolysate, soybean peptide, vitamins and minerals. Larvae fed on this new diet have grown to 50 to 60 mm in total length (TL), and have begun to metamorphose into glass eels approximately 250 days after hatching.

3) Kagawa, H., H. Tanaka, H. Ohta, T. Unuma, and K. Nomura. 2005. The first success of glass eel production in the world: basic biology on fish reproduction advances new applied technology in aquaculture. *Fish Physiol. Biochem.* 31, 193-199.

The eel has long been esteemed as an important food fish in the world, especially in Japan, and has been used as an experimental fish for many fields of fish physiology. However, the decreases in eel resources have been a serious concern in recent years. The catches of glass eels as seedlings for aquaculture have shown a long-term decrease in both Europe and East-Asia. To increase eel resources, the development of techniques for artificial induction of maturation and spawning and rearing their larvae have been eagerly desired. Recent progress of reproductive physiology of fish, especially mechanisms of oocyte maturation and ovulation in female and of spermatozoa maturation in male, facilitate to establish techniques for hormonal induction of maturation and spawning in sexually immature eels. With persistent effort to development of rearing techniques of larvae, we have first succeeded to produce glass eel. These applied techniques are may contribute to understand the basic reproductive physiology of the eel.



## **SPAWNING AND LARVAL REARING OF YELLOWTAIL AMBERJACK (*SERIOLA LALANDI*) IN SOUTHERN CALIFORNIA**

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Hubbs-SeaWorld Research Institute (HSWRI) has been rearing yellowtail amberjack (*Seriola lalandi*), known locally as California yellowtail, at its laboratory in San Diego since 2003. The broodstock have been spawning naturally in a 140 m<sup>3</sup> maturation pool since 2002. The sex ratio has been close to 1:1 and spawning typically occurs between 16.5 and 22.0°C (April thru August). The number of adults has ranged from 21 to 35 and associated individual weights from 8.2 to 20.8 kg. Our primary egg quality measures have shown inconsistent spawn quality within a spawning season, with hatch rates ranging from 10 – 90% and survival to first feeding ranging from 5 – 80%. Typically the highest quality spawns are seen early in the season. The numbers of spawning events and eggs per spawn have increased in recent years due to increasing female biomass and improvements to the broodstock feeding regime.

Recent research results on yellowtail have shown no significant differences in growth or survival at 10 dph among egg stocking densities of 50, 100, and 200 eggs per L. Greenwater was demonstrated to be beneficial to larvae up to 16 dph as was 24hr light and high light intensity of 5,000 to 13,000 lux. There were no significant differences in growth or survival when yellowtail larvae were fed at rotifer densities of 15, 30, and 45 rotifers per ml. Larvae were able to consume 1<sup>st</sup> instar *Artemia* (*Artemia franciscana*) as early as 6 dph. Finally, we determined that greenwater rearing conditions created using either SanoLife ALG (INVE), algae paste, or live algae all produced similar results.

Our current culture protocols for production include 1) stocking eggs at a density of 100 per L; 2) maintaining water temperature at 21.0 – 22.0 °C; 3) providing rotifers (*Brachionus rotundiformis*) from 2 to 9 dph at 20 per ml; 4) moving larvae from tall, narrow 1600L egg incubators to shallow, wide 8,000L tanks at 10 dph; 5) providing 1<sup>st</sup> instar *Artemia* (*Artemia franciscana*) from 6 to 10 dph and 2nd instar *Artemia* from 10 to 35 dph; 6) providing 24hr light at 5,000 to 13,000 lux above each tank; and 7) greening the water using SanoLife ALG from 2 to 17 dph.

These procedures yielded survival rates from egg to juvenile of up to 5% with a relatively high degree of variability. High larval mortality, from unidentified causes at around 17 - 20 dph and deformity rates as high as 40% are focal areas for improvement through future research.

### **Annotated Bibliography of Key Works**

Benetti, D. 1997. Spawning and larval husbandry of flounder (*Paralichthys woolmani*) and Pacific yellowtail (*Seriola mazatlanica*), new candidate species for aquaculture. *Aquaculture* 155, 307-318.

This paper describes the spawning and larval rearing of two new species for aquaculture in Ecuador. Experimental production of these species has been conducted for the first time from eggs in captivity. Survival of *Seriola* throughout larval rearing ranged from 0 – 70%, however high mortalities caused by diseases, cannibalism, and weaning onto artificial diets during and after metamorphosis reduced average survival rates through the juvenile stage to less than 1%. The basic broodstock and larval rearing information provided by the author is a good starting point for the culture of either *Paralichthys* or *Seriola* culture.

Moran, D., C.K. Smith, B. Gara, C.W. Poortenaar. 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). *Aquaculture* 262, 95-104.

The authors describe the spawning behaviour of wild caught brood stock as well as early egg and larval development of yellowtail kingfish (*Seriola lalandi*). Spawning occurred naturally when water temperature was above 17 C. Courtship behaviour involved one male and female, and consisted of a high-speed pursuit punctuated by stalling, nipping and touching. This lasted for approximately 0.5 – 1.5 h until, immediately prior to spawning, the male would nip at the female gonoduct, presumably to induce spawning. Spawning occurred in the early daylight hours at the start of the spawning season, but shifted to around dusk in the latter part. Spawned eggs were positively buoyant, had a high fertilization rate (>99%), ranged 1.33 – 1.50 mm in diameter with a single oil droplet 0.30 – 0.33 mm diameter. Information given in this paper is indicative of a healthy spawning population of *S. lalandi* and can be used as a reference or baseline for culture of this species.

Papandroulakis, N., C.C. Mylona, E. Maingot, P. Divanach. 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. *Aquaculture* 250, 155-161.

The authors apply the mesocosm method for larval rearing to the greater amberjack (*Seriola dumerili*). This paper gives a good description of larval rearing for *Seriola* in terms of weaning schedule, turbidity levels, and live feed levels. Along with the description of the rearing protocols, growth information is provided which is a good template for most *Seriola* species.

Sakakura, Y. and K. Tsukamoto. 1999. Ontogeny of aggressive behaviour in schools of yellowtail, *Seriola quinqueradiata*. *Environmental Biology of Fishes* 56, 231-242

The authors studies ontogenetic changes in social interactions, especially in aggressive behaviour of the yellowtail, *Seriola quinqueradiata*, and compared these to morphological and physiological changes. No agonistic interactions were observed during the larva period until 10 mm in total length (TL) at approximately 20 days after hatching. Typical shivering behaviour with 'J-posture' was observed during metamorphosis, when fin rays and calcification of vertebra were completed and there was an increase of tissue thyroid hormone. The onset of aggressive behaviour was just after metamorphosis to the juvenile period, and coincided with a significant increase in tissue cortisol levels. The onset of schooling behaviour was at 12 mm TL, slightly after the onset of aggressive behaviour. The authors provided a good basis for determining aggressive behaviour in *Seriola quinqueradiata* which can be used as a model for other *Seriola* species. This paper also presents possible solutions to aggressive interactions found in the culture environment.

Yoseda K., K. Yamamoto, K. Asami, M. Chimuea, K. Hashimoto, S. Kosaka. 2008. Influence of light intensity on feeding, growth, and early survival of leopard coral grouper (*Plectropomus leopardus*) larvae under mass-scale rearing conditions. *Aquaculture* 279, 55-62.

This study investigated the effect of different light intensities on feeding, growth, and survival of early stage leopard coral grouper *Plectropomus leopardus* larvae. Four different light intensities (0, 500, 1000, and 3000 lx) were used and larvae were kept under constant light conditions from 0 day after hatching (DAH) to 5 DAH. The results indicate that coral grouper larvae are visual feeders and their food intake increases with increasing light intensity and that light intensity is the factor affecting larval feeding, growth, and survival. The authors provide a sound experimental design and interesting results which can translate to any species whose larvae are visual predators. The comprehension of optimization of environmental rearing conditions such as light intensity as it relates to first feeding larvae is necessary in establishing stable mass-scale rearing technology for any species.

**A CHALLENGE TO TECHNOLOGY DEVELOPMENT OF THE YEAR ROUND EGG PRODUCTION IN KAN-PACHI AMBERJACK *SERIOLA DUMERILI*: PROPOSAL OF NEW SYSTEMATIZED AQUACULTURE**

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Recently, the demand for marine products has rapidly increased due to consumption expansion of marine products especially in developing countries in Southeast Asia and to global health-oriented request. However, the natural fishery resources show a decreasing tendency, and it is forecasted that marine food products will become greatly shortage in the future. Under this situation, the role and development of aquaculture that can supply secure and reliable marine products to consumers are and will be more and more essential for future.

For further development of aquaculture industry, both advanced seed production and, grow-out technologies (comprehensive systematized aquaculture) should be cooperatively established for meeting precisely and timely the needs from farmers to tables.

In the present paper, we will introduce a series of our trials on the year round egg production and so on in Kan-Pachi amberjack *Seriola dumerili*, an international species, as a model of new systematized aquaculture that we have challenged.

**SABLEFISH HATCHERY METHODS. AN OVERVIEW OF HATCHERY DESIGN, BROOD STOCK SPAWNING, AND EGG AND YOLK SAC LARVAE INCUBATION PROCEDURES AT THE MANCHESTER RESEARCH STATION**

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The sablefish (*Anoplopoma fimbria*) is a deep-water finfish species native to the north Pacific. They range from the Bering Sea, and adjacent waters from Hokkaido, Japan to Baja, California. Due to its high value and culture potential it has generated great interest as an aquaculture species. As with many marine fish the major bottleneck in culture is the production of juveniles. Currently, there are only a couple of commercial hatcheries producing juveniles in the US and Canada.

In this presentation, an overview of our sablefish hatchery, brood stock spawning and egg and yolk sac incubation methods are described. The hatchery system utilizes recirculated seawater and has precise temperature and salinity control. The water is filtered to 1 micron and UV sterilized before being introduced to the incubators or yolk sac silos.

Ultrasound technology is used to determine brood stock maturation status and timing of ovulation. Hormone injections are needed to induce final maturation in most cases. Hormones are administered at an oocyte diameter of 1.2 mm or greater. With the use of ultrasound technology a biopsy is no longer needed to determine oocyte diameter.

Improvements in egg and yolk sac larval incubation techniques have significantly increased survival during these stages. Key factors during incubation are maintaining hatchery water temperatures at  $6.0 \pm 0.5$  degrees Celsius and salinity at  $33 \text{ ppt} \pm 0.5 \text{ ppt}$ . Additional egg and yolk sac larval incubation methods are also discussed.

## **DEVELOPMENT OF A VISUALIZING METHOD FOR PHYSICAL INJURY TO FISH LARVAE**

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Groupers are new target species for aquaculture and stock enhancement in Japan because of their high commercial value. Early mortality is, however, a critical problem in larviculture of groupers. Early mortality of fish larvae is potentially caused by various factors: for example poor egg quality, problems related to first feeding success of larvae, quality of live feeds, disease and surface tension related death. Furthermore, physical injury to larvae is believed to be one of the critical factors causing early mortality. It is thought that physical injury occurs through collision with the fine net over the drain outlet, wall of rearing tank and the bubbles and turbulence due to aeration, and also through collisions within aggregations of larvae. However there are no reports proving those, because there is no method to evaluate physical injury in larvae. Here, we attempted to develop a method for visualizing whether physical injury occurs during larviculture and its prevalence. As an approach to overcome this, we searched for reagents that can be used to identify artificial physical injuries on the epidermis of a variety of larvae including groupers caused by a sharp glass needle used in microinjection. We checked about thirty kinds of reagents and antibodies for visualizing factors related to apoptosis, necrosis, wound healing, and change in cell membrane permeability: for example, acridine orange, DAPI, propidium iodide, Evans blue, TIMP, MMP, c-Jun, DiI, and DiO. As a result, we found five reagents for detecting artificial physical injury. To detect physical injury, considering the reagent's sensitivity, toxicity to larvae and ease of handling, of five reagents which were determined as suitable, one was found to be optimal. In addition, we could quantify the degree of physical injury using the reagent. In conclusion, we could develop a method to visualize and quantify physical injuries in larvae. We think that this method can answer the question of whether physical injury is a key cause of mortality in larviculture or not. Further, this method has potential to contribute greatly to maintaining a preferable environment for larvae in larviculture of not only groupers but other kind of fishes.

## **Program of Poster Presentations**

The 39<sup>th</sup> Scientific Symposium of UJNR Aquaculture Panel

Kagoshima University Inamori Hall, Kagoshima

11:30-14:00 October 26<sup>th</sup>, 2010

1. Source and Suitability of Adult Jack Mackerel (*Trachurus japonicus*) for Induction of Maturation and Spawning  
Mitsuo Nyuji (Kyushu University)
2. Captive Reproduction of Chub Mackerel : Experimental System and Analysis of Neuroendocrine Hormones  
Sethu Selvaraj (Kyushu University)
3. Characterization of a Newly Identified Anti-lipopolysaccharide Factor (ALF2) Gene in Kuruma Shrimp, *Marsupenaeus japonicus*  
Shogo Okugawa (University of Miyazaki)
4. Characterization of Class B Scavenger Receptor from Kuruma Shrimp *Marsupenaeus japonicus*  
Raja Sudhakaran (University of Miyazaki)
5. Identification and Characterization of Dicer2 Gene in Kuruma Shrimp, *Marsupenaeus japonicus*  
Jun-ichi Nishi (University of Miyazaki)
6. Myxosporean Parasites of Four Economic Grouper Species; Wild Life and Culture, from Thailand  
Kittichon U-taynapun (Prince of Songkla University, Thailand, Univ. Miyazaki)
7. Astakine, an Ancient Cytokine, in Kuruma Shrimp: RNA Expression and Sequence Analysis of *Mjastakine* Gene  
Mari Inada (University of Miyazaki)
8. Sequence and Expression Analyses of c-Jun N-terminal Kinase (JNK) Gene in Kuruma Shrimp *Marsupenaeus japonicus*  
Maki Yoshimine (University of Miyazaki)
9. Community Structure, Seasonal Changes and Temperature Tolerance of Four *Sargassum* Species (Fucales) at the Boundary Zone between Temperate and Subtropical Region of Japan, in Relation to Climate Changes  
Yutaro Tsuchiya (Kagoshima University)
10. Community Structure, Seasonal Changes and Temperature Tolerance of Edible Seaweed, *Sargassum fusiforme* (Fucales), at the Boundary Zone between Temperate and Subtropical Region of Japan, in Relation to Climate Changes  
Yuhei Amano (Kagoshima University)

11. Assessment of Effects of Starvation on Freshwater Carp by  $^1\text{H}$ -NMR Metabolomics Approach  
Emiko Kokushi (Kagoshima University)
12. Molecular Identification of *Anisakis simplex* from Fishes Caught along the Coast of Kagoshima and Human Gastric Anisakiasis  
Hiroshi Kita (Kagoshima University)
13. Immune Response against *Edwardsiella tarda* in Ginbuna Crucian Carp (*Carassius auratus langdorfii*)  
Masatoshi Yamasaki (Kagoshima University)
14. Effect of Soy Peptide Replacement Level on Growth Performance and Digestive Enzyme Activity in Juvenile Japanese Flounder, *Paralichthys olivaceus*  
Roger E.P. Mamauag (Kagoshima University)
15. Effects of Polychaete Extracts on Reproductive Performance and Tissue Lipid Compositions of Kuruma Shrimp, *Marsupenaeus japonicus* Bate  
Binh Thanh Nguyen (Kagoshima University)
16. Effect of Complete Replacement of Fishmeal with a Composite Mixtures of Soybean Meal and Sea Food Processing By-products on the Performances of Red Sea Bream, *Pagrus major*  
Md. Abdul Kader (Kagoshima University)
17. Effects of Different Supplementation Levels of Extracted Soy Peptide on Growth Performance and Tolerance to High Temperature Stress in Juvenile Japanese Flounder, *Paralichthys olivaceus*  
Janice A. Ragaza (Kagoshima University)
18. Effect of Dietary Oxidized Fish Oil with Vitamin E Supplementation on Growth Performance and Reduction of Oxidative Stress in Red Sea Bream (*Pagrus major*)  
Jian Gao (Kagoshima University)
19. Sediment Characterization and Remediation of Shiraishi Lake Oyster Farm  
Sheila Mae Santander (Kagoshima University)
20. Effect of ATP and IMP on the Quality of Fish and Fish Products  
Yuko Murata (National Research Institute of Fisheries Science, FRA)
21. How Do We Reduce the Impact of Global Warming on Aquaculture?  
— An Introduction to Projects to Develop Methods to Counter the Impacts of Global Warming —  
Tadashi Andoh (Hokkaido National Fisheries Research Institute, FRA)



## **SOURCE AND SUITABILITY OF ADULT JACK MACKEREL (*TRACHURUS JAPONICUS*) FOR INDUCTION OF MATURATION AND SPAWNING**

Mitsuo Nyuji<sup>1</sup>, Kazuki Fujisawa<sup>1</sup>, Hajime Kitano<sup>1</sup>, Mari Yoda<sup>2</sup>, Michio Yoneda<sup>3</sup>, Akio Shimizu<sup>3</sup>, Akihiko Yamaguchi<sup>1</sup>, and Michiya Matsuyama<sup>1\*</sup>

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The jack mackerel, *Trachurus japonicus*, is one of the most important commercially exploited fish. In response to increasing demand for this species, aquaculture of jack mackerel has recently commenced in southwestern regions of Japan. To stabilize its supply, it is necessary to develop techniques for the aquaculture of jack mackerel including seed production. The captive maintained adult jack mackerel do not spawn spontaneously. Our research group has examined the techniques for inducing final oocyte maturation (FOM), ovulation and spawning by hormone treatment in order to obtain high-quality eggs. In this presentation, we have compiled the information about how to obtain adult female jack mackerel for inducing FOM and spawning.

Generally, adult female fish possessing fully grown vitellogenic oocytes are selected for hormonal induction of FOM and spawning. In our study, using gonadal biopsy, we measured oocyte diameter, and observed the position of germinal vesicle. The jack mackerel possessing oocytes of over 500 µm diameter were determined to be suitable females as it indicated the completion of vitellogenesis. Further, to observe the position of nucleus using light microscopy, oocytes were cleared by Sera solution. When the nucleus was not clearly observed, including fish showing oocytes of over 500 µm diameter, those fish were not selected as it raised the possibility of start of atresia in the oocytes.

All adult jack mackerel used in this study were over 2 year old and caught from the wild. They were used for induced maturation experiment with the following set up in captive conditions: jack mackerel 1) reared for 2-3 months in sea pens, 2) reared for over a year in 3-ton tanks, 3) used soon after caught by purse seining and 4) used soon after caught by hook and line fishing. The results obtained from the above set up were as follows: 1) only 20 suitable females were obtained among 125 fish (about 50% for each sex), 2) a total of 285 adult fish were sampled regularly from rearing tanks. However, vitellogenic ovaries were observed in only four fish in June and July, and there were no fish with oocyte of over 500 µm diameter\*, 3) the majority of females showed atretic oocytes\*\*, 4) all fish were suitable and therefore, 4 females and 5 males were used for GnRH $\alpha$  (des-Gly<sup>10</sup>-[D-Ala<sup>6</sup>] LH-RH ethylamide, 400 µg/kg BW) injection. GnRH $\alpha$ -injected fish spawned for 18 consecutive days in a 3-ton tank. The number of eggs collected after each spawning was 145,000 on the 1st night, 92,000 on the 2nd night and 4,000-33,000 on the other days.

In conclusion, the results revealed that stress during fishing and captive rearing conditions can influence the progression of vitellogenesis in female jack mackerel. Further, it indicates that adult fish caught by low-stress fishing method, such as hook and line, can be effectively used for inducing FOM and spawning.

### References

- \*Nyuji M., Shiraishi T., Irie S., Nii S., Kitano H., Yamaguchi A., Matsuyama M. 2008. Annual changes in the gonadal histology of captive reared jack mackerel, *Trachurus japonicus*. Sci. Bull. Fac. Agr., Kyushu Univ. 63: 115-123 (in Japanese with English abstract).
- \*\*Yoda M., Mizuta K., Matsuyama M. 2006. Induction of ovarian maturation and ovulation in jack mackerel *Trachurus japonicus* by human chorionic gonadotropin. Bull. Fish. Res. Agen. 16: 15-18 (in Japanese with English abstract).

## **CAPTIVE REPRODUCTION OF CHUB MACKEREL: EXPERIMENTAL SYSTEM AND ANALYSIS OF NEUROENDOCRINE HORMONES**

Sethu Selvaraj<sup>1</sup>, Tetsuro Shiraishi<sup>1</sup>, Hajime Kitano<sup>1</sup>, Masafumi Amano<sup>2</sup>, Mitsuo Nyuji<sup>1</sup>, Hirofumi Ohga<sup>1</sup>, Akihiko Yamaguchi<sup>1</sup>, Michio Yoneda<sup>3</sup>, Akio Shimizu<sup>3</sup>, and Michiya Matsuyama<sup>1\*</sup>

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The chub mackerel (*Scomber japonicus*) belongs to the family Scombridae and is a key multiple batch-spawning pelagic fish contributing to global marine fish production. Due to unreliable and unpredictable wild catches, aquaculture of this species commenced recently in southwestern Japan. Young or adult fish are caught from the wild using purse-seine and reared for an appropriate duration in sea pens. This captive maintained male fish undergo normal spermatogenesis but female fish after the completion of vitellogenesis fails to undergo final oocyte maturation (FOM) and ovulation during spawning season (April-June).

The brain-pituitary-gonad (BPG) axis is a key neuroendocrine system involved in the reproductive processes, and gonadotropin-releasing hormone (GnRH) represents a key upstream signaling molecule in this system. Brain GnRH stimulates the synthesis and release of the pituitary gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which regulate the synthesis of sex steroids that are responsible for gonadal growth and maturation. In recent years, kisspeptins, encoded by the *Kiss1* gene have emerged as novel upstream regulator of GnRH secretion. Reproductive studies in chub mackerel is focused on understanding the role of different components of the BPG axis.

Our previous studies revealed the role of pituitary GtHs in reproductive cycle and steroidogenic pathways in the ovarian follicles of chub mackerel. However, the role of other upstream regulators such as brain kisspeptins and GnRHs has not yet been elucidated. As a first step, we isolated cDNAs encoding *Kiss* and *GnRH* forms and studied their quantitative changes in the expression of *Kiss* and *GnRH* mRNA in the brain and corresponding GnRH peptides in the brain and pituitary of females during different ovarian stages. Chub mackerel express two *Kiss* (*Kiss1*, *Kiss2*) and three GnRH forms (seabream GnRH, sbGnRH; chicken GnRH-II, cGnRH-II; salmon GnRH, sGnRH). *Kiss1* levels in brain did not vary significantly among the different ovarian stages. However, *Kiss2* expression declined from the immature stage (November) to the post-spawning (PS) season (August). Brain *sbGnRH* mRNA and corresponding peptide levels showed significant changes only during PS season; however, pituitary peptide levels increased gradually from the immature stage onward, peaking during late vitellogenesis (April) and the PS season. No significant differences in *cGnRH-II* mRNA or corresponding peptide levels were found. Likewise, brain *sGnRH* mRNA levels did not show significant changes; however, corresponding peptide levels in the brain and pituitary rose significantly during late vitellogenesis and the PS season, respectively. In conclusion, expression profiles of *Kiss2* mRNA in the brain and sbGnRH peptide in the pituitary suggest their possible involvement in the seasonal ovarian development of chub mackerel in captivity.

## Annotated Bibliography of Key Works

1. Selvaraj S., H. Kitano, Y. Fujinaga, H. Ohga, M. Yoneda, A. Yamaguchi, A. Shimizu, and M. Matsuyama. 2010. Molecular characterization, tissue distribution, and mRNA expression profiles of two *Kiss* genes in the adult male and female chub mackerel (*Scomber japonicus*) during different gonadal stages. Gen. Comp. Endocrinol. DOI:10.1016/j.ygcen.2010.07.011.

The full-length cDNAs of *Kiss1* and *Kiss2* in the chub mackerel were cloned and sequenced. Chub mackerel *Kiss1* and *Kiss2* cDNAs encode 105 and 123 amino acids, respectively. A comparison of the deduced amino acid sequences of chub mackerel *Kiss1* and *Kiss2* with those of other vertebrate species showed a high degree of conservation only in the kisspeptin-10 region (Kp-10). The Kp-10 of chub mackerel *Kiss1* (YNFNSFGLRY) and *Kiss2* (ENFNPFGRLR) showed variations at three amino acids. The mRNA expression profiles of *Kiss1* and *Kiss2* in the brain, pituitary, and gonads at different gonadal stages suggested the possible involvement of two *Kiss* genes in the brain and *Kiss1* in the gonads of chub mackerel during seasonal gonadal development.

2. Selvaraj S., H. Kitano, Y. Fujinaga, M. Amano, A. Takahashi, A. Shimizu, M. Yoneda, A. Yamaguchi, and M. Matsuyama. 2009. Immunological characterization and distribution of three GnRH forms in the brain and pituitary gland of chub mackerel (*Scomber japonicus*). Zool. Sci. 26: 828-839.

This study reports on the presence of three GnRH forms in the brain of the chub mackerel, *Scomber japonicus*, namely, salmon GnRH (sGnRH), chicken GnRH-II (cGnRH-II), and seabream GnRH (sbGnRH), as confirmed by combined high performance liquid chromatography (HPLC) and time-resolved fluoroimmunoassay (TR-FIA). Immunocytochemical localization of the three GnRH forms in the brain was investigated by using specific antisera, to elucidate possible roles of each GnRH form in reproduction in this species, and double immunolabeling was used to localize GnRH-ir (immunoreactive) fibers innervating the pituitary. The results of the study indicated that multiple GnRH forms serve different functions, with sbGnRH having a significant role in reproduction in stimulating FSH- and LH-producing cells, and sGnRH and cGnRH-II serving as neurotransmitters or neuromodulators.

3. Shiraishi T., S.D. Ketkar, H. Kitano, M. Nyuji, A. Yamaguchi, and M. Matsuyama. 2008. Time course of final oocyte maturation and ovulation in chub mackerel *Scomber japonicus* induced by hCG and GnRHa. Fish. Sci. 74: 764-769.

The question of whether the ovulation and spawning time in chub mackerel *Scomber japonicus* is entrained by a circadian rhythm was tested by administering human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone analogue (GnRHa) at two 'opposite' times, 14:00 and 02:00 hours, and the time courses of FOM and ovulation were compared. When hCG was injected, ovulation occurred 33 h post-injection in both groups. Similarly, ovulation began at 36 h post-injection of GnRHa, regardless of the timing of injection. These results indicated that timing of ovulation in chub mackerel depends on the timing of luteinizing hormone (LH) stimulation.

4. The following are published reports of our reproductive studies in chub mackerel:

- Matsuyama M., T. Shiraishi, J.K. Sundaray, M.A. Rahman, K. Ohta, and A. Yamaguchi. 2005. Steroidogenesis in ovarian follicles of chub mackerel, *Scomber japonicus*. Zool. Sci. 22: 101-110.
- Shiraishi T., K. Ohta, A. Yamaguchi, M. Yoda, H. Chuda, and M. Matsuyama. 2005. Reproductive parameters of the chub mackerel *Scomber japonicus* estimated from human chorionic gonadotropin induced final oocyte maturation and ovulation in captivity. Fish. Sci. 71: 531-542.
- Shiraishi T., K. Okamoto, M. Yoneda, T. Sakai, S. Ohshimo, S. Onoe, A. Yamaguchi, and M. Matsuyama. 2008. Age validation, growth and annual reproductive cycle of chub *Scomber japonicus* off the waters of northern Kyushu and in the East China Sea. Fish. Sci. 74: 947-954.
- Shiraishi T., S.D. Ketkar, Y. Katoh, M. Nyuji, A. Yamaguchi, and M. Matsuyama. 2008. Spawning frequency of the Tsushima Current subpopulation of chub mackerel *Scomber japonicus* off Kyushu, Japan. Fish. Sci. 75: 649-655.

**CHARACTERIZATION OF A NEWLY IDENTIFIED  
ANTI-LIPOPOLYSAHCHARIDE FACTOR (ALF2) GENE IN KURUMA SHRIMP,  
*MARSUPENAEUS JAPONICUS***

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Innate immunity is the important defense mechanism of invertebrates against harmful agents. Antimicrobial peptides (AMPs) play significant role in innate immunity of shrimp in the forms of anti-lipopolysaccharide factors (ALFs), crustins, penaeidins and lysozymes. AMPs have broad spectrum of antimicrobial activities such as killing or neutralizing bacteria, fungi, parasites and viruses.

So far, ALF genes have been found in different species of shrimp includes *Penaeus monodon* (*PmALF*), *Fenneropenaeus chinensis*, *Litopenaeus setiferus*, *L. vannamei*, *L. stylirostris* and *Marsupenaeus japonicus* (*MjALF1*). More than one isoform have been identified in *P. monodon*, and representation of *MjALF1* was up-regulated upon stimulation with LPS *in vitro*.

In this study, we have identified a new ALF isoform from kuruma shrimp *M. japonicus* (*MjALF2*). The full-length *MjALF2* gene consists of 558 bp with a 363 bp open reading frame, encoding 121 amino acids, which contains a putative signal peptide of 22 amino acids. The deduced amino acid sequence of *MjALF2* showed 83.3% identity with sequences of *PmALF2*. Phylogenetic analysis revealed *MjALF2* had been placed in the group closer to *PmALF1* and *PmALF2*. Expression analysis revealed that *MjALF2* had been constitutively expressed in brain, gill, heart, hemocytes, hepatopancreas, intestine, lymphoid organ, muscle, nerve, stomach. This study was supported by Research and Development Program for New Bio-industry Initiatives and Japan Society for the Promotion of Science.

## **CHARACTERIZATION OF CLASS B SCAVENGER RECEPTOR FROM KURUMA SHRIMP *MARSUPENAEUS JAPONICUS***

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The scavenger receptor, Croquemort is a member of the CD36 superfamily comprising transmembrane proteins involved in the recognition of polyanionic ligands. It has been proved by various researchers that member of CD36 superfamily are involved in immunity and developmental processes.

In the present study, we report a cDNA encoding the kuruma shrimp, *Marsupenaeus japonicus* Croquemort scavenger receptor (MjSCRBQ) obtained from the cDNA library of lymphoid organ and RACE amplification. The full length cDNA of 2098 bp contains an open reading frame of 1596 nucleotides that translates into a 532-amino acid putative peptide, with a 5' untranslated region of 323 bp and 3' UTR of 153 bp.

The MjSCRBQ is constitutively expressed in gills, heart, hemolymph, hepatopancreas, intestine, lymphoid organ, muscle, nerve, stomach and in the brain with high value. Expression analysis in lymphoid organs of WSSV post infection revealed the high levels of MjSCRBQ in 72 and 120 hrs durations. The MjSCRBQ contains putative functional domains including transmembrane domains and CD36 domain.

Multiple alignments of MjSCRBQ amino acid sequences showed significant identity with DmSCRBQ (31%), SsSCRBQ (29%), HsSCRBQ (28%) and RnSCRBQ (30%). In the phylogenetic analysis, MjSCRBQ is identified in the invertebrate scavenger receptor region. This is the first report in crustacean sector on identification and characterization of Croquemort scavenging receptor, MjSCRBQ which may lead to better understanding of the molecular events unexplored in shrimp system.

## **IDENTIFICATION AND CHARACTERIZATION OF DICER2 GENE IN KURUMA SHRIMP, *MARSUPENAEUS JAPONICUS***

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RNA silencing is a common antiviral defense mechanism in many eukaryotes from plants to animals. Generation of microRNA or small interfering RNA is dependent on the RNase III enzyme called Dicer. Dicer, which plays an important role in the RNA silencing, has been reported that there are two types of Dicer genes in insects. However, only one type of Dicer gene was reported in *Penaeus monodon* and *p. vannamei*. In the present study, we report the another type of Dicer gene (*MjDicer2*) in kuruma shrimp. This is the first report of presence of two types of Dicer gene in crustaceans.

The full length of *MjDicer2* cDNA was 4,967 bp in length, including an open reading frame of 4,527 bp encoding a polypeptide of 1,509 amino acids and molecular mass of about 172 kDa. Analysis of the deduced amino acid sequence indicated that the mature peptide showed the highest similarity to Dicer like protein of black cottonwoods (*Populus trichocarpa*) with a maximum identity of 28.7% containing all the six recognized functional domains.

Quantitative analyses of *MjDicer2* in different organs of healthy kuruma shrimp revealed high level expression in gills, whereas in all other organs are comparatively low. *MjDicer2* is found to be highly expressed in hemocytes at 2hours post stimulation with white spot syndrome virus.

This study was supported by Research and Development Program for New Bio-industry Initiatives and Japan Society for the Promotion of Science.

## **MYXOSPOREAN PARASITES OF FOUR ECONOMIC GROUPEL SPECIES; WILD LIFE AND CULTURE, FROM THAILAND**

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The phylum *Myxozoa* Grasse, 1970 are important parasites of cavities and tissues of fresh water and marine fish, however, they are uncommon in amphibians, reptiles and mammals. More than 2,180 species of these parasites have been reported, 2,160 of which have been found in fish. The worldwide knowledge on myxosporidia in fish is a fast developing field in biodiversity. Although there are numerous detailed descriptions of myxosporidian species from important fishery areas, in South East Asia the studies in Thai marine economic fish species have been scarce. During a survey on the high economic value of four grouper species, *Epinephelus coioides*, *E. malabaricus*, *E. fuscoguttatus* and *E. Bleekeri* from southern Thailand including the Gulf of Thailand, Songkhla Lake and Andaman Sea, the occurrence of four myxosporea species were found. The urinary parasite, *Sphaerospora epinepheli* was found in kidney of *E. malabaricus*, while unidentified *Ceratomyxa* sp. was presented from gall-bladder *E. coioides*. Plasmodia and spore of *Myxidium* sp. and myxobolus sp. were detected in gill filament of *E. fuscoguttatus* and *E. Bleekeri*, respectively. Morphometry and light microscopy (DIC) of mature spores of each species and some parts of histopathology are presented.



## **ASTAKINE, AN ANCIENT CYTOKINE, IN KURUMA SHRIMP: RNA EXPRESSION AND SEQUENCE ANALYSIS OF *MJASTAKINE* GENE**

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Cytokines have been known to regulate hematopoiesis and immune responses in vertebrates. However, information about cytokines or cytokine-like factors concerning the hematopoiesis was very few in invertebrates, although the importance of the hemocytes has been well known in crustacean immune responses. Astakine was found to induce the shrimp hematopoietic stem cell differentiation as the first cytokine-like factor in crustacean. We present here the entire cDNA sequence (1,589 bp) of the kuruma shrimp *Marsupenaeus japonicus* astakine (*Mjastakine*).

The open reading frame of *Mjastakine* encoded a protein of 124 amino acids with an estimated mass of 13.3 kDa. Amino acid sequence homology of *Mjastakine* were 79.8% and 52.4% to these of the tiger shrimp *Penaeus monodon* and freshwater crayfish *Pacifastacus leniusculus*. In the prokineticin domain of crustaceans, 11 cysteines were highly conserved. *Mjastakine* mRNA was highly expressed in the brain and hemocytes in healthy shrimp. On the other hand, *Mjastakine* mRNA was low level expression in hepatopancreas and ovary. In the hemocytes, *Mjastakine* expression reached its peak one day and decreased to its normal level 5 days after the virus injection when white spot syndrome virus (WSSV) was injected into the kuruma shrimp. This study was supported, in-part, by the University of Miyazaki's Program for the Support of Women in the Sciences and the Research and Development Program for New Bio-industry Initiatives and Japan Society for the Promotion of Science.

## SEQUENCE AND EXPRESSION ANALYSIS OF C-JUN N-TERMINAL KINASE (JNK) GENE IN KURUMA SHRIMP *MARSUPENAEUS JAPONICUS*

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Apoptosis, programmed cell death, is known as a defense mechanism against virus infection in animals, but the apoptosis mechanisms of shrimp are not well understood. In mammals, c-Jun N-terminal kinase (JNK) is activated by various stresses, such as osmotic shock, heat shock and ultraviolet (UV). In this study, we cloned JNK gene from kuruma shrimp *Marsupenaeus japonicus* (*MjJNK*) and this is the first report about JNK gene in crustacean. The full length cDNA of *MjJNK* consists of 2,388 bp with a 94 bp 5'-untranslated region (UTR), a 884 bp 3'-UTR and a 1,410 bp open reading frame (ORF) encoding 458 amino acids. The deduced amino acid sequence of *MjJNK* showed 87.5 % and 79.2 % identity with sequences of fruit fly (*Drosophila melanogaster*) *JNK* and human (*Homo sapiens*) *JNK*. *MjJNK* has S\_TKc domain (Serine/Threonine protein kinases, catalytic domain), that has an ATP binding activity. Phylogenetic analysis revealed that *MjJNK* is included in group of insects. Expression of *MjJNK* in the healthy kuruma shrimp was confirmed in muscle, hematopoietic tissue, intestine, nerve and lymphoid organ. The highest gene expression was found in a muscle and it was 30-fold higher than that in the ovary. This study was supported, in-part, by Research and Development Program for New Bio-industry Initiatives, the University of Miyazaki's Program for the Support of Women in the Sciences and Japan Society for the Promotion of Science.

## COMMUNITY STRUCTURE, SEASONAL CHANGES AND TEMPERATURE TOLERANCE OF FOUR *SARGASSUM* SPECIES (FUCALES) AT THE BOUNDARY ZONE BETWEEN TEMPERATE AND SUBTROPICAL REGION OF JAPAN, IN RELATION TO CLIMATE CHANGES

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Kyushu Island, which lies between Honshu and Okinawa Islands, Japan, is regarded as the boundary zone where temperate and subtropical seaweeds coexist. Moreover, the increase in the number of subtropical species has been suggested to be related to the effects of climate change. Mixed seaweed communities of temperate and subtropical species of *Sargassum* (Phaeophyceae) can be found distributed around Kagoshima Bay of southern Kyushu Is. The purpose of this study is to reveal the seasonal changes and photosynthetic characteristics of temperate species (*S. piluliferum* and *S. patens*) and subtropical species (*S. crispifolium* and *S. alternato-pinnatum*) at Kagoshima Bay.

Field surveys were conducted by SCUBA and skin diving from May 2009 to April 2010. The seasonal changes in plant size for four species were examined during the study period; additionally, line-transects were used to reveal their community structure in May 2010. Photosynthetic activity was determined at various temperatures (10 - 36 °C) by Imaging-PAM (Heinz Walz GmbH) and dissolved oxygen meter (YSI BOD5905).

Each species was found on rocky shores between the intertidal and sublittoral zones (0.0 m to 4.9 m in depth). Typically, the seaweed community was composed of these four species, however subtropical species dominated in the sublittoral zone below 2 m depth. Maximum dry weights of temperate species were observed in May or June, and was 30.0 g for *S. piluliferum* (May) and 17.0 g for *S. patens* (June). Meanwhile, for subtropical species maximum dry weights occurred in July and was 24.6 g for *S. crispifolium* and 3.7 g for *S. alternato-pinnatum*. After they matured, the two subtropical species disappeared from the substrata by September. Temperate species also decreased in the number of branches by September, however, holdfast, stem and regenerated shoots of the plants survived during the summer season, to grow for the next year.

The relative Electron Transport Rate (ETR) of the Photosystem II (water/plastoquinone oxido-reductase) of each species was highest from 28 °C to 30 °C. However, it decreased from 32 °C to 36 °C. Meanwhile, net photosynthetic rate of each species was highest at 20 °C. An ANOVA analysis indicated that there was no significant differences from 16 to 24 °C for all species, suggesting that the optimum temperatures of each species are related to the temperatures of growing season of each species.

**COMMUNITY STRUCTURE, SEASONAL CHANGES AND TEMPERATURE TOLERANCE OF EDIBLE SEAWEED, *SARGASSUM FUSIFORME* (FUCALES), AT THE BOUNDARY ZONE BETWEEN TEMPERATE AND SUBTROPICAL REGION OF JAPAN, IN RELATION TO CLIMATE CHANGES**

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The edible macro alga, *Sargassum fusiforme* (Fucales), is known as an important local fisheries resource in Kyushu and Okinawa Is., Japan. However, there is concern that this resource is decreasing as a result of climate and other environmental changes. Furthermore, knowledge of its ecological and physiological characteristics is required to establish the consistent cultivation of this seaweed in this region. The focus of this study was to reveal the phenology and temperature tolerance of *S. fusiforme* in Kagoshima and Okinawa Prefectures, where the community is close to its southern distributional limit.

A phenological study was conducted by skin diving at four sites around Nagashima Is. (Sasue, Karakuma, Shiomi and Shoura) and at a site at Sakurajima Island, which are both in Kagoshima Prefecture, from April 2009 through June 2010. Population density, maturation, length and weight were recorded, as well as appropriate environmental factors (e.g., temperature, salinity, dissolved inorganic nitrogen and phosphate). A physiological study, which examined photosynthetic activity at various temperatures was conducted using an Imaging-PAM Fluorometer (Heinz Walz GmbH) and Dissolved Oxygen meter (YSI Model 58). Materials were collected from the sites in Kagoshima as well from Okinawa Is. (i.e., Uken Beach). After collection, materials were cultured with aeration in an aquaculture tank within a few days, at temperatures similar to those during the collection date, and were prepared for the photosynthesis experiments.

Dry weight and length of *S. fusiforme* increased in spring at each site, and showed a peak around June. Furthermore, released egg and germlings were observed on the receptacles in June. It is relevant to note that local fisherman harvested *S. fusiforme* in May, suggesting that harvesting occurs before maturation. After maturation, plants including holdfasts disappeared from the substrata by September. Young shoots were confirmed in around October. According to reports from other areas of Japan, holdfasts of this species appear to be able to survive over the summer season, and maintain a perennial community for up to a few years. However, our result suggests that this species in Kagoshima generally cannot survive over the summer, and can be regarded as an annual community.

Net photosynthetic rate was highest at 24°C. The relative Electron Transport Rate (ETR) of the Photosystem II (water/plastoquinone oxido-reductase) was highest as 30°C, and quickly decreased at higher temperatures, suggesting that projected increases in summer seawater temperature might influence their community structure and the availability of this resource.

## **ASSESSMENT OF EFFECTS OF STARVATION ON FRESHWATER CARP BY <sup>1</sup>H-NMR METABOLOMICS APPROACH**

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<sup>1</sup>H-NMR metabolomics of biofluids produces clear and comprehensive biochemical profiles of low molecular weight metabolites that reflect the health and physiological condition of an organism. Hence, the application of this technique to various fields has grown rapidly. In the present study, the effects of starvation on the freshwater carp (*Cyprinus carpio* L.) were examined using <sup>1</sup>H-NMR metabolomics. Carp was starved for 14 days and the variations of the metabolites in the blood plasma of the control and starved groups were compared. Statistical analysis was performed using principal component analysis (PCA) of the NMR spectrum-derived data. PCA score plot showed that the starved group was significantly different to the control after 14 days of starvation although no significant difference was observed yet on the 7<sup>th</sup> day. The differences observed between control and starved groups at 14 days were the following: 1) glucose and several amino acid metabolites were significantly reduced in the starved group, and 2) ketone bodies of 3-D-hydroxybutyrate and acetoacetate were significantly elevated also in the starved group. These results suggest that starvation promotes fatty acid degradation in *C. carpio*.

## MOLECULAR IDENTIFICATION OF *ANISAKIS SIMPLEX* FROM FISHES CAUGHT ALONG THE COAST OF KAGOSHIMA AND HUMAN GASTRIC ANISAKIASIS

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*Anisakis simplex* is known as a parasitic nematode of human gastric anisakiasis. There have been 2000~3000 infection reports every year in Japan. Morphological identification using the third stage larvae (L3 larvae) can only determine type groupings; *Anisakis* Type I larvae or *Anisakis* Type II larvae. *Anisakis* Type I has longer ventriculus and has mucro while *Anisakis* Type II has short ventriculus and has no mucro. On the other hand, recent molecular studies of *Anisakis*, which launched from a series of studies using allozyme and mitochondrial DNA (mt DNA) *cox2* gene analyses, revealed that the *Anisakis* species consisted of 8 valid species, 5 species [*A. ziphidarum*, *A. typica* and three sibling species of *A. simplex* complex, namely *A. simplex* (sence stricto, s.s.), *A. pegreffii*, and *A. simplex* C] and 3 species [*A. paggiae*, *A. brevispiculate* and *A. physeteris*] of which are in the *Anisakis* Type I and *Anisakis* Type II groupings, respectively.

Recent study showed *A. simplex* identified from fish caught along the coast of Japan consisted of *A. simplex* (s.s.) and *A. pegreffii*, the former is dominant in fishes caught along the Pacific coast of Japan, the latter is dominant in fishes caught in the Japan Sea. The aim of this study is examined the species composition of *A. simplex* collected from fishes caught along the coast of Kagoshima and from patients with gastric anisakiasis. L3 larvae were collected from the visceral of anchovy (*Engraulis japonicus*), chub mackerel (*Scomber japonicus*), bluefin tuna (*Thunnus thynnus*) caught along the coast of Kagoshima in 2010. *Anisakis* larvae agent of anisakiasis were collected from patients in Kagoshima, Miyazaki and Kumamoto from 2009 to 2010. All *Anisakis* Type I larvae were examined by PCR-RFLP of the ITS region (ITS1-5.8-ITS2) of ribosomal DNA.

As a result, 38 individuals of *Anisakis* Type I larvae were collected from those fishes, 10 were identified as *A. simplex* (s.s.), 27 identified as *A. pegreffii*, and 1 identified as *A. typica*. 12 individuals of collected from anisakiasis patients and all were identified as *A. simplex* (s.s.).

**IMMUNE RESPONSE AGAINST *EDWARDSIELLA TARDA* IN GINBUNA CRUCIAN CARP (*CARASSIUS AURATUS LANGDORFII*)**

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*Edwardsiella tarda* is an intracellular pathogen with a broad host range and causes Edwardsiellosis in fish. Although there is no direct evidence, previous studies have suggested that cell-mediated immunity (CMI) has a major role in the protection from intracellular bacterial infection in fish, as in mammals. In order to verify the induction of CMI, immune response was evaluated in fish after infection with *E. tarda*.

Ginbuna crucian carp (*Caraccius auratus langsdorfii*) were infected with *E. tarda* at  $3.4 \times 10^4$  CFU/fish by intraperitoneal injection (control; PBS). Kidney leukocytes (KLs) were isolated after experimental infection and examined expression level of interferon-gamma (IFN-gamma) gene, perforin gene, CD8alpha gene and TCR-beta gene for monitoring CMI.

Results of the gene expression analysis, the expression level of all isotypes of IFN-gamma in KLs showed increase at 2 days post-experimental infection. In addition the expression level of IFN-gamma1-1 had decreased at 4days was up-regulated from 8days to 16days as compared to control. Expression of *perforin-1* and *perforin-3* were suppressed by infection with *E. tarda*. In contrast expression of *perforin-2*, *CD8alpha* and *TCR-beta* were up-regulated from 8 days to 16 days as compared to control. These results suggest that CMI was induced in the protection from infection with *E. tarda*.

**EFFECT OF SOY PEPTIDE REPLACEMENT LEVEL ON GROWTH PERFORMANCE AND DIGESTIVE ENZYME ACTIVITY IN JUVENILE JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS***

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The incorporation in aqua feed of a hydrolysate processed from a high quality protein source and containing a high proportion of peptide may be beneficial. To determine whether the incorporation of extracted soy peptide can improve the growth performance of juvenile Japanese flounder, *Paralichthys olivaceus*, a growth experiment was conducted for 45 days. Six isonitrogenous and isolipidic diets in which extracted soy peptide was substituted with 0, 5, 10, 15, 20% of the fish meal protein and the sixth diet serving as the negative control (soybean meal) were formulated and fed to the flounders. Twenty five juvenile Japanese flounder ( $0.517 \pm 0.016$  g, mean  $\pm$  SD) were stocked in a 100-L tanks and were fed *ad libitum* twice a day. Result showed that fish fed the diet containing 0, 5, 10 and 15% of protein from soy peptide did not significantly differ ( $P > 0.05$ ) in terms of final body weight, weight gain and specific growth rate. However, diets with an inclusion level of 20% soy peptide showed a significantly ( $P < 0.05$ ) marked decrease in body weight gain and specific growth rate. Fish fed diets with soybean meal as the main source of protein has the lowest observed growth performance parameters among diets. Survival and total feed intake were not significantly different ( $P > 0.05$ ) among diets.

Trypsin and chymotrypsin activity from the pancreas of the fish fed the diets with 0, 5, 10 and 15% extracted soy peptide showed similar levels ( $P > 0.05$ ) compared to a lower trypsin and chymotrypsin activity to fish fed 20% inclusion level extracted soy peptide and diet containing soy bean meal as the main source of protein.

The study showed that processing soybean ingredient can improve growth performance parameters of the fish and can be used to replace (30% inclusion ) fish meal as the main source of protein.



**EFFECTS OF POLYCHAETE EXTRACTS ON REPRODUCTIVE PERFORMANCE AND TISSUE LIPID COMPOSITIONS OF KURUMA SHIMP, *MARSUPENAEUS JAPONICUS*, BATE**

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A series of 3 experiments were set up to examine effects of marine polychaete extracts on reproductive performance and body tissue lipids of *Marsupenaeus japonicus*. In experiment 1, three polychaete extracts including trichloroacetic- soluble, neutral and polar lipids as supplements (0.5% of DM) to a basal diet given to unilaterally ablated wild caught female *M. japonicus*. Effects of polychaete extracts on *M. japonicus* ovarian maturation were observed and compared. Polychaete neutral lipid (PNL) extract probably containing some steroid hormonal substances was the most effective on *M. japonicus* ovarian maturation and followed by TCA-soluble extract. In experiment 2, four levels of PNL extract (0, 0.2%, 0.4% and 0.8%) incorporated with a basal diet were given to female *M. japonicus* for a 15-day period. The results showed that the sufficient supplemental PNL extract level in maturation diet to give positive effects on ovarian maturity of *M. japonicus* is from 0.4%. In experiment 3, a combination of polychaete TCA-soluble and PNL extracts was tested as supplements to a basal diet with comparison to fresh polychaetes and non-feeding. Synergetic effects of combined polychaete extracts in maturation diet on reproductive performance of *M. japonicus* were observed as significant higher hatching percentage and number of nauplii per spawn.

Polychaete extracts affected shrimp hepatopancreatic and ovarian lipid tissues through influences on sexual maturation process. Effects of polychaete extracts on total lipids, lipid class and fatty acid compositions of hepatopancreas and ovaries were discussed.

**EFFECT OF COMPLETE REPLACEMENT OF FISHMEAL WITH MIXTURES OF SOYBEAN MEAL AND SEA FOOD PROCESSING BY-PRODUCTS ON THE PERFORMANCES OF RED SEA BREAM, *PAGRUS MAJOR***

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An eight week feeding trial was conducted to evaluate the effect of animal and plant protein ratio for the development of non fishmeal (FM) practical diet of red sea bream, *Pagrus major*. Four diets were formulated to be isonitrogenous (50% crude protein), isolipidic (15% total lipid) and isocaloric (20 KJ g<sup>-1</sup>). The control diet was the FM based diet (FM100). The remaining three diets were prepared by replacing 60 (FM40), 80 (FM20) and 100% (FM0) FM protein with different proportions of sea food processing by-products and plant proteins. A commercial diet (19.3 KJ g<sup>-1</sup> gross energy) was used as the reference diet (COM). Fifteen fish with a mean initial weight of approximately 1.35g per fish were stocked in triplicate in each of 15 100-l polycarbonate circular tanks. The fish were fed to satiation by hand twice daily.

Results of the study indicated that weight gain (%) and specific growth rate (% day) of fish did not differ significantly ( $P>0.05$ ) with up to 80% FM protein replacement. In this level, growth was also comparable ( $P>0.05$ ) with the commercial diet (COM). Feed intake and utilization were significantly ( $P<0.05$ ) depressed in 100% FM replacement group (FM0). However, no difference was found among the rest. Similar trends were also found for protein gain and retention. Whole body composition was directly influenced by the nutritional composition of the diets. Whole body protein was comparatively higher and total lipid was significantly lower in COM group. On the other hand, fish fed FM0 showed significantly lower ash content. No difference was found in condition factor and hepatosomatic index among treatments. Apparent digestibility of dry matter and lipid had no significant differences compared to the control whereas apparent protein digestibility was significantly decreased in FM0 group. There was no abnormal signs observed in hematological parameters and oxidative stress conditions of fish and it was assumed that all the fish were in good physiological state.

It was concluded that 80% of the FM protein in a typical commercial diet could be replaced with a combination of sea food processing by-products and soybean proteins.

**EFFECTS OF DIFFERENT SUPPLEMENTATION LEVELS OF EXTRACTED SOY PEPTIDE ON GROWTH PERFORMANCE AND TOLERANCE TO HIGH TEMPERATURE STRESS IN JUVENILE JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS***

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The experiment was designed to examine whether different supplementation levels of extracted soy peptide (SPEP) to soy protein concentrate (SPC) diet could improve growth performance, amino acid body composition, and tolerance to high temperature stress in juvenile Japanese flounder (*Paralichthys olivaceus*). Four isonitrogenous and isolipidic SPC diets and one fishmeal (FM) diet (positive control D1) were prepared with increasing SPEP supplementation levels of 0% (D2), 2% (D3), 5% (D4), and 10% (D5), respectively. Triplicate groups of twenty juveniles ( $1.29 \pm 0.01$  g, mean  $\pm$  SD) were stocked in 100-L polypropylene tanks and were fed twice a day to apparent satiation level for 42 days.

The present study demonstrated that fish fed D5 grew faster than those fed diets 2, 3, and 4, respectively, although growth was significantly lower in fish fed D5 than that in fish fed D1. The feed conversion ratio and protein efficiency ratio showed the same trend as that of growth performance, but feed intake was not significantly ( $P > 0.05$ ) different among diets. Except for some amino acids, whole body total and free amino acid compositions of flounder were not altered by SPEP supplementation in the diets.

In the lethal heat stress test, fish fed D5 demonstrated the highest value of  $LT_{50}$ , which was significantly ( $P < 0.05$ ) longer than that of fish fed other diets. Furthermore,  $LT_{50}$  values of fish fed diets D3 and D4 were significantly ( $P < 0.05$ ) longer than fish fed D1. Heat shock protein 70 family (HSP70s) was highest in the gill, liver, and skin of fish fed D5, which was significantly ( $P < 0.05$ ) higher than that of fish fed other diets after sub-lethal stress exposure. Moreover, HSP70s values of fish fed D3 and D4 were significantly ( $P < 0.05$ ) higher than fish fed basal and control diets. Time-course sub-lethal heat shock exposure results demonstrated that level of HSP70s significantly ( $P < 0.05$ ) decreased among all groups during recovery period, but did not return to normal and initial state after 24 hours.

Based on the overall performance of the fish, SPEP can be efficiently absorbed and utilized by flounders, but more than 10% supplementation might be needed to catch up the performance of fishmeal based diet in the condition applied in this study.

**EFFECT OF DIETARY OXIDIZED OIL WITH VITAMIN E SUPPLEMENTATION ON GROWTH PERFORMANCE AND REDUCTION OF OXIDATIVE STRESS IN RED SEA BREAM (*PAGRUS MAJOR*)**

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Due to fish oil contains large amount of essential n-3 highly unsaturated fatty acids is used in the formulation of commercial aquafeeds. However, it is susceptible to lipid peroxidation which is highly deleterious, resulting in damage to biomembranes and it is implicated in several pathological conditions in fish. On the other hand, vitamin E, especially the  $\alpha$ -tocopherol, is fat-soluble antioxidant, due to its free radical quenching, lipid protection role. Therefore, the objective of this study was to examine the influence of dietary oxidized lipid with vitamin E supplementation on growth performance, oxidative stress in juvenile red sea bream.

A 8-week feeding trial on juvenile red sea bream (average weight 1.8g) was conducted in triplicate groups with seven test diets containing two degrees of oxidized fish oil (peroxide values of 83.8 and 159meq/kg) with different level of vitamin E (0, 100 and 200ppm) supplementation, respectively. Fresh fish oil (13.5meq/kg) with 100ppm vitamin E was employed as control group. Growth performance such as body weight gain (BWG), specific growth rate (SGR) and feed conversion ratio (FCR), thiobarbituric acid reactive substances (TBARS), reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) in plasma and blood chemical composition (T-Cho and TG) were taken at the end of trial.

No significant difference were observed on growth performance of fish fed diets containing two degrees of oxidized lipid with 100 and 200ppm vitamin E supplementation, TBARS in tissue and blood chemical composition showed same values compared to those in control group. Increased dietary vitamin E level led to decreased TBARS value, d-ROMs value, TG and T-Cho, and increased BAP value and BWG in both oxidized lipid group. However when fish fed diets containing oxidized fish oil without vitamin E supplementation indicated significantly lower growth performance and increase of TBARS value in tissues. In conclusion, oxidized lipid effect negatively on growth but 200ppm vitamin E supplementation improves dietary value for red sea bream.

## **SEDIMENT CHARACTERIZATION AND REMEDIATION OF SHIRAISHI LAKE OYSTER FARM**

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Environmental deterioration such as eutrophication and harmful algal blooms has been coupled with aquaculture to cause massive mortality of cultured organisms. Rapid developments in aquaculture have been observed for the past decades in Japan; however, increasing the production with sustainable aquaculture environment remains to be a challenge. Recently, sediments in Shiraishi Lake, located in Owase, Mie, Japan, have been shown to be affected by oyster aquaculture. The present study aims to characterize the condition of the sediments in Shiraishi Lake and suggest a possible measure to remediate the contaminated environments.

Sediment core samples were collected from three stations around the oyster farm of Shiraishi Lake in October and November 2009; and January 2010. Grain size, moisture contents, and acid volatile sulfide-sulfur (AVS-S) were measured. The surface 0-1 cm layer of the sediment was characterized by a black color, 93% of silt, and 69.94±4.54% of moisture content. Averaged AVS-S from three sampling months was 1.44±0.29 mg g<sup>-1</sup> dry weight. In the months of October and January, however, AVS-S concentrations in some sampling points reached 2.0-2.5 mg g<sup>-1</sup> dry weight, a threshold concentration based on the Japanese environmental criterion on the Law to Ensure Sustainable Aquaculture Production. The AVS-S also showed an increase in concentration from 10 cm layer depth to surface layer, an indication of accumulation of organic matter in the area. These results and the preceding massive oyster mortality in the area suggest that the sediment of Shiraishi Lake has been highly affected by organic pollutants. At present, a remediation experiment of the Shiraishi Lake sediment by potential chemical remediation agents including titanium oxide and magnesium hydroxide is being conducted.

## **EFFECT OF ATP AND IMP ON THE QUALITY OF FISH AND FISH PRODUCTS**

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ATP and its related compounds, that is ATP, ADP, AMP, IMP, HxR and Hx are the main nucleotides in fish muscle. The composition of nucleotide related compounds (Nu-ATP) in fish muscle can be considered as an indicator of freshness. The components of Nu-ATP in fish muscle dramatically change after death. In live fish muscle, most Nu-ATP is ATP and just after death, more than 90% of Nu-ATP in the muscle is ATP. After death however, ATP is degraded to ADP, AMP, and then to IMP. ATP has a suppressive effect on denaturation of the myofibrillar protein of muscle. Therefore, micro-sized meat with a high content of ATP from fishes immediately after instant killing has a good gel forming ability by addition of only a low concentration of salt and shows good freeze tolerance. These facts suggest that for processing, especially, for surimi processing and freezing, using fishes with a high content of ATP, i.e. using fishes immediately after killing, enables to keep a high quality of the products. However ATP is tasteless and the taste of such fishes with a high ATP content is negligible. On the other hand, IMP contributes to an umami taste and has a synergistic effect on sweet and/or umami tastes of various amino acids. IMP content is negligible just after death and accumulates depending on the degradation of ATP. Thus, according to the accumulation of IMP, the umami taste of fish muscle increases. Up to 7~10hr after death, more than 80% of Nu-ATP is IMP, and the umami taste of fish muscle is enriched. These results suggest that the best time to eat, that is fish when it is most delicious, is 7~10hr after death.

### **Annotated Bibliography of Key Works**

Kawai, M., Okiyama, A., Ueda, Y. 2002. Taste Enhancements Between Various Amino Acids and IMP. *Chem. Senses*, Vol.27, Pp 739-745.

It is well known that a strong synergistic interaction of umami occurs between L- $\alpha$ -amino acids with an acidic side chain, such as L-Glu or L-Asp, and 5'-mononucleotides, such as inosine 5'-monophosphate (IMP). The authors tested taste interactions between various L- $\alpha$ -amino acids and IMP by the psychophysical method and found that taste enhancement occurred when IMP was added to several sweet amino acids, such as L-Ala, L-Ser, and Gly. The enhanced quality of taste was recognized as umami, and was not blocked by the sweetness inhibitor  $\pm$ 2-(*p*-methoxyphenoxy)propanoic acid. The total taste intensities of various concentrations of the amino acid and IMP mixtures were measured using magnitude estimation. The results showed that the potentiation ratios were larger than 1 in the cases of L-Ala, L-Ser and Gly. However, the ratio was  $\sim$ 1 in the case of D-Ala, which had an enhanced taste of sweetness. Thus the umami taste enhancement of several sweet L- $\alpha$ -amino acids by

IMP was synergistic rather than additive as that of acidic amino acids.

Murata, Y., Beauchamp, G.K., Bachmanov, A.A. 2009. Taste perception of monosodium glutamate and inosine monophosphate by 129P3/J and C57BL/6ByJ mice. *Physiol. and Behav.* Vol. 98, Pp 481-488.

The author's previous studies have shown that in long-term two-bottle preference tests, mice from the C57BL/6ByJ(B6) inbred strain drink more monosodium glutamate (MSG) and inosine monophosphate (IMP) than mice from the 129P3/J (129) inbred strain. The goal of this study was to examine whether this variation in consumption could be attributed to strain differences in perception of the taste quality of MSG and IMP. The authors developed a conditioned taste aversion (CTA) in B6 and 129 mice to 100 mM MSG or 10 mM IMP and used a brief-access taste assay to examine CTA generalization. B6 and 129 mice did not differ in the generalization patterns following CTA to MSG: mice from both strains generalized CTA from MSG to NaCl. In contrast, strain differences in the generalization patterns were evident following the CTA to IMP: while mice from both strains generalized CTA from IMP to MSG, 129 mice tended to have stronger CTA generalization to saccharin and D-tryptophan, both of which are perceived as sweet by humans. These data suggest that the strain differences in MSG consumption are not due to variation in perception of the taste quality of MSG. Instead, the differential intake of IMP likely reflects strain differences in the way the taste quality of IMP is perceived. The author's data suggest that mice perceive MSG and IMP as complex taste stimuli: some taste components are shared between these two substances, but their relative intensity seems to be different for MSG and IMP. The amiloride-sensitive salt taste component is more prevalent in MSG than in IMP taste, and in B6 compared with 129 mice.

Murata, Y., Bachmanov, A.A., Beauchamp, G.K. 2008. Behavioral analysis of the taste of valine in C57BL/6J mice and distribution of valine in sea urchin gonads. *Jpn. J. Taste Smell Res.* Vol.15, Pp 463-446. (Japanese)

Valine (Val) has bitter and slightly sweet taste in human and contributes to taste of sea urchin (Komata, 1964). However, there are many unclear points in its taste quality. While, Val evoke only negligible activation of the mouse T1R1+T1R3 receptor, but activation of this receptor increases considerably when Val are mixed with IMP (Nelson et. al., 2002). This suggests that addition of IMP changes the taste quality of Val. The authors tested taste quality of Val and this hypothesis using a conditioned taste aversion (CTA) technique. Separate groups of C57BL/6J mice were exposed to 50 mM Val with or without 2.5 mM IMP, or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with five basic taste solutions, Met, Val, Lys and Arg with and without IMP, and their lick responses were recorded. An aversion to Val generalized to Val, Lys, Arg with and without IMP and quinine. An aversion to Val+IMP generalize to a mixture of 50 mM monosodium glutamate (MSG) and 30  $\mu$ M amiloride (Ami; added to block sodium taste) with and without 2.5 mM IMP, but not quinine. This suggests that, as predicted by the in vitro study, addition of

IMP changes the taste quality of Val in vivo. In addition the content of Val in mature and immature gonads (which are edible part) of sea urchin, *Hemicentrotus pulcherrimus* were examined. Val was 60-100mg/100g and its content was only 3-5% of total amino acids in the gonads. Thus there is no difference between mature and immature, also ovary and testis.

Murata, Y. and Unuma, T. 2009. Concentration of Val, Met and IMP in gonads of seven edible species of sea urchin in Japan. Jpn. J. Taste Smell Res. Vol.16, Pp 445-446. (Japanese)

Valine (Val) and Methionine (Met) have a bitter and slightly sweet taste for humans and contribute to the taste of sea urchins (Komata, 1964). In a previous CTA experiment with C57BL/6J mice, the results suggested that the taste quality of Val and Met changed to have umami after mixing with IMP. Also the concentration of Val in *Hemicentrotus pulcherrimus* gonads was examined. In Japan, there are 7 species of sea urchins that are mainly eaten. The taste of gonads differs among these species, however there are no scientific data to prove it. In order to clarify the cause of this difference we investigated the concentration of Val, Met and IMP in gonads from these 7 species of sea urchin. The content of Val and Met ranged 7~70mg/100g and 2~26mg/100g, respectively, and accounted for under 5% of total amino acids in the gonads of each species. The content of IMP was the same level as Met, ranging 1.2~26mg/100g. These data suggest that the small content of Val and Met may contribute to a pleasant bitterness and small content of IMP may change the taste quality of amino acids in the gonads of each species of edible sea urchin.

Murata, Y., Okazaki, E., Kimura, M., Imamura, S., Hiraoka, Y., Kimura, I. 2010. Gel forming ability and freeze tolerance of ground fish meat with a high content of ATP. Journal of Fisheries Technology. Vol.2, Pp 105-110. (Japanese)

Muscle from horse mackerel was ground with a cutter mill immediately after instant killing to prepare ground meat with high contents of ATP. The ATP level was kept high about 70% until 90 seconds after the grinding started. For comparison, the meat with high ATP was stored at 5°C for 6 hours to prepare one with a low content of ATP. The ground meat with high ATP was able to form a strong gel by addition of 3% or lower concentrations of NaCl. After storage at -20 and -80°C for 2.5 months, it formed a smooth gel by addition of NaCl and solubility of myofibrillar proteins was higher than that of the ground meat with low ATP. A gel forming ability of the ground meat with low ATP recovered to the same level of that with high ATP after addition of ATP. These results suggest that it is important to keep the level of ATP in ground fish meat at certain level for its gel forming ability and freeze tolerance.



## HOW DO WE REDUCE THE IMPACT OF GLOBAL WARMING ON AQUACULTURE?

— An introduction to projects to develop methods to counter the impacts of global warming —

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Global warming will have a big impact on aquaculture. A rise in water temperature could result in a variety of negative effects, such as: reduced food conversion efficiency due to a rise in catabolic activity, asphyxia due to low dissolved oxygen, and an increased risk of infectious diseases due to high temperature stress. Techniques to reduce the impact of global warming on aquaculture of marine fish are underway. An initial step has been clarification of the relationship between growth and temperature in each species. The second step has been quantitative and qualitative analyses of bodily and organ functions at high temperature. The third step has been estimations of individual variations in physiological status and growth at high temperature. The physiological characters for four marine fishes (flounder, sea bream, yellowtail and herring) at high temperatures have been analyzed to date. The results indicate that the temperature for maximum growth in each species is higher than that of the area where the species occurs naturally and that the variation in individual growth rate at high temperature is wider than at each species natural temperature. Notably, some individuals were even recorded showing growth close to the survival temperature limit. Selection and breeding of individuals for the ability to grow sufficiently at high temperature will be an important measure to counter the effects of global warming on aquaculture.