2010 Korea-Japan, Japan-Korea Joint Symposium on Aquaculture

“Eco-friendly Aquaculture and Branding Aquaculture Industry”

(9th Korea-Japan, Japan-Korea Joint Symposium on Aquaculture)

Program and Abstracts

2010 韓日・日韓 水産増養殖 SYMPOSIUM
(第9回韓日・日韓水産増養殖シンポジウム)

プログラム・講演要旨集

November 13, 2010
Busan Exhibition & Convention Center (BEXCO), Busan, Korea

Organized by
Aquaculture Chapter of the Korean Society of Fisheries and Aquatic Science (KOSFAS)
Japanese Society for Aquaculture Research (JSAR)
Presentation Program

November 13 (Saturday)

09:00 – Registration (2nd Floor, Multipurpose hall 202 – 1&2, Bexco)

09:30 – 10:00 Opening Ceremony

10:00 – 12:00 Special Lectures

14:00 – 16:50 Oral Presentation

16:50 – 18:00 Poster Presentation
Opening Address

Good morning, distinguished invited speakers, Dr. Yukinori Takahashi who is taking place of Dr. Ken-ichi Yamamoto, President, Japanese Society for Aquaculture Research (JSAR), Dr. Jeong-Yeol Lee, President, the Korean Society of Fisheries and Aquatic Science (KOSFAS), and Symposium participants, organizing committee members, Ladies and Gentlemen!

As the Aquaculture Chapter President of KOSFAS it is an honor and a privilege to give my opening address in this valuable and very important Symposium between the two countries. Welcome to Busan, Korea.

First of all, I would like to welcome again, on behalf of all the Korean participants, especially, Aquaculture Chapter members of KOSFAS, I would like to extend my warm welcome to Busan for all Japaness participants to attend this symposium. And, I thank you all of your JSAS leadership by hard work behind the symposium and successful cooperation with the leadership of our Aquaculture Chapter, KOSFAS to organize the last official symposium between the two organizations. As we know this symposium is organized by JSAS and Aquaculture Chapter of KOSFAS. These two organizations would be one of the best scientific organizations in the area of aquaculture in Japan and in Korea. Today's professional Symposium will provide the exchange of expert views and innovative ideas. I am sure that this symposium will accomplish the goals to exchange our new information in the view of two countries aquaculture industry.

For the 21st century, one of the most important challenges of Aquaculture Industry around the world would be “Environmentally Friendly Aquaculture” and “Branding Aquaculture Industry”. Therefore, today’s symposium title is on “Eco-friendly Aquaculture and Branding Aquaculture Industry”. Aquaculture professionals from all over the world have committed the same challenges for a long time to the progressive development of eco-friendly and branding global aquaculture industry through science, technology, education, and information exchange. Even though this symposium would be the last one between the two organizations, but our spirit and our theme will last forever.

In conclusion, Busan is a very beautiful metropolitan city on the south cost of Korean Peninsular, and it can provide you with lots of sea food dishes and Korean cultures. I hope that not only you can participate for the symposium but also you can enjoy the Korean traditional and sea food dishes and cultures. So that you can take lots of fond memories with you on the way back to your home. I wish that your stay in Busan will be a rewarding and pleasant one.

Thank you very much!

Sungchul C. Bai,
President, Aquaculture Chapter of KOSFAS
Professor/Director: Dept. of Marine Bio-materials and Aquaculture/Feeds and Foods Nutrition Research Center (www.ffnrc.com) at Pukyong Nat’l Univ.
SPECIAL LECTURE PROGRAM and ABSTRACTS

10:00 – 10:40  S-1  Historical trail and present status of marine finfish aquaculture in Japan and the branding strategy
Osamu Murata (Fisheries Laboratory of Kinki University)

10:40 – 11:20  S-2  Greenhouse gas (GHG) emissions reduction using seaweed
Ik Kyo Chung (Div. Earth and Environmental System, Pusan National University)

11:20 – 12:00  S-3  Development of environmental friendly aquaculture with closed recirculation techniques
Kotaro Kikuchi (Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry)
Historical trail and present status of marine finfish aquaculture in Japan and the branding strategy

Osamu Murata
(Fisheries Laboratory of Kinki Univ.)

Marine finfish aquaculture in Japan started in 1928 using yellowtail *Seriola quinqueradiata*, but it was not in widespread use due to the low productive efficiency. Since then, many efforts and technical innovation have been introduced into the aquaculture, and now it has become a main sector in the Japan fisheries. However, the aquaculture economy is stagnant nowadays in Japan. It is time to consider about the equilibrium between supply and demand, and to supply safety products to get the confidence from consumers. Moreover, branding strategy is also a critical part for a stable aquaculture operation.

1. Historical trail of marine finfish aquaculture in Japan

In 1927, Mr. Wasaburo Noami constructed an artificial pond by building a bank at small bay in Hiketa, Kagawa prefecture, and reared young yellowtail in the pond. This was the first finfish aquaculture in Japan. This banking method was not in widespread use due to the high cost and low productive efficiency. In 1954, Dr. Teruo Harada of Kinki University commenced to develop net-cage aquaculture of yellowtail, and he succeeded the cage culture in 1956. His cage culture method outperformed other methods and was widely used throughout the nation. Annual yield of yellowtail increased rapidly from 363 tons in 1958 to 154,872 tons in 1978. The aquaculture production of yellowtail has kept for more than 30 years. Therefore, Kinki University started selective breeding for rapid growth in red sea bream *Pagrus major* in 1964, and succeeded to ship the rapid growth strain to the fish farms from 1973. This red sea bream strain is used for the commercial aquaculture production until now.

2. Present status of marine finfish aquaculture in Japan

The aquaculture yield of amberjack including yellowtail amberjack and greater amberjack *Seriola dumerili* is the highest for the past 3 decades. In 2008, the yield was 158,600 tons. The second highest yield was red sea bream, 71,000 tons. Then followed by coho salmon *Oncorhynchus kisutch*, Pacific blufin tuna *Thunnus orientalis* (PBT), Japanese flounder *Paralichthys olivaceus* (JF), tiger puffer *Takifugu rubripes* and striped jack *Pseudocaranx dentex*. The aquaculture yield of PBT increased 10-fold in a decade, but that of JF decreased by half.

3. Branding strategy

In recent years, aquaculture economy is stagnant due to the low market price of fish, the rising prices of fish diet and labor cost, etc. So, cost down is the high-priority issue now. On the other hand, environment friendly, conservation of natural resources, and disease prevention are required for a sustainable aquaculture.

Introduction of traceability system, and quality control by management system such as HACCP (Hazard Analysis and Critical Control Point), ISO (International Organization for Standardization), SQF (Safe Quality Food), Organic JAS (Organic Certification of Japanese Agricultural Standard) are very important for a sound management in aquaculture industry. These traceability and quality control systems are possible to make the safety aquaculture products as a reliable brand.
The importance of ‘Blue Carbon’ in the global carbon cycle and the critical information on the carbon sequestration by the natural coastal vegetations in coastal ecosystems have been emphasized. Seaweeds as Blue Carbon are considered to be model organisms in research related to global warming because they fix a prodigious quantity of CO2. As seaweeds are proper and promising organisms for use in concert with adaptation and mitigation measures in the context of climate change, the Korean project - Algae and Global warming (AGW): Greenhouse Gas Emissions Reduction Using Seaweed - funded by the Ministry of Land, Transport and Maritime Affairs, has been ongoing since June 2006 (http://agw-seaweed.org/). In order to institute a practical seaweed mitigation and adaptation measures, the concept of the Coastal CO₂ Removal Belt (CCRB) has been newly developed and a pilot seaweed mitigation and adaptation farm is built and in operation. The Asian Pacific Phycological Association has launched a Working Group - ‘The Asian Network for Using Algae As a CO₂ Sink’ - for the purpose of collaborative R & D on the use of algae to remove CO₂. Recent development of a red algal pulp could provide an alternative to the use of trees and will thereby minimize further deforestation. Seaweeds can also be converted to useful fuels, including ethanol, which decrease our reliance on fossil fuels. As seaweeds have been recognized for their utility as CO₂ sinks, sustainable seaweed aquaculture has become an important prerequisite, and therefore, a seaweed based Integrated Multi-Trophic Aquaculture (IMTA) could be a practical way to synthesize a CCRB in coastal waters. In conclusion, seaweeds are promising organisms as adaptation and mitigation measures against global warming: Seaweed Solution; (1) Seaweed sink, (2) Red algae paper, and (3) Seaweed biofuel.
Development of environmental friendly aquaculture with closed recirculation techniques

Kotaro Kikuchi
(Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry)

Increasing productivity without serious impact on the natural environment is believed to be essential for sustainable development of aquaculture. Several efforts have been carried out to develop effective technologies. Fish production with closed recirculation system, which makes reduced discharge of organic and inorganic wastes possible, is considered to be one of the promising approaches. Closed aquaculture also has advantages of high productivity by preventing disease out-breaking, controlling culture environment, and receiving little damage from typhoons and red tides, and is considered to be highly traceable.

Closed recirculation system consists of fish tank, settling tank, mechanical and biological filters, heating-cooling unit, oxygen generator and supplier, blower, and circulation pump. UV and ozone are available for disinfection and degradation of organic substances in the culture water. The closed system must be designed and operated specifically to maximize the productivity based on the requirement of producing fish species. Development of closed recirculation techniques for Japanese flounder and tiger puffer, major marine aquaculture species in Japan, has been conducted since 1986. Research efforts were mostly focused on biological information of target fish such as optimum temperature and salinity for the growth, proper stocking density, urine and feces excretion, oxygen consumption, effective feed composition and etc. Feeding experiments with properly designed and operated system showed that tiger puffer can be grown to 1 kg size during 18 months with 90% survival and stocking density of 30 kg/m³ culture water with the closed system, much higher than for net cage aquaculture.

Utilization of fish feces and dissolved nitrogen in culture water with invertebrates and algae were tried for further decrease of aquaculture wastes. Polychaeta Perineris nuntia vallata is considered to be one of the promising species for feces treatment and the worm fed Japanese flounder feces retained 63% in their body, and excreted 21% and 16% as feces and ammonia, respectively, on nitrogen basis. Daily feeding rate of the feces was estimated to be 31.6 mg/g BW at 20°C. There seems to be technical issues to grow polychaeta practically in the closed system. Water quality changes considerably through biological and physicochemical treatments of excreted ammonia and organic substances in closed aquaculture. Accumulation of nitrate, phosphate and yellow substance are major characteristics of recycling culture water. Nitrate accounted for 40 to 60% of consumed feed nitrogen in Japanese flounder, and is seemed to inhibit feeding of fish at more than 600 mg-N/l. Utilization of nitrate and phosphate in the culture water with hydroponics of tomato and lettuce was tried for fresh water aquaculture to improve profitability. Nitrate fixation by Ulva sp. was examined for seawater system on laboratory scale, and absorption rate was 0.3 mg-N/g algae per day at 20°C under 3,000 lx fluorescent light. Ulva production was estimated to be 3 times the fish biomass when all of the nitrogen excreted from Japanese flounder was fixed with the algae.
ORAL PRESENTATION PROGRAM and ABSTRACTS

14:00 – 14:25  O-1  Effects of dietary inclusion of fermented soybean meal on the growth, body composition and immune responses of rockfish *Sebastes schlegeli*
Sang-Min Lee (Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University)

14:25 – 14:50  O-2  Bacterial diseases of marine fish and development of vaccine in Japan
Yukinori Takahashi (Department of Applied Aquabiology, National Fisheries University)

14:50 – 15:15  O-3  Development and characterization of EST-linked microsatellite markers for Manila clam *Ruditapes philippinarum*
Hyun-Sil Kang (Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University)

15:35 – 16:00  O-4  Life history and conservation of a catadromous sculpin *Trachidermus fasciatus* in Japan
Naohiko Takeshita (Department of Applied Aquabiology, National Fisheries University)

16:00 – 16:25  O-5  Feasibility study on offshore tuna aquaculture in Korea
Seung Cheol JI (Future Aquaculture Research Center, NFRDI)

16:25 – 16:50  O-6  Seasonal change in photosynthetic capacity of *Ecklonia kurome* Okamura (Phaeophyta) in the western Seto Inland Sea, Japan
Noboru Murase (Department of Applied Aquabiology, National Fisheries University)
Effects of dietary inclusion of fermented soybean meal on the growth, body composition and immune responses of rockfish *Sebastes schlegeli*

Sang-Min Lee, Ki-Min Bae and Hyun Ho Park
(Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, 1CJ Cheiljedang Corporation)

Introduction

Rockfish (*Sebastes schlegeli*) is a commercially important mariculture fish species in Korea. Rockfish aquaculture production in Korea has increased for the last decade and reached approximately 40,000 tons in 2009, and will increase rapidly in coming years.

Protein is the most expensive nutrient component in feeds and significantly affects growth performance of fish. Due to the shortage and expensiveness of fish meal, various protein sources including soybean meal, canola meal and lupin meal have been investigated and reported as potential alternatives for fish meal in feeds for fish. In addition, fermented protein source such as soybean meal could be used as a feed ingredient for growth of fish. Therefore, this experiment was to investigate the effects of dietary inclusion of fermented soybean meal (Soytide, CJ Cheiljedang Corporation) as an alternative for fish meal on the growth, body composition and immune responses of rockfish.

Materials and Methods

Three replicate groups of rockfish (averaging weight 148 g) were fed one of the five experimental diets (designated as ST0, ST8, ST16, ST24 and ST32) containing different fermented soybean meal levels (0, 8, 16, 24 and 32%) for 8 weeks.

Results and Discussion

Survival, weight gain, feed intake, feed efficiency, protein efficiency ratio, morphological parameters (condition factor, hepatosomatic index and visceralsomatic index), blood chemistry and chemical composition of rockfish were evaluated at the end of the feeding trial. Survival was over 90% and not affected by dietary fermented soybean meal levels. There were no significant differences in weight gain, feed intake, feed efficiency, protein efficiency ratio, condition factor, hepatosomatic index and visceralsomatic index. Proximate analysis and amino acid composition of muscle in rockfish were not affected by dietary fermented soybean meal levels. Superoxide dismutase (SOD) activity of plasma of rockfish fed the diet containing fermented soybean meal was significantly higher than that of fish fed the control diets. Glutathione peroxidase (GPx) activity of plasma of rockfish fed the diet containing 16–32% fermented soybean meal was significantly higher than that of fish fed the diets containing 0–8% fermented soybean meal.
Bacterial diseases of marine fish and development of vaccine in Japan

Yukinori Takahashi\(^1\), Kohei Fukuda\(^1\), Masakazu Kondo\(^1\), Shinya Yasumoto\(^1\)
Ikuo Hirono\(^2\) and Takashi Aoki\(^2\)
\(^1\)Department of Applied Aquabiology, National Fisheries University, \(^2\)Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology


A commercially available *L. garvieae* and *V. anguillarum* vaccine is used in yellowtail and amberjack by oral administration or injection. *S. iniae* vaccine is used in olive flounder by injection. Recently, oil-based bivalent vaccine against *P. damsela* and *L. garvieae* infection was developed in yellowtail and amberjack.

We have developed pentavalent vaccine against streptococciosis and edwardsiellosis in olive flounder. Efficacy of pentavalent vaccine with formalin-killed *S. parauberis* (serotype 1 and 2 strain), *S. iniae* and *E. tarda* (motile and non-motile strain) were tested by vaccination followed by intramuscular challenge with the each pathogen. The olive flounder vaccinated with pentavalent vaccine showed higher resistance against the each pathogen on two weeks post-vaccination.

These results revealed that pentavalent vaccine was effective in prevention against streptococciosis and edwardsiellosis in cultureed olive flounder.

Development and characterization of EST-linked microsatellite markers for Manila clam *Ruditapes philippinarum*

Hyun-Sil Kang, Bong-Kyu Kim, Hyun-Ki Hong and Kwang-Sik Choi
(Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University)

Introduction

The Manila clam or short-neck clam (*Ruditapes philippinarum*) is an important aquaculture species that inhabits on sandy or muddy tidal flats along the coastal areas in the world. Although this species have transplanted into several countries for aquaculture purpose, it is faced with several problems such as deterioration of habitat, an increase of infectious diseases, overfishing and the production declines. To resolve these issues, researchers have studied on genetic traits to conserve and manage for shellfish resources *R. philippinarum*. In this study, we developed eleven EST-linked microsatellite markers originated from Manila clam hemocyte cDNA library and characterized the genotypes of 115 clams sampled from Korea, China, Japan and Hong Kong.

Materials and Methods

Nineteen microsatellite loci were obtained from EST database of *Perkinsus olseni* challenged Manila
clam’s hemocyte cDNA library analyzed by the screening of micro repeat motifs, and examined the PCR amplification for marker optimization. Eleven PCR verified markers were determined the polymorphism in 15 clams. Of the 19 markers, five markers (KR2, 6, 14, 17 and 19) showed a high allele number. Using these 5 markers, we investigated genetic distance and phylogeny to 115 clams. The genotypes were scored using Genescan and Genotyper (Applied biosystems). The number of alleles, observed heterozygosity, expected heterozygosity and Hardy-Weinberg equilibrium were calculated using POWER MARKER 2.0 software.

Results and Discussion
Of the 19 microsatellite loci, eleven were successfully amplified in PCR as we expected and the remaining eight were rejected due to the nonspecific or no PCR output. Three loci (KR1, KR17 and KR 19) of the 11 markers were not amplified in some individuals, indicating that the null alleles are present in Manila clam. The characteristics of 11 microsatellite loci are as followed; the numbers of alleles per locus ranged from 3 to 11 with an average of 6.5. The observed heterozygosity was 0.0667 to 0.37692, while expected heterozygosity was 0.3571 to 0.8843. Significant departures from Hardy Weinberg equilibrium were found in two loci KR12 and KR17.

The genetic distance and phylogeny of 5 markers (KR2, 6, 14, 17 and 19) showed that Boryoung (Korea) and Yentai (Xiamen) was a phylogenetically closest than other populations. This finding suggested that the Boryong population could be a transplanted strain derived from China. The EST-linked microsatellites used in this study were found to be a useful resource for population genetic of *R. philippinarum*.

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**O-4**

**Life history and conservation of a catadromous sculpin**

*Trachidermus fasciatus* in Japan

Naohiko Takeshita

(Department of Applied Aquabiology, National Fisheries University)

In Japan, the catadromous roughskin sculpin *Trachidermus fasciatus* occurs only in Ariake Bay and the rivers flowing into this water bodies. Elsewhere, this species is found along the southern and western coasts of the Korean Peninsula, and the eastern coast of China.

The growth and migration of this fish were investigated using a mark-recapture method in the Kashima River, in Kyushu Island, Japan. Seasonal changes in total lengths of captured and recaptured individuals indicated an annual growth of ca. 130–190 mm TL, with a period of growth stagnation in summer. Because only one population mode was apparent, this sculpin is thought to have a single-year life span. Individuals showed primarily upstream migration between May to July, eleven recapture individuals having moved upstream during this period. From August to October, almost all recaptured individuals were taken at the same sites. Subsequently, all sculpins disappeared in the river by January.

The spawning grounds of this fish were investigated in the mud flat near the mouth of the Kashima River from January to March. The nests were found about 1.1–2.3 km off the mouth of this river, where the bottom salinity was 8-21 ppt at low tides. The empty Oyster shells were mainly used as their nests. The average number of guarded egg clusters per male in shells was 1.3 ± 0.4.

Recently, the empty oyster shells for the spawning nest of this sculpin were decreasing. We are setting the artificial nests in the mud flat, for conservation of *T. fasciatus*. 
Feasibility study on offshore tuna aquaculture in Korea

Kyong Min Kim, Seung Cheol JI, Jun Ho Koo, Nack Jung Choi and Seok Jung Han
(Future Aquaculture Research Center, NFRDI)

Introduction

The study is aimed to achieve stable supply of tuna and industrialization of tuna farming through Korean-typed offshore aquaculture technology, establish eco-friendly aquaculture system and advanced aquaculture engineering technology by relocating aquaculture farms from coastal to offshore waters and lay a foundation for the stable tuna culture industry through management of captive bluefin tuna broodstock.

Materials and Methods

We conducted feasibility study on the offshore culture by analyzing the biological and ecological characteristics of tuna, developed technology for catch, transportation, domestication and offshore cage rearing of tuna, conducted offshore cage maintenance and carried out environmental assessment for the optimal farming site selection.

Results and Discussion

Pacific bluefin tuna is evaluated as the optimal tuna species of offshore cage culture as it is priced high, grows fast and migrates to the Korean coastal waters, making it easy to obtain juveniles needed for farming. Its fast growth and dynamic swimming pattern requires a large-scaled farming system. Also to be the optimal farming site, it should have well flowing of tide and currents and be free of red-tide. The Pyosun and Hanrim, Jeju was selected as the optimal farming site as it was assessed to have more favorable farming conditions like warm water temperature of 14°C in winter, high transparency, less suspended solid and high DO. As for other candidate sites, Yokji-island of Tongyoung and Gujora of Geoje were assessed to be vulnerable to red-tide, Guryongpo of Pohang city vulnerable to hypoxic water mass and Geomoon-island of Yeosu to be less accessible.

We reviewed on the supply channel of juveniles needed for farming. Juveniles can be obtained through trolling, purse seine and import, though trolling is the most preferred as it costs less while offers a very high change for success. As such, trolling was evaluated as the most effective channel of seed supply with a potential to boost the income of fishing community. We captured 833 0-year juveniles through trolling and 371 among which were contained in the offshore cage. It was judged that securing a large amount of young tuna is practicable. The tuna juveniles captive in the offshore cage were caught in 2009 and fed by raw fish. As of June 2010, the survival rate was 91%. The growth investigation was conducted by the dual-frequency identification sonar and stereo camera, which enabled effective growth investigations with no direct contact to the body of fish and no stress on it.

The open-type offshore cage is evaluated as eco-friendly as it is not affected by sea currents, being free of land-based pollution and dealing with pollutants in a very fast way. Meanwhile, the coastal cage is not only susceptible to land-based pollution but has less capacity to handle the pollutants, leaving the land-based wastes accumulated around the farming cages.
Seasonal change in photosynthetic capacity of *Ecklonia kurome* Okamura (Phaeophyta) in the western Seto Inland Sea, Japan

Noboru Murase\(^1\), Mikio Noda\(^1\), Mahiko Abe\(^1\), Goro Yoshida\(^2\) and Kenji Tarutani\(^2\)

\(^1\)National Fisheries Univ. \(^2\)FRA, Natl. Res. Inst. Fisheries and Environment of Inland Sea

*Ecklonia kurome* Okamura (Phaeophyta) is a perennial species and is distributed from Chiba Prefecture located at central Honshu to Kyushu in the Pacific Oceans, from central Honshu to Kyushu in the Sea of Japan and the Seto Inland Sea, Japan. This species forms dense population called *Ecklonia* beds, which plays a major role as a primary producer in coastal ecosystems, and are important as nursery grounds for coastal fishes and other animals. The present study was carried out to clarify the photosynthetic capacity which is basis of growth, and the seasonal change in photosynthesis-light relationships of *E. kurome*.

The new bladelets of *E. kurome* for photosynthetic and respiratory measurements were collected from the population at 2-4m depth off Mashima Island, Yamaguchi Prefecture in the western Seto Inland Sea. Measurement of photosynthesis and respiration were carried out at six different photon irradiances (0, 12.5, 25, 50, 100, 200 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\)) at *in situ* water temperature with a Productometer, an improved differential gas-volumeter.

The monthly photosynthesis-light relationships of *E. kurome* were fitted using a hyperbolic tangent function. The photosynthetic rate of *E. kurome* showed a minimum value of 20 \(\mu\text{L O}_2\text{ cm}^{-2}\text{ h}^{-1}\) in February, thereafter increased gradually and reached a maximum value of 32 \(\mu\text{L O}_2\text{ cm}^{-2}\text{ h}^{-1}\) in September. The respiratory rates ranged from 1.9 to 4.9 \(\mu\text{L O}_2\text{ cm}^{-2}\text{ h}^{-1}\). It was suggested that the seasonal changes of respiratory rates correlated to not only the water temperatures but also the maturity of *E. kurome*. The present study provides fundamental data on physiological ecology of *E. kurome* and productivity of the population.
Poster PRESENTATION

P-1 Flavor is important for eating — feeding stimulants and deterrents in oranges for oriental weatherfish—
○ Taiko Miyasaki
(Department of Food Science and Technology, National Fisheries University)

P-2 Morphological and cytochemical characteristics of neutrophil from chub-mackerel
Scamber japonicus
○ Masakazu Kondo, Shinya Yasumoto and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University)

P-3 Granules of neutrophil in devil stinger Inimicus japonicus
○ Masakazu Kondo, Shinya Yasumoto, Itaru Ikeda and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University)

P-4 Development of a low-cost Fishway for various animals
○ Akira Araki¹ and Takenori Ozawa²
(¹ National Fisheries University, ² Yamaguchi Prefecture, River Section)

P-5 Blood acid-base balance in the pearl oyster Pinctada fucata martensii under hypoxic conditions
○ Takeshi Handa and Ken-ichi Yamamoto
(Department of Aquabiology, National Fisheries University)

P-6 Oral immunization of common carp with a liposome vaccine containing pathogenic antigen
○ Shinya Yasumoto, Masakazu Kondo and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University)

P-7 Porphyra spheroplast as a dietary protein source for fish: A review
○ Takao Yoshimatsu
(Graduate School of Bioresources, Mie Univ.)

P-8 Fertility of cryopreserved spermatozoa of the Japanese eel Anguilla japonica
○ Hiromi Ohta¹, Daiki Hamada¹, Ivan Chong Chu Koh¹, Daisuke Tanaka¹, Ken-Ichi Yokoi¹, Kazuharu Nomura² and Hideki Tanaka²
(¹ Department of Aquaculture, Graduate School of Agriculture, Kinki Univ., ² National Research Institute of Aquaculture)

P-9 Effects of cooling rate on post-thaw motility and fertility of spermatozoa from the Japanese pearl oyster Pinctada fucata martensii
○ Kahori Arita¹, Kiyoshi Isowa², Takashi Ishikawa², Hideo Aoki³ and Hiromi Ohta¹
(¹ Graduate School of Agriculture, Kinki Univ., ² Mie Prefectural Fish Farming Center, ³ Mie Prefecture Fisheries Research Institute)

P-10 The development of cryopreservation method for seven band grouper sperm with CASA evaluation
○ Ivan Chong Chu Koh¹, Ken-ichi Yokoi¹, Masaharu Tsuji², Yasushi Tsuchihashi² and Hiromi Ohta¹
(¹ Department of Fisheries, Graduate School of Agriculture, Kinki University, ² Owase Fisheries Research Laboratory, Mie Prefecture Fisheries Research Institute)

P-11 Microbial communities in the eelgrass bed of the Izu Peninsula, Japan
○ Chia-Hui Chen, Terukuni Kozaki, Yusuke Tanaka, Shiro Ito, Kiyoshi Yoshihara and Haruo Sugita
(Department of Marine Science and Resources, Nihon University)
P-12 The AHL-producing bacteria in the intestinal tracts of ayu \textit{Plecoglossus altivelis}  
\(^\circ\)Masayuki Ogawa, Shiro Itoi and Haruo Sugita  
(Department of Marine Science and Resources, Nihon University)

P-13 Microflora of \textit{Artemia} nauplii hatched under experimental conditions  
\(^\circ\)Haruka Kurihara, Hitomi Nimura, Konomi Sekita, Yusuke Tanaka, Shiro Itoi and Haruo Sugita  
(Department of Marine Science and Resources, Nihon University)

P-14 Microbial communities in the intestinal tracts of coastal fish  
\(^\circ\)Yusuke Tanaka, Chia-Hui Chen, Terukuni Kozaki, Shiro Itoi and Haruo Sugita  
(Department of Marine Science and Resources, Nihon University)

P-15 Effects of suspended sediment on stress hormone response in wild and cultured strains of ayu \textit{Plecoglossus altivelis}  
\(^\circ\)Satoshi Awata\(^1\), Hirohiko Takeshima\(^2\), Tetsuya Tsuruta\(^1\), Takashi Yada\(^3\) and Kei'ichiro Iguchi\(^1\)  
(\(^1\)Ueda Station, Freshwater Fisheries Res. Div., NRIFS, FRA, \(^2\)Atmosphere and Ocean Res. Inst., the Univ. of Tokyo, \(^3\)Nikko Station, Freshwater Fisheries Res. Div., NRIFS, FRA)

P-16 The preventive effect of oral administration of the bacteria derived from red sea bream \textit{Pagrus major} intestine on edwardsiellosis in red sea bream  
\(^\circ\)Keitaro Kato, Wataru Takahashi, Kensuke Tsukahara, Yumina Ishikawa, Yuki Morisaki, Katsuya Ishimaru and Osamu Murata  
(Fisheries Laboratory of Kinki Univ.)

P-17 Cells and the total number of principal ducts of digestive diverticula in Japanese scallop \textit{Patinopecten yessoensis}  
Susumu Asano, Masahiro Ebine, Yuji Mori and \(^\circ\) Takeshige Matsutani  
( Faculty of Science and Engineering, Ishinomaki Senshu University)

P-18 Effects of salinity on clove oil and lidocaine-HCl anesthesia in the marine medaka \textit{Oryzias dancena}  
In-Seok Park\(^1\), \(^\circ\)Hyun Woo Gil\(^1\), Yoon Kwon Nam\(^2\) and Dong Soo Kim\(^2\)  
(\(^1\)Division of Marine Environment and Bioscience, \(^2\)College of Ocean Science and Technology, Korea Maritime University)

P-19 Effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder \textit{Paralichthys olivaceus} at suboptimal temperature  
\(^\circ\)Young Jin Cho and Sung Hwoan Cho  
(Division of Marine Environment & Bioscience, Korea Maritime University)

P-20 Effects of various concentrations of \textit{Scutellaria baicalensis} extract in the diets on growth, body composition and immune response of juvenile olive flounder (\textit{Paralichthys olivaceus})  
\(^\circ\)Kyoung Tae Kim, In-Cheol Choi, Young Jin Cho and Sung Hwoan Cho  
(Division of Marine Environment & Bioscience, Korea Maritime University)

P-21 Evaluation of Protide\(^®\), a commercial nucleotide product, in diets for olive flounder (\textit{Paralichthys olivaceus}) and red sea bream (\textit{Pagrus major})  
\(^\circ\)Jin-Woo Song, Se-Jin Lim, Sung-Sam Kim, Jae-Hyeong Shin, Jin-Dong Kim\(^1\), Jung-Un Kim\(^1\) and Kyeong-Jun Lee  
(Department of Marine Life Science, Jeju National University, Department of Global Marketing, CJ CheilJedang Corporation)

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\(^\circ\) Sung-Sam Kim\(^1\), Ji-Hoon Cha\(^1\), Jin-Woo Song\(^1\), Kang-Woong Kim\(^2\), Maeng-Hyun Son\(^2\) and Kyeong-Jun Lee\(^1\)  
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○ Jeong Dae Kim¹, Se Min Choi², Seung Jun Shin², Bong Soo Lim³ and Jong Pyo Seo⁴
(¹Department of Animal Life System, Kangwon National University, ²Feed/Animal Research Institute, CJ JeilJedang Corporation, ³Marine and Environmental Research Insititute, Jeju National University, ⁴AquaGenoTech Co.)

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\(^{1}\)National Fisheries Research and Development Institute, \(^{2}\)Pukyong National University, \(^{3}\)Jeju National University

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\(^{\circledast}\) Kyong-Duck Kim, Jin Do Kim, Sang Gu Lim, Kang-Woong Kim and Maeng Hyun Son
(Aquafeed Research Center, National Fisheries Research and Development Institute)

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\(^{\circledast}\) Shin-Kwon Kim, Kyong-Duck Kim, Kang-Woong Kim and Maeng-Hyun Son
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\(^{\circledast}\) Hye Jung Lee and Sung Bum Hur
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\(^{\circledast}\) Hee Woong Kang, Duck Young Kang, Sang-Ho Baek and In Kwon Jang
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\(^{\circledast}\) Sang-Min Lee\(^1\), Joo-Young Seo\(^1\) and Gyu-Doek Hwang\(^2\)
\(^{1}\)Faculty of Marine Biosciences and Technology, Gangneung-Wonju National University, \(^{2}\)Inland Fisheries Research Institute, Chung Cheong Buk-Do

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\(^{1}\)Dept. of Aquaculture / Feeds and Foods Nutritional Research Center, Pukyong Nat’l University, \(^{2}\)Aquaculture Management Division, National Fisheries Research and Development Institute

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(¹Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, ²Marine Living Resources Research Department, Korea Ocean Research and Development Institute, ³Pearl Oyster Domestication Laboratory, IFREMER, French Polynesia)

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(¹Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, ²West Sea Fisheries Research Institute, National Fisheries Research and Development Institute (NFRDI), ³NFRDI)

P-50  Effects of artificial factors on the activity of storage sperm of wild-caught striped jewfish *Stereolepis deoderleini*
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(Department of Marine Bio-Materials and Aquaculture, Pukyong National University)

P-51  Effects of environmental factors on the activity of cryopreserved sperm of wild-caught striped jewfish *Stereolepis deoderleini*
Young Soo Kim¹, Young Sin Ko², Minh Hwang Le¹, Ki Tae Kim¹ and Young Jin Chang¹
(¹Department of Marine Bio-Materials and Aquaculture, Pukyong National University, ²Gyeongsang Nam-do Fisheries Institute)

P-52  Properties and activities of sperm contaminated with blood or seawater of wild-caught striped jewfish *Stereolepis deoderleini*
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P-53 Comparison of aging methods and growth for largemouth bass *Micropterus salmoides* in Goe-san Lake, Korea

*Ming-Ming Zhang, Chul-Woong OH, and Jong-Hun Na
(Department of Marine Biology, Pukyong National University)

P-54 Age determination and growth characters of the yellow catfish *Pelteobagrus fulvidraco* in Goe-san Lake, Korea

*Ming-Ming Zhang, Jong-Hun Na, and Chul-Woong OH
(Department of Marine Biology, Pukyong National University)

P-55 Estimation of optimum dietary organic copper (MINTREX®) levels in juvenile olive flounder (*Paralichthys olivaceus*)

*Mohseni, M.1, Park, G.H.1, Lee, J.H.1, Lee, S.1, Hwang, N.Y.1, Liu Yi, Yun1, Y.H.1, Lee, J.H.1, Yun, H.H.1, Okorie, O. E.1, Bae, J.Y.1, Browdy, C.2, Bharadwaj, A. S.2 and Bai S.C.1
(1Department of Aquaculture/feeds & foods Nutrition Research centre, College of Fisheries Science, Pukyong National University, 2Novus International, 20 Research Park Drive, St. Charles, MO 63304 USA)
Flavor is important for eating
—feeding stimulants and deterrents in oranges for oriental weatherfish—

Taiko Miyasaki
(Department of Food Science and Technology, National Fisheries University)

Objective
Water extracts of many fruits, vegetables and flowers attract fish and abalone. Attractive fruitages, vegetables and flowers are, for example, apple, Japanese medlar, onion and abelia for yellowtail, strawberry, cabbage and *Elaeagnus multiflora* for oriental weatherfish, cherry, soybean and *Camellia japonica* for black abalone. Oriental weatherfish is attracted by unshiuorange but not by iyoorange. There is little information on chemical attractants and stimulants in these samples. Therefore, feeding stimulating activity of several oranges for oriental weatherfish was investigated.

Methods
Twenty oriental weatherfish (*Misgurnus anguillicaudatus*) were placed in each of six glass test tanks. The test and control diets (2.0 g each) were placed in two bent glass tubes. The two tubes were fixed on the gravel bed of the tank to compare the feed ingestion of the test diet with that of the control. Average ingestion of the 6 tanks was measured to estimate the feeding stimulation activity for the fish. The test and control diets were formulated based on casein protein. The test diets were added with freeze dried whole oranges, *Citrus natsudaidai* sp., *C. iyo* sp., *C. hassaku* sp. and *C. limon* sp., or authentic flavonoids. Contents of major flavonoids in the oranges, hesperidin, neohesperidin, naringin and neohesperidin dihydrochalcone (NHDC), were analyzed with HPLC.

Results
Feeding stimulation activity was found in *C. limon* sp., whereas *C. natsudaidai* sp. and *C. hassaku* sp. suppress the feeding. Significant effect was not observed in *C. iyo* sp. Albedo of *C. limon* sp. and its extractive with dimethylformamide (DMS) showed high feeding stimulation activity. NHDC (300 µg/kg diet) and neohesperidin (85 mg/kg diet) showed feeding stimulation activity, whereas naringin (560 mg/kg diet) showed antifeedant activity. These findings showed that oriental weatherfish liked sweet (for human) flavonoid, NHDC and disliked bitter (for human) flavonoid, naringin. Preference for oranges was thought to be depended on the contents of stimulants and deterrents.

Morphological and cytochemical characteristics of neutrophil from chub-mackerel *Scomber japonicus*

Masakazu Kondo, Shinya Yasumoto and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University)

Morphological characteristics of neutrophil (phagocytic granulocyte) from chub-mackerel *Scomber japonicus* peripheral blood were examined using multiple Romanowsky-type stain valuation. Three types of granules, eosinophilic granule (αG), chromophobic granule (βG) and basophilic granule (γG) were observed in the cytoplasm of a single neutrophil. The αG was small and round in shape. The staining pattern of αG was influenced by pH, constituent and concentration of diluent of the staining solution. Eosinophil of αG was disappeared by long-time Giemsa stain after May-Grünwald. The βG was round to oval and was larger than αG. The γG was round to oval, smaller than βG and stained light blue. The γG was observed in Giemsa staining preparation, but not in May-Grünwald. Therefore, the color tone of the γG may be due to the orthochromatic reaction by azure B in Giemsa. These results indicate that the neutrophil of chub-mackerel belongs to the type 1 neutrophil similar to the neutrophil of Asian arowana (*Scleropages*...
formosus), common carp (Cyprinus carpio), Japanese eel (Anguilla japonica), Nile tilapia (Oreochromis niloticus), striped grunt (Parapristipoma trilineatum) and Japanese amberjack (Seriola quinqueradiata). Acid phosphatase, \( \beta \)-glucronidase, some eaterases (\( a \)-naphtyl acetate, \( a \)-naphtyl butyrare, naphthol AS-D chloroacetate) were detected in the neutrophil as granular positive. Peroxidase activity was observed in the \( \beta \)G.

Granules of neutrophil in devil stinger Inimicus japonicus

Masakazu Kondo, Shinya Yasumoto, Itaru Ikeda and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University)

Three types of granules, eosinophilic granule (\( a \)G), chromophobic granule (\( \beta \)G) and basophilic granule (\( \gamma \)G) were observed in the cytoplasm of a single neutrophil (phagocytic granulocyte) from devil stinger Inimicus japonicus. Therefore, the neutrophil belonged to type 1 neutrophil. However, staining characteristics of \( a \)G and \( \gamma \)G were different from those of typical type 1 neutrophil of Asian arowana, common carp, Japanese eel, Nile tilapia, striped grunt and Japanese amberjack. The \( a \)G of typical type 1 neutrophil was stained with May-Grünwald (MG), but not with Giemsa. However, the \( a \)G of devil stinger was stained with Giemsa, but not with MG and MG-Giemsa. The \( \gamma \)G of typical type 1 neutrophil was stained with Giemsa, but not with MG. However, the \( \gamma \)G of devil stinger was stained with not only Giemsa but also MG. From the difference between the devil stinger neutrophil and typical type 1 neutrophil, we classified the typical type 1 neutrophil and devil stinger neutrophil as new categories, type 1A neutrophil and type 1B neutrophil, respectively. Acid phosphatase and \( \beta \)-glucronidase were not detected in the devil stinger neutrophil. On the other hands, some eaterases (\( a \)-naphtyl acetate, \( a \)-naphtyl butyrare, naphthol AS-D chloroacetate) were detected in the neutrophil as granular positive. Peroxidase activity was observed in the \( \beta \)G.

Development of a low-cost Fishway for various animals

Akira Araki1 and Takenori Ozawa2
(1National Fisheries University, 2Yamaguchi Prefecture, River Section)

In Japan, many diadromous animals migrate for reproduction between the sea and the river in life history. They contain commercially important species of river fisheries, e.g. the ayu fish Plecoglossus altivelis, the Japanese eel Anguilla japonica, the Japanese mitten crab Eriocheir japonica.

Many artificial structures like weirs and dams are present in Japanese rivers and they become barrier walls for the migration. Most these structures have been modified fishways for big fish, e.g. salmon and trout. But those fishways are not suitable for small diadromous animals. We research on low-cost and simple method of construction to improve them with Yamaguchi Prefecture.

“Mizube-no-Kowaza” fishway developed form the joint research with Yamaguchi Prefecture is a slope type fishway with protruding boulders. “Mizube-no-Kowaza” means collection of the small scale devices for river improvement. Because the current economic situation is very bad, we cannot spend much money for repair dams and weirs. Therefore, we aiming to connect between fluvial upstream and downstream for aquatic organisms by accumulating “Mizube-no-Kowaza”. It can be constructed at low cost for its simple structure. “Mizuze-no-Kowaza” fishway is effective for increasing wild resource of these animals and can make network among habitats isolated by manmade structures.
Blood acid-base balance in the pearl oyster *Pinctada fucata martensii* under hypoxic conditions

Takeshi Handa and Ken-ichi Yamamoto
(Department of Aquabiology, National Fisheries University)

The pearl oyster *Pinctada fucata martensii* is used by pearl fisheries and is the most important species in Japan. In pearl aquaculture, a tissue fragment from the mantle lobe is implanted into the gonad of another pearl oyster, and pearl production ensues by a process of forming a pearl layer over the mantle lobe fragment. The process is the same as that of the growth of shell valves, and is directly related to metabolism. Oxygen is utilized during metabolic processes, and the carbon dioxide produced by the metabolism and calcium ion taken in from seawater form calcium carbonate (calcification for shell valves). The metabolism of the pearl oyster has been studied in relation to the regulation of ventilation volume, oxygen uptake and oxygen utilization. However, there are few previous studies on blood acid-base balance in respiration physiology. Therefore, we examined blood O2, CO2 and pH under hypoxic conditions. In order to analyze the blood acid-base balance, we determined the blood CO2 solubility coefficient and dissociation constant of carbonic acid, and calculated the CO2 partial pressure and concentration of bicarbonate ion. The experiments used 40 pearl oysters (mean total wet weight: 25.3 g). To collect blood from the animal while it is immersed in water, cannulation of the anterior aorta was conducted before 2hr inducing hypoxic conditions. After the first blood collection (O2 saturation: 100%), further blood collections were carried out under hypoxic conditions (O2 saturation: 10%). The blood pH, total CO2 concentration and O2 partial pressure were immediately measured after each blood collection. In order to calculate the blood CO2 partial pressure and the concentration of bicarbonate ion, the CO2 solubility coefficient and dissociation constant of carbonic acid were determined by the tonometer for equilibrating the blood with standard CO2 gases. Under hypoxic conditions, the blood O2 partial pressure decreased to the same level as that of environmental water. When the hypoxic condition continued, total CO2 concentration, CO2 partial pressure and the concentration of bicarbonate ion increased even though the blood pH decreased. Therefore, acidosis of the pearl oyster under hypoxic conditions was derived from hypoxemia, and was gradually compensated for by a rise in the bicarbonate ion.

Oral immunization of common carp with a liposome vaccine containing pathogenic antigen

Shinya Yasumoto, Masakazu Kondo and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries University.)

We made liposome vaccines in which formalin-inactivated pathogens was entrapped within the liposomal membrane compartment for oral vaccination in common carp. In this study, *Aeromonas hydrophila* and Koi herpesvirus (KHV) were used as a pathogen.

Oral administration of the vaccine was found to well-immunize common carp *Cyprinus carpio* against *A. hydrophila*. Two fish groups received the liposome vaccine (protein concentration, 33 μg/ml) at doses of 10 and 30 μl/fish/day for a period of 3 days. The levels of antigen-specific antibodies in the serum rose at 2 and 3 week post vaccination (p.v.) and then declined at 4 week p.v. Fish groups orally immunized with a total of 30 μl/fish for 3 days were challenged by an intracutaneous injection with live *A. hydrophila* at 22 days p.v., and the fish were protected against the bacterial infection.

In vaccination experiments for KHV, two fish groups (mean body weight, 30 g) received the
liposome vaccine (protein concentration of 20 μg/ml using an isolate of NKC03 from Nara Prefecture or an isolate of IKC03 from Ibaraki Prefecture) at a dose of 20 μl/fish/day for 3 days. Neutralization titers of vaccinated fish sera were significantly higher than control at 22 days after last vaccination. In addition, 22 days after last vaccination, the fish were challenged with NKC03 at 10^{2.3} TCID_{50}/100 μl/fish or IKC03 at 10^{2.2} TCID_{50}/100 μl/fish by dropping the inoculum onto their gills, and resulted RPS was 74.4% and 65.0%, respectively. These results proved the efficacy of oral vaccination with the KHV liposome vaccine against KHV infection in carp.

These results indicate that our liposome vaccine is highly useful and practical to immunize fish via oral route.

Porphyra spheroplast as a dietary protein source for fish: A review

○Takao Yoshimatsu
(Graduate School of Bioresources, Mie Univ.)

Red algae Porphyra spp. are widely cultured in the coastal lines of Japan, Korea and China as a human food. Porphyra includes high percentage of protein and also contains high amount of taurine, which is an important amino acid for marine larval fish, besides various minerals and vitamins. A new dietary ingredient was prepared by using single-cell material (i.e. spheroplasts) produced by the cell-wall digestion of Porphyra with three degrading enzymes (agarase, β-1, 3-xylanase and β-1, 4-mannanase). PS is estimated to be a suitable feed ingredient since digestive absorption has been improved by the removal of rigid cell-walls.

A series of experiments was conducted to evaluate the dietary value of Porphyra spheroplasts (PS) as a fish diet and/or alternative dietary protein source using white fish meal as the primary dietary protein for some important aquaculture targets: red sea bream Pagrus major, black sea bream Acanthopagrus schlegelii, flounder Paralichthys olivaceus and common carp Cyprinus carpio. Studies have revealed that growth performance, survival, feed utilization efficiency and nutrient retention were usually high in most species when PS was supplemented to the diets. Both groups of the PS and control diets had similar levels of body nutritional profile in terms of proximate compositions. From these experimental results, thus, it is comprehensible that supplementary diet containing PS can be used for maximizing not only growth of fishes and their survivals but also for the utilization of the feed ingredients. Porphyra spheroplasts therefore can be a good alternative dietary protein source for culturing finfish in future.

References
The Japanese eel Anguilla japonica is one of the most important fish for freshwater cultivation in Japan. As many scientists are involved in the development of methods for induction of maturation and larval rearing of the eel, the establishment of seed production method is expected in the near future. For the genetic improvement of aquaculture strains, the development of basic techniques for breeding is also essential. In this study, we developed the method for cryopreservation of the Japanese eel spermatozoa, and fertility of the cryopreserved spermatozoa was examined.

Cultivated male Japanese eels received weekly injections of human chorionic gonadotropin (hCG) for induction of spermatogenesis and spermiation. We examined the effects of the nature and concentration of cryoprotectant, the nature of the extender, the cooling rate and cooled temperature prior to immersion in liquid nitrogen (LN) on the post-thaw motility of the spermatozoa, and concluded that 10% methanol + 22.5% fetal bovine serum + 67.5% artificial seminal plasma for eel (K30-ASP) is a suitable diluent for cryopreservation of the spermatozoa and that samples should be cooled to \(-60^\circ C\) at \(-9^\circ C\) min\(^{-1}\) for effective storage.

To examine the fertility of post-thaw spermatozoa, we conducted the following experimental protocol. On the 1st day, we collected milt from males and diluted the milt with K30-ASP, which can increase the potential for sperm motility during incubation (Ohta et al., 2001; Aquaculture 198; 339-351), at 10 times and stored at 5\(^\circ C\). On the 2nd day, we cryopreserved half of the cool stored spermatozoa and stored in LN. On the 3rd day, we compared the motility and fertility of the post-thaw spermatozoa and two-days cool stored spermatozoa.

Motility values of post-thaw spermatozoa comparative to cooled stored spermatozoa were 77%, 76% and 91% for percent motility, velocity and linearity of movement, respectively. Fertility (percent cleavage and percent hatchability) of post-thaw spermatozoa and cool stored spermatozoa when inseminated with fresh eggs was not significantly different. These results indicate that for fertilization of the eggs obtainable from one female (200-400 g) with cryopreserved spermatozoa, cryopreservation of 0.5-1.0 ml of fresh milt is necessary.
suitable diluent for cryopreservation. The \( 250 \, \mu l \) straws containing spermatozoa diluted with diluent should be cooled at 10 cm or 12.5 cm from the LN surface to \(-50^\circ C\) for efficient storage (Kawamoto et al., 2007; Cryobiology 54; 19–26). When the sperm samples were cryopreserved under this method, post-thawed spermatozoa showed large differences in percent motility among straws. To clarify the reasons for the differences, we elucidated the effects of changes in cooling rate on post-thaw motility and fertility. We cooled 51 straws at 11 kinds of height (from 1.5 cm to 16 cm) from LN surface, and examined the cooling rate, post-thaw motility, and fertility of each straw.

Although a statistically significant \( (P<0.001) \) strong correlation \( (r = -0.94) \) was observed between height of straws and the cooling rates, the cooling rates showed individual differences even in the same height (the maximum difference of \(-14.4 \, \text{min}^{-1}\) was observed at 11.5 cm in height). Percent motility and fertility of the post-thaw spermatozoa showed high values only in the range with a narrow cooling rates around \(-20^\circ C \, \text{min}^{-1}\). Most of the fertilization rates showed 0% when the cooling rates shifted from the range of \(-15 \text{ to } -25^\circ C \, \text{min}^{-1}\).

These results indicate that the sperm of the Japanese pearl oyster has a very narrow range of suitable cooling rate, and the key to success of its cryopreservation depends on the control of the cooling rate.

**P-10** The development of cryopreservation method for seven band grouper sperm with CASA evaluation

Ivan Chong Chu Koh\(^1\), Ken-ichi Yokoi\(^1\), Masaharu Tsuji\(^2\), Yasushi Tsuchihashi\(^2\) and Hiromi Ohta\(^1\)

\(^1\)Department of Fisheries, Graduate School of Agriculture, Kinki University, \(^2\)Owase Fisheries Research Laboratory, Mie Prefecture Fisheries Research Institute

Seven band grouper (Epinephelus septemfasciatus) is a potential aquaculture candidate in Japan. The seven-band grouper is a protogynous hermaphrodite, maturing first as females before changing sex to males at a much later period. The large sizes of the males make for a limited amount of functional male broodfish, therefore collection of milt is always a problem. The development of cryopreservation methods will help synchronization for the availability of gamete for seed production and breeding study purposes.

In the present study, we examined methods for the cryopreservation of seven-band grouper spermatozoa. The concentration and type of cryoprotectant and extender, the cooling rate, and cooled temperature before immersion into liquid nitrogen (LN) was examined. The percent motility, average path velocity, and linearity of movement (LIN) of fresh and corresponding post-thaw sperm were used to evaluate sperm quality. Sperm motility was investigated using computer-assisted sperm analysis.

Milt was collected by abdominal massage from single males 48 h after hCG injection. The basic protocol developed from preliminary experiments was as follow: 10% dimethyl sulphoxide (DMSO) as cryoprotectant plus 90% fetal bovine serum (FBS) as extender; dilution ratio of milt with the diluents, 50 times; the height of the straw from LN surface, 5 cm; the cooled temperature before immersion into LN, \(-50^\circ C\). The effects of the following variations were then examined: (1) cryoprotectant [0 and 10% DMSO, N-N, dimethylformamide (DMF), methanol]; (2) concentration of methanol, DMSO and DMF (5, 10, 15%); (3) extender [FBS/artificial seminal plasma (ASP)/5.6% glucose/13% trehalose); (4) cooled temperature \((-30, -40, -50, -60, -70^\circ C)\); (5) cooling rate (from 27.1 ± 2.1, 31.4 ± 10, 54.7 ± 2.5 and 94.3 ± 25.7°C \, \text{min}^{-1})\).

Five percent DMSO with 95% FBS was the most successful cryoprotectant diluent with a comparative post-thaw motility of 77.6 ± 8.5%; 5% dimethyl formamide was also effective. Fetal bovine serum was significantly better as an extender when compared with artificial seminal plasma, glucose, and trehalose solution. Sperm tolerated a wide range of cooling rates (from 27.1 to 94.3°C \, \text{min}^{-1})\); however, the post-thaw motility of sperm cooled to \(-30^\circ C\) was significantly lower than that of other cooled temperatures.
(−40 to −70°C). The velocity of post-thaw sperm was significantly lower than that of fresh sperm, although LIN remained the same.

For effective cryopreservation of seven-band grouper sperm, samples should be diluted in 5% DMSO with 95% FBS and cooled to at least −40°C before immersion in liquid nitrogen. The results suggested that the methods for sperm cryopreservation of seven-band grouper described here are applicable for artificial seed production and breeding studies.

**P-11 Microbial communities in the eelgrass bed of the Izu Peninsula, Japan**

○Chia-Hui Chen, Terukuni Kozaki, Yusuke Tanaka, Shiro Itoi, Kiyoshi Yoshihara and Haruo Sugita (Department of Marine Science and Resources, Nihon University)

The eelgrass *Zostera marina* is a flowering plant and forms the eelgrass bed in shallow bottoms throughout the Atlantic, eastern Pacific and Mediterranean. Since the eelgrass bed can temper the tidal current, it plays an important role as breeding and nursing habitats for fish and shellfish. This study was performed to investigate the microbial communities of the thallus of eelgrass specimens, and seawater and sand samples using the clone library method.

The eelgrass specimens, and seawater and sand samples were collected in June and September 2008 at the Tanoura Inlet in Shimoda, Shizuoka, Japan. Microbial communities attached to the thallus of eelgrass specimens were recovered by swabbing with sterile cotton swabs. The DNA was extracted from each sample and 16S rDNAs were amplified by PCR to construct clone libraries. The 16S rDNA sequence of each clone was analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and identification to species was confirmed by phylogenetic analysis using Clustal W software package. Direct counts were determined by the epifluorescent staining method after fixation with Lugol iodine solutions.

The total count of microbial communities ranged from $3.1 \times 10^6$ to $1.6 \times 10^7$ cells/ml in seawater samples, from $1.2 \times 10^8$ to $2.4 \times 10^8$ cells/g in sand samples, and from $1.1 \times 10^7$ to $1.8 \times 10^7$ cells/cm$^2$ on the thallus of eelgrass specimens. A total of 315 clones were classified into 14 classes, 62 families, 126 genera and 158 species. Alphaproteobacteria and Gammaproteobacteria accounted for 30 and 35% of clones, respectively. Clones obtained from seawater, sand and thallus samples were 50 to 61, 50 to 60 and 44 to 50 species, respectively, suggesting that the microbial communities on the thallus of eelgrass consisted in a diverse of bacterial species.

**P-12 The AHL-producing bacteria in the intestinal tracts of ayu Plecoglossus altivelis**

○Masayuki Ogawa, Shiro Itoi and Haruo Sugita (Department of Marine Science and Resources, Nihon University)

Quorum sensing is known to be a mechanism for controlling gene expression of many pathogenic factors in response to an expanding bacterial population. In many Gram-negative bacteria, the diffusible quorum sensing signal molecule is a member of the N-acylhomoserine lactone (AHL) family. Therefore, the present study was carried out to examine the distribution of AHL-producing bacteria in the intestinal
tracts of ayu *Plecoglossus altivelis* for the better understanding of ecology of the fish intestinal bacteria.

The intestinal contents of cultured specimens of ayu were serially diluted and plated onto TS, MacConkey, GAM and FM agar media. The TS and MacConkey agars were incubated under aerobic conditions and the GAM and FM agars were incubated under anaerobic conditions, both at 20°C for five days. About 20 colonies were isolated from each agar plate and examined for the production of AHL using *Chromobacterium violaceum* CV026. Moreover, the isolated bacteria were subjected to the identification to species based on the 16S rDNA nucleotide sequences, which were analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and the identification to species was confirmed by phylogenetic analysis using the Clustal W software package.

Total viable counts were $5.9 \times 10^5$–$1.3 \times 10^9$ CFU/g in the ayu intestines, $4.2 \times 10^4$ CFU/ml in the rearing water and $8.9 \times 10^4$ CFU/g in the pelleted diets. The density of AHL-producers were $7.2 \times 10^2$–$2.8 \times 10^8$ CFU/g in the ayu intestines, $1.3 \times 10^4$ CFU/ml in the rearing water and < $4.5 \times 10^3$ CFU/g in the pelleted diets. Major AHL-producers derived from ayu were identified as *Aeromonas hydrophila*. In addition, the genera *Plesiomonas*, *Citrobacter*, *Vibrio*, *Shewanella* and *Cetobacterium* were isolated from the ayu intestines as major components.

**P-13** Microflora of *Artemia* nauplii hatched under experimental conditions

© Haruka Kurihara, Hitomi Nimura, Konomi Sekita, Yusuke Tanaka, Shiro Itoi and Haruo Sugita

(Department of Marine Science and Resources, Nihon University)

*Artemia* nauplii are widely used as live food for larvae and juveniles of fish and crustacean. However, since the *Artemia* nauplii are often contaminated by fish pathogens such as *Listonella anguillarum*, the nauplii are considered to be one of infection sources in cultured fish. Therefore, this study was performed to investigate the microflora of *Artemia* nauplii hatched under experimental conditions for the better understanding of the microbiological risks in the aquaculture management.

*Artemia* cysts were chemically decapsulated and incubated in both natural and sterile seawater. Hatched *Artemia* nauplii were homogenized, serially diluted and plated onto 1/20PYBG agar medium. The inoculated 1/20PYBG agar plates were incubated at 25°C for seven days under aerobic conditions. After then, approximately 20 bacteria were isolated from each plate and subjected to the identification to species. Concomitantly, rearing water samples were processed similarly. The 16S rDNA were amplified from each bacterial isolate by the PCR (polymerase chain reaction) method. The 16S rDNA nucleotide sequence was analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and the identification to species was confirmed by phylogenetic analysis using Clustal W software package.

Bacterial populations associated with *Artemia* nauplii were mainly composed of *Planococcus* and *Psychrobacter*, almost of which seemed to be halotolerant. The *Artemia* nauplii hatched in the sterile seawater were mainly colonized with *Psychrobacter* and *Bacillus* while those hatched in the natural seawater were colonized with *Vibrio*. This finding demonstrates that *Artemia* nauplii have high affinity with members of *Vibrio* rather than bacterial species associated with *Artemia* cysts, suggesting that *Artemia* nauplii are easily contaminated with pathogenic vibrios.
Microbial communities in the intestinal tracts of coastal fish

Yusuke Tanaka, Chia-Hui Chen, Terukuni Kozaki, Shiro Itoi and Haruo Sugita
(Department of Marine Science and Resources, Nihon University)

It is found by the plate count method that the intestinal tracts of marine fish are dominantly colonized by members of genus Vibrio. However, Sugita et al. (2005) reported that the direct counts of intestinal contents from coastal fish ranged from $10^9$ to $10^{10}$ cells/g, regardless of fish specimens and food habitat, while the viable counts ranged from $10^3$ to $10^9$ CFU/g. This finding demonstrated that the culturability of intestinal bacteria is different among fish specimens. This study therefore examined the microbial communities of the intestinal tracts of coastal fish using the clone library method.

Coastal fish were collected using conventional line fishing gears at the Tanoura Inlet in Shimoda, Shizuoka, Japan. The DNA was extracted from each intestinal sample of fish and 16S rDNAs were amplified by PCR to construct clone libraries. The 16S rDNA sequence of each clone was analyzed by a model ABI 3130xl Genetic Analyzer. The similarity in 16S rDNA sequences between a given isolate and other organisms was compared with all sequence data in the DDBJ/EMBL/GenBank databases using the BLAST algorithm and identification to species was confirmed by phylogenetic analysis using Clustal W software package. Direct counts were determined by the epifluorescent staining method after fixation with Lugol iodine solutions.

Direct counts of the intestinal contents from the file fish Stephanolepis cirrhifer and black rockfish Sebastes inermis were $2.2 \times 10^9$ and $2.7 \times 10^9$ cells/g, respectively. A total of 59 and 58 clones constructed from the file fish and black rockfish, respectively, were mainly composed of genera Pseudomonas, Propionibacterium, Sphingomonas, Methylobacterium, Haemophilus and Moraxella, which have not yet been isolated from coastal fish by the plate count method. Members of Vibrionaceae were not detected in both clone libraries. Microbial communities in the intestinal tracts of other coastal fish are in progress.

Effects of suspended sediment on stress hormone response in wild and cultured strains of ayu Plecoglossus altivelis

Satoshi Awata¹, Hirohiko Takeshima², Tetsuya Tsuruta¹, Takashi Yada³ and Kei'ichiro Iguchi¹
(¹Ueda Station, Freshwater Fisheries Res. Div., NRIFS, FRA, ²Atmosphere and Ocean Res. Inst., the Univ. of Tokyo, ³Nikko Station, Freshwater Fisheries Res. Div., NRIFS, FRA)

It is well known that excessive suspended sediment (SS) negatively affects the fitness of fish by causing gill damage, lowered feeding efficiency and physiological stress, all of which may impair growth, survival and reproduction. Despite numerous researches on the effects of SS on fishes, few studies have examined its impact on stress hormone response.

We conducted experiments to test whether increased levels of SS elevate stress hormone secretions in ayu Plecoglossus altivelis (Plecoglossidae) inhabiting clear rivers. First, we measured serum cortisol concentrations in wild and low- and high-inbred hatchery strains of ayu after exposure to the water containing 200 mg/l of kaolin. After 3 h, fish exposed to SS exhibited greater elevations in cortisol levels than control fish and initial groups.

Moreover, stress response to SS in high-inbred strains was significantly lower than those in wild and low-inbred strains. In the second experiment, we evaluated stress hormone response of wild fish to 3 h- or 24 h-exposure to different levels of SS (50–500 mg/l). Long-time exposure to low levels of...
SS as well as short-time exposure to high levels of SS elevated cortisol levels of ayu. These results suggest that SS is a significant environmental stressor that may increase the susceptibility of ayu to stress-related disease.

Further, highly domesticated ayu may be maladaptive in nature because of lower reactivity of their stress axes when compared with those of wild or low-inbred strains.

**P-16** The preventive effect of oral administration of the bacteria derived from red sea bream *Pagrus major* intestine on edwardsiellosis in red sea bream

Keitaro Kato, Wataru Takahashi, Kensuke Tsukahara, Yumina Ishikawa, Yuki Morisaki, Katsuya Ishimaru and Osamu Murata
(Fisheries Laboratory of Kinki Univ.)

Edwardsiellosis is one of the most serious diseases, which has caused a great economic loss in commercial aquaculture of red sea bream, *Pagrus major*. The pathogen of this disease is *Edwardsiella tarda*. The major symptoms are granulomatous inflammation and ulceration on the forehead, which causes ugly and undesirable appearance on the market size fish. *E. tarda* is an enteric bacterium and mainly thought to penetrate through intestine. In this study, we investigated the preventive effect of oral administration of the isomalt-oligosaccharide as a prebiotics and the bacteria with anti-*E. tarda* activity on edwardsiellosis in red sea bream.

One hundred eighty bacterial strains were isolated from red sea bream intestine by using agar medium containing isomalt-oligosaccharide as a carbon source. The bacterial strains were classified into 23 groups by whole-cell protein profiling using SDS-PAGE. The antibacterial activities against *E. tarda* of each representative strain of the 23 groups were tested by parallel streak method. Red sea bream (average body weight, 68 g) were fed diet containing the bacteria with antibacterial activity for 2 weeks, and then the fish were fed diet containing the bacteria and isomalt-oligosaccharide for 1 week. Finally, fish resistance to the *E. tarda* infection was tested by immersion challenge test (Immersing fish for 16 hours in sea water suspended 1.43 × 10¹⁰ CFU/ml of *E. tarda*). The effective dose of the bacteria was also investigated.

Four strains showed the antibacterial activity against *E. tarda*: *Vagococcus* sp. (2 strains), *Aeromonas* sp., and *Shewanella* sp. Oral administration of these 4 strains did not affect the growth of the red sea bream in the present study. The incidence rates of edwardsiellosis in the challenge test were the lower in the fish fed *Shewanella* sp. (57–67%) than other groups (82–100%). The results showed the oral administration of the *Shewanella* sp. is effective to prevent the edwardsiellosis. The effective dose of the *Shewanella* sp. was less than 0.14 g (9.24 × 10⁹ CFU/kg body weight).

**P-17** Cells and the total number of principal ducts of digestive diverticula in Japanese scallop *Patinopcten yessoensis*

Susumu Asano, Masahiro Ebine, Yuji Mori and Takeshige Matsutani
(Faculty of Science and Engineering, Ishinomaki Senshu University)

Cells and the total number of principal ducts of digestive diverticula in the scallop *Patinopcten yessoensis* were observed histologically. The digestive diverticula, monolayer epithelial tissue, were composed with 5 type of cell, type-A,-B,-C,-D,-E. Each type of cell contained various number and size of vacuole in its cytoplasm. Type-A cell had no vacuole and type-E cell contained many large vacuoles.
These morphology of the cells might show transporting process of materials via digestive diverticula.

The total number of principal ducts of the digestive diverticula was estimated in the scallop. Digestive gland surrounding stomach contains numerous digestive diverticula which open into stomach cavity. Cross sections of the principal ducts were oval (the mean major axis 40.2 μm). The mean density of digestive diverticula was 38.4/mm² in stomach epithelium (principal duct) and was 54.0/mm² near epidermis. Superficies of stomach cavity was estimated at 295 mm² using injected resin mold and paraffin mold that was produced with the resin mold. From these results, the total number of principal duct of digestive diverticula in the scallop was calculated at 11,311. Superficies of digestive diverticulum is thought to be wider than it of intestine. We may underestimate a physiological role of digestive diverticula that is a diagnostic apparatus of bivalves.

**P-18**

**Effects of salinity on clove oil and lidocaine-HCl anesthesia in the marine medaka Oryzias dancena**

In-Seok Park¹, Hyun Woo Gil¹, Yoon Kwon Nam² and Dong Soo Kim²

(¹Division of Marine Environment and Bioscience, ²College of Ocean Science and Technology, Korea Maritime University)

**Introduction**

Anesthesia can decrease stress levels when fish are subjected to blood sampling, immobilization, handling, vaccine injections and antibiotic substances, medical treatment for diseases, artificial spawning, transport, and sorting. In hyperosmotic environments, the marine medaka showed better tolerance than the Japanese medaka Oryzias latipes including survival rates of adult fish and hatching rates of oosperm. Optimum concentrations of anesthetic clove oil and anesthetic lidocaine-HCl were determined for a species of adult marine medaka Oryzias dancena over a range of salinity conditions.

**Materials and Methods**

The anesthetic effects of clove oil and lidocaine-HCl were tested at different salinities (0, 10, 20, 30, and 40 ppt) and concentrations (50, 75, 100, 125, and 150 ppm for clove oil; 200, 300, 400, 500, 600, 700, and 800 ppm for lidocaine-HCl). Until experiment termination, the water temperature was maintained at 26°C. All the fish were deprived of food for 24 hours before the study.

**Results and Discussion**

Research indicated that the higher the concentration of anesthetic at each salinity, the shorter the anesthesia time at each salinity. At each concentration, fish were anesthetized slower at water salinities over 10 ppt (P<0.05). Anesthesia time at 10 ppt was faster than any other salinity. The results from this study will contribute to safe laboratory handling of the marine medaka, which are commonly required by many research studies and experiments.

**P-19**

**Effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder Paralichthys olivaceus at suboptimal temperature**

Young Jin Cho and Sung Hwoan Cho

(Division of Marine Environment & Bioscience, Korea Maritime University)

**Introduction**

Temperature is one of the most critical environmental factors affecting not only availability of
nutrient content in the diets, but also growth of fish. Therefore, determining temperature condition for fish growth is critical for fish farmers because it eventually affects total production cost of fish. There is a report that juvenile olive flounder (*Paralichthys olivaceus*) averaging 10 g subjected to up to 8.5°C for 10 days followed rearing at 22°C for the next 30 days achieved full compensatory growth and concluded that a short period low temperature exposure might not affect annual growth of fish. We reported that growth of juvenile olive flounder averaging 38 g fed the extruded pellet (EP) for 5 days a week was comparable to that of fish fed the EP for six days a week at optimum temperature condition. However, no information on periodic feed deprivation and refeeding on performance of subadult olive flounder at suboptimal temperature are available yet. In this study, therefore, effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder fed EP at suboptimal temperature was determined.

**Materials and Methods**

Two hundred twenty five subadult averaging 272 g (fifteen fish per tank) were randomly distributed into fifteen of 300 L circular flow-through tanks and water source was sand-filtered natural seawater. Water temperature ranged from 9.8°C to 17.1°C (mean ± SD: 13.5 ± 2.10°C). Five treatments with different feeding regime were prepared in triplicate: Fish were hand-fed with EP containing 54.4% crude protein, 9.2% crude lipid and 11.8% ash (18.9 kJ/g diet gross energy) to apparent satiation once a day (09:00), seven days a week (7DF), which was used as the control group or consecutive six, five, four and three days a week, for 12 weeks at suboptimal temperature, referred to as 6DF, 5DF, 4DF and 3DF treatments, respectively.

**Results and Discussion**

Weight gain (*P* < 0.03) and SGR (*P* < 0.04) of fish in 6DF treatment were significantly higher than those of fish in 7DF, 4DF and 3DF treatments, but not significantly different from those of fish in 5DF treatment. This indicated that seven days of satiation feeding a week produced no positive effect on growth of fish and five days of feeding a week seemed to be recommendable when fish were fed with EP at suboptimal temperature. Daily feeding rate of fish in 6DF and 7DF treatments was significantly (*P* < 0.0001) higher than that of fish in 5DF, 4DF and 3DF treatments. However, feed efficiency ratio and protein efficiency ratio of fish were not significantly different among treatments. None of chemical composition of fish was significantly different among treatments. Because optimum days of feeding a week for fish could be largely affected by several factors such as fish species, fish size, water temperature and dietary nutrient composition, it must be carefully considered. Results of this study demonstrated that five days of feeding a week was recommendable for subadult olive flounder fed with extruded pellet to satiation once a day at suboptimal temperature.

**P-20 Effects of various concentrations of *Scutellaria baicalensis* extract in the diets on growth, body composition and immune response of juvenile olive flounder (*Paralichthys olivaceus*)**

©Kyoung Tae Kim, In-Cheol Choi, Young Jin Cho and Sung Hwoan Cho  
(Division of Marine Environment & Bioscience, Korea Maritime University)

**Introduction**

Dietary additives to improve survival, growth, immune resistance and/or muscle quality of olive flounder (*Paralichthys olivaceus*) have been developed; Obosan, lactic acid bacteria cultured in herb (*Acanthopanax koreanum*) extract, extract of mushroom mycelium *Phellinus linteus* and *Coriolus militaris*, *Chlorella* powder, green tea and chitosan. An extract of *Scutellaria baicalensis* in which baikalin and baicalein are the major active components has been known to have antimicrobial,
antibacterial, antiviral, antiinflammatory and/or antioxidant activities and recently developed as additive for aquafeed. In this study, therefore, effects of various concentrations of *S. baicalensis* extract (SBE) in the diets on growth, body composition and immune response of juvenile olive flounder were determined.

**Materials and Methods**

A commercially available SBE (Bioskintech Co. Ltd., Korea) containing ca. 100 ppm baicalin was used as a dietary additive. Six experimental diets (Crude protein: 55.4–57.2% and crude lipid: 8.7–9.9%) were prepared in triplicate: SBE-0, SBE-0.5, SBE-1, SBE-2, SBE-3 and SBE-5 diets containing SBE at the concentrations of 0, 0.5, 1, 2, 3 and 5%, respectively, at the expense of wheat flour. Fishmeal, dehulled soybean meal and corn gluten meal were used as protein sources for the experimental diets. Wheat flour, and fish and soybean oils were used as carbohydrate and lipid sources, respectively. Blood chemical analysis and lysozyme activity of fish were measured at the end of 8-week feeding trial. In addition, 20 fish from each tank were infected with *E. tarda* at $4 \times 10^7$ bacteria/ml per fish and their mortality was recorded for the next 96 h.

**Results and Discussion**

Survival and weight gain of fish were not significantly affected by dietary concentrations of SBE. However, specific growth rate (SGR) of fish fed the SBE-2 diet was significantly ($P<0.05$) higher than that of fish fed the SBE-0, SBE-1, SBE-3 and SBE-5 diets, but not significantly different from that of fish fed the SBE-0.5 diet. Feed consumption of fish fed the SBE-2 diet was significantly ($P<0.05$) higher than that of fish fed the SBE-0, SBE-0.5, SBE-1, SBE-3 and SBE-5 diets. Feed efficiency ratio and protein retention of fish was not significantly affected by dietary concentrations of SBE. Chemical composition and serum chemical analysis of fish were not significantly affected by dietary concentrations of SBE. No significant difference in lysozyme activity of fish fed the experimental diets with the various concentrations of SBE for 8 weeks was observed. Fish fed the SBE-0 diet showed 100% mortality at 96 h after *E. tarda* infection, but 77-87% for fish fed the other diets although no significant difference was found among the experimental diets. Results of this study indicated that dietary inclusion of 2% *S. baicalensis* extract seemed to be recommendable to improve SGR of olive flounder and *S. baicalensis* extract have the potential as a dietary additive to mitigate mortality of fish at *E. tarda* infection.
Protide® at 0, 3 and 5% replacing 0, 7 and 12% fish meal protein (designated as Pro-0%, Pro-3% and Pro-5%, respectively) for red sea bream (initial body weight 23.0 ± 0.01 g). In the two experiments, triplicate groups of fish were fed the experimental diets to apparent satiation (twice a day, 08:00 and 18:00 h) for 8 weeks.

Results
In Exp I, after 8 weeks of feeding trial, no significant differences were observed in weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival among all the dietary treatments. Blood parameters and non-specific immune responses were not different among all the dietary treatments.

In Exp II, after 8 weeks of feeding trial, no significant differences were observed in WG, FCR, SGR, PER and survival among all the dietary treatments. The results of glucose and triacylglycerol in plasma of fish fed pro-5% diet were significantly lower than those of fish fed the control and Pro-3% diets. Hematocrit, hemoglobin, cholesterol and alanine aminotransferase in plasma were not significantly different among all the dietary treatments. However, in non-specific immune responses, dietary supplementation of Protide® significantly enhanced phagocytic activity. Serum myeloperoxidase and lysozyme activities of fish fed the Pro-3% diet were also significantly higher than that of fish fed the control. This study indicates that the dietary supplementation of Protide® can enhance innate immunity of fish.

P-22 Optimum feeding rate by commercial expanded pellet for sub-adult olive flounder (Paralichthys olivaceus) in low water temperature season

Sung-Sam Kim1, Ji-Hoon Cha1, Jin-Woo Song1, Kang-Woong Kim2, Maeng-Hyun Son2 and Kyeong-Jun Lee1

(1Department of Marine Life Science, Jeju National University, 2Aquaculture Management Division, National Fisheries Research and Development Institute)

Introduction
Though marine finfish culture production in Korea has been steadily increased for the last 20 years, raw-fish based moisture pellet (MP) diet is mainly being used for marine fish species. Supplying MP for aquaculture led to several problems, such as water pollution, over-catch of small fish, inclusion of pathogen and increase in storage and labor costs. However, expanded pellet (EP) is not widely used for flounders in Korea due to lack of information on optimum feeding rates over the entire growth stages and water temperatures. Therefore, this study was conducted to investigate the optimum feeding rates of sub-adult flounder in low water temperature season.

Materials and Methods
Commercial feed used in the feeding trial was supplied by Suhyup Feed Co., Ltd. (Uiryeong, Gyeongsangnamdo, Korea). Three hundred sixty fish with initial body weight of 370±5.72 g (mean ± SD) for sub-adult stage of olive flounder were randomly distributed into 12 aquaria. Two aquaria (2 replicates/diet) were then randomly assigned to each of six feeding regimes: 0, 0.3, 0.4, 0.5, 0.6% BW/day and satiation. Fish were fed twice a day (9:00 and 17:00 h). The feeding trial was conducted in a flow-through system in line with 300-l aquaria receiving filtered seawater at a rate of 1.0 l/min.

Results and Discussion
At the end of the 4 weeks of feeding trial, weight gain (WG) and specific growth rate (SGR) were significantly higher ($P<0.05$) in fish fed at 0.6% BW/day compared to the fish fed no diet or at 0.3%. There were no significant differences in WG and SGR among fish fed at 0.4, 0.5, 0.6 and satiation. Hematocrit was significantly lower in the unfed fish (0%) than in other treatments except for fish fed to satiation. The optimum feeding rate in sub-adult olive flounder (~ 370 g) seemed to be 0.51% BW/day.
**P-23**

**Growth and feed utilization of slow growing juvenile flounder** (*Paralichthys olivaceus*) **fed either extruded or moist pellet under the subterranean seawater of 17°C**

Jeong Dae Kim¹, Se Min Choi², Seung Jun Shin³, Bong Soo Lim⁴ and Jong Pyo Seo⁴

¹Department of Animal Life System, Kangwon National University, ²Feed/Animal Research Institute, CJ JeilJedang Corporation, ³Marine and Environmental Research Insitutue, Jeju National University, ⁴AquaGenoTech Co.

**Introduction**

Growth of olive flounder (*Paralichthys olivaceus*) is greatly affected by various factors including water temperature, sex and seeds’ quality. Therefore, it is time consuming work to establish the standard feeding ratio for the fish cultured in a practical condition. It is well-known that male flounder shows growth lower than female one. The difference in growth is distinctly revealed as the fish reach a growing stage of 200 g. At this stage, male fish are selected and sold because their feeding is considered to be uneconomic. To date, however, nobody attempted to determine the growth and feed utilization of the slow growing male flounder. Furthermore, any data were not made on feeding rate for the fish when fed either extruded (EP) or moist pellet (MP) under the subterranean seawater of 17°C.

**Materials and Methods**

A three thousand olive flounder weighing 230 g were purchased and divided to two groups. They were adapted to either EP or MP in each of two rectangular concrete tanks for 2 weeks. Feeding trial was lasted for 8 weeks during which fish were hand-fed to apparent satiety twice a day for 6 days per week. Feed fed was daily recorded with the weight and the number of dead fish. At the beginning and the end of the trial fish were counted and bulk-weighed following a 24-h fast. Whole fish, muscle and blood were sampled for chemical analysis at the end of the experiment. Water temperature was maintained constant at 17°C. Dissolved oxygen, ammonia, pH and salinity were regularly checked and recorded.

**Results and Discussion**

At the end of the experiment the mortality of 4.0% and 4.1% was recorded for fish fed EP and MP, respectively. Fish showed the average final weight of 361.8 g for EP and 363.0 g for MP. As-fed basis, feed conversion of 0.94 and 2.61 was obtained in EP and MP, respectively. Feeding rate, daily gain, nutrient retentions, muscle composition and blood plasma changes are discussed in fish fed either EP or MP.

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**P-24**

**Development of material cycle model in brackish water (culture farm)** — carbon cycle model based on Box model —

Sang-ho Baek¹, Ki-Ho Choi¹ and Shinya Otake²

¹National Fisheries Research and Development Institute, ²Fukui Prefectural University, Japan

**Introduction**

Lake Mikata is an inland lake of five connected lakes linked with the sea called “Mikata Goko” in Japan and farthest lake from the sea. This water quantity shows a double structure by freshwater flowing from Hasu river and quite salty flowing from a connected lake. In this lake, a water bloom occurs recently and causes an environmental problem. Purpose of this study is to find out an occurrence mechanism of the water bloom quantitatively using a material cycle model in this lake.

The primary production model, from nutrient salts to phytoplankton, was built by measuring
carbon in the light and dark bottles. This model was verified by using the DO budget model. Finally, this primary production model was applied to the whole lake.

**Materials and Methods**

In order to calculate a material budget, we adopted both a Box model dealing the whole lake as a open system and a closed system model by a light and dark bottle method in July and September, 2002. This model consisted of DOC, POC, DIN, and Phytoplankton as a simple ecosystem model. The coefficients in the model, e.g. growth speed etc., between each terms were used Nakada model, and was adapted in the light and dark bottles.

The all existing quantities of POC, DOC, DIN, and Phytoplankton were calculated using both the coefficient and their time change. Phytoplankton calculated by this model was compared with the phytoplankton production calculated from the DO budget model. In these terms, DO produced by phytoplankton can be measured using light and bottle method and can transform to the phytoplankton. Finally, this primary production model was applied to the whole lake.

**Results and Discussion**

Discharge distribution in the lake by the Box model: According to these results in July, there were large exchange in the entrance of the lake, especially in middle and lower layer. It is shown that there was little exchange in the center of the lake. While, in September, large flow entered to the center of the lake and exchanged in upper layer water.

The primary production model: The phytoplankton carbon obtained by this model was smaller than the measured. The investigation period was a daytime. It can be considered that this short time be estimated the coefficients of the growth speed etc. in the model larger, when compared with the case that the Nakada mode was used.

The application of the primary production model to the whole lake Mikata: The phytoplankton carbon in two investigations was small relatively in total cycle and equal roughly. The POC and DOC in September were larger than the one in July. These trend agreed with light bottle method. We could take account of the carbon cycle in Lake Mikata in July and September.

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**P-25**

**Effect of zinc administration on physiological responses of rock bream *Oplegnathus fasciatus***

〇Young-Sug Kim, Hee-Ju Park, Young-Ju Lee and Ju-Chan Kang  
(Department of aquatic life medicine, Pukyong National University)

**Introduction**

Zinc is essential inorganic matter for metabolism of life, meanwhile having heavy metal characters that can be toxic. Since fish have a problem usually when this element is deficient, there were extensive researches about insufficiency of this element. However there are few researches about sufficient amount of zinc for fish species. This study employed for learning requirement and physiological responses of zinc in rock bream *Oplegnathus fasciatus*.

**Materials and Methods**

Healthy, cultured rock bream were obtained from a commercial farm in yeosu. Dietary 30, 60 120 and 240 mg/kg of zinc was exposed to the fish during first 40 days, and then feed without zinc was provided for 20 days. Growth was expressed as the weight growth rate and feed efficency. SOD, GSH and GPx activity of liver and gill measured. Significant differences between groups were determined using one-way ANOVAs and Duncan’s test for multiple comparisons.

**Results and Discussion**

There were significant differences in growth among control and 30, 60, 120, 240 mg/kg group.
However, there was no significant difference among 30, 60, 120 and 240 mg/kg group. Zinc toxicity was not observed during day 20 and day 40, when zinc was added to the feed. There was significant difference in SOD, GSH and GPx activities for the first 20, 40 and 50 days, but no significant difference was observed after 60 days exposure. This is considered due to the increase of antioxidant ability on the tissues of the rockbream. And antioxidation ability of fish increased when 30 mg/kg of zinc were fed for 20 days. These date indicate that optimal zinc supplement is 30–60 mg/kg.

**Changes of growth and hematological factors in catfish *Silurus asotus* exposed to paraquat**

Young-Ju Lee, Hee-Ju Park, Young-Sug Kim and Ju-Chan Kang

(Department of Aquatic Life Medicine, Pukyoung National University)

**Introduction**

Paraquat (PQ) is a non-selective, broad-spectrum herbicide widely used about 50 years for weed control, which has been known to be a highly toxic compound for humans and animals. Catfish *Silurus asotus* ranges all over the river in Korea. The objective of this study was to investigate the effects of 4 weeks exposure to PQ on growth, hematological parameters and antioxidant enzymes in the liver, kidney and gills of catfish.

**Materials and Methods**

Healthy, cultured catfish was obtained from a commercial farm in Chung-buk of Korea. The exposure took place in 150 l aquaria. After acclimatization for 4 weeks, catfish (total length 22.1 ± 0.2 cm, body weight 64.1 ± 1.5 g) were selected for the experiments. Catfish were exposed to various concentration of PQ (0, 1, 2, 5, 10 mg/l). Daily growth gain (length, weight), RBC count, Ht and Hb were measured in blood sample. For serum, organic (total protein, glucose and total bilirubin), inorganic (calcium and magnesium) parameters and enzymes (GOT, GPT, ALP, LDH) were detected. Moreover, several antioxidant enzymes (glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR) and superoxide dismutase (SOD)) were studied in the liver, kidney and gills of catfish. Significant differences between groups were determined using one-way ANOVAs and Duncan’s test for multiple comparisons.

**Results and Discussion**

Daily length gain and daily weight gain of catfish exposed to paraquat were significantly decreased in 5, 10 ppm groups of 4 weeks exposure compared to control. This study revealed that high PQ concentration reduced growth of catfish. In haematological factors, red blood cell count, hematocrit levels in catfish decreased in 10 mg/l. Therefore, PQ induced anemia. Above this, serum glucose, GOT, GPT, ALP, LDH elevated proportionally to PQ exposure level. It means liver damage. And PQ induced significantly elevated GSH, GST of liver, kidney and gill and GR, GPx of kidney. SOD activity of 1 or 2 mg/l showed an increase but that of 5 or 10 mg/l showed decreasing tendency in 5, 10 mg/l. Resultingly, most of the results show the catfish damaged above 2 mg/l of PQ.
Introduction
Dietary additives to improve survival, growth, immune resistance and/or muscle quality of olive flounder (Paralichthys olivaceus) have been developed; Obosan, lactic acid bacteria cultured in herb (Acanthopanax koreanum) extract, extract of mushroom mycelium Phellinus linteus and Coriolus militaris, Chlorella powder, green tea and chitosan. An extract of Scutellaria baicalensis in which baicalin and baicalein are the major active components has been known to have antimicrobial, antibacterial, antiviral, antiinflammatory and/or antioxidant activities and recently developed as additive for aquafeed. In this study, therefore, effects of various concentrations of S. baicalensis extract (SBE) in the diets on growth, body composition and immune response of juvenile olive flounder were determined.

Materials and Methods
A commercially available SBE (Bioskintech Co. Ltd., Korea) containing ca. 100 ppm baicalin was used as a dietary additive. Six experimental diets (Crude protein: 55.4–57.2% and crude lipid: 8.7–9.9%) were prepared in triplicate: SBE-0, SBE-0.5, SBE-1, SBE-2, SBE-3 and SBE-5 diets containing SBE at the concentrations of 0, 0.5, 1, 2, 3 and 5%, respectively, at the expense of wheat flour. Fishmeal, dehulled soybean meal and corn gluten meal were used as protein sources for the experimental diets. Wheat flour, and fish and soybean oils were used as carbohydrate and lipid sources, respectively. Blood chemical analysis and lysozyme activity of fish were measured at the end of 8-week feeding trial. In addition, 20 fish from each tank were infected with E. tarda at 4 × 10^7 bacteria/ml per fish and their mortality was recorded for the next 96 h.

Results and Discussion
Survival and weight gain of fish were not significantly affected by dietary concentrations of SBE. However, specific growth rate (SGR) of fish fed the SBE-2 diet was significantly (P<0.05) higher than that of fish fed the SBE-0, SBE-1, SBE-3 and SBE-5 diets, but not significantly different from that of fish fed the SBE-0.5 diet. Feed consumption of fish fed the SBE-2 diet was significantly (P<0.05) higher than that of fish fed the SBE-0, SBE-0.5, SBE-1, SBE-3 and SBE-5 diets. Feed efficiency ratio and protein retention of fish was not significantly affected by dietary concentrations of SBE. Chemical composition and serum chemical analysis of fish were not significantly affected by dietary concentrations of SBE. No significant difference in lysozyme activity of fish fed the experimental diets with the various concentrations of SBE for 8 weeks was observed. Fish fed the SBE-0 diet showed 100% mortality at 96 h after E. tarda infection, but 77-87% for fish fed the other diets although no significant difference was found among the experimental diets. Results of this study indicated that dietary inclusion of 2% S. baicalensis extract seemed to be recommendable to improve SGR of olive flounder and S. baicalensis extract have the potential as a dietary additive to mitigate mortality of fish at E. tarda infection.
Mitochondrial DNA analysis of the snow-crab in the South Korea East Coast

○ Sun Mee Hong, So-Jung Kim, Dong-Goong Choi, Jung-Hee Woo, Nyun-Ho Park, Deuk San Jeon and Choong-Gon Kim
(Gyeongbuk Institute for Marine Bioindustry, Korea)

Introduction
In recent years, different molecular techniques, using nuclear or mitochondrial DNA (mtDNA), have provided new information concerning the genetic variability of wild and cultivated populations of several fish species, permitting the delineation of management units and allowing assessment of conservation priorities. To date, there has been no literature on genetic stock characterization of Chionoecetes populations. This study therefore aims to provide the first screening of mtDNA variation of crab in South Korea East Coast.

Materials and Methods
First, total genomic DNA was obtained from crab tissues and DNA purification was performed with phenol-chloroform.

Second, to investigate and compare the COI gene sequences of each region in South Korea East Coast, we amplified approximately 750 bp that corresponds to the COI region of the mtDNA by PCR and sequenced (ABI 377). Sequences were subjected to BLASTn and BLASTx searches at the NCBI and they were aligned using ClustalW software.

Results and Discussion
Molecular variations were observed in the adults of crab, Chionoecetes opilio and Chionoecetes joponica, collected in the South Korea East Sea in February 2010. We obtained partial (about 700 bp) mitochondria DNA (mtDNA) Cytochrome c Oxidase-I (COI) sequences for 27 individuals of C. opilio from Uljin, Youngduk, Pohang, and 9 C. joponica from Uljin examined, and 10 individuals imported from Russia as a control. Single nucleotide polymorphisms (SNPs) within the partial COI gene differentiated C. opilio populations into 4, 0, 4, and 6 mtDNA hyplotypes from Uljin, Youngduk, Pohang, and Russia, respectively but the nucleotide variations of the COI gene in the C. joponica not indicated the presence of an intra-variation of mtDNA. In east korea sea, the mtDNA sequence of C. joponica was less polymorphic but identified SNPs variants of COI gene in C. opilio. Overall, the presence of COI SNPs was associated with silent substitution. Additional research will be needed to determine and obtain more correct variation data of these snow crabs in the South Korea East Coast.

Comparative efficacy of MS-222, 2-phenoxyethanol and clove oil as anesthetics in the mackerel Scomber japonicas

○ Seung Cheol Ji, Kyong Min Kim, Jun Ho Koo, Nack Jung Choi and Seok Jung Han
(Future Aquaculture Research Center, National Fisheries Research and Development Institute)

Introduction
Anesthesia was using for decrease stress level when fish are subjected to sampling, handling, transportation, sorting and injection of vaccines. In this study, the efficacy of three anesthetic agents (MS-222, 2-phenoxyethanol and clove oil) was evaluated in the mackerel.

Materials and Methods
Ten fish were subjected to 200, 400, and 800 ppm MS-222 (ethyl 3-aminobenzoate methanesulfonate), 2-phenoxyethanol and clove oil solution. The procedure was conducted three times. Fish were
then put back into seawater and recovery time was recorded with a chronometer. Recovery was established when fish exhibited normal equilibrium and swimming behavior. After anesthesia, five fish were randomly selected from the recovery group at 0, 0.5, 1, 2, 4, 8, 24, 32 and 48 h post-anesthesia to be used for blood glucose and cortisol level analysis. Blood was extracted from the caudal vein using a 1 ml heparinized disposable syringe. Plasma was obtained from blood samples by centrifugation (20,000 × g, 4°C), from which glucose was measured by analytical kit (Wako, Japan), while the ELISA method was applied for measurement of cortisol level following the manufacturer’s instructions (Oxford Biomedical Research, Inc. USA).

Results and Discussion

The acquisition of complete anesthesia in <2 min and recovery in <5 min, the optimal dose were determined to be 100 ppm of MS-222 (induction 70.7 and recovery 115.7 sec), 400 ppm of 2-phenoxyethanol (induction 86.7 and recovery 95.0 sec), and 50 ppm of clove oil (induction 71.3 and recovery 167.0 sec). Post-stress cortisol response reached a peak after 0.5 h. 2-Phenoxyethanol group had significantly lower plasma cortisol level than those of MS-222 and clove oil after 0.5 h. After 2 h of recovery, cortisol level returned back to normal levels in 2-phenoxyethanol and MS-222, but clove oil group returned back normal levels after 48 h. Glucose level of clove oil group was higher than those of 2-phenoxyethanol group during the recovery. The study demonstrated that 2-phenoxyethanol can be used as an effective anesthetic in mackerel aquaculture.

P-30 Vertical distribution and reproductive aspects of two crangonid shrimps in the deep-water of the East Sea, Korea

Hye-Min Park, Jung Nyun Kim, Byeong Gyu Hong, Myeong Ho Sohn, Hyeong-Gi Kim and Chul-Woong Oh

(Department of Marine Biology, Pukyong National University & Fisheries Resources Research Division, National Fisheries Research and Development Institute (NFRDI) & Dokdo Fisheries Research Center, NFRDI)

Introduction

In an ecosystem, there should be strong selection for animals to place themselves in suitable environments that supply needed resources: mates, shelter, food, etc. Vertical distance of deep sea is longer than coast, depth in the distribution will be more affected under these environments. These intermediated depths from surface to deep water have strong physical and biochemical gradients. Life history and reproductive strategies of a species appear to be selective compromises with respect to combination of biotic and abiotic factors in a particular environment. As their own reproductive strategy of each species, distribution will be able to vary according to sex and developmental stage. Distribution of ovigerous and non ovigerous females which released egg from body could provide insights into the reproductive strategies employed by a particular species. Despite their distribution in the East Sea of Korea as a unique deep-sea environment, there is a great lack of knowledge on the biological, ecological and distribution related to depth.

Materials and Methods

Neocrangon communis and Argis toyamaensis were collected from the East Sea off Samcheok, at depths ranging from 300 to 900 m. The shrimp were sampled by trawl over 5 years, during the following periods: June and December 2004, May and November 2005, March and September 2006, April and October 2007, and June and November 2008. The gonadosomatic index (GSI) was determined with the following formula: GSI=ovarian dry weight/(body dry weight-ovarian dry weight)*100. The eggs were treated as ellipsoids and the volume quantified with the formula: Volume=4/3πr1r2r3.
Results and Discussion

The distribution of the shrimps showed significant differences by depth. *N. communis* was found between 300 m and 900 m depth throughout the survey area with the highest number of individual at shallow water depth. *A. toyamaensis* occurred over the entire depth range with the highest number of individuals at deep water depth. Two species are distributed separately by depth for each sex during sampling period. Shallow-dwelling species, *N. communis* ovigerous females occurred mainly at shallow water depth during winter season. *A. toyamaensis* did show that ovigerous females were widely distributed in all depth ranges, GSI was not significantly different by depths. But deep dwelling species, *A. toyamaensis* had bigger eggs than *N. communis*.

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**P-31**

Effect of preservation method on hatching of resting egg of rotifer

*Brachionus plicatilis*

Joo-Yeon Youn and Sung Bum Hur  
(Department of Marine Bio-materials and Aquaculture, Pukyong National University)

Introduction

The rotifer *Brachionus plicatilis* is one of the most important food organism in aquaculture. The resting eggs produced by mictic female of the rotifer can be easily stored and hatched out. Therefore, the resting eggs are very useful as the starter for mass culture of rotifer in marine larval culture. This study was carried out to understand the optimum preservation method of the resting eggs for high hatching rate.

Materials and Methods

The marine rotifer *B. plicatilis* (CCUMP R-4, 8, 10) were received from the Culture Collection of Useful Marine Plankton in Pukyong National University. The rotifer to produce resting eggs were cultured with *Nannochloris oculata* (KMMCC C-31) received from Korea Marine Microalgae Culture Center in 5L beaker. The resting eggs (CCUMP R-4, 10) harvested were cryopreserved and tested on methanol (MeOH), dimethylsulfoxide (DMSO) and glycerol as a cryoprotectant agents (CPAs). The cryopreservation comprised two freezing procedures. Firstly, the samples containing the CPAs were kept at 4°C for 10min before being plunged into liquid nitrogen (−196°C). Secondly, freezing of samples containing CPAs was performed by Mr. Frosty Freezing Container (−1°C min⁻¹) before being plunged into liquid nitrogen. The resting eggs stored for one month. The resting eggs (CCUMP R-8) harvested were dried at different temperatures (30, 40 and 50°C) and drying hours (1hr, 2hrs and 3 hrs).

Results and Discussion

The resting eggs (R-4) without drying showed the highest hatching rate (69%) in 4°C preservation without CPA. But the resting eggs (R-10) dried at 30°C for 1 hour showed the highest hatching rate (89%) in liquid nitrogen preservation. With regard to drying temperature and hours of the rotifer (R-8), the highest hatching rate (92%) appeared in 1 hour drying at 30°C. In conclusion, drying method at 30°C for 1 hour is effective for high hatching rate. The effect of CPA was so variable by the rotifer strains. In general, the resting eggs dried at 30°C for 1 hour and preserved in liquid nitrogen was good preservation method for high hatching rate of the resting eggs of rotifer.
Influence of temperature and salinity on growth and size of rotifer

*Brachionus plicatilis*

Joo-Yeon Youn and Sung Bum Hur

(Department of Marine Bio-materials and Aquaculture, Pukyong National University)

**Introduction**

Rotifer *Brachionus plicatilis* are valuable live food for larval fish and crustacean. Several characteristics of rotifer, such as high nutritional quality, small body size and relatively slow motility have contributed to their usefulness as good prey for active larvae. The rotifer divides into three type: Large-type (280 μm), Small-type (180 μm) and Ultra small-type (120 μm). Each type of the rotifer provides to larvae according to mouth size. In this study, we examined the influence of temperature and salinity on growth and size of the rotifer.

**Materials and Methods**

The rotifers were received from the Culture Collection of Useful Marine Plankton (CCUMP). In this study, large-type (CCUMP R-3, 5, 18, 20) and small-type (CCUMP R-22, 27) were examined. Rotifers precultured at 24°C and 20 psu with *Nannochloris oculata* (KMMCC C-31). The growth and size of the rotifer were examined at different water temperatures (16°C, 24°C, 32°C) and salinities (20 psu, 25 psu, 30 psu, 35 psu) under continuous light (2,000 lx). One gravid female was cultured with *N. oculata* in 3 ml cell chamber with 2 ml saline water. Growth (no of inds./ml) of the rotifer was measured every two days. Lorica length and width of 50 individuals were measured after 20 days culture. The experiments were repeated eight times.

**Results and Discussion**

The maximum density for the large-type rotifer was 822 ind./ml in R-5 strain cultured at 32°C and 30 psu. That of the small-type was 1,246 ind./ml in R-27 strain cultured at 32°C and 35 psu. The density of the small-type rotifer were higher than that of the large-type rotifer. The small-type rotifer prefered higher salinity than large-type rotifer. The minimum lorica length in the large-type rotifer R-5 at 32°C and 25 psu was 210 μm and the maximum lorica length was 270 μm in R-18 cultured at 16°C and 25 psu. The minimum lorica length in the small-type rotifer R-27 cultured at 32°C and 20 psu was 154 μm. The maximum lorica length R-22 cultured at 16°C and 25 psu was 207 μm. The size of rotifer cultured at high temperature regardless of salinity was smaller than that at low temperature. The results demonstrated that size of rotifer was influenced by temperature and salinity.

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**P-33**

A fluorescence-producing transgenic fish model as exemplified by RFP-transgenics in marine medaka *Oryzias dancena*

Young Sun Cho, Dong Soo Kim and Yoon Kwon Nam

(Center for Risk Assessment of Marine Living Modified Organisms, Pukyong National University)

**Introduction**

Stable transgenesis in fish using ubiquitous promoters to drive the expression of desired transgene(s) continually from the embryonic development to adulthood is often valuable for (I) tracing the cell lineage and/or cell migration in vivo, (II) studying the mechanism of transgene silencing, (III) visualizing the process of tissue regeneration and/or (IV) identifying the sexual dimorphism or phenotypic sex in juveniles. In this study, we tested the functional utility of beta-actin-RFP transgene (RFP driven by cytoskeletal actin promoter) to visualize ubiquitously the fluorescence signals in whole
cells of marine medaka *Oryzias dancena* throughout its entire life cycle.

**Materials and Methods**

A F3 transgenic line carrying pod*β*-actRFP transgene was selected based on their capability of stable germ-line transmission. To examine the external characteristics, eye, muscle, gill, fin and scale were obtained, while various somatic and gonadic tissues were sampled to examine the RFP phenotypes of internal organs. Tissue samples were subjected to RFP assay under both daylight and fluorescent conditions. In addition, quantitative real-time RT-PCR amplification was performed in order to compare the transgenic expression (RFP regulated by *β*-actin promoter) with endogenous expression (*β*-actin gene) in various tissues.

**Results and Discussion**

RFP-positive organs could be distinguished from the corresponding counterparts from wild type individuals with unaided eye under normal daylight. Although there was slight variation in the intensity of red fluorescence among transgenic individuals, the most significant difference between transgenic and non-transgenic organs was found particularly in intestine. When considering the intestine is one of the organs to show the highest expression level of endogenous *β*-actin transcripts in this species, it suggests that the transgenic *β*-actin regulator inserted into the host chromosome may be regulated as similarly with its endogenous counterpart. RFP expression in eye lens, scale and fin, was well in agreement with the essential role of the *β*-actin in cytoskeletal structuring. Transgenic fishes also expressed RFP in red blood cells (RBC) at a moderate level, in which the expression pattern was uniform in cytoplasm.

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Detection of waterborne estrogenic chemical using a transgenic marine medaka (*Oryzias dancena*) carrying choriogenin H promoter-driven RFP transgene

Sang Yoon Lee, Yoon Kwon Nam and Dong Soo Kim

(Department of Marine Bio-Materials & Aquaculture, Pukyong National University)

**Introduction**

Fish choriogenins are precursor isoforms of egg envelop proteins and they have been given attention as useful biomarkers for estrogenic pollutants. Further when combined with the transgenic technique, transgenic fishes harboring the fusion construct between regulatory elements from choriogenins and living color protein genes allows the *in vivo* monitoring of waterborne estrogens in a real-time fashion. Herein, we isolated and characterized the promoter of choriogenin H gene from a euryhaline medaka (*Oryzias dancena*), generated a stable transgenic line and evaluate the functional utility of the transgenic detection of estrogens.

**Materials and Methods**

From the genome walking method, upstream regulatory region of choriogenin H gene was assembled; selected region was characterized; and spliced into the upstream of reporter gene (red fluorescent protein gene; *rfp*). Experimental transgenesis using the transgene construct (podchoH1RFP) was performed in *O. dancena* based on the examination of stable transmission up to F3 generations. Using the transgenic larvae and fry, various strengths and durations of estrogen exposures (particularly using estradiol-17*β*) were tested to evaluate the functional utility of this transgenic line.

**Results and Discussion**

Regulatory region of *O. dancena* choriogenin H gene was proven to possess various transcription factor binding motifs especially including ones associated with response to estrogenic chemicals. Microinjection-based gene transfer resulted in founder generation of mosaic transgenics, and a portion
of founders transmitted their transgene to F1 generation. Stable germ line was established by crossing F1 transgenics with control individuals based on the examination of Mendelian inheritance. Selected F2 transgenic progeny were able to express clear RFP signal in their livers during exposure to estrogens, in which generally the responsiveness or expression levels could be dose- and duration-dependent, although a certain saturation of the RFP signals have also been observable with the high concentrations of estrogen. Many of transgenic fry exposed to estrogen displayed a strong red fluorescence color in their livers, which could be easily detectable with unaided eye under normal daylight. Results from this study indicate that estrogenic pollutants would be sensitively detected using transgenic *O. dancena* carrying the podchoH1RFP, and also suggest that such a transgenic detection might be applicable to a wide range of salinity condition, since this species has a great osmoregulatory capability.

**P-35**

**Protein and lipid requirements of flounder *Paralichthys olivaceus* in different growth stages and water temperatures**

*Kang-Woong Kim¹, Kyong-Duck Kim¹, Shin-Kwon Kim¹, Maeng Hyun Son¹, Yong Jin Kang¹, Sungchul C. Bai² and Kyeong-Jun Lee³*

(¹National Fisheries Research and Development Institute, ²Pukyong National University, ³Jeju National University)

**Introduction**

These studies were conducted to investigate the protein and lipid requirements of flounder (*Paralichthys olivaceus*) in different growth stages and water temperatures. Growth stages were classified into three large groups of juvenile (less than 80 g), young (80–320 g) and sub-adult (more than 320 g), and rearing water temperatures were classified into two large groups of optimal (18–25°C) and suboptimal (less than 18°C).

**Materials and Methods**

The isocaloric experimental diets containing different protein levels from 35% to 56% and isonitrogenic (50% crude protein) diets containing different lipid level from 6% to 18% were designed for studies on dietary protein and lipid requirements, respectively.

**Results and Discussion**

Dietary protein requirements of flounder were determined using broken line model or ANOVA test based on the data of growth and feed efficiency in feeding trials. Optimum dietary protein levels are 50% and 51.7% for juvenile (28–45 g) and young (55–85 g) flounder in suboptimal water temperatures of 14.5 ± 0.1°C and 15.3 ± 0.9°C, respectively. And, optimum dietary protein levels are 49.7% and 50.1% for sub-adult flounder (300–500 g) in optimum (14.8 ± 0.7°C) and suboptimal (21.5 ± 1.9°C) water temperatures, respectively. These results suggest that optimum dietary protein levels are around 50% for flounder with no relation to growth stages and rearing water temperatures in these experimental conditions. Optimum dietary lipid levels were determined by the same methods as dietary protein requirements studies. Optimum dietary lipid levels are 9.1%, 10.1% and 10.5% for juvenile (18–60 g), young (85–280 g) and sub-adult (300–500 g), respectively, in optimal water temperatures of 18–25°C. In suboptimal water temperatures of 13 to 15°C, optimum dietary lipid levels are 11.8% and 13.4% for young (70–140 g) and sub-adult (280–360 g), respectively.
Effects of dietary lipid sources on the growth and body composition of the far eastern catfish Silurus asotus

Kyoung-Duck Kim, Jin Do Kim, Sang Gu Lim, Kang-Woong Kim and Maeng Hyun Son
(Aquafeed Research Center, National Fisheries Research and Development Institute)

Introduction
Dietary lipids are important sources of energy and of essential fatty acids (EFA) for fish. Providing desired amounts of EFA is necessary for the normal growth and survival of juvenile fish. EFA requirements of fish are largely affected by fish species, water temperature and salinity, and different to those of terrestrial animals. EFA affect the fluidity, permeability and enzyme activity of membrane, and are known as the precursors of the prostaglandins and leukotrienes. Therefore, this study investigated the effects of dietary lipid sources on growth performance and body composition of juvenile far eastern catfish Silurus asotus.

Materials and Methods
Three replicate groups of fish (average weight 3.6 g) were fed with one of the following experimental diets containing 10% beef tallow (BT), 5% BT plus 5% corn oil (CO), 5% BT plus 5% linseed oil (LO), or 5% BT plus 5% squid liver oil (SO) as the lipid source for 5 weeks.

Results and Discussion
No significant difference was observed in the survival among groups. The weight gain of fish fed the LO (high in 18:3n-3) and SO (high in n-3 highly unsaturated fatty acid) diets was significantly higher than that of the fish fed the CO (high in 18:2n-6) and BT diets \((P<0.05)\). The feed efficiency of fish fed LO and SO diets was significantly higher than that of the fish fed the BT diet \((P<0.05)\), but not significantly different from that of the fish fed the CO diet. The protein efficiency ratio of fish fed the SO diet was significantly higher than that of fish fed the CO and BT diets \((P<0.05)\), but not significantly different from that of fish fed the LO diet. The 18:1n-9 of whole-body polar lipid fraction in fish fed the BT diet increased compared to that of fish fed the other diets. Fish fed the CO and LO diets had significantly higher contents of 18:2n-6 and 20:4n-6, and 18:3n-3, than the fish fed the other diets in polar and non-polar lipid fractions, respectively \((P<0.05)\). Significantly higher contents of 20:5n-3 and 22:6n-3 were observed in the whole-body polar lipid fraction of fish fed the SO diet compared with fish fed the other diets \((P<0.05)\). The study results indicate that linseed oil and squid liver oil containing n-3 fatty acids are good dietary lipid sources for the growth of far eastern catfish.

Effect of dietary taurine levels on the growth performance of juvenile Korean rockfish Sebastes schlegeli

Shin-Kwon Kim, Kyoung-Duck Kim, Kang-Woong Kim and Maeng-Hyun Son
(Aquafeed Research Center, Aquaculture Research Institute, National Fisheries Research and Development Institute)

Introduction
Taurine is an amino acid that was first isolated from the bile of ox, Bos taurus. Taurine has been suggested to have important roles such as fat digestion of bile acid conjugator, development of retina and brain, function of osmoregulation. In previous study, we reported that the growth, feed efficiency and feeding behavior of juvenile flounder were improved with taurine supplementation in experimental diets. But it is not clear that the role of taurine on the growth of juvenile Korean rockfish. This study was made to investigate the effect of dietary taurine on the growth performance of juvenile Korean rockfish.
Materials and Methods

Five different taurine level diets were prepared by the supplementation of taurine to basal composition (T-0, 0.5, 1.0, 1.5 and 2.0%). The fish meal washed with 70% ethanol to remove taurine was used as a sole protein source. Feeding experiments were carried out at 15°C by using fish (BW: 13.4 g). Fish were fed the experimental diets for 3 months, respectively. At the end of experiments, fish were weighed and stored at -80°C for free amino acids analysis using amino acid analyzer.

Results and Discussion

The final average body weight of juvenile Korean rockfish was 23.7 g in the T-1.5% group and was significantly higher than the other groups. The feed efficiency ratio (%) was 82.4% in the T-1.5% group and this feed efficiency ratio was higher than the other groups. No significant difference was observed in the survival rate among groups. The taurine contents of the whole body increased with increase of the taurine supplementation. This means that the juvenile Korean rockfish were not receiving sufficient taurine contents from T-0% as compared with that of T-1.5%. In this study, taurine supplementation was found to be related to growth and feed efficiency ratio of juvenile Korean rockfish. This result indicates that juvenile Korean rockfish require at least 15 mg/g of taurine in their diets for normal growth performance.

Comparison between molecular phylogeny and growth by salinity of the genus Chlorella

Hye Jung Lee and Sung Bum Hur
(Korea Marine Microalgae Culture Center, Pukyong National University)

Introduction

The genus Chlorella Beijerinck is spherical or ellipsoidal, solitary living form, and do not possess mucilage or bristles. The Chlorella is one of the representative commercial microalgae for health food, aquaculture and other bio-industry.

Since the description of the type species of Chlorella vulgaris Beijerinck (1890), about 1,000 species have been described in Chlorella. However, these have been synonymized and revised according to comparative morphology and reproduction. In recently, evaluating biochemical and 18S rDNA sequence data have been accumulated, only four species (C. vulgaris, C. kessleri, C. lobophora and C. sorokiniana) of the genus Chlorella have been reported. In this study, we examined 70 strains of the Chlorella distributed in Korea using the 18S rDNA sequence data. The representative strains of each clade were cultured under different salinity to examine the phenotypic diversification.

Materials and Methods

The 70 strains of Chlorella were obtained from Korea Marine Microalgae Culture Center (KMMCC). Molecular phylogenetic tree was constructed and divided similar sequence groups. Two strains of Chlorella from each clade were examined on cell growth rate and cell size alteration. Chlorella strains were batch cultured in 100 ml of Jaworski’s medium with 0 psu and 100 ml of f/2 medium with 0 psu, 16 psu, 32 psu of at 25°C under continuous light with 100 μmol photons m⁻² s⁻¹. Cell numbers were counted using a hemocytometer per every day and the cell size of 40 specimens was measured by a CCD scientific camera with light microscope (×400). The experiments were repeated three times.

Results and Discussion

The almost complete 18S rDNA nucleotide sequences were determined for seventy strains of the genus Chlorella collected from Korea and foreign areas. The molecular phylogenetic tree was constructed using the 70 sequences and other known Chlorella 18S rDNA sequences, 70 strains were
divided into distinct five clades (Clade A, B, C, D, E). The feature of Chlorella strains in each clade can be summarized as follows:

- Clade A included 39 strains of seawater species which were almost collected from Korean coastal water. Growth rates of the strains in this group are the highest in 32 psu of f/2 medium.
- Clade B and C grew best in freshwater condition and sequence similarity between two clade was closely related than other clades.
- Clade D contained 15 strains and growth rate was the highest among four clades of freshwater species.
- Size of the strains in clade E was the largest. This clade contained group I intron sequence region.

**Sexual maturation and spawning characteristics of river puffer**

*Takifugu obscurus* indoor cultured in low salinity

○ Hee Woong Kang, Duck Young Kang, Sang-Ho Baek and In Kwon Jang
(=West Sea Fisheries Research Institute, National Fisheries Research and Development Institute)

**Introduction**

The puffer fish *Takifugu obscurus* it has been widely cultured in Korea as well as China because of easier to salinity acclimation in freshwater. In order to completely controlled aquaculture, the spawning, reproductive cycle and histological study of gonadal development must be discovered in fish. Subsequently, this species can be artificial seed production by induction of spawning and increasing the biomass of aquaculture seed supplies available to all year round as well as endangered species. The purpose of this study, to observe gonadal development and spawning period of male and female in *T. obscurus* in cultured of low salinity used by histological methods. Therefore, our results can contribute to aquaculture development for *T. obscurus*.

**Materials and Methods**

In *T. obscurus*, those were collected from July 2004 until June 2005, 30 individual (body length 21.5 ± 0.3 cm, weight 216.9 ± 3.3 g) per month and were purchased from the land water tank nursery of Kyonggi-Do Kimp’o. The breeding condition of *T. obscurus* was kept with recirculation system, solar salt in the recirculation system to be supplied regularly with salinity 1.8–3.2 psu. The temperature and luminous intensity were kept with 20.2–27.0°C and managed darkly below 100 lx.

For histological assay, tissues were fixed in Bouin’s solution for 24 hours and embedded in paraffin. Paraffin sections were made continuous section at 5–8 μm thickness. Which was stained with Hansen’s hematoxylin and 0.5% eosin, connective and muscle tissues were divided by Mallory, and was observed with the form and size of the germ cell. Moreover, our investigated to gonadosomatic index (GSI), hepatosomatic index (HSI) and condition factor (CF) for presume indirectly spawning season.

**Result and discussion**

The external morphology of the gonads in female and male *T. obscurus*, that was cultured in low salinity, is composed of a pair of saccular structure. Based on monthly changes in the GSI, it is assumed that in female *T. obscurus*, that was cultured in low salinity, spawn from March through May. Therefore, it showed a negative correlation relationship between changes in the GSI and HSI. On the whole, in females and males, it showed a similar pattern between wild and cultured *T. obscurus*.

The reproductive cycle with the gonad developmental phases can be classified into successive five stages in females: the early growing stage, late growing stage, mature stage, ripe and spent stage, and recovery and resting stage. In males, that can be divided into successive four stages: the growing
stage, mature stage, ripe and spent stage, and recovery and resting stage.

In case of wild *T. obscurus*, the spawning period has once a year, however, those cultured in the high water temperature (20–27°C)-low salinity (under 3.3 psu) condition have reproductive characteristics having possibilities of discharge of eggs and sperms year-round as a multiple spawner.

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**Optimal dietary protein and lipid level for growth of snail** *(Semisulcospira coreana)*

Sang-Min Lee¹, Joo-Young Seo¹ and Gyu-Doek Hwang²

(¹Faculty of Marine Bioscience and Technology, Gangneung-Wonju National University, ²Inland Fisheries Research Institute, Chung Cheong Buk-Do)

**Introduction**

The freshwater snail *Semisulcospira coreana* has been considered as a potential candidate for the freshwater shellfish culture in Korea due to its increasing market demand and high nutritional values. Aquaculture of this species has been developed in Korea and expected to expend in coming years, but available feeds are limited. Currently, a commercial abalone feed is employed in *S. coreana* aquaculture, but that is expensive and not adequate for this species in terms of nutrition and economic. The present study was aimed to determine optimal dietary protein and lipid levels for the growth of snail.

**Materials and Methods**

A factorial (4 × 2) feeding trial with three replications was conducted to determine the optimal dietary protein and lipid levels for growth of snail, *Semisulcospira coreana*. Eight experimental diets (designated as P10L5, P10L10, P20L5, P20L10, P30L5, P30L10, P40L5 and P40L10) were formulated to contain 10%, 20%, 30% and 40% protein with 5% and 10% lipid, respectively. Snails (average weight, 28 ± 1 mg/snail) were randomly distributed in twenty four 25 l rectangular aquaria at a density of 130 snails per aquarium. Three replicate groups of snails were fed one of the experimental diets *ad libitum* once a day for 10 weeks.

**Results and Discussion**

Weight gain, survival, fatty acid and chemical composition of snails were evaluated at the end of the feeding trial. Survival was over 91% and not affected by dietary protein and lipid levels. At each dietary protein level, although there were no significant differences in weight gain, the value in snails fed the diets containing 5% lipid numerically higher than that in snails fed the diets containing 10% lipid. At each lipid level, the weight gain of snails non-linearly increased with increasing protein level. The highest value was observed in snails fed the diet containing 30% protein, but was not different from that of snails fed the diet containing 20% protein. Within dietary protein level, lipid content in the edible portion of snails fed the diets containing 10% lipid was higher than that of snails fed the diets containing 5% lipid, except for the P10L5 and P10L10 diets. Fatty acid composition in the edible portion of snails significantly reflected the fatty acid composition of dietary lipid sources. The present results suggest that a feed containing 20% protein and 5% lipid consistent with a protein/energy ratio of 45 mg protein/kcal is sufficient for optimal growth of *S. coreana*. 
The effects of feeding rate in grower olive flounder *Paralichthys olivaceus* fed commercial diet at the low temperature season

Namyong Hwang¹, Jun-Ho Lee¹, Youn¹, Jun-Young Bae¹, Hyun-ho Yun¹, Jin-Hyeok Lee¹, Yi-Liu¹, Yong-Hyun Yun¹, Gun Hyun Park¹, Kang-Wong Kim², Maeng Hyun Son² and Sungchul C. Bai¹

(¹Dept. of Aquaculture / Feeds and Foods Nutritional Research Center, Pukyong Nat'l University, ²Aquaculture Management Division, National Fisheries Research and Development Institute)

Fish feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both growth efficiency and feed wastage. Moreover, knowledge of the optimum feeding rate is important not only for promoting best growth and feed efficiency, but also for preventing water quality deterioration as a result of excess feeding. Olive flounder is one of the most economically important fish species farmed in eastern Asia including Korea, Japan and China. But, From a practical point of view, flounders farmers haven’t conception exactly about fed their fish. In addition, they are not consideration for environmental conditions such as stocking density, temperature and size of fish. Low temperatures in the winter season cause stress; reduce resistance to disease; and decrease feed intake and digestibility. This ongoing study is conducted to investigated the effects of feeding rates in grower olive flounder fed commercial diet (extrude pellet) at the low temperature season (below 15°C). Three replicated groups of fish (initial weight 5.3 ± 0.22 g) are fed commercial diet at the feeding rates of 0, 0.25, 0.5, 0.75 and 1.0% body weight (BW)/day and satiation. The feeding trial is conducted by using a semi-recirculating system with eighteen 400-l aquaria receiving filtered seawater from a center tank. The duration of the trial is four weeks. The effects of feeding rates on growth performance, body composition and serological characteristics will be discussed later.

Physiological, ecological and carbon absorption investigation of algae cultured at the experimental aquafarm

Tae Ho Seo and Jong Ahm Shin
(Aquaculture Program, Chonnam National University)

Introduction

Background and Purpose: Based on the results of the study of seaweeds physiological changes caused by the climate changes, seaweeds biomass assessment and seaweeds belt for reducing CO₂, in order to study the possibility of reducing greenhouse gas by using seaweeds and find the mean technology of reducing CO₂ by seaweeds, the physiological and ecological researches of seaweeds cultured at the CDM experiment aquafarm and the carbon exchange were studied in this study, and the results will be the baseline data for the building of mathematic modeling for the CO₂ changes of the artificial seaweeds eco-system.

Materials and Methods

July 2009, 0.2 ha CDM experiment aquafarm was set at Nam-myeon Namhae-gun Gyeongsangnam-do Korea, and the object of study such as *E. cava* and *E. stolonifera* were transplanted to the point. The growth of the studied seaweeds were measured monthly from July 2009 to June. 2010, and the regression analysis were carried out on these results.

Results and Discussion

The growth of *E. cava* and *E. stolonifera* were steadily from July 2009 to June 2010. The total blade length and total weight of most sample reduced science July 2009, in May 2010, the total blade length of *E. cava* was 38.8 ± 8.53 cm, total weight was 156 ± 60.25 g; the total blade length of *E. stolonifera* was
108.5 ± 27.42 cm, total weight was 240.6 ± 97.45 g, *E. stolonifera* growed faster than *E. cava*.

The regression analysis result of total blade length and total weight showed that the determination coefficient of *E. cava* was 0.3027, and *E. stolonifera* was 0.8149; The regression analysis result of the blade area (blade length \( \times \) blade width) and total weight showed that the determination coefficient of *E. cava* was 0.7563, and *E. stolonifera* was 0.8859.

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**Effects of dietary soy protein concentrate (SPC) and soybean meal (SBM) on growth and body composition of the olive flounder (*Paralichthys olivaceus*)**

ⓒHaeYoung Moon Lee

(East Sea Fisheries Institute, National Fisheries Research & Development Institute)

**Introduction**

In republic of Korea, major species utilizing feeds are olive flounder and black rockfish in seawater aquaculture fish species in 2009. For marine finfish culture production (98,942 tons), about 472,000 tons of (78%) trash raw fish provide the main feed source; however, formulated diets are now being used with present production of 134,000 (22%) for fish species in 2008. Research on an environmentally-friendly and high efficient feed is mainly conducting including the development of new feed in flounder and rockfish. Finally there is a future trend towards the use of nutrition research to improve product quality in terms of producing more desirable culture products. These soy aquafeed developments for olive flounder are reviewed in detail. Two 6-week and 3-month feeding trials was conducted in a natural seawater flow-through aquarium system with juvenile and growing olive flounder to determine the maximum levels of soybean protein concentrate (SPC) and soybean meal (SBM) that may replace fishmeal (FM) in diets containing 50% crude protein, without reducing weight gain (WG), respectively.

**Materials and Methods**

Juvenile fish of about 56 g and growing fish of 165 g in initial body weight were fed diets to satiation twice daily, 6 days per week for 6 week at 16.0–24.9°C and 12.5–23.2°C, respectively. In the first experiment, the control diet contained 60% fishmeal as the protein source, while 15, 30, 45 or 60% fishmeal was replaced with SPC in these experimental diets (replacing approximate 25, 50, 75 or 100% of fishmeal protein in the control, respectively). In the second experiment, the control diet also contained 60% fishmeal as the protein source, while fishmeal was replaced with SPC and SBM in the experimental diets.

**Results and Discussion**

In the first experiment, fish fed the diets containing up to 75% of the protein from SPC gained as much WG as fish fed a diet with 100% of protein from FM, but fish fed the diet with 100% of protein from SPC gained significantly (\( P<0.05 \)) less. Fish fed the diets with 100% of their protein from SPC gained significantly (\( P<0.05 \)) less WG and feed efficiency (FE) than those fed the diet with all of its protein from FM with similar survival. A minimum of 25% of protein from FM appears necessary in practical diets containing most of their protein from SPC to prevent impaired growth and FE of juvenile olive flounder. WG, FE and survival and body composition are discussed in fish fed the different diets containing various soy protein levels and sources in the second experiment.
Effects of various concentrations of garlic powder and extract in the diets on growth and immune response of juvenile olive flounder (*Paralichthys olivaceus*)

○ Kyoung Tae Kim, In-Cheol Choi, Young Jin Cho, Sang-Min Lee and Sung Hwoan Cho
Division of Marine Environment & Bioscience, Korea Maritime University

**Introduction**

The use of natural resources as dietary additives has several advantages such as food safety for human consumption and minimizing the risk of side-effects. Garlic (*Allium sativum*) containing allicin, allyl cysteine, ajoene, allin and related components has been known to have antibacterial, antimicrobial, anti-inflammatory, antioxidant and/or antitumourigenic effects. Therefore, garlic seems to have high potential for aquafeed as well. In this study, effects of various concentrations of garlic powder and extract in the diets on growth and immune response of juvenile olive flounder were determined.

**Materials and Methods**

Garlic powder (crude protein: 19.0%, crude lipid: 0.8% and ash: 3.1%) and extract (Edentownfnb, Korea) were used as the additives. Seven experimental diets with various concentrations of garlic powder (GP) and garlic extract (GE) were prepared in triplicate: GP-0 without garlic supplementation, which was used as control diet, GP-0.5, GP-1, GP-2, GP-3 and GP-5 diets containing garlic powder at the concentrations of 0.5, 1, 2, 3 and 5%, respectively at the expense of wheat flour and finally, GE-0.4 diet containing 0.4% garlic extract were prepared. Fishmeal, dehulled soybean meal and corn gluten meal were used as protein sources for the experimental diets. Blood chemical analysis and lysozyme activity of fish were measured at the end of 8-week feeding trial. In addition, 20 fish from each tank were infected with *E. tarda* at $4 \times 10^7$ bacteria/ml per fish and their mortality was recorded for the next 96 h.

**Results and Discussion**

Weight gain of olive flounder fed GP-0 diet was significantly higher than that of fish fed GP-1, GP-2, GP-3 and GP-5 diets, but not different from that of fish fed GP-0.5 and GE-0.4 diets. Feed consumption of fish fed GP-0 diet was significantly higher than that of fish fed GP-1, GP-2, GP-3 and GP-5 diets, but not different from that of fish fed GP-0.5 and GE-0.4 diets. Feed efficiency ratio (FER) of fish fed GP-0 and GP-1 diets was significantly higher than that of fish fed GP-3 and GP-5 diets, but not different from that of fish fed GP-0.5, GP-2 and GE-0.4 diets. Serum total protein, glucose, glutamate oxaloacetate transaminase, cholesterol and triglyceride levels of fish was not different among the experimental diets. However, glutamate pyruvate transaminase of fish fed GP-1 diet was significantly higher than that of fish fed GP-0, GP-0.5, GP-3 and GP-5 diets, but not different from that of fish fed GP-0.5 and GE-0.4 diets. Lysozyme activity of fish fed GP-0, GP-1, GP-3 and GE-0.4 diets was significantly higher than that of fish fed GP-5 diet, but not different from that of fish fed GP-0.5 and GP-2 diets. Mortality of fish fed GP-0 diet was relatively high compared to that of fish fed the other diets containing various concentrations of garlic powder and extract after *E. tarda* infection, but no significant difference was observed. In conclusion, dietary inclusion of 0.5% garlic powder and 0.4% garlic extract seemed to have the potential as immunostimulant to lower mortality of fish at occurrence of *E. tarda*.
Effects of dietary composition on compensatory growth of subadult olive flounder (*Paralichthys olivaceus*) with different feeding regime at suboptimal temperature

In-Cheol Choi, Kyoung Tae Kim, Young Jin Cho and Sung Hwoan Cho
(Division of Marine Environment & Bioscience, Korea Maritime University)

Introduction

Optimum temperature condition for growth of olive flounder (*Paralichthys olivaceus*) were reported to be 20–25°C. However, temperature frequently rises above 30°C in summer and falls below 10°C during winter in Korea. Olive flounder overwinters to grow up to marketable size and grows slowly at low or suboptimal temperature due to its slow metabolism. At this condition, compensatory growth can be one of the most effective fish culture methods adapted by fish farmers. Lipid in fish body was primarily utilized for energy source for the basal metabolism and survival while fasting and body weight of fish decreased in proportion to feed deprivation. Therefore, manipulation of dietary nutrient composition can affect compensatory growth of fish. Effects of dietary nutrient composition on compensatory growth of subadult fish with different feeding regime were determined at suboptimal temperature.

Materials and Methods

Four hundred five fish (an initial body weight of 271.2 g) (fifteen fish per tank) were randomly distributed into 27 of 300 l flow-through tanks. Nine treatments were prepared in triplicate: fish were hand-fed with Con (C) diet to satiation twice a day, seven days a week, for 12 weeks (12WF-C), which was used as a control group; four groups of fish were starved for 1 week, and then fed with C, high protein (HP), high lipid (HL) and high protein and lipid (HPL) diets to satiation twice a day, seven days a week, for 11 weeks, referred to as 11WF-C, 11WF-HP, 11WF-HL and 11WF-HPL, respectively; and other four groups of fish were starved for 2 weeks, and then fed with C, HP, HL and HPL diets to satiation twice a day, seven days a week, for 10 weeks, referred to as 10WF-C, 10WF-HP, 10WF-HL and 10WF-HPL, respectively. The sand-filtered natural seawater was used and water temperature ranged from 9.8 to 16.9°C (mean ± SD: 12.9 ± 1.96°C) throughout the 12-week feeding trial.

Results and Discussion

Weight gain and specific growth rate of subadult olive flounder in 12WF-C treatment were significantly higher than those of fish in 11WF-C, 11WF-HP and 11WF-HL treatments among 1-week feed deprivation groups, and 10WF-C, 10WF-HP and 10WF-HL treatments among 2-week feed deprivation groups, but not different from those of fish in 11WF-HPL and 10WF-HPL treatments. Feeding rate of fish was not significantly different among treatments. Since 13.6% lower feeding rate in 10WF-HPL treatment produced 6.6% less weight gain of subadult olive flounder compared to those of fish in 12WF-C treatment, these two variables must be compromised to determine feasibility of compensatory growth of fish. Feed efficiency ratio of fish in 12WF-C, 11WF-HP, 11WF-HPL, 10WF-HL and 10WF-HPL treatments was significantly higher than that of fish in 11WF-C, 11WF-HL, 10WF-C and 10WF-HP treatments. In conclusion, subadult olive flounder subjected to 1- or 2-week feed deprivation were able to achieve full compensatory only when fish were fed with high protein and lipid (HPL) diet at suboptimal temperature.
Immunological parameters of Manila clam *Ruditapes philippinarum* analyzed using flow cytometer during post-spawning period

Hyun-Ki Hong¹, Kwang-Jae Park², Kwang-Sik Choi¹
(¹Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, ²Tidal Flat Research Institute, National Fisheries Research & Development Institute)

**Introduction**

Manila clam *Ruditapes philippinarum* is one of the most important shellfish resources Korea. Mass mortalities of Manila clam in late summer and early fall have been observed in clam beds on the west coast of Korea. The observed mass mortalities are believed to be closely associated with spawning activity of clam. After spawning, clams are physically and physiologically exhausted and their immune function is often retarded. Consequently, the weakened clams are susceptible to pathogens and environmental stresses. In an effort to understand impact of spawning on clam physiology, we investigated immunological parameters of *R. philippinarum* collected during post-spawning period using flow cytometer.

**Materials and Methods**

In October 2009, two hundred clams were sampled from a population in Goheung, on the southern coast of Korea. As physiological parameters, condition index (CI), tissue protein and carbohydrate contents were measured. The total hemocyte count, hemocyte mortality, phagocytosis activity, reactive oxygen species (ROS) production and hemocyte DNA damage were measured individually using a flow cytometry. All clams used in the analysis were subject to histological analysis to understand their reproductive condition. After collecting hemolymph, a cross-section was made in the middle of the body, fixed in the Davidson’s fixative. Reproductive condition of each clam was determined from the histological preparation.

**Results and Discussion**

In October 2009, most of clams used in the flow cytometry analysis were in spent stage (60.5%), followed by spawning stage (24.0%), resting stage (12.0%) and ripe and ready for spawning (3.5%). CI and tissue protein and carbohydrate level of spent clams were significantly lower than those of ripe or spawning clams (*P*<0.05) suggesting that spent clams were physiologically in poor condition. Total hemocyte count, DNA damage and ROS production of oysters in ripe condition were not significantly different from clams in spent and resting phases, while phagocytosis capacity and hemocyte viability of ripe clams were significantly higher (*P*<0.05), suggesting that immune parameters of spent clams were depressed compared to the ripe clams. Our data suggest that phagocytosis capacity and hemocyte viability are good measures to understand immunological status of clam.

Monitoring of *Marteilioides chungmuensis* infection in Pacific oyster *Crassostrea gigas* from Gamakman Bay, off the southern coast of Korea

M.R. Mondol¹, Bong-Kyu Kim¹, Hyun-Sil Kang¹, Chul-Won Kim² and Kwang-Sik Choi¹
(¹Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, ²Korea National College of Agriculture and Fisheries)

Protozoan parasite *Marteilioides chungmuensis* infects the oocytes of the Pacific oyster *Crassostrea gigas*, resulting in spawning failure. In this study we have surveyed *M. chungmuensis* infection in Pacific oysters suspended cultured in Gamakman bay over a year period. Histological observation performed on 87 of 3 years old and 1,004 of 1 year old juvenile oysters and revealed 24 infected
individuals. Higher prevalence (14.9%) was observed in 3 years old having 13 infected oysters but the prevalence was only 1.1% in the juvenile. The infection was observed only in ripe oocytes during the spawning and post spawning period. Histological survey results were confirmed by PCR assay using \textit{M. chungmuensis} specific primers (OPF-2/PD-18S R) where 100% of the infected Pacific oysters were successfully amplified by PCR. This study confirmed for the first time occurrence of \textit{M. chungmuensis} in juvenile Pacific oyster in Gamakman bay.

\textbf{Development of immunological probe to assess reproductive effort of black lip pearl oyster (\textit{Pinctada margaritifera}, Linnaeus 1758) from Chuuk State, Federated State of Micronesia and its application}

Hee-Do Jeung\textsuperscript{1}, Do-Hyung Kang\textsuperscript{2}, Heung-Sik Park\textsuperscript{2}, Jonathan Fournier\textsuperscript{3},
Le Moullac Gilles\textsuperscript{3} and Kwang-Sik Choi\textsuperscript{1}

\textsuperscript{1}Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, \textsuperscript{2}Marine Living Resources Research Department, Korea Ocean Research and Development Institute, \textsuperscript{3}Pearl Oyster Domestication Laboratory, IFREMER, French Polynesia)

\textbf{Introduction}

The black lip pearl (BLP) oyster, \textit{P. margaritifera} is widely distributed in the tropical and subtropical region. BLP oyster produce a good quality and high valued pearl. Recently pearl oyster population is depleting due to over exploitation. To protect the pearl population, its aquaculture, is emphasized. Knowledge about reproductive effort is fundamental for the management and understanding the life history of marine bivalves. Quantification of reproductive effort of marine bivalves is quite difficult where immunological method can easily solved this problem. Specifically, ELISA is the highly sensitive, low cost and successful egg protein quantification technique where specific immuno probes are used. Therefore in this study we reported the egg specific antibody development and measurement of reproductive effort by ELISA.

\textbf{Materials and Methods}

To develop anti-BLP oyster egg protein specific IgG, \textit{P. margaritifera} where collected from Chuuk State. The BLP oyster mature eggs were purified from ripe stage of female oysters and served as antigen. An egg protein extract was used to immunize a New Zealand white rabbit. Potential cross-reaction of the serum that weakly bound to the other tissue extracts was removed using immunoabsorbent. The anti-BLP oyster egg protein-specific IgG was isolated and purified by precipitation. The specificity and the sensitivity of our developed antibody were checked by western blotting, ELISA and immunofluorescence staining. Finally, the activity of our developed antibody was verified by estimating the reproductive effort by ELISA of \textit{P. margaritifera} collected from Chuuk State and Tahiti.

\textbf{Results and Discussion}

Potential cross-reaction of the serum was removed using immunoabsorbent. Western blotting showed that our developed antibody specifically bound to egg protein. Molecular mass of the polypeptide fractions of the eggs were determined approximately 270, 140, 100, 45, 40, and 23 kDa. Immunofluorescence staining and ELISA also showed the high sensitivity only to the egg. Finally, ELISA indicated that the Chuuk State BLP oysters produced 7.3 to 26.4% of their body weight as eggs in ripe stage whereas the respective values were 9.6 to 18.5% in Tahiti oysters. The results of this study suggest that our developed antibody was specifically and sensitively reacted to BLP oyster egg proteins unrelated the location and ELISA performed a rapid, affordable and sensitive method to assess reproductive effort of \textit{P. margaritifera}. 
Annual gametogenesis and reproductive effort of Pacific oyster (Crassostrea gigas) raised in Hebei sprit oil spill area on the west coast of Korea

Hee-Jung Lee¹, M.R. Mondol¹, Hyun-Jeong Lim², Sang-Ho Baek², Je-Cheon Jun³ and Kwang-Sik Choi¹

¹Faculty of Marine Biomedical Science (POST BK21) and Marine and Environment Research Institute, Jeju National University, ²West Sea Fisheries Research Institute, National Fisheries Research and Development Institute (NFRDI), ³NFRDI

Introduction
On 7th December 2007, 209,000 tons of crude oil was spilled from the oil tanker Hebei sprit off the coast of Taean on west coast of Korea. To understand long term impact of the spilled oil on physiology of Pacific oyster, we investigated growth and reproductive condition such as reproductive effort and gonad development of oysters in Taean area.

Materials and Methods
Oysters were collected monthly from the oil spill area from February 2008 to September 2010. As a control, a population of oyster was also sampled monthly from oyster farms in Incheon and the northern Taean. Condition index (CI) was measured as a ratio of oyster meat dry weight to the shell internal cavity volume. A cross-section was cut from the middle of oyster body for histology and gonad development was examined. Reproductive effort, the quantity of egg mass in an individual oyster was assessed using an oyster egg-specific antibody in enzyme-linked immunosorbant assay (ELIZA). The reproductive effort was expressed as percentage of egg mass per unit tissue weight as gonad-somatic index (GSI).

Results and Discussion
Histology revealed that oysters in the spilled area and the control area initiated gametogenesis in February. However, gonad development of oysters in the spilled area was delayed compared to the control oysters in spring. During spawning season, reproductive effort (GSI) of the control oysters was significantly higher (P<0.05) than that of oyster in the spilled area. In the control site, the mean GSI prior to spawning was 39% in 2008 and 38% in 2009, while GSI of oysters in the spilled area prior to spawning was 27% in 2008 and 32% in 2009. Annual mean CI of oysters in the spilled area was significantly lower (P<0.05) than the control oysters in 2008 and 2009. In July 2010, no significant difference in reproductive effort was found between the control oysters and the oysters in the spilled area (31%), although gametogenesis of oysters in the spilled area is somewhat delayed compared to the control oyster.

Effects of artificial factors on the activity of storage sperm of wild-caught striped jewfish Sterolepis deoderleini

Minh Hwang Le, Ki Tae Kim, Young Soo Kim and Young Jin Chang
(Department of Marine Bio-Materials and Aquaculture, Pukyong National University)

Storage of sperm is useful for genetic studies and artificial breeding of fish, yet few studies have been conducted in this area. The objectives of the present study were to find the best conditions and evaluate artificial factors on activity of stored sperm of wild-caught striped jewfish Sterolepis deoderleini. The best condition for cold storage sperm was dilution ratio of 1:4 in 0.3 M glucose extender at 0°C and the stored sperm sustained the activestate for 7 days. The spermatozoa motility and duration of spermatozoa motility of stored sperm contaminated with blood were higher than those
of stored sperm contaminated with seawater and were not significantly different from those of normal stored sperm. These results provide the basic knowledge about effects of environmental factors on cold storage sperm of striped jewfish. These results also demonstrate that sperm or sperm contaminated with blood of striped jewfish can be preserved.

**P-51  Effects of environmental factors on the activity of cryopreserved sperm of wild-caught striped jewfish *Stereolepis deoderleini***

Young Soo Kim¹, Young Sin Ko², Minh Hwang Le¹, Ki Tae Kim¹ and Young Jin Chang¹
(¹Department of Marine Bio-Materials and Aquaculture, Pukyong National University, ²Gyeongsang Nam-do Fisheries Institute)

Striped jewfish *Stereolepis deoderleini* is now regarded as being endangered in South Korea because of its continuous decline in recent year. Preservation and protection of its genetic resources were demanded in order to protect this rare fish species. In this project, cryopreservation technique for sperm of striped jewfish was developed. The effects of various extenders and cryoprotectants on spermatozoa motility (SM) and duration of spermatozoa motility (DSM) of cryopreserved sperm in striped jewfish were examined.

The SM and DSM of cryopreserved sperm in 0.3 M glucose were significantly higher than those in 0.5 M glucose, starry flounder artificial seminal plasma (ASP), filefish ASP or black porgy ASP and were not significantly different from those of fresh sperm. SM and DSM of cryopreserved sperm which was cryopreserved in 10% glycerol cryoprotectant were not significantly different from those of fresh sperm. Using the above method, striped jewfish sperm contaminated with blood or seawater was cryopreserved in 0.3 M glucose extender supplement 10% glycerol cryoprotectant. As a result, the SM and DSM of cryopreserved sperm contaminated with blood were significantly higher than those of cryopreserved sperm contaminated with seawater and were not significantly different from those of normal cryopreserved sperm. These results suggest that sperm or sperm contaminated with blood of striped jewfish can cryopreserve in 0.3 M glucose extender supplement 10% glycerol cryoprotectant and reject sperm contaminated with seawater. SM, DSM and delay time for activation (DT) of cryopreserved sperm activated by artificial seawater at different salinity were different. The highest SM, DSM and DT were observed with artificial seawater of 35, 25 and 20 psu, respectively. The most suitable temperature of artificial seawater to activate striped jewfish cryopreserved sperm was measured to be 20°C. These results demonstrate that DT of cryopreserved sperm of striped jewfish was affected by salinity and not by temperature.

**P-52  Properties and activities of sperm contaminated with blood or seawater of wild-caught striped jewfish *Stereolepis deoderleini***

Young Jin Chang, Minh Hwang Le, Young Soo Kim and Ki Tae Kim
(Department of Marine Bio-Materials and Aquaculture, Pukyong National University)

The objectives of the present study were to determine properties and activities of sperm contaminated with blood or seawater of wild-caught striped jewfish *Stereolepis deoderleini* and compare them with data reported in literature on other freshwater and marine fish species. The sodium, chloride, glucose, total protein concentrations of normal sperm were not significantly different from those of sperm contaminated with blood or seawater. However, the salinity and osmolality concentration of
semen contaminated with blood were lower than those of sperm contaminated with seawater and were not significantly different from those of normal sperm. In addition, the spermatozoa motility (SM) and duration of spermatozoa motility (DSM) in sperm contaminated with blood were higher than those in sperm contaminated with seawater and were not significantly different from those in normal sperm. The best condition for SM and DSM in normal sperm was dilution rate of 1:50. Sperm were immotile in distilled water. Cationic factors can stimulate the initiation of spermatozoa activation. The maximum SM and DSM were observed in each solution containing 0.4 M NaCl, 0.6 M KCl, 0.6 M CaCl$_2$ and 0.4 M MgCl$_2$. The present study provides some basic knowledge about striped jewfish sperm biosensitivity to cationic effects. In this regard, the Na$^+$ is major factor inhibitory factor of spermatozoa motility in this species.

Comparison of aging methods and growth for largemouth bass *Micropterus salmoides* in Goe-san Lake, Korea

Ming-Ming Zhang, Chul-Woong OH and Jong-Hun Na
(Department of Marine Biology, Pukyong National University)

Introduction

The largemouth bass is one of the most popular sport-fish, introduced to other countries from United States. The great demand and their relative high selling price have raised interest in their commercial culture. So far, it is cultured in some area of China. The aims of this study: 1) to investigate the biological characteristics of largemouth bass in Geo-san Lake, and 2) to determine the age and growth of it and to supply the fundamental information for fishermen and researchers.

Materials and Methods

Scale, otolith and vertebra were removed and stored in 5% KOH solution and 70% ethanol respectively. Scales were mounted between glass slides and viewed under the microscope. The distal surfaces of the otolith and vertebrae, immersed in pure glycerin, were examined on a black background with a binocular microscope under reflected light. Scale, otolith and vertebrae annuli were distinguished by different standards. Assigned numbers and compared. The reliability of the assigned numbers of annuli using each body part were ranked, firstly, by examining the correlation coefficients, indicating the level of agreement; and secondly, by examining the regression equation parameters. Growth was described by von Bertalanffy equation.

Results and Discussion

The total length observed was 89.8–472.0 mm. The weight grew isometrically ($b_M=3.0545$; $b_F=3.0640$) with the total length and there are no differences in growth patterns between males and females. The total length-weight relationship can be described by equation: $W = 9E^{-06} \times L^{3.0562}$. Age determination based on otolith reading showed that the estimated ages are between 0+ and 7+year. Growth could be described by von Bertalanffy equation with the growth parameters based on mean back-calculation total length: $L_{\infty}=680.76$ mm, $K=0.2341/y$, $t_0=-0.5725y$ and weight growth parameters: $W_{\infty}=1407.12$ g, $k=0.2608/y$, $t_0=-0.6125y$, $\Phi=4.24$. The dominant age was 3+-4+, and total length was abundant in 347.9–429.6 mm. Scale growth slows down at maturity while otoliths continue to grow in relation to length increases, and generally underestimate fish age.
Age determination and growth characters of the yellow catfish *Pelteobagrus fulvidraco* in Goe-san Lake, Korea

Ming-Ming Zhang, Jong-Hun Na and Chul-Woong OH

(Department of Marine Biology, Pukyong National University)

Introduction

Yellow catfish *Pelteobagrus fulvidraco* with both high economic and nutritional value, is one of the promising freshwater species for culture in East and South Asia, but no culture is known in Korea. Based on recent assessment of this species, the natural stock is probably at a reduced reproductive capacity and at risk of being harvested unsustainably. Knowledge of the age and growth are fundamental in the study of the population dynamics of fish species. The objective of this study is to determine the age and growth and supply the basic information for its population dynamics, then give some guide on its harvest.

Materials and Methods

Standard length and body weight were measured to the nearest 0.1 mm and 0.1 g. Got the 5-10th vertebrae, put them into 5% KOH solution for 24h, and degreased in the 95% ethanol. Check the annuli, the distance between the outer edges of the translucent zone. Body weight and body length have a relationship: \( W = a \times L^b \). Growth was described by von Bertalanffy equation.

Results and Discussion

During this period, 27 specimens were collected in Guai Shan Lake. Relationship between vertebra radius and body length for female and male was positive correlation. Those between body weight and body length for female and male were respectively expressed as \( W_\text{female} = 0.0034L^{2.51} \) and \( W_\text{male} = 0.0026 \times 10L^{2.24} \), so the both growth was allometric. The composition of age had 2+ to 5+ in males and females. The von Bertalanffy growth parameters could be expressed respectively, Male: \( L_\infty = 299.8 \text{ mm}, K = 0.2340/y, t_0 = 0.02875y, W_\infty = 220.4 \text{ g} \); Female: \( L_\infty = 268.1 \text{ mm}, K = 0.2341/y, t_0 = 0.5725y, W_\infty = 207.5 \text{ g} \); The growth index were respectively \( \Phi_M = 3.6175 \), \( \Phi_F = 3.8726 \). The inflexion point of age and body weight was estimated at 3.4 years and 62.08 g for males, correspondingly, 4.0 years and 56.74 g for female. Because the number of samples was small, the age composition showed simple.

As the relative regular form to other structural materials and high agreement and reliability, vertebra is usually chosen to determine the age and growth. The result of this study is similar with that of researches on catfishes.

Estimation of optimum dietary organic copper (MINTREX®) levels in juvenile olive flounder (*Paralichthys olivaceus*)

Mohseni, M.1, Park, G. H.1, Lee, J. H.1, Lee, S.1, Hwang, N. Y.1, Liu, Yi, Yun1, Y. H.1, Lee, J. H.1, Yun, H. H.1, Okorie, O. E.1, Bae, J. Y.1, Browdy, C.2, Bharadwaj, A. S.2 and Bai, S. C.1

1Department of Aquaculture/feeds & foods Nutrition Research centre, College of Fisheries Science, Pukyong National University

2Novus International, 20 Research Park Drive, St. Charles, MO 63304 USA

Introduction

Copper is an essential trace element for humans and animals for a number of biochemical functions (Davis and Mertz 1987; Lall 2002) which serves as a co-factor in many enzyme systems in the body. It plays an important role in metabolism and its concentration is well regulated. Improved availability of Cu from organic Cu complexes compared with the commonly used Cu salts recently has been
suggested. Chelates, complexes are the organic form of Cu and are usually considered for use in animal diet as alternatives to inorganic Cu source. More bioavailability of Cu is probably due to better absorption, which enhances its efficiency (Downs et al. 2000; Yu et al. 2000; Guo et al. 2001). The aim of the present study was designed to evaluate the effects of dietary Mintrex® copper on the growth, survival, carcass compositions and hematological parameters in juvenile olive flounder (Paralichthys olivaceus), and to determine the optimum dietary organic copper levels for this species.

**Materials and methods**

Juvenile olive flounder were fed diets containing 7 levels (0.89, 5.56, 10.11, 14.86, 18.96, 42.41 and 85.68 mg/kg diet) of MINTREX®, a chelate of copper and 2-hydroxy-4-methylthiobutanoic acid, as the copper source. Each diet was fed to triplicate groups of olive flounder (initial body weight: 6.82 ± 0.18 g, means ± SD) in an indoor static rearing system for 14. Total body weight in each aquarium was determined every two weeks and the amounts of diet fed to the fish were adjusted accordingly. Proximate composition analyses of experimental diets were performed by the standard methods of AOAC (1995). Copper content of diet, tissue, whole body and water was determined by digestion of samples in nitric acid (AOAC, 2000). The Cu concentration in rearing water was monitored regularly and remained less than 1.5 µg/l.

**Results and discussion**

Fish fed the diet containing 10.1 mg Cu/kg diet had the highest weight gain, specific growth rate and the best hematological values, but they were not significantly different from that of fish fed the 14.9 mg Cu/kg diets (P>0.05), but declined in both lower and higher levels (P<0.05). The lowest feed efficiency and protein efficiency ratio were observed in fish fed the diet containing the highest Cu content diet (P<0.05). Proximate composition of fish body was significantly affected by dietary copper level (P<0.05). The profile of Cu accumulation among tissue in olive flounder was dependent on the exposure periods and the Cu concentrations of diets. Liver could be the most important storage tissue, and the order of Cu accumulation in tissue was liver > Intestine > kidney > gill > muscle. Based on the one-way ANOVA test, the optimum dietary MINTREX® Cu level could be between 10.1 mg Cu/kg diet for maximum WG and SGR in juvenile olive flounder. Olive flounder show dietary Cu toxicity in terms of hepatic Cu accumulation, reduced growth and feed intake.