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|---|--------------------------|
| 3. Challenges for development of aquafeeds<br>Makoto Nakada (Japan)   | 10:25-10:50              |
|   | Break 10:50-11:05        |
| 4. Maintaining human health benefits in farmed fish: A focused review of the<br>seafood and human health literature<br>Walton Dickhoff (U.S.A.) | 11:05-11:30              |
| 5. NOAA/USDA Future of Feeds Initiative<br>Robert Iwamoto (U.S.A.)  | 11:30-11:55              |
|   | Announcement 11:55-12:00 |
|   | Lunch 12:00-13:00        |

## **Session II: Novel findings for the development of aquafeed research**

(Moderators: Robert Iwamoto and Shuichi Satoh)

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|--|-------------------|
| 6. Over view of the plant feeds in aquaculture working group<br>Michael Rust (U.S.A.)  | 13:00-13:25       |
| 7. An overview of progress toward developing an all plant-based diet for rainbow<br>trout<br>Gibson Gaylord (U.S.A.)                                       | 13:25-13:50       |
| 8. Dietary supplementation strategies to improve performance of rainbow trout<br><i>Oncorhynchus mykiss</i> fed plant-based diets<br>Wendy Sealey (U.S.A.) | 13:50-14:15       |
| 9. Taurine: An essential nutrient in fish<br>Shin-Kwon Kim (Japan)   | 14:15-14:40       |
|  | Break 14:40-14:55 |

(Moderators: Walton Dickhoff and Takao Yoshimatsu)

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| 10. Development of a new dietary material from unutilized algal resources using<br>fermentation skills<br>Motoharu Uchida (Japan) | 14:55-15:20              |
| 11. Approach to expand the use of Japanese anchovy as feed material<br>Noriko Ishida (Japan)                                      | 15:20-15:45              |
| 12. Larval feeds research in the United States<br>Michael Rust (U.S.A.)   | 15:45-16:10              |
| 13. Aquaculture feed and seafood quality<br>Cheng-Sheng Lee (U.S.A.)  | 16:10-16:35              |
| 14. Quality control of cultured fish by feed supplements<br>Heisuke Nakagawa (Japan)  | 16:35-17:00              |
|   | Announcement 17:00-17:05 |

Wednesday, October 29<sup>th</sup>, 2008

Registration 9:15-9:30

**Session III: Problems and new study approaches for breakthrough**

(Moderators: Cheng-Sheng Lee and Makoto Yamasaki)

15. Protein production advantages in the face of increasing feed costs: Identifying opportunities within the aquaculture industry 9:30-9:55  
Gina Shamsak (U.S.A.)
16. Availability of gene-modified feed ingredient to fish 9:55-10:20  
Shuichi Satoh (Japan)
17. Quantification of waste feed and fish feces using stable carbon and nitrogen isotopes 10:20-10:45  
Hisashi Yokoyama (Japan)
18. Measurement of swimming behavior and digestive process of cultured fish 10:45-11:10  
Toshinori Takashi (Japan)

Break 11:10-11:25

**Session IV: General discussion**

11:25-11:55

(Moderators: Panel members)

Closing remarks (Robert Iwamoto, U.S.A. Panel Chair) 11:55-12:00

## Presentation 1

### **PRESENT STATUS OF AQUAFEED RESEARCH IN JAPAN**

Takao Yoshimatsu

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Japan used to be the leading fish meal manufacturer in the world. For example, in 1980s there were constant 3-4 million mt or more of sardine *Sardinops melanostictus* fisheries production from the surrounding seas of Japan. At that time this huge catch of sardine substantially supported and maintained the large production of developing sector of yellowtail *Seriola quinqueradiata* and red sea bream *Pagrus major* feeding cultures as materials for fish meal and/or Oregon-type moist pellets. However after 1990s Japanese sardine production drastically decreased mainly due to the natural regime shift of oceanic environment with long-term intervals, and currently, we only have several hundred thousands mt level of annual production that was scant to support the ever growing requirements in Japanese aquaculture.

To compensate this collapse we have been importing fish meal products from Peru, Chile and many other countries, and its total amount reaches 400-500 thousands mt nowadays. Approximately 50% of those imported fish meal are used as a feed ingredient for cultured fish and eventually 400-500 thousands mt of formula fish feed is produced for finfish culture in Japan. Commercial formula feeds produced in Japan are supplied to yellowtail culture for about 40%, to red sea bream culture for about 30%, and to freshwater species like eel, trout and common carp for less than 20%.

For the development of aquaculture sector in Japan, NRIA played a very important role as a governmental research institute so far. Other than the basic nutritional and metabolic studies, recent research activities on aquafeeds in the Feed Research Group of NRIA can be categorized as follows: 1) Efficient utilization of plant-derived feed ingredients like soybean meal, 2) Efficient utilization of plant oil and carbohydrate as feed ingredients for finfish, 3) Necessity of taurine as an important nutrient for marine finfish, 4) Development of new fish feeds without fish meal using bile salts and taurocholic acid, 5) Development of new fish feeds utilizing single-cell materials from marine algae, 6) Dealing with the safety issues on aquafeed ingredients and additives, etc. Also many governmental/private universities, prefectural research institutes and private feed companies struggle to solve problems on aquafeeds for the development of aquaculture in Japan and the world.

### **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) Yamamoto, T., Suzuki, N., Furuita, H., Sugita, T., Tanaka, N., and T. Goto. 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed

utilization of rainbow trout *Oncorhynchus mykiss*. *Fisheries Science*, **73**: 123-131.

A feeding experiment was conducted to evaluate the supplemental effect of bile salts to a defatted soybean meal-based non-fish meal diet for rainbow trout *Oncorhynchus mykiss*. Experimental results suggest that bile salt supplementation to a soybean meal-based diet improves the nutrient utilization by normalizing digestive processes in rainbow trout.

2) Matsunari, H., Yamamoto T., Kim S. K., Goto T., and Takeuchi T. 2008. Optimum dietary taurine level in casein-based diet for juvenile red sea bream *Pagrus major*. *Fisheries Science*, **74**: 347-353.

This study was conducted to investigate the effect of dietary taurine and cholytaurine on growth and body composition of juvenile red sea bream *Pagrus major*. From the experimental results it can be inferred that the optimal dietary taurine requirement of juvenile red sea bream is 0.5% on a dry weight basis, and that the supplementation of taurine in the diet not only improves the growth but also increases hepatic lipid levels of red sea bream juveniles.

3) Khan M. N. D., Yoshimatsu T., Kalla A., Araki T., and S. Sakamoto. 2008. Supplemental effect of *Porphyra* spheroplasts on the growth and feed utilization of black sea bream. *Fisheries Science*, **74**: 397-404.

A 56-day feeding trial was conducted to evaluate the effects of *Porphyra* spheroplasts as a feed additive on growth, carcass composition and feed utilization of black sea bream. The experimental results obtained in this feeding trial suggest that the optimum dietary PS supplementation level up to 3% for black sea bream can be considered as improved consequence on growth performance, nutrient utilization or body composition were noticed.

## Presentation 2

### **THE ISSUES SURROUNDING AQUACULTURE FEED AND THE CURRENT MEASURES AGAINST THEM IN JAPAN**

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In recent years, production of the marine fish aquaculture in Japan has remained stable above the 250,000 t level, and occupied an important position in the Japanese marine fishery production. However, the high price of fish meal since 2006 has kept the inflationary price of aquaculture feed, which have afflicted fish farmers with the deterioration of managing conditions.

Therefore, it is necessary to promote the sustainable aquaculture production, for example, through development of aquafeed that contains a little or no fish meal and through introduction of self-feeding systems to prevent excessive administration of aquaculture feeds.

## **CHALLENGES FOR DEVELOPMENT OF AQUAFEEDS**

- What should be considered for the development of commercial aquafeeds now? –

Makoto Nakada

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Artificial aquafeeds which is used fishmeal as the major protein source, has been revealed just about perfect nutrition, good-taste for raising fishes and cheap enough for aquaculture industrial use. Since world production of fishmeal and fish oil has fluctuated dramatically further improvements in fish feed, including use of alternative protein source, soybean meal, poultry meal and a certain amount of fish oil, along with soybean oil, tallow and/or coconut oil, has been undertaken. We are developing the automatic feeding systems to improve the feeding efficiency and compensate poor nutritional value for the substitute type feeds, which are low palatability and growth. Aquaculture in no-feeding or fertilizing system will be the new trend for world food supply. We should investigate the fortification feeds to those basic aquaculture types to increase production and improve the product quality.

Nowadays, more research is needed to develop the cheap and subordinate feed, appropriate feeding techniques that will be adopted for growth stage and seasons. Very special attention should be taken to the group fish equalize feeding which are swimming under invisible water and difficult to care individually, refer to human and stockbreeding. Aquaculture farmer has lost profit because of raised feed prices. Aquafeeds producers also lose benefit because of too-many kind of EP size and brand for areas or seasons, which bring worst manufacture efficiency. Our important subject is to generalize the unification of aquafeeds size and C/P ratio and to pay attention to reasonable feeding test plan by the fish group and the facility environment such as water temperature and quality.

## **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) N. Ahmed, et al, Edited by Mohammad R. Hasan, Aquaculture Management and Conservation Service Fisheries and Aquaculture Management Division FAO Fisheries and Aquaculture Department, FAO FISHERIES TECHNICAL PAPER 505, 2007, *Economics of aquaculture feeding practices in selected Asian countries*, Pp1-219.

This technical paper provides an analysis of the economical implication of, and the reasons for, adopting various feeding practices for different fish species and aquaculture systems in Asia. It comprises of six selected country case study reports from Asia (Bangladesh, China, India, the Philippines, Thailand and Viet Nam) and an overall synthesis ending with conclusions and recommendations.

2) M. Nakada, (in press), Capture-Based Aquaculture of Yellowtail, FAO International

Workshop on Technical Guidelines for the Responsible use of wild fish and fishery sources for Capture-based Aquaculture production, Hanoi Viet Nam, 8-12 October 2007, Pp 213-229.

Yellowtail mariculture has developed remarkably due to the abundant supply and low price of wild-caught juveniles (Mojako). Ear yellowtail culture was depending upon the locally available trash fishes such as; Sand eel, Anchovy, Mackerel, and Sardine for feeds. However, it became apparent that use of trash fish as a sole feed for yellowtail, led to nutritional diseases because of putrefaction and unsuitable protein and energy levels. High quality and raised cost of artificial feed was acceptable to yellowtail culture, supported by expensive consumers' price of fishes in Japan. Yellowtail Production are stable with 60,000 tones of wild harvest and 150,000 of CBA in Japan.



**MAINTAINING HUMAN HEALTH BENEFITS IN FARMED FISH:  
A FOCUSED REVIEW OF THE SEAFOOD AND HUMAN HEALTH  
LITERATURE**

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It is well established that the fishmeal-fish oil component of fish feeds is beneficial to fish growth and to human health for consumers of seafood. Human health benefits of seafood include proper neurodevelopment and reducing cardiovascular disease. Animal studies and some epidemiological studies suggest that seafood consumption may also prevent a number of inflammatory diseases and promote mental health. A major focus on identifying factors contributing to human health benefits has been on the long-chain polyunsaturated fatty acids (LCPUFA) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), although other chemical components of seafood also provide human health benefits. Since aquaculture consumes approximately 50% of the global supply of fishmeal and 80% of the supply of fish oil, expansion of fish farming will be limited unless effective alternatives to fishmeal and fish oil are found. Fish reared on feeds containing vegetable oils tend to have less EPA and DHA and higher levels of other fats, including arachidonic acid (ARA), which may promote inflammation. Responsible aquaculture should strive to maintain human health benefits of farmed seafood by incorporating such nutritious components as LCPUFA into fish feeds.

The strongest case for human health benefit from seafood consumption is for reducing cardiovascular risks, including risk of heart attack and stroke. A meta-analysis of several dozen human epidemiological studies of cardiovascular disease risk indicates that seafood consumption to attain an intake of EPA plus DHA of 250 mg/day provides maximal benefit. Minimal levels of LCPUFA intake to achieve health benefits for neurodevelopment and reducing risk of inflammatory disease have not been broadly established. The appropriate ratios of EPA, DHA, and other LCPUFA to maximize health benefit are also unknown. More studies are needed to establish the relationships between human health parameters and seafood and LCPUFA consumption to provide better guidelines for fish nutritionists to maintain nutritious seafood. Also, if alternatives to fishmeal and fish oil are used, more studies of the dependence of fish growth and health on LCPUFA are needed.

**ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) Mozaffarian, D., and E. Rimm. 2006. Fish intake, contaminants, and human health: Evaluating the risks and benefits. *JAMA* **296**:1885-1899.

The authors present a meta-analysis of human studies relating cardiovascular events and mortality and neurological development to fish consumption, long chain n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), methyl mercury, PCBs and dioxins. They focused on randomized clinical trials and large prospective studies in many countries – 20 studies total. Intake of EPA + DHA of 250 mg/day decreased risk of coronary heart death by 36%. Very high rates of EPA + DHA (0.9 to 1.8 g/day as in Japan) did not further decrease risk of death, but lowered non-fatal cardiovascular events. For neurological development, maternal DHA levels during pregnancy positively correlated with behavioral attention, visual recognition memory, and language comprehension of infants. With regard to methylmercury intake with fish, the study support the advice that women of child bearing age should consume modest levels of fish but avoid four species with high mercury. Long-term exposure to PCBs and dioxins increases cancer risk. However levels of these contaminants in fish are equal to or lower than in beef, pork, chicken and dairy products. Higher levels of PCBs and dioxins have been found in farmed versus wild fish, but are below levels of concern. Benefits of fish consumption in reduction cardiovascular death outweigh cancer risk by 100- to 370-fold for farmed salmon and 300- to over 1000-fold for wild salmon. Implications for target nutrient and contaminate levels in farmed fish can be derived from the information presented in this paper. Table 2 is a particularly useful summary of EPA, DHA, and contaminants in fish, shellfish and other foods.

2) Weaver, K.L., P. Ivester, J.A. Chilton, M.D. Wilson, P. Pandey, and F.H. Chilton. 2008. The content of favorable and unfavorable polyunsaturated fatty acids found in commonly eaten fish. *J. Am. Diet. Assoc.* **108**:1178-1185.

The authors note that over the last century changes in the human diet have favored greater consumption of saturated fat and n-6 polyunsaturated fats (PUFA) and lower n-3 PUFA. Such changes may impact cardiovascular health and promote inflammatory diseases. With recent increases in farmed fish and changes in species of fish consumed, the profile of fatty acids in the diet is changing. A survey was made of the n-3 fatty acid composition of 30 species of the most commonly consumed farmed and wild fish, with highest concentrations in wild and farmed salmonids and lowest levels in tuna, red snapper, monkfish, and mahi-mahi. A more focused examination of fatty acids was made of farmed Atlantic salmon, trout, tilapia and catfish. Farmed salmon and trout contained higher levels (>3g/100g) of n-3 PUFA than tilapia and catfish (<0.5 g/100g). Ratios of n-6/n-3 PUFA were well below 1 in farmed salmon and trout, but greater than 2 in tilapia and catfish. Levels of saturated and monounsaturated fats and arachidonic acid were also higher in tilapia and catfish. Arachidonic acid levels were highly variable in samples depending on the source of the tilapia. Some tilapia samples from Central America had arachidonic acid levels greater than 300 mg/110 g, which is much higher than typically found in beef and pork. Although some laboratory and clinical studies indicate that high ratios of n-6/n-3 PUFA promote inflammation, the ideal ratio of n-6/n-3 in the human diet is controversial and may depend on genetic factors. Results of this work raise concern about what species of fish is most appropriate for human health benefit.

3) Fritsche, K. 2006. Fatty acids as modulators of the immune response. *Annu. Rev. Nutr.*

26:45-73.

The author provides an overview and history of the effects of n-3 polyunsaturated acids (PUFA) on such inflammation/immune-mediated human diseases as asthma, arthritis and infection. Both laboratory and clinical studies are critically reviewed. Laboratory and animal studies are clearly supportive of the anti-inflammatory nature of n-3 PUFA supplementation. Although some clinical studies clearly show some benefit of n-3 PUFA consumption in treating inflammatory diseases, in general the results of clinical studies are disappointing given the expectations of laboratory studies. Some of the discordance between expectation and evidence in this area may be due to problems with using mice as models for human disease and problems with design of animal and clinical trials. Recommendations are made to improve research approaches by increasing statistical power, improving immune assays and considering genetic variation.

4) J. Whelan and C. Rust. 2006. Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr.* **26**:75-103.

The authors briefly review scientific literature on human health benefits of n-3 fatty acids and discuss recommended levels of intake. Various potential sources of beneficial n-3 fatty acids are described, including vegetable oils (canola, soybean, flax), genetically modified vegetable oils, nuts, echium oil, black currant oil, fish and fish oil, and terrestrial meats. A large summary is made of foods that have been enhanced with n-3 fatty acids, including eggs, dairy products, margarines, salad dressings, pasta and breads, cereal, infant formulas, baby foods, and others. Commercial sources of n-3 fatty acids are also listed. This paper may provide a guide for fish nutritionists formulating diets to enhance n-3 fatty acids in farmed fish.

## Presentation 5

### **NOAA/USDA FUTURE OF FEEDS INITIATIVE**

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To meet the growing consumer demand for seafood in the United States, increasing supplies of finfish and shellfish will be needed. Most experts agree that development of aquaculture will be the only way to sustainably meet this increase in demand. The question that must be answered is how to ensure that aquaculture production increases are sustainable. The development and expansion of farming of carnivorous fish species will be constrained by a limited supply of fish meal and oil for feeds. Fishmeal and oil have traditionally made up a large part of the diet of farm-raised carnivorous fish. 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 primarily consists of expert and public consultations that will discuss the future of fish feeds and the benefits to the U.S. by the development of such alternative feeds.

Elements of the initiative include:

1. An invitation to the general public to comment on the issue, which will indicate the level of understanding and knowledge that the public has regarding fish feeds.
2. A consultation with experts who are active researchers in the area of fish feeds and related topics (Researcher Panel).
3. A consultation with experts who are active stakeholders in the area of fish feeds and related topics (Stakeholder Panel).
4. A White Paper answering the questions raised by the public comment process and summarizing the results of the two expert panels.
5. A public meeting to summarize the contents of the White Paper and to allow for comment by the broader stakeholder community.

A Steering Committee made up of scientists, federal policymakers, communications, public relations experts and logistics experts has been assembled to move the process through these five steps.

### **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) NOAA/USDA ALTERNATIVE FEEDS INITIATIVE Robert Iwamoto and Michael

Rust, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle, WA, 98102 USA. Alternative Feeds Initiative Web Site.

<http://aquaculture.noaa.gov/news/feeds.html>

The National Oceanic and Atmospheric Administration (NOAA) in partnership with the U.S. Department of Agriculture (USDA) launched the Alternative Feeds Initiative on November 15, 2007, to accelerate the development of alternative feeds for aquaculture. Details of meetings and public comments can be found at this site. This web site track it's progress and will be used to provide copies of the white paper when it is finished

2) NOAA 10-Year Plan for Marine Aquaculture, October 2007. Available at

<http://aquaculture.noaa.gov>

Takes input from reports by the Presidents Commission on Ocean Policy, the National Marine Aquaculture Summit and stakeholder input to develop a strategy and tactical plan for the NOAA aquaculture program.

3) Offshore Aquaculture in the United States: Economic Considerations, Implications & Opportunities, July 2008 (Pre-Publication Copy).

Available at <http://aquaculture.noaa.gov>

Ten authors cover 12 chapters on various aspects that impact the economic considerations that may apply to offshore aquaculture. Chapters cover: US potential, feeds, technologies, analogous industries, economic models, national impacts, interactions with wild capture fisheries, current status, markets and the issues surrounding the current debate in the US.

4) Summary of the National Marine Aquaculture Summit. June 2007. Available at

<http://aquaculture.noaa.gov>

Summary of a summit of aquaculture leaders, health professionals and seafood stakeholders addressing the needs for and ramifications of developing a significant marine aquaculture sector in the United States.

5) US Department of Commerce Aquaculture Policy. Available at

<http://aquaculture.noaa.gov>

Provides the policy framework for aquaculture research, development and regulation for NOAA and other agencies of the Department of Commerce.

6) Presendents Ocean Commission, 2004. Setting a course for marine aquaculture –

Chapter 22. An ocean blueprint for the 21st century. Pages 330-336. Available at

<http://aquaculture.noaa.gov>

Report of a special commission on the Oceans to recommend policy for the President of the United States. Chapter 22 deals with marine aquaculture and provides much of the rational for an invigorated US effort in marine aquaculture.

## **OVER VIEW OF THE PLANT FEEDS IN AQUACULTURE WORKING GROUP**

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A large number of researchers from several countries and representatives from US federal technical agencies came together on several occasions to develop an integrated (cross-disciplinary), strategic approach to improve understanding of the factors limiting inclusion of plant feedstuffs in the diet of carnivorous fishes, with the goal to increase inclusion of sustainable plant nutrients in feeds for aquaculture. A roadmap of the research required to reach that goal was drafted. The specific objectives of the working group were to define goals, performance measures, present status, and two and four year targets. The defined goals are; 1) Establish standardized research approaches and protocols for systematic evaluation of plant feedstuffs across carnivorous fish species. 2) Enhance fish germplasm and discover genes. 3) Enhance the inherent composition of crops to provide a beneficial balance of bioactive compounds in order to optimize their use in aquafeeds for carnivorous fish. 4) Improve understanding of the interactions between plant-based diets and intestinal microflora and their effects on the intestinal morphology, nutrient transport, gut-associated immune responses, and disease resistance in carnivorous fish species. 5) Improve and optimize ingredient processing, feed manufacturing technology, and feed formulations to increase inclusion of plant-derived ingredients in the diet of carnivorous fish. 6) Optimize the storage, nutritional and sensory quality of aquaculture species for human consumption. 7) Establish an international communications network for research on optimizing plant products in aquafeeds. This information has been written into a strategic plan which outlines research needs. In addition two reviews on the use of plant feeds in aquaculture have been produced by the working group, and a tactical plan is being developed. A web site (<http://www.aquafeed.com/ppa-about.php>) has been established to encourage coordination and international collaboration to improve the use of plant products in aquafeeds for species grown in all parts of the world. Participation is open to all researchers interested in this field. Those interested should visit the web site.

### **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) Over view of the plant feeds in aquaculture working group. Michael B. Rust, Aquaculture Program, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle, WA, 98102 USA. Plant Products in Aquafeeds Working group. 2005. Expanding the Utilization of Sustainable Plant Products in Aquafeeds White Paper. Available at <http://www.aquafeed.com/ppa-about.php>

This “white paper” considers various types of plant feedstuffs which currently are or potentially may be incorporated into aquafeeds to support the sustainable production of various fish species in aquaculture. The nutritional composition of these various feedstuffs are considered along with the presence of any bioactive compounds that may positively or negatively affect the target organism. Specific strategies and techniques to optimize the nutritional composition of plant feedstuffs and limit potentially adverse effects of bioactive compounds are also described.

2) Plant Products in Aquafeeds Working group. 2005. Aquafeed Working Group Strategic Research Plan available at <http://www.aquafeed.com/ppa-about.php>

This paper provides a guide for coordinating and enhancing the development of new research efforts. It is a comprehensive and coherent research agenda for addressing the problems encountered when increasing the amount of plant protein used in feed for carnivorous fish species. Seven writing teams drafted a “roadmap” of the research steps or performance measures needed to accomplish the seven goals identified by the group. With a plan that will stand up to review by peers and program managers, researchers have the competitive advantage when responding to solicitations for proposals, and when proposing new programs.

3) Gatlin, Delbert, M. III, Frederic T Barrows, Paul Brown, Konrad Dabrowski, T Gibson Gaylord, Ronald W Hardy, Eliot Herman, Gongshe Hu, Åshild Krogdahl, Richard Nelson, Kenneth Overturf, Michael Rust, Wendy Sealey, Denise Skonberg, Edward J Souza, David Stone, Rich Wilson, Eve Wurtele. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review *Aquaculture Research*, Volume **38**, Issue 6: 551-579

Based on the 2005 white paper “Expanding the Utilization of Sustainable Plant Products in Aquafeeds” this peer-reviewed publications expands on the original.

4) Barrows, Frederic T., Diane Bellis, Åshild Krogdahl, Jeffrey T. Silverstein, Eliot M. Herman, Wendy M. Sealey, Michael B. Rust and Delbert M. Gatlin III. 2008. Report of the Plant Products in Aquafeed Strategic Planning Workshop: An Integrated, Interdisciplinary Research Roadmap for Increasing Utilization of Plant Feedstuffs in Diets for Carnivorous Fish. *Reviews in Fisheries Science*, Volume **16**, Issue 4:449 – 455.

Based on the Plant Products in Aquafeeds Strategic plan, this peer-reviewed publication expands on the original.

## **AN OVERVIEW OF PROGRESS TOWARD DEVELOPING AN ALL PLANT-BASED DIET FOR RAINBOW TROUT**

T. Gibson Gaylord<sup>1\*</sup>, Frederic T. Barrows<sup>1</sup>, Kenneth E. Overturf<sup>1</sup>, Keshun Liu<sup>2</sup>, and Gongshe Hu<sup>2</sup>

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Feeds for rainbow trout have historically contained high quantities of fish meal and fish oil as cost effective sources of both essential amino acids and fatty acids. Research results from around the world have identified ingredients to replace fish meal and fish oils, but limits still exist in completely removing these ingredients from the diet.

The USDA-ARS Trout-Grains Project, officially titled “The integration of nutritional, genetic and physiological approaches to improve production efficiency of rainbow trout (*Oncorhynchus mykiss*)” was initiated with a primary goal to identify cost effective replacements for fish meal. The project team consists of two fish nutritionist, a fish geneticist, a cereal grain chemist and a plant geneticist. This group of scientist addresses a range of issues including; selection/development of cereal grains with improved nutritional profiles, development of methods to fractionate valuable components of the grains, and development of families of trout that are better able to utilize nutrients from plant-based feeds. The nutrition component of the project focuses on evaluation of new and traditional ingredients, nutrient requirements for specific life stages, and identification of nutrients in fish meal not present in plant meals. Finally we are developing a database of nutrient availability from plant-based ingredients that will have potential to supply the nutritional needs of rainbow trout in aquaculture.

Some highlights of our research findings have been:

- 1.) Taurine may be conditionally indispensable when only plant-derived protein sources are utilized.
- 2.) Vitamin premix formulations require modification when plant derived ingredients are utilized in lieu of fish meal in extruded trout feeds.
- 3.) Starch amylose/amylopectin ratios in energy feed ingredients will affect carbohydrate and energy digestibility.

Results of laboratory and pilot scale studies of feeding fish meal free diets to rainbow trout will be presented.

### **ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

- 1) USDA-ARS Trout-Grains Project. Developing Plant-Based Feeds for Rainbow Trout.



Barrows, F., Gaylord, T.G., Sealey, W.M., Smith, C.E., Porter, L. 2008. The effect of protein source and vitamin premix on growth efficiency of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **283**:148-155.

The authors tested the adequacy of current NRC guidelines for minimal vitamin inclusion levels in fishmeal-based and plant-based diets for rainbow trout. The authors estimated losses of vitamins due to cooking extrusion processing of the feeds and determined that vitamin premixes required adjustments based in dietary ingredients (fishmeal vs. plant-based). Fish performance variables showed an interaction between vitamin inclusion levels and plant vs. fish based diets. The authors therefore recommended a modified vitamin premix formulation that adjusted for processing and storage loss in plant-based trout feeds.

2) Lunger, A.N., Mclean, E., Gaylord, T.G., Kuhn, D., Craig, S.R. 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile coibia (*Rachycentron canadum*). *Aquaculture*. **271**:401-410

The authors found that fish meal levels could be reduced to less than 15% of the diet for juvenile coibia when amino acids and taurine were supplemented with increasing levels of yeast protein. Taurine supplementation appeared to be a major factor in contributing to the yeast protein replacement in the authors' first trial. The authors' second trial showed less positive results, but may have been due to other limiting amino acids inhibiting performance. The authors highlight the importance of quantitative amino acid requirements for coibia and the potential improvements in growth and feed efficiency that taurine supplementation may have in alternative ingredient diets fed to coibia.

3) Gaylord, T.G., Barrows, F., Teague, A.M., Johansen, K.A., Overturf, K.E., Shepherd, B.S. 2007. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **269**:514-525.

The authors tested the ability to supplement methionine and/or taurine to plant-based diets for rainbow trout. The authors observed a small improvement in weight gain with taurine supplementation while methionine supplementation inhibited growth. Taurine supplementation also improve fillet yield in trout when no methionine was supplemented. The authors discuss the confounding responses of methionine and taurine supplementation in plant-based trout feeds and the need for further research to address optimal supplementation levels of amino acids.

4) Gaylord, T.G., Teague, A.M., Barrows, F.T. 2006. Taurine supplementation of all-plant protein diets for rainbow trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*. **37**:509-517.

The authors address the potential for taurine supplementation in all-plant protein based diets for rainbow trout in a factorial design experiment utilizing diets with and without fishmeal protein. Taurine supplementation improved growth, feed conversion ratios, protein retention efficiencies and energy retention efficiencies of fish fed the plant protein diets. No effects of taurine supplementation were observed for these response factors in fish fed the diets containing fishmeal. The authors concluded that taurine is conditionally indispensable to trout fed plant-based feeds.

5) Gaylord, T.G., Barrows, F.T., Rawles, S.D., Liu, K., Bregitzer, P., Hang, A., Obert, D., and Morris, C. (2008) Apparent digestibility of nutrients in extruded diets from cultivars of barley and wheat selected for nutritional quality in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*. On-line early

The authors addressed the potential differences in feed value of lines of barley and wheat selected for differences in nutrient composition. The authors performed a digestibility experiment and found that phosphorus availability ranged from 17 to 78% and was influenced by starch type in wheat. Apparent protein digestibility ranged from 53 to 125% and differences were observed between wheat varieties based on starch type. Apparent energy digestibility ranged from 32 to 63%, with waxy barley varieties having higher energy digestibility coefficients than normal starch varieties. Waxy starch varieties had higher starch digestibility in both barley and wheat because of the greater digestibility of amylopectin than amylose. The authors concluded that the higher energy digestibility of waxy barley lines suggests that these varieties warrant further attention as feed ingredients for rainbow trout.

6) Barrows, F., Gaylord, T.G., Sealey, W.M., Haas, M.J., Stroup, R.L. 2008. Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*.**283**:143-147.

A new method of soybean meal processing has been developed, which may simplify the process of biodiesel production. This method, 'in situ transesterification', eliminates hexane extraction to remove the oil, combining the extraction and transesterification steps so as to synthesize biodiesel via a single treatment conducted directly on a lipid-bearing solid material. If the resulting meal is comparable in nutritional value to commercially available hexane extracted soybean meal (SE-SBM) the new process could become widely used in the bio-fuel industry. Two levels (17.5 and 35%) of each of three types of soybean meal were fed to triplicate lots of rainbow trout. The three types of soybean meal included SE-SBM, experimentally produced hexane extracted SE-SBM (ESE-SBM), and meal produced using in situ transesterification (IS-SBM). There was no effect of source of soybean meal on weight gain of trout. The fish fed the meal processed by the new method, IS-SBM, gained as much weight as fish fed either of the two control meals, within an inclusion level. The fish fed the diets containing IS-SBM, however, did have higher feed intakes (2.51% bw/d) as compared to fish fed the ESE-SBM or SE-SBM, 2.38 and 2.46% bw/d, respectively. Protein and energy retention values were lower for the fish fed the IS-SBM diets. ADC for protein was lower for the IS-SBM (85.9%) than for the ESE-SBM (89.3%). Feeding IS-SBM did not decrease weight gain in this study, but due to the increased feed intake and FCR, long term feeding trials should be conducted to further evaluate the meal.

**DIETARY SUPPLEMENTATION STRATEGIES TO IMPROVE PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss* FED PLANT-BASED DIETS**

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Higher inclusion levels of plant-based feedstuffs in diets for rainbow trout necessitate identification of methods to mitigate the negative effects on fish growth and health. Thus, an increasing number of novel and re-purposed dietary supplements are becoming commercially available to counteract the anti-nutritional effects of plant-based ingredients. Specifically, probiotic bacteria and yeast products, which have previously been primarily investigated for their anti-pathogenic effects in fish, are now promoted to aid in plant-based diet utilization and digestive tract health. Additionally, dietary supplementation of commercially produced anti-inflammatory antibodies used to reduce gastrointestinal tract inflammation in terrestrial animals may also have potential to improve digestive tract health and performance of aquatic animals fed plant-based diets. The present paper describes a series of studies conducted at the Hagerman Fish Culture Experiment Station that investigated the ability of dietary probiotics, yeast, and anti-phospholipase A2 antibody to improve performance of rainbow trout fed soybean meal-based diets.

**ANNOTATED BIBLIOGRAPHY OF KEY WORKS**

1) Bakke-McKellep, A.M., M.H. Penn, P.M. Salas, S. Refstie, S.Sperstad, T. Landsverk, E. Ringo and A. Krogdahl. 2007. Effects of dietary soybean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the telost Atlantic salmon (*Salmo salar* L.) *British Journal of Nutrition*, Volume 97. Pp 699-713.

The authors explore the role of the intestinal microbiota of salmon in the pathogenesis of soybean meal-induced enteritis. A comprehensive approach is described involving identification of both descriptive and functional indices of gut health. Detailed methodology for the varied indices is provided allowing for more thorough evaluation of the effects of alternative protein sources in salmonids as well as less studied species. Results of the study indicate that soybean meal-induced enteritis is accompanied by induction of distal intestine epithelial cell responses and changes in

microbiota. These results strengthen the putative linkage between gut health and microbiota and indicate that further research in this area is warranted.

2) Gatesoupe, F.J. 2007. Live yeasts in the gut: Natural occurrence, dietary introduction and their effects on fish health and development. *Aquaculture*. Volume **267**. Pp 20-30.

The author summarizes the present state of knowledge regarding the importance of yeast in the fish gut. The putative role of yeast is discussed in regards to both the natural occurrence and the use of “industrial” yeast in aquaculture. Functions discussed include pathogenicity, colonization and competition, modulation of immune responses and alteration of host (fish) metabolism. Species, and yeast subcomponents of importance (i.e. glucans) are identified and supporting references are included.

3) Kesarcodi-Watson, A., H. Kaspar, M. J. Lategan, and L. Gibson. 2008. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*. Volume **274**. Pp 1-14.

The authors review recent and historic literature regarding the use of probiotics in aquaculture. Special emphasis is placed on differences between the use of probiotics in mammalian and aquatic species. These differences illustrate and the authors described why the definition of probiotic as described by Fuller (1989), “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” must be expanded for aquatic species.

Definitions which more accurately reflect the mode of actions observed for probiotics in aquatic species are proposed. Modes of action including improving the use of feed or enhancing the nutritional value are discussed.

## **TAURINE: AN ESSENTIAL NUTRIENT IN FISH**

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Fish meal is the most important protein source in aquaculture feed for finfish. In addition to protein, fish meal contains many functional substances such as essential fatty acids, vitamins, minerals, and so on. Fish meal also contains much amount of taurine, which is recently considered to be a candidate nutrient having potential as a growth promoter for marine finfish. We conducted some dietary experiments by feeding taurine free diet for Japanese flounder to elucidate the nutritional essentiality of taurine in fish.

Different taurine level diets were prepared by the supplementation of taurine to the basal composition. Taurine was eliminated from fish meal as low as possible by washing with 70% ethanol. This fish meal was used as the sole protein source for experimental diets.

Abnormal feeding behavior was observed in the taurine deficient fish group, whereas normal in the taurine supplemented group, during experimental period. Fish fed taurine deficient diet showed low growth performance. The taurine contents of the whole body and tissues increased with increase of the taurine supplementation. The concentrations of taurine conjugated bile acids, such as taurocholic acid and taurochenodeoxycholic acid, in the gall bladder increased by feeding the high taurine level diet. These findings of the taurine deficiency indicate that taurine is essential nutrient for normal growth and development of Japanese flounder.

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This study was conducted to investigate taurine deficiency and the ability of taurine biosynthesis in both juvenile Japanese flounder (JF) and juvenile common carp (CC) in vivo using low taurine level diets. Three different taurine level diets were prepared by the supplementation of taurine to the basal composition (JF – 0, 0.5 and 1.5% in JF; CC – 0, 1, 3% in CC). The final average body weight and feed efficiency of JF fed the JF – 1.5% was significantly higher than those of fish fed on the JF – 0%. On the other hand, no significant difference was observed in CC fed with CC – 0, 1, and 3% diets. The taurine retention rate was negative in the case of JF-fed with the taurine-free supplement (JF – 0%). On the other hand, the taurine retention rate was about 280% in the case of CC-fed with the taurine-free supplement (CC – 0%). These

findings indicate that while taurine is essential for growth of JF, it is not essential for the growth of CC.

2) Kim, S.K., Matsunari, H., Nomura, K., Tanaka, H., Yokoyama, M., Murata, Y., Ishihara, K., Takeuchi, T. 2008. Correlation of dietary taurine levels and lipid contents in diets on the conjugated bile acid composition and growth performance of juvenile Japanese flounder *Paralichthys olivaceus*. *Fisheries Science*, Vol:74:4. Pp 875-881.

The effects of dietary taurine levels and lipid contents on the conjugated bile acid composition and growth performance of juvenile Japanese flounder *Paralichthys olivaceus* were investigated. Six types of diet (three different levels of taurine at two different levels of lipid) were fed to juveniles (average body weight, 0.04 g). Fishmeal that was washed with 70% ethanol to remove taurine was used as the sole protein source. Feeding experiments were carried out at 20°C for 6 weeks. The body weight and percent weight gain of the juveniles were improved by the dietary taurine supplementation. The taurine contents of the whole body and tissues increased with the increase of the dietary taurine contents. The conjugated bile acids in the gall bladder consisted of taurocholic acid and taurochenodeoxycholic acid, which increased with the increase of the dietary taurine level. Taurocholic acid accounted for more than 95% of the total conjugated bile acids. This indicates that taurine is the sole amino acid to conjugate bile acid in Japanese flounder.

3) Kim, S.K., Matsunari, H., Takeuchi, T., Yokoyama, M., Murata, Y., Ishihara, K. 2007. Effect of different dietary taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*, *Aquaculture*, Vol:273. Pp 595-601

This study investigated the effects of different taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder. Five level diets of dietary taurine were prepared by the supplementation of taurine (0, 0.1, 0.2, 0.5 and 1.5%) to a basal composition. Fish meal washed with 70% ethanol to remove taurine was used as the sole protein source. Two feeding experiments were carried out at 20 °C by using different sized fish (BW: 0.7 g and 9.6 g). Both size groups of fish were fed the experimental diets for 6 weeks. The body weight and feed efficiency of Japanese flounder were improved by taurine supplementation in the experimental diets. The taurine contents of the whole body and tissues increased with the increase of the taurine supplementation. The conjugated bile acids in gall bladder were composed with taurocholic acid (Expt.I: 33.0–146.8 mg/ml; Expt.II: 64.8–145.3 mg/ml) and taurochenodeoxycholic acid (Expt.I: 0.8–5.5 mg/ml; Expt.II: 3.5–4.9 mg/ml). These bile acids increased with the increase of the dietary taurine level. Taurocholic acid accounted for more than 95% of the total conjugated bile acids. No other conjugated bile acids except the taurine conjugated bile acids were detected in the gall bladder of Japanese flounder. This means that taurine is the sole amino acid to conjugate bile acid in Japanese flounder. Dietary taurine intake affects the conjugated bile acid composition in juvenile and fingerling Japanese flounder.

4) Kim, S.K., Takeuchi, T., Yokoyama, M., Murata, Y., Kaneniwa, M., Sakakura, Y. 2005. Effect of dietary taurine levels on growth and feeding behavior of juvenile

Japanese flounder *Paralichthys olivaceus*. *Aquaculture*, Vol:250. Pp 765-774.

This study was conducted to investigate the effect of dietary taurine levels on growth and feeding behavior of juvenile Japanese flounder. Three different taurine level diets were prepared by supplementation of taurine (T-0%, 0.5% and 1.5%) to the basal diet. Fish meal washed with 70% ethanol to remove taurine was used as the sole protein source. Feeding experiments were carried out twice at 20°C by using different size of fish (average body weight: 0.3 g in Experiment I and 3.7 g in Experiment II). The feeding behavior of fish was observed throughout the experimental period. The final average body weight and feed efficiency of juvenile Japanese flounder fed the T-1.5% diet was significantly higher than those of fish fed the T-0% diet in Experiments I and II. Abnormal feeding behavior such as multiple feeding while swimming in the water column was observed in the T-0% group in Experiment I. These findings indicate that taurine is essential for normal growth and development of normal feeding behavior of juvenile Japanese flounder.

## **DEVELOPMENT OF A NEW DIETARY MATERIAL FROM UNUTILIZED ALGAL RESOURCES USING FERMENTATION SKILLS**

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“Marine silage (MS)” is fish dietary materials prepared from algae by a fermentative processing. On the 34<sup>th</sup> U.S.-Japan Aquaculture Panel Symposium, we had reported the method to prepare the MS from seaweed by lactic acid fermentation. The objective of this work is to report the recent progress of this study.

The fermentation of seaweeds can be performed by the enzymatic saccharification by cellulase, followed by the fermentation process with the use of lactic acid bacteria. This method can be applied on any kind of seaweeds, but quantity of lactic acid production was varied at least more than six times among the different lot samples of seaweed. Lactic acid fermentation of microalgae is also possible, but decomposition of cell wall is only partial with presently available enzyme products.

Developing MS is interesting from three viewpoints. Firstly, it makes possible to convert unutilized algal biomass resources into dietary materials for aquaculture. Secondly, fermentation product is acidic and long-preservative at room temperature without energy cost. Therefore, for example, surplus microalgae culture can be preserved and supplied depending on a demand. Thirdly, fermented materials are usually expected to have some functions to contribute to keep health. Red sea bream that are challenged with iridovirus and fed a diet containing the MS at 10% (w/w) resulted in the significant promotion of the survival rate (%) against those fed the control diet. We believe that aquaculture with MS is eco-friendly and contribute to culture fish that is free from drugs.

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1) Uchida, M., and M. Murata. 2002. Fermentative preparation of single cell detritus from seaweed, *Undaria pinnatifida*, suitable as a replacement hatchery diet for unicellular algae. *Aquaculture*, **207**. 345-357.

Single cell detritus (SCD) is a product ca. 5.8-11.5 $\mu$ m in diameter prepared by decomposing seaweed to a cellular level, and is expected to be utilized as a fisheries-diet in place of unicellular algae. The present paper details the culture conditions for the effective preparation of SCD from *Undaria pinnatifida* based on a fermentative method. The presently developed method needs no facilities for temperature conditioning, lightening, and air supplying, but needs only a plastic tank for obtaining the SCD. Here, we suggest a novel fisheries-diet of “marine silage”, which is produced from the combination of two principles, conversion of seaweed to SCD and



induction of lactic acid fermentation utilizing activities of a microbial mixture composed of a lactic acid bacterium and yeast.

2) Uchida, M., and M. Murata. 2004. Isolation of a lactic acid bacterium and yeast consortium from a fermented material of *Ulva* spp. (Chlorophyta). *Journal of Applied Microbiology*, **97**. 1297-1310.

Three kinds of microorganisms composed of a lactic acid bacterium, *Lactobacillus brevis*, and yeasts, *Debaryomyces hansenii* and a *Candida zeylanoides*-related specimen, were isolated from a fermented material of *Ulva* spp. Lactic acid and ethanol fermentation could be induced in various kinds of seaweed by adding this microbial consortium along with cellulase. This is the first report of demonstrative performing of lactic acid and ethanol fermentation in seaweed, which is expected to provide a new material for food and dietary applications.

3) Uchida, M., K. Numaguchi, and M. Murata. 2004. Mass preparation of marine silage from *Undaria pinnatifida* and its dietary effect for young pearl oyster. *Fisheries Science*, **70**. 456-462.

Marine silage (MS) is a new dietary item prepared by decomposing seaweed to a cellular unit and performing lactic acid fermentation. The present paper demonstrated a large (10 L)-scale preparation and a long-term (18 months)-preservation of the MS using *Undaria pinnatifida* as a substrate. Furthermore, feeding trials demonstrate the dietary value of the MS to the Japanese pearl oyster *Pinctada fucata martensii*. The present study is the first to demonstrate a mass preparation of MS and its positive dietary effect for a bivalve.

4) Uchida, M., H. Amakasu, Y. Satoh, and M. Murata. 2004. Combinations of lactic acid bacteria and yeast suitable for preparation of marine silage. *Fisheries Science*, **70**. 508-518.

To examine a suitable combination of starter microorganisms, marine silage (MS) was prepared with different compositions of LAB and yeasts from a substrate of *Undaria pinnatifida*. *Lactobacillus brevis* showed the highest predominating ability in MS among the tested, while *L. acidophilus* (casei-type) IAM 10074 and *L. plantarum* IAM12477T also showed predominating ability with relatively inferior scores. The present study demonstrated that a single use of LAB, including food industry-familiar species, is suitable for preparation of MS, while the superiority of *L. brevis* B5201 was suggested in predominating ability in marine silage.

**APPROACH TO EXPAND THE USE OF JAPANESE ANCHOVY AS FEED MATERIAL** - Efficacy of thiamine supplementation -

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Japanese anchovy (*Engraulis japonicus*) resource is considered to be extensive, however not effectively utilized. In order to resolve this issue and eventually increase the anchovy catch, Fisheries Agency has launched a project study for effective utilization of anchovy to increase the value of anchovy.

Here, we introduce the project study schema and five research issues respectively, such as fish quality, fish machinery, surimi, fish meal, and feed material. Then, we would like to focus on the results of the feed material issue.

Moist pellets are still used as a major feed material in amberjack (*Seriola dumerili*) farms in western Japan. Among the fish species used for feed materials, anchovy is definitely the cheapest. However anchovy is not often applied to moist pellets for farming amberjack because of the development of thiamine deficiency. It is said that thiamine deficiency developed in farming yellowtail (*Seriola quinqueradiata*) which were fed anchovy. The amberjack farmers are concerned that the same disease development will occur in amberjack and do not use anchovy for moist pellets.

From these circumstances, we investigated the development of thiamine deficiency in farmed amberjack. We designed three experimental groups which were respectively fed anchovy-based moist pellets, thiamine supplemented anchovy-based moist pellets, and mackerel-based moist pellets that the farmers often use. These diets were adjusted to the same caloric value. We concluded that thiamine supplementation is necessary for anchovy-based moist pellets. The thiamine supplementation for anchovy pellets prevented thiamine deficiency disease, but also achieved as the same level of the growth, disease-resistance, and meat quality as the cultured fish which was fed mackerel-based moist pellets.

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Yellowtail fed on raw anchovy as a sole feeding stuff with or without thiamine was analyzed for thiamine, pyruvic acid, lipid and protein contents in various tissues. The results obtained may be summarized as follows: (1) In the fish kept on raw anchovy with no added thiamine, contents of thiamine, cholesterol and triglyceride in various

tissues were significantly decreased, while pyruvic acid in the blood increased. The pyruvic acid and thiamine contents however, were restored to normal levels when thiamine was added to the meat. (2) The fish reared on raw anchovy with added thiamine during the entire period showed a similar body composition to the fish given horse mackerel, mackerel and sand lance which contain no thiaminase. [In Japanese with English summary]

2) Masumoto, T., R. W. Hardy, and E. Casillas. 1987. Comparison of transketolase activity and thiamin pyrophosphate levels in erythrocytes and liver of rainbow trout (*Salmo gairdneri*) as indicators of thiamin status. *American Inst. Nutr.*, **117**: 1422-1426.

Yearling rainbow trout (*Salmo gairdneri*) were fed a purified diet with and without thiamin supplementation for 30 wk, at which time overt signs of thiamin deficiency appeared in the deficient group. Overt signs of thiamin deficiency were anorexia, darkening and ataxia. Death rapidly followed the development of overt thiamin deficiency. Transketolase activity and thiamin pyrophosphate levels were measured monthly in erythrocyte and liver samples. Significant differences in erythrocyte transketolase activity between fish fed the thiamin-deficient and control diets were measured after 24 wk of feeding. No significant difference in liver transketolase activity was found between trout fed diets with or without thiamin supplementation. Thiamin pyrophosphate levels were significantly lower in erythrocytes and liver of fish fed the thiamin-deficient diet after 16wk of feeding. Thiamin pyrophosphate levels in erythrocytes and liver were found to be a more sensitive indicator of thiamin status of rainbow trout than erythrocyte or liver transketolase activity.

3) Nyoman Adiasmara Giri, I., A. Kanazawa, S. Teshima, and S. Koshio. 1996. Effects of dietary thiamin on growth and thiamin content on the hepatopancreas of kuruma prawn (*Penaeus japonicus*) juveniles. *Suisanzoshoku*, **44** (3): 325-333.

*Penaeus japonicus* ( $0.99 \pm 0.06$ g) were fed with diets containing 0, 5, 15 and 20 mg thiamin per 100g dry diet for 12 weeks to investigate their effects on survival, growth and thiamin content in the hepatopancreas of prawns. Prawns fed thiamin-deficient diet showed significantly lower survival, weight gain, and thiamin pyrophosphate (TPP) content in the hepatopancreas than those from other treatments. While a significant difference in weight gain was first observed by the eighth week of feeding, the TPP content in the hepatopancreas of prawn fed thiamin-deficient diet was markedly decreased already by the fourth week of feeding, being also significantly lower compared to other treatments at the end of experiment. These results indicated that the TPP content in the hepatopancreas may be more sensitive indicator to detect the healthy condition of prawn than growth. A positive relationship between weight gain and TPP content in the hepatopancreas was found in the present study. Based on the weight gain and on TPP content in the hepatopancreas of prawn, the minimum thiamin requirements of kuruma prawn juveniles were estimated to be 13.98 mg and 14.31 mg per 100g dry diet, respectively.

## **LARVAL FEEDS RESEARCH IN THE UNITED STATES**

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This presentation will examine the various types of microparticulate diets currently being developed in the United States, several new tools to help improve microparticulate diets and some international effort to improve collaboration in this field. I will also spend the last few minutes on my thoughts on feeds for larval eel. Following on the pioneering work of Kanazawa and co-workers in Japan, there are three labs in the US currently working on a new generation of larval feeds. These labs have focused attention on a variety of microparticle types and combinations of these particles which are termed complex microparticles. In addition our lab has focused attention on developing tools that can be used to better test microparticulate diets. So far three tools have been developed. The first one allows marking diets and live feeds so that consumption and diet preference can be directly measured. The second one adapts the apparent digestibility methods used by fish nutritionists worldwide on larger fish to small larvae. The third method measures mass leaching in real time from microparticle diets. At the end of this presentation I will defend the idea that eel larvae are parasites and give my thought about how to test this hypothesis and potential improve larval nutrition for this important and delicious species.

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Details of producing two types of agglomerated microparticulate diets and the resulting performance of these diets when fed to larval walleye are presented. Dr. Barrow's has numerous other papers dealing with larval fish diets and nutrition.

2) Cook, M.A., Johnson, R.B., Nicklason, P.N., Rust, M.B. 2008. Marking live feeds with inert metal oxides for fish larvae feeding and nutrition studies, *Aquaculture Research*.

The authors developed a simple and accurate method to mark live and microparticulate feeds in order to quantify consumption. This method can be used to optimize a variety of conditions in order to maximize feeding success. The method also is the first step toward determining apparent digestibility in fish larvae.

3) Johnson, R.B., Nicklason, P.N., Cook, M.A., Rust, M.B. in press, Determination of apparent protein digestibility of live *Artemia* and a microparticulate diet in 8-week-old Atlantic cod *Gadus morhua* larvae. *Aquaculture*.

The study of larval nutrition has been hampered by a lack of tools to measure diet performance. In larger fish, one of the key tools is the determination of apparent digestibility. This paper for the first time, adapts and tests a procedure for determination of apparent digestibility to larval fish. In this case, the cod are pre-metamorphic, but the authors are in the process of extending the method back as far as possible to the first feeding stage.

4) Langdon, C., Nordgreen, A., Hawkyard, M., Hamre, K. in press, Evaluation of wax spray beads for delivery of low-molecular weight, water-soluble nutrients and antibiotics to *Artemia*. *Aquaculture*.

The latest work from Dr. Langdon's lab describes production and use of lipid walled microcapsules. Numerous authors have demonstrated that low-molecular weight, highly soluble nutrients leach within seconds of contact with water for the majority of microparticle types. Lipid walled beads are one of the few technologies that is capable of retaining highly soluble nutrients. This paper uses the lipid walled microcapsules to deliver highly soluble nutrients to enrich *Artemia*.

5) Nicklason, P.N., Johnson, R.B. in press, Real-time measurement of protein leaching from micro-particulate larval fish feeds, *Aquaculture Research*.

This paper describes a relatively inexpensive, quick and simple method to measure the leaching qualities of microparticulate diets produced by different processes or treated differently. This method could help to better define the physical characteristics of diets used across labs, experiments and species.

6) Finn, R. N. and B. G. Kapoor II (editors). 2008. Fish Larval Physiology. Science Publishers, Enfield, NH, 724 pages.

Treatise on various aspects on the physiology of larval fishes necessary for understanding the underlying biology as it may relate to larval nutrition. Written by 38 international experts in 20 chapters and seven sections. Sections address ontogeny, respiration and homeostasis, nutrition and energy, sensory physiology, movement, control and defense and functional changes in form.

## **AQUACULTURE FEED AND SEAFOOD QUALITY**

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Seafood has been an important additional animal protein source in many less developed countries. Contribution of seafood from aquaculture has been increasing annually over the past decades as the harvest from capture fisheries decreased. According to FAO projection, aquaculture has to continually increase to meet the increasing future demand of seafood. The challenges for aquaculture in the 21<sup>st</sup> century will include not only quantity to meet the increasing demand but also quality for health consideration. The consumers want to have safe and nutritious seafood.

Seafood quality has broad definition from texture, taste, nutritional value to safety etc. Unlike the seafood harvested from the sea, the quality of aquaculture products can be controlled by many factors from the production phase to the dinner table. This report will only discuss factors such as pond management and feeding contribute to the change of seafood quality.

Since farmed fish feed on the food provided by farmers, the final nutrients/chemical compositions can be manipulated in farmed fish through feeding. External appearance such as coloration of fillet can be determined by Astaxanthin contents in diet. Replacement of fish meal and oil by vegetable protein and oils or change of oil content in fish feed will change odor and fatty acid composition such as Polyunsaturated fatty acid (PUFA) levels and consequently, taste in farmed fish. Muscle lipid content affects the texture of fish as well. The decontamination procedures and environmental monitoring during the farming practice may also be a possible means of reducing some contaminant levels. This presentation is to overview the presentation at the workshop "Seafood Quality and Aquaculture" held in October, 2007 in Hawaii. Exchange the means of manipulating the final nutrient composition or boosting a selected chemical component will increase the product value and benefit the consumers and producers.

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## QUALITY CONTROL OF CULTURED FISH BY FEED SUPPLEMENTS

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Advancements in fish nutrition have progressed considerably with regard to growth performance. However, much concern has arisen over the quality of fish flesh and health in aquaculture. In response, a variety of feed supplements have been investigated with respect to their effects on sensory evaluation, protein metabolism, lipid metabolism, vitality and disease resistance of cultured fish.

Regular ingestion of substances by wild fish suggests positive function as feed supplements. The importance of feed supplements was determined in substances that are found in digestive tracts of wild fish. For example, a variety of wild fishes ingest algae, including some primarily carnivorous fishes. Crustaceans are important and often the major food organisms of larval fish and contain considerable amount of chitin. A small amount of medium-chain fatty acids was found in the stomach content of wild fish. Therefore, the substance was supplemented to the composed diet of ayu (*Plecoglossus altivelis*), red seabream (*Pagrus major*) and black seabream (*Acanthopagrus shlegelii*). The dietary supplements commonly improved physiological condition and normalized metabolism without depression of growth performance.

Whereas incorporated level of essential nutrients in cultured fish was found to be fairly low in comparing with those of wild fish, effects of fortification of essential nutrients such as EPA, DHA and vitamin C were discussed in terms of the above point of view.

Recently application of new technologies such as molecular biology has approached to evaluate fish quality and to confirm the effect of feed supplements. Therefore, some examples of effect of feed supplements determined by gene expression technique are introduced.

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Based on the fact that medium-chain triglycerides suppress accumulation of reserved lipids in mammals and some fish species, the effect of dietary laurate on lipid accumulation and vitality was further examined in red seabream. Ethyl laurate (0.5%) supplemented to the diet was fed to zero-year fish (0.3 g) for 47 days. While dietary laurate did not influence growth performance, increasing of muscle ratio and decreasing of liver weight were observed. The dietary laurate led to reducing liver triglycerides, intraperitoneal fat body ratio, adipocyte diameter, and liver lipid. In addition, DHA,

functioning as a physiologically active nutrient, tended to increase in the muscle, liver, heart, brain, and eyes. Dietary laurate activated resistance against air-dipping and liver function, as seen the case of dietary DHA fortification. It is concluded that dietary laurate could depress lipid accumulation and improve vitality, while the influence of laurate on the tissue DHA deposition might relate to its efficacy.

2) Nakagawa H., M Sato, and D.M. Gatlin III (eds.) 2007. Dietary Supplements for the Health and Quality of Cultured Fish, 1<sup>st</sup> ed. CABI Publisher, UK, p. 133-167 and 168-177.

The book addressed current information on the effects of non-macronutrients such as micronutrients and the efficacious substances from plants, animals, and bacteria, with regard to quality and health of cultured fish. Reports of the nutritional merits of various substances are based on evidence available in published scientific literature of from yet unpublished data presented at scientific meetings or acquired as personal communications from researchers. Although some feed supplements have been used in fish culture without scientific evidence to support their efficacy, the book focuses on substances that have been experientially assessed by fish culturists of proven to have beneficial effects in mammals.

The chapters of the book deal with four parts, as follows;

- I. How to evaluate and assess the quality
- II. Essential nutrients (amino acids, fatty acids, vitamins, minerals)
- III. Feed supplements originating from organisms  
(microorganisms, terrestrial plants, algae, chitin, plant saponins, nucleotides)
- IV. Nutrigenomics, Economics, and food safety

## **PROTEIN PRODUCTION ADVANTAGES IN THE FACE OF INCREASING FEED COSTS: IDENTIFYING OPPORTUNITIES WITHIN THE AQUACULTURE INDUSTRY**

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This presentation will provide an overview of the trends in the global production of raw feed materials. Recent trends impacting the production and prices of key raw feed ingredients will be highlighted and discussed. This will include a discussion of the current and future trends in production and prices for both agricultural sources of key feed ingredients (soybeans, corn) as well as marine based (fishmeal, fish oil) sources. The focus will then turn to a discussion of feed ingredients and feed costs as they relate to the production of key proteins, including both agriculture and aquaculture sources of production (e.g. beef, chicken, pigs, salmon, and catfish). It will then be argued that the aquaculture sector is in a strong position to provide quality protein at competitive prices despite increases in raw feed prices. A critical factor will be the degree to which the aquaculture sector, and further, certain species within the aquaculture sector, can substitute across key feed ingredients. Substitution gives farmers the opportunity to reduce feed costs in the face of rising feed prices by allowing them to find feed combinations that are cost effective and also nutritionally sufficient. Thus, given the potential for substitution within the aquaculture sector, producers have the opportunity to manage feed costs in a manner that maintains production of quality protein at competitive prices.

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The report by leading fisheries and resource economists and business experts, Offshore Aquaculture in the United States: Economic Considerations, Implications & Opportunities, examined a range of topics on the industry's potential and found that a significant domestic offshore aquaculture industry could develop and be successful over the next 20 years with a clear regulatory framework. One chapter in particular (Chapter 4) examines the future of aquaculture feeds and feed costs by examining the role of fish meal and fish oil. The authors suggest that the limited supply of fish meal and fish oil is not likely to be a constraint on the expansion of U.S. aquaculture as alternative feed ingredients from soybeans and other plants, algae, yeasts, fish processing waste, and other products are being developed.

2) Jackson, Andrew. 2007. Challenges and Opportunities for the Fishmeal and Fish oil Industry. *Feed Technology Update*. Volume 2, Issue 1. January 2007.  
[http://www.aquafeed.com/newsletter\\_pdfs/nl\\_000245.pdf](http://www.aquafeed.com/newsletter_pdfs/nl_000245.pdf)

This paper first examines the current state of the global fish meal and fish oil industry and then examines the claim that fish meal and fish oil might limit the future growth of the aquaculture industry. The author asserts that fishmeal and fish oil will become more strategic dietary ingredients, rather than limiting the growth of the aquaculture industry.

3) Kristofersson, Dadi. and James L. Anderson. 2006. "Is There a Relationship between Fisheries and Farming? Interdependence of Fisheries, Animal Production and Aquaculture" *Marine Policy*, Vol. 30, No. 6: 721-72

These authors examine the role of fishmeal as a critically important feed ingredient for use in animal (e.g., poultry and pork) and aquaculture production (e.g., salmon, trout, and shrimp). Their research suggests that since 1998 market for fishmeal has changed. An important explanation is likely to be the increasing use of specialized feed formulations in the pork, poultry and aquaculture sectors. It is clear that growth in aquaculture production alone is insufficient to explain the structural change since growth in the use of fishmeal in aquaculture has slowed considerably despite the continued rapid growth in global aquaculture sector. The increasing relative price of fishmeal is likely to increase costs for animal producers, act as a stimulus for innovation and have considerable implications for the management of pelagic fisheries.

## AVAILABILITY OF GENE-MODIFIED FEED INGREDIENTS TO FISH

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The feeding studies were conducted to assemble the information on the availability of gene-modified (GM) feed ingredient for rainbow trout, using diet containing GM defatted soybean meal (SBM) as an alternative protein source to provide and ensure good protein accessibility and product safety. The utilization of genetically modified defatted soybean meal (GM SBM) as feed by rainbow trout was investigated, in comparison with non genetically modified defatted soybean meal (non-GM SBM). The nutrient utilization showed that there was no significant difference in growth and feed performance between GM and non-GM SBM groups in 12 week feeding experiment. However, the cauliflower mosaic virus 35S promoter fragment of the GM SBM was detected in the muscle of fish receiving GM SBM diet by Nested-PCR. Additionally, the promoter fragment vanished by the 5<sup>th</sup> day after changing the diet to non-GM diet. Subsequently, the study was carried out to examine the degradation and the possible carryover of foreign DNA fragment by means of measuring it from transgenic plant and host plant contained in GM or non-GM SBM and evaluate the safety for fish. These foreign DNA fragments were not completely degraded in stomach and intestine and might be taken up into organ via the gastrointestinal (GI) tract. However, foreign DNA was not detected after the withdrawal period. Judging from these findings, the novel feed ingredients derived from GM SBM could be considered as having equivalent nutritional quality and verifying the safety as feed ingredient.

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The use of genetically modified(GM) defatted soybean meal as rainbow trout feed was investigated, in comparison with non-GM SBM. Both SBMs were included at levels of approximately 15 and 30% in four diets. The diets were fed to juvenile fish for 12 weeks. The nutrient use showed that there were no significant differences in growth and feed performance between GM and non-GM SBM groups at both inclusion levels at the end of 12th week. The cauliflower mosaic virus 35S promoter fragment of the GM SBM was detected in the muscle of fish receiving GM SBM diet by nested PCR. Additionally, the promoter fragment was not detected by the fifth day after changing the diet to non-GM. Conversely, the promoter fragment was not detected from fish fed the non-GM SBM diet. The results demonstrated that the availability of protein in GM

SBM was similar to that of non-GM SBM, and the promoter fragments found in the muscle of fish were not detectable after changing the diet to non-GM, verifying the availability of the GM SBM in rainbow trout feed.

2) P. Chainark, S. Satoh, I. Hirono, T. Aoki, and M. Endo, 2008. Availability of genetically modified feed ingredient II – Investigations of ingested foreign DNA in rainbow trout, *Oncorhynchus mykiss*. *Fisheries Science*, **74** (2), 380-390.

The foreign DNA fragment from genetically modified defatted soybean meal (GM SBM) in rainbow trout was traced by nested-PCR and located by *in situ* hybridization. GM or non-GM SBM formulated diet (42% protein) was fed to fish (Av. Wt. 50.5 g) for 2 weeks. The degradation results showed that the cauliflower mosaic virus 35S promoter (220 bp) fragment was detected in the contents of digestive system only in fish fed GM SBM diet, and there was not detected on the 3<sup>rd</sup> day after changing the diet to non-GM SBM diet. For the possible transferal results, the promoter fragment was detected in the leukocyte, head kidney and muscle of only fish fed GM SBM diet, afterward it was not detected on the 5<sup>th</sup> day after changing the diet to non-GM SBM diet. These results suggested that foreign DNA fragment was not completely degraded and might be taken up into organ via the gastrointestinal tract. However, foreign DNA was not detected after the withdrawal period. Thus, the data might be considered that uptake of DNA from GM SBM might not remain in the tissues of the fish fed GM SBM diet.

## QUANTIFICATION OF WASTE FEED AND FISH FECES USING STABLE CARBON AND NITROGEN ISOTOPES

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For improving the economic and environmental sustainability of fish farming, it is necessary to optimize feeding regimes and to decrease waste feed. We developed a method to quantify waste feed and fecal matter in sediment trap materials and sediments beside and beneath fish cages using stable carbon and nitrogen isotope ratios. The contribution ratio of three sources (waste feed, fecal matter and natural organic matter) in sedimentary organic matter in and around a red sea bream (*Pagrus major*) farm in Gokasho Bay, central Japan, was estimated using an isotopic mixing model. The result showed that waste feed (29% in the bulk organic matter) exceeded fecal matter (12%) in the fish farm area, suggesting the overfeeding in this farm. Then we monitored the growth and mortality of red sea bream, C and N fluxes and sediment chemistry at two commercial fish cages; cage 1 (conventional satiation feeding) and cage 2 (restricted feeding: same feeding frequency but 18% reduction in the feed amount) for 276 days. The restricted feeding achieved normal growth, increased feed conversion efficiency and reduced mortality of red sea bream and reduced contents of waste feed-derived organic matter in the sediment. Determining the relative amount of waste feed and fecal matter in settling and sedimentary organic matter is effective for the evaluation of the optimum ration level from the viewpoint of the minimization of waste feed.

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The stable carbon and nitrogen isotope ratios of the sedimentary organic matter (SOM) collected from 41 stations in and around a coastal fish farm in Japan were measured to quantify aquaculture-derived organic matter in the sediment. SOM in the fish-farm area (within 30 m from the edge of cages) is characterized by its reduced  $\delta^{13}\text{C}$  (mean  $\Delta\delta^{13}\text{C} = -0.4\text{‰}$ ) and enriched  $\delta^{15}\text{N}$  (mean  $\Delta\delta^{15}\text{N} = +0.9\text{‰}$ ) values, which reflect the deposition of  $\text{C}_3$ -plant-derived and fish-derived elements, respectively. Compositions of waste feed (WF) and fecal matter (FM) in SOM at each station was determined based on the isotopic compositions of feed ( $\delta^{13}\text{C} = -20.2\text{‰}$ ,  $\delta^{15}\text{N} = 9.7\text{‰}$ ), fish feces ( $\delta^{13}\text{C} = -24.3\text{‰}$ ,  $\delta^{15}\text{N} = 6.3\text{‰}$ ) and marine organic matter in the sediment ( $\delta^{13}\text{C} = -19.9\text{‰}$ ,  $\delta^{15}\text{N} = 5.5\text{‰}$ ). The sediment in the fish-farm area was characterized by high WF and FM ratios in SOM (28.8 and 11.9%). As the distance from the fish cages



increased, aquaculture-derived organic matter decreased exponentially. The spatial extent of waste dispersal extended to an area up to 300 m, whereas dissolved oxygen of the bottom water and acid volatile sulfides in the sediment were affected even at stations 600 m away from the fish farm. There was a significant negative relationship between the aquaculture-derived nitrogen content in the sediment and the mean current velocity, suggesting that areas (water depth = ca. 18 m) where the near-bed current velocity is >8 cm/s will not receive excessive accumulation of organic wastes.

2) Yokoyama, H., and Y. Ishihi. 2007. Variation in food sources of the macrobenthos along a land-sea transect : a stable isotope study. *Marine Ecology Progress Series*, Vol:346. Pp127-141.

To evaluate the relative importance for the macrobenthos of possible food sources, including riverine particulate organic matter, reeds, benthic microalgae, seaweeds, coastal phytoplankton and aquaculture-derived matter, over 194 macrofaunal species were collected from 9 intertidal and subtidal stations along the axis of Gokasho Bay, Japan, and their isotopic compositions analyzed. The  $\delta^{13}\text{C}$  values for terrestrial organic matter were very different from those of the consumers (>5‰), suggesting a negligible trophic role for this source. Differences in  $\delta^{15}\text{N}$  between seaweeds and primary consumers were small, suggesting a minor contribution of seaweeds. The  $\delta^{13}\text{C}$  values of the majority of consumers fell between coastal phytoplankton and benthic microalgae, allowing the calculation of the relative contribution of these microalgae to the diets of consumers. The estimated contribution ratios and the between-site comparison of the  $\delta^{13}\text{C}$  value for the same species along the bay axis suggest a shift of the main food source from benthic microalgae on the mudflat to coastal phytoplankton at the subtidal seaward stations. Several consumers collected from the fish-farm area had enriched  $\delta^{15}\text{N}$  and depleted  $\delta^{13}\text{C}$  values relative to samples of the same species that occurred outside of the farm area. The  $\delta^{13}\text{C}$  values of the consumers at the fish farm were significantly more depleted than those at the other stations. These findings suggest that the consumers inhabiting the farming area incorporate  $^{15}\text{N}$ -enriched fish meal and  $^{13}\text{C}$ -reduced cereals that are major constituents of fish feed.

3) Uede T., and T. Takeuchi. 2007. Postprandial changes in digesta, leftovers and feces, and their carbon and nitrogen contents in cultured red sea breams, *Pagrus major*. *Aquaculture Science*, Vol:55:3. Pp. 409-415.

For establishing a sustainable aquaculture for red sea bream, we examined postprandial changes in digesta and suspending matter discharged as leftovers and feces. Gastral digestion for extruded pellet (EP) needed 24 h in the fish weighing 158 g at water temperature range of 20.3-26.6°C. Enteral digestion for EP almost ceased at about 72 h after feeding. The C/N ratio of suspending matter was similar to EP until 3 h after feeding, and rapidly rose after then. This suggested that suspending matter until 3 h after feeding and after then was originated from leftovers and feces, respectively. EP given was discharged as leftovers and feces ranges of 3.6-15.4 and 4.1-14.7%, respectively. Moist pellet (MP) given was also discharged as leftovers and feces ranges of 10.1-19.2 and 2.6-10.8%, respectively. Feed intake, leftovers and feces of MP were 1.9, 3.5 and 1.5 times greater than those of EP, respectively. Until 3 h after feeding EP, N in leftovers was at the range of 48.3-57.4% of total N discharged. Thereafter, it is intensely

recommended that preferable feeding restriction with EP is effective for conducting nitrogen load reduction and sustainable aquaculture for red sea bream. [In Japanese with English summary]

## MEASUREMENT OF SWIMMING BEHAVIOR AND DIGESTIVE PROCESS OF CULTURED FISH

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The biotelemetry method, which can measure fish behavior and physiological conditions such as body temperature and cardiac beat, is a useful tool for aquaculture to manage health and feeding cultured fish. In this study, we introduce two topics which are about monitoring of cultured Pacific bluefin tuna (PBT) using micro data-logger. (I) Using the acceleration data logger, swimming depth, tail beat and body angle of PBT were monitored in two different sizes of net cage. The monitored data indicated influence of net case size on cultured PBT behavior. The swimming behaviors of PBTs were apparently different between in the large and small net cages. In the small net cage, the PBT kept their swimming speed slowly, and frequently change the body angle in nighttime. In contrast, the PBT in the large net cage swim faster and body angle was stable. (II) We also monitored abdominal cavity temperature of PBT to estimate basal physiological condition in a net cage. The PBT maintains higher body temperature in several degrees than ambient water temperature. However, the body temperature was changed by the feeding and temperature fluctuation. The results suggested that the abdominal cavity temperature fluctuation was influenced by the ambient temperature, swimming activity, and food digestion and assimilation.

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Bluefin tuna maintain a higher body temperature than ambient sea water. Body heat is derived mainly from metabolic heat to elevate and maintain regional body temperature that is higher than the ambient, while heat loss is caused by heat transfer throughout the whole body surface and gills. Retention of high body temperature is thought to differ at each growth stage, so that a larger body mass maintains a higher body temperature. We evaluated the whole-body heat transfer coefficient, thermal difference between each tissue and water temperature, and metabolic heat in tissues during swimming of juvenile bluefin tuna as a function of fork length (FL) using a small thermometer and a treadmill-type flow tank. A system for maintaining high body

temperature was well developed in fish with FL greater than 20.0 cm. Whole-body heat transfer coefficient was fitted to a  $-0.695$  power of mass. Juvenile bluefin tuna showed a transition speed of 3.0 FL/s at which they switched from aerobic to anaerobic motion.