10th Japan-Korea, Korea- Japan Joint Symposium on Aquaculture 2012

Program and Abstracts

第10回日韓・韓日水産増殖シンポジウム 2012
日本水産増殖学会第11回大会
講演要旨集

December 8-9, 2012
Nagasaki University, Nagasaki, JAPAN
2012年12月 8 ～ 9 日
長崎大学

Organized by
Japanese Society for Aquaculture Research (JSAR)
Aquaculture Chapter, The Korean Society of Fisheries and Aquatic Science (KSFAS)
# Symposium Schedule and Presentation Program

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**10th Japan-Korea, Korea-Japan Joint Symposium on Aquaculture 2012**

**Chairpersons:** Kiyoshi Soyano (Nagasaki University)
Seok-Jung Kang (Gyeongsang National University)

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<td>Nam-Gil Kim (Department of Marine Biology and Aquaculture, Gyeongsang National University, Korea)</td>
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<td>09:50-10:30</td>
<td>S2</td>
<td>Microorganisms Have Close Relations to the Culture of Brachionus Rotifers</td>
<td>Akinori Hino (Prof. Emeritus, The University of Tokyo, Japan)</td>
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<td>10:30-11:10</td>
<td>S3</td>
<td>Suminoe Oyster (Crassostrea ariakensis) Culture in Korea</td>
<td>Sang-Duk Choi (Aquaculture Program, Fisheries and Ocean Science, Chonnam National University, Korea)</td>
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<td>11:10-11:50</td>
<td>S4</td>
<td>The Current Status of Fish Culture in Nagasaki and Actions of the Nagasaki Prefectural Institute of Fisheries</td>
<td>Akihiko Fujii (Nagasaki Prefectural Institute of Fisheries, Japan)</td>
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**Interpreter:** Dr. Hee-Jin Kim (Nagasaki University, Japan)

11:50-12:00 General Discussion

12:00-13:00 Lunch
High Density Production of Seaweed Biomass in Korea

Nam-Gil Kim

(Department of Marine Biology and Aquaculture, Gyeongsang National University, Korea)

For the first time, experimental cultivation of *Saccharina* began in February 1969 in Bangeojin in the south eastern coast of Korea. Since then, farming grounds have expanded to the south coast due to successful re-transplanting from the east coast and successful adoption of the technique to the warm temperature environment of the region. Wando and Gijang are famous for the mass culture sites of kelp in Korea. Especially, various seaweeds (green, brown and red algae) are produced as food source and diet of marine invertebrate in Wando and adjacent area in the southern coast of Korea. The production of *Saccharina* from Wando ranges from about 80,000 to 100,000 tons annually and contributes about 80% to total production of Korea. The supply of *Saccharina* is now sufficient and no increased demand for edible materials is expected in the near future. However, due to the strong demand for good quality products and materials for industrial use, large scale, “mass culture system” methods for *Saccharina* are now being considered for further development. Experimental study for high density production of seaweed biomass was carried out at three *Saccharina* culture grounds (Gijang, Tongyeong and Wando) in Korean coast from December 2010 to July 2012. After the young fronds have grown to 1-2 cm in length, the seed strings are removed from the seed frame and attached to the main culture rope in one of several ways (insertion and winding of seed string). Popular method is to cut seed strings into ca. 3-4 cm length which are then inserted at 30-50 cm intervals into the twist of the main culture rope (long-line). In this study, seed strings are then inserted (10 cm, 25 cm and 50 cm in interval) to or wound the main culture rope. Maximum production of *Saccharina* by the inserted interval of seed string were 80.6 kg·wt·m⁻¹ in Gijang, 77.8 kg·wt·m⁻¹ in Wando and 49.6 kg·wt·m⁻¹ in Tongyeong in 10 cm. Maximal production of seaweed biomass per hectare were 161,200 kg in Gijang, 155,600 kg in Wando and 99,200 kg in Tongyeong respectively in legal culture system.
Microorganisms Have Close Relations to the Culture of *Brachionus* Rotifers

Akinori Hino

(Prof. Emeritus, The University of Tokyo, Japan)

Half a century has passed after the establishment of marine fish seedling production. Although the introduction of *Brachionus* rotifers into the food schedule is one of the most contributive techniques to the progress, there still are problems in the maintenance of the culture. This lecture reviews studies both on promoting and inhibiting functions of bacteria and/or protozoa, and expects to be a help to resolve problems.

**Bacteria as the producer of essential vitamin B$_{12}$** Yu, Hirayama et al. (Nagasaki Univ. 1988) isolated 39 strains from rotifer tanks, and detected that those eight strains which promoted rotifer growth were all B$_{12}$ producers.

**Energy feedback by bacteria in rotifer culture** From a thinning rotifer culture, Ushiro et al. (Univ. Tokyo. 1990) obtained surprising values that food-nitrogen recovery to rotifer exceeded 80%, and the daily sum of harvested and excreted N was larger than the N inputted as food. From the experiment determining size and number of suspending particles accurately, they suggested that the food chain [food $\rightarrow$ rotifer $\rightarrow$ excretion, feces $\rightarrow$ bacteria $\rightarrow$ rotifer] is a key to conventional rotifer culture.

**Rotifer-killing bacteria** Yu et al. (Univ. Tokyo. 1990) isolated one venomous strain of *Vibrio alginolyticus* of which LD50 is $2.5 \times 10^4$ c.f.u./ml. It grows rapidly in rotifer-tank water, which can induce sudden destruction of rotifer mass culture.

**Rotifer-killing Heliozoa (protozoa)** Cheng, et al. (Univ. Tokyo. 2005) isolated *Oxnerella maritima* (10–15 $\mu$ m$^3$) which ate detritus or bacteria. Rotifer became paralyzed, sunk and died after the contact with a projecting spine of the protozoa.

**Indirect effect of Ciliate (protozoa)** Hagiwara et al. (Nagasaki Univ. 1995) suggested that *Euplotes* the most popular one affects the rotifer through bacteriovory Ushiro et al. (1998) and Cheng et al. (2005) proved that *Euplotes* doesn’t ingest living algae but bacteria from fluorescent-microscopy and isotope techniques, respectively.

**Above studies indicate** that rotifer is also a member of micro-organismic ecosystem, and not independent. Even for continuous rotifer culture, which is becoming main current in Japan, we should isolate it from venomous bacteria and protozoa.
The Suminoe oyster *Crassostrea ariakensis* is considered a potential aquaculture species in Korea, potentially supplementing or supplanting culture of the Pacific oyster *Crassostrea gigas*, currently the focus of commercial production and research. Production of cultured Suminoe oysters in Korea is limited, in part due to limited information on its biology and ecology. Commercial production is presently restricted to two rivers (Seomjin and Kawha). Here we describe the current status of *C. ariakensis* in Korea, focusing on its ecology and factors affecting development of aquaculture for this species. Preliminary investigations suggest that the Suminoe oyster shows excellent potential for expanded cultivation. A comprehensive monitoring program is needed to detect natural and anthropogenic ecosystem changes affecting production of the Suminoe oyster.

**key words:** *Crassostrea ariakensis, oyster culture, Korea*
Nagasaki Prefecture is located in northwestern Kyushu, where there are many islands and peninsulas. It has 4,184 km of shoreline, the second longest in Japan. With its geographical characteristics, fish culture flourishes in many areas with calm seas. Fish culture began in Nagasaki in the 1960s, and the main species first cultured were Japanese amberjack, *Seriola quinqueradiata*, and red seabream, *Pagrus major*. In the 1990s, tiger puffer, *Takifugu rubripes*, culture flourished. At 17,628 tons, the amount of cultured production in Nagasaki was the fourth highest in Japan in 2010. By species, at 2,449 t, the puffer production was the highest in Japan (55% of the total), while at 7,234 t for amberjack and 3,359 t for seabream, the production of each species ranked fifth highest nationwide. However, cultured production has been decreasing because of the remarkable rise in bait fish costs and the slump of the price of fish. Therefore, various measures are being taken to increase profitability in Nagasaki. For the technical support, the Institute promotes the development of seed production and cultured techniques in order to introduce new cultured fish species with high market value and reduce production costs. One new species, the kelp grouper, *Epinephelus bruneus*, has high market value, and a technique for producing 310,000 fry was developed in 2010. In addition, we developed a mixed feed for seabream that lowered the fish meal content to reduce production costs; 20% of fish meal feed contained an added enzyme that resulted in growth and survival equaling that of fish meal-based feed. Currently, we are developing a closed-circuit land culture system and examining new varieties of plant material to produce high quality tiger puffer.
**Poster Presentation (incl. oral)** 13:00–14:00, December 8, 2012

**P01** The cellular change in the organ an ascidian, a sea squirt following the progress of tunic softness syndrome  
Jae Won Kim¹, Pyong Kih Kim¹, Dong geun Seo¹, Ka Yeon Ku² and Hye Jin Kim²  
(¹Department of Marine Life-Science, Gangwon Provincial College, Gangneung 210-804, Korea,  
²Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Korea)

**P02** Antioxidant Activity of Nudibranch (*Melibe viridis*) Glycosaminoglycans  
Zuliyati Rohmah, Bernadeth F. Ticar, Si-Hyang Park¹, Yeung Joon Choi, Seok-Jeong Kang and Byeong-dae Choi  
(Gyeongsang National University/Institute of Marine Industry, ¹Sunmarine Biotechnology, South Korea)

**P03** Preparation of enzymatic hydrolysate from oyster (*Crassostrea gigas*) and purification of angiotensin I-converting enzyme inhibitory peptides  
Eon Joo Jin, Jin-So Jo Kim, Jong-Myung Ha¹, Andre Kim¹, Se Young Choung², and Yeung Joon Choi  
(Department of Seafood Science and Technology/Institute of Marine Industry, Gyeongsang National Univ., Korea, ¹Department of Pharmaceutical Engineering, Silla Univ., Korea, ²Department of Hygienic Chemistry, Kyung Hee Univ., Korea)

**P04** Isolation and Identification of Microorganisms (*Bosea minatilantensis GNU-112*) for High-density Water Flea Culture  
U-Cheol Jung, Jong-Cheol Han, Jin Feng, Byeong-Dae Choi, Jong-duck Choi and Seok-Joong Kang  
(College of Marine Science, Gyeongsang National University, Korea)

**P05** Effects of diets supplemented with herb essential oils on infection of parasitic trichodinid ciliates and physiology in chum salmon *Oncorhynchus keta* fry  
Shinya Mizuno¹, Makoto Hatakeyama¹, Nobuhisa Koide¹ and Shigehiko Urawa²  
(¹Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization, Japan, ²Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Japan)

**P06** Sulfate-reducing bacteria in the intestinal tract of spotnape ponyfish *Leiognathus nuchalis*  
Kazuyuki Sagara, Chia-Hui Chen, Shiro Itoi and Haruo Sugita  
(Department of Marine Science and Resources, Nihon University, Japan)

**P07** Effect of oxytetracycline on the microbial community in the bottom sand of the pufferfish-rearing aquarium  
Ryo Nagamitsu, Shiro Itoi, Haruo Sugita  
(Department of Marine Science and Resources, Nihon University, Japan)

**P08** Morphological characteristics of hemocytes from parasitic crustaceans  
Masakazu Kondo, Shinya Yasumoto and Yukinori Takahashi  
(Department of Applied Aquobiology, National Fisheries University, Japan)

**P09** Neutrophil granules of Japanese whiting and redspotted grouper  
Masakazu Kondo, Shinya Yasumoto and Yukinori Takahashi  
(Department of Applied Aquobiology, National Fisheries University, Japan)

**P10** A passive immunity effect of anti-*Aeromonas hydrophila-*IgY for mortile aeromonad disease in gold fish *Carassius auratus auratus*  
Shinya Yasumoto¹, Yohei Iwatani¹, Masakazu Kondo¹, Yukinori Takahashi¹, Junichi Yamane² and Kouji Umeda³  
(¹Department of Applied Aquobiology, National Fisheries Univ., Japan, ²Japan Pet Design Co., Japan, ³Japan Pet Design Co., Japan)
P11 An attempt to primary tissue culture from tumor tissue in common carp Cyprinus carpio
Daiji Koga, Shinya Yasumoto, Masakazu Kondo and Yukinori Takahashi
(Department of Applied Aquabiology, National Fisheries Univ., Japan)

P12 Comparison of bile tolerance in Lactococcus lactis strains derived from different sources
Shihori Takanashi, Ai Miura, Koko Abe, Junya Uchida, Shiro Itoi and Haruo Sugita
(Department of Marine Science and Resources, Nihon University, Fujisawa, Kanagawa 252-0880, Japan)

P13 Isolation and characterization of lactic acid bacteria from intestinal contents of hard clam, Meretrix lamarckii
Junya Uchida, Shihori Takanashi, Tomoyo Narita, Satomi Naya, Shiro Itoi and Haruo Sugita
(1Department of Marine Science and Resources, Nihon University, Kanagawa, Japan, 2Ibaraki Prefecture Sea-Farming Association, Kashima, Ibaraki, Japan)

P14 Changes in the distribution of Streptococcus parauberis serotype II cells during the disease progression in artificially infected Japanese flounder
Yuji Ishii, Kousirou Suga, Yukitaka Sugihara, Kazuma Yoshikoshi and Kinya Kanai
(1Graduate School of Fisheries Science and Environmental Studies, Nagasaki Univ., Japan, 2Nagasaki Prefectural Institute of Fish., Japan, Faculty of Fish., Nagasaki Univ., Japan)

P15 Hypoosmotic shock adaptation by prolactin involves upregulation of arginine vasotocin and osmotic stress transcription factor 1 mRNA in the cinnamon clownfish Amphirion melanopus
Na Na Kim, Hyun Suk Shin, Gyung-Suk Kil and Cheol Young Choi
(1Division of Marine Environment & BioScience, Korea Maritime Univ., Korea, 2Division of Electrical and Electronic Engineering, Korea Maritime Univ., Korea)

P16 Expression of aquaporin 3 and 8 mRNAs in the parr and smolt stages of sockeye salmon, Oncorhynchus nerka: Effects of cortisol treatment and seawater acclimation
Young Jae Choi, Hyun Suk Shin, Na Na Kim, Yuzo Yamamoto, Hiroshi Ueda and Cheol Young Choi
(1Division of Marine Environment & BioScience, Korea Maritime Univ., Korea, 2Field Science Center for Northern Biosphere and Division of Biosphere Science, Hokkaido Univ., Japan)

P17 Effects of exogenous cortisol and seawater adaptation on thyroid hormone receptors and Na+/K+-ATPase in the smolt stage of the sockeye salmon, Oncorhynchus nerka
Hyun Suk Shin, Young Jae Choi, Na Na Kim, Yuzo Yamamoto, Hiroshi Ueda and Cheol Young Choi
(1Division of Marine Environment & BioScience, Korea Maritime Univ., Korea, 2Field Science Center for Northern Biosphere and Division of Biosphere Science, Hokkaido Univ., Japan)

P18 RIG-I like gene in disk abalone Haliotis discus discus: the first molecular characterization in mollusc
Qiang Wan and Jehee Lee
(Department of Marine Life Science, Jeju National University, Jeju-si, Jeju-do, Korea)

P19 Effect of 17α-hydroxyprogesterone administration on the ionic composition of seminal plasma in artificially matured male Japanese eel Anguilla japonica
Akira Miura, Kazuharu Nomura, Hideki Tanaka and Hiromi Ohta
(1Graduate School of Agriculture, Kinki Univ., Japan, 2National Research Institute of
P20 Function of gonadotropins in spermatogenesis of the wrasse *Pseudolabrus sieboldi*

Yashiki K¹, Takeshita M¹, Kitano H¹, Lee J.M², Kusakabe T², Yamaguchi A¹ and Matsuyama M¹

¹Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, ²Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

P21 Purification and characterization of follicle-stimulating hormone and luteinizing hormone in chub mackerel *Scomber japonicas*

Mitsuo Nyuji¹, Akio Shimizu², Hirofumi Ohga¹, Ryoko Kodama¹, Akihiko Yamaguchi¹ and Michiya Matsuyama¹

¹Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, ²National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama 236-8648, Japan

P22 Relationship between growth and age on pubertal development in *Epinephelus bruneus*

Keisuke Yamamoto¹, Masahiro Nakagawa², Tatsuo Tsuzaki² and Kiyoshi Soyano¹

¹Institute for East China Sea Research, Nagasaki University, Japan, ²Goto Station, Seikai National Fisheries Research Institute, Fisheries Research Agency, Nagasaki, Japan

P23 Characteristics of oocyte development and maturation in white-streaked grouper

Tomofumi Yamaguchi¹, Yuuki Kawabata¹, Takayuki Takebe², Atsushi Nanami², Taku Sato², Kazuhsa Teruya² and Kiyoshi Soyano¹

¹Institute for East China Sea Research, Nagasaki University, Nagasaki, Japan, ²Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency, Okinawa, Japan

P24 Effects of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder *Paralichthys olivaceus* in suboptimal temperature

Hee Sung Kim, Ka Hee Kim, Sung Hyun Byun, Sung Hwoan Cho

(Division of Marine Environment and BioScience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791, Korea)

P25 Effects of dietary inclusion of various concentrations of *Scutellaria baicalensis* Georgi extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*)

Gyu Ho Jeon, Hyun Jong Kim, A Reum Kim, Sung Hwoan Cho

(Division of Marine Environment and BioScience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791, Korea)

P26 Growth performance and feed utilization of juvenile red seabream (*Pagrus major*) fed diets containing distillers dried grain

Jin Choi, Md Mostafizur Rahman, Sang-Min Lee*

(Department of Marine Bioscience and Technology, Gangneung-Wonju National Univ., Gangneung 210-702, Korea)

P27 The optimum feeding rate at three different water temperature in juvenile Korean rockfish (*Sebastes schlegeli*)

Gun Hyun Park, Jun-Ho Lee, Hyeonho Yun, Jin-Hyuk Lee and Sungchul C. Bai*

(Dept. of Aquaculture / Feeds and Foods Nutritional Research Center (FFNRC), Pukyong Nat'l University, 599-1, Deayeon-3-dong, Nam-gu, Busan 608-737, Korea)

P28 The effect of rearing tank background color on feed intake, growth and MCH level of the olive flounder, *Paralichthys olivaceus*

YC Kim¹, BS Lim², HB JungG² and JH Lee¹

¹Department of Marine Life Science, School of Marine Biomedical Sciences, Jeju
P29 Self-feeding behavior of spotted knifejaw *Oplegnathus punctatus*: influences of photoperiod and luminous intensity on daily feeding pattern
Jun Kohbara\textsuperscript{1}, Daich Touyama\textsuperscript{1}, Mitsushi Yamashita\textsuperscript{1}, Hirofumi Yamashita\textsuperscript{2}, Satoru Matsuoka\textsuperscript{2}, Hiroshi Kume\textsuperscript{2} and Koji Shiota\textsuperscript{2}
\textsuperscript{1}Graduate School of Bioreources, Mie Univ., Japan, \textsuperscript{2}Fisheries Research Center, Ehime Research Institute of Agriculture, Forestry and Fisheries, Japan

P30 For survival experiments *Moina mongolica* salt concentration jump move
College of Marine Science, Gyeongsang National University, Korea

P31 Gene expression profile during resting egg hatching in the rotifer *Brachionus plicatilis* Müller
Hee-Jin Kim, Koushirou Suga and Atsushi Hagiwara
Graduate School of Fisheries Science and Environmental Studies, Nagasaki Univ., Japan

P32 Effect of CoSO\textsubscript{4} \cdot 7H\textsubscript{2}O supplementation on the reproduction of *Moina macrocopia* and *M. mongolica*
Huang Wei, Miho Hayashi, Akari Yoshida, Yoshihito Manabe and Takao Yoshimatsu
Graduate School of Bioreources, Mie University, Japan

P33 Sex difference in the effects of ascorbic acid on the toxicity of potassium dichromate to *Daphnia magna*
Koko Abe\textsuperscript{1}, Naoyuki Ishida\textsuperscript{1}, Chiharu Araoka\textsuperscript{1}, Takeru Matsumoto\textsuperscript{2}, Shiro Itoi\textsuperscript{1} and Haruo Sugita\textsuperscript{1}
\textsuperscript{1}Department of Marine Science and Resources, Nihon University, Fujisawa, Kanagawa 252-0880, Japan, \textsuperscript{2}Mitsubishi Chemical Medience Institute, Yokohama, Kanagawa 227-0033, Japan

P34 Optimal culture conditions of two marine copepod species as live food
Keisuke Iwasaki, Yoshitaka Sakakura and Atsushi Hagiwara
Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Japan

P35 Distribution pattern of *Musculista senhousia* (Bivalvia: Mytilidae) And Tidal-flat Sediments
Sang Duk Choi\textsuperscript{1}, Ho-Seop Yoon\textsuperscript{1}, Yun-Keun An\textsuperscript{1}, Sang-Ock Ryu\textsuperscript{2} and Hong-II Jeon\textsuperscript{1}
\textsuperscript{1}Division of Marine Science, Chonnam National University, Yeosu 550-749, Korea, \textsuperscript{2}Institute of Coastal Management and Technology, Doring-ri 466-7, Cheongggye-myeon, Muan, Jeonnam 534-729, Korea

P36 Diet composition of juvenile goby species, *Gymnogobius heptacanthus* and *Chaenogobius annularis*, in the coastal waters of Geoje, Korea
Hyeon Ji Kim\textsuperscript{1}, Sang Jin Ye\textsuperscript{1}, Joo Myun Park\textsuperscript{3}, Sung Hoi Huh\textsuperscript{4} and Gun Wook Baec\textsuperscript{1,2}
\textsuperscript{1}Department of Marine Biology & Aquaculture / \textsuperscript{2}Institute of Marine Industry, College of Marine Science, Gyeongsang National University, Tongyeong 650-160, Korea, \textsuperscript{3}Division of Marine Technology, Chonnam National University, Yeosu 550-749, Korea, \textsuperscript{4}Department of Oceanography, Pukyong National University, Busan 608-737, Korea

P37 Feeding habits and reproductive ecology of scorpion fish, *Sebastiscus marmoratus*, in the coastal waters of Tongyeong, Korea
Gun Wook Baec\textsuperscript{1,2}, Yeong Mi Yeo\textsuperscript{1}, Jae Mook Jeong\textsuperscript{1}, Joo Myun Park\textsuperscript{3} and Sung Hoi Huh\textsuperscript{4}
\textsuperscript{1}Department of Marine Biology & Aquaculture / \textsuperscript{2}Institute of Marine Industry, College of
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¹(Korea Institute of Ocean Science & Technology, ²Gyeongnam Fisheries Research Institute)

P39  **Puffers smell tetrodotoxin**

Kogen Okita¹, Hideki Yamasaki², Kazutaka Sakiyama², Hikaru Yamane¹, Shinya Niina¹, Tomohiro Takatani¹, Osamu Arakawa³ and Yoshitaka Sakakura¹

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P40  **Salinity selectivity of tiger puffer juveniles**

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P41  **Effects of ocean acidification on reproduction sea urchins**

Atsushi Ishimatsu¹, Rui Yin¹, Kyoung-Seon Lee², Guining Wang³, Mitsuharu Yagi¹, Jiro Okada⁴ and Haruko Kurihara⁵

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Masatoshi Ban, Hiromi Ito, Satoru Takahashi

(Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Japan)

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Jae-Gil Jang, Nam-Gil Kim and Hwa-Yeon Lee

(Department of Marine Biology and Aquaculture, Gyeongsang National University, Korea)

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Jong-Cheol Han, U-Cheol Jeong and Seok-Joong Kang

(College of Marine Science, Gyeongsang National University, Korea)

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Yoichi Minami¹,² and Michiyasu Yoshikuni²

¹Okinawa Prefectural Fisheries and Ocean Research Center, Japan, ²Fishery Research Laboratory, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Japan)
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Ô Yoshiaki Tsuji¹, Sachiko Sugimoto¹, Kiyoshi Isowa², Hideo Aoki³ and Hiromi Ohta¹
(¹Graduate School of Agriculture, Kinki Univ., Japan, ²Mie Prefectural Fish Farming Center, Japan, ³Mie Prefecture Fisheries Research Institute, Japan)

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(¹Toita Women’s College, Japan, ²Gakushuin Women’s College, Japan)

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(¹Korea Institute of Ocean Science & Technology, ²Gyeongnam Fisheries Research Institute)

P52 Comparison of characteristics between generations of hybrid strain in the self-fertilizing mangrove killfish
Ô Kyoko Horii¹, Atsuko Kurakake¹, Koushiro Suga¹, Akira Kanamori², Atsushi Hagiwara¹ and Yoshitaka Sakakura¹
(¹Graduate school of Fisheries Science and Environmental Studies, Nagasaki Univ., Japan, ²Division of Biological Science, Graduate School of Science, Nagoya Univ., Japan)

P53 Ploidy status of progeny from the crosses between naturally occurred triploid females and diploid males of spined loach (*Cobitis*, Teleostei)
Ô Olga Jablonska¹⁵, Dorota Juchno¹, Anna Nynca², Sylwia Swigonska², Magdalena Krol⁴, Anna Leska¹, Anna Grabowska¹, Roman Kujawa², Aneta Spóz³ and Alicja Boron¹
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P54(KO1) Oral Presentation
Effects of dietary probiotic on growth performance feed utilization, innate immunity and disease resistance against *Streptococcus iniae* in olive flounder (*Paralichthys olivaceus*)
Ô Ji-Hoon Cha¹, Si-Yong Yang², Kyeong-Jun Lee¹,*
(¹Department of Marine Life Sciences, Jeju National Univ., Korea, ²CJ Research Institute of Biotechnology, CJ Cheiljedang Corp., Korea)

P55(KO2) Oral Presentation
Effects of dietary supplementation of propolis on growth performance, feed utilization, non-specific immune response and disease resistance in juvenile olive flounder (*Paralichthys olivaceus*)
Ô Dae-Han Oh¹, Yong-kap Hur², Kyeong-Jun Lee¹,*
Effects of different lipid sources in the floating extruded pellet on growth performance, feed utilization, fatty acid composition and flesh quality of adult flounder *Paralichthys olivaceus*

Jin Choi¹, Kyoung-Duck Kim², Kang-Woong Kim², Sang-Min Lee¹ *

¹Department of Marine Life Sciences, Jeju National Univ., Korea, ²Korea Atomic Energy Research Institute Invested Company., Korea

Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview

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The cellular change in the organ an ascidian, a sea squirt following the progress of tunic softness syndrome

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Sea squirts with tunic softness syndrome were collected from the southern coastal region of Korea and divided into 4 stages (stage 0,1,2,3) by observing elasticity, external features and color. Histopathological analysis was made on the tunic, siphon, branchial sac, and body musculature. Sample preparations were fixed in Bouin’s solution for 24 hours. The specimens were embedded in paraplast and subsequently sectioned at 5μm thickness using a microtome, stained with haematoxylin-eosin(H-E), (AB-PAS pH2.5) and examined under a light microscope.

The tunic, derived from the epithelial layer, was composed of a cuticle, matrix and outer epidermis. During the progressive stages of tunic soft syndrome, degeneration went from the microfiber layer in the matrix region, to the epithelial layer and then the cuticle layer. The microfiber of the matrix region loosened its interval, after it transformed wave motion pattern into straight type and dissected. The epithelial layer separated at an early stage, indicating the epithelial layer was necrotic and losing its function of matrix synthesis. With increased progression of tunic soft syndrome, the epithelial layer became separated from muscular layer with connective tissue. The interval of microfiber in the matrix region become loose and necrotic with vacuolization. We observed ruptures in the dissected muscular fiber. The epithelial cells of branchial sac from infected tunicates were thinner while the diameter of the brachial sac decreased. We also observed a loss of lateral cilia as well as vacuolar deformation. The lumen of branchial sac had increased hemocytes, and we observed ruptures of branchial sac cells and degenerated cells of lumen of branchial sac. With an increased progression of tunic soft syndrome, the epithelial layer separated from muscular layer with connective tissue and we can confirm the deformation of a vacuole. We observed the dilation of blood lymph in muscular layer, cytoplasmic hypertrophy of muscular fiber and vacuolation and necrosis in a bundle of muscular fiber.

Antioxidant Activity of Nudibranch (Melibe viridis) Glycosaminoglycans

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The glycosaminoglycans (GAGs) from Melibe viridis were extracted and optimized using Multifactorial design. Four factors (Temperature, pH, incubation period, and enzyme ratio) were varied in the optimization experiment. The design used three enzymes namely; Alcalase, Flavourzyme, and Protamex. The optimum results were obtained using Flavourzyme at pH 8.0,
incubation temperature of 45°C, for 15 hours, and an enzyme ratio of 1.5% relative to sample weight. The extracted Melibe GAGs (prepared at different concentrations) were tested for DPPH scavenging effect, it showed that, it has a comparable activity with standard, the Ascorbic acid. It also showed a reductive capability in a concentration-dependent manner. For the hydroxyl radical scavenging effect, the results showed that the activity is very near with that of the standard too, butylated hydroxyanisole (BHA). From the data obtained in this study, it can be inferred that, Melibe GAGs has a potential in the field of medicine as well as they can be used as dietary supplements with specific functional properties.

P03  Preparation of enzymatic hydrolysate from oyster (Crassostrea gigas) and purification of angiotensin I-converting enzyme inhibitory peptides

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The objectives of this study were to prepare oyster hydrolysate using commercial proteases and to purify the angiotensin converting enzyme (ACE) inhibitory peptides. Oyster protein was cross-linked using transglutaminase and then hydrolyzed with Protamex and Neutrase. Degree of hydrolysis and potential ACE inhibitory activities improved with the combination of Protamex and Neutrase. ACE inhibitory activity was the highest in the range of 200–500 Da. Twelve peptides were purified from the oyster hydrolysate through multi-step chromatographic purification comprised of anion exchange, size exclusion, and reverse-phase liquid chromatography, and their sequences were identified using an amino acid sequencer and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. The IC₅₀ values of the synthetic peptides TAY, VK, KY, YA, AFY and MC were 2.18, 2.63, 5.90, 8.76, 11.18, and 14.36 µg, respectively, and no toxicities to the Hep-G2 cell line were detected for any of the synthetic peptides.

P04  Isolation and Identification of Microorganisms (Bosea minatillanensis GNU-112) for High-density Water Flea Culture

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Introduction
Artemia is an essential food organism for fish seed production worldwide. In Korea, Artemia cyst supply relies entirely on importation. Depending on the international demand and supply, the price of Artemia cyst could skyrocket. This concern prompted research for alternative prey organisms.
Freshwater and seawater flea as alternative prey organisms to Artemia are being studied for more than 10 years by our research team. The water flea can be cultured outdoors but at low density, and indoors at high density using the concentrated freshwater Chlorella as feed. However, the use of Chlorella gives rise to contamination of water quality at high density culture. Hence, isolation and identification of microorganisms as replacement to microalgae in water flea culture can be useful to resolve these issues.

**Materials and Methods**

Cladocerans *Moina macrocopa* used in the experiment were acquired from the Feed Engineering Laboratory of Marine Biology at Gyeongsang National University in Korea. Two hundred ten (210) strains of bacteria from poultry farm, rice paddy and coastal mudflat were isolated and cultured in PCA, PDA, TSA and LA. Four superior bacterial strains were selected and used to test survival of the Cladocerans in culture. 16S rDNA sequencing was carried out to identify the bacterial strains.

**Results**

1) Of the 210 bacterial isolates, two strains from poultry farm, one strain from rice paddy and one strain from coastal mudflat were selected.
2) Cladoceran was successfully cultured using the four bacterial strains. The bacterial strains a) improved the water quality by decreasing TAN, b) increased the survival rate, and c) increased the fecundity rate of the cladoceran.
3) The best bacterial strain was selected, and identified by 16S rDNA sequencing as *Bosea minatitlanensis* GNU-112.

**P05**

**Effects of diets supplemented with herb essential oils on infection of parasitic trichodinid ciliates and physiology in chum salmon *Oncorhynchus keta* fry**

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**Introduction**

Chum salmon *Oncorhynchus keta* are artificially propagated to enhance their coastal stock in Hokkaido of northern Japan. Infection of ectoparasitic trichodinid ciliates causes high mortalities in chum salmon fry, which are cultured with freshwater in the ponds of salmon hatcheries, for release of artificial seed into the river in the propagation. The ciliate found on the skin of the fry in northern Japan is identified as *Trichodina truttae* (Urawa and Arthur 1991). At present, the trichodinid ciliates are exterminated from the body surface of the infected fry by bath of the fry in the dilute vinegar or dilute salt. However, there have been no techniques to restrain their infection in the fry. It is well-known that essential oil of herbs including mint and lavender functions in moth proofing. In the present study, effects of herb essential oils dietary supplement on infection of trichodinid ciliates and physiology in chum salmon fry were studied.
Materials and methods

Hatchery-reared fry, which were not infected with trichodinid ciliates, were divided into 5 individual groups of 200 fry on May 21, 2010. The five groups were individually housed in a 60 l-acrylic tank and cultured with running springwater for 35 days. Each of the five groups was fed either commercial trout crumble diet (control) or diets enriched with essential oil of original Japanese (OJ-) mint, breeding Japanese (BJ-) mint, peppermint or lavender, respectively. All herb oils were added to respective control diet at 500 ppm. Total amount of the supplied diets were equalized between the 5 groups. Fry were randomly sampled a few times during the experiment from each group and used for counting total parasite number of trichodinid ciliates. In addition, survival rate in 48 hours after transferring from freshwater to seawater and gill Na+, K+-ATPase and serum lysozyme activities were examined using the fry samples on 35 days.

Results and summary

Total parasite number significantly increased during the experiment in the control, BJ-mint, peppermint and lavender groups. On the other hand, infection of trichodinid ciliates had not been found during the period in the fry of OJ-mint group. In the present study, dietary supplements of all herb oils significantly restrained infection of trichodinid ciliates in the fry on 21 and 35 days. Control group showed significantly higher serum lysozyme activity with the infection compared to other dietary supplement groups. However, there were no significant differences in the survival rate of the fry after seawater transfer and gill Na+, K+-ATPase activity between control and other herb dietary supplement groups. In consequence, the present study suggested that dietary supplements of mint and lavender oils suppressed infection of trichodinid ciliates and had no impacts on seawater adaptability of the fry. Besides, it is possible that effects of herb dietary supplement on control of the infection depends on variation of herb species.

Reference


Sulfate-reducing bacteria in the intestinal tract of spotnape ponyfish Leiognathus nuchalis

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In general, the intestinal tracts of marine fish are predominantly colonized by members of the family Vibrionaceae while obligate anaerobes have not been detected as major components. On the other hand, sulfate-reducing bacteria (SRB) are obligate anaerobes that are distributed widely in estuarine and coastal sediments. This fact strongly suggests that intestinal tracts of costal fish may be populated by SRB. Therefore, in this study SRB were examined to distribute in the intestinal tracts of costal fish including spotnape ponyfish Leiognathus nuchalis by the combination of real-time PCR technology and clone library analysis.

Coastal fish were collected by fishing lines in Kanagawa and Shizuoka Prefectures, Japan. DNA was extracted from microbial cells in the intestinal contents and purified using a FastDNA SPIN
kit (MP Biomedicals). Fragments of the dissimilatory sulfite reductase genes (*dsrA*) was PCR amplified, cloned into the pGEM-T vector system (Promega) and transformed into *Escherichia coli* DH5α. Partial clone sequences (approx. 500 bp) were compared with all sequence data in the DDBJ / GenBank / EMBL databases using the BLAST algorithm.

As a result, SRB was detected in one of five specimens of spotnape ponyfish with a density of $3.3 \times 10^8$ copies/g. Thirty-two clones in the library were composed of four families of SRB including *Desulfobacteraceae* (6 species, 13 clones) and *Desulfobulbaceae* (3 species, 13 clones), *Desulfomicrobiaceae* (1 species, 3 clones) and *Desulfovibrionaceae* (2 species, 3 clones). These results suggested that the intestinal tract of spotnape ponyfish was populated by diverse species of SRB.

Effect of oxytetracycline on the microbial community in the bottom sand of the pufferfish-rearing aquarium

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In the aquaculture facilities, bacterial infections sometimes occur, which results in serious economic losses. In such cases, antibacterial drugs are usually used to treat the bacterial diseases of fish. However, with long-term exposure to antibacterial drugs pathogenic bacteria often develop drug resistance, which makes infections more difficult to treat. Moreover, a large amount of antibacterial drugs used to treat the bacterial diseases are usually discharged into the water and sediment surrounding the aquaculture facilities, resulting in the reduction of microbial activities and possibly water quality problems. Oxytetracycline (OTC) is a broad-spectrum antibiotic, active against a wide variety of bacteria, including the family *Vibrionaceae*. Therefore, this study was undertaken to examine the effect of OTC on the microbial community in the bottom sand of a pufferfish (*Takifugu rubripes*)-rearing aquarium.

Eighty mL of seawater and 40 g of bottom sand were transferred from the pufferfish-rearing aquarium into a 100 mL of screw capped bottle, to which OTC was added at a final concentration of 20 μg/mL. Simultaneously, three sets of this system were prepared and incubated separately at 10, 20 and 30°C for 28 days. The seawater and bottom sand were collected after 0, 7, 14, 21 and 28 days of the incubation. The concentrations of OTC were determined by the disc diffusion method using *Staphylococcus aureus* subsp. *aureus* NBRC 14462 strain. Total numbers of bacterial cells in the seawater and sand were determined by epifluorescence microscopy. DNA was extracted from microbial cells in the sand samples and purified using a FastDNA SPIN kit (MP Biomedicals). The 16S rDNA was PCR amplified, cloned into the pGEM-T vector system (Promega) and transformed into *Escherichia coli* DH5α. The inserted DNA was analyzed by the Dye Terminator Cycle Sequencing method using a model 3130xl DNA sequencer (Applied Biosystems). The clones were identified using the Ez-taxon server on the basis of 16S rDNA sequence data.

As results, the concentrations of OTC in the seawater decreased at a rate of 0.6–0.7 and 1.5 μg/mL/day at 10/20°C, and 30°C, respectively. Microbial communities in the sand were populated by *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* on day 0, and Gram-positive bacteria such as *Actinobacteria* and *Firmicutes* after 7 and 14 days of the incubation. These results strongly suggest that microbial communities of bottom sand of aquaculture facilities were influenced remarkably by OTC.
Morphological characteristics of hemocytes from parasitic crustaceans

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In previous report, we made a speculation about the evolutionary process of crustacean hemocytes as follows. 1. Ancestor of crustacean had only a single type of hemocyte and this characteristic is taken over by the species in lower classes of crustaceans such as Branchiopoda and Maxillopoda up to the present. 2. Plural hemocyte types appeared in more advanced class, Malacostraca (Even in the malacostracans, primitive species (leptostraca) have only a single type of hemocyte). In the present study, morphological characteristics of hemocytes from the parasitic crustaceans, marine argulid Argulus caecus (Japanese name, HO-SO-U-MI-CHO-U; Arguloida, Branchiura, Maxillopoda) and cymothoid Mothocya sajori (Japanese name, SA-YO-RI-YA-DO-RI-MU-SHI; Flabellifera, Isopoda, Eumalacostraca, Malacostraca) collected from grass puffer Takifugu niphobles and halfbeak Hemirhamphus sajori respectively, were examined by light microscopy after staining with May-Grünwald stain using acid phosphate buffer (1/15 M, pH5.0) as a diluent. Only a single type of hemocyte was observed in the hemolymph of A. caecus. The hemocytes contained chromophobic granules. On the other hand, plural (eight) hemocyte types were detected in the hemolymph of M. sajori as below: basophilic fine granular cell, basophilic plasma cell, basophilic granulocyte, chromophobic small granulocyte, chromophilic large granulocyte, basophilic and eosinophilic granulocyte, eosinophilic granulocyte type 1 (with fine chromatin mesh) and eosinophilic granulocyte type 2 (with condensed nucleus).

Neutrophil granules of Japanese whiting and redspotted grouper

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We have classified fish neutrophil (phagocytic granulocyte) based on the granule composition. Primitive fish, hagfish, had only one type of granule, basophilic granule (γ G). On the other hand, various granule compositions were observed in advanced fish (teleost fish) as below: Type 1 neutrophil, three types of granules, eosinophilic granule (α G), chromophobic granule (β G) and γ G; type 2, α G and β G; type 3, β G only; type 4, β G and γ G. Furthermore, α G and γ G were classified into two and three subclasses respectively, based on the staining characteristics. Type 1 neutrophil was observed in Asian arowana (Scleropages formosus), common carp (Cyprinus carpio), Japanese eel (Anguilla japonica), Nile tilapia (Oreochromis niloticus), striped grunt (Parapristipomina trilineatum) and Japanese amberjack (Seriola quinqueradiata); type 2, tiger puffer (Takifugu rubripes) and red sea-bream (Pagrus major); type 3, ayu (Plecoglossus altivelis), northern pike (Esox lucius), gray mullet (Mugil cephalus), rudderfish (Girella punctata) and Japanese flounder (Paralichthys olivaceus); type 4, Japanese lates (Lates japonicus); marbled rockfish (Sebastiscus marmoratus) and oblong rockfish (Sebastes oblongus). Type 3 neutrophil was classified into two types based on the difference of
peroxidase positive sites (3-A, β G only; 3-B, β G and nucleus). The neutrophils from Japanese whit- ing Sillago japonica and redspotted grouper Epinephelus acaara were identified as type 3-B.

**P10**

**A passive immunity effect of anti-Aeromonas hydrophila-IgY for mortile aeromonad disease in gold fish Carassius auratus auratus**

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In this study, we introduce anti-Aeromonas hydrophila-IgY (egg yolk Immunoglobulin) to examine a passive immunity effect for protection of gold fish against artificial infection with A. hydrophila by direct cannulation to anterior intestine.

Anti-A. hydrophila-IgY was added to the commercial diet, and this diet was used for IgY diet (1280 titer/g). On the other hand, saline was added to commercial diet as a control (normal diet). Four experimental groups were established (n=15, mean body weight 5.0 g), and two fish groups (IgY groups) were orally administrated with IgY diet for experimental period (0.1 g/day). The other groups (control groups) were orally administrated with normal diet for same period. Two weeks after first oral administration, fish were challenged with live A. hydrophila suspension at 1.2 × 10⁷ cells per a fish by direct cannulation to anterior intestine. Challenged fish were held at 22°C for 2 weeks. Mortalities were recorded daily. Each day moribund and fish just after death were removed. All of the surviving fish were removed at the end of the experiments. In a challenge test, the IgY groups had 2 and 4 dead fish within 7 days after challenge and the cumulative mortality resulted in 13.3 and 26.7% by 2 weeks after challenge. On the other hand, the control groups had 6 and 8 dead fish within 10 days after challenge and the cumulative mortality resulted in 40.0 and 53.3%. As a result, total relative percent survival (RPS) was 57.2%.

**P11**

**An attempt to primary tissue culture from tumor tissue in common carp Cyprinus carpio**

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Ovarian tumor has been reported. Recently, we found the tumor structure in adult male common carp. In this study, we found newly diseased common carp which had huge tumor, and we performed histopathological study of tumor and attempted to primary tissue culture from tumor tissue.

Diseased common carp were removed from the tank and pieces of tumor, intestine, head kidney, body kidney, spleen, liver, heart, gills and brain were fixed in 10% formalin and processed for
histopathological examination. Tissue sections were stained with Mayer’s hematoxylin and eosin. The removed tumors bathed in 1% sodium hypochlorite solution for 1 minute and then rinsed in 70% ethylalcohol for a few seconds. The farther preparations followed standard methods using Eagle’s minimum essential medium supplemented with 20% fetal bovine serum, 0.5% carp serum and antibiotics for tissue culture. The tissues were grown at 23°C in a volume of 6 ml in cell culture flasks (25 cm²).

Diseased common carp showed the abdominal enlargement, exophthalmus and scale protrusion. At necropsy, a large tumor and abdominal ascites were found in body cavity. Histopathologically, the large tumor was composed of parenchyma which was satisfied with propagated tumor cells and stroma which was spongiform connective tissue and fibrin, and parenchyma and stroma mixed area. These histopathological changes were similar to previous report.

We attempted to primary tissue culture from three kinds of tumor tissues (parenchyma, stroma and mixed area). The cells from parenchyma and stroma mixed tissue grew out within 14 days forming small foci of fibroblastic cells in the flask. However, the cells from parenchyma and stroma tissue did not grow out.

P12

Comparison of bile tolerance in Lactococcus lactis strains derived from different sources

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Lactic acid bacteria (LAB) have been isolated from various environments such as the surface of plants and fermented dairy products, and used for long terms as probiotics for human. Recently, we successfully isolated Lactococcus lactis subsp. lactis from the intestinal tract of marine fish. In this study, we examined the bile tolerance of L. lactis strains derived from different sources because the high tolerance to gastric acid and bile of microorganisms may be required for probiotic use in aquaculture.

Three strains of L. lactis subsp. lactis used in this study were derived from marine fish (MFL), freshwater fish (FFL) and cheese starter culture O-114 (CSL), whereas a strain of L. lactis subsp. cremoris was derived from cheese starter culture O-114 (CSC). These strains were cultured under various conditions as follows. MRS broth containing various concentration of bile salts/acids including sodium cholate, sodium deoxycholate and sodium taurocholate; MRS agar containing 0 to 10% oxgall; PBS containing the fish bile collected from goldfish or pufferfish.

The surviving degree of the strains in the presence of sodium cholate and sodium deoxycholate were MFL and CSL > CSC > FFL, whereas that in the presence of sodium taurocholate was MFL > CSL > CSC > FFL. In oxgall tolerance, MFL and CSL could grow in 9% and 3%, respectively. On the other hand, CSC and FFL strains could not grow in more than 0.6%. The survival of MFL was not affected by the bile from goldfish or pufferfish although those of the other strains were significantly inhibited.
Isolation and characterization of lactic acid bacteria from intestinal contents of hard clam, *Meretrix lamarkii*

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Lactic acid bacteria had been isolated from various environments containing dairy products and plant surfaces. Recently, we isolated lactic acid bacteria (LAB) from the intestinal tracts of marine and freshwater fish, and identified as *Lactococcus lactis* subsp. *lactis*, based on the 16S rRNA gene sequence. As the *L. lactis* subsp. *lactis* strains derived from different sources showed unique phenotypic properties including halotolerance and fermentation profiles, the effective strains for probiotic use may be included. In this study, we newly attempted to isolate LAB from the intestinal contents of hard clam *Meretrix lamarkii*, which is one of important species of coastal fisheries.

Intestinal contents of *M. lamarkii* were pre-cultured in MRS broths and then a loopful of culture was incubated on MRS agar plates to isolate LAB. DNA was extracted from some colonies of candidate LAB and the partial DNA fragment of bacterial 16S rRNA gene was amplified by PCR. Sequencing of the amplified fragment showed that the isolates were identified as *L. lactis* subsp. *lactis*, *Lactobacillus* sp. and *Pediococcus* sp. The LAB were subjected to the phenotypic characterization including oxidase test, halotolerance test, gram staining, catalase test and fermentation test. As a result, the fermentation pattern of *L. lactis* subsp. *lactis* were highly similar to that of marine fish-derived *L. lactis* subsp. *lactis*. Fermentation patterns of *Lactobacillus* sp. and *Pediococcus* sp. were highly similar to that of *Lactobacillus plantarum* and *Pediococcus pentosaceus*, respectively.

Changes in the distribution of *Streptococcus parauberis* serotype II cells during the disease progression in artificially infected Japanese flounder

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Streptococcosis of Japanese flounder, *Paralichthys olivaceus*, caused by *Streptococcus parauberis* has occurred since the beginning of this century. Two serotypes, type I and type II, are known in *S. parauberis* isolated from flounder. Type I was frequently isolated from diseased fish from spring to autumn, and type II from summer to autumn. Generally the virulence of type II strains is lower than that of type I. Accordingly the knowledge on the pathogenicity of type II is scarce. However, studies on the pathogenicity of type II are necessary for the development of prevention measures such as vaccines.

First, we examined the virulence of six type II strains (NUF943, NUF945, NUF1042, NUF1076, NUF1089, PS08-8) by inoculating into the back muscle of Japanese flounder. As a result, NUF943 showed a high mortality rate. Next, the fish were challenged with NUF943 as above, and tissue
specimens including heart, kidney, spleen, liver and gills were collected from the fish at different points in time and made into paraffin sections. Then, using immunohistochemical techniques with rabbit anti-\textit{S. parauberis} type II serum and goat antibody against rabbit antibody labeled with peroxidase as a primary and secondary antibody, respectively, we examined the distribution of the bacterial cells in the tissues. As a result, it was found that the type II cells existed mainly in the vicinity of the blood vessels in each organ and that the bacteria were engulfed by phagocytes. From these observations, it is suggested that infected \textit{S. parauberis} type II cells spread into different organs through the blood circulatory system with phagocytic cells and then grow in the organs.

\textbf{P15} Hypoosmotic shock adaptation by prolactin involves upregulation of arginine vasotocin and osmotic stress transcription factor 1 mRNA in the cinnamon clownfish \textit{Amphiprion melanopus}

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We cloned cDNA-encoding arginine vasotocin (AVT) from the brain of the cinnamon clownfish \textit{Amphiprion melanopus}, and that was predicted to encode a protein of 153 amino acids. We examined changes in the expression of AVT mRNA in the brain and arginine vasotocin receptor (AVTR) mRNA and osmotic stress transcription factor 1 (OSTF1) mRNA in the gills of the cinnamon clownfish using quantitative real-time PCR in an osmotically changing environment (seawater (35 psu) $\rightarrow$ brackish water (BW, 17.5 psu) and BW with prolactin [PRL]). The expression of AVT, AVTR, and OSTF1 mRNA in the brain and gills increased after transfer to BW, and the expression was repressed by PRL treatment. AVT-immunoreactive cells were almost consistently observed in the telencephalon. The plasma \(Na^+\) and \(Cl^-\) levels decreased in BW, but the level of this parameter increased in BW with PRL treatment during salinity change. These results suggest that AVT, AVTR, and OSTF1 play important roles in hormonal regulation in osmoregulation organs, and that PRL improves the hyperosmoregulatory ability of cinnamon clownfish in BW environment.

\textbf{Acknowledgement} This research was supported by Technology Development Program for Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Korea.
Expression of aquaporin 3 and 8 mRNAs in the parr and smolt stages of sockeye salmon, *Oncorhynchus nerka*:
Effects of cortisol treatment and seawater acclimation

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²Field Science Center for Northern Biosphere and Division of Biosphere Science, Hokkaido Univ., Japan)

In this study, two aquaporin (AQP) isoforms (AQP3, 8) found in sockeye salmon (*Oncorhynchus nerka*) were classified and their tissue specificity and mRNA expression in response to a hyperosmotic challenge and during parr and smoltification. AQP3 mRNA was detected in the gill, esophagus, intestine, kidney and AQP8 mRNA was detected in the intestine and kidney. We examined the changes in the expression of AQPs mRNA in the gill and intestine of the parr and smolt stages of sockeye salmon increased transfer to SW using quantitative real-time PCR in an osmotically changing environment [freshwater (FW, 0 psu; practical salinity unit) to seawater (SW, 35 psu) and cortisol injection]. Correspondingly, AQPs peaked during smoltification than parr stages of sockeye salmon, respectively. Also, the plasma osmolality level increased in SW, but the level of this parameter decreased in SW with cortisol treatment during salinity change. These results suggest that the AQPs gene play important roles in water absorbing mechanism associated with multiple AQP isoforms in a hyperosmotic environment.

[Acknowledgement] This work was financially supported by KMU.

Effects of exogenous cortisol and seawater adaptation on thyroid hormone receptors and Na⁺/K⁺-ATPase in the smolt stage of the sockeye salmon, *Oncorhynchus nerka*

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(¹Division of Marine Environment & BioScience, Korea Maritime Univ., Korea, ²Field Science Center for Northern Biosphere and Division of Biosphere Science, Hokkaido Univ., Japan)

The objective of this investigation was to quantify how thyroid hormone receptors of the sockeye salmon, *Oncorhynchus nerka*, respond to salinity changes from freshwater (FW) to seawater (SW) conditions. Thyroid hormone receptors (TRs), Na⁺/K⁺-ATPase (NKA) mRNA and protein expressions, and T₃ and T₄ plasma levels significantly increased when the fish were transferred from FW to SW. Moreover, these parameters were significantly lower in the cortisol-injected groups than in the control. Hence, TRs, T₃, T₄, and NKA may play a role in SW adaptation, while TRs, T₃, and T₄ may be related to growth enhancement during the smolt stage, when the fish migrate from FW to SW environments. We showed a negative correlation between cortisol and thyroid hormone levels. We
also recorded a significant increase in ACTH and plasma K⁺ levels in the kidney when the fish were transferred to SW, with levels being significantly lower in the cortisol-injected group. Hence, cortisol appears to be a stress hormone in the fish. Moreover, Na⁺ and Cl⁻ plasma levels significantly increased when the fish were transferred to SW, with levels being significantly lower in the cortisol-injected group. These results indicate that cortisol modulates ion transportation by regulating NKA in the gills.

[Acknowledgement] This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2012-0001370).

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**RIG-I like gene in disk abalone Haliotis discus discus:** the first molecular characterization in mollusc

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The retinoic acid-inducible gene-I (RIG-I) like receptors (RLRs) are an evolutionarily conserved family of cytosolic pattern recognition receptors. They play a key role in the recognition of viral RNA, and are essential in the early induction of type I interferon (IFN) in innate antiviral defense of vertebrates. However, no IFN-like molecule has been described from molluscs and other non-vertebrates, in which defense against viral infection is considered to rely mainly on RNA interference mechanism. In the present study, we identified a RIG-I like gene in disk abalone transcriptome database and revealed its genomic structure through BAC library sequencing. Abalone RIG-I like gene contains 16 exons and 15 introns, encoding a protein with 115 kDa predicted molecular mass. Abalone RIG-I protein contains two repeats of the caspase recruitment domain (CARD)-like domains at N terminus, one DExD/H box-containing RNA helicase domain and one C-terminal repression domain, suggesting a similar primary structure as the RIG-I and MDA5 genes in vertebrates. The promoter analysis of the 2 kb 5' flanking region identified a classic TATA box located 26 bp upstream of the transcription start site (TSS) and a number of putative transcription factor binding sites involved in immune response, including three IRF, two NFKB, two NFAT, four STAT binding sites. The transcripts of abalone RIG were found to be expressed most abundantly in hemocytes by tissue distribution analysis. Further expression analysis was performed in hemocytes after various immune challenges by intramuscular injection of vibrio parahaemolyticus, Listeria monocytogenes, LPS, Poly I:C and viral hemorrhagic septicemia virus (VHSV). The expression of abalone RIG was significantly induced and peaked within 12 hours post injection of bacterial, LPS and VHSV. However, the response to Poly I:C was shown much slower with the highest induction at 48 hour post injection. The structure and expression analysis results suggested the involvement of abalone RIG in both anti-bacterial and antiviral defense. Together with the recent identification of other genes in IFN signaling cascade, such as Toll-like receptors, IRFs, TBK1 and Mx, in genomes and transcriptomes of numerous invertebrate species, our previous view to the evolution of host antiviral defense can be changed.
Effect of 17α-hydroxyprogesterone administration on the ionic composition of seminal plasma in artificially matured male Japanese eel *Anguilla japonica*

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Cultivated Japanese eel do not mature under rearing conditions. However spermatogenesis and spermiation in males is easily induced by repeated injections of human chorionic gonadotropin (hCG). To induce spontaneous spawning, males receive a priming injection of hCG two days before spawning, followed 24 h later by an injection of 17α-hydroxyprogesterone (OHP), the precursor of 17, 20β-dihydroxy-4-pregnen-3-one (DHP: the maturation inducing steroid in the eel). Naturally induced spawning with females which have received hormonal treatment will occur about 12-18 h after the OHP injection.

We found that high sperm motility (%) of the males was maintained until OHP injection, at which there is a significant decrease in less than 24 h, followed by recovery of motility a few days after. Coincidentally, the decrease and increase in potassium concentration of seminal plasma mirrored the changes in sperm motility (Miura et al., submitted). It is well known that the potential for motility of the eel spermatozoa changes with the potassium concentration of the seminal plasma which is usually high (10–30 mM) compared to that of the serum (3–6 mM).

In the present study, we tried to elucidate the origin of the high concentration of potassium ions in the seminal plasma and the whereabouts of potassium ions which disappeared from the seminal plasma after OHP injection. For this purpose, we incubated the sperm cells (SC), sperm duct (SD), and testicular tissues (TT) separately or together in the ASP containing various doses (0, 1, 5 μg/mL) of OHP or DHP and observed the changes in concentration of potassium and sodium ions in the ASP during 24 h incubation.

Concentration of potassium ions in the ASP incubating with SC or SD did not show significant changes during the incubation, but that in the ASP incubating with TT showed significant increase with the incubation time. In contrast, concentration of sodium ions in the ASP showed significant decrease only in the medium incubating with TT. Medium incubating with both SC and SD did not show significant change in concentration of both ions, but medium of co-culture with TT showed significant increase of concentration of potassium ions. These changes in ionic concentration did not differ irrespective of the hormonal concentration.

These results suggest that high concentration of potassium ions in the seminal plasma is maintained by the testicular tissue, and decrease of potassium ions after OHP injection is not caused by the direct action of OHP or DHP on the sperm cells, sperm duct, and testicular tissues.
**P20**

**Function of gonadotropins in spermatogenesis of the wrasse Pseudolabrus sieboldi**

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**Background:** The bambooleaf wrasse *Pseudolabrus sieboldi* is a protogynous hermaphroditic labrid fish. Females mate with sex-changed males (secondary males) every day during spawning season. In the testis of secondary male (secondary testes), the number of spermatozoa are peaked just before spawning (6:00-9:00), representing the diurnal patterns of sperm production. Gametogenesis in vertebrates including teleosts is controlled by endocrine hormones on the brain-pituitary-gonad (BPG) axis. We previously reported that mRNA of two pituitary gonadotropins (FSH: follicle stimulating hormone, LH: luteinizing hormone) show diurnal fluctuation in the secondary male, suggesting they stimulate spermatogenesis via sex steroid synthesis in the secondary testis. Recently we succeeded in producing the functional recombinant bambooleaf wrasse FSH (r-bwFSH) and LH (r-bwLH) using silkworm-baculovirus protein expression system. In the present study, to reveal the functions of FSH and LH in the spermatogenesis, steroidogenic potencies were analyzed by *in vitro* bioassay.

**Method:** The secondary testis were dissected and incubated for 24 hours in Leibovitz’s L-15 medium with (1-1000 ng/ml) or without r-bwFSH and r-bwLH, respectively. The concentration of 11-ketotestosterone (11-KT), Estradiol-17β (E2), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) in the culture media were determined by ELISA.

**Results and Discussion:** 11-KT synthesis was induced by 1000 ng/ml r-bwLH only. E2, 17,20β-P and 20β-S syntheses were not induced by both r-bwFSH and r-bwLH, respectively. These results suggest that LH stimulate spermatogenesis through 11-KT synthesis. On the other hand, FSH may act on spermatogenesis through the activation of non-steroidal factors (e.g. stem cell factor, insulin-like growth factor-1 and neuregulin.)

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**P21**

**Purification and characterization of follicle-stimulating hormone and luteinizing hormone in chub mackerel Scomber japonicus**

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**Background:** Bluefin tuna *Thunnus thynnus* is a commercially important cultured scombrid species, and reproductive problems such as the inhibition of oocyte maturation and a late age of sexual maturity is a serious issue. However, it is difficult to analyze the physiological mechanism regulating gametogenesis in reared bluefin tuna because the adult body size is very large. The chub mackerel *Scomber*
Japanese mackerel (Scomber japonicus) is a commercially exploited fish in Japan and considered to be a useful model for analyzing the physiological mechanisms in cultured scombrid species due to its small size. Two gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are key regulators of gametogenesis in vertebrates. Purified FSH and LH are available for analyzing the physiological mechanisms that control gametogenesis. Therefore, we have recently purified FSH and LH from chub mackerel pituitaries (Ohga et al., 2012; Reprod Biol Endocrin). In this study, we have modified purification method in order to get high quality of hormones and characterized their biological activities to evaluate availability.

Methods: Extracts of chub mackerel pituitaries were applied to HPLC, using a DEAE cellulose anion-exchange column. FSH and LH fractions were purified by gel filtration, respectively. LH extracts were further fractionated through agarose-bound concanavalin A (conA). Biological activity of purified FSH and LH (cmFSH and cmLH) were confirmed by an in vitro bioassay for steroidogenic activity and maturation-inducing potency. A portion of fully-vitellogenic ovaries was incubated in culture medium for 24 hr, with or without cmFSH or cmLH. The percentage of oocytes during germinal vesicle breakdown (GVBD) was estimated and E2 and 17,20\(\beta\)-P concentrations were measured.

Results and Discussion: The cmFSH and cmLH were separated by anion-exchange chromatography. The remaining contaminants were completely removed by gel filtration from both fractions and with conA-agarose from LH fraction. The cmLH induced GVBD of oocytes, whereas cmFSH did not induce maturation. The cmFSH significantly enhanced E2 secretion but did not 17,20\(\beta\)-P secretion, whereas cmLH significantly enhanced 17,20\(\beta\)-P secretion but did not E2 secretion. These purified intact hormones will enable future study, using chub mackerel, to reveal the physiological mechanisms regulating gametogenesis in reared scombrid species.

### Relationship between growth and age on pubertal development in *Epinephelus bruneus*

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The first breeding of longtooth grouper *Epinephelus bruneus*, which is an important species for aquaculture in Japan, starts at 3 or 4 years old. For artificial seed production, the development of new techniques to obtain the fertilized eggs from young fish less than 3 years old is desirable. However, it is difficult to induce puberty in young fish, although this approach is conducted in many species. Thus, in this study, we investigated the relationship between growth and age using physiological methods in order to understand the mechanism of initiation of puberty in the longtooth grouper.

Female longtooth grouper of 1 - 5 years old were used for the experiment. These fish were kept in a floating net cage under natural photoperiod and water temperature. Experiment fishes were sampled in July 2011 and June 2012. After total length and body weight were measured, blood and gonad were collected for analysis of plasma sex steroid concentrations and histological observation of gonadal development, respectively. Plasma steroids were analyzed by enzyme-linked immunosorbent assay.

Total length and body weight increased significantly in the fish of 3 years old compared with 2 years old. However, there was no significant difference in body size between the fish of 3 years old
and upward. GSI and appearance frequency of yolk vesicle, yolk globule, and mature stage oocytes increased with age. In the fish of 1 and 2 years old, only perinucleolus stage oocyte was observed in the ovary. In the fish of 3 years old, yolk vesicle and mature stage were observed, although the appearance frequency is lower than those of 4 and 5 year old fish. There is no significant difference between the body size of mature and immature fish. These results suggest that the puberty starts at 3 years old. However, the growth (body size) is not correlated with the onset of puberty.

Characteristics of oocyte development and maturation in white-streaked grouper

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¹Institute for East China Sea Research, Nagasaki University, Nagasaki, Japan,
²Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency, Okinawa, Japan

White-streaked grouper, *Epinephelus ongus*, is one of the most important fish resources in Okinawa, Japan. However, its resources have decreased rapidly in recent years. Then, the resource management in this species should be done as soon as possible. We examined the gonadal development and maturation during spawning season in order to collect the basic information that is required for resource management.

Experiment 1): The wild female grouper were purchased from Yaeyama fish market at intervals of 2–3 days from April 16 to May 20, 2012. The ovary was sampled and morphological changes in oocyte were observed histologically. Experiment 2): Captured males and females were divided into 3 groups and reared in 5t FRP tanks from April 22 to May 22, 2012. The ovaries and blood were sampled at intervals of 3 days. Morphological changes in oocyte were observed histologically. Plasma sex steroids were measured by enzyme-linked immunosorbsent assay (ELISA).

Result of exp. 1): Ovaries of all females developed toward the last quarter moon synchronously. Ripe stage oocytes appeared after the last quarter moon on May 13, while GSI reached to the peak level. Result of exp. 2): Tertiary yolk stage oocytes were observed during the experimental period. Ripe stage oocytes appeared in the last quarter moon on May 13, while GSI reached to the peak. Post-ovulatatory follicles were observed 6 days after the last quarter moon. These results suggest that white-streaked grouper spawn around the last quarter moon in both natural and rearing conditions.
Effects of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder *Paralichthys olivaceus* in suboptimal temperature

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(Division of Marine Environment and BioScience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791, Korea.)

Temperature is one of the most important environmental factors affecting not only availability of nutrient content in the diets, but also growth of fish. Kim et al. (2009, 2010) proposed that the optimum feeding frequency for both subadult olive flounder averaging 270 g and grower fish averaging 117 g was one meal a day at suboptimal temperature conditions, 12.1°C and 13°C, respectively when fish were fed with extruded pellet (EP) to satiation. However, there is no information available of periodic feed deprivation and refeeding on performance of subadult olive flounder under suboptimal temperature conditions. In this study, therefore, the effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder fed the EP in suboptimal temperature was determined.

Five treatments with different feeding regimes were prepared in triplicate: Fish in the control group were hand-fed with EP, seven days a week (7DF). The experimental groups were fed to apparent satiation for consecutive six (6DF), five (5DF), four (4DF) and three days (3DF) a week, for 12 weeks during winter season.

Survival ranged from 93.3% to 100% was not significantly different among treatments. The weight gain and SGR of subadult olive flounder 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. FE and PER of subadult olive flounder was not significantly different among treatments. PR of fish in 3DF treatment was significantly higher than that of fish in all other treatments. Neither CF nor HSI of fish was significantly affected.

In conclusion, five days of feeding a week was recommendable for subadult olive flounder fed extruded pellet in suboptimal temperature.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial weight (g/fish)</th>
<th>Final weight (g/fish)</th>
<th>Weight gain (g/fish)</th>
<th>SGR (%)/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>7DF</td>
<td>271.78 ± 0.22</td>
<td>355.00 ± 5.47</td>
<td>83.22 ± 5.26</td>
<td>0.30 ± 0.01a</td>
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<tr>
<td>6DF</td>
<td>271.67 ± 0.54</td>
<td>336.51 ± 4.69</td>
<td>64.84 ± 4.16</td>
<td>0.24 ± 0.01ab</td>
</tr>
<tr>
<td>5DF</td>
<td>271.67 ± 0.54</td>
<td>336.51 ± 4.69</td>
<td>64.84 ± 4.16</td>
<td>0.24 ± 0.01ab</td>
</tr>
<tr>
<td>4DF</td>
<td>271.87 ± 0.44</td>
<td>318.67 ± 4.72</td>
<td>46.80 ± 5.14</td>
<td>0.18 ± 0.02b</td>
</tr>
<tr>
<td>3DF</td>
<td>271.87 ± 0.44</td>
<td>318.67 ± 4.72</td>
<td>46.80 ± 5.14</td>
<td>0.18 ± 0.02b</td>
</tr>
</tbody>
</table>

<table>
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<th>Treatments</th>
<th>DFR</th>
<th>FE</th>
<th>PER</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>7DF</td>
<td>0.24 ± 0.00a</td>
<td>0.81 ± 0.09</td>
<td>1.50 ± 0.17</td>
<td>31.6 ± 3.13b</td>
</tr>
<tr>
<td>6DF</td>
<td>0.24 ± 0.00a</td>
<td>1.13 ± 0.10</td>
<td>2.07 ± 0.18</td>
<td>43.2 ± 4.36b</td>
</tr>
<tr>
<td>5DF</td>
<td>0.21 ± 0.00b</td>
<td>1.14 ± 0.08</td>
<td>2.11 ± 0.15</td>
<td>43.8 ± 1.06b</td>
</tr>
<tr>
<td>4DF</td>
<td>0.18 ± 0.00c</td>
<td>0.98 ± 0.10</td>
<td>1.80 ± 0.18</td>
<td>41.9 ± 1.62b</td>
</tr>
<tr>
<td>3DF</td>
<td>0.14 ± 0.00d</td>
<td>1.32 ± 0.18</td>
<td>2.44 ± 0.32</td>
<td>56.9 ± 6.97a</td>
</tr>
</tbody>
</table>
Effects of dietary inclusion of various concentrations of Scutellaria baicalensis Georgi extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (Silurus asotus)

Gyu Ho Jeon, Hyun Jong Kim, A Reum Kim, Sung Hwoan Cho
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Far eastern catfish (Silurus asotus) is a commercially important freshwater finfish for aquaculture in Korea because of its fast growth and high resistance to disease. An extract of Scutellaria baicalensis (SB) in which baicalin and baicalein are the major active components has been known to have antimicrobial, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and/or anticancer activities and recently developed as additive for aquafeed. In this study, effects of dietary inclusion of various concentrations of SB on performance of far eastern catfish were determined.

Eight hundred forty fish averaging 0.96 g were randomly distributed into 24 tanks (thirty five fish per tank). Eight experimental diets were prepared in triplicate: Con diet without supplementation of SB and SB-0.25, SB-0.5, SB-1, SB-2, SB-3 and SB-5 diets containing SB at the concentrations of 0.25, 0.5, 1, 2, 3 and 5%, respectively. In addition, 0.1% commercial product of immune enhancer was included into the diet (CP diet). At the end of the 8-week feeding trial, ten externally normal fish from each tank were infected by Vibrio anguillarum with 0.1 mL of culture suspension of pathogenic containing $3.9 \times 10^8$ cells/mL.

No significant difference in survival, weight gain and feed efficiency ratio (FER) of fish was found (Table 1). However, cumulative mortality of fish fed the Con diet was higher than that of fish fed the all other diets since 10 day after V. anguillarum infection (Fig. 1). Results of this study indicated that dietary inclusion of SB extract was effective to improve survival of fish after V. anguillarum infection, but the various concentrations of SB did not affect fish performance.

Table 1. Performance of far eastern catfish fed the experimental diets

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Initial weight (g/fish)</th>
<th>Final weight (g/fish)</th>
<th>Survival (%)</th>
<th>Weight gain (g/fish)</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0.96±0.01</td>
<td>43.5±1.17</td>
<td>98.1±0.95</td>
<td>42.6±1.17</td>
<td>0.91±0.00</td>
</tr>
<tr>
<td>SB-0.25</td>
<td>0.95±0.00</td>
<td>44.7±1.42</td>
<td>98.1±0.95</td>
<td>43.8±1.42</td>
<td>0.95±0.01</td>
</tr>
<tr>
<td>SB-0.5</td>
<td>0.96±0.00</td>
<td>44.8±1.06</td>
<td>98.1±0.95</td>
<td>43.8±1.05</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>SB-1</td>
<td>0.96±0.00</td>
<td>46.4±3.40</td>
<td>96.2±1.90</td>
<td>45.4±3.40</td>
<td>0.91±0.02</td>
</tr>
<tr>
<td>SB-2</td>
<td>0.96±0.00</td>
<td>45.4±1.73</td>
<td>93.3±5.30</td>
<td>44.5±1.73</td>
<td>0.89±0.03</td>
</tr>
<tr>
<td>SB-3</td>
<td>0.95±0.00</td>
<td>44.1±1.43</td>
<td>95.2±2.52</td>
<td>43.2±1.43</td>
<td>0.91±0.02</td>
</tr>
<tr>
<td>SB-5</td>
<td>0.95±0.01</td>
<td>45.9±2.60</td>
<td>95.2±0.95</td>
<td>45.0±2.61</td>
<td>0.90±0.01</td>
</tr>
<tr>
<td>CP</td>
<td>0.95±0.00</td>
<td>44.0±1.40</td>
<td>99.1±0.95</td>
<td>43.1±1.40</td>
<td>0.95±0.01</td>
</tr>
</tbody>
</table>

Fig. 1. Cumulative mortality of far eastern catfish fed the experimental diets and subsequently infected with V. anguillarum (* indicates that cumulative mortality of fish fed the Con diet was significantly higher than that of fish fed the other diets).
A 6-week feeding trial was designed to test the evaluation of dietary inclusion of distillers dried grain (DDG) on growth performance and body composition of juvenile red seabream (*Pagrus major*). DDG is solid residue obtained by filtration of an aqueous mixture of fermented rice with *Aspergillus oryzae* and yeasts. Five isonitrogenous and isocaloric diets were formulated to contain 0% (DDG0), 20% DDG (DDG1) replacing wheat flour, 20% DDG (DDG2) and 28% DDG (DDG3) replacing fish-meal and wheat flour and commercial feed (CF). Juvenile red seabream averaging 7.5 ± 0.02 g was randomly distributed in fifteen 50-L tanks in a flow through systems. Three replicate groups of fish fed one of the experimental diets to visual satiation three times a day for 6 weeks. Survival was not affected by dietary inclusion of DDG (P > 0.05). Weight gain and specific growth rate of fish fed the DDG1 and DDG2 diets were not different to those of fish fed DDG0 but these value of fish fed DDG3 diet were lower than those of fish fed DDG0 diet (P < 0.05). Feed efficiency and daily feed intake were not affected by dietary inclusion of DDG (P > 0.05). The results of this experiment indicated that DDG is good ingredient to replace plant origin such as wheat flour and corn gluten meal and could be used up to 20% for the optimum growth performance of juvenile red seabream.
Exp2, WG and SGR of fish fed to 6.00, 6.25, 6.5 and satiation were significantly higher than those of fish fed at 3.0 and 5.0% bw/day (p<0.05). but there were no significant differences in these parameters among fish fed at 5.5, 6.0, 6.25, 6.5 and satiation. Exp3, WG and SGR of fish fed at 6.0, 6.25% and satiation were significantly higher in fish fed at 3.0, 5.0, 5.5% bw/day. But there were no significant differences in WG and SGR among fish fed at 5.75, 6.0, 6.25% bw/day and those fed to satiation (P>0.05). Feed efficiency (FE) of all the Exps were tended to decrease when feeding rates increased.

In conclusion, growth performance and body composition of Juvenile olive flounder were significantly affected by feeding rate, and the optimum feeding rates based on Weight gain as determined by the broken line analysis were 2.87, 5.97 and 5.92% bw/day for 15°C, 17.5°C and 20°C, respectively.

**P28**

The effect of rearing tank background color on feed intake, growth and MCH level of the olive flounder, *Paralichthys olivaceus*.

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Olive flounder (*Paralichthys olivaceus*) is one of the major marine species cultured in Korea due to its good taste and great economical values. In this study, we performed an experiment to investigate the effect of rearing tank background colors on the visual appearance and growing performance of flounder. Flounders were reared in five tanks with different background colors (white, gray, yellow, green and blue) for 4 weeks. Brains of five flounders were sampled in each tank at 1, 3, 7 and 28 day. Total RNA was extracted and melanin-concentrating hormone (MCH) gene expression was determined by qRT-PCR. Meanwhile, feed conversion ratio (FCR), body weight and specific growth rate were also evaluated. MCH expression levels in the brains of flounders were shown significant differences in different colored tanks. Despite of similar mean body weight (38.40 ± 0.96 g) after 4-week experiment, flounder body weight was significantly higher in the white and significantly lower in gray tank, compared with other tanks. The lowest FCR was found in gray tank; however, no significant variation could be observed in the other tanks. In conclusion, different background colors may lead to different expression level of flounder brain genes and hence different growth performances of olive flounder.
Self-feeding behavior of spotted knifejaw *Oplegnathus punctatus*: influences of photoperiod and luminous intensity on daily feeding pattern

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Spotted Knifejaw *Oplegnathus punctatus* has lately attracted considerable attention as one of the valuable farmed fish for aquaculture in Japan. However, information concerning rearing technique, such as appropriate feeding time and/or suitable daily feed amount for this fish species is scattered. Recently, we applied self-feeding technique to several fish species and have found its efficacy on fish farming. In addition, to make a valid application of self-feeding method to fish farming, it is important to know about the characteristics of self-feeding behavior of the target fish species. In this study, we conducted an experiment to test if spotted knifejaw have ability to learn self-feeding under the operant conditioning and to clarify their characteristic of self-feeding behavior under different luminous intensities.

Twelve spotted knifejaws, weighing 20.7–122.8 g were used. They were kept individually in a 24 l PVC aquarium equipped with a circulate water filtrating system adjusting the water temperature at 24°C. Three tanks were placed in a small experimental room and a set of a pull-type switch and a food dispenser was placed above each tank. The illumination was provided by fluorescent lamps with a LD 12:12 (L: 06:00-18:00) photoperiod regime controlled by an electric timer. Two levels of luminous intensities, about 1.06W/m² (about 100 lx range, corresponds to water surface) and 0.03W/m² (about 1 lx range, corresponding to 25 m water depth) were established as a light condition of daytime. The Chronobiology Kit (Stanford Co. Ltd.) was used for the data acquisition and analysis.

Spotted knifejaw learned self-feeding easily within a few days. Under LD 12:12 condition in both luminous intensities, their self-feeding activities appeared to be strongly entrained by the photoperiod on a cycle of 24hr and these rhythms showed a typical diurnal pattern with feeding peak at the scotophase. In detail, high feeding frequencies were observed for two hours just after the light onset and for two hours just before the light offset. There was no significant difference in the self-feeding activities between the two levels of luminous intensities. The self-feeding method seems to be effective as one of feeding method on the spotted knifejaw culture.

For survival experiments *Moina mongolica* salt concentration jump move


(College of Marine Science, Gyeongsang National University, Korea)

Introduction

Fish Seed Production in Artemia worldwide is an essential food organisms. In case of Korea, the Artemia cyst relies entirely on imports. According to changes in the international environment, Artemia has continual concerns of imbalance between supply and demand and price skyrocketing.
Therefore, it is necessary to develop a new prey which be able to substitute for Artemia as live food for the stable seeding production of fish. This research team is making for several years research on fresh water flea and brackish water flea cultivation as Artemia principal parts food creature. *Moina mongolica* that inhabit in saline of latest China upcountry is done interest by Artemia live food organism. This experiment is part of the basic experiment for the first time in Korea. Experiments were performed on salinity due to breeding research.

**Materials and method**

1) *Moina mongoica* collected in China inner mongolia Bayinnaoer lake.
2) Domesticated were cultured in the salinity of the 20 psu
   After 0, 10, 30, 40, 60, 80, 100psu salinity jump experiments were conducted to investigate the effect of moving (saline culture conditions). And established 20 sample vials in each plot. As a 40 ml sample vial filled 25 ml of culture.
3) Incubation water after mixing Bosea sp. Bacteria culture water and Seawater chlorella 100 million cell / ml were used. Lighting used incandescent lamp, and the temperature was kept to 25 degrees.
4) Survival existence and nonexistence of *Moina mongolica* did that color of the body changes white under microscope.

**Result**

1) Optimal salt concentration of *Moina mongolica* is 20 psu.
2) Jump due to the salinity of *Moina mongolica* experimental results differ by more than a 10 psu if survival sharply declined.

![P31](image)

**Gene expression profile during resting egg hatching in the rotifer *Brachionus plicatilis* Müller**

©Hee-Jin Kim, Koushirou Suga and Atsushi Hagiwara

(Graduate School of Fisheries Science and Environmental Studies, Nagasaki Univ., Japan)

The resting egg of monogonont rotifer *Brachionus plicatilis* sensu stricto O. F. Müller is a product of sexual reproduction, and shows a remarkable tolerance to unfavorable conditions. These eggs can retain their viability as diapause phase under harsh environment (in complete darkness), but regenerate with light which is a trigger factor to lead hatching. Although the chemical mechanism of the resting egg hatching has been expected, there are no empirical researches. In this study, we investigated the gene expression of resting eggs associated with lighting period (total darkness, 30 minutes and 4 hours) using differential-display reverse transcription PCR. The resting eggs showed no significant differences in morphological embryonic development between the incubation in total darkness and with 4-hour illumination by microscopic observation. On the contrary, different 81 cDNA fragments were expressed among treatments: 20, 40 and 21 fragments were expressed with a dark preservation, 30-minute and 4-hour exposures, respectively. To analyze functional gene flow from dormancy to hatching, the BLAST data of 81 fragments were used (both >50 of score and <1.0xE-13 of E-value). The resting eggs kept in total darkness mainly expressed the genes with putative function of cell
defense and homeostasis such as heat-shock proteins (Hsp 90) to protect themselves from harsh environmental condition. The genes related to cell and embryo development were actively expressed with 30-minute illumination, while these genes were not expressed with 4-hour illumination. The resting eggs under 4-hour lighting expressed transport genes at a higher level. The gene of Acyl-CoA dehydrogenase related to fatty acid metabolism was expressed with the lighting for 30 minutes. The results obtained from this study are preliminary, whereas the expected chemical mechanism of hatching has a relation to the oxidation of highly unsaturated fatty acid and thus the further study of these gene species can clarify the hatching mechanism of resting eggs.

**P32**

**Effect of CoSO₄ · 7H₂O supplementation on the reproduction of Moina macrocopa and M. mongolica**

Huang Wei, Miho Hayashi, Akari Yoshida, Yoshihito Manabe and Takao Yoshimatsu
(Graduate School of Bioresources, Mie University, Japan)

_Moina_ spp., representative species of cladocerans, are excellent as well as important initial living food organisms for rearing of cultured freshwater and marine fishes. As for rotifer culture, it was reported previously that Co compounds supplementation to fish feed and/or culture water could enhance growth performance of zooplankton by synthesizing vitamin B₁₂. Our target in this trial is to improve the nutritional value of the diet for culturing _Moina_, through adding Co compound, so as to reduce costs in large scale production of live foods.

We used _Moina macrocopa_ (freshwater cladocera) and _M. mongolica_ (brackish cladocera) as tested zooplankton. Newly-born _Moina_ neonates were cultured individually using six well plastic micro-plates with two kinds of laboratory-cultured vitamin B₁₂ free microalgae (_Chlorella vulgaris_ and _Nannochloropsis oculata_) and supplementations of Cobalt (II) sulfate heptahydrate (10⁻³, 10⁻², 10⁻¹, 10⁰, 10¹ mg L⁻¹) or vitamin B₁₂ (10¹, 10², 10³, 10⁴, 10⁵ μg L⁻¹) under 16L and 8D in 24-28°C incubation chamber. We investigated daily the number of total neonates, life span and sex difference for the detection of the effects of various dietary conditions on _M. macrocopa_ and _M. mongolica_.

As expected, Vitamin B₁₂ supplementation to feed affected and promoted the reproduction performance and life span of both of _Moina_ spp. The supplementation of Cobalt (II) sulfate heptahydrate also showed similar responses in the population growth and reproduction performance of _Moina_. And concentration of two nutrients was also detected to be an important factor on reproduction performance and life span of _Moina_ spp.

**References:**


2) Preliminary trials on the effect of lighting for the population growth of the rotifer, _Brachionus plicatilis_. T. Yoshimatsu, T. Higuchi, Y. Hamasaki, K. Tanaka. _JARQ_, 42 (2) 131-136 (2008)
Sex difference in the effects of ascorbic acid on the toxicity of potassium dichromate to *Daphnia magna*

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*Daphnia magna*, the cladoceran crustacean, is a cyclical parthenogen producing asexually female neonates under favorable environmental conditions. Appearance of male neonates is observed under unfavorable environmental conditions such as decreasing photoperiod, decreasing food concentration and increasing population density. The previous study demonstrated that the toxicity of potassium dichromate, which is used for reference substance in the toxicity test for daphnids, was different between males and females. In the present study, we investigated the difference in the effects of ascorbic acid on the potassium dichromate between female and male, in order to clarify a sex difference on the influence of antioxidant.

Female neonates were prepared using daphnids in the parthenogenetic reproductive phase, whereas male counterparts were prepared by exposure to methyl farnesoate. Male and female neonates within 24 h after birth were used in acute toxicity tests of potassium dichromate under presence of ascorbic acid with various concentrations. Acute toxicity tests of potassium dichromate (0.57–60.55 mg/l) with various concentrations of ascorbic acid (0–60 mg/l) were performed to determine the median lethal concentrations (LC₅₀) of potassium dichromate in each concentration of ascorbic acid.

As the results of the tests, the values of LC₅₀ of potassium dichromate increased in ascorbic acid concentration-dependent. In addition, the LC₅₀ of potassium dichromate for male neonates was significantly higher than that for females after 24- and 48-hour exposure (*P*<0.01). On the other hand, the mitigation activity of ascorbic acid to the toxicity of potassium dichromate was more effective to female than male at least 24-hour exposure. These results suggest that the female daphnids have lower tolerance activity to oxidative stress than males and ascorbic acid positively affect the chemical resistance of daphnid neonates, especially male.
Optimal culture conditions of two marine copepod species as live food

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(Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Japan)

We examined culture conditions of two copepod species. One is harpacticoid Tigriopus japonicus, which can be mass cultured, but not appropriate as fish larval food because of its benthic behavior. Thus we tried to monitor its behavioral change under different lighting condition. Acartia tonsa (calanoida) is important prey for fish larvae in natural condition, but its rearing condition has not been well optimized.

In order to examine phototaxis of T. japonicus, 15 adults were incubated in seawater under four different light wavelengths (white, peak wavelength 460, 570 nm; blue, 470 nm; green, 525 nm and red 660 nm). Light intensity was regulated at 0.5, 2.0 and 3.5 W/m². Under these conditions, the phototaxis of T. japonicus was positive at 0.5, 2.0 W/m² and negative at 3.5 W/m² with all wavelengths. We also examined light absorption spectrum of the eyespot of T. japonicus (nauplius, copepodite and adult), it increased with their development, and better absorption was observed with blue and green light. Next, we investigated the appropriate microalgal species for the culture of A. tonsa. Among four microalgae including Tetraselmis tetrathele, Rhodomonas sp., Chaetoceros sp. and Isochrysis galbana, A. tonsa reached to adult by feeding Rhodomonas sp. and I. galbana. The feeding of Rhodomonas sp. resulted in the fastest growth. We also investigated the hatching condition (salinity and light wavelength) of resting eggs. The hatching rate was highest at 34 psu salinity and under 470–525 nm light wavelength.

Distribution pattern of Musculista senhousia (Bivalvia: Mytilidae) And Tidal-flat Sediments

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The analysis of grain size composition and organisms were carried out for the surface sediments in order to investigate the sedimentary environment and distribution pattern Musculista senhousia. The surface sediments are classified into three different types; gravel, gravelly muddy sand and silty sand. The patch distribution of mussel was observed in the middle part (St. B, St. C, St. D) of the tidal-flat in study area. The mean density of mussel was in the range from 17,184 to 39,456 inds./m² in surface. On the other hand, bottom fluctuated between 3,875 and 4,610 inds./m². The mean size of shell length varied from 7.40 mm to 19.44 mm during the study period. The relationship between shell length (SL) and shell height (SH) could be represented by following equation SH = 4.430SL - 0.192 (R² = 0.084) in surface and SH = 0.423SL - 0.158 (R² = 0.831) in bottom. And the relationship between shell length (SL) and total weight (TW) were represented by equation TW = 0.652 × 10⁻⁵SL².733 (R² = 0.801) in surface and TW = 0.672 × 10⁻⁵SL².733 (R² = 0.843) in bottom.
Diet composition of juvenile goby species, *Gymnogobius heptacanthus* and *Chaenogobius annularis*, in the coastal waters of Geoje, Korea

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The *Gymnogobius heptacanthus* and *Chaenogobius annularis* belonging to the Perciformes, Gobiidae, mainly distributed in the coastal waters of Korea, Japan and China. The gobid fishes is known to 212 genera, 1,875 species distribution in the worldwide, of which 27 genera, 59 species in Korean waters. This species occurred commonly in seagrass meadow. They are not important commercial fishes, from coastal ecosystems, that important role as a food resource. The objectives of this study are to investigate the diets of two goby species. Fish samples consisting of a total of 229 *G. heptacanthus* (from 14 to 29 mm SL) and 281 *C. annularis* (from 13 to 18 mm SL) were collected monthly during summer (June-August) from June 2011 to August 2011 using plankton net. All samples were fixed immediately with 5% formalin after collection. Stomachs were then removed, and the stomach contents were identified under a dissecting microscope. Diets were quantified by frequency of occurrence (%F), percentage of number (%N) and volume (%V), and index of relative importance (IRI). Index of relative importance (IRI) is calculated by Pinkas et al. (1971) equation. To assess the feeding habits of *G. heptacanthus*, we used the graphical analysis proposed by Amundsen et al. (1996). A total of 229 *G. heptacanthus* examined, 208 individuals contained food items and 21 were empty (9.2%). Of the prey items consumed, the major food item is copepods constituted 98.08% in frequency of occurrence, 77.14% in numbers, and 90.20% in volume and 96.74% in IRI. Barnacle larvae were the second largest dietary component. A total of 281 *C. annularis* examined, 250 individuals contained food items and 21 were empty (11.0%). Of the prey items consumed, the major food item is copepods constituted 68.40% in frequency of occurrence, 52.89% in volume and 61.61% in IRI. Barnacle larvae were the second largest dietary component. Graphical analysis of the diet composition showed that *G. heptacanthus* and *C. annularis* is an opportunistic and specialized predator characterized by strong individual feeding specialization. Copepods are located in the upper right corner of the diagram, suggesting a specialization for copepods by the *G. heptacanthus* population, and for copepods and barnacle larvae by the *C. annularis*. 
Feeding habits and reproductive ecology of scorpion fish, *Sebastiscus marmoratus*, in the coastal waters of Tongyeong, Korea

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The *Sebastiscus marmoratus* is scorpion fish (Scorpaenidae) that is mainly distributed in the coastal water of Western Pacific. They are important commercial species in Korea, Japan and China where they are caught primarily by gill net and pots. Their unique reproductive characteristics i.e. ovoviviparity has been drawn considerable interest, and many studies have been conducted on their physiology and ecology. Knowledge of the trophic and reproductive ecology of fish species is necessary for understanding the trophic level and/or niche of fishes within ecosystems and is very useful for understanding aspects of species biology and ecology for more sustainable management of stocks and the development of conservation measures. The aim of this study is examined of main prey items, reproductive ecology of *S. marmoratus* in the coastal waters of Tongyeong, Korea. The feeding habits and reproductive ecology of scorpion fish, *S. marmoratus*, were analyzed using 324 specimens collected in the coastal waters of Tongyeong, from January to December, 2009. The size of the specimens ranged from 9.8 to 30.1 cm (mean ± SD = 15.5 ± 2.9 cm) in standard length (SL). Diet was quantified by frequency of occurrence (%F), percentage of number (%N) and weight (%W), and index of relative importance (IRI). *Sebastiscus marmoratus* is a piscivore that consumes mainly teleosts such as *Engraulis japonicus*, *Clupea pallasii* and gobid fishes. Of the fish species *E. japonicus* was the most preferred prey. Its diet also includes shrimps and crabs. Hermit crabs, polychaetes, bivalves and euphausia were minor preys. Smaller individuals (<13 cm SL) fed mainly on shrimps, crabs and fishes. The proportion of shrimps and crabs decreased as body size increased, whereas the consumption of fishes gradually increased. Fishes accounted for almost stomach contents of larger individuals (more than 19 cm SL). The size of prey items also increased as growing their body size. The gonadosomatic index (GSI) of females was highest in April and decreased until August. The spawning season lasted from September to April, and the ratio of female to male did not differ significantly (χ²-test, p > 0.05). The length at first spawning was 13.0 cm SL, and the size at 50% maturity was estimated at 14.2 cm SL. All females more than 18.0 cm SL were sexually mature. Fishes was most common prey during spawning seasons, whereas crabs and shrimps were mainly consumed during spring.
Marking on Scales by Immersion in Chemicals for Estimate of the Early Survival Rate of Released Fish on Marine Ranching Area

Choong Hwan Noh¹ and Kyung Dae Park²

(¹Korea Institute of Ocean Science & Technology, ²Gyeongnam Fisheries Research Institute)

We evaluated the efficiency of chemical marking of black rockfish scales by immersion in oxytetracycline hydrochloride (OTC), alizarin red S (AS) and calcein (CAL) diluted rearing water. Immersion treatment of chemicals had no effects on both mortality and growth of black rockfish. Marking success was 100% in all treatment durations (24, 48, 72 hours.). Marking retention rates at 24 weeks after treatment were 100% in OTC and CAL treated group, but marking quality was higher in CAL treated group (brilliant 92%, bright 8%, dim 0%). Than in OTC treated group (brilliant 4%, bright 70% and dim 26%). AR treated group had lower marking retention rates and marking quality than OTC and CAL treated group. As a result, immersion treatment with OTC and CAL was effective in marking scales of fish and practical in releasing program.

Acknowledgement: This study was supported by a project (#PE98752) of KIOST.

Puffers smell tetrodotoxin

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Tetrodotoxin (TTX) is reported to attract tiger puffer Takifugu rubripes juveniles, but the sensing mechanism for recognition of TTX has not been clarified. We hypothesized that T. rubripes senses TTX by olfactory which senses odorants in water. In this study, olfactory ablated juveniles were tested for behavioral response to TTX in a Y-maze, to investigate whether recognition of TTX is accompanied by olfaction. Behavioral observation was conducted using a Y-maze channel (each arm is 50 cm). Agarose (2%, 5 mL) were placed at the each head of channels as a control. We acclimated 3 hatchery-reared juveniles (standard length 5.8 ± 0.5 cm) at the down stream of Y-maze for 30 min, then, behavior of each juvenile was observed for 3 hours. We also placed agarose containing 200 MU TTX or agarose at each head of channel and the same observation as control was conducted. We also tested the same procedure for olfactory ablated juveniles and for juveniles received sham operation. We observed the frequency of pecking agarose and calculated the average frequency per individual, following the comparison among 3 treatments. Juveniles were not attracted to agarose (0.0–3.7 times). In contrast, juveniles without surgery and juveniles received a sham operation showed significant selectivity to TTX (135.9 ± 85.1 times and 178.6 ± 103.0 times, respectively), while the olfactory ablated juveniles were not attracted to TTX (3.3 ± 5.8 times). These results indicate that T. rubripes juveniles detect TTX by olfactory organ.
Salinity selectivity of tiger puffer juveniles

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Tiger puffer (Takifugu rubripes) hatch at sand substrate in offshore, and they migrate from the spawning ground to estuary during the early life stage. It indicates that tiger puffer undergo various salinities their early stages. We examined the salinity-selectivity of juveniles by immersing fish to the salinity-gradient environment.

We made salinity gradient tank (98 cm diameter, 66 cm depth); we poured diluted-seawater (10 ppt) from the surface, diluted-seawater (20 ppt) from the middle layer and seawater (30 ppt) from the bottom. Another tank was filled with seawater (30 ppt) as a control. Five fish (3.8, 5.1, 5.3, 7.0 cm in average, respectively) were transferred into these tanks, and we observed swimming-depth (surface, middle, bottom) of juveniles for 3 hours with triplicate.

Juveniles of 3.8 ± 0.3 cm and 5.1 ± 0.2 cm in body length (BL) swam at the same swimming-depth in both control and salinity gradient tank. On the other hand, juveniles of 5.3 ± 0.6 cm and 7.0 ± 0.2 cm in BL swam different depth in these tanks. One hour after transfer, juveniles swam at 10–16 ppt (surface) in salinity gradient tank, but distributed equally in a control tank.

Effects of ocean acidification on reproduction sea urchins

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Our current research focuses on the effects of ocean acidification and warming on the reproductive profiles and selected physiological aspects (oxygen consumption, feeding, mobility etc) in Hemicentrotus pulcherrimus and more specifically on feeding in Strongylocentrotus purpuratus. Eighty H. pulcherrimus were divided into 4 groups of 20 individuals, and exposed to separated or combined conditions of elevated CO2 (1,000 ppm) and temperature (+2°C) for 9 months. Spawning was completely inhibited in sea urchins reared in both high CO2 conditions. Feed intake was reduced gradually in both high CO2 conditions, to a greater extent under the combined condition. Oxygen consumption was initially stimulated and then became ca. 40% of the control in both high CO2 conditions toward the end of exposure. Mobility as determined by light avoidance and food search was significantly
impaired under both high CO\(_2\) conditions. Sixty-three adult purple sea urchins (*Strongylocentrotus purpuratus*) were reared individually in running seawater bubbled with three different CO\(_2\) concentrations (control: 380 ppm, high CO\(_2\) treatments: 1000 ppm and 3000 ppm) at ambient temperature for 140 days. Feed intake was initially comparable between the 3 treatments, but became significantly lower in the two high CO\(_2\) groups than in the control after 90 days. The results of these two experiments indicate that various physiological activities of sea urchins could be significantly affected by the CO\(_2\) levels projected by the end of this century. It is crucial to verify if the impacts observed for the two species are seen also for other sea urchin species and for other echinoderms. If such is the case, we must develop adaptation measures against the possible threat to aquaculture of sea urchins (and possibly other animals) caused by the oceanic climate changes.

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**Restoration of wild chum salmon resources by artificial hatching and stocking**

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The wild population of chum salmon (*Oncorhynchus keta*) was functionally extirpated from the Ishikari River, which drains into the Ishikari Gulf, in 1964 because of construction of a dam in the middle reach. Recently however, a fish ladder was retrofitted at the dam to allow passage. In an attempt to re-establish a wild chum population, the Hokkaido National Fisheries Research Institute is conducting artificial hatching and stocking in the river. As part of this effort, 500,000 otolith marked fry (fertilized in late October) were transferred from the Chitose Hatchery to the Ishikari River in March of the year following fertilization. The stocking was repeated for 3 years between 2009-11. The fry were stocked above the dam and up to 150 km from the river mouth. The Chitose Hatchery, located on the Chitose River, a tributary of the Ishikari River, is ~80 km from the river mouth. In autumn of 2011, we captured two groups of 3-year-old chum, the first from the Ishikari River (IR-G) and the second from other rivers (OR-G), using nets that were set along the coast of the Ishikari Gulf. These two groups were distinguished based on otolith marks. We compared the migration timing, gonad somatic index (GSI), and lipid content of adult salmon in the two groups. In addition, we conducted spawning surveys for IR-G fish near the stocking areas.

The migration timing of both the IR-G and OR-G was similar and peaked in early October. The GSI was 6% and 15% in male and female fish, respectively, and was not different between the groups. In contrast, the lipid content was higher in IR-G than in OR-G. In particular, the lipid content of male IR-G individuals (4.2%) was significantly higher than in male OR-G individuals (2.8%). We speculate that fish stocked into the upper areas of the river may deposit more lipid to provide energy for the long distance upstream migration.

During our survey of the Ishikari River, we counted the number of redds and spawned fish around the stocking areas between October and December. Both these indices increased during October and plateaued by late November. At the Chitose Hatchery, the mature fish return to spawn at approximately the same time as their parents were fertilized. However, the peak in spawning for IR-G individuals occurred during early-mid November, about 2 weeks later than the time at which they were fertilized 3 years prior. We hypothesize that this temporal shift is a function of the transfer to the upper section of the basin. The total number of redds observed was ~300. Thus, we believe that at
least 300 pairs of adults returned and spawned. The redds were primarily constructed at the edge of a sandbar along the river where water temperatures were close to the mainstream.

In summary, we documented the return and spawning of stocked fry in the release area. In addition, we compared their run timing, maturation state, and GSI to wild chum salmon in a different system within the same region.

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Culture study of *Porphyra tenera* (Kjellman) f. *tamatsuensis* (Miura)

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The present work was carried out to make clear the life history of *Porphyra tenera* f. *tamatsuensis* from Korea. Culture study of this species was conducted at constant temperatures (5, 10, 15, 20 and 25°C), at photon flux densities (10, 20, 40 and 80 μmol m⁻²s⁻¹) under photoperiods (14L:10D and 10L:14D). Conchocelis grew fast at 20°C and 40 μmol m⁻²s⁻¹ under both photoperiods. Conchosporangial branches were produced at 10–15°C, 10–80 μmol m⁻²s⁻¹, and were abundant when the conchocelis were cultured at higher temperatures of 20–25°C under both photoperiods. Foliose thalli grew well at the conditions of 5°C, 14L:10D and 10°C, 10L:14D. At 25°C, the foliose thalli could not survived seven weeks of long daylength and five weeks of short daylength, respectively.

Archeospores were observed at all culture conditions except for 5°C, 14L: 10D. Spermatangial and zygotosporangial sori were formed along the upper to lower margins of mature thallus. Zygotospores were released at 5–20°C under both photoperiods. Anatomical examination revealed that the matured spermatangial sorus had was 64 (a/4, b/4, c/4) and the zygotosporangial sorus was 8 (a/2, b/2, c/2) or 16 (a/2, b/2, c/4) according to the calculation of Hus’s formula (1902). In these results, life history of this species was typical biphasic, and was in accordance with the type of *P. lacerata* type.

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Growth of Suminoe Oyster, *Crassostrea ariakensis* in Seomjin River estuary

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This study was carried out to obtain the biological fundamental data for the resources annexation of *Crassostrea ariakensis* in Seomjin River estuary. The Water temperature during the study periods was 4.8–27.7°C and there was a little difference between each depth. Salinity was ranged from 0.8 to 30.4 psu. Chlorophyll-a was ranged from 1.65 to 31.61 μg/L, its maximum in January 2006 and its minimum in April 2006. At each depth, the highest mean growth of 0.19 mm day⁻¹ (surface) and 0.18 mm day⁻¹ (bottom), respectively. Shell length had a mean absolute growth of 0.12 mm day⁻¹ (surface, bottom). The daily growth rate of shell height were 0.27% (Surface) and 0.26% (bottom) during the same
period, respectively. The daily growth rate of shell length were 0.26% (Surface) and 0.24% (bottom) during the same period, respectively. Relative growth equations between shell height and shell length of spat were ranged from 0.8494 to 0.8846. Survival rate of the spat was higher (60%) significantly for the bottom group than that at the surface (50%).

Growth of sea cucumber *Apostichopus japonicus* in pond bottom and lantern net culture

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Introduction

Sea cucumber is an important seafood in dietary life even years before. To date, the demand is increasing every year, which makes it a target species for coastal resource development. Faced with this necessity, technologies have been developed, including nursery production and insemination aquaculture. However, such aquaculture methods are low density rearing methods depending on natural feed, wherein productivity per unit area is very small. In addition, the catching of sea cucumber is prohibited during the months of July and August, which is before and after breeding season. Furthermore, sea cucumber aestivates in high temperature season and hibernates in low temperature season. With this, aquaculture production is limited due to short growth period in a natural environment in Korea. Problems with sluggish growth of sea cucumbers have been encountered in previous rearing experiments. To assess the importance of feeding ecology on sea cucumber growth, pond bottom and lantern net culture experiments were conducted.

Material and method

Culture experiments were conducted in a sea cucumber farm in Dongying City, Shandong Province, China. The experiment site is primarily known for shellfish farming in China. The area of culture pond was 22,500 m² (150 m × 150 m) with depth of four meters. For lantern net culture, round plastic baskets stacked in five layers were suspended one meter below water surface. Pond bottom and lantern net culture were carried out in the same pond. Sea cucumbers were reared without feeding. Water exchange was carried out on a regular basis.

Results

Experimental results showed that sea cucumber in the bottom culture grew from 3 g to 120 g. On the other hand, in the lantern culture, the sea cucumber grew from 25.6 g to 29.8 g.

Conclusion

Sea cucumber reared in pond bottom has better growth compared to those reared in lantern nets. This implies that culture of sea cucumber can be difficult to grow with filter-feeding aquaculture species like shellfish in open water system due to its detritus-feeding habit.
Neuronal peptides induce oocyte maturation and gamete spawning of sea cucumber, *Holothuria scabra*

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In recent years, the annual catch of tropical sea cucumbers in Okinawa has been increasing mainly in response to a growing demand for dried sea cucumber products for export to China. Therefore, the need for the biological research and fisheries resource surveys of the tropical sea cucumbers in Okinawa is increasing. In Okinawa, in the most part of fisheries, there are no fishing rights of sea cucumber, and are no closed season and size restriction for sea cucumber. Therefore, until today, “excessive catch – exhausted – leaving of fisheries – resuming of catch” has been repeated. It is necessary to take measures to maintain the stability of the tropical sea cucumbers resources in Okinawa. Simultaneously, it is important to to take measures to cope with basic researches for the promotion of propagation and aquaculture of the tropical sea cucumbers. The neuropeptide cubifrin has recently been shown to potent substances that induce in vitro oocyte maturation and spawning in the Japanese common sea cucumber *Apostichopus japonicas*, and the usage of it has already started in seedling production facilities.

In this research, for the purpose of maintaining the stability of the tropical sea cucumbers resources in Okinawa, we conducted the reproductive physiological research of the typical edible sea cucumber *Holothuria scabra*, and the research of the gonadal hormones like the cubifrin of the sea cucumber *A. japonicas*.

The hemolymph acid-base balance of pearl oyster (*Pinctada fucata martensii*) while the shell valves closing.

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We investigated the acid-base balance of the pearl oyster, *Pinctada fucata martensii*, while the shell valves closing by measuring the pH, total CO₂ content (Tco₂), and O₂ partial pressure (Po₂) of the hemolymph collected from the anterior aorta through a cannula at 20°C. The CO₂ partial pressure (Pco₂) and bicarbonate concentration ([HCO₃⁻]) were calculated using the Henderson-Hasselbalch equation with substitution of the values of the apparent dissociation constant of carbonic acid (pK) determined in this study. The pearl oysters (mean total wet weight 30.0 g) were obtained from a marine farm in Tsushima, Nagasaki Prefecture, Japan. After cleaning the shell valves, they were reared for one month at 20°C in aerated seawater containing cultivated phytoplankton. Twenty-four hours before collection of hemolymph, the pearl oysters were transferred to the seawater filtered for particles. After cannulation to the anterior aorta, we collected the hemolymph through the cannula under normoxic condition (0 hr, initial sampling). The shell valves of cannulated animals were then
forcibly shut and bound with rubber bands, and we collected the hemolymph at 12–48 hr from the
time of binding. A control group was placed in a respiratory chamber, and hemolymph was collected
at the same times as the bound animals. Approximately 0.2 ml hemolymph was anaerobically col-
clected from the anterior aorta through a cannula connected to a gas-tight syringe for each sample. Our
results showed that in the bound animals, the hemolymph Po2 decreased to 50% of the initial value.
The hemolymph pH decreased to 85%, and Tco2 increased 3-fold. The hemolymph Pco2 increased to
30-fold the initial value, while hemolymph [HCO3–] increased 2.5-fold. The Po2, pH, Tco2, Pco2 and
[HCO3–] which cancelled the restriction of the shell valves movement by removing the rubber band
recovered gradually, and reached to the initial levels for 12–24 hr.

P48 The effect of air exposure on the hemolymph CO2 partial pressure
in the black-lip pearl oyster (Pinctada fucata margaritifera)

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We determined the CO2 solubility coefficient and the apparent dissociation constant of carbonic
acid with tonometry and calculated the CO2 partial pressure (Pco2) and bicarbonate concentration
([HCO3–]) to elucidate the acid-base balance of black-lip pearl oyster (Pinctada margaritifera) during
the air exposure. Black-lip pearl oysters (n=30; mean total wet weight, 204 g) were obtained from
Kochi Prefecture, Japan. After cleaning the shell valves, they were reared for one month at 25–26°C
in aerated seawater containing cultivated phytoplankton. Twenty-four hours before collection of the
hemolymph, the pearl oysters were transferred to seawater filtered for particles 0.45 μm or more in
diameter. The hemolymph was anaerobically collected from the adductor muscle with polyethylene
tubing. Approximately 0.3 ml of hemolymph was collected at 0 hr (in seawater), 36 hr (during air expo-
sure) and 39–60 hr (in seawater, recovery). The hemolymph pH and total CO2 content (Tco2) were
immediately measured after each collection. To determine CO2 solubility coefficient, the hemolymph
acidified by lactic acid was equilibrated with the standard CO2 gas (CO2, 5%; O2, 21%; N2, balance).
The apparent dissociation constant of carbonic acid was analyzed with the hemolymph equilibrated
with the standard CO2 gases (CO2, 0.1–5.0%; O2, 21%; N2, balance). The hemolymph Pco2 was cal-
culated by rearrangement of the Henderson-Hasselbalch equation with the coefficient and the con-
stant determined in this study. The hemolymph pH of black-lip pearl oysters during air exposure fell
to 90% of the control value, while Tco2 increased to 3-fold of the control value. The calculated Pco2
showed 1.5–2.0 torr at 0 hr, and increased to 10-fold during air exposure. The hemolymph [HCO3–]
was 2.0 mM/l, and increased to 2.5-fold during air exposure. These facts demonstrated that the black-
lip pearl oysters were caused respiratory and metabolic acidosis due to inadequate discharge of CO2
during the prolonged air exposure.
Cool storage of Japanese pearl oyster sperm

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It is well known that pearl quality is determined by the genetic traits of pearl oyster. The purpose of this study is to develop a short-term cooling storage system for pearl oyster sperm in order to facilitate its efficient breeding. We examined appropriate characteristics of diluent, dilution ratio and storage temperature for short-term cooling storage of pearl oyster sperm.

Spermatozoa used in this study were obtained from testes of the oyster. Sperm motility was observed after dilution (final dilution ratio was 1000 times) with activating solution (seawater containing 2 mM NH3). The percentage of motility of the fresh semen used in the study was 81.5 ± 2.7% (n= 10).

Firstly, we evaluated the cool storage of undiluted spermatozoa. Spermatozoa obtained from testes were stored at 4°C or 20°C in a 2.2 ml cylindrical micro-tube. At room temperature (20°C), sperm became immotile after 3 days. In contrast, sperm stored at 4°C maintained motility of above 40% for up to 7 days.

We then examined the effects of pH of the artificial sea water (ASW) on the initiation of motility of the testicular spermatozoa to determine the diluent in which oyster sperm is immotile. Sperm was diluted 1000 times with ASW of different pH (from 6.0 to 10.0), and sperm motility in the ASW was observed during 180 minutes after dilution. Pearl oyster sperm was motile when the pH was 9.0 or greater and immotile at pH 8.0 and below.

Next, we attempted long term cool storage using the same ASW as diluents. The spermatozoa were diluted (1:99) with ASW of various pH in a 2.2 ml cylindrical micro-tube, and kept in a refrigerator at 4°C for 33 days. Sperm motility was observed after a further dilution with activating solution (final dilution ratio was 1000). Spermatozoa stored in ASW of pH from 6.5 to 7.5 maintained the high potential for motility (more than 40%) for 3 weeks.

Finally, we examined the appropriate dilution ratio in the diluent. Spermatozoa were diluted with ASW of pH 7.0 at 5, 10, 50, and 100 times, and stored at 4°C. The highest motility ratio was obtained at 50 times dilution.

We had previously reported that cryopreserved pearl oyster sperm show approximately 30-40% post-thaw motility (Kawamoto et al., 2007, Arita et al., 2011). From the results obtained in this study, we propose the following storage method for pearl oyster sperm; when the desired storage time is less than 7 days, undiluted cool storage of spermatozoa is a convenient and useful method. However, if the desired period is beyond 7 days but within 3 weeks, spermatozoa should be diluted with ASW of pH 7.0 and cooled. If more than 3 weeks storage is desired, cryopreservation of spermatozoa should be used.
Preliminary study on amino acid and organic acid compositions in Pacific oyster *Crassostrea gigas* cultured by two different methods in Nagasaki Prefecture

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Pacific oyster is conventionally cultured, in Japan, by the suspension method where oysters are cultured on scallop shells suspended in the sea. This method makes adult oyster shells deformed in shape as a result of congestion of growing larvae. On the other hand, in the ‘single-seed method (SSM)’ recently developed, oyster spat are attached to and grown on granules of fractured oyster shells in hatcheries and cultured in mesh baskets suspended in the sea. Oysters cultured by the SSM are round-shaped and suitable for half-shell trade. Oyster farmers employing the SSM say that oysters raised by the method taste better than those by the conventional suspension method (CSM). However, little data are available for the taste of oysters raised by different methods. In this study, we preliminarily compared the amounts and compositions of free amino acids and organic acids between oysters cultured by the two methods to know the effects of the culture methods on taste characteristics.

We obtained six oysters each cultured by the two methods off Konagai in Nagasaki Pref., and analyzed the amino acid and organic acid compositions in their extracts with HPLC. The mean of total amount of amino acids was higher by ca. 20% in the oysters cultured by the SSM than in those by the CSM, although no significant difference was detected. Some differences were found in the amino acid composition. The level of alanine, which is related to sweetness and umami, was significantly higher by ca. 90% in the oysters cultured using SSM than CSM. Also, in spite of no significant difference obtained, aspartic acid and glutamic acid in oysters cultured using SSM showed higher level by ca.70% and 40%, respectively. Although the total amount of organic acids varied much individually, the oysters cultured by SSM seemed to contain more than those by CSM. The present study suggested that the difference in culture methods affected the oyster tastes.

Genetic Improvement of Red Sea Bream (*Pagrus major*) in Korea: Growth Performance, Eggs Production and Health Assessment

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Red sea bream, *Pagrus major*, is an important aquaculture fish species both in Korea and Japan and many genetic studies of this species were carried out for aquaculture purpose. In this paper we report the effects of selection by fish size on growth enhancement of Korean strain of red sea bream and the comparative growth performance and health conditions of selected Korean strain, cultured Japanese strain and their reciprocal intraspecific hybrids. Offspring from selected line (5th generation of selection) showed significantly better performance in body weight, body length, weight gain, specific growth and feed consumption (but not in feed conversion ratio) than offspring from non-selected line.
(2nd generation of wild) in sea cages rearing trials with communal stocking. At 36 months after hatching offspring from selected line grew 1.49 times faster in body weight than offspring from non-selected line. Growth of selected Korean line (KORDI-F4), cultured Japanese line (JPN) and their reciprocal intraspecific hybrids lines were studied. In sea cages, offspring from JPN ♂ × KORDI-F4 ♀ showed significantly faster growth (P<0.05, ANOVA, n = 240) than other offspring groups (KORDI-F4 ♂ × KORDI-F4 ♀, KORDI-F4 ♂ × JPN ♀, JPN ♂ × JPN ♀). Offspring groups from mating with JPN females (JPN ♂ × KORDI-F4 ♀, JPN ♂ × JPN ♀) had shorter spawning period and less amount of fertilized eggs by natural mating in natural spawning season than Offspring groups from mating with KORDI-F4 females (JPN ♂ × KORDI-F4 ♀). Offspring of JPN ♂ × JPN ♀ showed poor health status compared to other three offspring groups, especially degradation tissue activation in November. This result implies the high mortality of offspring of JPN ♂ × JPN ♀ during low temperature period in Korea.

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**Comparison of characteristics between generations of hybried strain in the self-fertilizing mangrove killifish**

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Mangrove killifish (*Kryptolebias marmoratus*) is known to reproduce by self-fertilization and consists homozygous population. However some fish occasionally become males. We successfully produced one hybrid (PDHy) by artificial insemination between two genetically different clones (PAN-RS, DAN), which was revealed as heterozygote by AFLP. F\(_2\) generation of PDHy showed different growth and we selected 4 individuals (families). We investigated the growth, morphological proportion and behavior as phenotypes from F\(_2\) to F\(_5\) of PDHy and compared to parental strains. PDHy strain from F\(_2\) to F\(_5\) generations (n = 196) and their parental strains (PAN-RS, n = 19 and DAN, n = 9) were reared at the same environmental conditions (17 ppt salinity, 25°C and 14L:10D photoperiod). Measurements of 24 morphological characters and observation of attack behavior toward the mirror image of the 400 days old fish were conducted. Discrimination analysis was used to detect the differences in characteristics among 4 families. Between parental strains, total length and attack frequency of PAN-RS were significantly higher than DAN. Total length and attack frequency among individuals of F\(_3\) varied. Families from F\(_2\) with high growth or low growth showed the same traits until F\(_5\). Attack frequency of PDHy was not significantly different in F\(_3\), however, individuals of families with high attack frequency in F\(_2\) showed high attack frequencies. Hybrid strain and the parental strains were significantly different in the preanal length/standard length and body width/standard length. Discrimination analysis revealed that the parental strains and F\(_2\) generation of PDHy were significantly different. F\(_3\) and F\(_4\) generations of PDHy were discriminated significantly from the parental strains and from each family. In the F\(_5\), 4 families were discriminated each other, but a few families were not discriminated from the parental strains by the morphometric characters.
Ploidy status of progeny from the crosses between naturally occurred triploid females and diploid males of spined loach (*Cobitis*, Teleostei)

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In Polish *Cobitis* populations dominate gynogenetic allotriploid females (3n = 74) coexisting with low number individuals of parental species *C. taenia* and/or *C. elongatoides* as well as allotetraploids of both sexes. The aim of the study was to examine the ploidy status of progenies artificially produced from crossing between triploid females from diploid-polyploid population (Bug River, Poland) and males of the spined loach *C. taenia* (2n = 48) from an exclusively diploid population (Leginskie Lake, Poland).

All parental *Cobitis* taxa were karyologically identified. Parental females and males used for all 18 experimental crosses were hormonally stimulated. Ploidy status of progeny was determined by measurement of relative DNA content using flow cytometry and/or chromosomal counting. DNA content was measured in whole larvae or gills using Nuclear Isolation Media (NIM)-DAPI staining solution (Beckman Coulter). The samples were analyzed with MoFlo™ XDP flow cytometer (Beckman Coulter) and diploid progeny from cross between *C. taenia* females and males were used as an external diploid standard. Chromosome preparations from the kidneys were made according to routine procedure.

Flow cytometric analysis of 219 progenies indicated that 50.6% as well as 49.4% were tetraploids and triploids, respectively. Moreover, karyotype analysis of 107 individuals showed that 25% of them were tetraploids, whereas others were triploids. Differences between the number of identified tetraploids among small (hatching, fry) and older individuals have been observed. Among the progeny under 1 month the number of tetraploids were greater (61%) than among individuals from 1 to 15 months post-hatching (23%). Moreover, flow cytometry analysis demonstrated that 63% of one-two days post hatching progeny were tetraploids. The obtained results revealed indirectly that c. 60% of eggs of triploid *Cobitis* were fertilized by the sperm of *C. taenia*.

Presented study for the first time documented that tetraploid of *Cobitis* are commonly produced, but their viability seems to be lower in comparison with triploid ones.

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Effects of dietary probiotic on growth performance, feed utilization, innate immunity and disease resistance against *Streptococcus iniae* in olive flounder (*Paralichthys olivaceus*)

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Probiotics are defined as microbial cell preparations that have beneficial effects on general health condition including non-specific immune response and antioxidant activity in host animals including fish. Probiotics are suggested as a way to step into a more environment friendly aquaculture by reducing the use of chemicals including antibiotics.

Four experimental diets were formulated to contain *Bacillus subtilis* at levels of 0 (as control), 0.1, 0.3 and 0.5%. Triplicate groups of fish were fed the experimental diets to apparent satiation (twice a day) for 12 weeks.

Growth performance of fish fed 0.5% *B. subtilis* were significantly (P<0.05) higher than that of fish fed the control diet. Among immune parameters, NBT, MPO and SOD activities were not significantly different among all the fish groups. Meanwhile, groups of fish fed 0.5% *B. subtilis* had significantly higher lysozyme activity than fish fed the control diet. Hematological analysis indicated the positive effects of dietary probiotic on fish stress resistance.

Cumulative mortality of fish after challenge with *S. iniae* was less than 10% in the groups fed 0.3 and 0.5% of *B. subtilis* indicating increased disease resistance of olive flounder following probiotic administration.

**Figure 1.** Survival of olive flounder (*Paralichthys olivaceus*) after challenge with *S. iniae* by immersion (1×10⁹ CFU/ml). Survivals are presented as mean of triplicate groups.

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Effects of dietary supplementation of propolis on growth performance, feed utilization, non-specific immune response and disease resistance in juvenile olive flounder (*Paralichthys olivaceus*)

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This study was conducted to investigate the effects of dietary supplementation of propolis on growth performance, feed utilization, non-specific immune response and disease resistance against *Streptococcus iniae* and viral hemorrhagic septicemia virus (VHS) for juvenile olive flounder. Eight experimental diets were formulated to contain propolis in both powder and liquid forms at levels of 0, 0.25, 0.5, 0.75, 1.0, 0.25, 0.5, 1.0% respectively (designated as Con, P0.25, P0.5, P0.75, P1.0 and L0.25, L0.5, L1.0). All diets were formulated to be isonitrogenous and isocaloric. Fish (initial body weight 8.94 ± 0.02 g) were randomly distributed into polyvinyl circular tanks. Triplicate groups of fish were fed the experimental diets to apparent satiation (2–3 times a day, from 08:00 to 18:00 h) for 8 weeks. At the end of the feeding trial, growth performance, feed utilization, survival and hematocrit of fish were not significantly affected by the supplementation of propolis. Hemoglobin values in the groups fed L0.25, L0.5 and L1.0 diets were significantly higher than the group fed the Con diet. Respiratory burst, lysozyme, superoxide dismutase, myeloperoxidase and antiprotease activities were not significantly different among all the fish groups. Cumulative mortality of fish fed the P0.75 diet was lower than other groups after 26 and 24 days of challenge with VHS and *Streptococcus iniae* respectively. In conclusion, optimum supplementation level of propolis diets for juvenile olive flounder is suggested to be 0.5–0.75% diet.

Acknowledgment: This work were supported by the Seol Propolis Co., Ltd and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012-0005394).
Effects of different lipid sources in the floating extruded pellet on growth performance, feed utilization, fatty acid composition and flesh quality of adult flounder Paralichthys olivaceus

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This study was conducted to investigate the effects of oil source on the growth performance, body composition, fatty acid profiles and flesh quality of adult flounder. Four extruded pellets (designated as CON, FO, LO and SO) were formulated to contain 0% oil (CON), 4% fish oil (FO), 4% linseed oil (LO), 4% and soybean oil (SO), respectively. Experimental flounder (average weight, 552.6 ± 1.73 g) were randomly distributed in twelve 10 ton tanks in a flow-through tank system. Three replicate groups of fish were fed one of the experimental diets to visual satiation once a day for 23 weeks. At the end of the feeding trial, survival of fish was above 93% and not significantly different among the dietary treatments. No significant differences were observed in weight gain and feed efficiency of adult flounder fed the experimental diets. Protein efficiency ratio of fish fed the LO diet was significantly higher than that of fish fed the CON and SO. No significant differences were observed in condition factor, hepatosomatic and viscera somatic indices between fish fed the experimental diets. Proximate compositions of dorsal muscle and liver were not affected by dietary lipid sources. Fatty acid composition of the muscle, liver, viscera, brain, kidney and heart of adult flounder were significantly reflected those of the feed oils. Higher concentration of C18:2n-6 was observed in fish fed the SO diets. Fish fed the LO diet had the highest concentration of C18:3n-3. Concentrations of n-3HUFA such as EPA and DHA in fish fed the FO diet were higher than those of fish fed other diets.
Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview

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The utilization of seaweed production holds great promise not only in its provision of a significant CO2 sink, but also in meeting to some extent global food, fodder, fuel and pharmaceutical requirements. The Korean project has been an innovative research on seaweeds in developing new baseline and monitoring methodologies for the mitigation and adaptation measures in the context of climate change. The new concept of the Coastal CO2 Removal Belt (CCRB) has been established for natural and/or man-made plant communities in the coastal region, to accomplish CO2 removal in the manner of a forest, and that is implementable on various spatial-temporal scales. About 10 tons of CO2 per ha per year could be drawn down in the pilot scale CCRB farm with a perennial brown alga *Ecklonia* estimated by the biomass increment and decrease in the dissolved inorganic carbon in the water column.
日本水産増殖学会第11回大会プログラム　2012年12月8日14:00～17:30

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Effects of dietary probiotic on growth performance feed utilization, innate immunity and disease resistance against *Streptococcus iniae* in olive flounder (*Paralichthys olivaceus*)
Ji-Hoon Cha¹, Si-Yong Yang², Kyeong-Jun Lee¹,*

P55(KO2)　Oral Presentation
Effects of dietary supplementation of propolis on growth performance, feed utilization, non-specific immune response and disease resistance in juvenile olive flounder (*Paralichthys olivaceus*)
Dae-Han Oh¹, Yong-kap Hur², Kyeong-Jun Lee¹,*

P56(KO3)　Oral Presentation
Effects of different lipid sources in the floating extruded pellet on growth performance, feed utilization, fatty acid composition and flesh quality of adult flounder *Paralichthys olivaceus*
Jin Choi¹, Kyoung-Duck Kim², Kang-Woong Kim², Sang-Min Lee¹,*

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鈴木崇史（東海大学海洋）・秋山信彦（東海大学海洋）

O-02　飼料中の塩分がアオリイカの摂餌に与える影響
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O-03　低分子化アルギン酸のアサリ稚貝に対する成長促進効果
山崎康裕（水大校）・多賀　茂・岸岡正伸（山口水研）

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O-05　養殖ウマツラハギの低塩分および干出ストレス耐性
土橋靖史・宮本敦史・中村紘司（三重水研）

O-06　人工干潟における塩分変動
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O-07　降海性コイ科魚類ウサギ属マルタニ型の形態的分化と地理的分布
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O-08　ウイルス性神経壊死症ウイルスに対する抗体検査法の高精度化
小川真依・笠井久会・吉水　守（北大院水）

O-09　マダイ腸内細菌の経口投与によるマダイのエドウジエラ症予防-1
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家戸敬太郎・石原俊介・石丸克也・山本篤司・村田　修・宮下　盛（近大水研）

O-10　マダイ腸内細菌の経口投与によるマダイのエドウジエラ症予防-2
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Installing kelp forests/seaweed beds for mitigation and adaptation against global warming: Korean Project Overview

I. K. Chung¹, J. H. Oak², J. A. Lee³, J. A. Shin⁴, J. G. Kim⁵ and K. S. Park⁶

14:30～15:15  座長：山﨑康裕（水大）(Chair: Ass. Prof. Y. Yamasaki, Natl. Fish. Univ.)
O-11  シオミズツボワツミの飼育と光環境が付着行動に与える影響
○各務 諒・阪倉良孝・薮原篤志（長大/水環）
O-13  光照射条件が VB₁₂ 生成とワツミの増殖に及ぼす影響
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O-14  異体類 4 種の EIRG 分析と緑体配列の比較
○柴田玲奈（水研水工研）・清水大輔（水研/東北水研）・長谷川英一（水研/水工研）

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O-15  低水温（13.5℃）飼育によるトラgeführt性化の検討
○服部亘宏・澤田好史・宮下 盛・山本篤司・升岡主計・村田 修（近大/水研）
O-16  チョウサメ卵成熟度および排卵能の経時的変化
○石原 学・徳井文平・安部智貴・井尻成保・足立伸次（北大/水水）
O-01
カワハギに対する焼きニホルエチの 飼料原料としての有効性
○鈴木崇史（東海大学海洋）・秋山信彦（東海大学）

【目的】廃棄焼きニホルエチの飼料原料としての有効性をカワハギで検証した。
【方法】試験飼料には、魚粉を焼きニホルエチで代替し、脂質とタンパク質の摂取量を評価するため、計10種類を用いた。また、干しニホルモも比較した。これらの飼料で4週間飼育し、成長と飼料効率を比較した。また、飼料のアミノ酸组成を比較した。
【結果】11～67%魚粉を焼きニホルエチで代替した飼料では、11、34%代替飼料で平均10.4, 12.6g増加し、飼料効率は43と42%で対照飼料の36%より高かった。これからの飼料の必須アミノ酸量は全て対照飼料より多かった。脂質とタンパク質を摂取した場合、代替率が高いほど成長し、対照飼料の3.1gが増加に対して、67%代替飼料で10.3gと最も増加した。干しニホルモを焼きニホルエチで代替する飼料では、差は見られなかった。以上の結果、焼きニホルエチは、必須アミノ酸組成が改善されることでカワハギ用飼料原料として有効と考えられた。

O-02
飼料中の塩分がアオリイカの 摂餌に与える影響
○飯田雅之（東海大学海洋）・秋山信彦（東海大学）

【目的】アオリイカは、海水に長時間放置された鮮魚を摂餌しないことが観察された。そこで飼料の塩分と摂餌率の関係を調査した。
【方法】7～121日飼育までに7日にごとに、飼育海水と、海水を塩分4%を換算溶液、及び蒸留水に15分浸漬したシャンブスを与え、摂餌数を比較した。飼育飼料1.0gと蒸留水10 mlをホモジナイスし、溶液中の塩分を比較した。さらに、吸盤の組織切片を観察した。
【結果】シャンブスを飼育海水、NaCl, MgC1₂, KCl, CaC1₂溶液に浸漬した場合にアオリイカは最大87.3%を摂餌した。摂餌率は、日数と共に増加したが、70日以降低下した。摂餌された飼料から溶出した塩分は0.11～0.14%と、摂餌されなかった飼料の0.01～0.09%より高かった。吸盤基底部の化学受容細胞は、0日飼育では確認できなかったが、日数と共に化学受容細胞が伸長し、7日飼育で15.1±3.5μm, 28日飼育では32.4±6.6μmとなった。
以上の結果から、本種は捕捉時に吸盤の化学受容細胞で飼の塩分を感応することが示唆された。

O-03
低分子化アルギン酸の アサリ稚貝に対する成長促進効果
○山崎康裕・多賀茂・岸塚正伸
（1水大校, 2山口水研）

【目的】近年、有効藻類生産性であるヘテロシージマ・アカシオが、アサリの成長を促進し、その効果には本種に含まれる酸性糖の関与が示唆されている。本研究では、アサリ稚貝に対する低分子化アルギン酸の実用規模における飼料効果を調べた。
【方法】低分子化アルギン酸の飼料効果判定のために、10日間の短期飼育試験（1L）及び30日間の実用規模（0.5L）での飼育試験を行い、複合飼料（低分子化アルギン酸とキートセロス）とキートセロス単独飼料（対照区）の飼料効果を比較した。
【結果】短期飼育試験の結果、低分子化アルギン酸とキートセロスの複合飼料は、対照区よりも成長を促進する有効な成長促進効果が認められなかったものの、殻成長の良好な個体が増加する傾向にあった。一方、実用規模飼育試験の結果、低分子化アルギン酸とキートセロスの複合飼料は、対照区と比較してアサリ稚貝の成長を有意に促進した。

O-04
海水の塩分濃度が稚魚期マサバの 成長に及ぼす影響
○北野隆・長野直樹・坂口光史・松山倫也
（1九大院農水系水研, 2九大院水）

【目的】マサバの種苗生産において、飼育海水の塩分濃度の違いが稚魚の成長に及ぼす影響を、異なる給餌条件下で評価した。
【方法】飼育海水の塩分濃度が34%（原海水）、24%お
および13%となるように調整した1トン水槽に孵化後34日目のマサバを収容し、さらにそれぞれの試験区において高給餌区（10%/体重/日）と低給餌区（3%/体重/日）を設け、40日間の飼育を行った。各試験区における成長は、10日おきに取り上げた魚体の測定結果（尾叉長、体重および肥満度）を比較することで評価した。
【結果】高給餌条件では、34%の塩分濃度海水区に比べ、24%および13%区で肥満度の有意な増加が認められたが、尾叉長には差がみられなかった。一方、低給餌条件では、全ての塩分濃度海水区において肥満度、尾叉長ともに差がみられなかった。以上の結果より、稚魚期のマサバは低塩分海水飼育により肥満度を増加するが、それにはある一定量以上の給餌が必要であることが示された。

【0-05】
飼殖ウマツラハギの低塩分および干出ストレス耐性
○土橋靖史、宮本敦史、中村砂帆子（三重水研）
【目的】海産魚飼殖の新魚種であるウマツラハギについて、低塩分および干出ストレス耐性の把握を目的として、陸上水槽での試験を行った。
【方法】供試魚にはウマツラハギ、および比較対象としてマダイ、トラフグ種苗を用いた。低塩分試験は、塩分33の海水で満たした30L水槽に各供試魚を10尾ずつ収容し、1時間毎に塩分を20、10、および5まで低下させた後、横転および死亡までの時間を測定した。干出試験は、各供試魚を10尾ずつ網子に取り上げ、10分間干出した後、30L水槽へ収容し、24時間後の生残率を測定した。
【結果】低塩分試験のウマツラハギの半数致死時間は7.7±0.9時間で、マダイの9.8±1.1時間よりも短かった。トラフグの死亡は認められなかった。干出ストレス試験のウマツラハギの24時間後の生残率は35±5%で、マダイの20±10%よりも高くなり、トラフグの95±5%よりも低かった。

【0-06】
人工干潟における塩変動
○佐野雅基、有山啓之、中嶋昌紀（大阪環農水研）
【目的】阪南2区人工干潟は大阪府和田市沖の人工島内に造成された。この付近には河川流入がないが、これまでの調査で、ハゼ、チチイ、ヨシエビ、タカノケトサイソガニ、エビナガスジエビなどの淡水性種が優占的に出現することが判っている。この原因を明らかにするために詳細な塩分観測を実施した。
【方法】2009年6月、7月に海底0.2m上層、海底直下、地下間隙水の塩分を満潮時と干潮時に測定するとともに、7月には2定点で地下間隙水の塩分測定を3時間毎に実施した。
【結果】2回の観測では海底0.2m上層と海底直上よりも地下間隙水で時間的、空間的な塩分制が大きくなり、6月には地下間隙水の塩分最高値が満潮時30.40から干潮時33.15に上昇した。地表50cm下層で定点採水した地下間隙水は3時間で28.46から25.47に低下した。このように塩分が短時間に変動する環境条件が、海水性種の優占的出現の要因の一つであると推察された。

【0-07】
降海性コイ科魚類ウグイ属マルタニ型の形態的分化と地理的分布
○天野翔太、廣田祐佑輔、中井博紀
酒井治己（水大校）
【目的】ウグイ属 Tribolodon は、コイ科魚類の中で唯一降海型を含む属で、中でもマルタ T. brandti は全てが降海型である。中村（1969）は、関東に生息するマルタ（マルタ型）と、青森県等のものを（ジュウサンウグイ型）とし、側線鱗数等の形態が若干異なることが、その詳細は明らかではない。そこで本研究では、各地のマルタの形態を比較し、形態的差異とその分布を明らかにすることの目的とした。
【材料と方法】日本および周辺のマチ標本18種地232個体を使用し、19計測形質の相対値と11計数形質を調査し、比較した。
【結果と考察】太平洋側の東京湾から大船渡湾の個体群と、その他の地域の個体群との間で、特に鱗数4形
質、脊椎骨数において大きな差異が認められた（t 検定、p < 0.0001）。両個体群の差異は明確であるため、前者（マルタ型）と後者（ジュウサンウダイ型）は別の資源と考えるべきである。これら二型の分類学的検討は残された課題である。

ウイルス性神経壊死症ウイルスに対する抗体検査法の高精度化
○小川真依・笠井久和・吉水守
（北大院水）

【目的】種苗生産施設や孵化場の防疫対策として、病
原体、特にウイルスフリー親魚の選別は重要である。マサカワではウイルス性神経壊死症対策に、親魚検
補を施設に導入するに際し、抗体検査による感染履
歴の把握を行っている。従来法は発病の有無を指標にELISA の設定を行うが、今回、中和抗体価を基に条
件設定を行い抗体検出 ELISA の精度向上を図った。

【方法】抗原として His tag を付加したウイルス外被
タンパク質を大腸菌発現系により作製した。抗マサカ
ワ IgM 血清は、イオン交換クロマトグラフィおよびゲル濁過後、ウサギに免疫して得た。基質発色液に
は TMBZ を用い、反応条件を最適化した。

【結果】発現タンパク質のアフィニティー精製より、
分子量約36 kDa のタンパク質を得た。得られたタン
パク質をマサカワに接種し、中和抗体が認められた血
清を陽性として抗体検出 ELISA の条件設定を行った。
既存の系に比べ、陰性・陽性個体の吸光値の差が明確
になり、抗体検出精度が向上した。

マダイ腸由来細菌の経口投与による
マダイのエドワジエラ症予防-1
－投与量および期間の影響－
○家戸敬太郎・石原俊介・石丸克也・山本薫司
村田修・宮下盛（近大水研）

【目的】マダイ養殖において、エドワジエラ症が大
きな問題となっている。原因菌は Edwardsiella tarda で、
腸が主要な感染門戸であり、腸内細菌叢を制御するこ
とで本症を予防できる可能性がある。本研究では、エ
ドワジエラ症予防に有効なマダイ腸由来抗菌性細菌の
添加量および投与期間について検討した。

【方法】E. tarda に対する抗菌活性を示すマダイの腸
由来細菌 5 株について、経口投与によるエドワジエラ
症予防効果の再現性を確認した。安定した効果を示し
た 1 種菌株を濃度および期間を変えてマダイに経口投
与し、予防に必要な投与量および期間について検討した。

【結果】復数回の攻撃試験において Shewanella sp. J 株
が安定して予防効果を示した。本菌株は魚体重 1 kg
あたり 1 日に 125 mg（8.25×10^7 CFU）以上の投与で
予防効果を示した。投与期間は 2 週間以上必要であっ
た。

マダイ腸由来細菌の経口投与による
マダイのエドワジエラ症予防-2
－海上生賀における予防効果－
○石原俊介・家戸敬太郎・石丸克也・山本薫司
村田修・宮下盛（近大水研）

【目的】これまでの研究でマダイの腸由来細菌 J 株の経
口投与がエドワジエラ症に有効であることを確認し
た。本研究では、海上生賀での予防効果を検討した。
また、J 株経口投与マダイにおけるエドワジエラ症原
因菌 Edwardsiella tarda 浸漬攻撃時の体内動態を調査
した。

【方法】実験 I ：平均体重 614 g のマダイを海上生賀に
収容し、魚体重 1 kg あたり 4.2 および 42 mg の J 株
を飼料に添加して投与した。実験開始から 30 日毎に発
症率を調べた。実験 II ：投与開始152日後に E. tarda
を浸漬攻撃して発症率を調べるとともに、腸、脾臓、
肝臓および血液中の E. tarda 生菌数を計数した。

【結果】実験 I ：対照区で発症率 1.5%であったが、J 株
添加区では感染魚は認められなかった。実験 II : 発症
率は J 株添加区で 4%および2%と、対照区の 20%よりも
有意に低かった。浸漬攻撃10日後の腸において J 株
添加区で E. tarda の減少がみられた。
高水温で培養したタネタサマアマノリ葉状体の生長
○玉城泉也・藤原雄二・藤吉宗次・小林正裕
（水研西海開研）

ノリ養殖業においては、秋季の高水温の影響による養殖...

光照射条件がVB₁₂生成とワムシの増殖に及ぼす影響
○黒 川村宏之・篠原孝仁・古川享司・一色 正
吉松隆夫（三重大学院生）

シオミツボワムシの加齢と光環境が付着行動に与える影響
○各務 諒・阪倉良孝・菅原篤志
（長大院水環）

異種類4種のEIRG分析と錐形体列の比較
○水野玲子・清水大輔・長谷川英一
（水研水工研，水研東北水研）
の分光感度と生態的特性との関係を考察した。
【方法】4種成魚を水工研に搬入後、暗順応下で眼球を摘出し、EIRG分析を行った。刺激光として、350～700 nm波長（10 nm間隔）の色光を同じ強度で網膜に照射して電位変化を記録した。EIRG分析終了後、速やかに眼球をブラン液で固定し、組織切片を作成し、染色を施し検覧した。
【結果】EIRG分析の結果、ヒラメ、マツカワ、ホシガレイ3種の相対感度ピーク波長が510～521 nmであったのに対し、パハガレイのみ485 nmで、他の3種に比べ、短波長寄りにピークが現れた。4種とも椎体は正方型モザイクを構成していた。分光感度の差違は生息水深（3種：200 m、パハガレイ450 m）に反映されたものと推定された。

O-16
チョウザメ卵成熟能および排卵能の経時的変化
○石原 学・徳井文平・安部智貴・井尻成保
足立伸次（北大院水）
【目的】魚類の卵成熟過程においては、卵母細胞が卵成熟（卵成熟誘起ホルモン：MIHに反応して卵成熟し得る能力）を獲得後に、卵卵巢は排卵能（MIHに反応して排卵し得る能力）を獲得する。本研究ではパステルチョウザメを用いて卵成熟能および排卵能の経時的変化を調べた。
【方法】同一個体から通年卵卵巢を摘出し、17α-hydroxyprogesteroneを添加して培養した。培養後の卵成熟率および排卵率を調べた。5月にはその個体にホルモン（LHRH）を投与し採卵を試みた。
【結果】卵成熟能については、採卵10ヶ月前の7月には一部の卵母細胞がすでに獲得しており、9月以降ではほとんどの卵母細胞が有していた。排卵能については、卵成熟能獲得後10ヶ月以降に一部の卵卵巢が獲得し始め、一旦、冬季に失った後、春季には多くの卵卵巢が獲得した。5月に排卵能が高かった個体は、孵化率が高い傾向がみられた。

O-15
低水温（13.5℃）飼育によるトラフグ雄性化の検討
○服部亘宏・澤田好史・宮下 盛・山本義司・井間主計・村田 修（近大水研）
【目的】孵化後20日のトラフグを水温15℃で100日間飼育すると約8割の個体が雄性化することが分かっている。本実験では、より高い雄性化率を期待して、より低水温となる水温13.5℃でトラフグの飼育を行い成長、生殖、および生殖腺の発達について調べた。
【方法】水温18℃で飼育した孵化後20日のトラフグを平均全長6.4±0.5 mmを200尾透明パラライト水槽3基にそれぞれ300尾収容し、水槽内の水温が13.5℃となるまで1日あたり約2℃ずつ水温を下降させ、以後13.5℃を維持するよう温調した。魚の成長にあわせてワムシ、アルテミア、および配合飼料を与え、孵化後135日まで飼育した。
【結果】孵化後100日には平均全長14.7±1.5 mmまで成長し、試験開始時の生长率は98.3±1.5%であった。しかしながら、孵化後100日目以降はほとんどの成長せず孵化後135日の平均全長および生殖腺は13.1±2.7 mmおよび84.3±5.5%であった。また、その時点での生殖腺は未分化であった。

O-17
ロシアチョウザメの分子的性分化期における卵巣関連遺伝子の発現
○山下量平・白濱将人・銃本和美・井尻成保
足立伸次（北大院水）
【目的】チョウザメ類は外見から雌雄を判別出来ず、また成熟するまでに長期間を要するため、キャピア養殖には高い飼育コストがかかる。そこで早期性別判別法の確立のため、生殖腺の形態的分化の先立って起こる、分子的性分化の解明が求められている。数種の魚種では、形態的未分化生殖腺において、卵巣関連遺伝子であるfox2およびcyp19a1aの発現の二型性が確認されている。本研究ではチョウザメを用いて両遺伝子の発現動態を調査した。
【方法】ロシアチョウザメ（Acipenser gueldenstaedti）の孵化後3、4、5および9ヶ月目の生殖腺におけるfox2およびcyp19a1a mRNA量を測定した。
【結果】孵化後4および5ヶ月目の形態的未分化生殖腺では、foxl2およびcyp19a1a発現の二型性がみられ
た。孵化後9ヶ月目には、一部の個体で形態的卵巣分化の兆候が現れ、それらの個体では両遺伝子発現が高
い値を示した。従って、両遺伝子発現を指標とした早期性判別が可能であることが示唆された。

【目的】カシモハタEpinephelus merraの最終成熟および排卵には、雄からのフェロモンの存在が不可欠であ
る。本研究では、1）雄フェロモンの産生場所および2）卵におけるフェロモン感受のタイミングを調べた。
【方法】1）成熟雌の精巣、腎臓、およびそれ以外の全内臓をそれぞれリン酸緩衝液内で破砕し、これを雌
のみの飼育水槽に添加して排卵の有無を調べた。2）雌のみの飼育水槽に成熟雌の飼育水を期間を変えて注
水し、排卵の有無を調べた。また、雌雄混合飼育する群も設定した。
【結果】1）雌雄混同飼育群では産卵が行われたもの
ので、雄由来の各種器識溶液添加によって最終成熟の誘
導はできなかった。2）雌は満月の2日前にはフェロ
モンに対する感受性を獲得しており、雄飼育水に暴露
されることで最終成熟および排卵をすることが可能で
ある。

【結果】5月から7月にかけて本種成熟魚（1〜2
歳魚）にHCGおよびTの投与を行った。その後、卵
巣および血液を採取し、卵巣の組織学的観察および
血中エストラジオール17-β(E2)の測定を行った。
また、ホルモン投与を行っていない個体の卵巣の一部
を、HCGおよびTを単独あるいは複合添加した培養
液で24時間培養した後、培養液中のE2濃度の測定
を行った。
【結果】HCGおよびT投与において卵巣細胞の発達
は誘導されず、血中E2濃度の変化は見られなかった。
培養実験において、Tを添加した培養液中のE2濃度
が高い値を示した。以上の結果より、本種未成熟魚の
卵巣はTをE2に変換する能力を有するもので、下垂
体-生殖腺系における内分泌系は十分に発達していな
いことが明らかとなった。

【目的】カシモハタの初回成熟開始前に及ぼす
ヒト経毛性生殖腺刺激ホルモン(HCG)およ
びテストステロン(T)の影響
【目的】カシモハタの初回成熟開始前に及ぼす  HCG およ
びTの影響について検討した。

【方法】5月から7月にかけて本種未成熟魚（1〜2
歳魚）にHCGおよびTの投与を行った。その後、卵
巣および血液を採取し、卵巣の組織学的観察および
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卵巣はTをE2に変換する能力を有するもので、下垂
体-生殖腺系における内分泌系は十分に発達していな
いことがあることが明らかとなった。
山口県の平生湾で採集されたノコギリガザミについて

○荒木 晶（水大校）
重田利拓（水研・瀬戸内水研）
小澤幹生・鈴木啓大・藤森健太（水大校）

ノコギリガザミの仲間は、日本では沖縄県をはじめとして、九州・本州の特に暖流の影響を受ける河口や河床水で捕獲されていることが多い。日本海側では鳥取県、京都府、石川県、山口県津波湾から、太平洋側では利根川河口以南の静岡県浜名湖、高知県浦戸湾、宮崎県、鹿児島県などからの報告があるが、瀬戸内海沿岸からの本種の報告はないと考えられる。

本研究では、山口県熊毛郡平生町の平生湾内の泥・泥砂場において、2009年（1個体）と2011年（2個体）に刺網（チヌ建）にて漁獲されたノコギリガザミ類の標本を入手することができた。また、山口県が平生湾近辺の定点で観測している海水温の経年変化との関連について調べた。これら標本の形態的特徴を観察したところ、2009年の個体はトゲノコギリガザミ（甲幅152 mm）、2011年の個体はアミメノコギリガザミ（甲幅125 mm、123 mm）ではないかと考えられた。

山口県の深川川上井手堰に施工された「水辺の小わざ」魚道

○荒木 晶・鈴木啓大・藤森健太
小澤幹生（水大校）
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中村好希・藤本 護（山口県河川課）

山口県では「水辺の小わざ」という理念に基づいて、河川に生息する生物の往来に配慮しつつ、安価に河川生態系を回復・保全するための堰堤や魚道の改修を行っている。深川川上井出堰は、中央部には魚道があり、右岸側には農業用取水路、余水吐きが設けてある。下流へは、魚道および余水吐きの2ヶ所から水が流れている。堰堤の魚道下部は河床が低下して大きな落差があり、さらに魚道内も毎年の川の流動の影響が激しかった。昨年度、魚道の改修工事前に魚道および余水吐きにおいて生物の調査を行ったが、下流から上流へ魚道内を移動する生物の観察はできなかった。本研究では、深川川の上井出堰魚道において、昨年度の調査結果に基づき、「水辺の小わざ」理念に基づく改修方法の提案を行い、実際に既設魚道の改修と細石付き斜路式魚道（「水辺の小わざ」魚道）の施工を行った。