제2차 한중 황해광역생태계 해수양식 학술회의

2nd YSLME Regional Mariculture Conference

TOWARDS SUSTAINABILITY IN YELLOW SEA MARICULTURE

Organized by West Sea Mariculture Research Center (NFRDI) of Korea, Yellow Sea Fisheries Research Institute (CAFS) of China, Jeju National University and UNDP/GEF YSLME project

Place and Date: Jeju KAL Hotel, Korea, 16-18 June 2009

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Welcome Address

Mi Seon PARK

Vice-President, National Fisheries Research & Development Institute, Busan, Republic of Korea
Email: parkms@nfrdi.go.kr

Good morning, distinguished speakers, Dr. Wang, Qing Ying, General Director of Yellow Sea Fisheries Research Institute of China, Drs. Fang, Jiang Guang and Jang, In Kwon in charge of YSLME mariculture section, Dr Mark Walton of YSLME PMO, conference participants, and organizing committee members.

Ladies and Gentlemen!

It is honor to give my welcoming address in the conference on behalf of National Fisheries Research & Development Institute (NFRDI).

First of all, I would like to convey my heartfelt congratulations on holding the Yellow Sea Large Marine Ecosystem 2nd Regional Mariculture Conference on "Toward Sustainability in the Yellow Sea Mariculture". Also, I would like to express my best wishes for the success of each one of all your deliberations at the conference in Jeju. This Yellow Sea Project is so important for both countries, as its mission statement indicates: "to protect, conserve, and manage the Yellow Sea through sustainable use of the waters, by reducing environmental stress and by promoting sustainable exploitation of the resources".

For the 21st century, one of the most important challenges of Aquaculture Industry around the world would be "Environmentally Friendly and Sustainable Aquaculture". National Fisheries Research & Development Institute of Korea has been committed to the same challenges for a long time since it was established, to the progressive and sustainable development of Korean aquaculture industry through promotion of excellence in science, technology, education, and implementation to private sectors.

To solve and overcome the problems resulting from unexpected and unsustainable mariculture in the Yellow Sea, we need to exchange information. In this sense, I believe this conference will be a golden opportunity to share knowledge and experience on sustainable aquaculture between two countries.

Recently, the Korean government is making an innovative and long-term 'Green Technology (GT) Project' to promote sustainability in agriculture and aquaculture and to cope with global warming. Also, the Aquatic Animal Disease Control Law (AADCL)

was enacted last year. We are sure that this law and GT project will greatly contribute to the enhancement of sustainability in the Yellow Sea mariculture in the future.

Again, on behalf of all the Korean participants, especially, members of National Fisheries Research & Development Institute, I would like to extend my warm welcome to all Chinese participants who have traveled from the other side of Yellow Sea to here in Jeju to attend this meeting. And, I thank you and congratulations again for all of your hard work behind the scenes and successful initiation and organizing of the conference.

Jeju is a very beautiful island and one of the most famous tourist places in Korea, and it can provide you with lots of exotic foods and cultures which are somewhat different from the other provinces of Korea. I hope that not only you can work hard for the meeting but also you can enjoy the Korean foods and cultures, so that you can take lots of fond memories with you on the way back to your home. I wish that your stay in Jeju will be a rewarding and pleasant one.
Thank you very much!

Opening remark of China

Qingyin WANG
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences
106 Nanjing Road, Qingdao, 266071, China
Email: qywang@public.qd.sd.cn

Welcome Speech of Local Host

Chi-Kyu CHOI

Cheju National University, 66 Jejudaehakno, Jeju 690-756, Republic of Korea
Email:

The Role of Sustainable Mariculture in the Yellow Sea

Mark Walton and Yihang Jiang

UNDP/GEF Project "Reducing environmental stress in the Yellow Sea". KORDI compound, 1270, Sa2-dong, Sangnok-gu, Ansan-si, Gyeonggi-do, 426-744, R. Korea
Email: mark@yslme.org

Vice president Park, Director Wang, President Choi, Ladies and Gentlemen, welcome to this lovely island Jeju and thank you for participating in this the second YSLME regional mariculture conference. I want to express my gratitude to Dr Jang In Kwon, Dr Choi Kwang-sik, Dr Fang Jianguang and Dr Zhang Jihong for their enormous help in organising this conference. Over the next two days we will hear what advances have been made in rearing techniques, in the field of genetics and in disease diagnosis, prevention and control and what these advances mean to the increased sustainability of the mariculture in this region.

This drive towards sustainability is going to become ever more important as increasing production is required from the industry. By endorsement of the YSLME Strategic Action Programme both governments will have jointly accepted the need to reduce fishing effort by between 25 to 30% by the year 2020 to reduce the exploitation rates of wild fish stocks to a sustainable level. Both governments have already spent millions of dollars to this end. However, reducing fishing effort is going to reduce the amount of fish caught at a time when the per capita income in the region is increasing and with it the demand for fish protein. It is estimated that the impact of this reduction in fishing effort will reduce the Yellow Sea fish catch by about a million tonnes.

Mariculture has grown enormously in the Yellow Sea over the last 10 years increasing from 400,000 tonnes to 6.2 million tonnes in 2004 and there is high probability of it continuing to grow. However, finfish/crustacean production has only reached 310,000 tonnes. Just in order to compensate for the decreased wild catch, production needs to rise by over a million tonnes by 2020. Moreover, to keep pace with the likely increase in demand for fish, shrimp and other crustaceans from an increasingly wealth population could require a six or seven fold rise in production. This is going to be quite a challenge given the spatial and environmental constraints.

We all know of the mounting problems that are facing the industry as result of the increasing disease frequency and decline in environmental quality, and although finfish/crustacean production has made substantial improvements in recent times, their culture has one of the worst environmental records in the industry.

Already, there are two shinning examples that we are using to demonstrate to others how this might be achieved. In Sanggou Bay with the integrated multi-trophic

aquaculture (IMTA) where different trophic levels are cultured together so nutrient requirement of one species are balanced by another's waste production. And at the West Sea Mariculture Research Station where heterotrophic shrimp culture is being trialled using bio-floc technology. This is where uneaten food and shrimp waste products are recycled by bacterial flocs that are a food source for the shrimp. This technology promises so much, reduced reliance on fish meal, almost zero nutrient outflows, increased stability of water parameters, reduced mortality from disease and the very high stocking densities can reduce the amount of coastal land used for culture.

But these are only two examples, over the next two days I am looking forward to hearing what other improvements to sustainability in the mariculture industry are being made. Thank you all for sharing your knowledge with us and I wish you a good conference.

Key Note Speeches

The forces driving sustainability in Yellow Sea mariculture in Republic of Korea

In Kwon JANG

Director of West Sea Mariculture Research Center, National Fisheries Research & Development Institute, Taean, Chungnam, Republic of Korea
Email: jangik@nfrdi.go.kr

Mariculture production in the Yellow Sea (YS) coast of South Korea reached 208 × 10^3, or 22.7% of total national mariculture production in 2004. Of these, the yield of seaweeds was 145.9 × 10^3 MT, or 70.1% of the total mariculture production in the Yellow Sea. The farmed production of finfish, crustaceans, and molluscs occupied 3.9%, 0.5% and 25.5% respectively. Of the mariculture production, shellfish is of most interest in the Yellow Sea coast of Korea. Total mariculture production in the Yellow Sea was not significantly changed during the last decade, decreased by 3.2% only. However, the production of seaweeds which reduce nutrients decreased by 17.4% and that of finfish increased 671% during the same period. Although it is not as serious as in the southern area of South Korea, the Yellow Sea area is also facing environmental stress including outbreaks of diseases, coastal eutrophication and harmful algal blooms in the farming ground**.**

Recently, the government is giving an impetus to development of new innovative technologies, so called GT (green technology) which can enhance sustainability of agriculture and aquaculture and reduce CO_2 and environmental pollution. GT project was established by the National Science and Technology Committee and the National Future Planning Committee in January, 2009 and its sub-projects in different fields have been preparing. For the fisheries and aquaculture a total of 28 major sub-projects are under preparation and enormous budget will be invested for it with the next decade. Of these, many projects are related to that of enhancing sustainability of aquaculture and reducing environmental stress in the Yellow Sea. Regardless of this project, some studies have showed excellent achievements and have already been implemented to private sectors, so that it has contributed to sustainability of aquaculture in the Yellow Sea. For example, limited water exchange shrimp culture technology has been developed and the technology is now implemented by private farmers; in the offshore aquaculture technology, several sets of commercial cages have been built and are now operating in Jeju island; big-scale sea ranching projects are successfully progressing in several locations funded by central and local governments; and different kinds of vaccines are commercially produced and provided to finfish farms.

In addition, the Aquatic Animal Disease Control Law (AADCL) and its enforcement regulations were took effect in December, 2008. This law includes surveillance, monitoring, quarantine, disease control and jurisdiction and so on. Infectious diseases designated by law includes red sea bream iridovirus (RSIV), spring viraemia of carp virus (SVCV), Koi herpesvirus (KHVD), viral nervous necrosis (VNN), viral hemorrhagic septicemia virus (VHSV), epizootic ulcerative syndrome (EUS), infectious pancreatic necrosis virus (IPNV), yellow head virus (YHV), Taura

syndrome virus (TSV), white spot syndrome virus (WSSV). For operation of the law, one Aquatic Animal Disease Control Center and other seven reference laboratories were established in this year. These laboratories will work for epizootiological surveillance, warning, reporting, destruction and disposal of the infected animals from the aquatic farms in the country.

Green technologies of aquaculture and recently enacted AADCL are expected to act as a strong forces driving sustainability in the Yellow Sea mariculture of the South Korea in future.

Key words: mariculture, sustainability, green technology, AADCL, Yellow Sea

The forces driving sustainability in Yellow Sea mariculture in China

Qingyin WANG
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences
106 Nanjing Road, Qingdao, 266071, China
Email: qywang@public.qd.sd.cn

Aquaculture has become one of the important sources of protein food supply for China. In 2004, mariculture production in Yellow Sea coastal areas of China reached 603×10^4 tons or 45.8% of total national mariculture output. Of which shellfish stood for 78.7%, seaweed took 15.4%, and finfish, crustacean and others accounted for 2.5%, 2.5% and 0.9%, respectively. 2006 was the first year when the total national mariculture output became overtook the capture fishing yield in China. The mariculture production in Yellow Sea and its ratio in national output in China are shown in Figure 1.

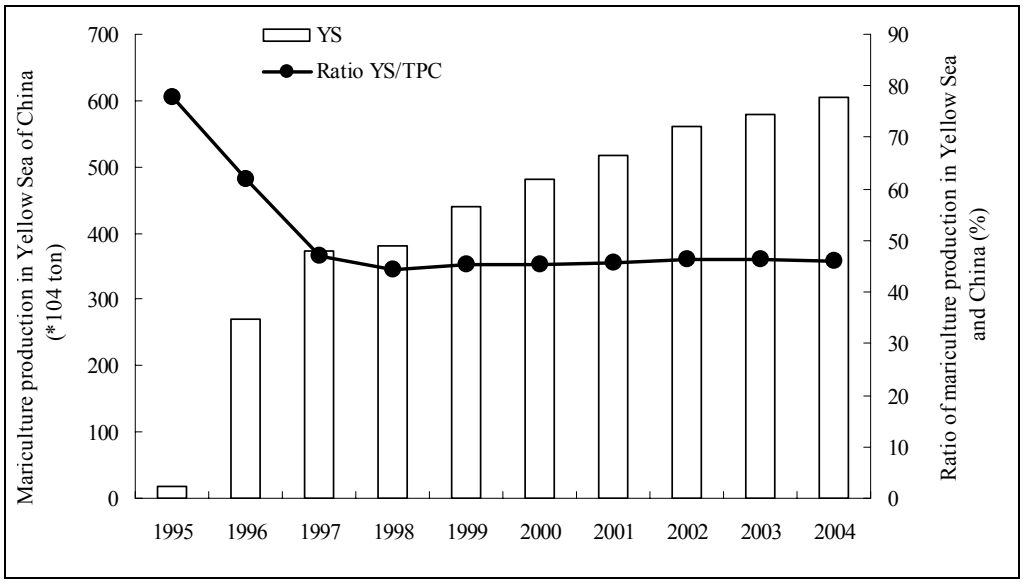


Figure 1: Mariculture production in Yellow Sea and its ratio in national output

The growth of world population has put more demand on aquaculture for ever higher production. However, with the rapid increase in mariculture densities and scale, problems such as environment pollution, outbreaks of diseases, harmful algal blooms have limited its sustainable development in Yellow Sea of China. Therefore, it necessary to recognize that sustainable development of mariculture is essential in the Yellow Sea.

In this presentation, the current status of mariculture in Yellow Sea of China is analyzed and the main constraints that threaten the sustainable development of mariculture are discussed. The forces driving sustainability in Yellow Sea mariculture in China are mainly focused on:

Research on basic theory and mechanism of mariculture are emphasized---

The government of China at different levels provides much more financial supports than ever on basic research, such as the fundamental biology and physiology of cultivated animals, the interaction of mariculture activity and ecosystem, the assessment of ecological carrying capacity, the mechanisms to keep food production sustainable. Several national projects including the National Key Basic Research Program, National Natural Science Foundation of China are implemented in Yellow Sea.

Setting up mariculture techniques of environment friendly are promoted ---

In order to improve maricultural production and seafood quality and to minimize the impact of mariculture on natural environment, different kinds of maricultural techniques and modes have been setup or are now being studied. Genetic improvement for new variety to improve productivity, disease diagnosis, prevention and control technique, environment friendly cultivated technique, such as integrated multi-trophic aquaculture (IMTA), offshore culture, seaweed forest reconstruction, design and construction of artificial reefs etc. have seen remarkable progress.

Management on Mariculture industry is reinforced---

The management reinforcement strategies include:

- The ecosystem approach to mariculture, such as artificial reefs' ecological function at habitat restoration, enhancement of biodiversity;
- Management of the release of hatchery raised juveniles for promotion of stock enhancement;
- Revolution of the legislation and policy on the mariculture certification, classification on the mariculture area, etc.;
- Seafood safety and quality control system etc.

Key words: Mariculture; Yellow Sea of China; Sustainable development

Session 1: Advances in mariculture rearing: techniques to reduce environmental impacts

Challenge of Offshore Aquaculture in Korea

Lee Jung Uie
Aquaculture Industry Department, National Fisheries Research & Development Institute,
Republic of Korea
Email: fishkorea@nfrdi.go.kr

Korean aquaculture activities for marine finfish have been traditionally performed in relatively small-scaled pens in naturally protected locations along the south coast. Recently, local failures to keep the activities within carrying capacity have hindered further progress. To make matters worse, many fish farmers, especially in the southern Korean coastal sea, have suffered annually from big damages caused by seasonal typhoons and red tides.

This project is aimed to introduce developed offshore cages worldwide and test on offshore aquaculture trial off Korean water. Our major concerns in this project are to secure cage safety, develop potential target fish species for offshore waters that are biologically and economically attractive, and monitor the environmental impacts by the aquaculture activities. We also have tried to develop offshore cage system suitable to Korean environment and local fish species.

Results and discussion:
In an attempt to replace inshore traditional pens with offshore cages which are considered more environmentally friendly, The National Fisheries Research and Development Institute (NFRDI) launched a pilot study to develop offshore aquaculture from 2005 to 2007. After a sequential survey of potential culture sites around south-eastern Jeju water by SONAR, we installed six offshore SeaStation cages (OceanSpar LLC) from the United Sates in the south coastal water off Jeju Island from 2005 to 2006. Parrot fish, *Oplegnathus fasciatus* was selected as a target fish on the basis of its adaptability to offshore cages, availability for hatchery-based mass seed production, and economic efficiency. Two age groups of parrot fish produced artificially in 2004 and 2005 (average body weight of 123 g and 5 to 10 g) were stocked in three cages in early July 2005. The fish were fed with formulated pellets once or twice per day using semi-auto feeder except on stormy days. Average weights of the fish at three cages on May 20, 2006 were 180 g, 210 g, and 380 g, respectively, showing different growth rates accordingly to initial fish sizes and stocking densities. Survival rates were relatively high except for one cage where accidental mortality occurred during the stocking procedure. In total, 27 tons of parrot fish (17 tons in 2006, 10 tons in 2007) were harvested, and around 80 tons are currently in rearing. After the harvest, fish species such as red sea bream, yellow croaker, and grouper will be stocked to investigate the suitability of other target fish for offshore cages.

We periodically monitored environmental parameters in the water mass and bottom sediment beneath the cages. But no noticeable changes were detected. The water velocities in the culture site were 32 to 121 cm per second depending on the direction of the tidal current. To date, the cage system was not damaged by the current. However, unexpectedly the mooring lines were extended by 20%, probably by the currents. Two typhoons directly hit the culture site, for a week each, with waves of over 10 m in height. The system was found to be safe against the typhoons with no animal mortality, but some damage to surface facilities like floating buoys was noted.

During this project we have faced with some difficulties such as under-water fish cultivation skill, feeding, monitoring of cages and culture fish, sorting, etc. Of particular difficulty were the sociological and legal concerns. As offshore aquaculture in Korea is only now being just launched, many challenges will need to be faced and new ones will undoubtedly emerge in the future.

Perspectives

Korean aquaculture has so far developed very fast and achieved many technical achievements in terms of seedling production and on-growing sectors. But its techniques have been mostly depended on the experience of fish farmers not on scientific data, or with technological difficulties, especially those from environment-friendly and modern culture systems.

This project will play a pivotal role, technologically and economically, for the Korean aquaculture industry which has traditionally used inshore water.s This paper can provide an effective management manual on coastal water by dividing inshore and offshore water. Inshore water can be guided by integrated coastal zone management (ICZM) which promotes ecosystem-based aquaculture with polyculture and/or multi-trophic aquaculture. While offshore waters, which has greater carrying capacity potential, should be developed for fed aquaculture that gives high economic returns such as finfish aquaculture. Also we can speed up development of offshore cage systems fit for Korean oceanic environment and culture animals with scientific data from this project. The final goal in this project is to give ideas and knowledge to administrators and fish farmers for shifting the Korean aquaculture paradigm.

Integrated Multi-Trophic Aquaculture (IMTA) of Sea Cucumber, Abalone and Kelp in Sunggou Bay, China

Fang Jian-guang[1*], Jon Funderud[2], Zhang Ji-hong[1], Jiang Zeng-jie[1], Qi Zhan-hui[1] and Wang Wei[1]
[1]Yellow Sea Fisheries Research Institute, CAFS, Qingdao, 266071. China
[2]Norwegian University of Science and Technology (NTNU), Trondheim, Norway
*Email: fangjg@ysfri.ac.cn

The sea cucumber is a detritivore species feeding on decaying organic matter in coastal sediments. This makes it an interesting candidate for use in integrated multi-trophic aquaculture (IMTA), where its feeding on sedimented particulate matter is an unused ecological niche compared to other extractive organisms such as filter-feeding bivalves (extracting suspended particulate matter) and seaweeds (extracting dissolved inorganic nutrients). In China, sea cucumbers (*Apostichopus japonicus*) have a high market price and are commonly cultured in tidal zone, ponds or indoor tanks. Abalone (*Haliotis discus hannai* Ino) and kelp (*Laminaria japonica*) are co-cultured on a large scale from suspended longlines in the coastal waters of North China. In this study, sea cucumbers were added directly to abalone cages without any modification of the culture equipment, for a simple and low cost production. To evaluate the feasibility of this co-culture model, growth of sea cucumbers was studied during a 7 month field experiment in Lidao, near Sanggou Bay, Shandong Province, North China.

Standard abalone cages (60x50x50 cm) were used containing 3 layers, suspended from kelp longlines to a depth of 5 m. Abalones (52.3±0.9 mm) were stocked at normal commercial density (250 cage^{-1}) and fed with kelp according to normal production procedures. Sea cucumbers (65.5+2.0 g) were added to the cages at 4 densities: 1, 2, 4 and 6 individuals per layer (treatments named ”1SC”, ”2SC, ”4SC” and ”6SC”). One control cage contained 1 sea cucumber per layer, but no abalones (”1SC-0A”). There were 3 replicate cages for each treatment, except for 6SC and 1SC-0A, which had only one replicate. The experiment lasted from October 2008 to May 2009. Wet body weight of all sea cucumbers was recorded monthly. Water was sampled monthly for analysis of particulate organic matter (POM) and total nitrogen (TN). In March, April and May, sediment traps were deployed at culture depth for calculation of sedimentation rate.

During the 7 month experiment, average sea cucumber body weight increased by 96 %, from 65.5 g in October to 128.8 g in May. Average specific growth rate (SGR) for all treatments during the whole experiment, calculated as average percent growth per day, was 0.33 % day^{-1}(Table 1). Growth was highest during the first month (October-November) with an average SGR of 1.00 % day^{-1}. As the temperature dropped from 18 °C in October, to 3 °C in January the growth rate decreased. During the winter (November-April) growth was slow, with an average SGR of 0.14 % day^{-1}. In May, the temperature had increased to 11 °C, and during the last month (April-

May) average SGR increased to 0.48 % day^{-1}.

The small number of replicates (n=3), a large variance in sea cucumber initial body weight, and a highly variable growth rate for individual sea cucumbers, makes it difficult to distinguish between treatments. From the initial and final size distribution (Figure 1) it is clear that sea cucumber growth performance is highly uneven and it appears that many of the smaller individual grew very little, while the bigger individuals grow fast.

The available food sources for sea cucumber includes abalone faeces, kelp detritus and "background" sediments from the water. The high sedimentation rate and the high growth rate of the sea cucumbers control group (1SC-0A), indicates that background sediments may be an important food sources. Large amounts of sediments were observed in the cages when they removed from the water.

The high overall growth rate of sea cucumbers measured in this experiment shows that adding sea cucumbers directly to abalone cages may be a feasible production technique. Compared to production in land based facilities, tidal ponds, or extensive bottom culture, this method is simple and requires a minimum of extra labour or additional investments. Considering the high market price of sea cucumber, it should be an economically interesting idea for abalone farmers. In addition to adding income to farmers and increasing the production output, the sea cucumbers may reduce the aquauculture impact on the local environment by assimilating nutrients and organic matter wasted by other farmed species. Further study and cooperation with local farmers should be conducted to determine year-round growth performance and survival, optimal culture densities, optimal stocking size and time, suitable culture locations, and product quality.

Keywords: Sea cucumber, IMTA, Abalone, Kelp

Table 1. Sea cucumber body weight and average specific growth rate (SGR) from October to May in each treatment.

Treatment	Initial BW (g)	Final BW (g)	SGR (% day^{-1})	Total growth (%)
0A-1SC	98.7 ± 12.5^{a}	186.7 ± 11.5^{1}	0.32 ± 0.07^{A}	89.2
1SC	60.6 ± 3.6^{b}	137.8 ± 5.7^{2}	0.40 ± 0.04^{A}	129.2
2SC	63.4 ± 5.3^{b}	120.3 ± 4.1^{2}	0.32 ± 0.02^{A}	90.4
4SC	66.2 ± 2.8^{b}	132.4 ± 9.6^{2}	0.34 ± 0.05^{A}	100.0
6SC	63.4 ± 5.7^{b}	118.5 ± 13.1^{2}	0.30 ± 0.01^{A}	78.3

Values are means (±SE, n=3) of replicate cages (1SC, 2SC and 4SC) or layers (0A-1SC and 6SC). Different superscripts indicate significant difference between means ($p<0.05$).

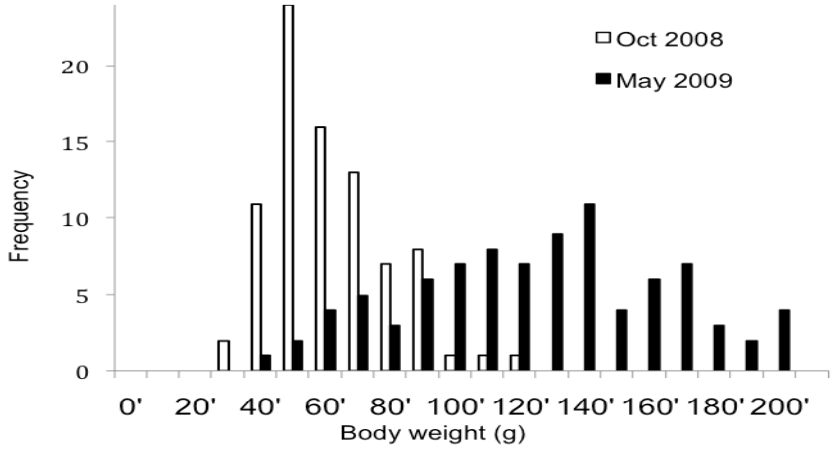


Figure 1: Size distribution histogram of sea cucumber body weight in October 2008 and May 2009 in 10 g intervals.

Lantern net

Sediment from lantern net

In Sanggou bay

Sea cucumber in the abalone lantern nets

Switchover of moist pellet to extruded pellet, the first step for sustainable development of olive flounder, *Paralichtys olivaceus* culture in Jeju Island, Korea.

Jeong-Dae Kim
Department of Animal Life System, College of Animal Life Sciences, Kangwon National University, Chuncheon 200-701, Korea
E-mail: jdekim@kangwon.ac.kr

Marine fish culture in Korea has relatively short history and its production is no more than 10% of the country's total mariculture production of 1,382,257 metric tons (MT). Olive flounder, *Paralichthys olivaceus* is major fish species and the production reached 46,329 MT last year (Fig. 1). Due to optimal water temperature ranging from 14 to 25 ^{o}C and plentiful subterranean sea water of 17 ^{o}C, Jeju Island is the top flounder producing region in Korea since 1990 when the practical farming was started. However, flounder feeding is still predominantly carried out using moist pellet (MP) composed of trash fish and mash feed. It is estimated that 119,000 MT of trash fish and 5,350 MT of mash feed were employed to culture the flounder in the island in 2008 (Fig. 2). Only 5,000 MT of extruded pellet (EP) were used, suggesting that fish farmers are still prepossessed with the idea that feeding EP could result in retarded growth and high mortality due to digestive disorder. However, according to the results from a recent on-farm experiment conducted in Jeju Island, no significant differences were recorded in growth rate, muscle composition, sensory evaluation and production profits between fish groups fed EP and MP. On the other hand, much higher water pollution (1.5 to 10 folds) was observed in MP groups (Kang et al., 2009). A tremendous quantitative development, leading to large increases in production, has been achieved in flounder farming industry during last two decades. Now the industry should progress qualitatively to improve the sustainability of the industry. For this, a compulsory use of EP should be urgently established, while the use of trash fish should be strictly prohibited in order to protect natural seed stocks as future food for the next generation and at the same time to prevent water pollution as much as possible. Vision and strategies for qualitative development of flounder industry in Jeju Island will be discussed.

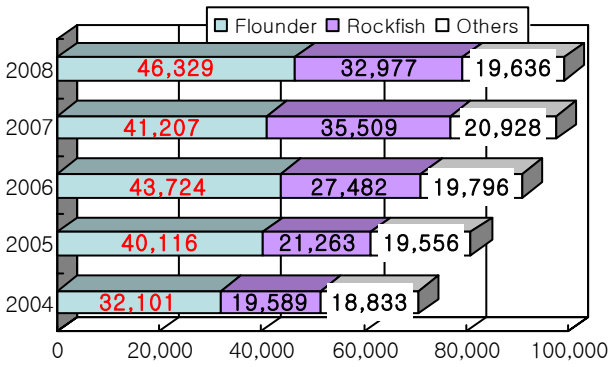


Fig.1. Mariculture fish production (MT) in Korea

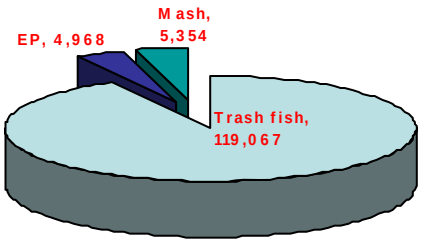


Fig. 2. Feed used in Jeju 2008 for flounder culture (unit: MT)

Development of sea ranching in the Yellow Sea

Chang Gil Kim*, Byung Gyun Kim, Yong Suk Choi, Kee Chae Cho

West Sea Fisheries Research Institute, National Fisheries Research & Development Institute, Eulwang, Incheon 400-420, Republic of Korea.
Email: cg0925@nfrdi.go.kr

This study describes the project of sea (marine) ranching in the western coastal waters of Korea in the yellow Sea. Marine ranching projects in Korea began in 1998, and four of five projects are currently progressing (Fig. 1). They will be promoted for a period of 8-11 years under the cultivation-based fishery rearing law, with a total funding commitment of approximately $128 million (US). One of these projects, the Taean marine ranching project in the Yellow Sea began in 2002 with a projected funding commitment of $ 33.7 million (US) over a period of 11- year.

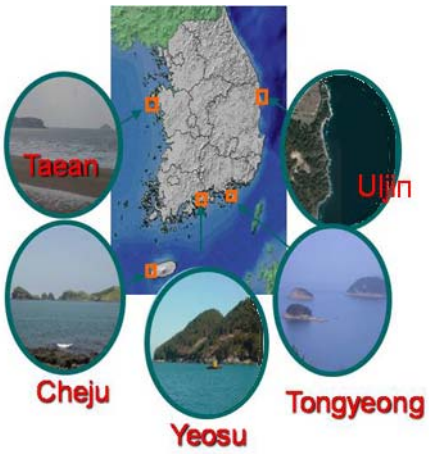


Fig. 1. Locations of marine ranching areas in Korea.

Marine ranching is a type of fishing industry where the seed of target fish species are released and harvested when required, like in terrestrial farming when livestock are put out to graze on pasture land to be later collected for food. Similarly, in this marine ranching project, hatchery raised fish species are released into a sea area where the environment has been enhanced through the deployment of artificial reefs, the area is then continuously managed to build up fishing productivity through sustainable fishing. Although the terminology of the marine ranching was introduced from Japan, the meaning differs between Korea and Japan. In Japan, marine ranching implies the technique of taming fish by acoustics. The technique is based upon a conditioned reflex of fish where the fish habituated to expect food at certain times. When fry or young fish are released, they are inclined to easily be preyed upon by predators. This conditioning technique was developed to decrease losses associated with predation.

In general, two strategies are used in marine ranching. One is through enhancing the

resources potential by increasing the primary productivity in the given waters or through habitat enhancement using artificial reefs, so as to promote the regeneration of resident fish population. The other is the resource-added type using artificially produced the seed of the resident fish populations and releasing them. Using this method, it's possible to increase the resources over a shorter period. For the marine ranching project in Taean coastal waters, a mix of both type was adapted. The former is a technology to enhance the reproductivity of resources from spawning to juvenile and adult fish by improving the current food chain system artificially at Taean coastal area in the Yellow Sea. It is achieved by both increasing the feed organism needed for the fish and creating spawning ground & nursery ground. The latter is a technology to increase the resources for a short period by releasing the seed of target fish species. Rockfish and flounder, which are widely used as aquaculture species in Korea, were selected as the target fish species for marine ranching in Taean's coastal waters.

To develop artificial reef grounds in Taean marine ranching waters, various surveys have been conducted. Data are analyzed from both fishing grounds and mariculture areas. According to the former's results, natural reefs exist diversely in Taean marine ranching waters, and sediment composes chiefly of muddy sand. Primary and secondary productivity is relatively rich, and spawning and nursery grounds lie scattered. Number of fish species caught in 2004 were 36. Of them, 15 fish species were associated with artificial reefs, 21 with other habitats. Currents are relatively strong, and scouring and accretion around reefs tend to be caused by bed-load or suspended load. As a result of this sediment the number of seaweed species decrease from a depth of 2 m. Also, the sediment particles carried by the strong currents are apt to collide with the attachment surfaces for seaweeds resulting in low attachment of seaweeds and low growth.

Based upon these results, both fishing and mariculture grounds are building up over an area of 5,000 ha in Taean coastal waters in the Yellow Sea. Artificial reefs for fishing grounds are installed to enhance the existing fishing grounds, either natural or artificial, rather than through the creation of new fishing grounds. The shapes of artificial reefs vary according to their objective; some are made a frame structure which contains over 80 % void space (Fig. 2). On the other hand, artificial reefs designed for shellfish like abalone have a structure with increased surface area that provides for both the attachment of seaweed and crevices for the refuge of shellfish. Up until 2007, approximately 10,009 m^3 of artificial reefs were installed and 780,000 fish were stocked at Taean marine ranching area in the Yellow Sea. The main target fish species released are *Paralichthy olivaceus* and *Sebastes schlegeli*.

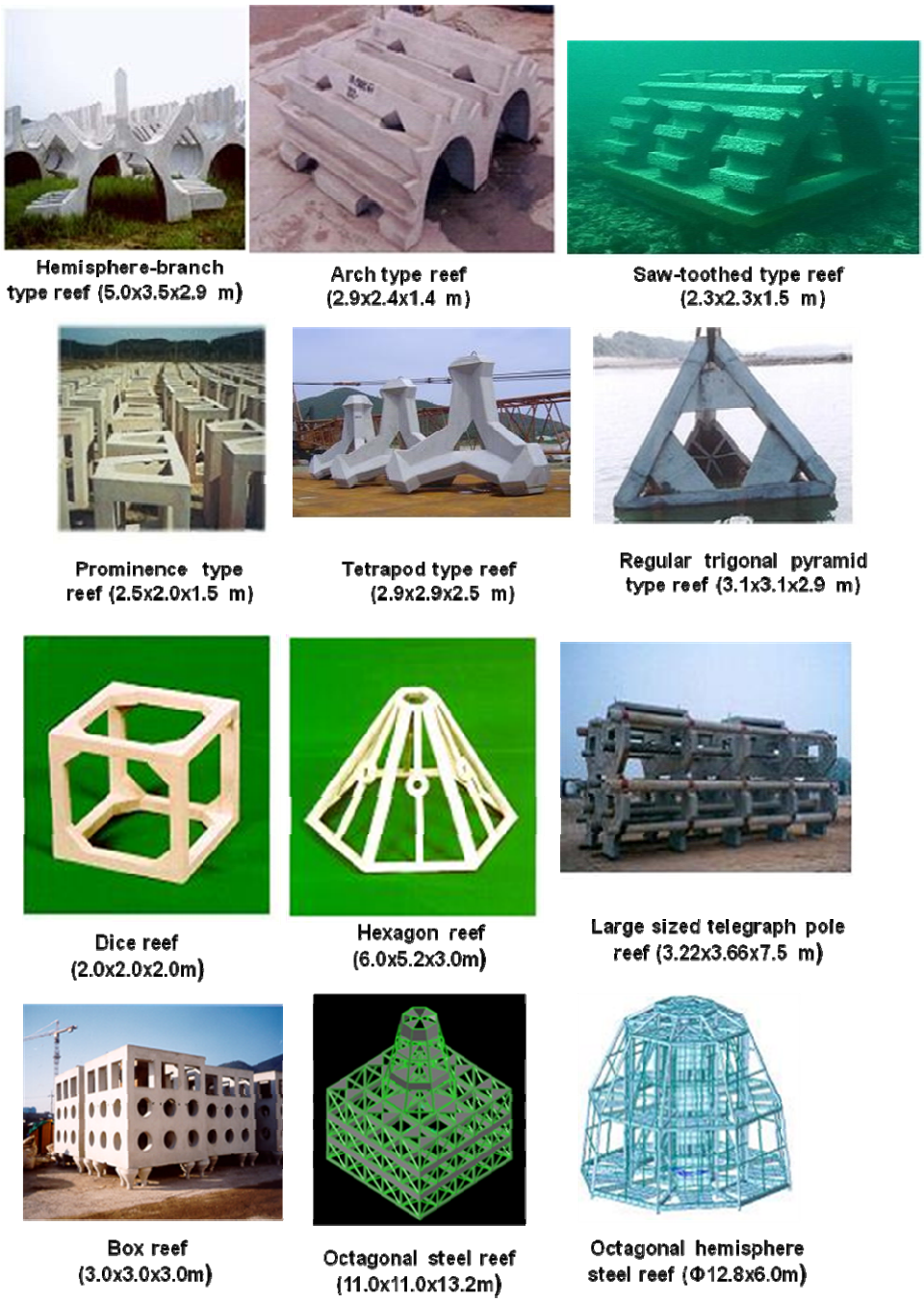


Fig. 2. Popular types of artificial reefs used in Korea. More than 50 reef designs are installed in Korea.

The progress of offshore longline culture in Sungo Bay

Jihong Zhang, Jianguang Fang, Zhanhui Qi, Wei Wang, Zengjie Jiang

Department of Mariculture Ecology and Carrying Capacity Laboratory, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071. P. R. China.
E-mail: zhangjh@ysfri.ac.cn

Culture of finfish, shellfish, and seaweeds in offshore waters is now technically feasible and is becoming one of the new frontiers of marine aquaculture production. Despite considerable interest in its potential, offshore aquaculture remains in the early stages of development. Moving to the offshore area would be a way to overcome the conflicts of space allotment and utilization in coastal waters and reducing the environmental impact of the mariculture industry. Currently, most of the emphasis worldwide is on the offshore farming of finfish. However, to date, there is very limited experience with offshore shellfish aquaculture. In this paper, the progress of offshore longline culture in Sungo Bay China is briefly discussed. The potential of suspended longline mariculture of scallop *Chlamys farreri* in offshore areas are evaluated. Growth and survival of the scallop *C. farreri*, was compared in inshore and offshore culture areas of Sungo Bay, China. Lantern nets were used in the scallop suspended culture from May 2007 to March 2008 at three different initial densities (20, 30 and 40 individuals/disk). Shell height and tissue weight were estimated monthly. Survival rate, muscle weight and chemical composition of scallops were measured at the end of this experiment. To account for growth variation, water quality data (e.g. temperature, salinity, current speed, chlorophyll a and total particulate materials) were collected by in situ instruments and direct water sampling every month. The results showed that after 10 months cultivation, the scallop *Chlamys farreri* had reached the commercial size. Average survival rates for the same initial densities were lower at inshore area than those at offshore area, and had negative relationship with the initial densities. Scallops at offshore area with initial densities of 20 and 30 individuals had significant higher shell height, tissue weights and muscle weight in the end than other groups (ANOVA, $p<0.05$). Tissue weight increased rapidly from May to September at both inshore and offshore area, because at that time the temperatures were optimal and phytoplankton was abundant. Scallops had a second growth peak during October to November in the offshore area, the average growth rate was 7.39 ± 1.12 mg d^{-1}, which was 2.4 times higher than those cultured in the inshore area. The parameters of the von Bertalanffy growth function were estimated, simulation results showed the asymptotic shell height (H_∞) of OS-30 group was about 100 mm, significantly higher than that of IS-30 and IS-40 groups (65.76 and 68.9 mm respectively). A tight coupling between chlorophyll a and scallop tissue growth are evident at inshore culture groups, suggesting that food limitation is characteristic of inshore areas. Whereas, scallops in the offshore area had higher growth and survival rates, which may be due to the increased food supply resulting

from the higher current speeds. The results indicated that scallop *C. farreri* is a potential species that could be cultured at offshore area.

Key words: scallop *chlamys farreri*; suspended longline mariculture; offshore; Sungo Bay

Nutrient budget in marine fish cage culture system and Integrated Multi-Trophic Aquaculture (IMTA) scheme

Jiang Zengjie, Fang Jianguang, Zhang Jihong
Yellow Sea Fisheries Research Institute, CAFS, Qingdao, 266071
Email: jiangzj@ysfri.ac.cn

Aquaculture has grown rapidly in the past two decades all over the world. Cage culture, the practice of farming of aquatics in cages and nets, ranks amongst the fastest growing industries in the world. In China, marine fish cage farming has proved to be a quite promising productive sector of industrial economy and has already been became the main fish culture method in coastal zone. Following the rapid development and application of marine fish cage culture, the large amounts of organic wastes, uneaten food, faeces, and excretory products deriving from fish farm activities has been systematically accused of being a potential source of serious impacts to the aquatic environment.

A case study was carried out from January 2007 to November 2007 at the farm site in Nansha bay, Ningbo city, China, to estimate the balance of nitrogen and phosphorus during cage culture of fish. In Nasha Bay there were total 4000 polyethylene fish cages in use with dimensions 3m×3m×6m. Sea bass *Lateolabrax japonicus* and large yellow croaker *Pseudosciaena crocea* were the main culture species. Based on the results of the investigation and monitoring, the balance of nitrogen and phosphorus had been estimated (Fig.1, 2). Results showed that the nitrogen and phosphorus content of the harvested adult fish was only 18.8% and 12.4% of the contents of nitrogen and phosphorus in trash-fish diet and the stocked fry, suggesting that more than 81% of nitrogen and 87% of phosphorus in fed trash fish became marine pollutants.

In order to reduce those effects, a fish-shellfish-seaweed model was built based on the integrated multi-trophic aquaculture (IMTA) theory. *Ostrea plicatula* and *Laminaria/Gracilaria* were selected as the component species of shellfish and seaweed respectively in this system. And the best layout and proportion of different components was also taken into account.

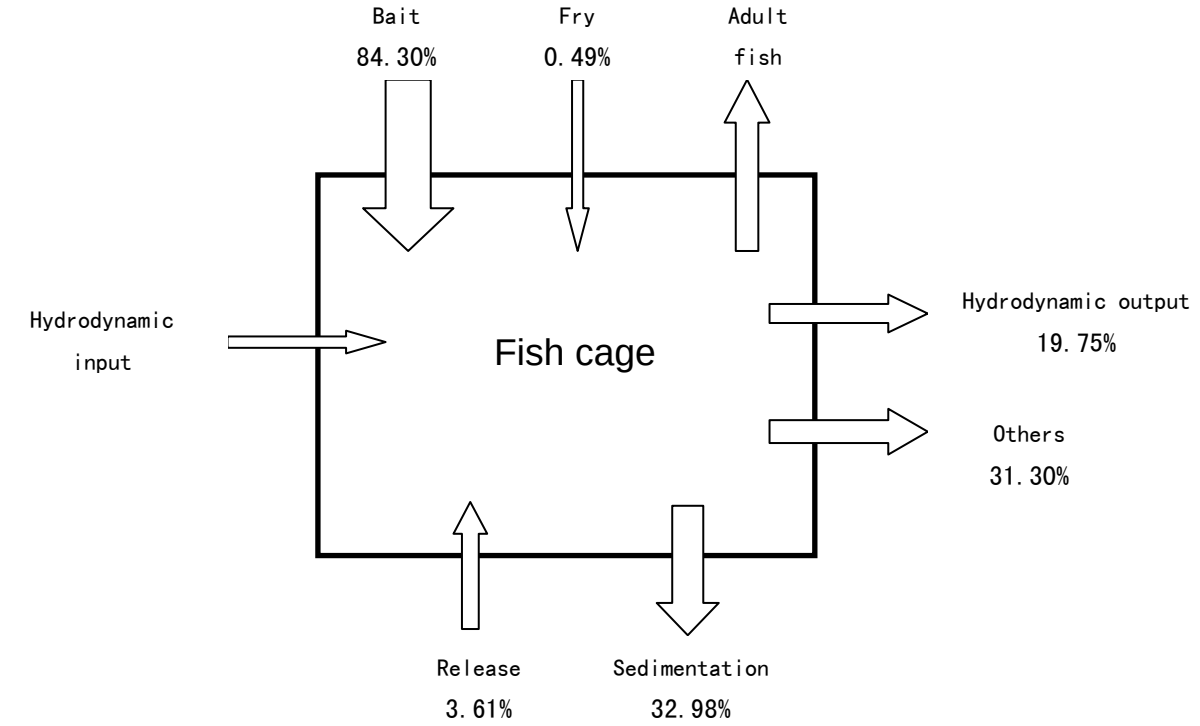


Figure 1: Nitrogen budget in marine fish cage culture system

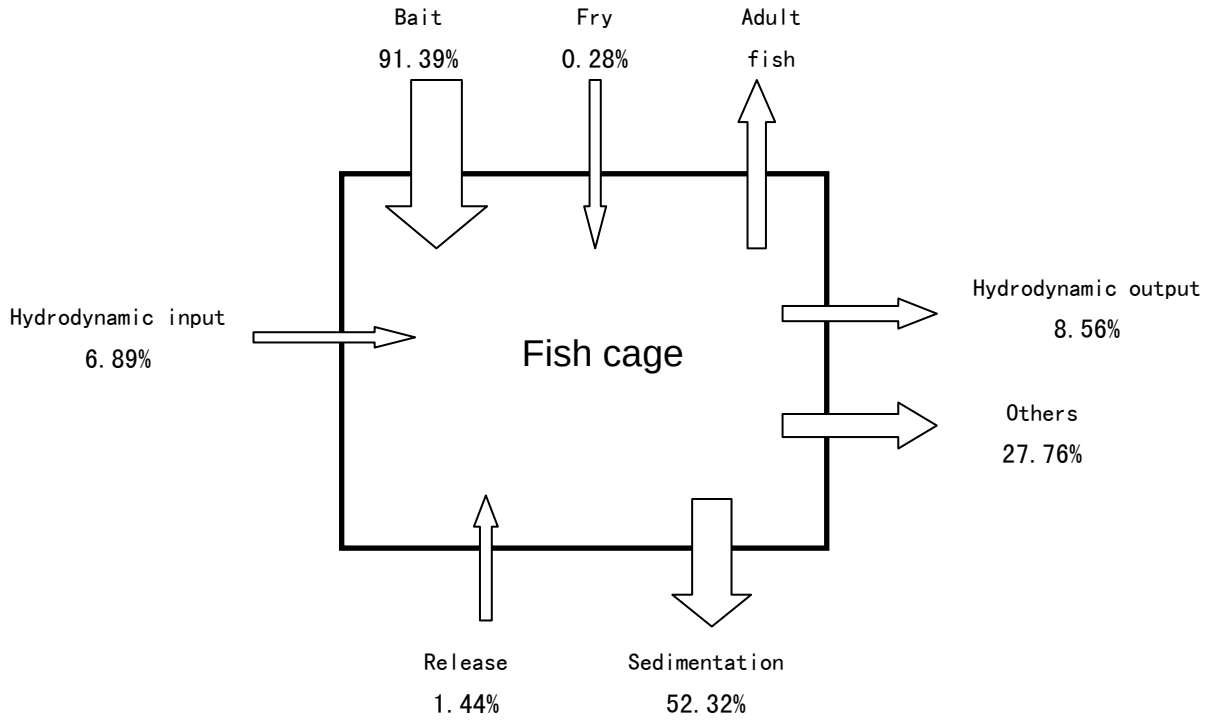


Figure 2: Phosphorus budget in marine fish cage culture system

Application of earlier seedling nursing for the large scale production of Sargassum thunbergii in northern coast of China

LI Mei-zhen, ZHAN Dong-mei and DING Gang
(Shandong Mariculture Institute, Qingdao, China 266002)
Email: li-meizhen@163.com

Sargassum thunbergii is a species of brown (Phaeophyta) macroalgae distributed widely in China Sea, which is of great economic and ecological value, and has potential of exploitation in the domains of medicine and mariculture as well as chemical and food industries. In recent years with the rapid development of abalone and sea cucumber culture, the wild resources of *S. thunbergii* has been over-exploited as high-grade feedstuff, leading to its gradual decline, and currently there is not sufficient supply to face the demand from various fields. Currently several institutes have started research on artificial breeding of *S. thunbergii* seedlings, but as yet some unsolved problems such as the shedding of small-sized seedlings after deployment in the sea, have limited its large-scale breeding and cultivation.

Table 1: Influences of seedling collecting times on the growth of thallus

group	seedling collecting times	parental thalli come from	Egg quality, rate of attachment (%)	times (d)	Growth of thallus		
					Rate of seedlings sheding (%)	length (mm)	Density (seedlings /cm^2)
1	30 May	Cultivation in Zhejiang	normal，98	30	5	3.2	110
2	5 June	Cultivation in Zhejiang	normal, 95	30	7	3.0	104
3	15 June	Cultivation in Zhejiang	normal，86	30	15	2.5	77
4	28 June	Native species with artificial maturation	normal，88	30	12	2.5	93
5	10 July	Native species with artificial maturation	normal，95	30	13	2.8	103
6	22 July	Wild species in Shandong	normal，95	30	10	2.8	83
7	28 July	Wild species in Shandong	normal，60	30	40	2.5	30
8	5 Aug	Wild species in Shandong	Few zygote, abnormal	—	—	—	—

To combat this technical shedding problem, an advanced large-size *Sargassum thunbergii* seedling breeding method was developed through advancing the seed collection by 50 days, shortening the time of indoor cultivation and artificial

acceleration of the maturation of parental thalli. This method should be used in combination with a set of off-shore culturing and managing measures. It is applicable for the northern sea area of China and was evidenced by productive breeding trials for three continuous years. About 3.3737 millions of seedlings with vigor and of uniform size have been bred with this method, which grew into the vertical young thalli (4-6cm in length) with 3-4 branches after being cultured off-shore for more than three months. This method overcomes the difficulty of breeding large-size seedlings met in previous *S. thunbergii* seedling breeding trials, and will certainly facilitate the large scale seedling breeding and cultivation of *S. thunbergii*.

Plate I. Observation of growth and development of artificial seedlings of *S. thunbergii*

A breeding for 40 days, 2~3 new-born leaves
B breeding for 50 days, 4~5 new-born leaves
C breeding for 60 days, 6~8 new-born leaves
D breeding for 65 days, upright branch sprouting
E breeding for 70 days, upright branch reached 0.5 cm
F breeding for 80 days, upright branch reached 1~2 cm
G breeding for 100 days, upright branch reached 3cm
H breeding for over 100 days, upright branch reached 4~6 cm

Aquaculture of the seaweeds in China and the usage for reducing marine eutrop hication

Guang-Ce WANG
Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
Email: gcwang@ms.qdio.ac.cn

The aquaculture of seaweeds in China and the reduction of marine eutrophication with seaweed aquaculture are introduced in this paper with the history of seaweed cultivation in China. The two most important economic seaweeds, *Laminaria japonica* and *Undaria pinnatifida,* were introduced into China in 20 century, and triggered both the seaweed aquaculture and the marine aquaculture in China. The techniques for the two seaweeds are described in detail, which include 5 parts: spore collection and indoor cultivation of sporeling, sporeling transplantation, setting up cultivation raft in the field, cultivation management in the field, and harvesting. The second algal aquaculture industry which was developed in China was *Porphyra* cultivation. *Porphyra* is different from *Laminaria* since *Porphya* has a very special character, being able to survive desiccation. Thus, there 3 ways for *Porphya* aquaculture, fixed pillars, semi floating method and fully floating. The third algal cultivation industry is the *Gracilaria* which is appreciated as a food and used as a feed for culturing marine animals. The most important use of *Gracilaria*, however, is the production of agar. It is known that the finfish aquaculture results in the production of solid wastes (uneaten food, faeces) and dissolved metabolic wastes (CO2, NH4, PO4) , the heavy eutrophication will greatly decrease the production of marine animals such as finfish and scallop, so measures for the integrated cultivation of animal and seaweeds are strongly suggested.

Key words: *Laminaria japonica*, *Undaria pinnatifida*, *Porphyra*, *Gracilaria*, seaweed aquaculture, reduction of marine eutrophication

Limited Water Exchange Shrimp Culture Technology (in Korea)

Jong Sheek Kim*, In Kwon Jang, Hyung Chul Seo, Yeong Rok Cho and Bong Rae Kim

West Sea Mariculture Research Center, National Fisheries Research & Development Institute
Taean, Chungnam, Republic of Korea
Email: philllip@nfrdi.go.kr

There are many shrimp farms using semi-intensive or intensive culture methods on the Yellow Sea coast of Korea. Water exchange is a common practice in semi-intensive and intensive shrimp culture. The rationale for water exchange is to improve pond dissolved oxygen concentrations and to flush out nutrients before they reach toxic levels. The possible introduction with incoming water of harmful pathogens, and the release of nutrient rich wastewater into receiving seas are both issues of particular concern. The shrimp farming industry will be required to meet tougher standards for effluent water release in the near future.

The super-intensive, limited water exchange shrimp culture based on suspended bio-floc communities in outdoor lined ponds and greenhouse enclosed raceway systems can be considered environmentally friendly in that containment of water within the system prevent potential spread of disease between the wild population and cultured animals and avoid nutrient rich waste from polluting coastal waters during the production period.

Strategies were demonstrated in West Sea Mariculture Research Center (WSMRC) for HDPD-lined pond production of up to 2.7 kg/m^2 of Pacific white-leg shrimp, *Litopenaeus vannamei* with limited water exchange in 2007. This result was as much as ten times compared with the average yield of other semi-intensive shrimp farms in Korea. Although high levels of productivity can be achieved in open lined ponds, production is limited to only one crop a year in temperate locations such as Korea and biosecurity still remains dubious.

During the last few years, researchers have been developing newer production methods which move shrimp production indoors, into greenhouse enclosed raceway systems. Like the recent improvements in pond production, covered raceways operate with no water exchange and benefit from the enhanced natural productivity that this strategy provides. Additionally, covered raceway systems offer an increased level of biosecurity, being an essentially closed system, and the capacity to produce shrimp year-round.

The yields on the commercial shrimp farm that have adopted these newer techniques from WSMRC were 6.7 kg/m^2 per crop in 2007 and 6.9 kg/m^2 per crop in 2008. Shrimp densities were 400 and 500 inds./m^2 with survival rates of 85 and 70% in 2007 and 2008 respectively. These results showed that production of the Pacific white-leg shrimp can be done successfully in lined pond and greenhouse enclosed raceways with limited water exchange. This technology can be conducted in shrimp

farms in Korea currently available as a tool to minimize losses to viral disease outbreaks and to reduce environmental impact by shrimp farming.

However, as a relatively new strategy for shrimp production, there is much still to be learned about the potential biological and economic benefits of producing shrimp in suspended bio-floc based culture system in Korea.

Keywords: Yellow Sea, Limited water exchange, super-intensive shrimp culture, bio-floc, *Litopenaeus vanammei*

Practice of recirculating aquaculture system technology in Korea

In-Bae Kim and Jae-Yoon Jo*
Pukyong National Unviersity, #599-1, Daeyon-dong, Busan, 608-737 Korea
E-mail: jyjo@pknu.ac.kr

In 1979, a pilot scale recirculating fish culture system was constructed on the campus of Pukyong National University (former name was National Fisheries University of Pusan), and this facility has been used for various research. A couple of other units of the recirculating aquaculture system based on the same principle have been set up elsewhere in Korea and they are now under practical fish production or used for educational purposes. This recirculating aquaculture system has been designated as 'Intensive Bio-production Korean System (IBK System) and the description herewith presented is mainly an explanation of this system.

Structural Characteristics of the IBK System

- Employment of only trouble-free components (low sophistication),
- Low pumping head at the pumping station (energy saving),
- Large filter unit (stable and marginal filter capacity)
- Integration of water treatment processes in the same component (simplification)

The system design is very simple and does not employ highly sophisticated parts. The system structurally consists of rearing tanks, small sedimentation tanks, a pumping station, and multiple sections of the biological filter. Open channels connect these components.

The rearing tanks, in use at present, are circular tanks and have dual drains, one for the main recirculation of the water in the system, and the other one for the quick separation of solid waste materials, which have been produced in the rearing tank.
Pumps to move the water are located at one place, and multiple pumps are installed to prevent any loss of water movement in case of mechanical failure of any pump. Laymen can operate the system because the routine work is quite simple and most work is manually operated, thus minimizing any damage caused by instrumental failure during the operation.

Micro Bubbles for Aquaculture Water Reuse System

JUN, Je Cheon[1], KANG Hee Woong[1] and KIM, Tschung il[2]
[1]West Sea Fisheries Research institute, NFRDI. Email: boojada@nfrdi.go.kr
[2] Seoul National University

1. Definition and Applications of Micro Bubbles

Micro bubbles (sized tens of micrometer) sometimes cause decompression sickness for divers and it had also resulted in the catastrophic collapse of Glen Canyon Dam in the United States.

In the 1900's, micro bubbles started to be used for water treatment technology. This technology combines micro bubbles with pollutants in water, pollutants attach to the micro bubble and float to the water surface facilitating their removal. The process is so effective that their application has become expanded using varying bubble sizes. In a test, 95% of micro algae and suspended particles (over 10 ㎛) was removed using this technology. Another application is micro bubble diffusing. By diffusing micro bubbles in pollutants which are settled on the bottom of water, the pollutant particles are combined with the bubbles and go up on the surface before treatment. A test shows a high removal rate of 77 %. The third application is to clear pollutants on land. In cleanup efforts after the oil spill accident in Taean, on the west coast of the Korean peninsula, micro bubbles were used to treat the oil pollutants, showing 97.5% of clearing rate. Finally, they were also proved to be effective on pathogens like E. coli. Though the mechanism is not identified, micro bubbles were 99% effective on removing bacillus with 20 minutes of diffusing.

Based on the successful applications described above, micro bubbles can be applied to aquaculture technology. Moreover micro bubbles can boost DO concentration in water up to 15-20 ㎎/L. It shows that micro bubbles can minimize the amount of water for an aquaculture tank and the amount of discarded water when they are applied in aquaculture water reuse system. It can be said that micro bubbles can contribute to reducing energy consumption for water supply and minimizing environmental pollution, leading to the "Low Carbon, Green Growth", one of key policy goals of the Korean government.

2. Lab Test on Aquaculture Water Reuse System Applied by Micro Bubbles.

The researcher cultured tongue sole (*Cynoglossus semilaevis*) in a round PP tank (size: 19.6 ㎡, depth: 80 ㎝) of water reuse system with micro bubbles at a density of 5.1 units/㎡ for 9 months from June 4, 2008 to March, 2009.

2.1 Conditions

Water temperature : 12.1-28.5℃ (average: 20.6±4.8℃); Salinity : 20.0~37.0 (average: 27.2±4.1); DO : 5.09-18.9 ㎎/L (average: 9.01±2.48 ㎎/L); pH : 5.40~8.60 (average: 6.71±0.72); NH_4-N : 0-1.50 ㎎/L; and NO_2-N : 0.2-10.0 ㎎/L.

2.2 Results

At the start of the test, total length(TL) was 12.6±1.5 ㎝ and average weight(AW) was 9.6g.

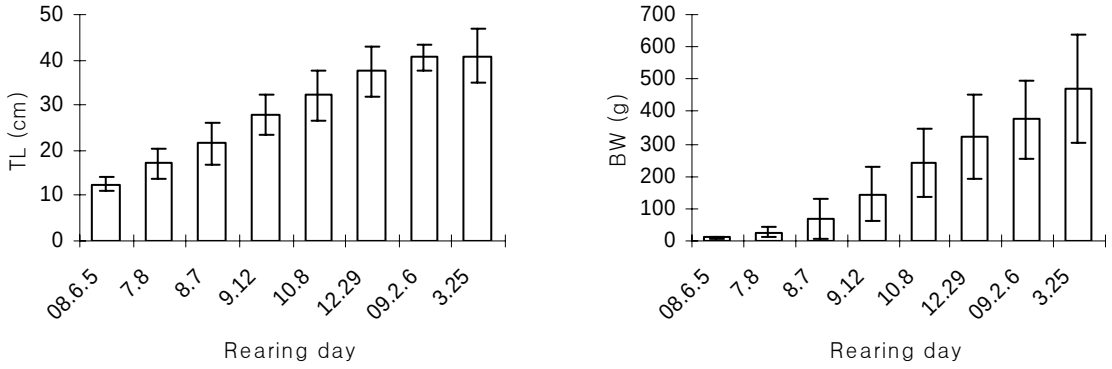
3 months from the start, TL was 28.0±4.5 ㎝ and AW was 125.5g.

6 months from the start, TL was 37.5±5.6 ㎝ and AW was 322.0g.

8 months from the start, TL was 40.5±3.0 ㎝ and AW was 375.8g.

In March 2009 or, 9 months from the start, TL was 36.0~46.6 ㎝ and weight was 252.0-642.0g with 75% of survival rate (Fig. 1). The test indicates that it is possible to grow tongue soles to a marketable size with only 1 year of farming in aquaculture water reuse system. Selective farming of female tongue soles which grow 3 times faster than male stock is expected to produce better results.

Fig 1. Changes of total length (left) and body weight (light) of *Cynoglossus semilaevis* in *a*quaculture water reuse system during culture period.



To develop a sustainable sea cucumber aquaculture industry in China

SUN, Huiling
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences
sunhl@ysfri.ac.cn

Sea cucumbers are important economically aquaculture species in Asian countries, especially China. There are about 20 species considered as traditional tonic products, and *Apostichopus japonicus* is of the highest commercial value among them. Culture of sea cucumber has developed rapidly in recent years and substantially contributes to Chinese aquaculture industry with a total production over 77000 tons (fresh weight) in 2007. The cultivation area covers from the northern Liaoning Province to Hainan Island in the southern China. In Shandong province, the aquaculture area reached 29000 ha, output over 54000 tons and valued at 10.8 billion Yuan (RMB) respectively in 2007.

The important cultivation methods are pond culture and sea ranching in inshore waters. Since 1990's, farming sea cucumber in abandoned shrimp pond is common in Northern China. Sea cucumber mainly feeds on natural foods, so the negative impacts of its aquaculture to eco-system are minimal. In recent years, environment-harmony and/or eco-system safety have been paid much more attention in aquaculture practice in China, that's why stock enhancement and/or integrated multi-trophic aquaculture (IMTA) are strongly promoted for the sustainable development of sea cucumber aquaculture industry in China.

The sustainable development of sea cucumber industry depends upon systematic researches on farming technologies and basic theories. Nowadays, research is focused on high-quality seed rearing, disease prevention and control, artificial feed formulation and process technology. Of special note is a new project to improve genetically the growth performance of the sea cucumber. The aims are to select new varieties with faster growth rates and resistance to stress from environment change. Tolerance to higher temperature in summer also is an important trait for selection. Some theoretical research is also in progress that covers nutrition composition, basal biology, physiological ecology, ecological immunology.

Progress in Aquaculture and Breeding of Scallop in China

Jingjie Hu and Zhenmin Bao*
*Key Laboratory of Marine Genetics and Breeding (Ocean University of China), Ministry of education. Ocean University of China, 5 Yushan Road, Qingdao, 266003, People's Republic of China
Email: zmbao@ouc.edu.cn,

Scallop farming in China started in the late 1970s. With the successful production of hatchery seedling, scallop aquaculture developed rapidly. Until the mid-1990s, the farming area of scallop had expanded to more than 30,000 ha. and the yield was about 1 million tons per year. During that period, referred to as the golden age of scallop aquaculture in China, the major species was the native Zhikong scallop *Chlamys farreri* (more than 80%) cultured along the coast of Yellow sea and Bohai sea (Shandong and Liaoning provinces). In southern Fujian and Guangdong provinces, there was small scale farming of *C. nobilis*. The bulk of hatchery produced *C. farreri*, *Argopecten irradians* and some *Patinopecten yessoensis* scallop spat were reared using lantern cage techniques. Whereas, *P. yessoensis* spat was bottom seeded, especially in the Dalian area.

Due to the rapid expansion of scallop farming, the aquaculture capacity, disease prevention and treatment, and development of superior stocks has become essential for sustainable growth of scallop aquaculture. From 1996 scallop farming suffered from serious diseases, and the output dropped to approximately 300 thousand tons per year by the end of 1990's, devastating the scallop farming industry. In order to recover the scallop aquaculture, many efforts have been made in China. The bay scallop, *A. irradian*s, was first introduced from the United States in 1982, followed by the introduction of several other foreign species. Now, bay scallop farming has become one of the most successful aquaculture species in China and contributes about 70% of total production. Also, *P. yessoensis* bottom seeding and stock enhancement have been successfully carried out in Dalian area and spread fast. At present, the major aquaculture species *C. farreri, A. irradians and P. yessoensis* accounts for 15%, 70% and 10% of the total production respectively. According to the statistics for 2005, the annual output of scallop reached 1,030,000 tons and farming area was 170,000 ha, indicating the accelerated restoration of scallop aquaculture. The coast along the Yellow sea and Bohai Sea is still the main farming area.

In recent years, studies were conducted on aquaculture capacity, polymariculture, disease pathology and epidemiology, amongst others. The genetic improvement for productive traits has been the focus of practical interest of the scallop farming industry. Selective breeding of *C. farreri* and *A. irradiant* began in late 1990s and great achievements were obtained. The BLUP breeding value estimation technique was carried out in scallop selection. Two new varieties "Penglaihong" (*C. farreri*) and "Zhongkehong" (*A. irradians*) exhibit outstanding characters with fast growth and stress resistance in the farming. Some molecular biological techniques，such as MAS, are applied to scallop breeding. Three genetic linkage maps of *C. farreri* based on AFLP markers were constructed, and SSR and SNP linkage maps are now under construction; *C. farreri* cDNA library, BAC library and fosmid library have been completed and a large amount of ESTs were released; High-throughput gene and SNP discovery is being carried out using 454 pyrosequencing and SOLiD sequencing. Some important functional genes have been cloned and characterized. These studies offered a solid foundation for further genetic breeding in scallop aquaculture.

Keyworlds：Scallop, Aquaculture, Genetics, Breeding, China

Session 2: Genetic advances that improve productivity

Exploitation and application of DNA markers in the breeding program of Chines e fleshy shrimp Fenneropenaeus chinensis

Jie Kong *, Xian-hong Meng, Jian Li, Qing-yin Wang
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences,
Nanjing Road 106, Qingdao, 266071, People's Republic of China
Email: kongjie@sina.com

The fleshy shrimp, *Fenneropenaeus chinensis*, used to be of economic importance for Chinese farming industries in the past. The break out of white spot syndrome viral (WSSV) disease destroyed a large number of shrimp farms and almost caused the collapse of the whole shrimp industry in China in the early 1990s. A breeding program was initiated to improve the performance of shrimp and a number of molecular markers were employed to facilitate the breeding program.

Random amplified polymorphism of DNA (RAPD) and simple sequence repeats (SSR) were used to evaluate the genetic variation of *F. chinensis* natural populations. The investigation results of these two markers are identical and support the conclusion that *F. chinensis* are composed of three natural populations in different geographical areas: the Bohai and Yellow Seas along the China coast, the west coast of Korea peninsula, and the south coast of Korea peninsula, which is of critical importance for the construction of base population in the breeding program.

SSR markers were aslo used for pedigree tracing to avoid inbreeding in the program. The simulation results showed that four loci were required to assign 95% and five loci to assign 97% of the progeny. A triplex PCR system was set up and the probabilities of paternity exclusion was 0.968, and the discrimination power was 0.999. Fig. 1 presents the amplification results of the triplex PCR in the parentage 35191.

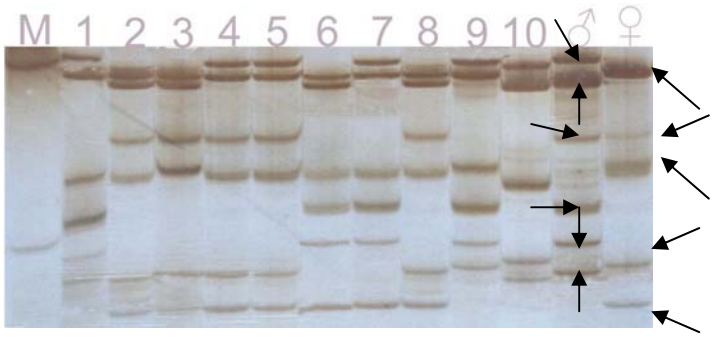


Fig. 1. Amplification results of the triplex PCR in the parentage 35191. M was the DL2000Marker, and the arrows showed the 500 bp (top) and 250 bp (bottom) . For male and female parents, the arrows from top to bottom showed the amplified bands of RS1101, RS0683 and H081 in turn.

RAPD, SSR and amplified fragment length polymorphism (AFLP) were used for genetic linkage map construction. A total of 52 microsatellite loci, 94 RAPD and 4530 AFLP markers were genotyped in a F_2 family, including parents and 100 progenies. The female framework map was composed of 231 markers and male framework map of 204 markers in 44 linkage groups. Fifty-nine putatively QTLs (LOD>2.5) of growth traits were located (Fig 2, 3) in the map and 14 major QTLs can explain 11.5–35.6% phenotypic variation.

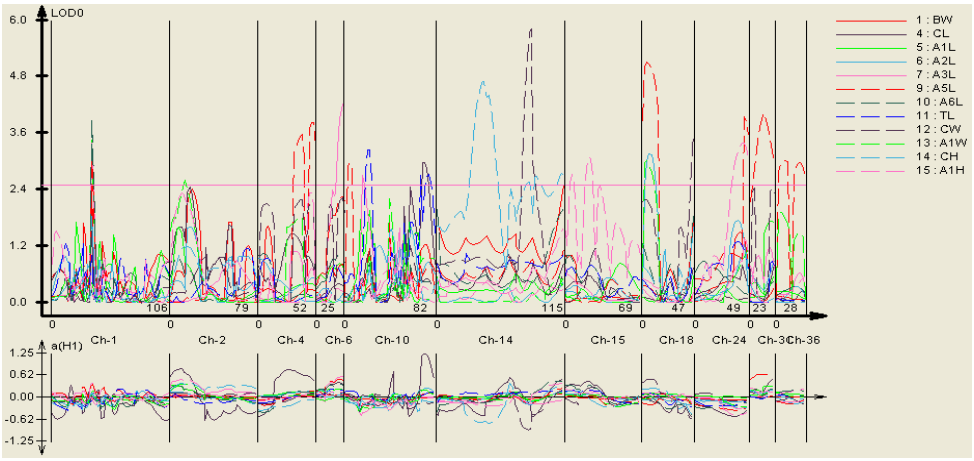


Fig. 2 Growth-related QTL detected on the 1, 2, 4, 6, 10, 14, 15, 18, 24, 31 and 36 linkage group of female

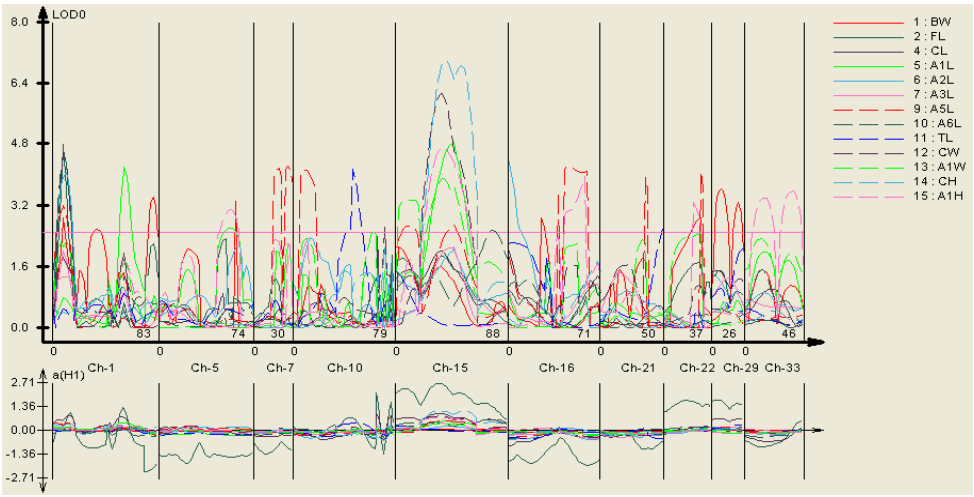


Fig. 3 Growth-related QTL detected on the 1, 5, 7, 10, 15, 16, 21, 22, 29 and 33 linkage group of male

Genetic breeding for shrimp is becoming more and more important, especially for selection of traits relating to disease-resistance. Because of the inefficiency of traditional breeding technology, DNA marker-assistant selection is believed to be a promising way to select desirable traits fast and efficiently.

Current status and future of selective breeding in Korea

Kyung-Kil Kim,[1] Hyun chul Kim[2], Jae-koo Noh[2], Jong-hyun Kim[1], Jeong-Ho Lee[2], Woo-jin Kim[1], and Jeong-In Myeong[1]
Biotechnology Research Division [1], Genetics & Breeding Research Center[2],
National Fisheries Research & Development Institute (NFRDI), Republic of Korea
Email: k48722@nfrdi.go.kr

Animal breeding theory has only recently been applied to fish culture, and is not yet widely practiced. Research in quantitative genetics of fish is mostly restricted salmonids (Norway, North America and France) and carp (Israel and U.S.S.R). The genetic improvement obtained in selection programs for fish and shellfish is remarkable and much higher than what has been achieved in territorial farm animals. Selection response is usually higher in fish and shellfish than in farm animals.

The ultimate goal of any genetic improvement programme is to increase biological productivity. Selection for increased growth has been used to indirectly improve feed efficiency in fish. Results showed significantly higher relative feed intake, growth and feed efficiency in offspring from Atlantic salmon selected for increased growth for five generations (Selected line)

Only a small number of broodstock are necessary to breed enough offspring for the next generation. As a result, inbreeding depression (and along with it disease and poor performance) can be accumulated rapidly. Inbreeding depression in fish is manifested in terms of poor growth, viability and survival and presence of abnormalities. The breeding regimen of these fish thus should be designed so as to avoid possible sib mating, that is unrelated pairs should be selected and mated to minimize inbreeding.

In Korea, olive flounder, *Paralichthys olivaceus* is a commercially important aquaculture species. Production ratio of olive flounder is about half of total farmed marine fish production. Thus, genetically based improvements in performance for this species have generated substantial interest among fish farms. NFRDI is carrying out a breeding study based on the genetic variation for an improvement of growth, body shape and disease resistance of olive flounder.

Efficient breeding programs will be crucial to this development, not only to reach production goal but also to reduce product cost, improve disease resistance, improve feed utilization of feed resources and improve product quality.

Genetic breeding studies of sea cucumbers and sea urchins in China

Yaqing Chang
Key Lab of Mariculture, Ministry of Agriculture, Dalian Fisheries University, P. R. China 116023
Email: yqchang@dlfu.edu.cn

Various species of sea urchins and sea cucumbers are consumed in China. As wild stocks decreased since 1980s, research efforts on genetics and breeding developed the technology of echinoderms aquaculture. Consequent echinoderm culture developed significantly, making it a large industry in China.

1. Sea cucumber

Supported by the Chinese national 863 major program, we carried out a research study on interspecies cross-breeding between Chinese and Russian population of sea cucumbers (*Apostichopus japonius*). Chinese sea cucumbers have higher nutrition quality and better environmental adaptability. Russian sea cucumbers, on the other hand, have thicker body wall with six lines of more and longer papillaes. We achieved improvements of traits in hybrid via hybridization among the two populations. Family breeding is another simple and effective breeding method we are studying on. Full-sib families of sea cucumbers were established in our lab. To breed special strains against high temperature and low salinity, we established special strains against high temperature and low salinity.

2. Sea urchin

We investigated growth, development and viability at the larval, juvenile and adult stages of three sea urchin species and their hybrids and achieved improvements of traits related to growth in hybrid F1 via hybridization among the parents. Two batches of full-sib families (52 full-sib families and 161 full-sib families) of sea urchins (*Strongylocentrotus intermedius*) were established using unbalanced nested design (1 ♂ :3 ♀) in October 2006 and November 2007, respectively. After the establishment of F1 families, 72 full-sib F2 families of sea urchins were established for different growth performance using unbalanced nested design in June 2008. The growth performance among families was obviously different. Moreover, Sea urchin phenotypes prediction models were established using ISSR marker technology.

Unusual mortality of edible echinoderms has been reported in China since 2000, which largely reduced the production of economically important echinoderms. Since then, various studies were carried out to deal with the industrial problems of both sea cucumbers and sea urchins in China. Although diseases have been adequately addressed through studies focused on genetics and breeding to improve the quantity and quality of cultured echinoderms, additional efforts should look at making the industry more environmentally friendly.

Key words: sea cucumber, sea urchin, *Apostichopus japonicus*, *Strongylocentrotus intermedius*, genetic breeding, aquaculture

Characterisation of genetic markers and its application in selective breeding of olive flounder

Jeong-Ho Lee*, Jae Koo Noh, Hyun Chul Kim, Choul-Ji Park, Byung Hwa Min, Kyung-Kil Kim, and Jeong-In Myeong
Genetics and Breeding Research Center, National Fisheries Research & Development Institute, Geoje, Gyeongnam, 656-842, Republic of Korea
E-mail: jhlee@nfrdi.go.kr

With the recent progress made in large-scale genome sequencing projects a vast amount of novel data is becoming available. A comparative sequence analysis, exploiting sequence information from various resources, can be used to uncover hidden information, such as genetic variation. With DNA markers, it is theoretically possible to observe and exploit genetic variation in the entire genome. Popular genetic markers in the aquaculture community include allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNP, and EST markers. The application of DNA markers has allowed rapid progress in aquaculture investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, and the construction of high-resolution genetic linkage maps for aquaculture species.

In this study, genetic divergence within and between farmed and wild olive flounder (*Paralichthys olivaceus*) was assessed by means of microsatellite DNA markers. Marked reductions of genetic variability in the farmed olive flounder compared with the wild olive flounder were observed in terms of number of microsatellite alleles. We conclude that, based on the reduced genetic variability observed in the farmed olive flounder, bottleneck effects occurred when the farmed olive flounder was founded. The useful information on the genetic variation in cultured and farmed populations obtained in this study has been applied for genetic improvement by selective breeding, and to design suitable management guidelines for olive flounder.

In addition, we present a strategy that allows rapid identification of candidate SNPs using publicly available EST databases. Single-nucleotide polymorphisms (SNPs) represent a new form of functional marker, particularly when they are derived from expressed sequence tags (ESTs). These polymorphisms can provide a rich source of useful molecular markers in genetic analysis, and can be used as excellent markers for genetic mapping because of their representation of functional genes and potential for high throughput genotyping. In this study, a collection of 9,525 olive flounder ESTs was assembled into contiguous sequences (contigs), and then visually inspected to identify primer pairs capable of amplifying specific alleles. Using the Tm-shift SNP genotyping method and the TaqMan SNP genotyping assay, we have been able to perform genetic association studies aimed at characterizing the genetic factors underlying inherited traits. Finally, family-based association studies are carrying out to examine the genetic association of genotyped SNPs and haplotypes in the olive flounder.

Genetic evaluation for growth traits and survival rate of brine shrimp Artemia si nica

Sheng LUAN
Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences,
Nanjing Road 106, Qingdao, 266071, People's Republic of China
Email: luansheng@ysfri.ac.cn

Artemia sinica is an unique Chinese species in the world, which includes 12 different strains coming from in different places in China. *A. sinica* has the advantages of a small body，short life cycle，being easy to produce a full-sib family. This makes it ideal for a selective breeding program. Necessary genetic improvements should be carried out to avoid inbreeding depression, germplasm depression occurring in the area of *A. sinica*.

Body length data were obtained from the *A. sinica* breeding program at the National Marine genetic breeding center, China. The G0 was formed using a diallel cross experiment involving four wild parental strains. The G1 generation was produced from the mating of G0 individuals, which had breeding values for body length above the population, mean in the actual population. In the G0 and G1 generations, full and half sib families were produced by a nested mating design, in which two dams were mated to the same sire. Half-sibs were produced by moving the sire to the second dams after mating with the first dam. In each generation, after selection, the mating of full-sib and half-sibs was avoided during mate allocation in the plastic breeding bottle. The analyzed body length records were obtained from the full- and half-sib families in the G0 and G1.

A control population was established for the G1 generations using single broodstock pairs from G0. This control broodstock had breeding values around the population mean in each generation. Each family of selected and control population was reared separately for a period ranging from 10 to 12 days at 500ml beaker in two 400L illumination incubators. Then individuals were transferred from the breeding beaker to one 50ml plastic transparent rearing bottle. The larva was cultured separately at the 50ml plastic bottle. The individual were harvested after a growth testing period of 20 days.

Estimate of the realized genetic gain for harvest body lengths from G0 to G1 was obtained from the following linear model;

$$Y_{ijk} = \mu + TS_i + bA_k(TS_i) + P_j + e_{ijk}$$

where Y_{ijk} is the observed harvest body length of the kth individual of the selected or the control population in G1; μ is the overall mean; TS_i is the fixed effect of the ith level of test tank (two levels) by sex (two levels) subclass (i=1,2,3,4); P_j is the fixed effect of the jth population (two levels: control and selected populations); and e_{ijk} is the random residual error of the kth individual. The covariate age at harvest, nested within the TS effect, on harvest body length was significantly different from zero ($P<0.05$) and was therefore included in the model.

An estimate of the realized correlated genetic gain for harvest body length from G0 to G1 was obtained as the difference in the least square means of harvest body length of the selected and control populations in the G1. The genetic gain was expressed as a percentage of the least squares mean of the selected population in the previous generation.

Variance components and heritability estimates of the body length data were analyzed using the *following* mixed model, which in matrix notation can be written as

$$y = Xb + Z_1a + Z_2c + e$$

where, y is a vector of observed body length at harvest; b is a vector of a fixed effect of the generation (two levels) by tank (three levels) by sex (two levels) subclass (with a total of four levels), and including a covariate of age at harvest for each level of the fixed effect; a is a vector of the animal additive genetic effect, $a \sim (0, A\sigma_a^2)$, where A is the additive genetic relationship matrix among all brine shrimps; c is a vector of random common full-sib effects, $c \sim (0, I\sigma_a^2)$; e is a vector of random residual errors, $e\, e \sim (0, I\sigma_a^2)$. X, Z_1, Z_2 are design or incidence matrices assigning harvest body length observations to the levels of b, a and c respectively. Phenotypic variance was calculated as $\sigma_p^2=\sigma_a^2+\sigma_c^2+\sigma_e^2$. Heritability ($h^2$) was calculated as $h^2=\sigma_a^2/\sigma_p^2$, while the common full-sib effect (c^2) was calculated as $c^2=\sigma_c^2/\sigma_p^2$.

BLUP *breeding* values for harvest body length within and across generations were obtained using the estimates of heritability and common full-sib effect across generation. The predicted genetic gain for harvest body length from G0 to G1 was obtained as the difference in the mean predicted breeding values for harvest body length of individuals in the G0 and G1. The genetic gain per generation was expressed as a percentage of the mean body length of the selected population in the previous generation.

Table 1 gives for each generation, population and test environment, the number of brine shrimp stocked, the mean and coefficient of variation for harvest body length and survival from tagging to harvest. The mean harvest body length of the G1 selected population was higher than that of the G0 base population at each test tank. At each test tank in G1, both the mean harvest body length and survival of the selected individual were not high than those of the control brine shrimp.

Table 1 the number of brine shrimp stocked (N), the mean observed harvest body length and survival rate for each generation, population and test tank of *Artemia sinica*

Generation	Population	Tank	N	Harvest body length(um)		Survival(%)
				Mean (um)	CV(%)	
G0	Base	PY0100	805	9417	16.33	73.10
		PY0200	875	9024	20.72	83.44
G1	Selected	PY0100	450	10282	12.54	93.95
		PY0200	467	9716	11.71	94.92
	Control	PY0100	186	9623	13.14	96.38
		PY0200	186	9444	12.75	95.38

For the harvest body length, the realized genetic gains are shown in Table 2. The least squares mean for control and selection population is 9517.53 and 10042.47, respectively. The difference between two populations was 524.95um. The realized genetic gain from generation G0 to G1 was 5.52%.

Table 2 Estimates of realized genetic gain for harvest body length from G0 to G1 generation

Generation	Population	Least squares mean (um)	Realized genetic gain	
			Mean (um)	(%)
G1	Control	9517.53		
	Selection	10042.47	524.94	5.52

Estimates of variance components, heritability and common full-sib effect for harvest body length within and across generations are shown in Table 3. Within generation, heritability estimates (0.48 to 0.68) were of high. Across generation, the heritability estimate was also high (0.32±0.08). The common full-sib effects within each generation were low. Across generations, the common full-sib effect was very moderate (0.12±0.05).

Table 3 Estimates of the additive, common full-sib, residual and phenotypic variances, heritability and common full-sib effect for harvest body length of *Artemia sinica* within and across generation

Generation		Variance components			Heritability	Common environment effect
		σ_c^2	σ_e^2	σ_p^2	h^2	c^2
G0	720306	4582	786108	1511000	0.48±0.24	0.0030±0.11

The role of antioxidant enzymes of the disk abalone (Haliotis discus discus) and t heir transcriptional responses to physical and biological stress

Mahanama De Zoysa, and Jehee Lee*

School of Applied Marine Science, Jeju National University, Republic of Korea

*E-mail: jehee@jejunu.ac.kr

Oxidative stress results when production of reactive oxygen species (ROS) exceeds the capacity of cellular antioxidant defenses to remove these toxic species. Diverse antioxidant system is essential for marine invertebrates to overcome the oxidative stress. The present study investigates the role of antioxidant enzymes of disk abalone under various physical and biological stress factors.

We observed that several antioxidant enzymes such as MnSOD, CuZnSOD, catalase, TPx, SeGPx, PRx-6 and TRx-2 were expressed in abalone, which respond collectively as a classical enzymatic antioxidant defense system. Comparative analysis of expression profiles indicated that catalase, TPx and SeGPx transcripts were significantly up-regulated ($p<0.05$) by all three physical stress conditions namely thermal ($28^{o}C$), salinity (25‰) and hypoxia. Interestingly, all the antioxidant transcripts exhibited significant up-regulation in response to salinity-related stress. Meanwhile, hypoxia caused up-regulation of the MnSOD, catalase, TPx and SeGPx, but not the CuZnSOD and TRx-2 transcripts.

Also, our preliminary results showed that all the antioxidant enzymes were induced after UV-B (312 nm) radiation for 10 min (100 mJ/cm^2) in abalone hemocytes. Further more these antioxidant enzymes were induced under biological stress by viral hemorrhagic septicemia virus (VHSV) infection. The salinity, hypoxia and VHSV treatments increase the transcription of immune response genes such as SOCS-2 and Mx. Based upon these results, we postulate that abalone utilizes the antioxidant and immune defense mechanisms together to overcome oxidative stress.

Key words: Abalone; antioxidant enzymes; biomarker; immune response; oxidative stress

Gene and SNP discovery associated with White Spot Syndrome Virus (WSSV) in Chinese shrimp Fenneropenaeus chinensis via 454 transcriptome sequencing

Xianhong Meng, Jie Kong, Qingyin Wang
Yellow Sea Fisheries Research Institute, CAFS, PR China
Email: mengxh@ysfri.ac.cn

In this study, a high throughput pyro-sequencing approach was applied to sequence the transcritptome of Chinese shrimp *Fenneropenaeus chinensis*. We present the preliminary analysis of assembling, annotation, gene ontology (GO) and single nucleotide polymorphisms (SNP) based on 451,637 expressed sequence tags (EST) that generated from WSSV-sensitive and WSSV-resistant shrimp by Roche 454 GS FLX system.

Large-scale WSSV infection of *F. chinensis* was conducted in Aug. 21, 2008. A total of 1,413 individuals (mean body weight 1.99±0.67g) from 77 full-sib or half-sib families were artificially infected by feeding dead WSSV infected shrimp after two days starvation in a pond. After 28 days (662 hrs) with the water temperature 24.8-27.4℃, there were 59 individuals alive. Candidates for sequencing were selected from the most WSSV-sensitive shrimp (Group S, three earliest moribund individuals) and WSSV-resistant shrimp (Group R, three of survivals). Total RNA was isolated from muscles and hepatopancrease of six individuals respectively. Before sequencing, cDNA of group S and R were labeled with different adaptors.

Table 1. Basic result of cDNA 454 sequencing of *F. chinensis*

ESTs	Group R	Group S
Reads No.(ave len)	268,511 (205 bp)	229,335 (235 bp)
Reads No. after trimming (ave len)	240,871(200 bp)	210,766 (225 bp)
Total bases (bp)	48,231,158	47,352,259
reads assembled in contigs	220,652	195,637
Contigs No.	11,750	11,218
Max length of Contig	3,588 bp	3,919 bp
Mean length of Contigs	321 bp	355 bp
Singletons No.	20,219	15,129
Unigenes No.	31,969	26,347

A total of 268,511 ESTs averaging 205 bp in group R and 229,335 averaging 235 bp in group S were obtained from 454 sequencing runs (Table 1). Of these, 240,871 (mean 200 bp) in group R and 210,766 (mean 225 bp) in group S entered the assembly with CAP3 software after trimming adaptors and low quality bases. In group R, 220,652 ESTs formed 11,750 contigs of average length of 321 bp, the longest contig length was 3,588 bp, and the remaining 20,219 ESTs were retained as singletons, for a total of 31,969 unigenes. In group S, 195,637 ESTs formed 11,218 contigs of average length of 355 bp, the longest contig length was 3,919 bp, and the

remaining 15,129 ESTs were retained as singletons, for a total of 26,347 unigenes.

We use GETORF and GoPipe standalone package to predict genes and analyze gene ontology in *F. chinensis*. More than 99% unigenes can be found ORF, 17.39% (5,536) in group R and 20.72% (5,443) in group S can be annotated (Fig. 1). A total of 2,773 unigenes in group R and 2,692 in group S can be predicted with different function of biological process, molecular function and cellular components. There were 4,979 specific contigs in group R and 4,725 in group S based on comparison of BLASTN (alignment ratio > 35% and *e* value > 1e-10). Of these, 968 with annotation and 427 with GO in group R and 1156 with annotation and 465 with GO in group S. All these specific contigs were considered very meaningful in analyzing the mechanism of WSSV resistance in shrimp.

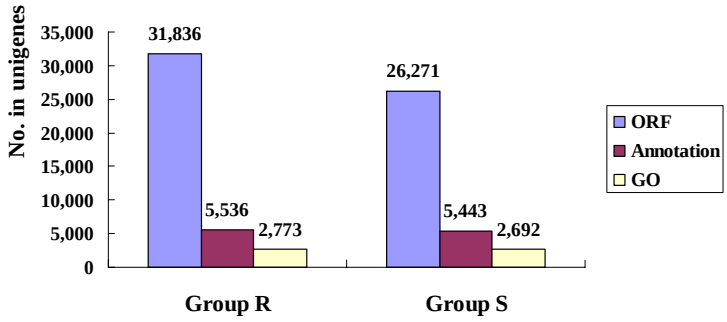


Fig. 1 Annotation and GO analysis in unigenes in *F. chinensis*

A total of 71,724 putative SNPs were detected within 18,560 contigs (unigenes) that were assembled by combination of group R and S. Stringent post-processing reduced this number to 4,241, including same-sense SNPs 2,495, mis-sense 1,704 and nonsense 42. The ratio of transition to transversion (ts/tv) was 1.918. Further experiment need to be conducted to confirm the facticity of putative SNPs. Once discovered, SNPs have a wide variety of applications in biological research, such as construction of high dense genetic linkage map, association analysis of growth or disease-resistance traits and so on. Especially those mis-sense and nonsense types of SNPs may reveal the truth of WSSV resistance in shrimp.

It's interesting that when we BLAST ESTs of group S and R to WSSV whole genome sequence (GenBank accession No. AF332093) respectively, there were as many as 9,402 reads in Group S and only 204 in group R hit to WSSV whole sequence. This result indicated that when shrimp were initially infected by WSSV, large amount of nucleotides were taken usage by virus to assemble its DNA in WSSV-sensitive shrimp. By comparison, in WSSV-resistance shrimp, some unknown mechanism was working to prevent virus replication, resulting some individuals survive long time under the water and food condition of full of virus. We hope based on the high throughput EST sequence, molecular mechanism or genetic markers of WSSV-resistance can be revealed to assist new variety breeding, which would solve the worldwide WSSV disease and decrease the economic loss in near future.

Research on artificial reproductive and genetic diversity of Octopus ocellatus

Jian-min YANG & Wei-jun WANG
Shandong Marine Fisheries Research Institute, Yantai, China 264006
Email: ladderup@126.com

Systematic observations and research were preformed into the physical and biological characteristics of *Octopus ocellatus,* such as the, living and reproductive habits, larval feeding, as well as the reproductive systems of male and female *Octopus ocellatus* by anatomy. Main genital organs were observed and studied by paraffin section, in order to determine developmental phases and time of fertilization. Shape and internal structure of sperm was recorded by scanning and transmission electron microscope (TEM), and embryo development observed by microscope. AFLP molecular marker system of *Octopus ocellatus* was established, in order to analyze their genetic diversity in different geographic populations.

The optimal temperature for *Octopus ocellatus* ranged from 10 to 26 ℃. If it was below 10 ℃, they would reduce food intake. They were mainly benthic and gregarious in sea areas in the temperate zone by north, having a liking for troglodytism and preying clams and little crabs. With adequate shelters, their culture density could be raised to 8 - 10 ones per cubic meter. The appropriate spawning temperature for *Octopus ocellatus* was between 11 to 14 ℃, and shell-dwelling behavior was particularly evident in mating season, when the males had distinct territorial behavior, and frequently fought for mating opportunities.

Food intake of *Octopus ocellatus* was significantly reduced before spawning, and almost none after. The females exhibited egg-caring behavior, and the males died shortly after spawning. Fecundity ranged from 230 to 270 granules, which was in positive correlation with body weight: a female weighing 130g had between 650 to 700 eggs. Average fecundity was 512 eggs. In shelters such as pottery jars, eggs became mature and were spawned in batches. Spawned eggs were appeared similar to cooked rice, many eggs wound together by the handle of the eggs like strings of grapes. The major axis of each egg was 4.5 - 6.3mm, and minor axis was 2.6 - 3.0mm. Spawning rate of *Octopus ocellatus* was comparatively low between 40% and 60%, which was probably related to their physical stamina. Hatchability was around 30%.

The just hatched larvae were fed on artemia, and then copepods and cladocera. After 20 days crabmeat and clam meat, etc. could be used. The juveniles were cannibalistic, perhaps due be lack of shelter and the low palatability of food. High culture density and temperature as well as size discrepancy all induced and aggravated cannibalism.

Anatomical studies showed that the male reproductive system of *Octopus ocellatus*, lying in mantle cavity, was composed of testis, sperm duct, Needham's sac, spermatophoric gland and penis. The Needham's sac could be divided into 3 parts —

sperm mass, cement body and ejaculatory apparatus. Total length of the spermatophore was approximately 30mm, where mature sperm were packed in the sperm mass, which was about 10mm long, 420μm in diameter and in regular spiral. The female reproductive system of *Octopus ocellatus* was simple in structure, including ovary, oviducal duct and oviducal gland.

Paraffin section study showed that the spermatophore was surrounded by a transparent membrane. The oviducal gland was comprised of 3 parts – spermatheca, central gland and peripheral gland. Spermatophore was the storage for mature sperms. Of the group bred in Spring, males reached sexual maturity around October, while eggs of the females became mature in May of the following year. Mature males could mate with females, yet sperms, stored in oviducal gland, did not enter the ovary and eggs remained unfertilized.

Scanning and TEM showed that a mature sperm consisted of head, neck and tail. The head was comprised of the acrosome and nucleus. The neck was mainly made up of basal body. The tail was comprised of the middle piece, the principal piece and the end piece. A sperm was linear with an unobvious head and a spiral acrosome, stretching 600 to 750μm with a diameter of 0.3μm. There was an acrosomal cavity between acrosome and nucleus, the end of which formed sleeve-shaped structure with a microtube inserted into. The basal body was made up of typical 9+2 structure, while the tail of typical 9+9+2 structure. The middle piece was surrounded by mitochondria; the principal piece, the longest, began at the part where mitochondria disappeared; the end piece changed to 9+2 structure.

According to whether the embryo had an eyespot or not, embryonic development of *Octopus ocellatus* could be divided into 2 periods: the early one and the late one. In early period, a reverse from animal polar to the opposite occurred to the embryo in egg membrane. It occurred in the late period as well. It took 35 to 45 days to hatch when water temperature at 19 to 22℃.

By genetic diversity analysis of 4 geographic populations of *Octopus ocellatus* via AFLP, 6 pairs of primers selected were totally amplified to 303 loci, in which the Dalian population (DL) had the highest ratio of polymorphic loci (67.93%), while the Qingdao population (QD) the lowest (62.03%). Shannon diversity index varied in the range of 0.3467 to 0.3893; Nei's index (H) changed in the range of 0.2353 to 0.2617; genetic distance between populations ranges from 0.0497 to 0.085. The ratio of polymorphic loci, Shannon diversity index and Nei's gene diversity index of the Dalian population were all higher than the other three populations. AMOVA analysis showed that 88.28% of the variation of *Octopus ocellatus* was within population, while variation among populations accounted for only 11.72%, which indicated that the genetic variations derived from inside each population. However, there was a certain degree of genetic differentiation among the populations.

Session 3: Advances in disease diagnosis, prevention and control, and new strains

Status of Fish Vaccine Development in Korea

Myoung-Ae Park
Pathology Research Division, National Fisheries Research & Development Institute,
152-1, Haeanro, Gijang-up, Gijang-gun, Busan 619-705, Republic of Korea
Email: mapark@nfrdi.go.kr

Infectious diseases, such as Edwardsiellosis, Streptococcosis and Iridovirus, pose the biggest single threat to aquaculture in Korea.

Vaccines have become one of the major approaches to combat fish diseases in recent years and have made a major contribution to improvements of fish health in aquaculture. Developing fish vaccines could potentially save aquaculture producers money worldwide by preventing this disease. Reduction of disease outbreak has a flow on effect to decreased antibiotic usage, aiding in the retention of the state's "clean green" image. Other advantages are reducing antibiotic use to control this bacterium in culture fish, making a safer, more environmentally friendly consumer product.

There has been much research into the development of effective vaccines, immunostimulants and adjuvants in fish. The introduction of a new generation of both oil- and non-oil adjuvant has greatly improved the efficacy of bacterial vaccines and has resulted in an impressive reduction in mortalities.

The vaccination strategies for the control of viral and bacterial diseases in aquaculture are being studied. For a more efficient disease control, National Fisheries Research and Development Institute (NFRDI) has been conducting research into the development of fish disease vaccine to convert the existing disease treatment system to disease prevention system. NFRDI has developed a Streptococcosis, Edwardsiellosis, Vibriosis, Streptococcosis-Edwardsiellosis mixed inactivated bacterial vaccine for flounder and recombinant protein vaccine against parrot fish iridovirus in 2005. The disease prevention efficiency rate of a developed vaccine against a target disease was estimated to be 70 percent since the start of the application.

Nodavirus and a triple mixed vaccine for Streptococcosis, Edwardsiellosis, and Vibriosis will be developed that can prevent these diseases by NFRDI in 2010.

Turbot iridovirus research in China

Cheng-Yin Shi

Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences,
Qingdao 266071, China
Email: shicy@ysfri.ac.cn

Turbot: Turbot, *Scophthalmus maximus*, was introduced to China in 1992. It has become an important aquaculture species in coastal areas of northern China, especially in the Shandong Peninsula. The annual production value of farmed turbot in China achieved US$400 million in 2006.

VRBS: The 'viral reddish body syndrome' (VRBS) of turbot was found in both juveniles and adults from 2001. The gross signs of diseased turbot were pale gills with local haemorrhages, petechial haemorrhages in fins and fin bases, especially haemorrhages in muscle and skin. Research results showed that the pathogen of VRBS was a new iridovirus which was temporary named as 'turbot reddish body iridovirus' (TRBIV).

Pathology: By light microscope, many enlarged cells were observed in the spleen and kidney of VBRS turbot. Infected cells became hypertrophic and the cytoplasm was homogeneous. Many TRBIV particles were detected by TEM examination in various organs of diseased turbot. The virions were mostly in cells of gill, intestinal submucosa, spleen and basement membranes of capillaries in glomeruli of the diseased fish. The spleen was the major target organ for the virus. In the affected splenic cells, cytoplasmic vacuolation was distinct while ballooning endoplasmic reticula also can be seen. The mitochondria were mild enlarged with disrupted cristae.

Morphology: The virion of TRBIV had an envelope. The viral particles were only present in the cytoplasm of infected cells. Transmission electron microscopy (TEM) showed virions were icosahedral in symmetry, measuring 120 to 130 nm from vertex to vertex and 110 to 116 nm from face to face. Complete virions consisted of three layers: the outer layer was an icosahedral capsid 10 to 14 nm in thickness, the intermediate layer was an 11 to 15 nm thick translucent space and the inner spheroid core was a homogeneously electron-dense nucleoid measuring 64 to 70 nm in diameter.

Genome: The complete genome of TRBIV was sequenced. The TRBIV genome was a linear dsDNA of 110,104 bp in length with a base composition of 54.99% G+C. About 115 open reading frames (ORFs) were identified with coding capacities for polypeptides ranging from 40 to 1,168 amino acids. The analysis of amino acid sequences deduced from above ORFs revealed that 39 of them were significant homology (75-99%) to known genes of other *Megalocystivirus*. The phylogenetic analysis indicated that TRBIV belongs to the genus *Megalocystivirus*, family *Iridoviridae*.

Detection: Based on the sequence of major capsid protein (MCP) gene, a one-step PCR method was developed for TRBIV detection. Viral DNA extracted from 100 ng of spleen or kidney tissues of infected turbot, or ~10^3 copies of virus DNA can be

detected. A nested-PCR based on the sequence of adenosine triphosphatease (ATPase) gene was also optimized to detect TRBIV. The sensitivity of the nested-PCR was 10^3 to 10^4 times higher than that of one-step PCR method. A loop-mediated isothermal amplification (LAMP) method for TRBIV detection was developed recently. The LAMP method can be conducted in 1 h and requires only a simple heating device for incubation. It has great potential for use in the detection of TRBIV in both the laboratory and the farm.

Prevalence: The prevalence of TRBIV was investigated by developed one-step PCR and nested-PCR methods. The results revealed that TRBIV has spread among farmed turbot in China. Turbot iridovirus was also found among farmed turbot in Korea in 2004. Pairwise alignment showed that TRBIV MCP shared 99% amino acid identity with that of turbot iridovirus in Korea. These two isolates should be considered as variants of one iridovirus. This meant that TRBIV prevails not only in China, but also in Korea.

MCP: The MCP of TRBIV was expressed successfully by the *Pichia* expression system. The amount of expressed MCP could reach to 60 μg/ml in the supernatant of recombinant yeast. It was a candidate for the recombinant vaccine of TRBIV. The protein interaction between MCP of TRBIV and splenic proteins of turbot was also investigated by yeast two hybrid system. The protein p2 of turbot was identified and it gave positive signals of protein interaction with MCP of TRBIV. This result suggested that the interaction between these two proteins was one of the molecular mechanism of infection and pathogenesis of TRBIV.

National Clam (Ruditapes philippinarum) Watch Project of Korea; the Methodology and Results

Kwang-Sik*, Hyun-Sung Yang, Bong-Kyu Kim, Hee-Jung Lee, Yanin Limpanont, Kwang-Jae Park[+] and Young-Je Park[++]
School of Applied Marine Science, Cheju National University (* skchoi@cheju.ac.kr)
+West Fisheries Research Institute, NFRDI Korea
++Tidal Flat Research Institute, NFRDI Korea

Introduction

Manila clam *Ruditapes philippinarum* is one of the most important shellfish resources supporting Korean fisheries industry. Incidence of mass mortalities of clams has been reported from major clam beds on the west coast for the past decade. Changes in the environmental quality, over-harvesting, abnormal weather and pathogens such as the *Perkinsus olseni* were reported to be responsible for the mortalities and the declining of clam landings. To understand current status of clams along the Korean coasts, a nation-wide surveillance program has been launched since 2007. The present study reports the program and the survey results.

Materials and methods

Several commercial clam beds on the west and south were selected for the monitoring. On the west, Padori, Sunjae and Hwangdo clam beds were selected for a long-term monitoring program, while other clam beds were visited twice in the spring and fall of 2007. The surveillance program investigates the fatness (i.e., condition index, CI) as a ratio of total tissue weight to the shell dry weight, total body burden of *Perkinsus olseni*, *Vibrio tapetis* infection (brown ring disease, BRD), degree of the conchiolin deposit (CDS) on the inner shell surface, the gametogenic stage (GI) and trematod infection from histology, and quantification of female reproductive efforts using ELISA. Total body burden of *P. olseni* was assessed using Ray's fluid thioglycollate medium assay (RFTM) and Choi's 2M NaOH digestion. For histology, a thin longitudinal section was cut in the middle of the body and fixed in the Davidson's. Quantity of the eggs in individual clam was measuring using rabbit-anti clam egg antibody using ELISA.

Results and conclusion

From January 2007 to September 2008, *P. olseni* infection intensity ranged from 2,000 to 2,700,000 cells/g tissue among the 3 clam beds. Hwangdo clams showed the highest infection intensity, while it was lowest in Padori. *Perkinsus* infection intensity

was higher in fall, indicating that the infection was closely related to the post-spawning condition of clams. Prevalence and infection intensity of BRD monitored from the 3 clam beds were very low; the prevalence was less than 10% in most cases and the intensity remained below 3 in 0-7 infection scales. CI varied from 0.267 to 0.931 annually and highest CI observed in spring just prior to a major spawning (Figure 4). Although the Hwangdo clams exhibited the highest *Perkinsus* infection, CI was also highest among 3 clam beds. Higher CI and *P. olseni* infection observed in Hwangdo was in part explained with the higher biomass of phytoplankton in Hwangdo.

Nation-wide survey conducted in spring and fall of 2007 indicated that spatial distribution of *Perkinsus* infection was closely associated with salinity and environmental contamination. It was noticeable that *Perkinsus* infection was none among clams collected from river mouth areas. Highest *Perkinsus* infection was recorded in Masan on the south coast where the environmental quality is known to be poor. Prevalence of BRD was very low; only 4 clams out of 1,384 clams collected in spring and fall did show BRD. CI of clam varied widely, 0.274-0.840 in spring and 0.255-0.552 in autumn. CIs of clams from commercial beds were significantly higher that clams from natural habitats. Histology indicated that clams was in early developing to ripe in late April to mid May and the degree of gonad maturation also varied among clam beds. Quantification of the egg biomass using ELISA showed that some clams collected in May exhibited over 20% of their body weight as eggs and they were ready for spawning. In contrast, most clams in natural habitats did show 3-5% of GSI, indicating that they were in the developing stage. The data collected in this surveillance program were considered to be crucial in understanding current declining in clam production and can be used in proper management of the clam beds.

Flow cytometry used in the immune parameter study of marine molluscs.

Ludovic Donaghy, Hyun-Ki Hong, Bong-Kyu Kim, Kwang-Sik Choi
School of Applied Marine Sciences (POST BK-21), Jeju National University, 66 Jejudaehakno, Jeju 690-756, Republic of Korea
Email: ludovic.donaghy@gmail.com

Molluscan immune defense relies on cells freely circulating in hemolymph and tissues, reported as hemocytes. Characterization of hemocytes under natural ambient conditions is essential to further understand cellular mediated response of molluscs to environmental, anthropogenic-related and pathological stresses. Types and functions of molluscan hemocytes are not yet fully appreciated. Classification of hemocytes was commonly based on morphological and biochemical features analysed through light and electron microscopy. Alternatively, flow cytometry was more recently applied to determine molluscan hemocyte populations and immune-related activities. Contrastingly with microscopy, flow cytometry allows rapid, accurate and quantitative analysis of morphology and activities, at the single cell level. Nowadays, flow cytometry has successfully been applied in the investigation of cellular immune functions in several marine mollusc species. Understanding immune system of marine molluscs is crucial to prevent disease associated mortality and subsequently improve management of farms and fisheries.

Detection of aquatic animal viruses by loop-mediated isothermal amplification (LAMP)

Quingli Zhang
Yellow Sea Fisheries Research Institute
Email: zhangql@ysfri.ac.cn

Aquatic animal viruses are a constant threat to the sustainability and economic viability of aquaculture. Early diagnosis plays a vital role in management of fish and shellfish virus diseases. Traditionally, biochemical, serological and molecular biological tests have been used for aquatic animal virus diseases diagnosis. However, the high cost, precision type instruments required for such diagnoses makes it difficult for aqua-culturists to easily adopt them under production conditions. Recently, a novel technique called loop-mediated isothermal amplification (LAMP) has been developed. LAMP is a simple, rapid, specific and cost-effective nucleic acid amplification method compared to nucleic acid sequence-based amplification (PCR) and has been widely used in the detection of bacterial, viral, fungal and parasitic diseases in both animal and plants at present. In this study, we established LAMP detection method of several viruses of fish and crustaceans including turbot reddish body iridovirus (TRBIV), white spot syndrome virus (WSSV), Taura syndrome virus (TSV), yellow head disease (YHV), hepatopancreatic parvovirus (HPV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), Monodon baculovirus disease (MBV), acute viral necrosis virus (AVNV). Also, the LAMP detection methods of these viruses have been developed as commercial kits. The above viruses LAMP detection method are promising assay for extensive application for the rapid diagnosis of aquatic viruses in the laboratory and the field, especially in countries that lack the resources needed for molecular diagnostic techniques.

Key words: loop-mediated isothermal amplification, LAMP, aquatic viruses, detection method.

Special Session: Oil Spill impacts on coastal aquaculture

Unusual environmental changes caused by the Hebei Spirit oil spill in the Yellow Sea coastal ecosystem of Korea.

Hyung Chul Kim*, Wan Seok Lee*, Chung Il Lee**

*West Sea Fisheries Research Institute, National Fisheries Research & Development Institute, Incheon, 400-420, Republic of Korea
E-mail: hckim@nfrdi.go.kr
**Faculty of Marine Bioscience & Technology, Kangnung National University, Gangneung, 210-702, Republic of Korea

Oil concentration in seawater was investigated around the West Sea coastal ecosystem immediately after the Hebei Spirit oil spill occurred in December 2007, Korea. Comprehensive surveys on the marine impacts were conducted over a large subtidal area almost every day for two months. Follow up surveys were performed on a weekly and monthly basis for a year afterwards, after which they were conducted seasonally until now. The initial concentrations of oils were over 100 ㎍/L seawater and this lasted for several days, concentrations then decreased to about 10 ㎍/L, which is marine water quality standard in Korea, and these levels persisted until the end of December 2007. Polycyclic aromatic hydrocarbons (PAHs) in sediment were also analyzed to evaluate the contamination of the oils. The investigation was mainly conducted at the intertidal and subtidal regions every month around Taean coast, which are famous as marine culture grounds of species such as oysters and clams. The oil concentration of seawater in tidal flat culture grounds revealed that oil concentration had stabilized at below 0.1 ㎍/L in February(2.48~3.67℃) 2008, although these increased slightly through May to July as the temperature increased (16.59~26.47℃). The oils in pore water remained 6 to 16 times higher compared to the seawater overlying the tidal flat, which indicates that residual oils will continue to influence the impacted regions for the foreseeable future.

Temporary and unusual changes in the West Sea coastal ecosystem of Korea were revealed from the national monitoring data of Korean seas using ocean colour data. This data indicated that there were significant changes in chlorophyll a concentration due to oil spill, and chlorophyll a decreased about 45 to 50% compared concentrations before the spill, and this decrease continued about for two weeks. However, by January-February 2008, one or two months after the spill, the concentration had increased to higher than average when compared with the same period during 1998 to 2007.

Changes in benthic fauna caused by the oil spill in the Taean Coast in the Yellow Sea

Byoung-Seol KOH[1], Yong-Hyun KIM[1], Rae-Hong JUNG[2], Sang-Pil YOON[2], Dong-Woon HWANG[1], Jae-Hee SONG[1], Yeong-Jo JO[3] and Young-Je PARK[1]
[1]Tidal Flat Research Institute, National Fisheries Research & Development Institute, Soryongdong, Gunsan, Jeonbuk 573-882, Republic of Korea
E-mail: bskoh@nfrdi.go.kr
[2] Environment Research Division, National Fisheries Research & Development Institute
[3]South Sea Fisheries Research Institute, National Fisheries Research & Development Institute

After the Oil Spill (Hebei Spirit) occurred in Taean Coast in the Yellow Sea in 7th December 2007, the changes of macro benthic fauna were monitored over one year (430 days) by Tidal Flat Research Institute, NFRDI. Study sites were 2 sand beaches (Malipo, Shinduri) and 1 mud flat (Sogeunri) that were directly impacted by the oil spill. Quantitative samples were obtained with can corer (0.025 square meter & 30 cm of depth × 2 times) at each station that were situated at high, mid and low intertidal levels. To look at the initial change of macro benthic fauna, sampling was carried out every one or two weeks. And then, to look at the seasonal change, it was carried out every one or two months.

In the case of the sand beaches (Malipo, Shinduri), number of species was maintained below 20 species but abundances have slightly increased with time. Big changes of diversity index were not found during study period. At the mud flat (Sogeunri), the number of species was stayed below 15 species, although abundances have slightly increased over time with seasonal fluctuations. Big changes of diversity index were not been found at the either.

On the other hand, some sensitive species after oil spill accident were observed in the sand beaches as like Polychaeta (*Haploscoloplos elongatus, Armandia lanceolata, Pygospio* sp.), Crustacea (*Scopimera bitympana, Scopimera globosa, Eohaustorius spingerus*), Bivalvia (*Cycladicama cumingii*). These were abruptly appearing or disappearing species after the Oil Spill accident. In the mud flat station, Polychaeta (*Perinereis aibuhitensis*) and Crustacea (Mysidacea, *Macrophthalmus japonicus, Hemigrapsus penicillatus*) were sensitive species.

Additionally, the composition of Crustacean species and Errantian benthic polychaeta group gradually decreased during the initial period in all study sites after the oil spill. The increase in total abundance over time could be considered as caused by the new settlement of certain opportunistic species.

List of Participants
YSLME 2nd Regional Mariculture Conference, Jeju Island, Republic of Korea
16 – 18 June 2009

Alphabetical order

Zhenmin BAO 包振民
Key Laboratory of Marine Genetics and Breeding, Ocean University of China, Ministry of education. Ocean University of China, 5 Yushan Road,
Qingdao 266003,
People's Republic of China,
Tel: 86-532-82031960
Fax: 86-0532-82031960
Email: zmbao@ouc.edu.cn

Yaqing CHANG 常亚青
Key Lab of Mariculture,
Ministry of Agriculture,
Dalian Fisheries University,
Dalian 116023
Liaoning Province
People's Republic of China
Tel:
Fax:
Email: yqchang@dlfu.edu.cn

Chi-Kyu CHOI
President,
Cheju National University
66 Jejudaehakno, Jeju 690-756,
Republic of Korea
Tel: 82-64-754-3422
Mob:
Fax:
Email:

Kwang-Sik CHOI
Professor
School of Applied Marine Science
Cheju National University,
66 Jejudaehakno, Jeju 690-756,
Republic of Korea
Tel: 82-64-754-3422
Mob: 011-699-9000
Fax: 82-64-756-3493
Email: skchoi@cheju.ac.kr

Mahanama DE ZOYSA
PhD student
School of Applied Marine Science
Jeju National University,
Jeju, 690-756,
Republic of Korea
Tel: +82-64-754-3472
Fax: +82-64-756-3493
Email: mahanama_dezoysa@yahoo.com

Ludovic DONAGHY
Researcher
School of Applied Marine Science
Cheju National University,
66 Jejudaehakno, Jeju 690-756,
Republic of Korea
Tel: 82-64-754-3422
Fax: 82-64-756-3493
Email: ludovic.donaghy@gmail.com

Jiang Guang FANG 方建光
Director
Mariculture Ecology and Carrying Capacity Division
Yellow Sea Fisheries Research Institute (YSFRI), CAFS
106 Nanjing Road,Qingdao 266071,
People's Republic of China
Tel: 86-532-8582-2957
Fax: 86-532-8581-1514
Email: fangjg@ysfri.ac.cn

In Kwon JANG
Director
West Sea Mariculture Research Center, NFRDI
Sinjindo-ri 75, Geunheung-myeon,
Taean-gun, Chungnam 357-945,
Republic of Korea
Tel: 82-41-675-3773
Mob: 010-6477-3511
Fax: 82-41-675-7077
Email: jangik@nfrdi.go.kr

Zengjie JIANG 蒋增杰
Mariculture Ecology and Carrying Capacity Division,
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fisheries Science,
Qingdao, 266071,
People's Republic of China
Tel: 86-532-8582-2957
Fax: 86-532-8581-1514
Email: jiangzj@ysfri.ac.cn

Jae Yoon JO
Professor
Dept. of Aquaculture
Pukyong National University,
599-1 Daeyeon 3-dong,
Nam-gu, Busan 608-737,
Republic of Korea
Tel: 82-51-629-5913
Mob: 011-839-6131
Fax: 82-51-629-5992
Email: jyjo@pknu.ac.kr

Je Cheon JUN
Scientist
Dept of Resource Enhancement Research
West Sea Fisheries Research Institute,
NFRDI, 707 Eulwang-dong, Jung-gu,
Incheon 400-420,
Republic of Korea
Tel: 82-32-745-0631
Mob: 016-771-3587
Fax: 82-32-745-0619
Email: jcj21@nfrdi.go.kr

Chang Gil KIM
Senior Scientist
Dept of Resource Enhancement Research
West Sea Fisheries Research Institute,
NFRDI
707 Eulwang-dong, Jung-gu, Incheon 400-420, Republic of Korea
Tel: 82-32-675-0631
Mob: 010-5195-7192
Fax: 82-32-745-0619
Email: cgkim@nfrdi.go.kr.

Hyung Chul KIM
Researcher
West Sea Fisheries Research Institute,
National Fisheries Research &
Development Institute,
Incheon, 400-420,
Republic of Korea
Tel: 82-32-745-0675
Mob: 010-5064-5686
Fax: 82-32-745-0678
E-mail: hckim@nfrdi.go.kr

Jeong-Dae KIM
Professor
Department of Animal Life System,
College of Animal Life Sciences,
Kangwon National University, Chuncheon
200-701, Republic of Korea
Tel: 82-33-250-8634
Fax: 82-33-255-5482
Mob: 011-360-5172
Email: jdekim@kangwon.ac.kr

Jongsheek KIM
Scientist
West Sea Mariculture Research Center,
NFRDI, Sinjindo-ri 75, Geunheung-
myeon, Taean-gun, Chungnam 357-945,
Republic of Korea
Tel: 82-41-675-3773
Mob: 010-5195-7192
Fax: 82-41-675-7077
Email: philllip@nfrdi.go.kr

Kyung-Kil KIM
Scientist
Biotechnology Research Division ,
National Fisheries Research &
Development Institute (NFRDI),
Republic of Korea
Tel: 82-51-720-2440
Mob: 011-9551-0628
Fax: 82-51-720-2456
Email: k48722@nfrdi.go.kr

Byoung-Seol KOH
Scientist
Tidal Flat Research Institute,
National Fisheries Research &
Development Institute,
Soryongdong,
Gunsan, Jeonbuk 573-882,
Republic of Korea
Tel: 82-63-465-2675
Mob: 010-3109-3838
Fax: 82-63-467-2675
E-mail: bskoh@nfrdi.go.kr

Jie KONG 蒋增杰
Professor
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
Nanjing Road 106,
Qingdao, 266071
People's Republic of China
Tel : 86-532-8582-3291
Mob: 13605426806
Fax: 86-532-85811514
Email: kongjie@sina.com

Jeong-Ho LEE
Scientist
Genetics and Breeding Research Center,
National Fisheries Research &
Development Institute,
Geoje, Gyeongnam, 656-842,
Republic of Korea
Tel: 82-55-633-1272
Mob: 010-6258-9939
Fax: 82-55-633-0891
Email: jhlee@nfrdi.go.kr

Jung Uie LEE
Director,
Aquaculture Industry Department,
National Fisheries Research &
Development Institute,
Republic of Korea
Tel: 82-51-720-2401
Mob: 011-9661-2234
Fax: 82-51-720-2439
Email: fishkorea@nfrdi.go.kr

Mei-zhen LI 李美真
Professor
Shandong Mariculture Institute,
Qingdao, 266002
People's Republic of China
Tel: 86-532-86558291
Fax: 86-532-82675569
Email: li-meizhen@163.com

Sheng LUAN 栾生
Researcher
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
Nanjing Road 106,
Qingdao, 266071
People's Republic of China
Tel : 86-532-8582-3291
Email: luansheng@ysfri.ac.cn

Xianhong MENG 孟宪红
Scientist
Researcher
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
Nanjing Road 106,
Qingdao, 266071
People's Republic of China
Tel : 86-532-8582-3291-803
Mob: 13697679828
Fax : 86-532-85811514
Email: mengxh@ysfri.ac.cn

Mi Seon PARK
Vice-President
National Fisheries Research &
Development Institute,
408-1 Shirang-ri, Gijang-eup,
Gijiang-gun, Busan 619-902,
Republic of Korea
Tel: 82-51-720-2800
Mob: 011-9339-7483
Fax: 82-51-720-2828
Email: parkms@nfrdi.go.kr

Myong Ae PARK
Director,
Dept of Pathology Research,
National Fisheries Research &
Development Institute, 408-1 Shirang-ri,
Gijang-eup, Gijiang-gun,
Busan 619-902,
Republic of Korea
Tel: 82-51-720-2470
Mob: 011-579-1308
Email: mapark@nfrdi.go.kr

Cheng-Yin SHI
Professor
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences,
Nanjing Road 106,
Qingdao, 266071
People's Republic of China
Tel: 86-532-8582-3062
Fax: 86-532-8582-1514
Email: shicy@ysfri.ac.cn

Huiling SUN 孙惠玲
Professor,
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
Qingdao 266071,
People's Republic of China
Tel: 86-532-8581-9199
Fax: 86-532-8581-1514
Email: sunhl@ysfri.ac.cn

Guang-Ce WANG 王广策
Institute of Oceanology,
Chinese Academy of Sciences,
Qingdao 266071,
People's Republic of China
Tel: 13505326219
Fax:
Email: gcwang@ms.qdio.ac.cn

Qinyin WANG
Director of YSFRI
Yellow Sea Fisheries Research Institute,
CAFS, Nanjing Road 106, Qingdao,
Shandong province 266071,
People's Republic of China
Tel: 86-532-8582-2959
Fax: 86-532-8581-1514
Email: qywang@public.qd.sd.cn

Jihong ZHANG 张继红
Associate professor
Department of Mariculture Ecology and
Carrying Capacity, YSFRI, Nanjing Road
106, Qingdao, Shandong province 266071,
People's Republic of China
Tel: 86-532-8582-2957
Fax: 86-532-8581-1514
Email: zhangjh@ysfri.ac.cn

Qingli ZHANG 张庆利
Researcher
Yellow Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences
Nanjing Road 106, Qingdao, 266071
People's Republic of China
Tel: 86-532-8582-3062
Fax: 86-532-8582-1514
Email: zhangql@ysfri.ac.cn

SECRETARIAT

Ms Junghwa KIM
Secretary
UNDP/GEF "Reducing environmental stress in the Yellow Sea Large Marine Ecosystem.
KORDI Compound, 1270, Sa2-dong,
Sangnok-gu, Ansan-si,
Gyeonggi-do, 426-744
Republic of Korea
Tel: (82-31) 400 7820
Fax: (82-31) 400 7826
email: junghwa@yslme.org

Mark WALTON
Fisheries Officer
UNDP/GEF "Reducing environmental stress in the Yellow Sea Large Marine Ecosystem.
KORDI Compound, 1270, Sa2-dong,
Sangnok-gu, Ansan-si,
Gyeonggi-do, 426-744,
Republic of Korea
Tel: (82-31) 400 7832
Fax: (82-31) 400 7826
email: mark@yslme.org